CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

205718Orig1s000

PHARMACOLOGY REVIEW(S)

ADDENDUM TO PHARMACOLOGY/TOXICOLOGY REVIEW OF NDA 205,718 DATED June 19, 2014

Two excipients in the drug product (AKYNZEO), [redacted] polyglyceryl oleate [redacted] were described as "Not present in Database" (i.e. FDA Inactive Ingredient Database) in table 2, on page 16 of the Pharmacology/Toxicology review of this NDA (this table also appears in the CMC review). The following safety assessment of these excipients is provided on page 16 of the Pharmacology/Toxicology review:

[redacted] and polyglyceryl oleate are present in the Aloxi capsules ([redacted]/capsule and [redacted] capsule, respectively) at amounts [redacted] than those in AKYNZEO [redacted] mg/capsule, respectively). Therefore, there is a reasonable assurance of safety for these excipients based on the previous human experience with Aloxi capsules."

However, after revisiting the database, it was discovered that both of these excipients are listed under different names. [redacted] is listed as "CAPRYLIC/ CAPRIC MONO/DI-GLYCERIDES" at the maximum amount of [redacted] per capsule. Polyglyceryl oleate is listed as [redacted] at the maximum amount of [redacted] per capsule, and [redacted] at the maximum amount of [redacted] per capsule. The specific polyglyceryl oleate used in AKYNZEO [redacted] is identified on the website of its supplier [redacted]. Therefore, we amend here that these two excipients are in fact present in the FDA Inactive Ingredient Database at the amounts mentioned above, which are much higher than those in the drug product ([redacted] capsule for [redacted] and [redacted] capsule for polyglyceryl oleate).

Patients will ingest only one AKYNZEO capsule on the first day of each cycle of chemotherapy, based on the recommended dosage and administration. Therefore, the information cited from the FDA Inactive Ingredient Database provides a reasonable assurance of safety for [redacted] and [redacted] ([redacted] Our original safety assessment of these excipients remains valid (i.e. a reasonable assurance of safety is derived from previous human experience).

Ke Zhang, Ph.D.
Pharmacologist
Division of Gastroenterology and Inborn Errors Products

David B. Joseph, Ph.D.
Lead Pharmacologist
Division of Gastroenterology and Inborn Errors Products

cc:
NDA 205,718
DGIEP
DGIEP/PM
DGIEP/D. Griebel
DGIEP/D. Joseph
DGIEP/K. Zhang
ONDQA/M. Kowblansky
ONDQA/R. Frankewich
R/D Init.: D. Joseph 9/15/14

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/s/

KE ZHANG
09/18/2014

DAVID B JOSEPH
09/18/2014
I concur. The issues described above were also addressed in an addendum to the CMC review.

MEMORANDUM

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

FROM: David B. Joseph
Lead Pharmacologist

DATE: July 17, 2014

SUBJECT: NDA 205,718 (SD # 1 dated September 25, 2013)

Sponsor: Helsinn Healthcare SA

Drug Product: AKYNZEO™ (netupitant and palonosetron) capsules

Comments:

I concur with Dr. Zhang's recommendations for conducting a juvenile rat toxicity study with netupitant alone to support the proposed pediatric development program (see section 1.3.2 in the Executive Summary and page 167 of the Pharmacology/Toxicology review dated June 19, 2014). Based on the age range (0 < 17 years) of patients to be included in the proposed safety and efficacy trial of netupitant + palonosetron fixed dose combination, dosing in the juvenile rat study should begin at a developmental stage comparable to the human neonatal stage. Juvenile animal studies with the drug combination are not considered as necessary to support the pediatric development program for Akynzeo, based on results of the combination toxicity studies of netupitant + palonosetron in adult rats and dogs, and the toxicity profile of palonosetron alone in neonatal rats and dogs (see Pharmacology/Toxicology review of NDA 21,372 dated July 11, 2003 by Dr. Yash Chopra).

Recommendations:

There are no nonclinical issues which preclude the approval of Akynzeo. I concur with Dr. Zhang's recommendation for approval and his recommended revisions in the label.

_____ _____
David B. Joseph, Ph.D. Date
Lead Pharmacologist
Division of Gastroenterology and Inborn Errors Products

cc:
NDA 205,718
DGIEP
DGIEP/PM
DGIEP/D. Joseph
DGIEP/K. Zhang
DGIEP/R. He
OND IO/A. Jacobs

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/s/

DAVID B JOSEPH
07/17/2014

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:	205,718
Supporting document/s:	1
Applicant's letter date:	September 25, 2013
CDER stamp date:	September 25, 2013
Product:	AKYNZEO™ (netupitant and palonosetron) capsules
Indication:	Prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy and moderately emetogenic cancer chemotherapy in adults
Applicant:	Helsinn Healthcare SA Pazzallo – Lugano, Switzerland
	U.S. Agent August Consulting, Inc. Austin, TX
Review Division:	Division of Gastroenterology and Inborn Errors Products (DGIEP)
Reviewer:	Ke Zhang, Ph.D.
Supervisor/Team Leader:	David Joseph, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Mary Chung

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 205,718 are owned by Helsinn Healthcare SA or are data for which Helsinn Healthcare SA has obtained a written right of reference. Any information or data necessary for approval of NDA 205,718 that Helsinn Healthcare SA 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 this NDA.

TABLE OF CONTENTS

1. Executive Summary

1.1 Introduction

AKYNZEO capsule is a fixed-dose combination of two active drug substances, netupitant and palonosetron HCl. Netupitant is a substance P/NK-1 (neurokinin-1) receptor antagonist. Palonosetron is a 5-HT3 receptor antagonist. Oral palonosetron, ALOXI (palonosetron HCl) Capsule is an approved drug in the U.S. for prevention of acute nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy (NDA 22,233). The sponsor developed AKYNZEO as a fixed-dose combination since the mechanism of action of the two drugs is exerted on different neuropathways (5-HT3 receptors and NK1 receptors) and both drugs show a similar pharmacokinetic profile in terms of extended plasma half-life. The sponsor seeks market approval for AKYNZEO for prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy and moderately emetogenic cancer chemotherapy in adults.

1.2 Brief Discussion of Nonclinical Findings

Netupitant alone was tested in oral toxicity studies of up to 26 weeks in rats and 9 months in dogs. Treatment with netupitant induced phospholipidosis at doses of 10 mg/kg/day or higher in both rats and dogs. The clinical significance of phospholipidosis in these studies is not clear. The calculated animal to human AUC multiples for netupitant based on the AUC values at 10 mg/kg/day in both rats and dogs ranged from 0.4 to 1.8. Oral toxicity studies with the combination of netupitant and palonosetron were performed in rats and dogs for up to 13 weeks. The combination did not produce any additional toxicity as compared with either drug tested alone.

Netupitant was negative in the Ames test, mouse lymphoma cell mutation assay, and *in vivo* rat micronucleus test. Long-term studies in animals to evaluate the carcinogenic potential of netupitant are not needed to support approval for the proposed indication (see Executive CAC meeting minutes from July 1, 2008 in Appendix). Therefore, no carcinogenicity studies with netupitant were conducted.

Daily oral administration of netupitant at 10 mg/kg/day and higher during the period of organogenesis increased the incidence of external and skeletal abnormalities in rabbit fetuses. These abnormalities included positional abnormalities in the limbs and paws, and fused sternebrae.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical standpoint, this NDA should be approved for the proposed indication.

1.3.2 Additional Nonclinical Recommendations

An oral toxicity study with netupitant alone of at least 8 weeks duration in juvenile rats is needed to support the proposed pediatric clinical efficacy study in patients age 0 to < 17 years. The juvenile rat study should include evaluation of developmental parameters, neurobehavioral effects, and fertility. The sponsor should submit the juvenile rat study protocol for review and evaluation prior to initiation of this study. The sponsor's proposed timeline for the pediatric study plan should be adjusted according to these recommendations.

1.3.3 Labeling

Established Pharmacologic Class (HIGHLIGHTS and section 11)

The EPC text phrase for netupitant in the Sponsor's proposed label is: "substance P/neurokinin 1 (NK_1) receptor antagonist", which is very similar to the EPC text phrase shown for aprepitant in the PRPLLR, and identical to the EPC text phrase in the aprepitant label. Therefore, the proposed EPC text phrase for netupitant is acceptable.

The EPC text phrase for palonosetron in the Sponsor's proposed label is: (b) (4) However, this should be changed to "serotonin-3 ($5\text{-}HT_3$) receptor antagonist", which is the EPC text phrase shown for palonosetron in the PRPLLR.

Sponsor's Version:

8.1. Pregnancy

(b) (4)

Evaluation:

(b) (4)

Recommended Version:

8.1. Pregnancy

Pregnancy Category C

Risk Summary

Adequate and well-controlled studies with AKYNZEO have not been conducted in pregnant women. In animal reproduction studies, no effects on embryo-fetal development were observed following daily administration of netupitant in pregnant rats during the period of organogenesis at doses up to 3.7 times the human AUC at the recommended single human dose to be given with each cycle of chemotherapy. However, a dose-dependent increase in adverse effects on embryo-fetal development was observed following daily administration of netupitant in pregnant rabbits during the period of organogenesis with doses at least 0.2 times the human AUC at the recommended single human dose to be given with each cycle of chemotherapy. Daily administration of netupitant in rats up to 3.7 times the human AUC at the recommended human dose during organogenesis through lactation produced no adverse effects in the offspring. In animal reproduction studies with palonosetron, no effects on embryo-fetal development were observed following oral administration during the period of organogenesis at doses up to 921 and 1841 times the recommended human oral dose in rats and rabbits, respectively. AKYNZEO should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal Data

Daily administration of up to 30 mg/kg netupitant in rats (3.7 times the human AUC at the recommended single human dose to be given with each cycle of chemotherapy) during the period of organogenesis produced no effects on embryo-fetal development. However, an increased incidence of external and skeletal abnormalities in rabbit fetuses was observed following daily administration of netupitant in rabbits at 10 mg/kg/day and higher (0.2 times the human AUC at the recommended single human dose to be given with each cycle of chemotherapy) during the period of organogenesis. These

abnormalities included positional abnormalities in the limbs and paws, and fused sternebrae. Reduction in fetal rabbit weight occurred at 30 mg/kg/day. Maternal toxicity in rabbits (i.e. loss of bodyweight during the treatment period) was also observed at 30 mg/kg/day. Daily administration of up to 30 mg/kg netupitant (3.7 times the human AUC at the recommended human dose) in rats during organogenesis through lactation produced no adverse effects in the offspring.

In animal reproduction studies with palonosetron, no effects on embryo-fetal development were observed in pregnant rats given oral doses up to 60 mg/kg/day (921 times the recommended human oral dose based on body surface area) or pregnant rabbits given oral doses up to 60 mg/kg/day (1841 times the recommended human oral dose based on body surface area) during the period of organogenesis.

Sponsor's Version:

12.1 Mechanism of action

Netupitant is a selective (b) (4) antagonist of human substance P/neurokinin 1 (NK_1) receptors. (b) (4)

Palonosetron is a 5-HT_3 receptor antagonist with a strong binding affinity for this receptor and little or no affinity for other receptors. Cancer chemotherapy may be associated with a high incidence of nausea and vomiting, particularly when certain agents, such as cisplatin, are used. 5-HT_3 receptors are located on the nerve terminals of the vagus in the periphery and centrally in the chemoreceptor trigger zone of the area postrema. Chemotherapeutic agents produce nausea and vomiting by stimulating the release of serotonin from the enterochromaffin cells of the small intestine. Serotonin then activates 5-HT_3 receptors located on vagal afferents to initiate the vomiting reflex. The development of acute emesis is known to depend on serotonin and its 5-HT_3 receptors have been demonstrated to selectively stimulate the emetic response.

Delayed emesis has been largely associated with the activation of tachykinin family neurokinin 1 (NK_1) receptors (broadly distributed in the central and peripheral nervous systems) by substance P. As shown in *in vitro* and *in vivo* studies, netupitant (b) (4) (b) (4) inhibition of substance P mediated response. (b) (4)

Evaluation:

The follow sentence should be deleted: (b) (4) Although the sponsor submitted

(b) (4) that is supportive of this statement, the review team determined that the statement does not add important information to the labeling. The review team also recommended deletion of the following sentence: (b) (4) The rest of the statements relevant to mechanism of action for palonosetron are similar to those included in the labels for ALOXI Injection and ALOXI Capsules, and are therefore acceptable.

Recommended Version:

12.1 Mechanism of Action

Netupitant is a selective (b) (4) antagonist of human substance P/neurokinin 1 (NK_1) receptors.

Palonosetron is a 5-HT_3 receptor antagonist with a strong binding affinity for this receptor and little or no affinity for other receptors. Cancer chemotherapy may be associated with a high incidence of nausea and vomiting, particularly when certain agents, such as cisplatin, are used. 5-HT_3 receptors are located on the nerve terminals of the vagus in the periphery and centrally in the chemoreceptor trigger zone of the area postrema. Chemotherapeutic agents produce nausea and vomiting by stimulating the release of serotonin from the enterochromaffin cells of the small intestine. Serotonin then activates 5-HT_3 receptors located on vagal afferents to initiate the vomiting reflex. The development of acute emesis is known to depend on serotonin and its 5-HT_3 receptors have been demonstrated to selectively stimulate the emetic response.

Delayed emesis has been largely associated with the activation of tachykinin family neurokinin 1 (NK_1) receptors (broadly distributed in the central and peripheral nervous systems) by substance P. As shown in *in vitro* and *in vivo* studies, netupitant (b) (4) (b) (4) inhibition of substance P mediated response. (b) (4)

Sponsor's Version:

12.3 Pharmacokinetics

(b) (4)

(b) (4)

Evaluation:

The following sentence should be deleted: (b) (4)

(b) (4)

(b) (4) the review team recommended that a statement be added to indicate that M1, M2 and M3 metabolites bind to NK-1 receptors.

Other revisions were recommended by the Clinical Pharmacology team, as shown below.

Recommended Version:

Metabolism

Once absorbed, netupitant is extensively metabolized to form three major metabolites: desmethyl derivative, M1; N-oxide derivative, M2; OH-methyl derivative, M3. M1, M2 and M3 metabolites were all shown to bind to the substance P/neurokinin 1 (NK_1) receptor.

(b) (4) Mean C_{max} was approximately 11 %, 47 % and 16 % of netupitant for metabolites M1, M2 and M3, respectively. Mean AUC for metabolites M1, M2, and M3 was 29%, 14% and 33% of netupitant, respectively.

Sponsor's Version:

13.1 Carcinogenesis, Mutagenesis and Impairment of Fertility

Netupitant

(b) (4)

Palonosetron
In a 104-week carcinogenicity study in CD-1 mice, animals were treated with oral doses of palonosetron at 10, 30 and 60 mg/kg/day. Treatment with palonosetron was not tumorigenic. The highest tested dose produced a systemic exposure to palonosetron (Plasma AUC) of about 90 to 173 times the human exposure (AUC= 49.7 ng•h/mL) at the recommended oral dose of 0.5 mg. In a 104 week carcinogenicity study in Sprague-Dawley rats, male and female rats were treated with oral doses of 15, 30 and 60 mg/kg/day and 15, 45 and 90 mg/kg/day, respectively. The highest doses produced a systemic exposure to palonosetron (Plasma AUC) of 82 and 185 times the human exposure at the recommended dose. Treatment with palonosetron produced increased incidences of adrenal benign pheochromocytoma and combined benign and malignant pheochromocytoma, increased incidences of pancreatic Islet cell adenoma and combined adenoma and carcinoma and pituitary adenoma in male rats. In female rats, it produced hepatocellular adenoma and carcinoma and increased the incidences of thyroid C-cell adenoma and combined adenoma and carcinoma.

Palonosetron was not genotoxic in the Ames test, the Chinese hamster ovarian cell (CHO/HGPRT) forward mutation test, the ex vivo hepatocyte unscheduled DNA synthesis (UDS) test or the mouse micronucleus test. It was, however, positive for clastogenic effects in the Chinese hamster ovarian (CHO) cell chromosomal aberration test. Palonosetron at oral doses up to 60 mg/kg/day (about 921 times the recommended human oral dose based on body surface area) was found to have no effect on fertility and reproductive performance of male and female rats.

Evaluation:

The proposed subsection for netupitant should be revised as shown below. The rat to human AUC ratios for netupitant were calculated based on the following AUC values: [redacted] (b) (4) (this value was recommended for inclusion in subsection 12.3 by the Clinical Pharmacology team). The proposed subsection for palonosetron is the same as shown in the label for ALOXI Capsules, and is therefore acceptable.

Recommended Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Netupitant

Long-term studies in animals to evaluate carcinogenic potential have not been performed with netupitant. Netupitant was not genotoxic in the Ames test, the mouse lymphoma cell mutation test, or the *in vivo* rat micronucleus test.

Daily oral administration of netupitant in rats at doses up to 30 mg/kg (1.9 times the human AUC in male rats and 3.7 times the human AUC in female rats at the recommended human dose) had no effects on fertility or reproductive performance.

Palonosetron

In a 104-week carcinogenicity study in CD-1 mice, animals were treated with oral doses of palonosetron at 10, 30 and 60 mg/kg/day. Treatment with palonosetron was not tumorigenic. The highest tested dose produced a systemic exposure to palonosetron (Plasma AUC) of about 90 to 173 times the human exposure (AUC= 49.7 ng·h/mL) at the recommended oral dose of 0.5 mg. In a 104-week carcinogenicity study in Sprague-Dawley rats, male and female rats were treated with oral doses of 15, 30 and 60 mg/kg/day and 15, 45 and 90 mg/kg/day, respectively. The highest doses produced a systemic exposure to palonosetron (Plasma AUC) of 82 and 185 times the human exposure at the recommended dose. Treatment with palonosetron produced increased incidences of adrenal benign pheochromocytoma and combined benign and malignant pheochromocytoma, increased incidences of pancreatic Islet cell adenoma and combined adenoma and carcinoma and pituitary adenoma in male rats. In female rats, it produced hepatocellular adenoma and carcinoma and increased the incidences of thyroid C-cell adenoma and combined adenoma and carcinoma.

Palonosetron was not genotoxic in the Ames test, the Chinese hamster ovarian cell (CHO/HGPRT) forward mutation test, the *ex vivo* hepatocyte unscheduled DNA synthesis (UDS) test or the mouse micronucleus test. It was, however, positive for clastogenic effects in the Chinese hamster ovarian (CHO) cell chromosomal aberration test.

Palonosetron at oral doses up to 60 mg/kg/day (about 921 times the recommended human oral dose based on body surface area) was found to have no effect on fertility and reproductive performance of male and female rats.

2 Drug Information

2.1 Drug

Trade Name: AKYNZEO™ (300 mg netupitant and 0.5 mg palonosetron free base equivalent)

Code Name:

Netupitant: RO0673189, RO0673189-008, 14-NETU
Palonosetron HCl: RS-25259-197

Chemical Name:
Netupitant: 2-[3,5-Bis(trifluoromethyl)phenyl]-N,2-dimethyl-N-[4-(2-methylphenyl)-6-(4-methylpiperazin-1-yl)pyridin-3-yl]propanamide

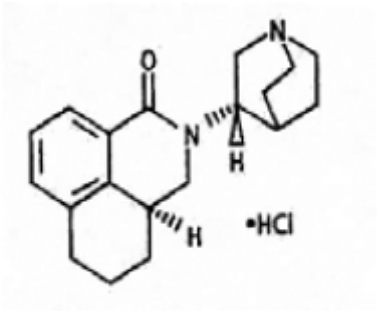
Palonosetron HCl: (3aS)-2-[(S)-1-Azabicyclo[2.2.2]oct-3-yl]-2,3,3a,4,5,6-hexahydro-1-oxo-1Hbenz[de]isoquinoline hydrochloride

Molecular Formula/Molecular Weight:

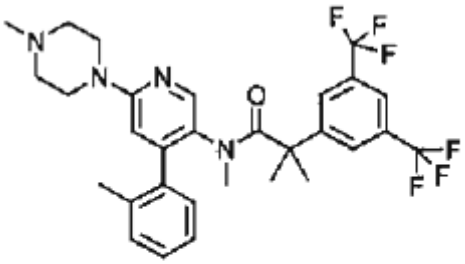
Netupitant: $C_{30}H_{32}F_6N_4O$ / 578.61

Palonosetron HCl: $C_{19}H_{24}N_2O$•HCl / 332.87

Structure or Biochemical Description:



Palonosetron HCl



Netupitant

Pharmacologic Class:

Netupitant is a NK1 receptor antagonist

Palonosetron is a 5-HT3 receptor antagonist.

2.2 Relevant INDs, NDAs, and DMFs: IND 73,493

2.3 Drug Formulation

AKYNZEO is a fixed-dose combination product composed of one hard gelatin capsule, Size 0, which contains three netupitant tablets (100 mg each) and one palonosetron HCl softgel (0.50 mg free base equivalent).

Table 1 Composition of the Netupitant-Palonosetron Combination Capsule

Ingredient	Reference	Function	%w/w	Quantity (mg)
Intermediate Netupitant Tablet				
Netupitant	Internal (b) (4)	Active ingredient (b) (4)		(b) (4)

Ingredient	Reference	Function	%w/w	Quantity (mg)
Intermediate Palonosetron Softgel				
(b) (4)				
Palonosetron HCl	Internal	Active ingredient		(b) (4)
Glycerol (b) (4) (b) (4)	Ph. Eur.	(b) (4)		
Glycerin (b) (4)	USP/Ph. Eur			
Polyglyceryl oleate (b) (4) (b) (4)	Internal			
Purified water	USP/Ph. Eur			
Butylated hydroxyanisole (BHA)	NF/Ph. Eur			
(b) (4)	NF/Ph. Eur.			
Theoretical Fill Weight	---	---		(b) (4)
(b) (4)				
Netupitant-Palonosetron Combination Capsule				
Size 0 hard gelatin capsule, white body/caramel cap, HE1 printed in black on the white body[6]	Internal	Capsule shell	---	1 capsule

(b) (4)

Table 2 from CMC Review of NDA 205718 – Excipients in Netupitant / Palonosetron Capsules

Excipient	Reference	Function	Amount per day (one capsule) (mg)	Database[1] maximum per capsule[2] (mg)
Microcrystalline cellulose (b) (4)	NF/Ph. Eur	(b) (4)		
Sucrose (b) (4) acid esters	Internal			
Povidone K-30	USP/Ph. Eur.			
Croscarmellose sodium	NF / Ph. Eur.			
Silicon dioxide (b) (4)	NF / Ph. Eur.			
Sodium stearyl fumarate	NF / Ph. Eur.			
Magnesium stearate (b) (4)	NF / Ph. Eur.			
(b) (4)	Ph. Eur.			
Glycerin (b) (4))	USP/Ph. Eur.			
Polyglyceryl oleate (b) (4)	Internal			
Butylated hydroxyanisole (BHA)	NF / Ph. Eur.			

1: FDA Inactive Ingredient Search for http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm

2: Maximum amount of this material in any dosage form recognized in the database for a name beginning with "oral: capsule".

(b) (4)

(b) (4) and polyglyceryl oleate are present in the Aloxi capsules ((b) (4) /capsule and (b) (4) /capsule, respectively) at (b) (4) than those in AKYNZEO ((b) (4) /capsule, respectively). Therefore, there is a reasonable assurance of safety for these excipients based on the previous human experience with Aloxi capsules.

Sucrose (b) (4) acid esters are accepted for use as a food additive within the conditions described in 21 CFR 172.859. Thus, there is reasonable assurance of safety for this excipient.

2.4 Comments on Novel Excipients:

Although sucrose (b) (4) acid esters is considered as a novel excipient, it is used as a food additive within the conditions described in 21 CFR 172.859. Therefore, there is reasonable assurance of safety for this excipient.

2.5 Comments on Impurities/Degradants of Concern: None

2.6 Proposed Clinical Population and Dosing Regimen

AKYNZEO is indicated for prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy and for prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy.

The proposed dose is one capsule administered approximately one hour prior to the start of chemotherapy. One capsule contains 300 mg netupitant and 0.5 mg palonosetron free base equivalent.

Regulatory Background

AKYNZEO (netupitant and palonosetron) capsule was developed under IND 73,493. In the pre-NDA meeting on April 16, 2013, the sponsor agreed to submit final study reports of all required nonclinical studies. All needed study reports were submitted in this NDA (see below).

Netupitant is an NME. Oral palonosetron, ALOXI (palonosetron HCl) Capsule, is an approved drug product in the U.S. for prevention of acute nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy (NDA 22,233). ALOXI Injection is an approved drug product for intravenous administration, indicated for the prevention of acute nausea and vomiting associated with initial and repeat courses of moderately and highly emetogenic cancer chemotherapy, and the prevention of delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy (NDA 21,372).

3 Studies Submitted

Pharmacology
Pharmacokinetics/ADME
Toxicology

Table 1: Overview of Toxicology Studies Performed with Netupitant and Netupitant/Palonosetron Combination

Study No.	Species	Route	Duration of treatment	Doses (mg/kg/day)	GLP
Netupitant Acute Studies					
NETU-07-23	Mouse	p.o	Single dose	1000, 2000	Yes
1009566	Rat	p.o	Single dose	500,1000,1500,2000	Yes
1009567	Dog	p.o	Single dose	200,300,400	Yes
B-167720	Dog	p.o	Single dose	0, 3, 10, 30, 30, 60, 100 and 150	No
Netupitant Repeated Dose Studies (Non-pivotal)					
NETU-07-24	Mouse	p.o.	7-day	0, 30, 120, 450	No
NETU-07-25	Mouse	p.o.	4-week	3,10,30	Yes
NETU-07-26	Mouse	p.o.	13-week (plus 8-week recovery)	0, 1, 3, 10	Yes

Study No.	Species	Route	Duration of treatment	Doses (mg/kg/day)	GLP
Netupitant Repeated Dose Studies (Non-pivotal)					
1007326	Rat	p.o.	14-day 1,3,5,9,14-day (plus recovery)	0, 30, 100, 300	No
B-167719	Rat	p.o.	15-day	0, 30, 100, 300	No
B-167723	Rat	p.o.	14-day	0, 30, 100	No
1003562	Rat	p.o.	4-week	0, 10,100	No
1006011	Rat	p.o.	4-week (plus 4-week recovery)	0, 3, 10,30	Yes
1006679	Dog	p.o	7-day (plus 2-month recovery)	0, 5, 15	Yes
1006010	Dog	p.o	4-week (plus 4-week recovery)	0, 1, 3, 5, 15, 50	Yes
Netupitant Repeated Dose Studies (Pivotal)					
161-578S	Rat	p.o.	13-week continuous and intermittent (plus 8-week recovery)	3,10,30	Yes
NETU-07-21	Rat	p.o.	26-week (plus 8-week recovery)	0,1,3,10	Yes
1009175	Dog	p.o.	13-week continuous and intermittent (plus 8-week recovery)	0, 1, 3, 10	Yes
NETU-07-22	Dog	p.o.	9 months (plus 8-week recovery)	0, 1, 3, 10	Yes
Netupitant/palonosetron Combination Repeated Dose Toxicity Studies (Non-pivotal)					
NETU-06-20	Rat	p.o.	7-day	18P/10N, 60P/30N	Yes
NETU-06-03	Rat	p.o.	4-week (plus 8-week recovery)	10P/3N,18P/10N, 60P/30N	Yes
NETU-06-21	Dog	p.o.	7-day	10P/3N, 20P/15N	Yes
NETU-06-05	Dog	p.o.	4-week (plus 8-week recovery)	10P/3N, 15P/7.5N, 20(15**)P/15N	Yes

Study No.	Species	Route	Duration of treatment	Doses (mg/kg/day)	GLP
Netupitant Intravenous Studies					
1006118	Rat	i.v.	3-day	1,5,10,2.5-25	No
1007386	Rat	i.v.	1-week	3,10,30	No
1008327	Rat	i.v.	14 -day	0, 3, 9, 30	Yes
1006263	Dog	i.v.	3-day	1,2,3	No
1008498	Dog	i.v.	3-day	0.50.100	Yes
1008328	Dog	i.v.	14-day	0,1,3,10	Yes
Netupitant/palonosetron Combination Repeated Dose Toxicity Studies (Pivotal)					
NETU-07-19	Rat	p.o.	13-weeks (plus 8-week recovery)	0,2P/1N,6P/3N, 18P/10N 18P only, 10N only	Yes
NETU-07-18	Dog	p.o.	13-weeks (plus 8-week recovery)	0,3P/1N, 5P/3N, 10P/10N	Yes
Netupitant, specified impurities and metabolite M4 Genotoxicity Studies					
1004078	Ames in vitro	-	-	2 – 200 µg/plate	Yes
1006128	Ames in vitro	-	-	1 – 500 µg/plate	Yes
Metabolite M4 NETU-12-41	Ames in vitro	-	-	1 – 5000 µg/plate	Yes
Specified impurity: (b) (4)	Ames in vitro	-	-	1 – 5000 µg/plate	Yes
Specified impurity: (b) (4)	Ames in vitro	-	-	1 – 5000 µg/plate	Yes
1013157	Mouse Lymphoma in vitro	-	-	5-30 µg/plate	Yes
1002925	Micronucleus Test Rat	p.o.	2-day	160,400,1000	Yes

Study No.	Species	Route	Duration of treatment	Doses (mg/kg/day)	GLP
Netupitant Reproductive Toxicity Studies					
1008497	Rat	p.o.	3-6-weeks (Fertility)	3,10,30	Yes
1007325	Rat	p.o.	2-weeks (Pilot embryo /fetal, DG 6-17)	10,30,100	No
1007930	Rat	p.o.	2-weeks (Embryo/fetal, DG 6-17)	3,10,30	Yes
NETU-10-23	Rat	p.o.	from DG 6 to Postnatal day 20 of lactation	3,10,30	Yes
1006571	Rabbit	p.o.	2-weeks (Pilot embryo /fetal, DG 6-18)	10,30,100	No
1007931	Rabbit	p.o.	2-weeks (Embryo/fetal, DG 6-18)	3,10,30	Yes
Netupitant Local Tolerance Studies					
1007324	Rabbit	i.v.	3-day	0, 1,3,10	No
Skin irritation 1007172	Rabbit	e.c.	Single dose	0.5 g per animal	Yes
Skin sensitization 1007171	Guinea pig	i.d./e.c.	Single dose/challenge	10% / 50 %a	Yes
Eye irritation 1007173	Rabbit	local	Single dose	0.1 g per animal	Yes
In vitro hemolysis and plasma precipitation and turbidity studies 1008464	Rat heparin-ated blood and plasma	In vitro	-	-	No
In vitro hemolysis and plasma precipitation and turbidity studies 1006117	human heparinated blood and plasma	In vitro	-	-	No

Study No.	Species	Route	Duration of treatment	Doses (mg/kg/day)	GLP
Netupitant and metabolite M4 Other Toxicity Studies					
Antigenicity 1007385	Guinea pig	s.c./i.v.	Single dose/challenge	6 mg	Yes
Phototoxicity - Mouse Fibroblasts 1003848	in vitro	-	1h	0.75 - 96 µg/mL	No
Phospholipidosis in vitro 1009769	Bovine corneal fibroblasts	In vitro	72 hours	up to 20 µM in culture medium	No
Metabolite M4 BALB/C 3T3 cell toxicity assay NETU-12-42	Fibroblast -like cell	In vitro	24 hours	up to 5000 µg/mL	No

[a] Netupitant in polyethylene glycol 300
M Male
F Female
i.v. Intravenous administration
i.d. Intradermal administration
e.c. Epicutaneous administration
p.o. Oral administration
i.v. Intravenous administration
P Palonosetron
N Netupitant
DG Day of Gestation
** Based on severe clinical signs noted in Group 4 animals, the high dose level of Palonosetron was reduced from 20 to 15 mg/kg/day from Day 12 onwards
M4 Metabolite M4

3.1 Studies Reviewed

All studies mentioned above were reviewed except those mentioned under 3.2.

3.2 Studies Not Reviewed

All nonclinical studies related to dependence, self-administration, and discrimination were reviewed by the Controlled Substance Staff.

The following studies are not reviewed:

Skin irritation study in rabbits
Skin sensitization in guinea pigs
Eye irritation in rabbits
Toxicity study of metabolite M4 in BALB/C 3T3 cells

3.3 Previous Reviews Referenced

The following pharmacology reviews under IND 73,493 were referenced. Full reviews of individual studies are included in this review verbatim:

1. Pharmacology Review (#003 and 004) by Ke Zhang, Ph.D. dated 7/2/2008

4 Pharmacology

4.1 Primary Pharmacology

Netupitant is a substance P/neurokinin-1 (NK_1) receptor antagonist and palonosetron is a serotonin-3 ($5\text{-}HT_3$) receptor antagonist. Some published reports suggest that a combination of a NK_1 receptor antagonist and a $5\text{-}HT_3$ receptor antagonist is more effective in the control of acute and delayed emesis as compared to $5\text{-}HT_3$ receptor antagonist alone.

In vitro studies:

Netupitant binds to the recombinant human NK_1 receptor with a pKi of 9.0, to the hNK_2 receptor with a pKi of 5.8, and the hNK_3 receptor with a pKi of 7.5. Netupitant also binds to the canine and rodent NK_1 receptors with a pKi of 8.6 and 8.1, respectively.

Three major metabolites, M1 (Ro 68-1133), M2 (Ro 71-3001), and M3 (Ro 73-1519), also bind to the hNK_1 receptor, with pK_i values of 9.0, 9.0, and 9.1, respectively. A minor metabolite M4 was identified at the level of 2.7% (AUC) or 5.5% (C_{max}) of the plasma exposure to netupitant in clinical studies. The results of a binding study indicated that M4 binds to hNK_1 receptors with a K_i of 1.6 nM, and to hNK_3 receptors with a K_i of 8 μM. M4 was not detected in animals.

The effect of netupitant on NK_1 receptors can be modulated by palonosetron (C. Rojas and B. Slusher, European Journal of Pharmacology, 2012). In this study, it was demonstrated that palonosetron inhibited the substance P-mediated responses *in vitro* in NG108-15 cells, and *in vivo* in nodose ganglia collected from rat treated with cisplatin.

In vivo studies:

In an emesis model in ferrets, oral administration of netupitant at 3 mg/kg completely blocked the emesis induced by apomorphine (0.125 mg/kg s.c.), morphine (0.5 mg/kg s.c.), ipecacuanha (1.2 mg/kg p.o.), or copper sulfate (100 mg/kg intragastric) (see the sponsor's Table 3 below).

Table 3: Effect of Netupitant on Various Emetogens in the Ferret

	No. of Retches and Vomits			
	Apomorphine (0.125 mg/kg s.c.)	**Morphine (0.5 mg/g s.c.)**	**Ipecacuanha (1.2 mg/kg p.o.)**	**Copper sulphate (100 mg/kg i.g.)**
Vehicle	26 ± 3	47 ± 4	38 ± 7	78 ± 6
Netupitant (3 mg/kg)	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Oral administration of netupitant (1-3 mg/kg) almost completely inhibited the acute (<24 hr) and delayed (24-72 hr) phases of the emesis induced by cisplatin in ferrets. The ID_{50} (inhibitory dose in 50% of animals) for oral netupitant for prevention of emesis induced by apomorphine and cisplatin was about 0.1-0.2 mg/kg. Oral administration of netupitant also inhibited the motion-induced emesis in shrews with an ID_{50} of ~0.1 mg/kg. Combined oral administration of palonosetron (0.1 mg/kg), netupitant (1 mg/kg), and dexamethasone also prevented emesis induced by cisplatin in ferrets.

Netupitant inhibited NK_1 receptor agonist-induced foot-tapping behavior in gerbils in a dose-dependent manner. The metabolites M1, M2, and M3, also blocked the foot-tapping activity in this study. However, the relevance of the effect on foot-tapping activity to the pharmacodynamic activity for the proposed indication is unknown.

4.2 Secondary Pharmacology

The selectivity of netupitant was investigated by testing its binding affinity to a number of G-protein coupled receptors, monoamine transporters, and ion channels. It was found that netupitant produced approximately 78-100% displacement at the histamine (H2), adenosine (A3), and the L-type Ca2+ channel binding sites. Netupitant binds to the diltiazem binding site on the Ca^{2+} channel with a pKi of 5.9. The metabolite M1 also binds to the L-type Ca2+ channel with an a K_i of 1.4 µM.

4.3 Safety Pharmacology

Central Nervous System:

Oral administration of netupitant in rats resulted in difficult breathing at 300 mg/kg or higher. Body weight and food consumption were reduced at these doses.

Oral administration of netupitant at doses of 3, 30 and 100 mg/kg did not have a significant effect on the time to convulsion induced by pentylenetetrazol in rats.

Cardiovascular System:

In Vitro studies:

Netupitant and its metabolites (M1, M2, and M3) significantly inhibited the potassium currents of the hERG channel expressed in the CHO cells, with IC_{50} values of 0.76, 0.84, 43, and 4.4 µM, respectively.

Netupitant and metabolite M1 inhibited the rapid component of delayed rectifier potassium current (IKr) by ~21-25% at 3 µM in isolated canine ventricular myocytes ("M" mid-myocardial cells). Metabolite M3 inhibited IKr by ~14% and 57% at 3 and 30 µM, respectively. However, metabolite M2, has no effect on this potassium current at concentrations up to 30 µM.

Netupitant (up to 1 µM), M1 (up to 0.3 µM), M2 (up to 3 µM), and M3 (up to 3 µM) had no significant effect on cardiac action potentials in isolated canine cardiac Purkinje fibers. At higher concentrations (3 µM), both netupitant and M1 shortened the action potential duration at 50% of repolarization (APD50), and the action potential duration at 70% and 90% of repolarization (APD70 and APD90, respectively).

Netupitant (up to 3 µM), M1 and M2 (up to 30 µM) had no significant effect on cardiac action potential parameters on isolated canine papillary muscle. Metabolite M3, however, significantly prolonged APD50, APD70 and APD90 at 3 and 30 µM in the isolated canine papillary muscle.

In Vivo studies:

Anesthetized guinea pigs

Intravenous administration of netupitant (0.01-1 mg/kg), M1 (0.01, 0.03, 0.1, 0.3, 1 and 3 mg/kg), M2 (0.01, 0.03, 0.1, 0.3, 1 and 3 mg/kg) had no significant effect on the epicardial monophasic action potential in anesthetized guinea pigs. M3 slightly prolonged the monophasic APD70 and APD90 at IV doses of 0.01, 0.03, 0.1, 0.3, 1, and 3 mg/kg in the same animal model.

Combined intravenous administration of palonosetron and netupitant (0.3 mg/kg palonosetron and 0.03, 0.1, 0.3, or 1 mg/kg netupitant prolonged the monophasic APD70 and APD90 by 19-38%.

Anesthetized rabbits

Netupitant at 30 mg/kg IV decreased the mean, systolic and diastolic arterial blood pressure in anesthetized rabbits. However, netupitant at 30 mg/kg had no effects on QT interval. M1 and M3 (1 mg/kg IV) and M2 (30 mg/kg IV) transiently decreased arterial blood pressure in anesthetized rabbits. Premature ectopic beats and tachycardias were noted in 2 of 6 rabbits treated with M3 at 1 mg/kg IV in the anesthetized rabbits. Combined intravenous administration of palonosetron at 3 mg/kg

and netupitant at 30 mg/kg significantly decreased mean, systolic and diastolic arterial blood pressure, and increased heart rate in anesthetized rabbits.

Conscious Dogs

Oral administration of netupitant at 2, 10 and 50 mg/kg/day for 5 days had no effects on blood pressure, heart rate, or ECGs in six male telemetered beagle dogs. However, 100 mg/kg netupitant produced increases in blood pressure (↑20%) and heart rate (↑ ~26%), and decreases in RR, PR and QT_c intervals. The shortening of QT_c was about 5%, and this small reduction may not be of any clinical significance.

Oral administration of netupitant in conscious beagle dogs at 50 mg/kg/day for 14 days produced a decrease (↓15-19%) in heart rate and prolonged PR, RR, and QT intervals as compared to the control group. The prolonged corrected QT intervals were approximately 5-8% as compared to the control group. Similarly, oral administration of M1 at 30 mg/kg/day for 14 days produced a decrease in heart rate (↓~37%) and prolonged PR, RR, and QT intervals. The prolonged corrected QT intervals were approximately 11-12% as compared to the control group.

Combined oral administration of palonosetron and netupitant had no clear treatment effects on heart rate, blood pressure, and ECGs following treatment at doses of 10/2 mg/kg, 10/10 mg/kg and 10/50 mg/kg of palonosetron/netupitant on day one in conscious telemetered dogs. However, the combination of 10 mg/kg palonosetron and 50 mg/kg netupitant increased heart rate (+17-20%) on treatment day 7 and prolonged the corrected QT intervals (+15-23%) as compared to the pretreatment values on treatment day14.

Respiratory system:

Oral administration of netupitant had no effects on respiratory parameters including respiratory rate, tidal volume, and minute volume at doses of 2, 10, and 50 mg/kg in dogs.

Renal/Urinary systems:

Netupitant had no clear effects on renal and urinary systems in female Sprague-Dawley rats following single oral doses of 3, 30 and 100 mg/kg.

Gastrointestinal system:

Netupitant had no effect on gastrointestinal motility or gastric emptying in female Sprague-Dawley rats following single oral doses of 3, 30 and 100 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption:

Rat

To study the pharmacokinetics of netupitant, Wistar rats (8 males) were treated by oral gavage with one of two netupitant suspensions: Standard Suspension Vehicle (SSV) and thixotropic vehicle (GKM0022). Plasma netupitant concentrations were determined using an LC-MS method. The results are summarized in the following sponsor's table.

Table 1: Pharmacokinetic parameters (mean ± SD) of Netupitant following single oral administration of 10 mg/kg in two different vehicles to male Wistar rats

Vehicle	C_{max} (ng/mL)	T_{max} (h)	$AUC_{(0\text{-}24h)}$ (h.ng/mL)
SSV	1450 ± 591	2.3	20400 ± 8220
GKM0022	1350 ± 175	4.1	19000 ± 1200

The oral bioavailability was calculated using the results from an IV study (studies #1000990 and #B-0168943, described below). The bioavailability was 71% for the suspension in SSV and 66% for the suspension in the thixotropic vehicle.

The pharmacokinetic profile of netupitant was also assessed in rats following a single intravenous, intraperitoneal and oral administration (studies #1000990 and #B-0168943) using an LC-MS method. The results were summarized in the following sponsor's table.

Table 2: Mean (n=2) pharmacokinetic parameters of single oral, intraperitoneal and intravenous administration of netupitant di-hydrochloride salt in water (9.4 mg/kg free base) to rats

Administration route	Oral	Intraperitoneal	Intravenous
C_{max} (ng/mL)	837	1670	-
T_{max} (h)	3	0.25	-
$AUC_{(0-24h)}$ (h.ng/mL)	13800	23350	26800
CL[1)] (mL/min/kg)	-	-	5.9
T½ (h)	18.3	67.1	24.2
Vz[1)] (L/kg)	-	-	11.8
F %	52	87	-

1) Calculated with AUC (0-24h)

Table 3: Mean (n=2) pharmacokinetic parameters of netupitant following single oral administration of netupitant free base in SSV (10 mg/kg), as well as single oral and intravenous administration of netupitant dihydrochloride salt (9.4 mg/kg free base) in water to rats

Administration route Formulation	Oral SSV	Oral water	Intravenous water
C_{max} (ng/mL)	706	997	-
T_{max} (h)	3.1	5.2	-
$AUC_{(0-inf)}$ (h.ng/mL)	14300	35950	40800[2)]
CL [1)] (mL/min/kg)	-	-	6.2
$T_{½}$ (h)	10.9	26.8	21.9 [3)]
Vz [1)] (L/kg)	-	-	11.3
F %	48.1	87.2	-

1) Calculated with AUC (0-48h), 2) AUC (0-72h), 3) without 72h concentration

Netupitant exhibited a long terminal half-life (11-67 hr). The oral bioavailability was 52-87%% for netupitant dihydrochloride salt given in water and 48% for netupitant free base in SSV.

Dog

The pharmacokinetic profile of netupitant was assessed in dogs following intravenous (lactate salt, 3 mg/kg) and oral doses (study #2000698). Various salt forms and netupitant free base were administered orally at doses of 70 or 120 mg free base equivalent, which was approximately 5.4 or 8.5 mg/kg, respectively. Plasma netupitant concentrations were determined using an LC-MS method. The results are summarized in the following tables (taken from the study report).

Table 5: Pharmacokinetic parameters following single intravenous administration of 3 mg/kg of netupitant (lactate salt) to dogs

Dog	Mean	SD
AUC $_{(0-\infty)}$ (h.ng/mL)	13900	2200
T½ (h)	42.0	15.3

Table 6: Mean pharmacokinetic parameters of netupitant after single oral administration to dogs (n = 2)

	Hydrochloride	Malate	Tartrate	Base	Base	Tartrate	Mesylate
Oral Capsule Dose (mg)	120	120	120	120	70	70	70
Dose (mg/kg)	8.7	7.9	10.2	8.0	5.0	5.3	5.9
C_{max} (ng/mL)	922	1260	1565	686	627	739	304
T_{max} (h)	2.5	3.5	4.5	2.0	5	2.5	3.5
$AUC_{(0-\infty)}$ (h.ng/mL)	31650	42200	42300	17650	16900	21650	7625
T½ (h)	56.1	54.2	38.8	47.9	38.1	44.0	34.0
F (%)**	79	116	90	48	74	90	28

* Dose expressed in mg of free base
**Calculated with $AUC_{(0-\infty)}$

Netupitant exhibited a long terminal half-life (34 to 56 hours) following an oral dose. The oral bioavailability of netupitant varied among the free base and salts that were tested.

The oral bioavailability of netupitant was also determined in another study in fasted dogs (study #1006336). In this study, dogs were fasted overnight before drug administration and fed 30 min before dosing (oral gelatin capsules). Netupitant plasma concentrations were determined using an LC-MS method. Based on the AUC determined from the intravenous dose in the above study, the oral bioavailability was 55-57% at a dose of 70 mg free base. It appears that concomitant administration of netupitant with food did not have a major effect on the oral bioavailability.

Monkey

The pharmacokinetics of netupitant was characterized following a single intravenous administration (mesylate salt) and a single oral administration (free base or tartrate salt) in cynomolgus monkeys (study #1006445), using an LC-MS method for determination of plasma netupitant concentrations. The results are summarized in the following tables (taken from the study report).

Table 8: Pharmacokinetic parameters of netupitant following single intravenous administration of 2 mg/kg netupitant to Cynomolgus monkeys

Formulation	Mesylate salt
AUC(0-inf) ng·h/mL	1785
T½ h	11.6

Table 9: Pharmacokinetic parameters of netupitant following single oral administration of different formulations of netupitant to Cynomolgus monkeys

Formulation	Base		Tartrate salt	
Animal number	1	2	3	4
Body weight (kg)	4.0	8.4	4.3	7.0
Dose (mg/kg)	6.3	3.0	5.8	3.6
C_{max} (ng/mL)	253	N.R.	202	81.8
T_{max} (h)	4	N.R.	4	4
C_{last} (ng/mL)	13.1	N.R.	11.2	13.9
Tlast (h)	48	N.R.	48	24
$AUC_{(0\text{-}last)}$ (ng·h/mL)	3320	N.R.	2770	1010
$T_{1/2}$ (h)	11.8	N.R.	11.9	8.55
F%	61.52[2)]	N.R.	55.42[2)]	37.01[1)]

[1)] Calculated with $AUC_{(0\text{-}24)}$ after i.v. administration for a dose of 2 mg/kg (1520 ng.h/mL)
[2)] Calculated with $AUC_{(0\text{-}48)}$ after i.v. administration for a dose of 2 mg/kg (1720 ng.h/mL)
N.R.: no result

Netupitant exhibited a terminal half-life in the range of 8-12 hours following oral or intravenous administration. The oral bioavailability of netupitant was 37-62% among the three monkeys with results reported.

Distribution:

Rat

To study the tissue distribution, [^{14}C]-netupitant was given orally (10 mg/kg) and intravenously (5 mg/kg) to albino or pigmented male rats (study #1007004). The total radioactivity in blood, plasma and urine was measured using quantitative whole-body autoradiography. The plasma concentrations of the unchanged drug and its metabolites M1, M2, and M3 were determined using an LC-MS method.

Following intravenous administration, the radioactivity was extensively distributed to almost all tissues, with the highest radioactivity levels in the lung, followed by adrenal cortex, spleen, brown fat, liver and pancreas. After oral administration, the radioactivity was found to be highest in the Harderian gland, followed by lung, adrenal, spleen, pituitary, exorbital and intraorbital lachrymal glands, and thyroid. The radioactivity levels were generally higher in tissues than in plasma, except for the brain and spinal cord.

The plasma radioactivity was mainly associated with metabolite M1 at 24 hours after dosing, suggesting that the terminal distribution and elimination of radioactivity was with M1, not the parent drug.

The tissue levels of radioactivity at 24 hours were higher following repeated dosing as compared to a single dose, suggesting that netupitant is accumulated over time. The tissue distribution profile in pigmented rats was similar to that in albino rats.

Distribution in brain was also assessed following intravenous administration in rats (studies #1000990 and #B-168943). A single intravenous dose of 9.4 mg/kg netupitant was given to rats, and the netupitant concentration in brain was then determined using an LC-MS method. The results are summarized in the following sponsor's table.

Table 11: Plasma CSF and Brain Concentration of netupitant after intravenous administration of 9.4 mg/kg to rats (n=1)

Time (h)	CSF (ng/mL)	Plasma (ng/mL)	Brain (ng/g)	Ratio (Brain/plasma)
0.30	4.86	2250	6692	3.0
0.33	16.0	1805	7532	4.2
0.5	BLQ	1489	6384	4.3
0.5	BLQ	1472	7224	4.9
1	BLQ	1329	3220	2.4
1	BLQ	1375	5292	3.8
2	BLQ	1062	2694	2.5
2	6.34	904	2548	2.8

The data indicates that drug concentrations were higher in brain than in plasma.

Dog

The distribution of netupitant and metabolites in heart was determined in dogs in toxicity studies (see the sponsor's table below).

Table 12: Heart Tissue Concentrations of Parent and Metabolites in Dogs

Study	Dose (p.o.) (mg/kg)	Heart C_{24h} (ng/g) M/F			
		Netupitant	M1	M2	M3
4-week Study 1006010	1 3 5 15 50	214/300 807/711 -/992 -/12900 -/40400	1260/1360 4960/4620 -/7000 -/77300 -/128000	BLQ BLQ BLQ BLQ BLQ	BLQ BLQ BLQ -/1070 -/5060
4-week Study NETU-06-05	10P/3N 15P/7.5N 20(15#)P/15N	414/731 1657/3649 10093/ 9296 439/389 $	1899/ 3053 7155/13070 53626/45353 228/136 $	BLQ BLQ BLQ BLQ	64.6/ 95.4 191.5/542.2 975/ 909 82.4/66.4 $
Telemetry 1006422	2 ≥ 10 ≥ 50	16610/- (C72h)	94179/- (C72h)	BLQ	1426/- (C72h)

N: Netupitant
#: Based on severe clinical signs noted in Group 4 animals, the high dose level of palonosetron was reduced from 20 to 15 mg/kg/day from Day 12 onwards
$: values from the high dose recovery group

Netupitant and the metabolites M1 and M3 were identified in the heart tissues with higher level of M1.

Protein binding studies

The plasma protein binding study was conducted with netupitant using human, dog, rat, and gerbil blood samples (study #1006047). The protein concentrations were determined using liquid scintillation counting. The mouse plasma protein binding was also studied using a dialysis method (study #NETU-08-08), and the protein concentrations were determined using LC-MS/MS. Netupitant exhibited high levels of binding (>99%) to plasma proteins in all species. The fraction of drug in erythrocytes was ~13% in human and dog, and 17-37% in rat.

The plasma protein binding of the major metabolites M1, M2, and M3 was also determined using the blood samples from human, dog, and Wistar rat (study #1010388). The results indicated that plasma protein binding was >99% for M1, and 97-99% for M2 and M3 in all species. The blood/plasma concentration ratios for M1, M2, and M3 were 1.1, 0.69, and 0.61, respectively, in humans, 0.73, 0.66, and 0.76, respectively, in dogs, and 0.94, 0.69, and 0.82, respectively, in rat. The blood/plasma concentration ratios for the parent drug were 0.69 in human, 0.77 in dog, and 0.77 in rats.

Metabolism:

Rat

To determine the netupitant metabolite profiles, plasma, urine, bile and feces were collected from bile duct cannulated male Wistar rats (study #1009719) following a single oral (4 mg/kg) and intravenous (2 mg/kg) dose of ^{14}C-netupitant. The results indicated that ~9-15% of the radiolabeled dose was recovered from feces, 12 to 40% in bile, and < 1% in urine through the end of the study (96 hours).

In plasma samples, parent drug accounted for over 50% of the total radioactivity, with up to 31% associated with metabolite M1, up to 7% associated with M2, up to 3% associated with M3, and up to 6% associated with a mono-hydroxylated metabolite.

In bile samples, the parent drug accounted for 27 to 40% of the total biliary drug-related material. The metabolites in bile included M1 (7-13%), M2 (5-12%), M3 (9-13%), and M8 (2.5-7%).

In feces, the parent drug accounted for 50-70% of the total fecal drug-related material, and the metabolites M1 and M2 accounted for 14-24% and 8-16%, respectively. Other metabolites accounted each for < 7% of the total fecal drug-related material.

Dog

To determine the netupitant metabolite profiles, plasma, urine, bile, and feces were collected from bile duct cannulated female dogs (study #1009870) following a single oral (6 mg/kg) and intravenous (2 mg/kg) dose of ^{14}C-netupitant. The results indicated that following intravenous administration, the parent drug accounted for 33-73% of the total radioactivity in plasma, M2 accounted for 27-58%, and M1 accounted for 20.6%.

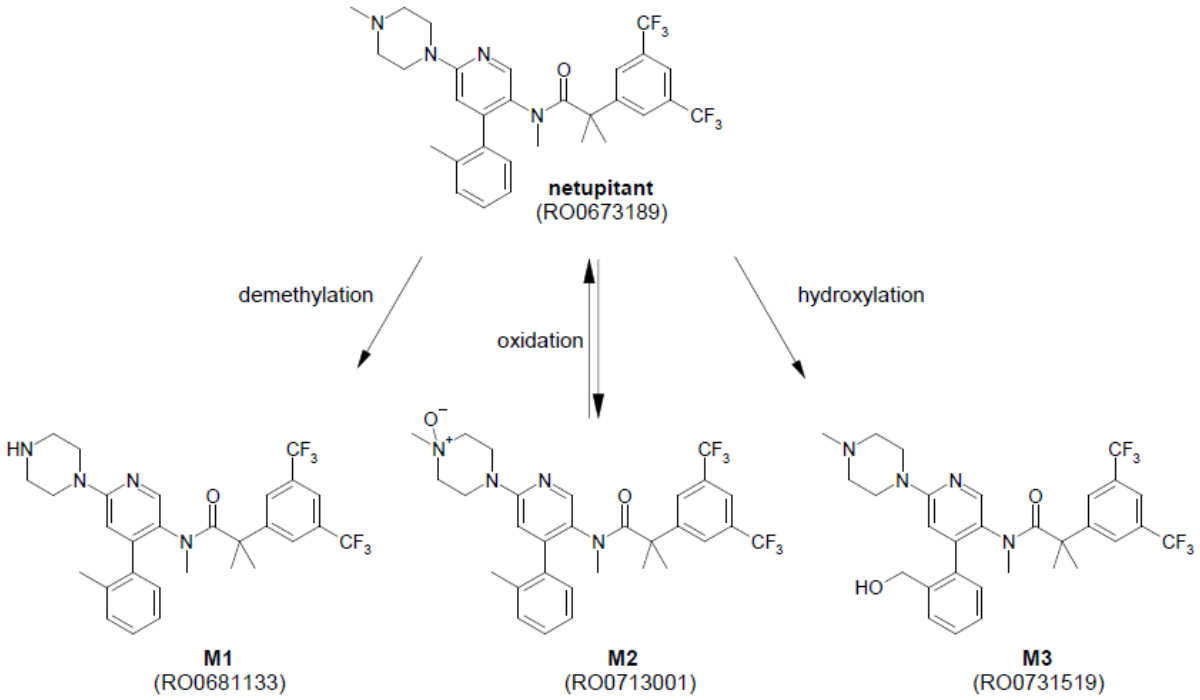
Following oral administration, the parent drug accounted for 3-40% of the total radioactivity in plasma, M2 accounted for 10-58%, M1 accounted for 3-16%, and M3 and additional secondary polar metabolites each accounted for 2-9%.

The biliary excretion of radioactivity accounted for 59% and 30-39% of the intravenous and oral dose, respectively. A small fraction of the dose was found in urine (0.4 to 3%).

In bile, the parent drug accounted for 2 to 4% of the total radioactivity, M2 accounted for 30-43%, the further hydroxylated M5 accounted for 10-14%, a glucuronide of the oxidative metabolite M6 (M6-G) accounted for 8-11%, and the glucuronide of M8 accounted for 3-5%. In urine, a small fraction of the dose was found, which consisted mainly of polar metabolites (M5, M6, and M8) conjugated to glucuronic acid.

The metabolic pathway for netupitant is presented in the sponsor's figure below.

Figure 1: Proposed Pathway for the Formation of Major Metabolites of Netupitant



A metabolism study with netupitant was performed using human, rat, dog, minipig and marmoset liver microsomes (studies #1003832 and #NETU-10-34). The results indicated that three major metabolites including an N-demethylation product (M1), an N-oxidation product (M2), and a hydroxylation product (M3) were present in all species.

The results from the study with recombinant expressed human CYP450 isoenzymes indicated that the oxidative steps were catalyzed by CYP3A4, but not by CYP2C9, CYP2C19, or CYP2D6. The apparent K_m of 11.3 µM and V_{max} of 1.5 nmol/min/mg protein were for the oxidative metabolism of netupitant. The apparent K_m of 2.8-9.1 µM was for the N-demethylation of the parent compound to the metabolite M1.

Excretion:

Rat

In a mass balance study in rats, [^{14}C]netupitant was given orally (4.7 mg/kg) and intravenously (4.9 mg/kg) to normal male rats (study #1001466). The excretion of [^{14}C]netupitant following both routes of administration was not completed after one week, with 6.0 ± 1.5% of the oral dose and 7.2 ± 0.8% of the intravenous dose still remaining in the carcass and gastrointestinal tract. Biliary elimination was the major route of excretion, as indicated by the recovery of ~87% of the radioactive dose in feces following intravenous administration. Similarly, ~86% of the dose was recovered in feces following oral administration. The urinary excretion accounted for <1% of the dose. Following intravenous administration, high levels of radioactivity were found in the stomach about one hour after dosing (study #1007004).

Dog

The netupitant excretion was studied in beagle dogs following oral (5 mg/kg) and intravenous (2 mg/kg) doses of [^{14}C]netupitant (study #1009956). Urine, feces, and cage washings were collected up to 1008 hours (42 days) after dosing. The results indicated that the total excretion was approximately 88% of the dose within two weeks following oral or intravenous administration. The majority of the radioactivity was found in feces, accounting for 88-90% of the administered dose; <2% was recovered in urine.

5.2 Toxicokinetics

See each individual toxicity studies.

6 General Toxicology

6.1 Single-Dose Toxicity

In the acute oral toxicity study in mice (# NETU-07-23), netupitant was lethal at 1000 and 2000 mg/kg.

In a study in Wistar rats (# 1009566), netupitant was administered orally at 0, 500, 1000, 1500 and 2000 mg/kg. The dose of 2000 mg/kg was lethal. Histopathological examination revealed foamy/vacuolated macrophages in the liver, lungs, skeletal muscle, mesenteric lymph nodes, duodenum, spleen, and stomach at doses of 1500 and 2000 mg/kg, suggesting phospholipidosis.

In a study with Beagle dogs (# 1009567), netupitant was administered orally at 0, 200, 300 and 400 mg/kg using a capsule formulation. Body weight loss and/or a decrease in body weight gain were noted at all dose-levels. Histopathological examination revealed increased incidence of vacuolated macrophage infiltration in the gall bladder and foamy alveolar macrophage at 400 mg/kg, suggesting phospholipidosis.

6.2 Repeat-Dose Toxicity

Mouse:

There were three oral toxicity studies in Crl:CD-1(ICR) mice including 7-day (# NETU-07-022), 4-week (# NETU-07-025), and 13-week (# NETU-07-026) oral toxicity studies. In the 7-day oral toxicity, netupitant was given by oral gavage to CD-1 mice at 0, 30, 120, and 450 mg/kg/day. The doses of 120 mg/kg/day or higher resulted in body weight loss, and the dose of 450 mg/kg/day was lethal. The dose of 30 mg/kg/day was selected as the high dose for the subsequent 4-week oral toxicity study in mice. In the 4-week oral toxicity, netupitant was given by oral gavage to CD-1 mice at 0, 3, 10, and 30 mg/kg/day. At 30 mg/kg/day, the histopathological exam revealed increased incidence of foamy histiocytes in the liver, lungs, and mandibular lymph nodes. Based on the results, the dose of 10 mg/kg/day was selected as the high dose for the 13-week oral toxicity study. In the 13-week oral toxicity study, netupitant was given by oral gavage to CD-1 mice at 0, 1, 3, and 10 mg/kg/day. There were no clear treatment-related changes. The dose of 10 mg/kg/day was the no observed effect level (NOEL).

Rat:

Oral toxicity studies two weeks and four weeks duration were conducted in rats (# 1007326, # B-167719, # B-167723, # 1006011, and # 1003562). The highest dose tested in these studies was 300 mg/kg/day (2-week studies). Animals were sacrificed prior to study termination at doses of 300 mg/kg/day in the 2-week studies and 100 mg/kg/day in a 4-week study due to body weight loss. Thus, the dose of 100 mg/kg/day exceeded the MTD. Phospholipidosis was noted at doses of 30 mg/kg/day or higher.

13-Week Oral Gavage Toxicity Study with RO0673189 in Rats (161/578)

Conducting Laboratory and Location: Hoffmann-La Roche Ltd
Basal, Switzerland

Date of study initiation: November 15, 2002
Report date: March 23, 2007

GLP compliance: This study was conducted in accordance with Good Laboratory Practice Regulations of the United States Food and Drug Administration (21 CFR Part 58).
QA-Report Yes (x) No ()

Methods: RO0673189 was given to rats (12 rats/sex/group) by oral gavage at 0, 3, 10, and 30 mg/kg/day for 13 weeks. Another group of rats (intermittent dosing) received RO0673189 at 30 mg/kg/day for 7 days with a 2-week of drug free period. This treatment regimen was repeated during 13 weeks.

species/strain: Wistar rats:Ico-WI(IOPS AF/Han)
- **#/sex/group or time point:** 12/sex/group
- **age:** ~6 weeks old

weight: males: 165-210 g, females: 134-163 g.
satellite groups used for toxicokinetics or recovery: Toxicokinetic parameters were determined on day 0 and during weeks 4, 7, and 13 before doing and at 1, 3, 5, 8,

24, 96, 192, 288, and 384 hour after dosing (3 rats/sex/group). The blood samples at 96, 192, 288, and 384 hour after dosing were collected only during week 13.

- **Dosing Volume**: 5 ml/kg

Drug lot# and % purity: BS0204SA01 and 99.8%

Formulation/vehicle:

Composition for 100 ml	
Polysorbate 80	0.20%
Cellulose Avicel RC 591	1.60%
Nipagin	0.18%
Nipasol	0.02%
Demineralized water	Filled up to 100 ml
Citric acid 0.1 N	Quantity necessary for adjustment to pH 6.0

Observations and times:

- Clinical signs: Clinical signs of toxicity were observed daily.
- Body weights: Body weights were determined weekly.
- Food consumption: Food consumption was determined weekly.
- Hematology, Clinical Chemistry, and Urinalysis: at termination.
- Gross pathology: Animals were necropsied at termination.
- Organ weighed: Following organs were weighed at termination: adrenal gland, brain, heart, kidney, liver, ovary, pituitary gland, prostate gland, spleen, testis, and thyroid gland.
- Histopathology: Following organs or tissues were examined histopathologically from each animal in all groups: adrenal glands, aorta, bone (femur) and articulation bone (sternum) with bone marrow, bone marrow smears, brain, bronchi (mainstem), caecum, colon, duodenum, epididymides, eyes, heart, ileum, jejunum, kidneys and ureters, larynx, liver, lungs, lymph node (mandibular), lymph node (mesenteric), mammary gland, oesophagus, optic nerves, ovaries and oviducts, pancreas, parathyroid glands, Peyer's patches, pituitary gland, prostate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerves, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid glands, tongue, trachea, urinary bladder, uterus (horns + cervix), vagina, and all gross lesions.

Results:

Clinical Signs: Hairloss was noted in all groups including control with higher incidence in the treated females.

Mortality: There were no deaths.

Body Weights: The mean body weight, terminal body weight gain, and % change of the terminal body weight gain are summarized in the following table.

Males

	control	3 mg/kg	10 mg/kg	30 mg/kg
Mean body weight (g) Initial Final	 185.8 455.2	 183.5 434.9	 186 456.4	 186.5 422.8
Body weight gain (g)	269.4	251.4	270.4	236.3
% change of body weight gain		~-7%	~+0.4%	~-12%

Females

	control	3 mg/kg	10 mg/kg	30 mg/kg
Mean body weight (g) Initial Final	 148.3 265	 149.4 268.2	 149.7 260.1	 151.5 246.2
Body weight gain (g)	116.7	118.8	110.4	94.7
% change of body weight gain			~-5%	~-19%

The mean terminal body weights in the low, mid, and high dose groups were 95.5%, 100.3%, and 93% of the control for males, and 101%, 98%, and 93% of the control for females, respectively.

Food Consumption: The mean food consumption in males was 27.1-30.9, 25.8-29.5, and 26.1-30.7, and 25.1-28.4 g/animal/day for the control, low, mid, and high dose groups, respectively. The mean food consumption in females was 20.4-22.5, 20.2-21.8, 18.8-21.6, and 18.1-20.2 g/animal/day for the control, low, mid, and high dose groups, respectively.

Ophthalmologic Examination: There were no treatment related changes.

Hematology: Slight decrease (4-7%) in hemoglobin level, mean cell volume, and mean cell hemoglobin was noted in the high dose group as compared to the control. Slightly

shortened APTT and PT were noted in the high dose group as compared to the control.

Clinical Chemistry: The protein and globulin levels were higher in the low, mid, and high dose females and in the high dose males as compared to the control. Also, in the high dose females, lower albumin level and higher cholesterol level were noted.

Urinalysis: Specific gravity was statistically lower in the mid and high dose groups as compared to the control.

Organ Weights: The absolute liver and kidney weights were increased by 22% and 8% in the high dose males, respectively. The absolute liver and kidney weights were increased by 42% and 8% in the high dose females, respectively.

Gross Pathology: Alopecia was noted in all groups with higher incidences in the treatment groups.

Histopathology: Treatment-related microscopic changes included hepatocellular vacuolation, increased pulmonary infitration by foamy macrophages, increased severity and/or incidence of diffuse histiocytosis and histiocytic aggregates in mesenteric and mandibular lymph nodes, and splenic histiocytosis, suggesting that treatment with netutipant induced phospholipidosis. Minimal necrosis was noted in the liver in four high dose females (none in other groups). High incidence of glandular stomach erosion was noted in the high dose females. Hepatocellular hypertrophy, follicular cell hypertrophy in the thyroids, and corporea lutea inflammation in the ovaries were also noted with higher incidences in the high dose group.

The results were presented in the following tables.

Groups 1, 2, 3, and 4 = control, 3, 10, and 30 mg/kg/day, respectively.
Group 5 = intermittent dosing (30 mg/kg/day).

Treatment-related microscopic findings – Liver

Sex	Males							Females						
Status at necropsy	K0					R1		K0					R1	
Group	1	2	3	4	5	1	4	1	2	3	4	5	1	4
No. of animals	12	12	12	12	12	4	4	12	12	12	12	12	4	4
- Centrilob. hypertrophy	-	2	4	11	9	-	2	-	-	-	5	1	-	-
Minimal	-	2	4	11	9	-	2	-	-	-	5	1	-	-
- Vacuolation	-	3	9	11	3	-	1	1	1	-	7	-	-	-
Minimal	-	3	9	5	3	-	1	1	1	-	6	-	-	-
Slight	-	-	-	6	-	-	-	-	-	-	1	-	-	-
- Necrosis	-	-	-	-	-	-	-	-	-	1	4	-	-	-
Minimal	-	-	-	-	-	-	-	-	-	-	4	-	-	-
Slight	-	-	-	-	-	-	-	-	-	1	-	-	-	-
- Single cell necrosis	2	1	-	2	-	2	2	1	-	1	2	1	-	-
Minimal	1	1	-	2	-	2	2	1	-	-	2	1	-	-
Slight	1	-	-	-	-	-	-	-	-	1	-	-	-	-
- Granuloma	-	-	-	-	-	-	-	-	-	-	2	-	-	-
Minimal	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Moderate	-	-	-	-	-	-	-	-	-	-	1	-	-	-

K0: terminal sacrifice; R1: recovery

-: Observation not recorded in group

Treatment-related microscopic findings – Lungs

Sex	Males							Females						
Status at necropsy	K0					R1		K0					R1	
Group	1	2	3	4	5	1	4	1	2	3	4	5	1	4
No. of animals	12	12	12	12	12	4	4	12	12	12	12	12	4	4
- Foamy macrophages	2	3	5	5	5	1	3	2	1	3	10	8	-	1
Minimal	2	3	5	5	5	1	3	2	1	3	6	7	-	-
Slight	-	-	-	-	-	-	-	-	-	-	2	1	-	1
Moderate	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Marked	-	-	-	-	-	-	-	-	-	-	1	-	-	-
- Peribronch.Histiocytes	-	-	-	4	1	-	-	-	-	-	1	-	-	-
Minimal	-	-	-	4	1	-	-	-	-	-	1	-	-	-

K0: terminal sacrifice; R1: recovery

-: Observation not recorded in group

Treatment-related microscopic findings – Mesenteric lymph node

Sex	Males							Females						
Status at necropsy	K0					R1		K0					R1	
Group	1	2	3	4	5	1	4	1	2	3	4	5	1	4
No. of animals	12	12	12	12	12	4	4	12	12	12	12	12	4	4
- Diffuse histiocytosis	12	12	12	12	11	4	4	10	12	12	12	12	4	4
Minimal	11	5	1	2	4	-	-	4	7	1	1	3	2	-
Slight	1	4	8	5	4	3	1	6	4	7	3	7	2	3
Moderate	-	3	3	4	3	1	3	-	1	3	8	2	-	1
Marked	-	-	-	1	-	-	-	-	-	1	-	-	-	-
- Histiocytic aggregates	6	9	12	10	9	3	4	8	12	12	11	11	2	4
Minimal	6	8	5	2	3	2	-	7	9	1	-	1	2	-
Slight	-	1	4	2	2	1	2	1	3	5	1	4	-	1
Moderate	-	-	3	6	4	-	2	-	-	6	8	6	-	3
Marked	-	-	-	-	-	-	-	-	-	-	2	-	-	-

K0: terminal sacrifice; R1: recovery

-: Observation not recorded in group

Treatment-related microscopic findings – Mandibular lymph node

Sex	Males							Females						
Status at necropsy	K0					R1		K0					R1	
Group	1	2	3	4	5	1	4	1	2	3	4	5	1	4
No. of animals	12	12	12	12	12	4	4	12	12	12	12	12	4	4
- Diffuse histiocytosis	9	10	11	11	12	3	4	10	11	12	12	12	4	2
Minimal	7	3	5	7	9	2	2	8	10	8	7	10	2	1
Slight	2	7	4	4	3	1	2	2	1	4	4	2	2	1
Moderate	-	-	2	-	-	-	-	-	-	-	1	-	-	-
- Histiocytic aggregates	-	3	-	4	1	-	2	1	3	5	5	1	-	-
Minimal	-	3	-	3	1	-	2	1	3	5	5	1	-	-
Slight	-	-	-	1	-	-	-	-	-	-	-	-	-	-

K0: terminal sacrifice; R1: recovery

-: Observation not recorded in group

Treatment-related microscopic findings – Thyroid glands

Sex	Males													
Status at necropsy	K0					R1		K0					R1	
Group	1	2	3	4	5	1	4	1	2	3	4	5	1	4
No. of animals	12	12	12	12	12	4	4	12	12	12	12	12	4	4
- Foll. cell hypertrophy	4	1	2	10	3	-	-	-	-	-	8	3	-	1
Minimal	4	1	2	10	3	-	-	-	-	-	8	3	-	1

K0: terminal sacrifice; R1: recovery

-: Observation not recorded in group

Treatment-related microscopic findings – Trachea and larynx

Sex	Males							Females						
Status at necropsy	K0					R1		K0					R1	
Group	1	2	3	4	5	1	4	1	2	3	4	5	1	4
No. of animals	12	12	12	12	12	4	4	12	12	12	12	12	4	4
Larynx														
- Inflammation	-	-	-	1	-	-	-	-	-	-	2	2	-	-
Minimal	-	-	-	1	-	-	-	-	-	-	2	1	-	-
Slight	-	-	-	-	-	-	-	-	-	-	-	1	-	-
- Epithelial ulceration	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Minimal	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Trachea														
- Inflammation	-	-	1	1	-	-	-	-	-	-	6	-	-	-
Minimal	-	-	1	1	-	-	-	-	-	-	3	-	-	-
Slight	-	-	-	-	-	-	-	-	-	-	2	-	-	-
Moderate	-	-	-	-	-	-	-	-	-	-	1	-	-	-

K0: terminal sacrifice; R1: recovery

-: Observation not recorded in group

Treatment-related microscopic findings – ovaries

Sex	Females						
Status at necropsy	K0					R1	
Group	1	2	3	4	5	1	4
No. of animals	12	12	12	12	12	4	4
- C. lutea inflammation	1	-	4	7	9	-	2
Minimal	1	-	2	5	8	-	2
Slight	-	-	2	1	1	-	-
Moderate	-	-	-	1	-	-	-

K0: terminal sacrifice; R1: recovery

-: Observation not recorded in group

Treatment-related microscopic findings – Stomach

Sex	Males							Females						
Status at necropsy	K0					R1		K0					R1	
Group	1	2	3	4	5	1	4	1	2	3	4	5	1	4
No. of animals	12	12	12	12	12	4	4	12	12	12	12	12	4	4
- Mucosal erosion	1	-	-	-	2	-	-	1	1	1	5	3	-	-
Minimal	1	-	-	-	-	-	-	-	1	-	2	2	-	-
Slight	-	-	-	-	1	-	-	1	-	1	1	1	-	-
Moderate	-	-	-	-	-	-	-	-	-	-	2	-	-	-
Marked	-	-	-	-	1	-	-	-	-	-	-	-	-	-
- Muscle degeneration	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Minimal	-	-	-	-	1	-	-	-	-	-	-	-	-	-

K0: terminal sacrifice; R1: recovery

-: Observation not recorded in group

Toxicokinetics:

The toxicokinetic data of the parent compound were presented in Table 5 and this table is attached below.

Table 5 **Toxicokinetic parameters of RO0673189 following oral administration of 3, 10 and 30 mg/kg/day RO0673189-008 to rats**

Dose mg/kg	Gender	Day	Tmax h	Cmax ng/mL	C(24h) ng/mL	Tlast h	AUC(0-last) h.ng/mL	AUC(0-24h) h.ng/mL	AUC(0-24h)/dose h.ng/mL/mg/kg
3	Male	0	3	212	59.9	24	3480	3480	1160
		28	3	432	173	24	6310	6310	2100
		49	3	552	222	24	8160	8160	2720
		91	3	426	182	24	7150	7150	2380
	Female	0	8	293	167	24	5660	5660	1890
		28	3	544	380	24	10400	10400	3470
		49	3	940	481	24	16800	16800	5600
		91	3	553	417	24	11100	11100	3700
10	Male	0	3	633	180	24	9580	9580	958
		28	3	884	362	24	13700	13700	1370
		49	3	968	383	24	15500	15500	1550
		91	1	764	400	24	14000	14000	1400
	Female	0	8	709	413	24	14200	14200	1420
		28	5	1450	944	24	27100	27100	2710
		49	3	1560	1020	24	29700	29700	2970
		91	5	1220	981	24	26600	26600	2660
30	Male	0	3	1370	246	24	21100	21100	703
		28	5	1570	704	24	27500	27500	917
		49	5	1630	808	24	29300	29300	977
		91	5	1490	980	288	106000	30200	1010
	Female	0	5	1590	1210	24	31500	31500	1050
		28	3	2680	1720	24	51300	51300	1710
		49	3	2330	1670	24	47000	47000	1570
		91	5	2080	2050	384	296000	49200	1640
30int.*	Male	0	8	1400	501	24	24600	24600	820
		28	3	1860	742	24	28200	28200	940
		49	3	1770	767	24	30800	30800	1030
		91	5	1310	969	288	91100	26500	883
	Female	0	5	1690	1330	24	33800	33800	1130
		28	3	2790	1700	24	49000	49000	1630
		49	3	2700	1700	24	49600	49600	1650
		91	3	2540	2040	384	251000	49400	1650

*intermittent administration

The plasma levels of the major metabolites (RO0681133=M1, RO0713001=M2, and RO0731519=M3) were presented in Tables 6, 7, and 8 and these tables are attached below.

Table 6 **Toxicokinetic parameters of RO0681133 following oral administration of 3, 10 and 30 mg/kg/day RO0673189-008 to rats**

Dose mg/kg	Gender	Day	Tmax h	Cmax ng/mL	C(24h) ng/mL	Tlast h	AUC(0-last) h.ng/mL	AUC(0-24h) h.ng/mL	AUC(0-24h)/dose h.ng/mL/mg/kg
3	Male	0	24	90.8	90.8	24	1660	1660	553
		28	5	445	410	24	9170	9170	3060
		49	5	563	514	24	11900	11900	3970
		91	3	622	592	24	14300	14300	4770
	Female	0	24	95.3	95.3	24	1440	1440	480
		28	5	437	423	24	10200	10200	3400
		49	8	572	435	24	12000	12000	4000
		91	24	599	599	24	12500	12500	4170
10	Male	0	24	257	257	24	3990	3990	399
		28	0	937	937	24	21600	21600	2160
		49	5	1030	1000	24	22800	22800	2280
		91	24	1150	1150	24	25700	25700	2570
	Female	0	24	209	209	24	3130	3130	313
		28	5	1030	922	24	21300	21300	2130
		49	5	1200	1080	24	23800	23800	2380
		91	3	1280	1150	24	27700	27700	2770
30	Male	0	24	408	408	24	7350	7350	245
		28	5	1560	1420	24	34600	34600	1150
		49	5	1770	1580	24	37600	37600	1250
		91	3	1910	1840	384	454000	43800	1460
	Female	0	24	429	429	24	5170	5170	172
		28	3	1520	1270	24	31000	31000	1030
		49	5	1630	1440	24	33700	33700	1120
		91	96	1680	1530	384	496000	36700	1220
30int.*	Male	0	24	596	596	24	8260	8260	275
		28	3	1440	1330	24	30000	30000	1000
		49	24	1590	1590	24	33600	33600	1120
		91	3	1560	1480	384	249000	32700	1090
	Female	0	24	427	427	24	5500	5500	183
		28	3	1320	1300	24	26700	26700	890
		49	5	1500	1490	24	29700	29700	990
		91	3	1520	1420	384	366000	32000	1070

*intermittent administration

Table 7 Toxicokinetic parameters of RO0713001 following oral administration of 3, 10 and 30 mg/kg/day RO0673189-008 to rats

Dose mg/kg	Gender	Day	Tmax h	Cmax ng/mL	C(24h) ng/mL	Tlast h	AUC(0-last) h.ng/mL	AUC(0-24h) h.ng/mL	AUC(0-24h)/dose h.ng/mL/mg/kg
3	Male	0			MNC		NC	NC	NC
		28	3	20.1	MNC	5	63.7		
		49	3	20.8	MNC	5	69		
		91	1	14.8	MNC	3	32.6		
	Female	0			MNC		NC	NC	NC
		28			MNC		NC	NC	NC
		49			MNC		NC	NC	NC
		91			MNC		NC	NC	NC
10	Male	0	3	19.2	MNC	5	57.1	NC	NC
		28	3	32.3	MNC	8	147	NC	NC
		49	3	30.4	MNC	8	136	NC	NC
		91	1	28.6	MNC	5	88.9	NC	NC
	Female	0	1	16.0	MNC	1	8.0	NC	NC
		28	5	11.5	MNC	5	36.5	NC	NC
		49	3	20.5	MNC	3	30.8	NC	NC
		91	1	14.7	MNC	8	39.3	NC	NC
30	Male	0	3	63.1	MNC	8	373	NC	NC
		28	3	55.7	MNC	8	266	NC	NC
		49	3	67.6	18.0	24	720	720	24.0
		91	5	40.6	13.4	24	581	581	19.4
	Female	0	1	36.9	MNC	5	102	NC	NC
		28	3	35.9	18.6	24	640	640	21.3
		49	3	44.3	21.3	24	739	739	24.6
		91	1	57.7	31.5	96	2430	841	28.0
30int.*	Male	0	5	70.4	MNC	8	434	NC	NC
		28	3	65.3	MNC	8	287	NC	NC
		49	3	67.2	14.1	24	683	683	22.8
		91	5	28.7	17.6	24	512	512	17.1
	Female	0	1	54.5	MNC	5	147	NC	NC
		28	3	50.2	18.2	24	691	691	23.0
		49	5	44.3	20.9	24	772	772	25.7
		91	3	47.3	25.8	24	689	689	23.0

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Table 8 Toxicokinetic parameters of RO0731519 following oral administration of 3, 10 and 30 mg/kg/day RO0673189-008 to rats

Dose mg/kg	Gender	Day	Tmax h	Cmax ng/mL	C(24h) ng/mL	Tlast h	AUC(0-last) h.ng/mL	AUC(0-24h) h.ng/mL	AUC(0-24h)/dose h.ng/mL/mg/kg
3	Male	0			MNC		NC	NC	NC
		28	5	25.7	20.1	24	528	528	176
		49	5	30.5	24.8	24	666	666	222
		91	8	24.7	21.5	24	552	552	184
	Female	0			MNC		NC	NC	NC
		28			MNC		NC	NC	NC
		49	8	21.4	14.5	24	420	420	140
		91	5	11.7	10.9	24	138	138	46
10	Male	0	8	25.8	20.5	24	487	487	48.7
		28	5	56.9	45.0	24	1230	1230	123
		49	5	64.2	47.1	24	1350	1350	135
		91	5	54.2	47.7	24	1190	1190	119
	Female	0			MNC		NC	NC	NC
		28	8	47.0	46.3	24	1110	1110	111
		49	5	57.3	54.0	24	1310	1310	131
		91	24	55.1	55.1	24	1260	1260	126
30	Male	0	8	55.1	30.4	24	934	934	31.1
		28	8	110	85.2	24	2350	2350	78.3
		49	5	131	113	24	2860	2860	95.3
		91	3	116	116	96	8560	2720	90.7
	Female	0	24	21.4	21.4	24	NC	NC	NC
		28	3	148	129	24	3260	3260	109
		49	5	153	150	24	3530	3530	118
		91	24	169	169	384	31600	3750	125
30int.*	Male	0	24	55.6	55.6	24	1090	1090	36.3
		28	8	114	91.3	24	2330	2330	77.7
		49	8	139	109	24	2790	2790	93.0
		91	24	115	115	96	7620	2520	84.0
	Female	0	24	21.8	21.8	24	NC	NC	NC
		28	3	110	108	24	2340	2340	78.0
		49	24	131	131	24	2650	2650	88.3
		91	24	128	128	192	15700	2610	87.0

intermittent administration

Key study findings: In the 13-week oral toxicity study, rats were treated with netupitant at 0, 3, 10, and 30 mg/kg/day for 13 weeks. Treatment with netupitant decreased the terminal body weight gain by 12% in males and 19% in females in the high dose group. Slight change of terminal body weight gain was noted in the mid dose group (10 mg/kg/day). Treatment with netutipant induced phospholipidosis in a dose dependent manner. Minimal necrosis was noted in the liver in four high dose females (none in other groups). Based on the effects on the body weight and histopathological changes (minimal necrosis in the liver in high dose females), the MTD is estimated between doses of 10 and 30 mg/kg/day or 20 mg/kg/day for females. The decreasing terminal body weight gain at high dose of 30 mg/kg/day in males (12%) suggests that the MTD is about 30 mg/kg/day. There was no other dose limiting

toxicity found in males at this dose. Therefore, the dose of 30 mg/kg/day is considered as MTD for males.

Study title: 26-week Oral Toxicity Study with Netupitant in Rats

Study no.:	NETU-07-21
Study report location:	n/a
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 16, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Netupitant, 27003139, 98.7%

Key Study Findings:

Rats were treated with netupitant at 0, 1, 3, or 10 mg/kg/day by oral gavage for 26 weeks. Treatment with 10 mg/kg/day resulted in periacinar hepatocytic hypertrophy, thyroid follicular epithelial hypertrophy (males), thymic atrophy, increased aggregations of alveolar macrophages, and syncytial macrophages in mandibular and mesenteric lymph nodes and spleen. The NOAEL is considered to be 3 mg/kg/day based on the microscopic findings, although the high dose of 10 mg/kg/day was well tolerated.

Methods

Doses:	0, 1, 3, and 10 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	Thixotrope vehicle
Species/Strain:	Crl:WI(Han) rats
Number/Sex/Group:	20/sex/group and additional 10/sex/group in control and high dose groups
Age:	~7 weeks
Weight:	Males: 228-232 g Females: 163-166 g
Satellite groups:	3, 5, 5, and 5/sex/group
Unique study design:	No
Deviation from study protocol:	No deviation occurred which adversely affected the quality of the study.

The dose selection was based on the results of the previous studies including a

28-day oral toxicity study (# 1006011) and 13-week oral toxicity study (# 161-578) in rats. In the 13-week oral toxicity study, netupitant produced a 12-19% decrease in terminal body weight gain in the high dose group (30 mg/kg/day). The sponsor selected 30 mg/kg/day as the high dose in the current study. There were also 14-day to 4 week oral toxicity studies in rats (# 1007326, # B-167719, # B-167723, and # 1003562). In these studies, the doses tested were up to 300 mg/kg/day. Phospholipidosis was noted at doses of 30 mg/kg/day or higher.

Thixotrope vehicle; this vehicle was used in previous studies and was selected by the sponsor.

This vehicle contains: 1.60% Avicel RC591 (provided by the sponsor, obtained from (b) (4) 0.20% Tween 80 (b) (4) 0.18% p-Hydroxybenzoic acid methyl ester, 0.02% p-Hydroxybenzoic acid propyl ester (b) (4) Citric acid solution, 0.1N (Citric acid from (b) (4) to adjust the pH to 6.0 and demineralised water.

Observations and Results

Mortality

One female in high dose group died during blood sampling on week 13.

Clinical Signs

There were no clearly treatment-related changes.

Body Weights

A slight reduction in body weight gain was noted in the high dose females (20-53% gain in high dose females, compared to 22-59% in control females).

Feed Consumption

Slightly lower food consumption was noted in the high dose females.

Ophthalmoscopy

There were no clearly treatment-related changes.

ECG

Not conducted.

Hematology

Slight decreases in hemoglobin, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin were noted in the high dose females.

Clinical Chemistry

In high dose group, increased total globulin, total protein, cholesterol, triglycerides (females only), phospholipids and decreased albumin/globulin ratio and total bilirubin were noted.

Urinalysis

There were no clearly treatment related changes.

Gross Pathology

An accentuated lobular pattern of the liver was noted in one high-dose male and foci on the lungs was observed in 5 high-dose females.

Organ Weights

Increased absolute and relative liver weights occurred in the high-dose males and females (relative liver weights ~115-121% of the control), decreased absolute and relative thymus weights were noted in the middle and high-dose females, and a minimal increase in relative kidney weight was observed in the high-dose females.

Histopathology

Liver:	There was periacinar hepatocytic hypertrophy (minimal to mild) in the livers of male and female rats of the 10mg/kg b.w. group.
Lungs:	There were aggregations of alveolar macrophages in the lungs of male rats (minimal to mild) and female rats (minimal to moderate) increased in the 10mg/kg b.w. group.
Mandibular Lymph Nodes:	Syncytial macrophages (minimal to mild) were increased in male and female rats of the 3 and 10mg/kg b.w. group.
Mesenteric Lymph Nodes:	Syncytial macrophages were increased in male rats (minimal to moderate) and in female rats (minimal to marked) of the 10mg/kg b.w. group.
Spleen:	Syncytial macrophages (minimal) were present in male and female rats of the 10mg/kg b.w. group.
Thyroid:	Thyroid follicular epithelium hypertrophy (minimal) was increased in male rats of the 3 and 10mg/kg b.w. group.
Thymus:	There was thymic atrophy (minimal to mild) in male and female rats of the 10mg/kg b.w. group.

Toxicokinetics

The toxicokinetic data for netupitant and the metabolites M1, M2, and M3 were summarized in the following sponsor's tables. The AUC values for netupitant and M1 were generally comparable, whereas the plasma exposure to M2 and M3 was much lower.

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Data are summarized below:

Toxicokinetic parameters of Netupitant

		Group 2: 1 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} @	hr	24	24	24	24	24	24	24	24
T_{max}	hr	4.0	2.0	0.5	2.0	8.0	4.0	1.0	4.0
C_{max}	ng/mL	55.9	113	134	126	82.7	269	305	318
# C_{max}	kg·ng/mL/mg	55.9	113	134	126	82.7	269	305	318
AUC_{0-24}	hr·ng/mL	877	1740	2430	2550	1580	5660	6710	6070
# AUC_{0-24}	hr·kg·ng/mL/mg	877	1740	2430	2550	1580	5660	6710	6070
AUC_{last}	hr·ng/mL	877	1740	2430	2550	1580	5660	6710	6070
# AUC_{last}	hr·kg·ng/mL/mg	877	1740	2430	2550	1580	5660	6710	6070
AUC_{∞}	hr·ng/mL	1000	n/a	n/a	n/a	n/d	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	1000	n/a	n/a	n/a	n/d	n/a	n/a	n/a
$t_{1/2}$	hr	8.12	15.0*	194*	115*	n/d	106*	358*	41.5*
		Group 3: 3 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} @	hr	24	24	24	24	24	24	24	24
T_{max}	hr	4.0	2.0	0.5	2.0	2.0	2.0	24	24
C_{max}	ng/mL	181	291	571	458	211	575	764	1050
# C_{max}	kg·ng/mL/mg	60.2	97.0	190	153	70.2	192	255	350
AUC_{0-24}	hr·ng/mL	2780	4690	6950	6510	4110	12800	15500	17200
# AUC_{0-24}	hr·kg·ng/mL/mg	927	1560	2320	2170	1370	4270	5170	5730
AUC_{last}	hr·ng/mL	2780	4690	6950	6510	4110	12800	15500	17200
# AUC_{last}	hr·kg·ng/mL/mg	927	1560	2320	2170	1370	4270	5170	5730
AUC_{∞}	hr·ng/mL	3200	n/a	n/a	n/a	8810*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	1070	n/a	n/a	n/a	2940*	n/a	n/a	n/a
$t_{1/2}$	hr	8.23	16.2*	21.0*	25.8*	27.2*	188*	n/d	n/d
		Group 4: 10 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} @	hr	24	24	24	168 @	24	24	24	336 @
T_{max}	hr	2.0	4.0	2.0	2.0	2.0	4.0	4.0	4.0
C_{max}	ng/mL	609	2060	923	829	613	1250	1180	1340
# C_{max}	kg·ng/mL/mg	60.9	206	92.3	82.9	61.3	125	118	134
AUC_{0-24}	hr·ng/mL	8880	20700	14700	16000	13300	24800	24100	27500
# AUC_{0-24}	hr·kg·ng/mL/mg	888	2070	1470	1600	1330	2480	2410	2750
AUC_{last}	hr·ng/mL	8880	20700	14700	48400	13300	24800	24100	138000
# AUC_{last}	hr·kg·ng/mL/mg	888	2070	1470	4840	1330	2480	2410	13800
AUC_{∞}	hr·ng/mL	13500*	n/a	n/a	n/a	260000*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	1350*	n/a	n/a	n/a	26000*	n/a	n/a	n/a
$t_{1/2}$	hr	14.8*	15.1*	25.4*	51.6	305*	91.4*	503*	112

: dose-normalised to 1 mg/kg, * : approximation, n/a : not applicable, n/d : not determined

@ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals at the high dose, a true value for T_{last} was obtained after the final dose was administered.

Data are summarized below:

Toxicokinetic parameters of metabolite M1									
		Group 2: 1 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} @	hr	24	24	24	24	24	24	24	24
T_{max}	hr	8.0	8.0	24	8.0	24	24	predose	4.0
C_{max}	ng/mL	33.2	123	203	190	29.7	157	217	184
# C_{max}	kg·ng/mL/mg	33.2	123	203	190	29.7	157	217	184
AUC_{0-24}	hr·ng/mL	657	2420	4110	4010	522	3490	4530	3960
# AUC_{0-24}	hr·kg·ng/mL/mg	657	2420	4110	4010	522	3490	4530	3960
AUC_{last}	hr·ng/mL	657	2420	4110	4010	522	3490	4530	3960
# AUC_{last}	hr·kg·ng/mL/mg	657	2420	4110	4010	522	3490	4530	3960
AUC_{∞}	hr·ng/mL	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
$t_{1/2}$	hr	n/d	n/d	n/d	n/d	n/d	n/d	n/d	89.9*
		Group 3: 3 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} @	hr	24	24	24	24	24	24	24	24
T_{max}	hr	24	predose	2.0	predose	24	24	24	24
C_{max}	ng/mL	93.9	440	582	652	89.4	532	774	1050
# C_{max}	kg·ng/mL/mg	31.3	147	194	217	29.8	177	258	351
AUC_{0-24}	hr·ng/mL	1850	8280	12200	13700	1460	11100	14300	16900
# AUC_{0-24}	hr·kg·ng/mL/mg	617	2760	4070	4570	487	3700	4770	5630
AUC_{last}	hr·ng/mL	1850	8280	12200	13700	1460	11100	14300	16900
# AUC_{last}	hr·kg·ng/mL/mg	617	2760	4070	4570	487	3700	4770	5630
AUC_{∞}	hr·ng/mL	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
$t_{1/2}$	hr	n/d	n/d	148*	429*	n/d	n/d	n/d	n/d
		Group 4: 10 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} @	hr	24	24	24	336 @	24	24	24	336 @
T_{max}	hr	24	24	2.0	24	24	24	24	24
C_{max}	ng/mL	407	1830	1070	1350	272	1110	1080	1170
# C_{max}	kg·ng/mL/mg	40.7	183	107	135	27.2	111	108	117
AUC_{0-24}	hr·ng/mL	5940	31000	24400	27100	3740	20800	22700	24600
# AUC_{0-24}	hr·kg·ng/mL/mg	594	3100	2440	2710	374	2080	2270	2460
AUC_{last}	hr·ng/mL	5940	31000	24400	169000	3740	20800	22700	203000
# AUC_{last}	hr·kg·ng/mL/mg	594	3100	2440	16900	374	2080	2270	20300
AUC_{∞}	hr·ng/mL	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
$t_{1/2}$	hr	n/d	n/d	n/d	56.5	n/d	n/d	n/d	176*

: dose-normalised to 1 mg/kg, * : approximation, n/a : not applicable, n/d : not determined
@ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals at the high dose, a true value for T_{last} was obtained after the final dose was administered.

Parameters were not determined for Group 2. Data for Groups 3 and 4 are summarised below:

Toxicokinetic parameters of metabolite M2									
		Group 3: 3 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} @	hr	8.0	8.0	8.0	8.0	2.0	8.0	2.0	24
T_{max}	hr	2.0	0.5	0.5	0.5	0.5	0.5	0.5	24
C_{max}	ng/mL	21.0	26.3	35.8	35.1	23.5	21.3	13.9	20.1
# C_{max}	kg·ng/mL/mg	7.00	8.77	11.9	11.7	7.83	7.09	4.62	6.70
AUC_{0-24}	hr·ng/mL	153	388	288	268	40.4	194	203	346
# AUC_{0-24}	hr·kg·ng/mL/mg	51.0	129	96.1	89.5	13.5	64.7	67.7	115
AUC_{last}	hr·ng/mL	108	161	149	143	27.7	113	22.3	346
# AUC_{last}	hr·kg·ng/mL/mg	36.0	53.7	49.7	47.7	9.23	37.7	7.43	115
AUC_{∞}	hr·ng/mL	157*	n/a	n/a	n/a	40.4*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	52.4*	n/a	n/a	n/a	13.5*	n/a	n/a	n/a
$t_{1/2}$	hr	4.33*	18.0*	8.53*	7.55*	1.04*	7.83*	16.6*	n/d
		Group 4: 10 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} @	hr	24	24	24	96 @	8.0	24	24	48 @
T_{max}	hr	1.0	4.0	2.0	2.0	2.0	2.0	0.5	1.0
C_{max}	ng/mL	54.1	165	37.0	53.5	71.5	34.2	32.0	37.6
# C_{max}	kg·ng/mL/mg	5.41	16.5	3.70	5.35	7.15	3.42	3.20	3.76
AUC_{0-24}	hr·ng/mL	628	1380	504	726	235	644	546	650
# AUC_{0-24}	hr·kg·ng/mL/mg	62.8	138	50.4	72.6	23.5	64.4	54.6	65.0
AUC_{last}	hr·ng/mL	628	1380	504	1990	212	644	546	1090
# AUC_{last}	hr·kg·ng/mL/mg	62.8	138	50.4	199	21.2	64.4	54.6	109
AUC_{∞}	hr·ng/mL	743*	n/a	n/a	n/a	236*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	74.3*	n/a	n/a	n/a	23.6*	n/a	n/a	n/a
$t_{1/2}$	hr	8.72	12.7*	21.6*	49.8*	2.13*	n/d	80.4*	26.4*

: dose-normalised to 1 mg/kg, * : approximation, n/a : not applicable, n/d : not determined
@ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals at the high dose, a true value for T_{last} was obtained after the final dose was administered.

Parameters were not determined for Group 2. Data for Groups 3 and 4 are summarised below:

Toxicokinetic parameters of metabolite M3									
		Group 3: 3 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} [@]	hr	n/d	24	24	24	n/d	24	24	24
T_{max}	hr	n/d	8.0	4.0	8.0	n/d	24	24	24
C_{max}	ng/mL	n/d	20.1	35.5	34.0	n/d	19.0	34.8	44.1
# C_{max}	kg·ng/mL/mg	n/d	6.70	11.8	11.3	n/d	6.34	11.6	14.7
AUC_{0-24}	hr·ng/mL	n/d	434	681	695	n/d	401	616	722
# AUC_{0-24}	hr·kg·ng/mL/mg	n/d	145	227	232	n/d	134	205	241
AUC_{last}	hr·ng/mL	n/d	434	681	695	n/d	401	616	722
# AUC_{last}	hr·kg·ng/mL/mg	n/d	145	227	232	n/d	134	205	241
AUC_{∞}	hr·ng/mL	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
$t_{1/2}$	hr	n/d	n/d	36.6*	n/d	n/d	n/d	n/d	n/d
		Group 4: 10 mg Netupitant/kg/day							
		Males				Females			
		D1	W4	W13	W26	D1	W4	W13	W26
T_{last} [@]	hr	24	24	24	96 [@]	n/d	24	24	168 [@]
T_{max}	hr	8.0	4.0	2.0	24	n/d	24	24	8.0
C_{max}	ng/mL	40.2	113	68.5	71.5	n/d	61.9	82.9	79.4
# C_{max}	kg·ng/mL/mg	4.02	11.3	6.85	7.15	n/d	6.19	8.29	7.94
AUC_{0-24}	hr·ng/mL	760	1870	1470	1590	n/d	1290	1740	1860
# AUC_{0-24}	hr·kg·ng/mL/mg	76	187	147	159	n/d	129	174	186
AUC_{last}	hr·ng/mL	760	1870	1470	4340	n/d	1290	1740	8020
# AUC_{last}	hr·kg·ng/mL/mg	76	187	147	434	n/d	129	174	802
AUC_{∞}	hr·ng/mL	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	n/d	n/a	n/a	n/a	n/d	n/a	n/a	n/a
$t_{1/2}$	hr	n/d	n/d	118*	56.3*	n/d	n/d	n/d	93.3*

: dose-normalised to 1 mg/kg, * : approximation, n/a : not applicable, n/d : not determined
[@] If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals at the high dose, a true value for T_{last} was obtained after the final dose was administered.

Dosing Solution Analysis

The analysis of drug concentration in samples of dosing formulations from each dose-group was in agreement with target concentrations (between 90% and 110%).

Dog:

Study title: RO0673189: Thirteen week oral (capsule) toxicity study in dogs

Study no.:	1009175
Study report location:	n/a
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 13 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RO0673189-008, BS0204SA01, 99.8%

Key Study Findings:

Dogs were treated with netupitant at 0, 1, 3, and 10 mg/kg/day via oral capsule for 13 weeks. Treatment with netupitant at 10 mg/kg/day resulted in vacuolated macrophages or vacuolated tingible body macrophages in the lymphoid tissue (mesenteric or mandibular lymph nodes, Peyer's patches or colonic lymphoid tissue), suggesting a drug-induced phospholipidosis. Since the microscopic lesions are not considered to be adverse, the NOAEL is 10 mg/kg/day.

Methods

Doses:	0, 1, 3, and 10 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral capsule
Dose volume:	N/A
Formulation/Vehicle:	Empty capsule
Species/Strain:	beagle dogs
Number/Sex/Group:	3 or 5/sex/group
Age:	~5-6 months
Weight:	Males: 9-11 kg Females: 7-10 kg
Satellite groups:	none
Unique study design:	No
Deviation from study protocol:	No deviation occurred which adversely affected the quality of the study.

Dose selection was based on the results of the 4-week oral dose toxicity study in dogs (#1006010). Body weight loss was noted at doses of 15 mg/kg/day or higher in this study. The sponsor selected 10 mg/kg/day as the high dose in the current study. There was also a 7-day oral toxicity study in female Beagle dogs with doses up to 15 mg/kg/day (#1006679). In this study, slight body weight loss and aggregates of foamy or vacuolated macrophages in lymphoid tissue and intestinal mucosa, suggesting phospholipidosis, were noted at 15 mg/kg/day.

Observations and Results

Mortality

No deaths occurred.

Clinical Signs

There were no clearly treatment-related changes.

Body Weights

There were no clear treatment effects on body weight.

Feed Consumption

Slightly lower food consumption was noted in the high dose groups.

Ophthalmoscopy

There were no clearly treatment-related changes.

ECG

There were no clear treatment effects on blood pressure, heart rate, or ECGs. These cardiovascular parameters were determined before study initiation and on days 1, 25, 88, and 142 at pre-dose and 1, and 6 hours after dosing.

Hematology

There were no clearly treatment-related changes.

Clinical Chemistry

There were no clearly treatment-related changes.

Urinalysis

There were no clearly treatment-related changes.

Gross Pathology

Darkness or dark areas were seen in the jejunum in one male each from low dose and high dose groups, and in one male and one female from middle dose group.

Organ Weights

The absolute and relative liver weights were higher in the high dose group (+18% and +19%, respectively, in males and (+40% and +52%, respectively, in females as compared to the controls.

Histopathology

Vacuolated macrophages or vacuolated tingible body macrophages were observed in the lymphoid tissue (mesenteric or mandibular lymph nodes, Peyer's patches or colonic lymphoid tissue) in the high dose group, suggesting a drug-induced phospholipidosis. These effects are not considered to be adverse.

Toxicokinetics

The maximum plasma concentrations of netupitant (C_{max}) in males were 84.6, 229 and 843 ng/mL on day 0 and 169, 342 and 819 ng/mL on day 87 following doses of 1, 3 and 10 mg/kg netupitant, respectively.

The C_{max} values for netupitant in females were 89.7, 244 and 635 ng/mL on day 0 and 137, 324 and 420 ng/mL on day 87 following doses of 1, 3 and 10 mg/kg netupitant, respectively.

The AUC_{0-24hr} values for netupitant in males were 880, 2140 and 7850 ng•hr/mL on day 0, and 2230, 3970 and 11,000 ng•hr/mL on day 87 following doses of 1, 3 and 10 mg/kg netupitant, respectively.

The AUC_{0-24hr} values for netupitant in females were 771, 2350 and 7310 ng•hr/mL on day 0, and 1640, 3520 and 6470 ng•hr/mL on day 87 following doses of 1, 3 and 10 mg/kg netupitant, respectively.

In males, C_{max} values for the main metabolite (M1) were 139, 261 and 1130 ng/mL on day 87 following doses of 1, 3 and 10 mg/kg netupitant, respectively. AUC values for M1 in males were 130%, 146%, and 231% of that of the parent compound on day 87 following doses of 1, 3 and 10 mg/kg, respectively.

In females, C_{max} values for the main metabolite (M1) were 106, 280 and 716 ng/mL on day 87 following doses of 1, 3 and 10 mg/kg netupitant, respectively. AUC values for M1 in females were 127%, 163%, and 241% of that of the parent compound on day 87 following doses of 1, 3 and 10 mg/kg, respectively.

The AUC_{0-24hr} values for metabolite M2 were 41% and 48% of that of the parent compound on day 87 in females and males, respectively. The AUC_{0-24hr} values for metabolite M3 were 14% and 11% of that of the parent compound on day 87 in females and males, respectively.

Dosing Solution Analysis

Oral capsules were used. The test article was packed in capsules on the day before the day of dosing.

Study title: 9-Month oral gavage toxicity study in dogs

Study no.:	NETU-07-22
Study report location:	n/a
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 15, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Netupitant, 27003139, 98.9%

Key Study Findings:

Dogs were treated with netupitant at 0, 1, 3, and 10 mg/kg/day by oral gavage for 9 months. Treatment with netupitant at 10 mg/kg/day slightly increased the QT_c and PQ intervals (males), and produced minimal periacinar hepatocytic hypertrophy (males). Plasma exposure to the metabolite M1 was approximately 2 times that of netupitant. The NOAEL was 10 mg/kg/day.

Methods

Doses:	0, 1, 3, and 10 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	1 ml/kg
Formulation/Vehicle:	Thixotrope vehicle
Species/Strain:	beagle dogs
Number/Sex/Group:	4 or 7/sex/group (see sponsor's table below)
Age:	~5-6 months
Weight:	Males: 8.0-8.2 kg Females: 5.7-5.9 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No deviation occurred which adversely affected the quality of the study.

Group	Dose Level (mg/kg b.w.)	Number of Animals		Animal Numbers	
	Netupitant	Males	Females	Males	Females
1 Main	0 (vehicle)	4	4	1-4	23-26
1 Recovery		3	3	5-7	27-29
2 Main	1	4	4	8-11	30-33
3 Main	3	4	4	12-15	34-37
4 Main	10	4	4	16-20[#]	38-41
4 Recovery		3	3	21-22	42-44

[#] Animal 20 was transferred from the Recovery to the Main group, and necropsied at the end of the treatment period, to compensate for animal 16 which died intercurrently.

Dose selection was based on the results of the 4-week oral dose toxicity study in dogs (# 1006010). Body weight loss was noted at doses of 15 mg/kg/day or higher in this study. The sponsor selected 10 mg/kg/day as the high dose in the current study. There was also a 7-day oral toxicity study in female Beagle dogs (# 1006679), with doses up to 15 mg/kg/day. In that study, slight body weight loss and aggregates of foamy or vacuolated macrophages in lymphoid tissue and intestinal mucosa were observed at 15 mg/kg/day, suggesting phospholipidosis.

Observations and Results

Mortality

One high-dose male was sacrificed due to moribund condition.

Clinical Signs

There were no clearly treatment-related signs.

Body Weights

The terminal body weight gains were 41% for the control males and 28% for the high-dose males, and 53% for control females and 36% for high-dose females.

Feed Consumption

Slightly lower food consumption was noted in the high-dose females.

Ophthalmoscopy

There were no clearly treatment-related changes.

ECG

Slightly prolonged QT_c intervals were noted in the high-dose males during treatment weeks 13 and 26 (231-240 msec measured post-dose, compared with 213-215 msec at pre-dose).

Slightly prolonged PQ intervals were also noted in the high-dose males during treatment weeks 13 and 26 (98-104 msec measured post-dose, compared with 83-93 msec at pre-dose).

There were no clear treatment effects on blood pressure.

Hematology

There were no clearly treatment-related changes.

Clinical Chemistry

Alkaline phosphatase was increased (131-262 U/L in high dose group compared to 50-118 U/L in control group).

Urinalysis

There were no clearly treatment-related changes.

Gross Pathology

An enlarged liver was noted in one high-dose male.

Organ Weights

The absolute and relative liver weights were approximately 139% and 151% of mean control weight in the high-dose males.

Histopathology

Minimal periacinar hepatocytic hypertrophy was noted in the high-dose males.

Toxicokinetics

The toxicokinetic data for netupitant and the metabolites M1, M2, and M3 are summarized in the following sponsor's tables. The AUC values for M1 were approximately 2-fold greater than the AUC values for netupitant. The AUC values for the parent compound and its metabolites in the high-dose group were higher in males.

Toxicokinetic data of Netupitant are summarised below:

Parameters		Group 2 1 mg/kg		Group 3 3 mg/kg		Group 4 10 mg/kg	
		M	F	M	F	M	F
Day 1							
$T_{last}^{\$@}$	hr	24	24	24	24	24	24
$T_{max}^{\$}$	hr	2.0	1.5	2.0	2.0	4.0	2.0
C_{max}	ng/mL	59.2	124	320	257	1010	576
# C_{max}	kg·ng/mL/mg	59.2	124	107	85.9	101	57.6
AUC_{last}	hr·ng/mL	640	1080	3090	2350	13000	5730
# AUC_{last}	hr·kg·ng/mL/mg	640	1080	1030	782	1300	573
AUC_{∞}	hr·ng/mL	633*	1380*	3710*	3090*	16900*	7930*
# AUC_{∞}	hr·kg·ng/mL/mg	633*	1380*	1230*	1030*	1690*	793*
$t_{1/2}$	hr	7.37	10.3*	8.73	11.5*	11.8*	13.3*
Week 4							
$T_{last}^{\$@}$	hr	24	24	24	24	24	24
$T_{max}^{\$}$	hr	1.0	2.0	2.0	2.0	2.0	2.0
C_{max}	ng/mL	151	175	484	410	1320	812
# C_{max}	kg·ng/mL/mg	151	175	161	137	132	81.2
AUC_{last}	hr·ng/mL	1460	1720	5180	4560	17100	10100
# AUC_{last}	hr·kg·ng/mL/mg	1460	1720	1730	1520	1710	1010
$t_{1/2}$	hr	10.3*	11.1*	11.9*	13.4*	13.0*	18.0*
Week 13							
$T_{last}^{\$@}$	hr	24	24	24	24	24	24
$T_{max}^{\$}$	hr	2.0	1.5	2.0	2.0	2.0	2.0
C_{max}	ng/mL	154	183	470	411	1310	854
# C_{max}	kg·ng/mL/mg	154	183	157	137	131	85.4
AUC_{last}	hr·ng/mL	1750	1950	5650	4980	18800	11600
# AUC_{last}	hr·kg·ng/mL/mg	1750	1950	1880	1660	1880	1160
$t_{1/2}$	hr	13.4*	11.7*	13.1*	12.9*	16.3*	16.8*
Week 26							
$T_{last}^{\$@}$	hr	24	24	24	24	24	24
$T_{max}^{\$}$	hr	2.0	2.0	2.0	2.0	2.0	2.0
C_{max}	ng/mL	165	216	460	562	1240	981
# C_{max}	kg·ng/mL/mg	165	216	153	187	124	98.1
AUC_{last}	hr·ng/mL	1730	2120	5470	6320	20100	14100
# AUC_{last}	hr·kg·ng/mL/mg	1730	2120	1820	2110	2010	1410
$t_{1/2}$	hr	10.5*	14.2*	12.8*	13.6*	19.0*	18.4*
Week 39							
$T_{last}^{\$@}$	hr	24	24	24	24	$24^{@}$	$24^{@}$
$T_{max}^{\$}$	hr	2.0	1.5	2.0	2.0	2.0	2.0
C_{max}	ng/mL	147	202	466	417	1200	848
# C_{max}	kg·ng/mL/mg	147	202	155	139	120	84.8
$AUC_{last}^{‡}$	hr·ng/mL	1880	2290	6280	5520	20500	12400
# $AUC_{last}^{‡}$	hr·kg·ng/mL/mg	1880	2290	2090	1840	2050	1240
$t_{1/2}$	hr	14.0*	13.7*	13.8*	12.8*	20.9*^	21.8*^

: dose-normalised to 1 mg/kg, * : approximation, $^{\$}$: median for Tlast and Tmax, ‡ : $AUC_{0\text{-}24h}$ was used for Recovery animals of Group 4, ^ : Recovery animals excluded, $^{@}$ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered: it was 168hr for males and 96hr for females at the high dose

Toxicokinetic data of M1 are summarised below:

Parameters		Group 2 1 mg/kg		Group 3 3 mg/kg		Group 4 10 mg/kg	
		M	F	M	F	M	F
Day 1							
T_{last} $^{\$ @}$	hr	24	24	24	24	24	24
T_{max} $^{\$}$	hr	8.0	6.0	8.0	8.0	8.0	8.0
C_{max}	ng/mL	45.0	67.6	183	131	714	333
# C_{max}	kg·ng/mL/mg	45.0	67.6	60.9	43.6	71.4	33.3
AUC_{last}	hr·ng/mL	831	1360	3570	2580	14000	6360
# AUC_{last}	hr·kg·ng/mL/mg	831	1360	1190	858	1400	636
AUC_{∞}	hr·ng/mL	n/d	6770*	9300*	n/d	n/d	n/d
# AUC_{∞}	hr·kg·ng/mL/mg	n/d	6770*	3100*	n/d	n/d	n/d
$t_{1/2}$	hr	n/d	60.6*	33.8*	n/d	n/d	n/d
Week 4							
T_{last} $^{\$ @}$	hr	24	24	24	24	24	24
T_{max} $^{\$}$	hr	4.0	4.0	6.0	8.0	8.0	8.0
C_{max}	ng/mL	146	168	484	415	1850	1070
# C_{max}	kg·ng/mL/mg	146	168	161	138	185	107
AUC_{last}	hr·ng/mL	2820	3280	9910	8720	40800	23100
# AUC_{last}	hr·kg·ng/mL/mg	2820	3280	3300	2910	4080	2310
$t_{1/2}$	hr	21.5*	29.0*	32.6*	43.7*	n/d	n/d
Week 13							
T_{last} $^{\$ @}$	hr	24	24	24	24	24	24
T_{max} $^{\$}$	hr	4.0	8.0	8.0	6.0	8.0	8.0
C_{max}	ng/mL	161	201	544	445	1980	1290
# C_{max}	kg·ng/mL/mg	161	201	181	148	198	129
AUC_{last}	hr·ng/mL	3170	3850	11700	9220	42600	26700
# AUC_{last}	hr·kg·ng/mL/mg	3170	3850	3880	3070	4260	2670
$t_{1/2}$	hr	27.1*	26.2*	83.0*	47.7*	106*	44.1*
Week 26							
T_{last} $^{\$ @}$	hr	24	24	24	24	24	24
T_{max} $^{\$}$	hr	6.0	8.0	5.0	2.0	4.0	4.0
C_{max}	ng/mL	166	199	526	560	2110	1310
# C_{max}	kg·ng/mL/mg	166	199	175	187	211	131
AUC_{last}	hr·ng/mL	3310	4160	10700	11900	45000	28900
# AUC_{last}	hr·kg·ng/mL/mg	3310	4160	3560	3960	4500	2890
$t_{1/2}$	hr	33.2*	30.2*	55.8*	59.1*	138*	64.0*
Week 39							
T_{last} $^{\$ @}$	hr	24	24	24	24	24$^{@}$	24$^{@}$
T_{max} $^{\$}$	hr	4.0	4.0	8.0	8.0	8.0	4.0
C_{max}	ng/mL	192	225	555	457	1990	1200
# C_{max}	kg·ng/mL/mg	192	225	185	153	199	120
AUC_{last} ‡	hr·ng/mL	3890	4650	12200	9920	45400	26100
# AUC_{last} ‡	hr·kg·ng/mL/mg	3890	4650	4070	3310	4540	2610
$t_{1/2}$	hr	33.4*	39.6*	n/d	37.0*	125*^	132*^

: dose-normalised to 1 mg/kg, * : approximation, $: median for Tlast and Tmax, n/d : not determined as the elimination phase could not be modelled with the data available, ‡ : $AUC_{0\text{-}24h}$ was used for Recovery animals of Group 4, ^ : Recovery animals excluded, @ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered: it was 336hr for males and females at the high dose

Toxicokinetic data of M2 are summarised below:

Parameters		Group 2 1 mg/kg		Group 3 3 mg/kg		Group 4 10 mg/kg	
		M	F	M	F	M	F
Day 1							
T_{last} $ @	hr	8.0	8.0	24	16.0	24	24
T_{max} $	hr	3.0	1.5	2.0	2.0	4.0	2.0
C_{max}	ng/mL	25.9	80.2	135	142	553	529
# C_{max}	kg·ng/mL/mg	25.9	80.2	45.0	47.3	55.3	52.9
AUC_{last}	hr·ng/mL	228	452	1150	937	5180	3410
# AUC_{last}	hr·kg·ng/mL/mg	228	452	383	312	518	341
AUC_{∞}	hr·ng/mL	192*	659*	1430*	1160	7950*	3950*
# AUC_{∞}	hr·kg·ng/mL/mg	192*	659*	477*	387	795*	395*
$t_{1/2}$	hr	4.78*	4.90*	9.25*	5.92	14.9*	7.74*
Week 4							
T_{last} $ @	hr	24	24	24	24	24	24
T_{max} $	hr	2.0	2.0	2.0	3.0	2.0	2.0
C_{max}	ng/mL	67.8	56.2	163	140	607	585
# C_{max}	kg·ng/mL/mg	67.8	56.2	54.4	46.8	60.7	58.5
AUC_{last}	hr·ng/mL	652	554	1580	1280	6330	5160
# AUC_{last}	hr·kg·ng/mL/mg	652	554	527	427	633	516
$t_{1/2}$	hr	11.3*	11.5*	20.0*	15.9*	14.6*	10.3*
Week 13							
T_{last} $ @	hr	24	16.0	24	24	24	24
T_{max} $	hr	2.0	2.0	2.0	2.0	2.0	2.0
C_{max}	ng/mL	50.3	60.7	139	124	593	600
# C_{max}	kg·ng/mL/mg	50.3	60.7	46.3	41.2	59.3	60.0
AUC_{last}	hr·ng/mL	585	524	1550	1310	6630	4980
# AUC_{last}	hr·kg·ng/mL/mg	585	524	515	437	663	498
$t_{1/2}$	hr	15.7*	13.7*	17.5*	18.3*	21.5*	12.5*
Week 26							
T_{last} $ @	hr	24	24	24	24	24	24
T_{max} $	hr	2.0	2.0	2.0	2.0	2.0	2.0
C_{max}	ng/mL	53.4	75.9	133	161	396	334
# C_{max}	kg·ng/mL/mg	53.4	75.9	44.2	53.6	39.6	33.4
AUC_{last}	hr·ng/mL	737	900	1600	1750	5840	4200
# AUC_{last}	hr·kg·ng/mL/mg	737	900	532	583	584	420
$t_{1/2}$	hr	20.5*	16.9*	19.9*	20.5*	34.8*	24.2*
Week 39							
T_{last} $ @	hr	24	24	24	24	24@	24@
T_{max} $	hr	2.0	2.0	2.0	2.0	2.0	2.0
C_{max}	ng/mL	68.8	85.2	149	120	437	440
# C_{max}	kg·ng/mL/mg	68.8	85.2	49.5	40.1	43.7	44.0
AUC_{last} ‡	hr·ng/mL	984	1160	1990	1630	6380	4940
# AUC_{last} ‡	hr·kg·ng/mL/mg	984	1160	663	543	638	494
$t_{1/2}$	hr	21.4*	17.7*	21.6*	24.6*	34.5*^	15.6*^

: dose-normalised to 1 mg/kg, * : approximation, $: median for Tlast and Tmax, ‡ : $AUC_{0\text{-}24h}$ was used for Recovery animals of Group 4, ^ : Recovery animals excluded, @ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered: it was 168hr for males and 96-168hr for females at the high dose

Toxicokinetic data of M3 are summarised below:

Parameters		Group 2 1 mg/kg		Group 3 3 mg/kg		Group 4 10 mg/kg	
		M	F	M	F	M	F
Day 1							
T_{last} $ @	hr			8.0	16	24	24
T_{max} $	hr			4.0	4.0	8.0	4.0
C_{max}	ng/mL			18.0	24.0	78.5	44.4
# C_{max}	kg·ng/mL/mg			6.00	7.98	7.85	4.44
AUC_{last}	hr·ng/mL	insuff.	data	106	313	1280	789
# AUC_{last}	hr·kg·ng/mL/mg			35.4	104	128	78.9
AUC_{∞}	hr·ng/mL			n/d	965*	1990*	1830*
# AUC_{∞}	hr·kg·ng/mL/mg			n/d	322*	199*	183*
$t_{1/2}$	hr			n/d	21.0*	13.2*	37.7*
Week 4							
T_{last} $ @	hr			24	24	24	24
T_{max} $	hr			4.0	4.0	8.0	8.0
C_{max}	ng/mL			31.5	31.0	117	69.2
# C_{max}	kg·ng/mL/mg			10.5	10.3	11.7	6.92
AUC_{last}	hr·ng/mL	insuff.	data	485	525	2190	1350
# AUC_{last}	hr·kg·ng/mL/mg			162	175	219	135
$t_{1/2}$	hr			17.7*	20.8*	11.2*	17.7*
Week 13							
T_{last} $ @	hr			24	24.0	24	24
T_{max} $	hr			4.0	4.0	8.0	4.0
C_{max}	ng/mL			30.6	33.2	117	73.8
# C_{max}	kg·ng/mL/mg			10.2	11.1	11.7	7.38
AUC_{last}	hr·ng/mL	insuff.	data	592	638	2390	1460
# AUC_{last}	hr·kg·ng/mL/mg			197	213	239	146
$t_{1/2}$	hr			21.9*	19.9*	43.2*	28.0*
Week 26							
T_{last} $ @	hr			24	24	24	24
T_{max} $	hr			6.0	4.0	8.0	4.0
C_{max}	ng/mL			30.3	47.1	119	82.3
# C_{max}	kg·ng/mL/mg			10.1	15.7	11.9	8.23
AUC_{last}	hr·ng/mL	insuff.	data	566	858	2460	1640
# AUC_{last}	hr·kg·ng/mL/mg			189	286	246	164
$t_{1/2}$	hr			22.6*	20.1*	53.3*	59.2*
Week 39							
T_{last} $ @	hr			24	24	24@	24@
T_{max} $	hr			8.0	8.0	8.0	8.0
C_{max}	ng/mL			32.7	34.5	115	78.2
# C_{max}	kg·ng/mL/mg			10.9	11.5	11.5	7.82
AUC_{last} ‡	hr·ng/mL	insuff.	data	648	679	2470	1590
# AUC_{last} ‡	hr·kg·ng/mL/mg			216	226	247	159
$t_{1/2}$	hr			19.3*	n/d	80.7*^	35.1*^

: dose-normalised to 1 mg/kg, * : approximation, $: median for Tlast and Tmax, n/d : not determined as the elimination phase could not be modelled with the data available, ‡ : $AUC_{0\text{-}24h}$ was used for Recovery animals of Group 4, ^ : Recovery animals excluded, @ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered: it was 96hr for males and 48-96hr for females at the high dose, insuff.data: Concentrations were near or below LLOQ, no calculations performed.

Percentage of Netupitant exposure per period in each dose group																
	M1						M2						M3			
Group	2	2	3	3	4	4	2	2	3	3	4	4	3	3	4	4
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
C_{max}																
Day 1	76	55	57	51	71	58	44	65	42	55	55	92	6	9	8	8
Week 4	97	96	100	101	140	132	45	32	34	34	46	72	7	8	9	9
Week 13	105	110	116	108	151	151	33	33	30	30	45	70	7	8	9	9
Week 26	101	92	114	100	170	134	32	35	29	29	32	34	7	8	10	8
Week 39	131	111	119	110	166	142	47	42	32	29	36	52	7	8	10	9
AUC_{last} ‡																
Day 1 *	130	99	116	110	108	111	36	33	37	49	40	60	3	13	10	14
Week 4	193	191	191	191	239	229	45	32	31	28	37	51	9	12	13	13
Week 13	181	197	207	185	227	230	33	27	27	26	35	43	10	13	13	13
Week 26	191	196	196	188	224	205	43	42	29	28	29	30	10	14	12	12
Week 39	207	203	194	180	221	210	52	51	32	30	31	40	10	12	12	13

* AUC_{last} was used when AUC_{∞} was reported as approximation
‡ : $AUC_{0\text{-}24h}$ was used for Recovery animals of Group 4.

Dosing Solution Analysis

The analysis of drug concentrations in samples of dosing formulations from each dose-group was in agreement with target concentrations (between 96% and 101%).

Study title: 13-Week oral gavage toxicity study with palonosetron and netupitant in rats

Study no.: NETU-07-19
Study report location: n/a
Conducting laboratory and location: (b) (4)
Date of study initiation: July 2, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Palonosetron, 26004786, 99.5%
Netupitant, BS0307SA01, 99.5%

Key Study Findings:

Rats were treated with netupitant at 1, 3, and 10 mg/kg/day in combination with palonosetron at 2, 6, and 18 mg/kg/day, respectively, by oral gavage for 13 weeks. Additional groups were treated orally with 10 mg/kg/day netupitant or 18 mg/kg/day

palonosetron. Slight increases in absolute and relative liver weights were noted in the high-dose combination group, the netupitant only group, and the palonosetron only group (up to 120% of the mean control weight). Histopathological examination revealed adrenal zona fasciculata hypertrophy, hepatocytic hypertrophy, and syncytial macrophages in the mesenteric lymph nodes, mainly in the high-dose combination and netupitant only groups. These effects are not considered to be adverse. Therefore, the NOAEL was 10 mg/kg/day netupitant + 18 mg/kg/day palonosetron.

Methods

Doses:	See sponsor's table below
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	Thixotrope vehicle
Species/Strain:	Crl:WI(Han)
Number/Sex/Group:	10/sex/group
Age:	~6 weeks
Weight:	Males: 160-167 g Females: 130-133 g
Satellite groups:	3 or 5/sex/group
Unique study design:	Combination treatment with both palonosetron and netupitant, and individual drugs
Deviation from study protocol:	No deviation occurred which adversely affected the quality of the study.

Group	Dose Level (mg/kg b.w.)		Number of Animals		Animal Numbers	
	Palonosetron	Netupitant	Males	Females	Males	Females
1 Main	0 (vehicle)		10	10	1-10	121-130
1 Satellite			3	3	11-13	131-133
1 Recovery			5	5	16-20	136-140
2 Main	2	1	10	10	21-30	141-150
2 Satellite			5	5	31-35	151-155
2 Recovery			5	5	36-40	156-160
3 Main	6	3	10	10	41-50	161-170
3 Satellite			5	5	51-55	171-175
3 Recovery			5	5	56-60	176-180
4 Main	18	10	10	10	61-70	181-190
4 Satellite			5	5	71-75	191-195
4 Recovery			5	5	76-80	196-200
5 Main	18	0	10	10	81-90	201-210
5 Satellite			5	5	91-95	211-215
5 Recovery			5	5	96-100	216-220
6 Main	0	10	10	10	101-110	221-230
6 Satellite			5	5	111-115	231-235
6 Recovery			5	5	116-120	236-240

The dose selection was based on the results of the previous toxicity studies including 7-day and 28-day studies in rats with combination treatment (# NETU-06-20 and # NETU-06-03, respectively). Doses tested in the 28-day study were:

10 mg/kg/day Palonosetron and 3 mg/kg/day Netupitant
18 mg/kg/day Palonosetron and 10 mg/kg/day Netupitant
60 mg/kg/day Palonosetron and 30 mg/kg/day Netupitant

The group treated with 60 mg/kg/day palonosetron and 30 mg/kg/day netupitant exhibited hunched posture, reduced body weight, and increased incidence and severity of syncytial macrophages in mesenteric lymph nodes. The sponsor selected 18 mg/kg/day palonosetron and 10 mg/kg/day netupitant as the high-dose combination for the current study.

Observations and Results

Mortality

Two main study toxicity rats and 3 satellite rats died during blood sampling. These deaths were not treatment related.

Clinical Signs

Increased salivation was noted in the high-dose combination group.

Body Weights

There were no clearly treatment-related changes.

Feed Consumption

Slightly lower food consumption was noted in the high dose group during weeks 1 and 2.

Ophthalmoscopy

There were no clearly treatment-related changes.

ECG

Not performed.

Hematology

A minimal decrease in hemoglobin and hematocrit was noted in the high dose combination group.

Clinical Chemistry

There were no clearly treatment-related changes.

Urinalysis

There were no clearly treatment-related changes.

Gross Pathology

There were no clearly treatment-related changes.

Organ Weights

Slight increases in absolute and relative liver weights were noted in the high-dose combination group, netupitant only group, and palonosetron only group (up to 120% of control mean weight). A slight increase in relative spleen weight was also noted in the males of the high-dose combination group.

Histopathology

Adrenal zona fasciculata hypertrophy in the high-dose combination females and the 10 mg/kg/day netupitant only females appeared to be treatment related. Periacinar hepatocytic hypertrophy appeared to be treatment related in the high-dose combination females, the 10 mg/kg/day netupitant only males and females, and the 18 mg/kg/day palonosetron only females. Syncytial macrophages in the mesenteric lymph node appeared to be treatment related in the mid-dose combination females, high-dose combination males and females, and the 10 mg/kg/day netupitant only males and females. The effects in adrenals and liver were not seen in the recovery animals, whereas syncytial macrophages in mesenteric lymph nodes were still present in the recovery animals.

The group mean severity gradings of selected lesions are tabulated:

Lesion	1M	2M	3M	4M	5M	6M		1F	2F	3F	4F	5F	6F
Dosage	0 & 0	2P & 1N	6P & 3N	18P & 10N	18P & 0N	0P & 10N		0 & 0	2P & 1N	6P & 3N	18P & 10N	18P & 0N	0P & 10N
Adrenals Zona fasciculata hypertrophy	0.4	-	-	0.2	0.2	0.3		0.0 [0.0]	0.0 [0.0]	0.0 [0.0]	**1.0** [0.0]	0.1 [0.0]	**0.8** [0.0]
Liver Periacinar hepatocytic hypertrophy	0.3 [0.0]	0.3 [0.0]	0.3 [0.0]	0.1 [0.0]	0.5 [0.0]	**1.0** [0.20]		0.1 [0.0]	0.0 [0.0]	0.1 [0.2]	**1.2** [0.0]	**0.6** [0.0]	**1.6** [0.0]
Lymph Node Mesenteric Syncytial macrophages	0.9 [1.4]	1.2 [1.4]	1.1 [1.2]	**2.5** **[2.4]**	1.0 [1.6]	**1.5** **[2.0]**		0.2 [1.6]	0.1 [1.2]	**0.5** **[2.2]**	**1.8** [1.8]	0.3 [1.4]	**1.5** **[2.4]**

Dosage expressed as mg/kg b.w. of P (Palonosetron) and of N (Netupitant)
Figures in [ ] are recovery results.

Toxicokinetics

The toxicokinetic data for palonosetron, netupitant, and the netupitant metabolites M1, M2, and M3 are summarized in the following sponsor's tables.

Toxicokinetic data of Palonosetron are summarised below.

Parameters		Summary Palonosetron data							
		Group 2: 2 mg/kg PS and 1 mg/kg NT				Group 3: 6 mg/kg PS and 3 mg/kg NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
T_{last}	hr					4.0	2.0	2.0	2.0
T_{max}	hr					0.5	0.5	0.5	0.5
C_{max}	ng/mL					4.87	3.90	3.77	7.88
# C_{max}	kg•ng/mL/mg					0.812	0.649	0.628	1.31
AUC_{last}	hr•ng/mL		insuffic.	data		6.93	4.06	3.71	5.45
# AUC_{last}	hr•kg•ng/mL/mg					1.16	0.677	0.618	0.908
AUC_{∞}	hr•ng/mL					7.60	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg					1.27	n/a	n/a	n/a
$t_{1/2}$	hr					1.04	0.945	0.645	0.505
Females									
T_{last}	hr					4.0	4.0	2.0	4.0
T_{max}	hr					0.5	0.5	0.5	0.5
C_{max}	ng/mL					22.7	29.2	18.2	25.7
# C_{max}	kg•ng/mL/mg					3.78	4.87	3.04	4.28
AUC_{last}	hr•ng/mL		insuffic.	data		18.0	21.1	11.7	20.5
# AUC_{last}	hr•kg•ng/mL/mg					3.00	3.52	1.95	3.42
AUC_{∞}	hr•ng/mL					18.8	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg					3.13	n/a	n/a	n/a
$t_{1/2}$	hr					1.01	1.12	0.467	0.968
		Group 4: 18 mg/kg PS and 10 mg/kg NT				Group 5: 18 mg/kg PS			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
T_{last}	hr	8.0	8.0	8.0	8.0	4.0	8.0	8.0	8.0
T_{max}	hr	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
C_{max}	ng/mL	80.8	67.3	66.6	37.2	67.9	69.3	54.4	62.6
# C_{max}	kg•ng/mL/mg	4.49	3.74	3.70	2.06	3.77	3.85	3.02	3.48
AUC_{last}	hr•ng/mL	82.9	70.2	77.6	40.4	69.8	87.3	82.3	77.6
# AUC_{last}	hr•kg•ng/mL/mg	4.61	3.90	4.31	2.24	3.88	4.85	4.57	4.31
AUC_{∞}	hr•ng/mL	84.4	n/a	n/a	n/a	72.0	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	4.69	n/a	n/a	n/a	4.00	n/a	n/a	n/a
$t_{1/2}$	hr	1.66	6.14	1.72	4.96	0.797	1.55	1.53	1.79
Females									
T_{last}	hr	4.0	8.0	8.0	8.0	8.0	8.0	24.0	8.0
T_{max}	hr	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
C_{max}	ng/mL	126	138	145	104	260	86.4	107	166
# C_{max}	kg•ng/mL/mg	7.02	7.64	8.04	5.80	14.5	4.80	5.96	9.20
AUC_{last}	hr•ng/mL	136	111	124	107	230	133	168	202
# AUC_{last}	hr•kg•ng/mL/mg	7.56	6.17	6.89	5.94	12.8	7.39	9.33	11.2
AUC_{∞}	hr•ng/mL	141	n/a	n/a	n/a	232	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	7.81	n/a	n/a	n/a	12.9	n/a	n/a	n/a
$t_{1/2}$	hr	0.804	2.79	2.12	2.16	1.37	1.37	5.97	1.35

PS = Palonosetron, NT = Netupitant, # = dose-normalised to 1 mg/kg, xx = approximation, n/a = not applicable
insuff.data: Concentrations were near or below LLOQ, no calculations performed.

Toxicokinetic data of Netupitant are summarised below.

Parameters		Summary Netupitant data							
		Group 2:2 mg/kg PS and 1 mg/kg NT				Group 3: 6 mg/kg PS and 3 mg/kg NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
T_{last} @	hr	8.0	24.0	24.0	96.0@	24.0	24.0	24.0	168.0@
T_{max}	hr	4.0	2.0	1.0	2.0	4.0	8.0	8.0	0.5
C_{max}	ng/mL	61.0	113	109	161	171	286	301	467
# C_{max}	kg•ng/mL/mg	61.0	113	109	161	57.0	95.4	100	156
AUC_{last}	hr•ng/mL	424	1720	1860	*3240*	2480	5630	6000	*7520*
# AUC_{last}	hr•kg•ng/mL/mg	424	1720	1860	*3240*	827	1877	2000	*2510*
AUC_{last}	hr•ng/mL				7490				16300
# AUC_{last}	hr•kg•ng/mL/mg				7490				5433
AUC_{∞}	hr•ng/mL	NC	n/a	n/a	n/a	2920	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	NC	n/a	n/a	n/a	974	n/a	n/a	n/a
$t_{1/2}$	hr	NC	20.4	12.3	30.9	8.74	18.1	21.4	43.1
Females									
T_{last} @	hr	24.0	24.0	24.0	168.0@	24.0	24.0	24.0	336.0@
T_{max}	hr	8.0	8.0	8.0	24.0	8.0	4.0	4.0	4.0
C_{max}	ng/mL	83.8	237	237	332	238	576	506	546
# C_{max}	kg•ng/mL/mg	83.8	237	237	332	79.3	192	169	182
AUC_{last}	hr•ng/mL	1590	4920	5290	*7500*	4490	11900	10500	*12500*
# AUC_{last}	hr•kg•ng/mL/mg	1590	4920	5290	*7500*	1497	3967	3500	*4150*
AUC_{last}	hr•ng/mL				20900				70000
# AUC_{last}	hr•kg•ng/mL/mg				20900				23333
AUC_{∞}	hr•ng/mL	2900	n/a	n/a	n/a	7570	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	2900	n/a	n/a	n/a	2520	n/a	n/a	n/a
$t_{1/2}$	hr	19.3	27.8	NC	38.8	17.2	37.1	47.3	60.6
		Group 4: 18 mg/kg PS and 10 mg/kg NT				Group 6: 10 mg/kg NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
T_{last} @	hr	24.0	24.0	24.0	168.0@	8.0	24.0	24.0	168.0@
T_{max}	hr	4.0	4.0	4.0	4.0	4.0	4.0	2.0	2.0
C_{max}	ng/mL	455	685	662	680	571	683	847	669
# C_{max}	kg•ng/mL/mg	45.5	68.5	66.2	68.0	57.1	68.3	84.7	66.9
AUC_{last}	hr•ng/mL	6820	12200	12300	*12100*	3590	11800	12900	*13800*
# AUC_{last}	hr•kg•ng/mL/mg	682	1220	1230	*1210*	359	1180	1290	*1380*
AUC_{last}	hr•ng/mL				37000				41600
# AUC_{last}	hr•kg•ng/mL/mg				3700				4160
AUC_{∞}	hr•ng/mL	8440	n/a	n/a	n/a	8880	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	844	n/a	n/a	n/a	888	n/a	n/a	n/a
$t_{1/2}$	hr	9.92	12.7	20.9	59.3	8.80	14.6	17.2	54.5
Females									
T_{last} @	hr	24.0	24.0	24.0	336.0@	8.0	24.0	24.0	336.0@
T_{max}	hr	4.0	8.0	4.0	8.0	2.0	8.0	24.0	2.0
C_{max}	ng/mL	621	1170	1150	1190	861	1210	1690	1230
# C_{max}	kg•ng/mL/mg	62.1	117	115	119	86.1	121	169	123
AUC_{last}	hr•ng/mL	11400	25100	24100	*27100*	5440	24700	30600	*22600*
# AUC_{last}	hr•kg•ng/mL/mg	1140	2510	2410	*2710*	544	2470	3060	*2260*
AUC_{last}	hr•ng/mL				130000				99800
# AUC_{last}	hr•kg•ng/mL/mg				13000				9980
AUC_{∞}	hr•ng/mL	31900	n/a	n/a	n/a	57400	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	3190	n/a	n/a	n/a	5740	n/a	n/a	n/a
$t_{1/2}$	hr	35.3	NC	59.5	63.1	47.5	27.7	NC	87.3

PS = Palonosetron, NT = Netupitant, # = dose-normalised to 1 mg/kg, xx = approximation, n/a = not applicable,
italics = $AUC_{0\text{-}24h}$ used for Week 13, NC = could not be calculated
@ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered.

Toxicokinetic data of M1 are summarized below.

Parameters		Summary M1 data							
		Group 2:2 mg/kg PS and 1 mg/kg NT				Group 3: 6 mg/kg PS and 3 mg/kg NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
T_{last} [@]	hr	24.0	24.0	24.0	168.0[@]	24.0	24.0	24.0	168.0[@]
T_{max}	hr	8.0	24.0	8.0	24.0	24.0	24.0	24.0	24.0
C_{max}	ng/mL	30.6	131	150	296	85.6	408	495	608
# C_{max}	kg•ng/mL/mg	30.6	131	150	296	28.5	136	165	203
AUC_{last}	hr•ng/mL	623	2450	3110	*5120*	1610	8600	10800	*13200*
# AUC_{last}	hr•kg•ng/mL/mg	623	2450	3110	*5120*	537	2870	3600	*4380*
AUC_{last}	hr•ng/mL				20700				44900
# AUC_{last}	hr•kg•ng/mL/mg				20700				14967
AUC_{∞}	hr•ng/mL	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
$t_{1/2}$	hr	NC	NC	NC	32.0	NC	NC	NC	37.6
Females									
T_{last} [@]	hr	24.0	24.0	24.0	168.0[@]	24.0	24.0	24.0	336.0[@]
T_{max}	hr	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0
C_{max}	ng/mL	30.9	160	197	265	73.5	422	397	775
# C_{max}	kg•ng/mL/mg	30.9	160	197	265	24.5	141	132	258
AUC_{last}	hr•ng/mL	524	3700	4120	*4850*	1370	9380	9070	*12300*
# AUC_{last}	hr•kg•ng/mL/mg	524	3700	4120	*4850*	457	3130	3023	*4090*
AUC_{last}	hr•ng/mL				17900				79800
# AUC_{last}	hr•kg•ng/mL/mg				17900				26600
AUC_{∞}	hr•ng/mL	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
$t_{1/2}$	hr	NC	NC	NC	33.6	NC	NC	NC	53.8
		Group 4: 18 mg/kg PS and 10 mg/kg NT				Group 6: 10 mg/kg NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
T_{last} [@]	hr	24.0	24.0	24.0	336.0[@]	8.0	24.0	24.0	336.0[@]
T_{max}	hr	24.0	24.0	8.0	4.0	8.0	24.0	24.0	24.0
C_{max}	ng/mL	194	781	1010	1110	201	959	971	1020
# C_{max}	kg•ng/mL/mg	19.4	78.1	101	111	20.1	95.9	97.1	102
AUC_{last}	hr•ng/mL	3410	18100	23300	*22300*	921	20000	22200	*23200*
# AUC_{last}	hr•kg•ng/mL/mg	341	1810	2330	*2230*	92	2000	2220	*2320*
AUC_{last}	hr•ng/mL				164000				160000
# AUC_{last}	hr•kg•ng/mL/mg				16400				16000
AUC_{∞}	hr•ng/mL	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
$t_{1/2}$	hr	NC	NC	NC	39.0	NC	NC	NC	59.3
Females									
T_{last} [@]	hr	24.0	24.0	24.0	336.0[@]	8.0	24.0	24.0	336.0[@]
T_{max}	hr	24.0	0.5	0.5	24.0	8.0	0.0	24.0	48.0
C_{max}	ng/mL	194	887	899	1170	116	879	1640	808
# C_{max}	kg•ng/mL/mg	19.4	88.7	89.9	117	11.6	87.9	164	80.8
AUC_{last}	hr•ng/mL	2820	19500	19800	*23400*	518	19600	25300	*17800*
# AUC_{last}	hr•kg•ng/mL/mg	282	1950	1980	*2340*	52	1960	2530	*1780*
AUC_{last}	hr•ng/mL				221000				145000
# AUC_{last}	hr•kg•ng/mL/mg				22100				14500
AUC_{∞}	hr•ng/mL	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
$t_{1/2}$	hr	NC	NC	NC	99.2	NC	NC	NC	88.5

PS = Palonosetron, NT = Netupitant, # = dose-normalised to 1 mg/kg, xx = approximation, n/a = not applicable,
italics = $AUC_{0\text{-}24h}$ used for Week 13, NC = could not be calculated
[@] If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered.

Toxicokinetic data of M2 are summarised below.

Parameters		Summary M2 data							
		Group 2:2 mg/kg PS and 1 mg/kg NT				Group 3: 6 mg/kg PS and 3 mg/kg NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
T_{last} @	hr					4.0	8.0	8.0	24.0@
T_{max}	hr					2.0	0.5	0.5	0.5
C_{max}	ng/mL					15.0	13.4	19.2	35.2
# C_{max}	kg•ng/mL/mg					4.99	4.48	6.41	11.7
AUC_{last}	hr•ng/mL		insuffic.	data		43.7	99.5	89.2	*325*
# AUC_{last}	hr•kg•ng/mL/mg					14.6	33.2	29.7	*108*
AUC_{last}	hr•ng/mL								325
# AUC_{last}	hr•kg•ng/mL/mg								108
AUC_{∞}	hr•ng/mL					NC	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg					NC	n/a	n/a	n/a
$t_{1/2}$	hr					NC	77.1	NC	22.3
Females									
T_{last} @	hr								24.0@
T_{max}	hr								0.5
C_{max}	ng/mL								15.0
# C_{max}	kg•ng/mL/mg								5.01
AUC_{last}	hr•ng/mL		insuffic.	data			insuffic.	data	*238*
# AUC_{last}	hr•kg•ng/mL/mg								*79.2*
AUC_{last}	hr•ng/mL								238
# AUC_{last}	hr•kg•ng/mL/mg								79.2
AUC_{∞}	hr•ng/mL								n/a
# AUC_{∞}	hr•kg•ng/mL/mg								n/a
$t_{1/2}$	hr								NC
		Group 4: 18 mg/kg PS and 10 mg/kg NT				Group 6: 10 mg/kg NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
T_{last} @	hr	8.0	8.0	24.0	24.0@	8.0	24.0	8.0	24.0@
T_{max}	hr	2.0	8.0	0.5	0.5	1.0	2.0	2.0	1.0
C_{max}	ng/mL	39.2	24.2	29.2	28.8	43.2	35.1	52.0	30.1
# C_{max}	kg•ng/mL/mg	3.92	2.42	2.92	2.88	4.32	3.51	5.20	3.01
AUC_{last}	hr•ng/mL	239	174	383	*324*	250	472	247	*480*
# AUC_{last}	hr•kg•ng/mL/mg	23.9	17.4	38.3	*32.4*	25.0	47.2	24.7	*48.0*
AUC_{last}	hr•ng/mL				324				480
# AUC_{last}	hr•kg•ng/mL/mg				32.4				48.0
AUC_{∞}	hr•ng/mL	408	n/a	n/a	n/a	461	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	40.8	n/a	n/a	n/a	46.1	n/a	n/a	n/a
$t_{1/2}$	hr	5.95	NC	11.8	12.2	7.00	13.8	5.42	52.2
Females									
T_{last} @	hr	24.0	24.0	24.0	48.0@	8.0	24.0	24.0	48.0@
T_{max}	hr	1.0	8.0	1.0	0.5	1.0	1.0	2.0	2.0
C_{max}	ng/mL	26.0	20.3	25.9	26.8	33.7	35.7	40.4	36.7
# C_{max}	kg•ng/mL/mg	2.60	2.03	2.59	2.68	3.37	3.57	4.04	3.67
AUC_{last}	hr•ng/mL	324	439	469	*495*	133	628	672	*478*
# AUC_{last}	hr•kg•ng/mL/mg	32.4	43.9	46.9	*49.5*	13.3	62.8	67.2	*47.8*
AUC_{last}	hr•ng/mL				807				754
# AUC_{last}	hr•kg•ng/mL/mg				80.7				75.4
AUC_{∞}	hr•ng/mL	1100	n/a	n/a	n/a	220	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	110	n/a	n/a	n/a	22.0	n/a	n/a	n/a
$t_{1/2}$	hr	36.1	NC	68.0	22.6	5.06	40.8	NC	28.8

PS = Palonosetron, NT = Netupitant, # = dose-normalised to 1 mg/kg, xx = approximation, n/a = not applicable,
italics = $AUC_{0\text{-}24h}$ used for Week 13, NC = could not be calculated
insuff.data: Concentrations were near or below LLOQ, no calculations performed.
@ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered.

Toxicokinetic data of M3 are summarised below.

Parameters		Summary M3 data							
		Group 2:2 mg/kg PS and 1 mg/kg NT				Group 3: 6 mg/kg PS and 3 mg/kg NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
T_{last} [@]	hr					NC	24.0	24.0	24.0[@]
T_{max}	hr					NC	24.0	24.0	4.0
C_{max}	ng/mL					NC	20.5	24.0	30.2
# C_{max}	kg•ng/mL/mg					NC	6.83	7.99	10.1
AUC_{last}	hr•ng/mL		insuffic.	data		NC	447	540	*643*
# AUC_{last}	hr•kg•ng/mL/mg					NC	149	180	*214*
AUC_{last}	hr•ng/mL								643
# AUC_{last}	hr•kg•ng/mL/mg								214
AUC_{∞}	hr•ng/mL					NC	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg					NC	n/a	n/a	n/a
$t_{1/2}$	hr					NC	NC	NC	95.1
Females									
T_{last} [@]	hr					NC	24.0	24.0	96.0[@]
T_{max}	hr					NC	4.0	24.0	24.0
C_{max}	ng/mL					NC	17.5	18.8	36.2
# C_{max}	kg•ng/mL/mg					NC	5.82	6.28	12.1
AUC_{last}	hr•ng/mL		insuffic.	data		NC	312	350	*529*
# AUC_{last}	hr•kg•ng/mL/mg					NC	104	117	*176*
AUC_{last}	hr•ng/mL								1870
# AUC_{last}	hr•kg•ng/mL/mg								623
AUC_{∞}	hr•ng/mL					NC	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg					NC	n/a	n/a	n/a
$t_{1/2}$	hr					NC	NC	NC	38.3
		Group 4: 18 mg/kg PS and 10 mg/kg NT				Group 6: 10 mg/kg NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males									
$T_{last\ v}$	hr	24.0	24.0	24.0	96.0[@]	8.0	24.0	24.0	96.0[@]
T_{max}	hr	8.0	8.0	4.0	4.0	8.0	8.0	8.0	24.0
C_{max}	ng/mL	23.7	49.0	54.1	58.1	31.0	54.5	59.2	59.2
# C_{max}	kg•ng/mL/mg	2.37	4.90	5.41	5.81	3.10	5.45	5.92	5.92
AUC_{last}	hr•ng/mL	436	1010	1220	*1230*	147	1090	1260	*1290*
# AUC_{last}	hr•kg•ng/mL/mg	44.0	101	122	*123*	15.0	109	126	*129*
AUC_{last}	hr•ng/mL				3190				3540
# AUC_{last}	hr•kg•ng/mL/mg				319				354
AUC_{∞}	hr•ng/mL	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
$t_{1/2}$	hr	NC	NC	141	52.3	NC	29.4	43.5	32.4
Females									
T_{last} [@]	hr	NC	24.0	24.0	168.0[@]	NC	24.0	24.0	168.0[@]
T_{max}	hr	NC	0.5	0.0	24.0	NC	8.0	24.0	24.0
C_{max}	ng/mL	NC	44.7	61.2	74.9	NC	55.0	123	62.7
# C_{max}	kg•ng/mL/mg	NC	4.47	6.12	7.49	NC	5.50	12.3	6.27
AUC_{last}	hr•ng/mL	NC	1010	1250	*1630*	NC	1250	1920	*1380*
# AUC_{last}	hr•kg•ng/mL/mg	NC	101	125	*163*	NC	125	192	*138*
AUC_{last}	hr•ng/mL				7020				5520
# AUC_{last}	hr•kg•ng/mL/mg				702				552
AUC_{∞}	hr•ng/mL	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
# AUC_{∞}	hr•kg•ng/mL/mg	NC	n/a	n/a	n/a	NC	n/a	n/a	n/a
$t_{1/2}$	hr	NC	NC	NC	92.5	NC	NC	NC	49.7

PS = Palonosetron, NT = Netupitant, # = dose-normalised to 1 mg/kg, xx = approximation, n/a = not applicable,
italics = $AUC_{0\text{-}24h}$ used for Week 13, NC = could not be calculated
insuff.data: Concentrations were near or below LLOQ, no calculations performed.
[@] If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered.

Percentage of Netupitant exposure per period in each dose Group	M1 %				M2 %				M3 %			
Group	2	3	4	6	2	3	4	6	2	3	4	6
Day 1												
Males												
C_{max}	50	50	43	35		9	9	8			5	5
AUC_{∞}	147[1]	65	50	26		2	4	7			6	4
Females												
C_{max}	37	31	31	13			4	4				
AUC_{∞}	33	31	25	10			3	2				
Week 4												
Males												
C_{max}	116	143	114	140		5	4	5		7	7	8
AUC_{last}	142	153	148	169		2	1	4		8	8	9
Females												
C_{max}	68	73	76	73			2	3		3	4	5
AUC_{last}	75	79	78	79			2	3		3	4	5
Week 7												
Males												
C_{max}	138	164	153	115		6	4	6		8	8	7
AUC_{last}	167	180	189	172		1	3	2		9	10	10
Females												
C_{max}	83	78	78	97			2	2		4	5	7
AUC_{last}	78	86	82	83			2	2		3	5	6
Week 13												
Males												
C_{max}	184	130	163	152		8	4	4		6	9	9
AUC_{last}	158	175	184	167		4	3	3		9	10	9
Females												
C_{max}	80	142	98	66		3	2	3		7	6	5
AUC_{last}	65	98	86	79		2	2	2		4	6	6

[1] Outlying value due to relatively low value for AUC_{last} of Netupitant on Day 1 in males of Group 2.

Dosing Solution Analysis

For palonosetron, the concentrations measured in dosing formulations for groups 2 to 5 were in the range of 87–111% of the nominal concentrations. For netupitant, the concentrations measured in dosing formulations for groups 2 to 4 and group 6 were between 89% and 107% of the nominal concentrations. The mean values of the duplicate samples were within the range of 90-110%.

Study title: 13-Week oral gavage toxicity study with palonosetron and netupitant in dogs

Study no.:	NETU-07-18
Study report location:	n/a
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 11, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Palonosetron, 25004427 and 99.7%26004786, 99.5% Netupitant, BS0307SA01, 99.5%

Key Study Findings:

Dogs were treated with netupitant at 1, 3, and 10 mg/kg/day combined with palonosetron at 3, 5, and 10 mg/kg/day, respectively, by oral gavage for 13 weeks. Slight decreases in body weight gain and food consumption were noted in the high-dose group. The ST-interval, uncorrected QT-interval, and corrected QT intervals according to Van de Water and Fridericia were slightly prolonged in the high dose females as compared to the control females. No treatment-related histopathological changes were noted. The NOAEL was the combination dose of 3 mg/kg/day netupitant and 5 mg/kg/day palonosetron.

Methods

Doses:	See table below
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	1 ml/kg
Formulation/Vehicle:	Thixotrope vehicle
Species/Strain:	beagle dogs
Number/Sex/Group:	4 or 6/sex/group (see sponsor's table below)
Age:	~5-6 months
Weight:	Males: 8.0-8.1 kg Females: 6.9-7.1 kg
Satellite groups:	none
Unique study design:	Treatment with both palonosetron and netupitant
Deviation from study protocol:	No deviation occurred which adversely affected the quality of the study.

Group	Dose Level (mg/kg b.w.)		Number of Animals		Animal Numbers	
	Palonosetron	Netupitant	Males	Females	Males	Females
1 Main 1 Recovery	0 (vehicle)		4 2	4 2	1-4 5-6	21-24 25-26
2 Main	3	1	4	4	7-10	27-30
3 Main	5	3	4	4	11-14	31-34
4 Main 4 Recovery	10	10	4 2	4 2	15-18 19-20	35-38 39-40

The dose selection was based on results of the previous toxicity studies, including a 7-day and 28-day study in dogs with combination treatment (# NETU-06-21 and # NETU-06-05, respectively). The doses tested in the 28-day dog study were:

10 mg/kg/day palonosetron and 3 mg/kg/day netupitant
15 mg/kg/day palonosetron and 7.5 mg/kg/day netupitant
15 mg/kg/day palonosetron and 15 mg/kg/day netupitant.

The groups treated with 15 mg/kg/day palonosetron + 7.5 mg/kg/day netupitant and 15 mg/kg/day palonosetron + 15 mg/kg/day netupitant exhibited tremors and seizure-like episodes. Therefore, the sponsor selected 10 mg/kg/day as the high dose for both palonosetron and netupitant in the current study.

Observations and Results

Mortality

No deaths occurred.

Clinical Signs

There were no clearly treatment-related signs.

Body Weights

The terminal body weight gain was reduced in the combination high-dose females (0.9 kg, as compared to 2 kg in the control females).

Feed Consumption

A slight reduction in food consumption was noted in the combination high-dose group during weeks 1 and 2.

Ophthalmoscopy

There were no clearly treatment-related changes.

ECG

ECGs were measured before study initiation and on day 2 and weeks 4, 8, and 13 (pre-dose, 2, and 6 hours after dosing).

The ST-interval, uncorrected QT-interval, and corrected QT intervals according to Van de Water and Fridericia were slightly prolonged in the high-dose females, as compared to the control females.

Hematology

There were no clearly treatment-related changes.

Clinical Chemistry

A slight decrease in albumin concentration occurred in the mid- (31.4 g/L) and high-dose (31.5 g/L) males at the end of treatment, as compared to 34.2 g/L in the control males.

A slight increase in glucose was noted in the mid- (6.56 mmol/l) and high-dose (6.5 mmol/l) males at the end of treatment, as compared to 5.81 mmol/l in the control males.

A slight decrease in triglycerides was seen in high-dose females (0.48 mmol/l) at the end of treatment, as compared to 0.62 mmol/l in the control females.

Urinalysis

There were no clearly treatment-related changes.

Gross Pathology

There were no clearly treatment-related changes.

Organ Weights

Relative liver weights were increased slightly in the high-dose.

Histopathology

There were no clearly treatment-related changes.

Toxicokinetics

The toxicokinetic data for palonosetron, netupitant, and the netupitant metabolites M1, M2, and M3 are summarized in the following sponsor’s tables.

Toxicokinetic data of Palonosetron are summarised below:

Parameters		Summary Palonosetron data											
		Group 2: 3 mg/kg b.w. PS and 1 mg/kg b.w. NT				Group 3: 5 mg/kg b.w. PS and 3 mg/kg b.w. NT				Group 4: 10 mg/kg b.w. PS and 10 mg/kg b.w. NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males													
T_{last} $ @	hr	8.0	8.0	24.0	16.0	8.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0@
T_{max} $	hr	0.8	0.8	1.0	1.0	1.5	1.0	0.8	1.0	1.0	1.0	1.0	1.0
C_{max}	ng/mL	182	176	175	198	238	465	527	402	649	752	673	697
# C_{max}	kg·ng/mL/mg	60.6	58.6	58.4	66.1	47.7	93.1	105	80.4	64.9	75.2	67.3	69.7
AUC_{last} ‡	hr·ng/mL	374	576	584	644	926	1810	1680	1500	2170	2590	2540	2720
#AUC_{last} ‡	hr·kg·ng/mL/mg	125	192	195	215	185	363	335	300	217	259	254	272
AUC_{∞}	hr·ng/mL	378	n/a	n/a	n/a	1050	n/a	n/a	n/a	2180	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	126	n/a	n/a	n/a	210	n/a	n/a	n/a	218	n/a	n/a	n/a
$t_{1/2}$	hr	1.17	2.12	2.56	2.29	2.30	3.11	3.44	2.63	2.27	3.54	3.12	4.06
Females													
T_{last} $ @	hr	8.0	24.0	24.0	24.0	8.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0@
T_{max} $	hr	1.0	1.0	1.0	1.0	1.0	1.0	0.5	0.8	1.0	1.0	1.0	1.0
C_{max}	ng/mL	209	239	309	252	360	477	502	473	620	594	638	560
# C_{max}	kg·ng/mL/mg	69.7	79.8	103	84.1	72.0	95.5	100	94.5	62.0	59.4	63.8	56.0
AUC_{last} ‡	hr·ng/mL	612	816	936	828	974	1220	1500	1360	2080	2290	2320	1900
#AUC_{last} ‡	hr·kg·ng/mL/mg	204	272	312	276	195	243	299	271	208	229	232	190
AUC_{∞}	hr·ng/mL	628	n/a	n/a	n/a	984	n/a	n/a	n/a	2090	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	209	n/a	n/a	n/a	197	n/a	n/a	n/a	209	n/a	n/a	n/a
$t_{1/2}$	hr	1.40	3.34	3.11	2.44	1.68	3.62	3.26	3.24	2.27	3.38	3.42	3.37

PS = Palonosetron; NT = Netupitant, $: median, # : dose-normalised to 1 mg/kg b.w., ‡: AUC_{0-24h} was used for the recovery animals, @ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered: it was 24-48hr for high dose animals, n/a: not applicable

Toxicokinetic data of Netupitant are summarised below:

Parameters		Summary Netupitant data											
		Group 2: 3 mg/kg b.w. PS and 1 mg/kg b.w. NT				Group 3: 5 mg/kg b.w. PS and 3 mg/kg b.w. NT				Group 4: 10 mg/kg b.w. PS and 10 mg/kg b.w. NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males													
T_{last} $ @	hr	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0@
T_{max} $	hr	2.0	2.0	2.0	2.0	3.0	2.0	2.0	2.0	2.0	2.0	2.0	3.0
C_{max}	ng/mL	123	134	149	167	345	586	571	527	621	945	1280	883
# C_{max}	kg·ng/mL/mg	123	134	149	167	115	195	191	175	62.1	94.5	128	88.3
AUC_{last} ‡	hr·ng/ml	945	1530	1620	1900	3760	6720	6220	6760	6740	13600	17500	15300
#AUC_{last} ‡	hr·kg·ng/mL/mg	945	1530	1620	1900	1250	2240	2070	2250	674	1360	1750	1530
AUC_{∞}	hr·ng/mL	1090	n/a	n/a	n/a	1460*	n/a	n/a	n/a	10600*	n/a	n/a	n/a
# AUC_{∞}	hr kg ng/mL/mg	1090	n/a	n/a	n/a	1480*	n/a	n/a	n/a	1060*	n/a	n/a	n/a
$t_{1/2}$	hr	7.75	10.4	11.3*	12.1*	8.21	11.5*	12.2*	11.8*	16.2	47.4*	14.9*	26.2*
Females													
T_{last} $ @	hr	8.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0@
T_{max} $	hr	2.0	1.5	2.0	2.0	2.0	1.5	1.5	1.0	2.0	2.0	2.0	2.0
C_{max}	ng/mL	83.2	122	143	154	246	336	390	374	942	1150	1070	795
# C_{max}	kg·ng/mL/mg	83.2	122	143	154	81.8	112	130	125	94.2	115	107	79.5
AUC_{last} ‡	hr·ng/mL	507	1360	1650	1670	2220	3610	3950	4070	9470	14100	13600	11500
#AUC_{last} ‡	hr·kg·ng/mL/mg	587	1360	1650	1670	738	1200	1320	1360	947	1410	1360	1150
AUC_{∞}	hr·ng/mL	769*	n/a	n/a	n/a	2790*	n/a	n/a	n/a	11700*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	769*	n/a	n/a	n/a	928*	n/a	n/a	n/a	1170*	n/a	n/a	n/a
$t_{1/2}$	hr	5.39	13.9*	12.4*	12.5*	10.2*	13.0*	11.4	12.8*	9.51	14.0*	13.8*	21.7

PS = Palonosetron; NT = Netupitant, $: median, # : dose-normalised to 1 mg/kg b.w., ‡: AUC_{0-24h} was used for the recovery animals, @ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered: it was 168hr for males and 96hr for females at the high dose, * : approximation, n/a: not applicable

Toxicokinetic data of M1 are summarised below:

Parameters		Summary M1 data											
		Group 2: 3 mg/kg b.w. PS and 1 mg/kg b.w. NT				Group 3: 5 mg/kg b.w. PS and 3 mg/kg b.w. NT				Group 4: 10 mg/kg b.w. PS and 10 mg/kg b.w. NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males													
T_{last} $^{\$@}$	hr	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0$^{@}$
T_{max} $^{\$}$	hr	8.0	4.0	4.0	5.0	8.0	8.0	4.0	5.0	8.0	1.5	6.0	1.0
C_{max}	ng/mL	60.2	140	147	156	237	669	616	633	455	1360	1710	1510
# C_{max}	kg·ng/mL/mg	60.2	140	147	156	78.9	223	205	211	45.5	136	171	151
AUC_{last} ‡	hr·ng/mL	1110	2730	2820	3060	4310	13600	12600	13500	8220	30200	36000	32600
#AUC_{last} ‡	hr·kg·ng/mL/mg	1110	2730	2820	3060	1440	4550	4210	4490	822	3020	3600	3260
AUC_{∞}	hr·ng/mL	1990*	n/a	n/a	n/a	10900*	n/a	n/a	n/a	27900*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	1990*	n/a	n/a	n/a	3630*	n/a	n/a	n/a	2790*	n/a	n/a	n/a
$t_{1/2}$	hr	18.8*	23.8*	26.3*	31.9*	29.3*	26.3*	33.5*	40.4*	32.5*	244*	63.2*	80.6*
Females													
T_{last} $^{\$@}$	hr	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0$^{@}$
T_{max} $^{\$}$	hr	8.0	6.0	4.0	4.0	6.0	4.0	4.0	4.0	8.0	8.0	4.0	4.0
C_{max}	ng/mL	43.3	131	141	147	130	335	350	324	592	1350	1320	1180
# C_{max}	kg·ng/mL/mg	43.3	131	141	147	43.2	111	117	108	59.2	135	132	118
AUC_{last} ‡	hr·ng/mL	833	2570	2810	2870	2530	6790	6890	6700	11300	29200	27400	24700
#AUC_{last} ‡	hr·kg·ng/mL/mg	833	2570	2810	2870	843	2260	2300	2230	1130	2920	2740	2470
AUC_{∞}	hr·ng/mL	2970*	n/a	n/a	n/a	9660*	n/a	n/a	n/a	30300*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	2970*	n/a	n/a	n/a	3220*	n/a	n/a	n/a	3030*	n/a	n/a	n/a
$t_{1/2}$	hr	42.7*	28.3*	34.4*	27.9*	47.2*	34.2*	33.0*	34.0*	33.9*	51.4*	52.6*	47.5*

PS = Palonosetron; NT = Netupitant, $^{\$}$: median, # : dose-normalised to 1 mg/kg b.w., ‡: $AUC_{0\text{-}24h}$ was used for the recovery animals, $^{@}$ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered: it was 168-336hr at the high dose, * : approximation, n/a: not applicable

Toxicokinetic data of M2 are summarised below:

Parameters		Summary M2 data											
		Group 2: 3 mg/kg b.w. PS and 1 mg/kg b.w. NT				Group 3: 5 mg/kg b.w. PS and 3 mg/kg b.w. NT				Group 4: 10 mg/kg b.w. PS and 10 mg/kg b.w. NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males													
T_{last} $^{\$@}$	hr	8.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0$^{@}$
T_{max} $^{\$}$	hr	2.0	2.0	2.0	2.0	3.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
C_{max}	ng/mL	55.4	46.5	47.2	41.9	146	219	194	198	400	519	533	432
# C_{max}	kg·ng/mL/mg	55.4	46.5	47.2	41.9	48.7	72.9	64.7	66.0	40.0	51.9	53.3	43.2
AUC_{last} ‡	hr·ng/mL	227	520	568	548	1510	2560	2200	2310	2550	5370	5210	4680
#AUC_{last} ‡	hr·kg·ng/mL/mg	227	520	568	548	504	853	733	770	255	537	521	468
AUC_{∞}	hr·ng/mL	304*	n/a	n/a	n/a	2000*	n/a	n/a	n/a	3250*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	304*	n/a	n/a	n/a	668*	n/a	n/a	n/a	325*	n/a	n/a	n/a
$t_{1/2}$	hr	3.22*	14.2*	15.7*	15.9*	10.9*	17.7*	16.4*	15.7*	9.30*	47.4*	13.3*	23.2*
Females													
T_{last} $^{\$@}$	hr	8.0	24.0	8.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0$^{@}$
T_{max} $^{\$}$	hr	2.0	2.0	2.0	2.0	2.0	2.0	1.5	2.0	2.0	2.0	2.0	2.0
C_{max}	ng/mL	36.6	50.2	50.8	45.4	207	183	125	119	590	563	453	381
# C_{max}	kg·ng/mL/mg	36.6	50.2	50.8	45.4	68.9	61.0	41.7	39.8	59.0	56.3	45.3	38.1
AUC_{last} ‡	hr·ng/mL	185	484	313	527	1140	1370	1110	1060	3930	4500	4030	3720
#AUC_{last} ‡	hr·kg·ng/mL/mg	185	484	313	527	379	456	371	353	393	450	403	372
AUC_{∞}	hr·ng/mL	278*	n/a	n/a	n/a	1270*	n/a	n/a	n/a	4580*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	278*	n/a	n/a	n/a	424*	n/a	n/a	n/a	458*	n/a	n/a	n/a
$t_{1/2}$	hr	3.86*	10.7*	6.63*	17.0*	5.57*	10.6*	10.2*	11.7*	7.34*	9.74*	11.9*	17.1*

PS = Palonosetron; NT = Netupitant, $^{\$}$: median, # : dose-normalised to 1 mg/kg b.w., ‡: $AUC_{0\text{-}24h}$ was used for the recovery animals, $^{@}$ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered: it was 96-168hr for males and 48-96hr for females at the high dose, * : approximation, n/a: not applicable

Toxicokinetic data of M3 are summarised below:

Parameters		Summary M3 data											
		Group 2: 3 mg/kg b.w. PS and 1 mg/kg b.w. NT				Group 3: 5 mg/kg b.w. PS and 3 mg/kg b.w. NT				Group 4: 10 mg/kg b.w. PS and 10 mg/kg b.w. NT			
		Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13	Day 1	Week 4	Week 7	Week 13
Males													
T_{last} $ @	hr	n.c.	n.c.	n.c.	n.c.	8.0	24.0	24.0	24.0	24.0	24.0	24.0	24.0@
T_{max} $	hr	n.c.	n.c.	n.c.	n.c.	8.0	4.0	4.0	4.0	6.0	6.0	6.0	8.0
C_{max}	ng/mL	n.c.	n.c.	n.c.	n.c.	23.9	45.3	43.8	38.3	41.4	82.3	105	79.1
# C_{max}	kg·ng/mL/mg	n.c.	n.c.	n.c.	n.c.	7.96	15.1	14.6	12.8	4.14	8.23	10.5	7.91
AUC_{last} ‡	hr·ng/mL	n.c.	n.c.	n.c.	n.c.	113	794	762	636	601	1660	2100	1700
#AUC_{last} ‡	hr·kg·ng/mL/mg	n.c.	n.c.	n.c.	n.c.	37.6	265	254	212	60.1	166	210	170
AUC_{∞}	hr·ng/mL	n.c.	n/a	n/a	n/a	n.c.	n/a	n/a	n/a	1200*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	n.c.	n/a	n/a	n/a	n.c.	n/a	n/a	n/a	120*	n/a	n/a	n/a
$t_{1/2}$	hr	n.c.	n.c.	n.c.	n.c.	n.c.	16.9*	15.7*	18.9*	15.5*	32.0*	27.7*	82.4*
Females													
T_{last} $ @	hr	n.c.	n.c.	n.c.	n.c.	8.0	24.0	16.0	16.0	24.0	24.0	24.0	24.0@
T_{max} $	hr	n.c.	n.c.	n.c.	n.c.	8.0	4.0	4.0	3.0	4.0	4.0	4.0	8.0
C_{max}	ng/mL	n.c.	n.c.	n.c.	n.c.	17.7	32.1	28.4	24.5	66.1	100	86.5	75.7
# C_{max}	kg·ng/mL/mg	n.c.	n.c.	n.c.	n.c.	5.90	10.7	9.46	8.15	6.61	10.0	8.65	7.57
AUC_{last} ‡	hr·ng/mL	n.c.	n.c.	n.c.	n.c.	105	556	380	367	1090	1860	1630	1480
#AUC_{last} ‡	hr·kg·ng/mL/mg	n.c.	n.c.	n.c.	n.c.	35.1	185	127	122	109	186	163	148
AUC_{∞}	hr·ng/mL	n.c.	n/a	n/a	n/a	n.c.	n/a	n/a	n/a	1570*	n/a	n/a	n/a
# AUC_{∞}	hr·kg·ng/mL/mg	n.c.	n/a	n/a	n/a	n.c.	n/a	n/a	n/a	157*	n/a	n/a	n/a
$t_{1/2}$	Hr	n.c.	n.c.	n.c.	n.c.	n.c.	17.8*	16.2*	152*	13.1*	21.7*	20.8*	27.7*

PS = Palonosetron; NT = Netupitant, $: median, # : dose-normalised to 1 mg/kg b.w., n.c.: could not be calculated ‡: AUC_{0-24h} was used for the recovery animals, @ If T_{last} was 24hours, this was the maximum time between subsequent doses. Only in Recovery animals, a true value for T_{last} was obtained after the final dose was administered: it was 48-96hr at the high dose, * : approximation, n/a: not applicable

Percentage of Netupitant exposure per period in each dose group									
	M1			M2			M3		
Day 1	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4
males									
C_{max}	49	69	73	45	42	64	-	7	7
AUC_{last}	102	115	122	21	40	38	-	3	9
females									
C_{max}	52	53	63	44	84	63	-	7	7
AUC_{∞} or AUC_{last}*	142	114	119	32	51	41	-	5	12
Week 4	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4
males									
C_{max}	104	114	144	35	37	55	-	8	9
AUC_{last}	178	202	222	34	38	39	-	12	12
females									
C_{max}	107	100	117	41	54	49	-	10	9
AUC_{last}	189	188	207	36	38	32	-	15	13
Week 7	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4
males									
C_{max}	99	108	134	32	34	42	-	8	8
AUC_{last}	174	203	206	35	35	30	-	12	12
females									
C_{max}	99	90	123	36	32	42	-	7	8
AUC_{last}	170	174	201	19	28	30	-	10	12
Week 13	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4	Group 2	Group 3	Group 4
males									
C_{max}	93	120	171	25	38	49	-	7	9
AUC_{last} ‡	161	200	213	29	34	31	-	9	11
females									
C_{max}	95	87	148	29	32	48	-	7	10
AUC_{last} ‡	172	165	215	32	26	32	-	9	13

* AUC_{last} was used when AUC_{∞} was reported as approximation
‡: AUC_{0-24h} was used for the recovery animals

Dosing Solution Analysis

For palonosetron, the concentrations analyzed in the dosing formulations were in the range of 90-115% of the nominal concentration. The values slightly above 110% were noted.

For netupitant, the concentrations analyzed in the dosing formulations were in the range of 87-102% of the nominal concentration. The values slightly below 90% were noted.

2-Week IV toxicity study in rats (# 1008327)

Netupitant was given intravenously via 5-hour infusions to Wistar rats (10/sex/group) at 0 (vehicle, 5% glucose solution), 3, 9 or 30 mg/kg/day for 14 days. At 30 mg/kg/day, body weight loss and decreased food consumption were noted. Local reactions were observed, including necrosis of the vessel wall, mural thrombi, inflammation of blood vessels or adjacent tissue, and endothelial hyperplasia at the injection sites in all treatment groups. These effects were more severe at 30 mg/kg/day. There were also 3-day (# 1006118) and 7-day (# 1007386) intravenous non-GLP toxicity studies in rats which were used as dose ranging studies for the 2-week IV study in rats.

2-Week IV toxicity study in dogs (# 1008328)

Netupitant was given intravenously to 3 male Beagle dogs per group at 0 (vehicle control), 1, 3, and 10 mg/kg/day for 14 days. The vehicle was 5% glucose solution. Local reactions including swelling and thrombi at the injection sites and decreased body weight gains were noted in all treatment groups, but these effects were more severe at the high dose. There were also two 3-day IV toxicity studies in dogs (# 1006263 and 1008498) which were used as dose ranging studies for the 2-week IV study in dogs.

7 Genetic Toxicology

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

Study title: Evaluation of RO0673189-008 for mutagenic activity in the Ames test

Study no.:	330M01
Study report location:	n/a
Conducting laboratory and location:	Non-Clinical Drug Safety F.Hoffmann La-Roche Ltd., Basel, Switzerland
Date of study initiation:	8/20/2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RO0673189-008, batch #: GPM0403, 99.6%

Key Study Findings: Treatment with the test article did not significantly increase the number of revertant colonies/plate.

Methods:

Strains:	*Salmonella typhimurium* strains TA1535, TA97, TA98, TA100, and TA102
Concentrations in definitive study:	5 to 500 µg/plate and 1 to 100 µg/plate (see sponsor's tables 1 and 2 below)
Basis of concentration selection:	Toxicity were observed at concentrations of 200 µg/plate (-S9) and 632 µg/plate (+S9) based on reduction of background growth and reduction or absence of revertant colonies.
Negative control:	DMSO
Positive control:	See below.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The plates were incubated at 37°C for 2 days.

Positive controls

Sodium azide ([(b) (4)] Lot 357183/144998)	Concentration: 1.0 µg/plate
ICR 191 ([(b) (4)] Lot. 295485190)	Concentration: 1.0 µg/plate
2-Nitrofluorene ([(b) (4)] Lot 34435-128)	
Abbreviation: 2-NF	Concentration: 0.5 µg/plate
Mitomycin C ([(b) (4)] Lot 117H2505)	
Abbreviation: MMC	Concentration: 0.4 µg/plate
2-Aminoanthracene ([(b) (4)] Lot 13H3454)	
Abbreviation: 2-Aminoanthr.	Concentration: 4.0 µg/plate

Study Validity: The study is valid based on the positive response in the positive control group.

Results: Treatment with the test article did not significantly increase the number of revertant colonies/plate, whereas the positive control compounds produced the expected increase in revertants. The results were summarized in the sponsor's table below.

TABLE 1) Summary of the results of the reverse mutation assay using bacteria of the indicated strains. Mean values and standard deviations. For detailed data see the APPENDIX TABLES

Study No.: 330M01 Experiment No.: 1 Experiment Start: 21.08.01 Method: AME
Test Compound: Ro 67-3189/008

Strain	TA1535	TA1535	TA97	TA97	TA98	TA98	TA100	TA100	TA102	TA102
Activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Concentration µg/plate										
0	13 ± 2	7 ± 2	157 ± 13	196 ± 10	13 ± 1	16 ± 1	102 ± 6	88 ± 8	322 ± 30	287 ± 5
5	15 ± 6	4 ± 3	146 ± 4	199 ± 25	12 ± 2	17 ± 2	97 ± 15	94 ± 7	318 ± 11	268 ± 9
15.8	18 ± 1	8 ± 2	144 ± 9	201 ± 14	10 ± 3	15 ± 4	105 ± 3	102 ± 11	318 ± 30	305 ± 38
50	16 ± 4	7 ± 2	167 ± 17	207 ± 8	13 ± 5	13 ± 3	69 ± 10	99 ± 12	133 ± 24 t	289 ± 19
158	8 ± 2 t	9 ± 1	152 ± 8	179 ± 17	6 ± 3	21 ± 5	13 ± 4 t	84 ± 19	-t	219 ± 22 t
500	3 ± 2 t	2 ± 1 t	138 ± 19 a	159 ± 24 a	2 ± 1 t	13 ± 3 a	-T	6 ± 3 t	-T	-t

Codes: a = precipitation of the test compound without influence on automatic counting; t, T : Toxic Effect (see corresponding tables)

TABLE 2) Summary of the results of the reverse mutation assay using bacteria of the indicated strains. Mean values and standard deviations. For detailed data see the APPENDIX TABLES

Study No.: 330M01 Experiment No.: 2 Experiment Start: 28.08.01 Method: PRE
Test Compound: Ro 67-3189/008

Strain	TA1535	TA1535	TA97	TA97	TA98	TA98	TA100	TA100	TA102	TA102
Activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Concentration µg/plate										
0	10 ± 1	7 ± 5	151 ± 13	183 ± 13	13 ± 4	15 ± 2	54 ± 5	60 ± 5	297 ± 11	273 ± 5
1	16 ± 6	7 ± 2	165 ± 18	180 ± 4	15 ± 3	19 ± 4	57 ± 11	64 ± 13	297 ± 10	272 ± 23
3.16	15 ± 5	7 ± 2	148 ± 11	168 ± 14	17 ± 2	17 ± 7	58 ± 5	53 ± 6	283 ± 13	296 ± 21
10	13 ± 4	7 ± 0	168 ± 11	168 ± 3	23 ± 3	18 ± 7	53 ± 4	59 ± 3	248 ± 23 t	245 ± 12
31.6	11 ± 3	8 ± 6	146 ± 11	166 ± 2	19 ± 4	18 ± 1	36 ± 6	60 ± 6	-t	284 ± 27
100	9 ± 3 t	6 ± 1	116 ± 14	169 ± 16	10 ± 4 t	24 ± 7	-t	63 ± 2	-T	248 ± 23

t, T : Toxic Effect (see corresponding tables)

TABLE A1 Reverse mutation assay using bacteria
Number of revertant colonies per plate, mean values and standard deviations.

Study No.: 330M01 Experiment No.: 1
Experiment Start: 21.08.01 Method: AME
Test Compound: Ro 67-3189/008

Strain	TA1535	TA1535	TA97	TA97	TA98	TA98
Activation	-S9	+S9	-S9	+S9	-S9	+S9
Positive Controls (µg/plate)						
2-nitrofluorene					187	
0.5					176	
					182 ± 8	
ICR 191			702			
1			737			
			720 ± 25			
sodium azide	874					
1	849					
	862 ± 18					
2-aminoanthracene	23	296	182	1434	17	2253
4	24	262	154	1381	24	2427
	24 ± 1	279 ± 24	168 ± 20	1408 ± 37	21 ± 5	2340 ± 123

Strain	TA100	TA100	TA102	TA102
Activation	-S9	+S9	-S9	+S9
Positive Controls (µg/plate)				
mitomycin C			801	
0.4			833	
			817 ± 23	
sodium azide	581			
1	562			
	572 ± 13			
2-aminoanthracene	96	1598	332	455
4	110	1633	349	517
	103 ± 10	1616 ± 25	341 ± 12	486 ± 44

TABLE A2 Reverse mutation assay using bacteria
Number of revertant colonies per plate, mean values and standard deviations.

Study No.: 330M01 Experiment No.: 2
Experiment Start: 28.08.01 Method: PRE
Test Compound: Ro 67-3189/008

Strain	TA1535	TA1535	TA97	TA97	TA98	TA98
Activation	-S9	+S9	-S9	+S9	-S9	+S9
Positive Controls (µg/plate)						
2-nitrofluorene					231	
0.5					214	
					223 ± 12	
ICR 191			1230			
1			1008			
			1119 ± 157			
sodium azide	825					
1	898					
	862 ± 52					
2-aminoanthracene	22	214	177	962	18	1585
4	23	204	196	1024	23	1863
	23 ± 1	209 ± 7	187 ± 13	993 ± 44	21 ± 4	1724 ± 197

Strain	TA100	TA100	TA102	TA102
Activation	-S9	+S9	-S9	+S9
Positive Controls (µg/plate)				
mitomycin C			731	
0.4			671	
			701 ± 42	
sodium azide	524			
1	537			
	531 ± 9			
2-aminoanthracene	74	983	348	473
4	75	1098	325	401
	75 ± 1	1041 ± 81	337 ± 16	437 ± 51

Ames test (study #023M99)

The sponsor also conducted another Ames test at lower concentrations with RO67-3189/001 with a negative result. Since the concentrations of the test drug in the above

Ames test (study # 330M01) covers those in the current study, the full review on this study is not performed. Following paragraph is from the sponsor's report.

Ro 67-3189/001 was dissolved in DMSO. Upon addition of aliquots to the aqueous medium formation of milky suspensions and precipitation was apparent at concentrations ≥166 µg/plate. In a range finder assay with TA100 (plate incorporation), toxic effects were observed starting at 166 µg/plate. On this basis the dose range of 2 to 200 µg/plate was selected for the main experiments (plate incorporation and preincubation tests, respectively). Toxic effects were generally visible at the highest dose level, with variation depending on strain, presence or absence of metabolic activation, and test version. Toxicity was slightly more pronounced without metabolic activation in tester strains TA100 and TA102.

No increase in the number of revertant colonies was apparent for any of the five tester strains after treatment with Ro 67-3189/001.

Thus it can be concluded that neither Ro 67-3189/001 *per se*, nor any of the metabolites formed is mutagenic in the Ames test under the described experimental conditions.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: R00673189-008: Mouse Lymphoma Cell Mutation Test

Study no.:	312M01
Study report location:	n/a
Conducting laboratory and location:	Non-Clinical Drug Safety department F. Hoffmann-La Roche Ltd, Basel, Switzerland
Date of study initiation:	August 8, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	R00673189-008 / GPM0403 / 99.5%

Key Study Findings: Treatment with test article did not significantly increase the mutant frequencies as compared to the control.

Methods:

Cell line:	mouse lymphoma L5178Y tk+/-
Concentrations in definitive study:	See sponsor's table 3
Basis of concentration selection:	Cytotoxicity was observed at higher concentrations.
Negative control:	DMSO
Positive control:	See sponsor's table below
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Incubation times were 3 and 24 hours

The positive control chemicals were supplied and used as tabulated below:

Chemical	Supplier	Batch/Lot	Molecular weight	Vehicle	Stock conc. [µg/mL]	Final conc. [µg/mL]	S9
4-nitroquinoline 1-oxide (NQO)	(b) (4)	84F0572	190.2	DMSO	10	0.1	-
Benzo[a]pyrene (BP)		110H0096	252.3	DMSO	200	1 and 2	+
Methylmethan sulfonat(MMS)		C1B	110.1	DMSO	500	5	-

Study Validity: The study is valid based on the positive response in the positive control group.

Results:

Treatment with test article did not significantly increase the mutant frequencies as compared to the control. However, the positive control compounds produced significant increases in mutant frequencies. The results are summarized in the following sponsor's tables.

Table 3 **Summary Table**

Small and large colony mutant frequencies and viabilities of negative controls, positive controls and treatment groups of RO0673189-008 after 3 or 24 h exposure in absence of metabolic activation and 3 h exposure in presence of metabolic activation.

Experiment	Test article concentration (μg/mL)		S9	RS Day 0 (%)	ADRS Day 0 (%)	PE Day 2 (%)	MF (Mutants per 10^6 viable cells) small	large	total	Proportion small/large colonies
312M01/2	DMSO (1%)	0	−	104	99	77	46	141	189	0.33
	DMSO (1%)	0		96	101	79	44	90	139	0.49
treatment		5.0		109	102	70	40	115	163	0.35
time: 3h		5.0		92	91	87	37	89	131	0.42
		7.5		84	71	87	23	86	109	0.27
		7.5		99	95	92	34	74	113	0.46
		10.0		84	67	101	25	79	107	0.32
		10.0		73	59	94	21	103	127	0.20
		12.5		55	35	89	39	107	153	0.36
		12.5		50	33	80	38	109	149	0.35
		15.0		27	14	61	59	109	177	0.54
		15.0		26	13	75	34	107	145	0.32
		17.5		10	3	†	†	†	†	†
		17.5		9	3	†	†	†	†	†
	Linear trend		NS							
	NQO	0.1		56	45	72	264	424	847	0.62
312M01/3	DMSO (1%)	0	+	89	88	73	41	138	184	0.30
	DMSO (1%)	0		113	114	71	36	138	177	0.26
treatment		5		102	110	73	56	119	186	0.47
time: 3h		5		87	88	79	41	92	135	0.45
		10		95	96	83	31	104	141	0.30
		10		101	98	70	23	111	138	0.21
		15		119	121	72	33	140	179	0.24
		15		107	97	59	29	147	182	0.20
		20		89	70	75	32	101	139	0.32
		20		76	60	62	61	157	231	0.39
		25		64	36	67	40	141	182	0.28
		25		64	33	73	33	136	174	0.24
		30		26	9	†	†	†	†	†
		30		20	6	†	†	†	†	†
	Linear trend		NS							
	BP	1		102	105	77	191	327	632	0.58
	BP	2		27	27	45	594	720	1'625	0.83

NS : No significant increases of mutant frequency *;**, *** : Significant at 5, 1, 0.1 % level

†: Adapted relative survival after treatment below 10 %

Table 3 cont. Summary Table

Small and large colony mutant frequencies and viabilities of negative controls, positive controls and treatment groups of RO0673189-008 after 3 or 24 h exposure in absence of metabolic activation and 3 h exposure in presence of metabolic activation.

Experiment	Test article concentration (µg/mL)		S9	RS Day 0 (%)	ADRS Day 0 (%)	PE Day 2 (%)	MF (Mutants per 10^6 viable cells) small	large	total	Proportion small/large colonies
312M01/4	DMSO (1%)	0	−	97	102	96	22	83	109	0.27
	DMSO (1%)	0		103	98	80	32	119	158	0.27
treatment		1		93	93	94	13	78	91	0.17
time: 24h		1		103	98	101	18	103	125	0.17
		3		100	103	101	14	78	95	0.18
		3		94	94	-	-	-	-	-
		5		56	47	80	40	74	119	0.54
		5		82	64	103	17	84	104	0.20
		6		64	37	76	33	71	108	0.46
		6		62	37	75	39	83	128	0.47
		7		46	8	73	33	54	90	0.61
		7		53	15	60	47	91	135	0.52
	Linear trend		NS							
	MMS	5		58	54	54	398	521	1'142	0.76
312M01/5	DMSO (1%)	0	+	96	94	80	30	108	143	0.28
	DMSO (1%)	0		105	106	71	38	92	131	0.41
treatment		5		123	125	74	27	117	150	0.23
time: 3h		5		103	105	80	26	87	117	0.30
		10		103	104	89	35	120	161	0.29
		10		77	72	72	47	116	165	0.41
		20		82	70	79	35	105	147	0.33
		20		54	44	85	35	91	127	0.38
		25		57	28	84	27	108	141	0.25
		25		48	28	87	45	91	144	0.49
		27.5		38	20	85	35	70	109	0.50
		27.5		36	18	67	48	111	168	0.43
		30		32	13	87	28	87	120	0.32
		30		35	10	89	27	71	100	0.38
	Linear trend		NS							
	BP	1		89	82	77	139	281	485	0.49
	BP	2		37	34	44	530	995	2'073	0.53

NS : No significant increases of mutant frequency *;**, *** : Significant at 5, 1, 0.1 % level

†: Adapted relative survival after treatment below 10 %

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

Study title: RO0673189-008: Micronucleus test in rat bone marrow and toxicokinetics monitoring - Oral administration (gavage)

Study no:	302M01
Study report location:	n/a
Conducting laboratory and location:	Non-Clinical Drug Safety F.Hoffmann La-Roche Ltd., Basel, Switzerland
Date of study initiation:	July 24, 2001
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RO0673189-008, batch #: GPM0403 / 99.6%

Key Study Findings: Treatment with RO0673189-008 did not significantly increase the frequency of micronucleated polychromatic erythrocytes.

Methods:

Doses in definitive study:	160, 400 and 1000 mg/kg
Frequency of dosing:	Daily for two days
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Vehicle suspension containing the following ingredients: 0.2% Polysorbate 80, 1.6% Avicel RC 591, 0.18% Nipagin, 0.02% Nipasol, 0.1 N citric acid
Species/Strain:	Wistar HanBrl rats
Number/Sex/Group:	10 (12/sex in high dose group only)
Satellite groups:	none
Basis of dose selection:	High dose produced clinical signs of toxicity such as hypoactivity.
Negative control:	vehicle suspension
Positive control:	Cyclophosphamide monohydrate

At least 4000 polychromatic cells were evaluated per animal.

Study Validity: The study is valid based on the positive response in the positive control group.

Results:

Treatment with RO0673189-008 did not significantly increase the frequency of micronucleated polychromatic erythrocytes. However, the positive control compound

(cyclophosphamide) produced the expected increase. The results are summarized in the sponsor's table below.

Table 1 Micronucleus Study: Summary Data

Group median ratio of polychromatic to normochromatic erythrocytes (PCE/NCE) Group median frequency of polychromatic (MN-PCE) erythrocytes and significance levels after oral administration(s) at a single harvest time (24 h).

Test item	Dose (mg/kg/day)	Ratio PCE/NCE	MN-PCE (%)	p-values
Oral doses on two consecutive days, harvest on the following day				
Negative control	0	**1.07**	**0.19**	
RO0673189-008	160	**1.32**	**0.20**	n.s.
RO0673189-008	400	**1.26**	**0.14**	n.s.
RO0673189-008	1000	**0.76**	**0.10**	n.s.
Positive control: single oral dose, harvest on the following day				
Cyclophosphamide	30	**0.78**	**1.22**	**

n.s.: no significance, **: $p < 0.01$

Table 2 Micronucleus Study: Group Median Values

Ratio of polychromatic to normochromatic erythrocytes (PCE/NCE), frequency of micronucleated polychromatic erythrocytes (MN-PCE) and median values per animal, sex and treatment group.

Test item and	Dose (mg/kg/day)	Animal No	Sex	Ratio PCE/NCE	Median/ Sex	Median/ Group	MN-PCE (%)	Median/ Sex	Median/ Group
Oral doses on two consecutive days, harvest on the following day									
		1	m	1.19			0.25		
		2	m	0.79			0.20		
		3	m	0.50	**0.94**		0.17	**0.25**	
		4	m	1.31			0.25		
		5	m	0.94			0.25		
Negative control	**0**					**1.07**			**0.19**
		6	f	1.23			0.15		
		7	f	1.31			0.10		
		8	f	1.38	**1.23**		0.07	**0.10**	
		9	f	0.72			0.07		
		10	f	0.87			0.20		
Positive control: single oral dose, harvest on the following day									
		1	m	1.54			1.79		
		2	m	1.03			0.95		
		3	m	0.98	**0.98**		1.15	**1.32**	
		4	m	0.88			1.32		
		5	m	0.47			1.35		
Cyclophosphamide	**30**					**0.78**			**1.22**
		6	f	1.03			0.89		
		7	f	0.62			1.22		
		8	f	0.31	**0.62**		0.82	**1.22**	
		9	f	0.34			1.27		
		10	f	0.68			1.22		

Table 2 (cont.) Micronucleus Study: Group Median Values

Ratio of polychromatic to normochromatic erythrocytes (PCE/NCE), frequency of micronucleated polychromatic erythrocytes (MN-PCE) and median values per animal, sex and treatment group.

Test item and	Dose (mg/kg/day)	Animal No	Sex	Ratio PCE/NCE	Median/ Sex	Median/ Group	MN-PCE (%)	Median/ Sex	Median/ Group
Oral doses on two consecutive days, harvest on the following day									
		1	m	2.37			0.30		
		2	m	1.64			0.17		
		3	m	1.11	**1.64**		0.30	**0.27**	
		4	m	2.01			0.22		
		5	m	1.04			0.27		
RO0673189-008	160					**1.32**			**0.20**
		6	f	1.27			0.02		
		7	f	1.37			0.22		
		8	f	1.86	**1.27**		0.15	**0.08**	
		9	f	1.06			0.07		
		10	f	0.98			0.08		
		1	m	2.23			0.12		
		2	m	2.08			0.15		
		3	m	1.18	**1.18**		0.20	**0.17**	
		4	m	0.91			0.17		
		5	m	0.88			0.20		
RO0673189-008	400					**1.26**			**0.14**
		6	f	2.14			0.07		
		7	f	1.33			0.10		
		8	f	0.80	**1.33**		0.13	**0.10**	
		9	f	1.60			0.15		
		10	f	0.89			0.10		
		1	m	0.76			0.07		
		2	m	0.66			0.05		
		3	m	2.08	**0.76**		0.20	**0.13**	
		4	m	1.02			0.25		
		5	m	0.60			0.13		
RO0673189-008	1000					**0.76**			**0.10**
		6	f	0.62			0.05		
		7	f	0.84			0.10		
		8	f	0.82	**0.75**		0.10	**0.10**	
		9	f	0.75			0.15		
		10	f	0.50			0.10		

7.4 Other Genetic Toxicity Studies

Study title: BACTERIAL MUTATION ASSAY with (b) (4) (impurity)

Study no.:	(b) (4)
Study report location:	n/a
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 18, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4), batch #: 200706120045

Key Study Findings: Treatment with (b) (4) did not significantly increase the number of revertant colonies/plate. (b) (4) is a process impurity.

Methods:

Strains:	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and WP2 *uvr*A
Concentrations in definitive study:	5000, 1580, 500, 158 and 50 µg/plate. (see sponsor's tables below)
Basis of concentration selection:	Highest concentration of 5000 µg/plate was used.
Negative control:	DMSO
Positive control:	See below.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The plates were incubated at 37°C for 72 hours.

Positive controls

Tester strain	*Absence of S9*	*Presence of S9*
TA1535	sodium azide 1 µg/plate	2-aminoanthracene 1 µg/plate
TA100	sodium azide 1 µg/plate	2-aminoanthracene 1 µg/plate
TA1537	9-amino-acridine 50 µg/plate	2-aminoanthracene 1 µg/plate
TA98	2-nitrofluorene 2 µg/plate	2-aminoanthracene 1 µg/plate
WP2 *uvr*A	methylmethanesulphonate 500 µg/plate	2-aminoanthracene 10 µg/plate

Study Validity: The study is valid based on the positive response in the positive control group.

Results:

Treatment with the test article did not significantly increase the number of revertant colonies/plate, whereas the positive control compounds produced the expected significant increases in the number of revertants. The results are summarized in the sponsor's tables below.

TABLE 3 - Experiment I - Plate incorporation method - TA1535

STUDY NO.: 91710

SOLVENT: DMSO

Strain: TA1535 Titre: 252

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	24	14	25	21	3.5	20	19	17	19	0.9
0.00	20	14	20	18	2.0	24	19	24	22	1.7
313	14	16	19	16	1.5	16	19	12	16	2.0
625	19	20	18	19	0.6	19	17	20	19	0.9
1250	17	15	12	15	1.5	15	27	18	20	3.6
2500	17	23	15	18	2.4	17	21	18	19	1.2
5000	21	19	15	18	1.8	24	16	14	18	3.1

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.142	0.0002	0.18434	0.4962	0.63493
1 - 4	-	4.258	-0.0003	-0.38166	1.3058	0.22087
1 - 5	-	4.139	0.0000	0.00589	0.0213	0.98337
1 - 6	-	4.121	0.0000	0.12808	0.5166	0.61252
1 - 3	+	4.527	-0.0006	-0.39228	1.1283	0.29636
1 - 4	+	4.384	-0.0001	-0.05566	0.1763	0.86358
1 - 5	+	4.372	0.0000	-0.05718	0.2065	0.83961
1 - 6	+	4.375	0.0000	-0.12362	0.4983	0.62506

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	24	14	25	21	3.5
Sodium Azide	1 µg/pl	-	470	499	489	486	8.5
DMSO	100 µL/pl	+	24	19	24	22	1.7
2-Aminoanthracene	1 µg/pl	+	91	93	108	97	5.4

TABLE 4 - Experiment I - Plate incorporation method - TA1537

STUDY NO.: 91710

SOLVENT: DMSO

Strain: TA1537 Titre: 234

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	18	20	20	19	0.7	26	22	23	24	1.2
0.00	20	20	21	20	0.3	24	20	27	24	2.0
156	25	24	21	23	1.2	NT	NT	NT		
313	21	17	21	20	1.3	27	26	20	24	2.2
625	18	16	21	18	1.5	27	26	26	26	0.3
1250	19	23	19	20	1.3	18	28	25	24	3.0
2500	20	18	15 *	18	1.5	22	17	29	23	3.5
5000	14	19	20 *	18	1.9	27	25	24 *	25	0.9

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.629	-0.0003	-0.13776	0.3680	0.72375
1 - 4	-	4.667	-0.0006	-0.48424	1.7502	0.11065
1 - 5	-	4.570	-0.0001	-0.21964	0.8117	0.43156
1 - 6	-	4.577	-0.0001	-0.44877	2.0087	0.06175
1 - 7	-	4.535	-0.0001	-0.45348	2.2178	0.03895
1 - 3	+	4.832	0.0004	0.40219	1.1622	0.28323
1 - 4	+	4.940	0.0000	-0.00303	0.0096	0.99253
1 - 5	+	4.975	-0.0001	-0.18986	0.6972	0.49795
1 - 6	+	4.908	0.0000	0.03556	0.1423	0.88858

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µL/pl	-	20	20	21	20	0.3
9-Aminoacridine	50 µg/pl	-	205	239	92	179	44.4
DMSO	100 µL/pl	+	24	20	27	24	2.0
2-Aminoanthracene	1 µg/pl	+	82	93	109	95	7.8

NT: not tested
*: thinning of the background lawn

TABLE 5 - Experiment I - Plate incorporation method - WP2 uvrA

STUDY NO.: 91710

SOLVENT: DMSO

Strain: WP2 uvrA Titre: 362

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	33	32	34	33	0.6	34	31	36	34	1.5
0.00	31	35	32	33	1.2	37	38	33	36	1.5
313	35	34	37	35	0.9	36	34	33	34	0.9
625	24	27	34	28	3.0	37	32	39	36	2.1
1250	33	33	24	30	3.0	34	35	27	32	2.5
2500	24	20	35	26	4.5	30	34	30	31	1.3
5000	39	28	34	34	3.2	34	35	32	34	0.9

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	5.857	-0.0006	-0.45772	1.3621	0.21539
1 - 4	-	5.775	-0.0003	-0.37274	1.2702	0.23276
1 - 5	-	5.759	-0.0003	-0.49978	2.0805	0.05782
1 - 6	-	5.561	0.0000	-0.01989	0.0796	0.93756
1 - 3	+	5.951	0.0000	-0.00518	0.0137	0.98946
1 - 4	+	6.011	-0.0002	-0.42864	1.5003	0.16444
1 - 5	+	5.975	-0.0002	-0.53325	2.2727	0.04066
1 - 6	+	5.881	0.0000	-0.26934	1.1187	0.27979

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	33	32	34	33	0.6
MMS	500 µg/pl	-	199	154	163	172	13.7
DMSO	100 µL/pl	+	37	38	33	36	1.5
2-Aminoanthracene	10 µg/pl	+	187	199	188	191	3.8

TABLE 6 - Experiment I - Plate incorporation method - TA98

STUDY NO.: 91710

SOLVENT: DMSO

Strain: TA98 Titre: 265

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	30	37	34	34	2.0	38	35	45	39	3.0
0.00	30	33	39	34	2.6	38	33	35	35	1.5
156	25	33	22	27	3.3	NT	NT	NT		
313	28	29	30	29	0.6	36	40	34	37	1.8
625	31	29	28	29	0.9	36	33	37	35	1.2
1250	22	25	25	24	1.0	39	34	40	38	1.9
2500	24	23	30	26	2.2	35	38	35	36	1.0
5000	25	26	30 *	27	1.5	33	35	46	38	4.0

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	5.669	-0.0014	-0.42071	1.2270	0.25950
1 - 4	-	5.545	-0.0004	-0.23546	0.7661	0.46130
1 - 5	-	5.576	-0.0005	-0.56904	2.4951	0.02684
1 - 6	-	5.465	-0.0002	-0.47133	2.1377	0.04832
1 - 7	-	5.368	-0.0001	-0.29534	1.3475	0.19368
1 - 3	+	5.978	0.0000	0.00195	0.0052	0.99603
1 - 4	+	5.947	0.0001	0.29049	0.9600	0.35969
1 - 5	+	5.994	0.0000	0.09587	0.3473	0.73394
1 - 6	+	5.987	0.0000	0.20074	0.8196	0.42446

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µL/pl	-	30	33	39	34	2.6
2-Nitrofluorene	2 µg/pl	-	160	177	167	168	4.9
DMSO	100 µL/pl	+	38	33	35	35	1.5
2-Aminoanthracene	1 µg/pl	+	382	395	354	377	12.1

NT: not tested
*: thinning of the background lawn

TABLE 7 - Experiment I - Plate incorporation method - TA100

STUDY NO.: 91710

SOLVENT: DMSO

Strain: TA100 Titre: 243

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	159	166	153	159	3.8	162	132	142	145	8.8
0.00	148	128	142	139	5.9	136	137	162	145	8.5
156	164	147	128	146	10.4	NT	NT	NT		
313	137	145	163	148	7.7	149	147	138	145	3.4
625	164	147	143	151	6.4	154	149	159	154	2.9
1250	139	127	145	137	5.3	159	138	155	151	6.4
2500	136	123	132 *	130	3.8	141	145	136	141	2.6
5000	139	140	146 *	142	2.2	134	154	169	152	10.1

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	11.831	0.0012	0.29965	0.8310	0.43340
1 - 4	-	11.887	0.0007	0.34442	1.1601	0.27294
1 - 5	-	12.079	-0.0001	-0.13512	0.4917	0.63114
1 - 6	-	12.121	-0.0003	-0.44236	1.9730	0.06603
1 - 7	-	11.995	-0.0001	-0.21504	0.9598	0.34922
1 - 3	+	11.967	0.0006	0.41822	1.2182	0.26262
1 - 4	+	12.057	0.0002	0.28672	0.9464	0.36623
1 - 5	+	12.193	-0.0001	-0.19210	0.7058	0.49279
1 - 6	+	12.117	0.0000	0.09330	0.3748	0.71270

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	159	166	153	159	3.8
Sodium Azide	1 µg/pl	-	564	537	583	561	13.3
DMSO	100 µL/pl	+	136	137	162	145	8.5
2-Aminoanthracene	1 µg/pl	+	911	925	940	925	8.4

NT: not tested
*: thinning of the background lawn

Study title: BACTERIAL MUTATION ASSAY with (b) (4) (impurity)

Study no.:	(b) (4)
Study report location:	n/a
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 18, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4), batch #: TES015

Key Study Findings: Treatment with (b) (4) did not significantly increase the number of revertant colonies/plate. (b) (4) is an impurity.

Methods:

Strains:	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and WP2 *uvr*A
Concentrations in definitive study:	5000, 1580, 500, 158 and 50 µg/plate. (see sponsor's tables below)
Basis of concentration selection:	Highest concentration of 5000 µg/plate was used.
Negative control:	Acetone
Positive control:	See below.
Formulation/Vehicle:	Acetone
Incubation & sampling time:	The plates were incubated at 37°C for 72 hours.

Positive controls

Tester strain	*Absence of S9*	*Presence of S9*
TA1535	sodium azide 1 µg/plate	2-aminoanthracene 1 µg/plate
TA100	sodium azide 1 µg/plate	2-aminoanthracene 1 µg/plate
TA1537	9-amino-acridine 50 µg/plate	2-aminoanthracene 1 µg/plate
TA98	2-nitrofluorene 2 µg/plate	2-aminoanthracene 1 µg/plate
WP2 *uvr*A	methylmethanesulphonate 500 µg/plate	2-aminoanthracene 10 µg/plate

Study Validity: The study is valid based on the positive response in the positive control groups.

Results:

Treatment with the test article did not significantly increase the number of revertant colonies/plate, whereas the positive control compounds produced the expected significant increases in the number of revertants. The results are summarized in the sponsor's tables below.

TABLE 3 - Experiment I - Plate incorporation method - TA1535

STUDY NO.: 91720

SOLVENT: ACETONE

Strain: TA1535 Titre: 252

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	24	14	25	21	3.5	20	19	17	19	0.9
0.00	23	16	18	19	2.1	17	22	13	17	2.6
313	17	19	16	17	0.9	19	15	11	15	2.3
625	16	17	15	16	0.6	16	19	14	16	1.5
1250	14	22	16	17	2.4	18	21	17	19	1.2
2500	19	14	16	16	1.5	18	25	19	21	2.2
5000	23	20	18 P*	20	1.5	14	20	15 P	16	1.9

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.342	-0.0006	-0.54401	1.7153	0.12999
1 - 4	-	4.243	-0.0001	-0.22533	0.7314	0.48134
1 - 5	-	4.215	-0.0001	-0.24893	0.9267	0.37096
1 - 6	-	4.114	0.0001	0.27975	1.1655	0.26089
1 - 3	+	4.061	-0.0002	-0.10808	0.2876	0.78195
1 - 4	+	3.967	0.0002	0.25779	0.8437	0.41855
1 - 5	+	3.962	0.0002	0.48133	1.9799	0.06929
1 - 6	+	4.114	0.0000	0.09410	0.3781	0.71035

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	24	14	25	21	3.5
Sodium Azide	1 µg/pl	-	470	499	489	486	8.5
DMSO	100 µL/pl	+	24	19	24	22	1.7
2-Aminoanthracene	1 µg/pl	+	91	93	108	97	5.4

P: precipitation
*: thinning of the background lawn

TABLE 4 - Experiment I - Plate incorporation method - TA1537

STUDY NO.: 91720

SOLVENT: ACETONE

Strain: TA1537 Titre: 234

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	18	20	20	19	0.7	26	22	23	24	1.2
0.00	22	15	16	18	2.2	25	21	28	25	2.0
156	24	26	19	23	2.1	NT	NT	NT		
313	23	24	28	25	1.5	26	21	27	25	1.9
625	21	21	23	22	0.7	22	29	30	27	2.5
1250	21	25	22	23	1.2	25	18	23	22	2.1
2500	20	24	26 *	23	1.8	25	21	30	25	2.6
5000	19	17	15 P*	17	1.2	25	25	31 P*	27	2.0

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.253	0.0026	0.72285	2.7677	0.02779
1 - 4	-	4.495	0.0006	0.34078	1.1463	0.27838
1 - 5	-	4.578	0.0002	0.25296	0.9427	0.36302
1 - 6	-	4.617	0.0001	0.24818	1.0248	0.32071
1 - 7	-	4.736	-0.0001	-0.35551	1.6580	0.11374
1 - 3	+	4.921	0.0004	0.28514	0.7871	0.45705
1 - 4	+	5.060	-0.0002	-0.27010	0.8871	0.39587
1 - 5	+	4.975	0.0000	-0.03880	0.1400	0.89080
1 - 6	+	4.933	0.0000	0.19425	0.7921	0.43990

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µL/pl	-	20	20	21	20	0.3
9-Aminoacridine	50 µg/pl	-	205	239	92	179	44.4
DMSO	100 µL/pl	+	24	20	27	24	2.0
2-Aminoanthracene	1 µg/pl	+	82	93	109	95	7.8

NT: not tested
P: precipitation
*: thinning of the background lawn

TABLE 5 - Experiment I - Plate incorporation method - WP2 uvrA

STUDY NO.: 91720

SOLVENT: ACETONE

Strain: WP2 uvrA Titre: 362

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	33	32	34	33	0.6	34	31	36	34	1.5
0.00	36	34	31	34	1.5	29	37	31	32	2.4
313	30	30	29	30	0.3	30	34	38	34	2.3
625	34	31	36	34	1.5	34	34	29	32	1.7
1250	37	30	31	33	2.2	34	36	35	35	0.6
2500	36	27	39	34	3.6	36	27	36	33	3.0
5000	34	36	30 P*	33	1.8	33	36	39 P	36	1.7

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	5.682	0.0000	-0.00069	0.0018	0.99859
1 - 4	-	5.676	0.0000	0.04410	0.1396	0.89175
1 - 5	-	5.662	0.0001	0.16484	0.6026	0.55715
1 - 6	-	5.686	0.0000	0.13791	0.5570	0.58525
1 - 3	+	5.726	0.0000	0.00590	0.0156	0.98799
1 - 4	+	5.690	0.0002	0.28477	0.9394	0.36965
1 - 5	+	5.751	0.0000	0.05240	0.1892	0.85288
1 - 6	+	5.728	0.0000	0.28811	1.2035	0.24629

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	33	32	34	33	0.6
MMS	500 µg/pl	-	199	154	163	172	13.7
DMSO	100 µL/pl	+	37	38	33	36	1.5
2-Aminoanthracene	10 µg/pl	+	187	199	188	191	3.8

P: precipitation
*: thinning of the background lawn

TABLE 6 - Experiment I - Plate incorporation method - TA98

STUDY NO.: 91720

SOLVENT: ACETONE

Strain: TA98 Titre: 265

Dose level [µg/pl]	Without metabolic activation Plate counts				Mean	S. E.	With metabolic activation Plate counts				Mean	S. E.
Untreated	30	37	34		34	2.0	38	35	45		39	3.0
0.00	29	33	35		32	1.8	39	44	35		39	2.6
156	29	31	37		32	2.4	NT	NT	NT			
313	29	31	27		29	1.2	33	30	35		33	1.5
625	24	30	22		25	2.4	32	34	37		34	1.5
1250	27	22	30		26	2.3	36	39	38		38	0.9
2500	24	22	32	*	26	3.1	39	35	34		36	1.5
5000	33	34	27	P*	31	2.2	37	39	42	P*	39	1.5

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	5.731	-0.0010	-0.45304	1.3445	0.22072
1 - 4	-	5.753	-0.0011	-0.70129	3.1109	0.01104
1 - 5	-	5.614	-0.0005	-0.57609	2.5412	0.02460
1 - 6	-	5.510	-0.0002	-0.47362	2.1510	0.04710
1 - 7	-	5.369	0.0000	-0.01072	0.0467	0.96322
1 - 3	+	6.149	-0.0007	-0.51595	1.5936	0.15506
1 - 4	+	5.988	0.0000	0.01312	0.0415	0.96771
1 - 5	+	5.990	0.0000	0.01183	0.0427	0.96661
1 - 6	+	5.953	0.0001	0.32815	1.3895	0.18371

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µL/pl	-	30	33	39	34	2.6
2-Nitrofluorene	2 µg/pl	-	160	177	167	168	4.9
DMSO	100 µL/pl	+	38	33	35	35	1.5
2-Aminoanthracene	1 µg/pl	+	382	395	354	377	12.1

NT: not tested
P: precipitation
*: thinning of the background lawn

TABLE 7 - Experiment I - Plate incorporation method - TA100

STUDY NO.: 91720

SOLVENT: ACETONE

Strain: TA100 Titre: 243

Dose level [µg/pl]	Without metabolic activation Plate counts				Mean	S. E.	With metabolic activation Plate counts				Mean	S. E.
Untreated	159	166	153		159	3.8	162	132	142		145	8.8
0.00	163	146	150		153	5.1	156	138	152		149	5.5
313	123	135	128		129	3.5	153	170	160		161	4.9
625	120	123	122		122	0.9	156	149	142		149	4.0
1250	136	131	129		132	2.1	169	154	133		152	10.4
2500	145	131	124		133	6.2	147	149	139		145	3.1
5000	143	127	111	P*	127	9.2	134	119	117	P*	123	5.4

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	12.247	-0.0021	-0.89590	5.3354	0.00108
1 - 4	-	11.865	-0.0006	-0.48882	1.7719	0.10683
1 - 5	-	11.675	-0.0001	-0.22640	0.8381	0.41714
1 - 6	-	11.642	-0.0001	-0.27789	1.1571	0.26421
1 - 3	+	12.351	0.0000	0.01821	0.0482	0.96292
1 - 4	+	12.365	0.0000	-0.03350	0.1060	0.91768
1 - 5	+	12.409	-0.0001	-0.27645	1.0372	0.31856
1 - 6	+	12.503	-0.0003	-0.73386	4.3213	0.00053

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	159	166	153	159	3.8
Sodium Azide	1 µg/pl	-	564	537	583	561	13.3
DMSO	100 µL/pl	+	136	137	162	145	8.5
2-Aminoanthracene	1 µg/pl	+	911	925	940	925	8.4

P: precipitation
*: thinning of the background lawn

Study title: BACTERIAL MUTATION ASSAY with METABOLITE M4

Study no.:	NETU-12-41
Study report location:	n/a
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 11, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	METABOLITE M4 / 4-NETU.i24 (M4), batch #: ALC1159-02-A

Key Study Findings: Treatment with the test article did not significantly increase the number of revertant colonies/plate.

Methods:

Strains:	*Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and WP2 *uvr*A
Concentrations in definitive study:	5000, 1580, 500, 158 and 50 µg/plate. (see sponsor's tables below)
Basis of concentration selection:	Highest concentration of 5000 µg/plate was used.
Negative control:	DMSO
Positive control:	See below.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The plates were incubated at 37°C for 72 hours.

Positive controls

Tester strain	*Absence of S9*	*Presence of S9*
TA1535	sodium azide 1 µg/plate	2-aminoanthracene 1 µg/plate
TA100	sodium azide 1 µg/plate	2-aminoanthracene 1 µg/plate
TA1537	9-amino-acridine 50 µg/plate	2-aminoanthracene 1 µg/plate
TA98	2-nitrofluorene 2 µg/plate	2-aminoanthracene 1 µg/plate
WP2 *uvr*A	methylmethanesulphonate 500 µg/plate	2-aminoanthracene 10 µg/plate

Study Validity: The study is valid based on the positive response in the positive control groups.

Results:

Treatment with the test article did not significantly increase the number of revertant colonies/plate, whereas the positive control compounds produced the expected significant increases in the number of revertants. The results are summarized in the sponsor's tables below.

TABLE 3 - Experiment 1 - Plate incorporation method - TA1535

STUDY NO.: 92940

SOLVENT: DMSO

Strain: TA1535 Titre: 272

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	17	18	18	18	0.3	20	20	17	19	1.0
0.00	13	18	20	17	2.1	20	19	14	18	1.9
313	15	16	16	16	0.3	16	15	14	15	0.6
625	17	15	19	17	1.2	22	15	16	18	2.2
1250	24	15	20	20	2.6	18	19	18	18	0.3
2500	18	16	16	17	0.7	24	20	15	20	2.6
5000	17	19	18	18	0.6	19	13	14	15	1.9

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.055	0.0000	0.01831	0.0485	0.96270
1 - 4	-	3.988	0.0003	0.40053	1.3823	0.19697
1 - 5	-	4.101	0.0000	0.10596	0.3842	0.70703
1 - 6	-	4.107	0.0000	0.16955	0.6882	0.50121
1 - 3	+	4.085	0.0000	-0.00436	0.0115	0.99112
1 - 4	+	4.044	0.0002	0.25517	0.8345	0.42346
1 - 5	+	4.050	0.0001	0.38312	1.4954	0.15867
1 - 6	+	4.177	0.0000	-0.10757	0.4328	0.67094

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	17	18	18	18	0.3
Sodium Azide	1 µg/pl	-	473	445	451	456	8.5
DMSO	100 µL/pl	+	20	19	14	18	1.9
2-Aminoanthracene	1 µg/pl	+	116	122	125	121	2.6

TABLE 4 - Experiment 1- Plate incorporation method - TA1537

STUDY NO.: 92940

SOLVENT: DMSO

Strain: TA1537 Titre: 274

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	16	25	24	22	2.8	29	23	26	26	1.7
0.00	19	21	26	22	2.1	25	22	26	24	1.2
78.1	28	24	19	24	2.6	23	22	27	24	1.5
156	22	19	23	21	1.2	26	28	25	26	0.9
313	21	29	26	25	2.3	22	29	23	25	2.2
625	20	14	15	16	1.9	28	22	22	24	2.0
1250	12	21	11	15	3.2	25	28	26	26	0.9
2500	5	8	8 *	7	1.0	8	15	13 *	12	2.1

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	4.747	-0.0004	-0.08298	0.2203	0.83192
1 - 4	-	4.667	0.0009	0.30373	1.0081	0.33717
1 - 5	-	4.878	-0.0010	-0.49051	2.0295	0.06340
1 - 6	-	4.852	-0.0009	-0.65620	3.4784	0.00310
1 - 7	-	4.855	-0.0009	-0.87243	7.7810	0.00000
1 - 3	+	4.885	0.0013	0.40086	1.1577	0.28498
1 - 4	+	4.950	0.0002	0.09939	0.3159	0.75860
1 - 5	+	4.983	-0.0001	-0.08510	0.3080	0.76300
1 - 6	+	4.945	0.0001	0.19552	0.7975	0.43685
1 - 7	+	5.152	-0.0005	-0.74927	4.9316	0.00009

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µL/pl	-	19	21	26	22	2.1
9-Aminoacridine	50 µg/pl	-	198	129	257	195	37.0
DMSO	100 µL/pl	+	25	22	26	24	1.2
2-Aminoanthracene	1 µg/pl	+	139	134	120	131	5.7

*: thinning of the background lawn

METABOLITE M4:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 5 - Experiment 1 - Plate incorporation method - WP2 uvrA

STUDY NO.: 92940

SOLVENT: DMSO

Strain: WP2 uvrA Titre: 347

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	32	30	28	30	1.2	35	34	33	34	0.6
0.00	34	33	27	31	2.2	34	34	34	34	0.0
313	33	33	35	34	0.7	25	34	35	31	3.2
625	32	35	28	32	2.0	32	29	32	31	1.0
1250	26	27	24	26	0.9	27	29	27	28	0.7
2500	23	27	21	24	1.8	27	24	26	26	0.9
5000	18	23	20	20	1.5	21	26	20	22	1.9

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	5.656	0.0000	0.05229	0.1385	0.89371
1 - 4	-	5.786	-0.0005	-0.65866	2.7681	0.01985
1 - 5	-	5.735	-0.0004	-0.78079	4.5057	0.00059
1 - 6	-	5.646	-0.0003	-0.83949	6.1799	0.00001
1 - 3	+	5.792	-0.0004	-0.38937	1.1184	0.30029
1 - 4	+	5.793	-0.0004	-0.65757	2.7601	0.02013
1 - 5	+	5.734	-0.0003	-0.76199	4.2424	0.00096
1 - 6	+	5.671	-0.0002	-0.82673	5.8778	0.00002

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	32	30	28	30	1.2
MMS	500 µg/pl	-	192	196	229	206	11.7
DMSO	100 µL/pl	+	34	34	34	34	0.0
2-Aminoanthracene	10 µg/pl	+	190	216	217	208	8.8

TABLE 6 - Experiment 1 - Plate incorporation method - TA98

STUDY NO.: 92940

SOLVENT: DMSO

Strain: TA98 Titre: 274

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	22	32	36	30	4.2	47	48	43	46	1.5
0.00	21	37	28	29	4.6	42	49	44	45	2.1
313	31	31	39	34	2.7	38	36	40	38	1.2
625	22	28	25	25	1.7	35	38	45	39	3.0
1250	22	27	24	24	1.5	32	35	35	34	1.0
2500	29	33	35	32	1.8	41	37	35	38	1.8
5000	19	26	30	25	3.2	34	34	33	34	0.3

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	5.531	-0.0005	-0.24713	0.6748	0.52147
1 - 4	-	5.522	-0.0005	-0.43394	1.5231	0.15871
1 - 5	-	5.283	0.0001	0.11368	0.4126	0.68666
1 - 6	-	5.373	-0.0001	-0.18722	0.7624	0.45693
1 - 3	+	6.598	-0.0007	-0.53560	1.6781	0.13723
1 - 4	+	6.575	-0.0006	-0.74263	3.5067	0.00566
1 - 5	+	6.384	-0.0002	-0.43892	1.7613	0.10168
1 - 6	+	6.341	-0.0001	-0.56693	2.7529	0.01415

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
DMSO	100 µL/pl	-	21	37	28	29	4.6
2-Nitrofluorene	2 µg/pl	-	141	131	152	141	6.1
DMSO	100 µL/pl	+	42	49	44	45	2.1
2-Aminoanthracene	1 µg/pl	+	445	567	480	497	36.3

METABOLITE M4:
BACTERIAL MUTATION ASSAY (*S. typhimurium* and *E. coli*)

TABLE 7 - Experiment 1 - Plate incorporation method - TA100

STUDY NO.: 92940

SOLVENT: DMSO

Strain: TA100 Titre: 258

Dose level [µg/pl]	Without metabolic activation Plate counts			Mean	S. E.	With metabolic activation Plate counts			Mean	S. E.
Untreated	165	154	127	149	11.3	156	140	161	152	6.3
0.00	132	128	155	138	8.4	162	140	140	147	7.3
39.1	146	132	145	141	4.5	136	153	176	155	11.6
78.1	130	125	163	139	11.9	166	164	150	160	5.0
156	143	128	132	134	4.5	133	145	141	140	3.5
313	133	176	162	157	12.7	139	153	143	145	4.2
625	131	104	106 *	114	8.7	110	155	134 *	133	13.0

Regression analysis:

Points	S9	Intercept	Slope	Corr. coeff.	t	P-value
1 - 3	-	11.786	0.0004	0.02515	0.0665	0.94880
1 - 4	-	11.838	-0.0013	-0.15784	0.5055	0.62419
1 - 5	-	11.649	0.0021	0.38329	1.4963	0.15847
1 - 6	-	11.983	-0.0014	-0.40317	1.7623	0.09711
1 - 3	+	12.145	0.0066	0.40140	1.1595	0.28427
1 - 4	+	12.417	-0.0024	-0.25605	0.8376	0.42182
1 - 5	+	12.354	-0.0012	-0.26992	1.0107	0.33060
1 - 6	+	12.368	-0.0014	-0.47027	2.1315	0.04890

Positive and negative controls

Treatment		S9	Plate counts			Mean	S. E.
Untreated		-	165	154	127	149	11.3
Sodium Azide	1 µg/pl	-	562	553	582	566	8.6
DMSO	100 µL/pl	+	162	140	140	147	7.3
2-Aminoanthracene	1 µg/pl	+	1440	1456	1402	1433	16.0

*: thinning of the background lawn

8 Carcinogenicity

On July 1, 2008, the Executive Carcinogenicity Assessment Committee (E-CAC) reviewed the sponsor's proposal for a 2-year carcinogenicity study of netupitant in rats. The combination of netupitant and palonosetron was developed for prevention of chemotherapy-induced acute and delayed nausea and vomiting. The Committee noted that this indication would not normally trigger the need for a carcinogenicity study; however, a carcinogenicity evaluation may be needed to support other indications (see E-CAC meeting minutes in Appendix). Therefore, the sponsor did not conduct carcinogenicity studies with netupitant to support approval of netupitant + palonosetron combination for the proposed indication.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Segment I: Oral Study of Fertility and Early Embryonic Development in the Rat

Study no.:	257R02
Study report location:	n/a
Conducting laboratory and location:	Non-Clinical Drug Safety F. Hoffmann La-Roche Ltd., Basel, Switzerland
Date of study initiation:	June 6, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RO0673189-008 / lot # BS0204SA01, 99.8%

Key Study Findings:

RO0673189-008 was given by oral gavage at doses of 3, 10, and 30 mg/kg/day to male and females rats starting at two weeks prior to mating, throughout a two-week mating period, and through gestation day 7 in females. Dosing of males continued until necropsy of partnered females, at least until gestation day 14. Treatment with RO0673189-008 at 30 mg/kg/day decreased body weight gain and food consumption. The number of corpora lutea, implantation sites, and fetuses per dam were slightly but statistically lower in the high dose group (30 mg/kg/day) relative to the control group. The sponsor stated that the number of corpora lutea in the high-dose group is within normal limits for this strain of rat. RO0673189-008 did not clearly produce adverse effects on fertility in either sex or early embryonic development.

Methods

RO0673189-008 was given by oral gavage at doses of 3, 10, and 30 mg/kg/day to males starting two weeks prior to mating, continuing throughout the mating period and

up to the day prior to sacrifice. Females were administered the same doses starting at 14 days prior to mating, continuing throughout the mating period and through day 7 of gestation

Doses:	0 (vehicle), 3, 10, and 30 mg/kg/day
Frequency of dosing:	Once a day
Dose volume:	5 ml/kg/day
Route of administration:	Oral gavage
Formulation/Vehicle:	Thixotrope vehicle as aqueous suspensions (for composition of the thixotrope vehicle, see table below)
Species/Strain:	Wistar rats (HanIbm:WIST)
Number/Sex/Group:	25
Satellite groups:	None
Study design:	See Sponsor's table below
Deviation from study protocol:	No deviation occurred which adversely affected the quality of the study.

APPEARS THIS WAY ON ORIGINAL

Group	Dose (mg/kg/day)	Dose volume (ml/kg/day)	No. of males / females at start	Animal numbers M= males / F= corresponding females
1	0 (control)	5	25 / 25	M101-125 / F101-125
2	3	5	25 / 25	M201-225 / F201-225
3	10	5	25 / 25	M301-325 / F301-325
4	30	5	25 / 25	M401-425 / F401-425

The age of the rats was not stated. The doses used in the current study were used previously in other toxicity studies, and the high-dose (30 expected to produce adverse effects such as reduced food consumption and mg/kg/day) was body weights.

Composition of Thixotrope Vehicle

	An. No.: /Lot	**100 L**	**100.00 %**
Polysorbate 80	7067666	0.200 kg	0.20 %
Cellulose Avicel RC 591	9622421	1.600 kg	1.60 %
Nipagin	Charge 25262	0.180 kg	0.18 %
Nipasol	Charge 49902	0.020 kg	0.02 %
Aqua demin. ad 100.0 L		ad 100 L	ad 100 %
Citric acid 0.1 N ad pH 6.0	Lot:49485	0.1060 kg	0.1060 %

Observations and Results

Mortality: None.

Clinical Signs: There were no treatment-related changes.

Body Weight: During premating period of 14 days, the initial (day 1) and final (day 14) body weights in the control groups were 197 and 220 g for females and 285 and 330 g for males, respectively. The body weight gains at day 14 were 23 g in the control females, and 3 g in the high dose females. The body weight gains at day 14 were 45 g in the control males, and 34 g in the high dose males.

Feed Consumption: Decreased food consumption was observed in the high dose groups.

Toxicokinetics: Not conducted.

Dosing Solution Analysis: The analysis of drug concentration in samples of dosing formulations from each dose-group was satisfactory. The analysis results were summarized in the following table (taken from the study report).

Study group	Target concentration	Concentration measured	% measured/target
1	0.0 mg/mL	0.00 mg/mL	-
2	0.6 mg/mL	0.596 mg/mL	99 %
3	2.0 mg/mL	2.00 mg/mL	100 %
4	6.0 mg/mL	5.86 mg/mL	98 %

Necropsy: There were no treatment-related changes.

Fertility and Reproduction Data

There were no treatment-related effects on fertility parameters including mating index, fertility index, mating days, and estrus stage. The results are summarized in the following sponsor's table.

257R02

TABLE 16
RO0673189-008: SEGMENT I: ORAL STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN THE RAT (STUDY NO. 257R02)
MATING PERFORMANCE AND MATING SUCCESS

		CONTROL	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
Females placed with males	N	25	25	25	25
Females with defined day 0 pc	N	25	25	25	25
female mating index	%	100.0	100.0	100.0	100.0
pregnant	N	24	24	25	23
female fertility index	%	96.0	96.0	100.0	92.0
Males placed with females	N	25	25	25	25
mated	N	25	25	25	25
male mating index	%	100.0	100.0	100.0	100.0
with females pregnant	N	24	24	25	23
male fertility index	%	96.0	96.0	100.0	92.0
Mating days until day 0 pc	MEDIAN	2.0	2.0	2.0	3.0
	Q1	1.0	1.0	1.0	2.0
	Q3	3.0	3.0	3.0	4.0
Day 1 to 4	N	24	24	23	23
	%	96.0	96.0	92.0	92.0
Day 5 to 8	N	1	1	2	1
	%	4.0	4.0	8.0	4.0
Day 9 to 14	N	0	0	0	1
	%	0.0	0.0	0.0	4.0

Statistical evaluation with procedure of E. Luedin (1985); no statistically significant results

The effects on reproduction parameters including the number of corpora lutea, implantation sites, fetuses per female, or pre- and post-implantation loss are summarized in the following sponsor's table.

257R02

TABLE 17
RO0673189-008: SEGMENT I: ORAL STUDY OF FERTILITY AND EARLY EMBRYONIC DEVELOPMENT IN THE RAT (STUDY NO. 257R02)
SUMMARY OF REPRODUCTION DATA (C-SECTION)

		CONTROL	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
Pregnant, used for calculation	N	24	24	25	23
Corpora Lutea	N	317	324	318	265
No. per animal	MEDIAN	14.0	14.0	13.0	11.0jj
	Q1	13.0	13.0	11.5	10.0
	Q3	15.8	15.0	14.0	13.0
Preimplantation Loss	N	13	21	12	16
% per group	%	4.1	6.5	3.8	6.0
% per animal	MEDIAN	0.0	0.0	0.0	0.0
	Q1	0.0	0.0	0.0	0.0
	Q3	6.6	14.0	8.6	10.0
Implantation Sites	N	304	303	306	249
No. per animal	MEDIAN	14.0	13.0	13.0	11.0jj
	Q1	11.3	12.0	10.5	9.0
	Q3	15.8	14.8	13.5	12.0
Fetuses	N	282	294	289	236
No. per animal	MEDIAN	13.0	13.0	12.0	10.0jj
	Q1	11.0	11.3	10.0	9.0
	Q3	14.8	14.0	13.0	12.0
Alive	%	100.0	100.0	100.0	100.0

Statistical key: Jonckheere test: j<0.05, jj<0.01

The numbers of corpora lutea, implantation sites, and fetuses per dam were slightly but statistically lower in the high dose group (30 mg/kg/day) than those in the control group. The sponsor stated that the number of corpora lutea in the high-dose group is within

normal limits for this strain of rat. The Sponsor provided the following explanation for the presentation of median values in the table above: “For statistical analysis the median was used instead of the mean, because it is more appropriate for non-normally distributed data. For the sake of consistency the median was also used for normally distributed data.”

There were no treatment-related effects on sperm motility, weight of the testis, and sperm counts.

9.2 Embryonic Fetal Development

Study title: Segment II: Oral Study for Effects on Embryo-Fetal Development in the Rat

Study no.:	148R02
Study report location:	n/a
Conducting laboratory and location:	Non-Clinical Drug Safety F. Hoffmann La-Roche Ltd., Basel, Switzerland
Date of study initiation:	May 15, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RO0673189-008, lot#: BS0204SA01, 99.8%

Key Study Findings:

Treatment with RO0673189-008 at 30 mg/kg/day decreased body weight gain and food consumption. RO0673189-008 did not adversely affect embryo-fetal development. RO0673189-008 was not teratogenic in this study. The maternal NOAEL was 10 mg/kg/day, based on the decrease (31%) in body weight gain in the 30 mg/kg/day females. The NOEL for embryo-fetal development was 30 mg/kg/day.

Methods

RO0673189-008 was given to pregnant rats by oral gavage at 0 (vehicle), 3, 10 and 30 mg/kg/day during gestation days 6-17. The dose selection was based on the results of a dose ranging study in rats (study # 041R02). In this study, RO0673189-008 was given by oral gavage to pregnant rats during gestation days 6-17 at oral doses of 0, 10, 30 and 100 mg/kg/day. The high dose resulted in clinical signs of toxicity and body weight loss. Reduction of body weight gain was also noted in the mid-dose group (30 mg/kg/day). Therefore, the dose of 30 mg/kg/day was selected as the high dose for the Segment II study.

Doses:	0 (vehicle), 3, 10, and 30 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Thixotrope vehicle as aqueous suspensions
Species/Strain:	Wistar rats (HanIbm:WIST)
# Females/Group:	24
Satellite groups:	2 females/group
Study design:	See sponsor's table below
Deviation from study protocol:	No deviation occurred which adversely affected the quality of the study.

Group	Dose (mg/kg/day)	Dose volume (ml/kg/day)	No. of inseminated females		Animal numbers	
			repro	kinetics	repro	kinetics*
1	0 (control)	5	24	0	101-124	-
2	3	5	24	2	201-224	225, 226
3	10	5	24	2	301-324	325, 326
4	30	5	24	2	401-424	425, 426

* data of kinetic (satellite) animals are excluded from summary tables for body weight, food consumption and reproductive data.

The age of the females was not stated. The females were sacrificed on day 21 of gestation and assessment of embryo-fetal development was conducted.

Observations and Results

Mortality: None.

Clinical Signs: There were no treatment-related clinical signs of toxicity.

Body Weight: Body weights on gestation days 6 and 18 were 228 g and 296 g, respectively, in in the control females. The body weight gains from days 6 to 18 were 68 g in the control females and 47 g in the high dose females. Slightly lower body weight gain was also noted in the mid dose group, but the weight gain in the low-dose group was similar to the control group.

Feed Consumption: Decreased food consumption was observed in the middle and high dose groups.

Toxicokinetics: TK results are summarized in the following sponsor's table.

Table 2 Toxicokinetic parameters following oral administration of 3, 10 and 30 mg/kg/day RO0673189-008 to pregnant rats

Dose mg/kg/day	Gestation Day	Tmax h	Cmax ng/mL	C (24h) ng/mL	AUC (0-24h) ng.h/mL	AUC(0-24h)/ Dose ng.h/mL	Accum. Factor*
3	6	8	331	89.7	5000	1670	
	15	8	956	315	15600	5200	3.1
10	6	5	824	494	13100	1310	
	15	5	1370	1020	27400	2740	2.1
30	6	3	1590	950	29800	993	
	15	8	2490	1770	52600	1750	1.8

*calculated with AUC(0-24h) ratios

Dosing Solution Analysis: The analysis of drug concentration in samples of dosing formulations from each dose-group was satisfactory. The analysis results are summarized in the following table (taken from the study report).

Study group	Nominal content	Measured content	% measured/nominal
1	**0.0 mg/mL**	**0.00 mg/mL**	-
2	**0.6 mg/mL**	**0.58 mg/mL**	**97 %**
3	**2.0 mg/mL**	**1.96 mg/mL**	**98 %**
4	**6.0 mg/mL**	**5.75 mg/mL**	**96 %**

Necropsy: There were no treatment-related changes.

Cesarean Section Data

There were no treatment-related effects on the number of implantations or fetuses. The results are summarized in the sponsor's table below.

148R02

TABLE 7
RO0673189-008: SEGMENT II: ORAL STUDY FOR EFFECTS ON EMBRYO-
FETAL DEVELOPMENT IN THE RAT (STUDY NO. 148R02)
SUMMARY OF REPRODUCTION DATA (C-SECTION)

		CONTROL	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
Pregnant, used for calculation	N	22	21	20	23
Corpora Lutea	N	297	275	239	284
No. per animal	MEDIAN	14.0	13.0	12.5	13.0 j
	Q1	13.0	12.0	12.0	11.0
	Q3	15.0	14.0	14.0	13.0
Preimplantation Loss	N	21	29	30	33
% per group	%	7.1	10.5	12.6	11.6
% per animal	MEDIAN	6.7	7.1	8.0	7.7
	Q1	0.0	0.0	0.0	0.0
	Q3	10.2	13.0	22.9	15.4
Implantation Sites	N	276	246	209	251
No. per animal	MEDIAN	13.0	12.0	12.0	12.0 j
	Q1	11.8	11.0	9.3	10.0
	Q3	14.0	13.5	13.8	13.0
Fetuses	N	262	228	198	235
No. per animal	MEDIAN	13.0	11.0	11.5	11.0
	Q1	10.8	9.0	7.8	9.0
	Q3	13.0	12.5	13.0	13.0
Alive	%	100.0	100.0	100.0	100.0

Statistical key: Jonckheere test: j<0.05, jj<0.01

There were no treatment-related effects on fetal body weight or the fetal sex ratio. The results are summarized in the sponsor's table below. The Sponsor provided the following explanation for the presentation of median values in the table above: "For statistical analysis the median was used instead of the mean, because it is more appropriate for non-normally distributed data. For the sake of consistency the median was also used for normally distributed data."

148R02

TABLE 7
RO0673189-008: SEGMENT II: ORAL STUDY FOR EFFECTS ON EMBRYO-
FETAL DEVELOPMENT IN THE RAT (STUDY NO. 148R02)
SUMMARY OF REPRODUCTION DATA (C-SECTION)

		CONTROL	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
Pregnant, used for calculation	N	22	21	20	23
Resorbed Implants: Total	N	14	18	11	16
No. per animal	MEDIAN	0.5	0.0	0.0	0.0
	Q1	0.0	0.0	0.0	0.0
	Q3	1.0	1.0	1.0	1.0
% of impl. per group	%	5.1	7.3	5.3	6.4
% of impl. per animal	MEDIAN	3.1	0.0	0.0	0.0
	Q1	0.0	0.0	0.0	0.0
	Q3	8.5	8.3	8.3	10.0
Resorbed Implants: Early	N	12	18	10	16
% of resorp. per group	%	85.7	100.0	90.9	100.0
Resorbed Implants: Late	N	2	0	1	0
% of resorp. per group	%	14.3	0.0	9.1	0.0
Viable Male Fetuses	N	140	111	102	117
	%	53.4	48.7	51.5	49.8
Female Fetuses	N	122	117	96	118
	%	46.6	51.3	48.5	50.2
Fetal Body Weight (g)	MEDIAN	5.0	4.9	5.0	5.0
	Q1	4.8	4.8	4.8	4.9
	Q3	5.1	5.1	5.1	5.1
	N LITTERS	22	21	19	23

Statistical evaluation with procedure of E. Luedin (1985); no statistically significant results

Offspring (Malformations, Variations, etc.)

The treatment did not affect the total incidence of abnormalities, variations and retardations. The results are summarized in the sponsor's table below.

148R02

TABLE 8
RO0673189-008: SEGMENT II: ORAL STUDY FOR EFFECTS ON EMBRYO-FETAL DEVELOPMENT IN THE RAT (STUDY NO. 148R02)
SUMMARY OF EXTERNAL, VISCERAL AND SKELETAL ABNORMALITIES, VARIATIONS AND RETARDATIONS

		CONTROL	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
Litters Evaluated	N	22	21	19	23
Fetuses Evaluated	N	262	228	198	235
Live	N	262	228	198	235
Dead	N	0	0	0	0
TOTAL ABNORMALITIES					
Fetal Incidence	N	4	4	1	2
	%	1.5	1.8	0.5	0.9
Litter Incidence	N	4	4	1	2
	%	18.2	19.0	5.3	8.7
Affected Fetuses/Litter	MEAN%	2.6	2.0	0.4	0.9
	S.D.	7.39	4.89	1.91	2.93
TOTAL VARIATIONS					
Fetal Incidence	N	69	57	62	62
	%	26.3	25.0	31.3	26.4
Litter Incidence	N	20	19	19	21
	%	90.9	90.5	100.0	91.3
Affected Fetuses/Litter	MEAN%	26.3	25.2	35.7	24.5
	S.D.	12.90	17.41	20.77	15.26
TOTAL RETARDATIONS					
Fetal Incidence	N	80	82	55	73
	%	30.5	36.0	27.8	31.1
Litter Incidence	N	22	21	16	21
	%	100.0	100.0	84.2	91.3
Affected Fetuses/Litter	MEAN%	29.9	36.5	29.7	28.8
	S.D.	14.12	14.79	24.14	13.60

The incidence of fetuses with limb variation (tarsal hyperextension) was 1, 2, and 3 in the control, middle, (2 liters), and high-dose (3 liters) groups, respectively. This change is not considered to be drug-related, given the absence of statistical significance and incidence in the control group. Two fetuses with limb variations (pes adductus) were observed in one litter in the middle dose group. The results are summarized in the sponsor's table below. These variations were not seen at a higher dose of 100 mg/kg/day in the dose ranging study in rats (study # 041R02).

148R02

TABLE 9
RO0673189-008: SEGMENT II: ORAL STUDY FOR EFFECTS ON EMBRYO-FETAL DEVELOPMENT IN THE RAT (STUDY NO. 148R02)
SUMMARY OF FETAL EXTERNAL ABNORMALITIES, VARIATIONS AND RETARDATIONS

		CONTROL	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
Litters Evaluated	N	22	21	19	23
Fetuses Evaluated	N	262	228	198	235
Live	N	262	228	198	235
Dead	N	0	0	0	0
TOTAL ABNORMALITIES					
Fetal Incidence	N	1	1	0	0
	%	0.4	0.4	0.0	0.0
Litter Incidence	N	1	1	0	0
	%	4.5	4.8	0.0	0.0
Affected Fetuses/Litter	MEAN%	1.5	1.0	0.0	0.0
	S.D.	7.11	4.36	0.00	0.00
TOTAL VARIATIONS					
Fetal Incidence	N	1	0	4	3
	%	0.4	0.0	2.0	1.3
Litter Incidence	N	1	0	3	3
	%	4.5	0.0	15.8	13.0
Affected Fetuses/Litter	MEAN%	0.3	0.0	1.5	1.1
	S.D.	1.64	0.00	3.75	2.96

148R02

TABLE 11
RO0673189-008: SEGMENT II: ORAL STUDY FOR EFFECTS ON EMBRYO-FETAL DEVELOPMENT IN THE RAT (STUDY NO. 148R02)
SUMMARY OF FETAL VISCERAL ABNORMALITIES, VARIATIONS AND RETARDATIONS

		CONTROL	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
Litters Evaluated	N	22	21	19	23
Fetuses Evaluated	N	139	118	105	123
Live	N	139	118	105	123
Dead	N	0	0	0	0
TOTAL ABNORMALITIES					
Fetal Incidence	N	0	1	0	1
	%	0.0	0.8	0.0	0.8
Litter Incidence	N	0	1	0	1
	%	0.0	4.8	0.0	4.3
Affected Fetuses/Litter	MEAN%	0.0	0.7	0.0	0.7
	S.D.	0.00	3.12	0.00	3.48
TOTAL VARIATIONS					
Fetal Incidence	N	7	9	7	10
	%	5.0	7.6	6.7	8.1
Litter Incidence	N	5	7	7	6
	%	22.7	33.3	36.8	26.1
Affected Fetuses/Litter	MEAN%	6.3	7.4	10.3	7.3
	S.D.	13.52	12.81	23.02	14.34
TOTAL RETARDATIONS					
Fetal Incidence	N	8	5	5	9
	%	5.8	4.2	4.8	7.3
Litter Incidence	N	6	4	5	7
	%	27.3	19.0	26.3	30.4
Affected Fetuses/Litter	MEAN%	7.0	4.6	8.6	6.5
	S.D.	13.54	11.78	23.11	10.77

148R02

TABLE 13
RO0673189-008: SEGMENT II: ORAL STUDY FOR EFFECTS ON EMBRYO-FETAL DEVELOPMENT IN THE RAT (STUDY NO. 148R02)
SUMMARY OF FETAL SKELETAL ABNORMALITIES, VARIATIONS AND RETARDATIONS

		CONTROL	3 MG/KG/DAY	10 MG/KG/DAY	30 MG/KG/DAY
Litters Evaluated	N	22	21	18	21
Fetuses Evaluated	N	123	110	93	112
Live	N	123	110	93	112
Dead	N	0	0	0	0
TOTAL ABNORMALITIES					
Fetal Incidence	N	4	2	1	1
	%	3.3	1.8	1.1	0.9
Litter Incidence	N	4	2	1	1
	%	18.2	9.5	5.6	4.8
Affected Fetuses/Litter	MEAN%	6.7	1.5	0.9	1.2
	S.D.	21.57	4.67	3.93	5.46
TOTAL VARIATIONS					
Fetal Incidence	N	61	48	52	49
	%	49.6	43.6	55.9	43.8
Litter Incidence	N	19	17	18	20
	%	86.4	81.0	100.0	95.2
Affected Fetuses/Litter	MEAN%	47.4	44.5	61.1	46.7
	S.D.	27.27	32.76	31.46	25.44
TOTAL RETARDATIONS					
Fetal Incidence	N	72	77	50	64
	%	58.5	70.0	53.8	57.1
Litter Incidence	N	21	21	15	21
	%	95.5	100.0	83.3	100.0
Affected Fetuses/Litter	MEAN%	55.0	71.0	49.8	59.7
	S.D.	26.16	24.78	33.42	23.62

Study title: Segment II: Oral Study for Effects on Embryo-Fetal Development in the Rabbit

Study no.:	843489
Study report location:	n/a
Conducting laboratory and location:	Non-Clinical Drug Safety F. Hoffmann La-Roche Ltd., Basel, Switzerland
Date of study initiation:	May 15, 2002
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	RO0673189-008, lot #: GPM0449, 99.8%

Key Study Findings:

Pregnant rabbits were treated with the test article by oral gavage at 0 (vehicle), 3, 10 and 30 mg/kg/day during gestation days 6-18. A loss of 70 g in bodyweight occurred in the high dose females during gestation days 6 to 19, as compared to a body weight gain of 88 g in the control females. Three females had abortions (1 in middle dose group

and 2 in the high dose group). One female at 30 mg/kg had total litter death. Fetal body weights at 30 mg/kg were decreased by 8% in males, as compared to the control males.

Limb and paw anomalies (position anomalies) were observed with higher incidence in the middle and high dose groups. The combined incidence of all findings was 1/135, 1/126, 3/114, and 9/107 among fetuses, and 1/22, 1/22, 3/20, and 6/17 among litters, in the control, low, middle, and high-dose groups, respectively. An increased number of minimally/partially fused sternebrae was noted at 10 (9/114 fetuses, 8/20 liters) and 30 mg/kg/day (16/107 fetuses, 10/17 litters), as compared to the control group (none). Based on the increased incidence of malformations (limb and paw anomalies, minimally/partially fused sternebrae) at 10 and 30 mg/kg/day, the NOAEL for embryo-fetal development is considered to be 3 mg/kg/day. The NOAEL for maternal toxicity was 10 mg/kg/day, based on the loss of bodyweight in the 30 mg/kg/day group during gestation days 6-19.

A consultation was sent to the Reproductive and Developmental Toxicology Subcommittee (RDTS) on March 18, 2014. The committee concluded that the limb and paw anomalies, and minimally/partially fused sternebrae were treatment-related and evidence of developmental toxicity.

Methods: RO0673189-008 was given to rabbits by oral gavage during gestation days 6-18 at 0 (vehicle), 3, 10 and 30 mg/kg/day. Dose selection was based on the dose ranging study in rabbits (#1006571), in which, doses of 0, 10, 30, and 100 mg/kg/day were tested. The dose of 100 mg/kg/day was lethal. The dose of 30 mg/kg/day was tested as high dose in other toxicity studies.

Doses:	0 (vehicle), 3, 10, and 30 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	2 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Thixotrope vehicle as aqueous suspensions
Species/Strain:	Himalayan Rabbits
# Females/Group:	20
Satellite groups:	2 females/group
Study design:	See sponsor's table below
Deviation from study protocol:	No deviation occurred which adversely affected the quality of the study.

Group	Female Numbers	Dose* mg/kg body weight
1	1 - 20**	0 (vehicle control)
	21 - 22***	
2	23 - 42**	3
	43 - 44***	
3	45 - 64**	10
	65 - 66***	
4	67 - 86**	30
	87 - 88***	

* = Dose levels were based on information from a pilot study in the same strain of rabbits performed by the sponsor.

** = Animals for evaluation of effects on embryo-fetal development

*** = Animals for toxicokinetic analysis; the animals were also evaluated for effects on embryo-fetal development

These rabbits were 18-31 weeks old at the time of pairing and were sacrificed on day 28 post coitum.

Observations and Results

Mortality: None.

Clinical Signs: There were no treatment-related clinical signs of toxicity.

Body Weight: Body weights on day 6 and day 19 were 2654 g and 2742 g, respectively, in the control females. The body weight gain from days 6 to 19 was 88 g in the control females, whereas the high dose females lost 70 g (see sponsor's table below).

DIFFERENCES IN MEAN BODY WEIGHT GAIN OF DAMS POST COITUM (G)

Group	Days post coitum									
	0 - 6		6 - 11		11 - 15		15 - 19		6 - 19*	
(mg/kg)	g	(%)	g	(%)	g	(%)	g	(%)	g	(%)
1 (0)	54	(+2.1)	17	(+0.6)	42	(+1.6)	29	(+1.1)	88	(+3.3)
2 (3)	37	(+1.4)	18	(+0.7)	35	(+1.3)	21	(+0.8)	74	(+2.8)
3 (10)	39	(+1.5)	7	(+0.3)	46	(+1.8)	28	(+1.0)	81	(+3.1)
4 (30)	41	(+1.6)	-36	(-1.4)	-31	(-1.2)	-3	(-0.1)	-70	(-2.7)

* = The calculations of body weight gain during the treatment period started on day 6 post coitum (immediately prior to the first administration) and ended on day 19 post coitum (approximately 24 hours after the last administration). Percentages of change refer to the initial body weight of the respective time interval.

Feed Consumption: Decreased food consumption was observed in the middle and high dose groups.

Toxicokinetics: TK results are summarized in the following sponsor's table.

Table 2 Toxicokinetic parameters of RO0673189 following oral administration of 3, 10 and 30 mg/kg/day RO0673189-008 to pregnant rabbits

Dose mg/kg/day	Day	Subject	Tmax h	Cmax ng/mL	C(24h) ng/mL	AUC(0-24h) ng.h/mL
3	6	43	7	42.9	22.0	712
		44	2	67.6	18.1	936
		Mean	**4.5**	**55.3**	**20.1**	**824**
	17	43	2	70.7	20.1	892
		44	5	43.7	14.6	740
		Mean	**3.5**	**57.2**	**17.4**	**816**
10	6	65	2	210	50.6	2910
		66	5	247	60.2	3320
		Mean	**3.5**	**229**	**55.4**	**3120**
	17	65	2	193	63.4	2740
		66	5	124	53.2	2260
		Mean	**3.5**	**159**	**58.3**	**2500**
30	6	87	5	566	112	7450
		88	5	530	131	7120
		Mean	**5**	**548**	**122**	**7290**
	17	87	5	329	126	5520
		88	7	290	162	5390
		Mean	**6**	**310**	**144**	**5460**

Dosing Solution Analysis: The drug concentrations were within the desired range (80-120%). The analysis results are summarized in the following table (taken from the study report).

Sample Idendification	Declared concentration	Content RO0673189-008	% of declared value
Group 1	0 mg / ml	**0.00 mg / g** (0.00; 0.00)	--
Group 2	1.5 mg / ml	**1.47 mg / g** (1.48; 1.46)	98
Group 3	5.0 mg / ml	**4.92 mg / g** (4.89; 4.94)	98
Group 4	15.0 mg / ml	**14.77 mg / g** (14.75; 14.75; 14.81 14.72; 14.87; 14.73)	98

Samples stored in the refrigerator (2-8°C) until the analysis was performed on May 21, 2002

Necropsy: There were no clearly treatment related changes.

Cesarean Section Data

Three females had abortions (1 in the middle dose group and 2 in the high dose group).

Fetal body weights in the high dose group were decreased by 8% in males, as compared to the control males (see sponsor's table below).

REPRODUCTION DATA SUMMARY

	GROUP 1 0 MG/KG	GROUP 2 3 MG/KG	GROUP 3 10 MG/KG	GROUP 4 30 MG/KG
NUMBER OF DAMS	22	22	20	17
CORPORA LUTEA	157	152	144	132
MEAN (+)	7.1	6.9	7.2	7.8
ST.DEV.	1.6	1.9	1.0	1.2
PRE-IMPLANTATION LOSS	13	17	22	17
% OF CORP. LUTEA (#)	8.3	11.2	15.3 #	12.9
MEAN (+)	0.6	0.8	1.1	1.0
ST.DEV.	0.6	0.9	1.0	0.7
NUMBER OF DAMS AFFECTED	12	11	14	13
IMPLANTATION SITES	144	135	122	115
% OF CORP. LUTEA (#)	91.7	88.8	84.7 #	87.1
MEAN (+)	6.5	6.1	6.1	6.8
ST.DEV.	1.7	1.8	1.2	1.6
POST-IMPLANTATION LOSS	9	9	8	8
% OF IMPL. SITES (#)	6.3	6.7	6.6	7.0
MEAN (+)	0.4	0.4	0.4	0.5
ST.DEV.	0.8	0.7	0.7	0.6
NUMBER OF DAMS AFFECTED	6	7	6	7
IMPLANTATION SITE SCARS	0	0	0	0
EMBRYONIC/FETAL DEATHS TOTAL	9	9	8	8
EMBRYONIC RESORPTIONS	9	6	6	8
% OF IMPL. SITES (#)	6.3	4.4	4.9	7.0
MEAN (+)	0.4	0.3	0.3	0.5
ST.DEV.	0.8	0.6	0.6	0.6
NUMBER OF DAMS AFFECTED	6	4	5	7
FETAL RESORPTIONS	0	3	2	0
% OF IMPL. SITES (#)		2.2	1.6	
MEAN (+)		0.1	0.1	
ST.DEV.		0.4	0.3	
NUMBER OF DAMS AFFECTED		3	2	
FETUSES				
TOTAL FETUSES	135	126	114	107
% OF IMPL. SITES (#)	93.8	93.3	93.4	93.0
MEAN (+)	6.1	5.7	5.7	6.3
ST.DEV.	1.9	1.7	1.5	1.8
LIVE FETUSES	135	126	114	107
DEAD FETUSES	0	0	0	0
ABNORMAL FETUSES	1	1	3	9
% OF FETUSES (#)	0.7	0.8	2.6	8.4 ##
MEAN (+)	0.0	0.0	0.2	0.5
ST.DEV.	0.2	0.2	0.4	0.8
NUMBER OF DAMS AFFECTED	1	1	3	6
ABNORMAL LIVE FETUSES AT EXTERNAL EXAMINATION	1	1	3	9
ABNORMAL DEAD FETUSES AT EXTERNAL EXAMINATION	0	0	0	0

REPRODUCTION DATA SUMMARY

	GROUP 1 0 MG/KG	GROUP 2 3 MG/KG	GROUP 3 10 MG/KG	GROUP 4 30 MG/KG
NUMBER OF DAMS	22	22	20	17
SEX OF FETUSES				
TOTAL MALES	61	45	45	40
% OF FETUSES (#)	45.2	35.7	39.5	37.4
MEAN (+)	2.8	2.0	2.3	2.4
ST.DEV.	1.2	1.4	0.9	1.2
TOTAL FEMALES	74	81	69	67
% OF FETUSES (#)	54.8	64.3	60.5	62.6
MEAN (+)	3.4	3.7	3.5	3.9
ST.DEV.	1.6	1.5	0.9	1.3
LIVE MALES	61	45	45	40
LIVE FEMALES	74	81	69	67
WEIGHTS OF LIVE FETUSES (LITTER BASIS)				
TOTAL FETUSES				
N (LITTERS)	22	22	20	17
MEAN (*)	32.5	33.3	32.9	30.8
ST.DEV.	2.9	2.9	2.9	3.6
MALES				
N (LITTERS)	21	18	20	16
MEAN (*)	32.6	32.2	33.2	30.3
ST.DEV.	3.0	3.0	3.1	3.2
FEMALES				
N (LITTERS)	21	22	20	17
MEAN (*)	32.6	33.5	32.9	30.8
ST.DEV.	3.3	2.9	3.2	3.8
WEIGHTS OF LIVE FETUSES (INDIVIDUAL BASIS)				
TOTAL FETUSES				
N (FETUSES)	135	126	114	107
MEAN (*)	32.1	32.9	32.7	30.2 **
ST.DEV.	3.2	3.3	3.7	4.2
MALES				
N (FETUSES)	61	45	45	40
MEAN (*)	32.6	32.3	32.7	30.1 **
ST.DEV.	3.3	3.3	3.5	4.4
FEMALES				
N (FETUSES)	74	81	69	67
MEAN (*)	31.7	33.2 *	32.7	30.3
ST.DEV.	3.1	3.3	3.9	4.2

*/** : Dunnett-Test based on pooled variance significant at level 5% (*) or 1% (**)
#/## : Fisher's Exact Test significant at level 5% (#) or 1% (##)
+ : Steel Test significant at level 5%

Offspring (Malformations, Variations, etc.)

Limb and paw position anomalies were observed with higher incidence in the middle and high dose groups. The combined incidence of all findings was 1/135, 1/126, 3/114, and 9/107 among fetuses, and 1/22, 1/22, 3/20, and 6/17 among litters, in the control, low, middle, and high-dose groups, respectively. The results are summarized in the sponsor's table below.

ABNORMAL FINDINGS FROM EXTERNAL AND FRESH VISCERAL EXAMINATION OF FETUSES - SUMMARY

	Group 1 0 mg/kg		Group 2 3 mg/kg		Group 3 10 mg/kg		Group 4 30 mg/kg	
Number of litters	22		22		20		17	
Number of fetuses	135		126		114		107	
Incidences of fetuses with	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Forelimbs								
- paw position anomaly, bilateral	1	0.7					2	1.9
- paw position anomaly, right					1	0.9	5	4.7
- paw position anomaly, left					1	0.9	2	1.9
- paw short, bilateral			1	0.8				
Forelegs and hindlegs								
- position anomaly, bilateral			1	0.8	1	0.9		
Skull, sutural bone between frontal bones	1	0.7	1	0.8	2	1.8		
Thymus								
- right side elongated	22	16	11	8.7	15	13	6	5.6
- left side elongated	4	3.0	3	2.4	4	3.5	2	1.9
- bilaterally elongated	3	2.2	1	0.8				
- fragmented	1	0.7						
- mottled	12	8.9	3	2.4	1	0.9	3	2.8
Trachea, disorganized cartilage	1	0.7						

ABNORMAL FINDINGS FROM EXTERNAL AND FRESH VISCERAL EXAMINATION OF FETUSES - SUMMARY (CONT'D)

	Group 1 0 mg/kg		Group 2 3 mg/kg		Group 3 10 mg/kg		Group 4 30 mg/kg	
Number of litters	22		22		20		17	
Number of fetuses	135		126		114		107	
Incidences of fetuses with	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Diaphragm, locally thinned			1	0.8				
Lung								
- accessory lobe absent	14	10	12	9.5	28	25	31	29
Heart and blood vessels								
- ductus arteriosus absent; right chamber absent, pulmonary artery thinned					1	0.9		
- right subclavian artery branched							1	0.9
- additional artery arising from innominate artery	1	0.7	1	0.8	3	2.6		
- additional artery below left subclavian arising from aortic arch	1	0.7	1	0.8				
- left subclavian artery, doubled							1	0.9
- additional artery arising from right common carotid artery			2	1.6				
- left cranial vena cava, abnormal pathway					1	0.9		
- left renal vein, branched	1	0.7						
- additional left venal vein	3	2.2						
Stomach								
- distended, gaseous content	25	19	6	4.8	1	0.9		
Kidney absent, right							1	0.9
Kidney and ureter absent, left					1	0.9		

ABNORMAL FINDINGS FROM EXTERNAL AND FRESH VISCERAL EXAMINATION OF FETUSES - SUMMARY (CONT'D)

	Group 1 0 mg/kg		Group 2 3 mg/kg		Group 3 10 mg/kg		Group 4 30 mg/kg	
Number of litters	22		22		20		17	
Number of fetuses	135		126		114		107	
Incidences of fetuses with	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Right testis, enlarged; besides cysts with clear liquid							1	0.9
Liver								
- small white lesion at right lateral lobe	7	5.2	2	1.6				
Gall bladder								
- clear contents	34	25	19	15	9	7.9	12	11
- dark content	1	0.7						
- small	2	1.5	2	1.6	3	2.6	1	0.9
- absent	1	0.7						

ABNORMAL FINDINGS FROM FETAL HEAD SECTIONS - SUMMARY

	Group 1 0 mg/kg		Group 2 3 mg/kg		Group 3 10 mg/kg		Group 4 30 mg/kg	
Number of litters	22		22		20		17	
Number of fetuses	135		126		114		107	
Incidences of fetuses with	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Lateral ventricles, Dilation	2	1.5	3	2.4	7	6.1	3	2.8
Third ventricle, Dilation	6	4.4	9	7.1	9	7.9	2	1.9
Clotted blood between skull and cerebral hemispheres	1	0.7	2	1.6			2	1.9
Microglossia					1	0.9		
Brain thalamic region, cystic dilation							1	0.9

An increased number of minimally/partially fused sternebrae was noted at 10 and 30 mg/kg and was considered treatment-related (9/114 fetuses in the middle-dose group and 16/107 fetuses in the high-dose group, as compared to none in the control group). The results are summarized in the following sponsor's table.

ABNORMAL FINDINGS FROM SKELETAL EXAMINATION OF FETUSES - SUMMARY

	Group 1 0 mg/kg		Group 2 3 mg/kg		Group 3 10 mg/kg		Group 4 30 mg/kg	
Number of litters	22		22		20		17	
Number of fetuses	135		126		114		107	
Incidences of fetuses with	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Fore- and/or hindpaw, position anomaly	1	0.7	1	0.8	3	2.6	8	7.5
Rib, absent	1	0.7	1	0.8				
Rib, bifurcated					1	0.9	1	0.9
Cervical or thoracic vertebral body, absent	1	0.7	1	0.8				
Cervical of thoracic vertebral body, fused	1	0.7						
Sternebrae, minimally or partially fused					9	7.9	16	15.0
Sternebrae, misshapen					1	0.9		
Pelvic girdle, unilateral or bilateral displaced	3	2.2	3	2.4	2	1.8		

The incidence of unilateral or bilateral supernumerary 13th ribs (half to full length of 12th rib) and/or rudimentary 13th ribs (less than half length of 12th rib) was higher in the middle and high dose groups. The incidence of supernumerary 13th ribs was 4/114 fetuses and 4/20 litters in the middle-dose group, and 7/107 fetuses and 5/17 litters in the high-dose group, as compared to 2/135 fetuses and 2/22 litters in the control group. The incidence of rudimentary 13th ribs was 3/114 fetuses and 2/20 litters in the middle-dose group, and 5/107 fetuses and 3/17 litters in the high-dose group, as compared to 1/135 fetuses and 1/22 litters in the control group.

SKELETAL EXAMINATIONS SUMMARY
STAGE OF DEVELOPMENT AND VARIANTS
ON A LITTER BASIS

	GROUP 1 0 MG/KG	GROUP 2 3 MG/KG	GROUP 3 10 MG/KG	GROUP 4 30 MG/KG
NUMBER OF LITTERS EXAMINED	22	22	20	17
ABNORMAL FINDING(S) (SHOWN ON PREVIOUS PAGE(S))	3 14%	5 23%	11 55% ##	11 65% ##
CERVICAL VERTEBRAE				
INCOMPLETELY OSSIFIED				
CERVICAL VERTEBRAL BODY 1	1 5%	1 5%	0	0
CERVICAL VERTEBRAL BODY 5	0	0	0	1 6%
ADDITIONAL OSSIFICATION				
CERVICAL VERTEBRAL ARCH 7, LEFT	0	0	1 5%	0
CERVICAL VERTEBRAL ARCH 7, RIGHT	0	0	1 5%	0
STERNUM				
INCOMPLETELY OSSIFIED				
STERNEBRA 1	0	1 5%	2 10%	0
STERNEBRA 2	1 5%	0	1 5%	2 12%
STERNEBRA 4	1 5%	1 5%	2 10%	0
STERNEBRA 5	22 100%	21 95%	20 100%	17 100%
STERNEBRA 6	0	1 5%	1 5%	0
NON-OSSIFIED				
STERNEBRA 5	8 36%	11 50%	10 50%	6 35%
ABNORMALLY OSSIFIED				
STERNEBRA 2	1 5%	0	0	0
STERNEBRA 3	1 5%	0	0	0
STERNEBRA 4	0	1 5%	0	1 6%
RIBS				
SHORTENED				
RIB 12, LEFT	1 5%	0	1 5%	0
RIB 12, RIGHT	0	0	1 5%	0
RIB(S), LEFT				
SUPERNUMERARY, ONE				
RIB(S), LEFT	2 9%	2 9%	2 10%	3 18%
SUPERNUMERARY, ONE RUDIMENTARY				
RIB(S), LEFT	0	1 5%	2 10%	3 18%
RIB(S), RIGHT				
SUPERNUMERARY, ONE				
RIB(S), RIGHT	0	1 5%	3 15%	4 24% #
SUPERNUMERARY, ONE RUDIMENTARY				
RIB(S), RIGHT	2 9%	0	2 10%	5 29%
LEFT FORELIMB				
INCOMPLETELY OSSIFIED				
METACARPALIA 1, LEFT	19 86%	21 95%	19 95%	16 94%
DIGIT 1 PROXIMAL PHALANX, LEFT	5 23%	5 23%	3 15%	7 41%
DIGIT 2 MEDIAL PHALANX, LEFT	1 5%	0	2 10%	0
DIGIT 3 MEDIAL PHALANX, LEFT	2 9%	3 14%	3 15%	1 6%
DIGIT 4 MEDIAL PHALANX, LEFT	16 73%	13 59%	10 50%	14 82%
METACARPALIA 5, LEFT	0	0	1 5%	0
DIGIT 5 PROXIMAL PHALANX, LEFT	0	0	1 5%	0
DIGIT 5 MEDIAL PHALANX, LEFT	22 100%	22 100%	20 100%	17 100%
NON-OSSIFIED				
DIGIT 4 MEDIAL PHALANX, LEFT	0	0	1 5%	0

#/## : Fisher's Exact Test significant at level 5% (#) or 1% (##)

SKELETAL EXAMINATIONS SUMMARY
STAGE OF DEVELOPMENT AND VARIANTS
ON A LITTER BASIS

	GROUP 1 0 MG/KG	GROUP 2 3 MG/KG	GROUP 3 10 MG/KG	GROUP 4 30 MG/KG
NUMBER OF LITTERS EXAMINED	22	22	20	17
LEFT FORELIMB				
NON-OSSIFIED				
DIGIT 5 MEDIAL PHALANX, LEFT	1 5%	0	2 10%	1 6%
ADDITIONAL OSSIFICATION				
HUMERUS LEFT	15 68%	17 77%	18 90%	15 88%
RIGHT FORELIMB				
INCOMPLETELY OSSIFIED				
METACARPALIA 1, RIGHT	19 86%	20 91%	20 100%	16 94%
DIGIT 1 PROXIMAL PHALANX, RIGHT	5 23%	3 14%	3 15%	8 47%
DIGIT 2 MEDIAL PHALANX, RIGHT	1 5%	0	3 15%	1 6%
DIGIT 3 MEDIAL PHALANX, RIGHT	5 23%	6 27%	6 30%	9 53%
DIGIT 4 MEDIAL PHALANX, RIGHT	20 91%	19 86%	19 95%	16 94%
METACARPALIA 5, RIGHT	0	0	1 5%	0
DIGIT 5 PROXIMAL PHALANX, RIGHT	0	0	1 5%	0
DIGIT 5 MEDIAL PHALANX, RIGHT	22 100%	22 100%	20 100%	17 100%
NON-OSSIFIED				
DIGIT 4 MEDIAL PHALANX, RIGHT	0	0	1 5%	0
DIGIT 5 MEDIAL PHALANX, RIGHT	1 5%	0	2 10%	2 12%
ADDITIONAL OSSIFICATION				
HUMERUS RIGHT	16 73%	17 77%	18 90%	15 88%
LEFT HIND LIMB				
INCOMPLETELY OSSIFIED				
TALUS LEFT	0	0	1 5%	0
TOE 3 MEDIAL PHALANX, LEFT	1 5%	1 5%	0	0
TOE 4 MEDIAL PHALANX, LEFT	22 100%	22 100%	20 100%	17 100%
NON-OSSIFIED				
TOE 4 MEDIAL PHALANX, LEFT	0	0	1 5%	0
ADDITIONAL OSSIFICATION				
FEMUR LEFT	16 73%	18 82%	17 85%	11 65%
TIBIA LEFT	3 14%	1 5%	4 20%	1 6%
RIGHT HIND LIMB				
INCOMPLETELY OSSIFIED				
TALUS RIGHT	0	0	1 5%	0
TOE 3 MEDIAL PHALANX, RIGHT	1 5%	0	0	0
TOE 4 MEDIAL PHALANX, RIGHT	22 100%	22 100%	20 100%	16 94%
NON-OSSIFIED				
TOE 4 MEDIAL PHALANX, RIGHT	0	0	1 5%	0
ADDITIONAL OSSIFICATION				
FEMUR RIGHT	15 68%	16 73%	15 75%	11 65%
TIBIA RIGHT	3 14%	1 5%	2 10%	1 6%

SKELETAL EXAMINATIONS SUMMARY
STAGE OF DEVELOPMENT AND VARIANTS
ON A FETUSES BASIS

	GROUP 1 0 MG/KG		GROUP 2 3 MG/KG		GROUP 3 10 MG/KG		GROUP 4 30 MG/KG	
NUMBER OF FETUSES EXAMINED	135		126		114		107	
ABNORMAL FINDING(S)								
(SHOWN ON PREVIOUS PAGE(S))	5	4%	5	4%	14	12% #	24	22% ##
CERVICAL VERTEBRAE								
INCOMPLETELY OSSIFIED								
CERVICAL VERTEBRAL BODY 1	1	1%	1	1%	0		0	
CERVICAL VERTEBRAL BODY 5	0		0		0		1	1%
ADDITIONAL OSSIFICATION								
CERVICAL VERTEBRAL ARCH 7, LEFT	0		0		1	1%	0	
CERVICAL VERTEBRAL ARCH 7, RIGHT	0		0		1	1%	0	
STERNUM								
INCOMPLETELY OSSIFIED								
STERNEBRA 1	0		1	1%	2	2%	0	
STERNEBRA 2	1	1%	0		1	1%	3	3%
STERNEBRA 4	1	1%	1	1%	3	3%	0	
STERNEBRA 5	89	66%	96	76% #	67	59%	65	61%
STERNEBRA 6	0		1	1%	1	1%	0	
NON-OSSIFIED								
STERNEBRA 5	15	11%	12	10%	19	17%	9	8%
ABNORMALLY OSSIFIED								
STERNEBRA 2	1	1%	0		0		0	
STERNEBRA 3	1	1%	0		0		0	
STERNEBRA 4	0		1	1%	0		1	1%
RIBS								
SHORTENED								
RIB 12, LEFT	1	1%	0		1	1%	0	
RIB 12, RIGHT	0		0		1	1%	0	
RIB(S), LEFT								
SUPERNUMERARY, ONE								
RIB(S), LEFT	2	1%	2	2%	2	2%	4	4%
SUPERNUMERARY, ONE RUDIMENTARY								
RIB(S), LEFT	0		1	1%	4	4% #	4	4% #
RIB(S), RIGHT								
SUPERNUMERARY, ONE								
RIB(S), RIGHT	0		1	1%	3	3%	5	5% #
SUPERNUMERARY, ONE RUDIMENTARY								
RIB(S), RIGHT	2	1%	0		2	2%	6	6%
LEFT FORELIMB								
INCOMPLETELY OSSIFIED								
METACARPALIA 1, LEFT	89	66%	79	63%	69	61%	83	78% #
DIGIT 1 PROXIMAL PHALANX, LEFT	6	4%	5	4%	3	3%	9	8%
DIGIT 2 MEDIAL PHALANX, LEFT	1	1%	0		2	2%	0	
DIGIT 3 MEDIAL PHALANX, LEFT	3	2%	3	2%	3	3%	1	1%
DIGIT 4 MEDIAL PHALANX, LEFT	26	19%	27	21%	16	14%	41	38% ##
METACARPALIA 5, LEFT	0		0		1	1%	0	
DIGIT 5 PROXIMAL PHALANX, LEFT	0		0		1	1%	0	
DIGIT 5 MEDIAL PHALANX, LEFT	133	99%	125	99%	112	98%	104	97%
NON-OSSIFIED								
DIGIT 4 MEDIAL PHALANX, LEFT	0		0		1	1%	0	

#/## : Fisher's Exact Test significant at level 5% (#) or 1% (##)

47

SKELETAL EXAMINATIONS SUMMARY
STAGE OF DEVELOPMENT AND VARIANTS
ON A FETUSES BASIS

	GROUP 1 0 MG/KG		GROUP 2 3 MG/KG		GROUP 3 10 MG/KG		GROUP 4 30 MG/KG	
NUMBER OF FETUSES EXAMINED	135		126		114		107	
LEFT FORELIMB								
NON-OSSIFIED								
DIGIT 5 MEDIAL PHALANX, LEFT	2	1%	0		2	2%	2	2%
ADDITIONAL OSSIFICATION								
HUMERUS LEFT	35	26%	48	38% #	67	59% ##	47	44% ##
RIGHT FORELIMB								
INCOMPLETELY OSSIFIED								
METACARPALIA 1, RIGHT	88	65%	79	63%	70	61%	83	78% #
DIGIT 1 PROXIMAL PHALANX, RIGHT	6	4%	3	2%	3	3%	10	9%
DIGIT 2 MEDIAL PHALANX, RIGHT	1	1%	0		3	3%	1	1%
DIGIT 3 MEDIAL PHALANX, RIGHT	8	6%	6	5%	7	6%	11	10%
DIGIT 4 MEDIAL PHALANX, RIGHT	60	44%	51	40%	43	38%	62	58% #
METACARPALIA 5, RIGHT	0		0		1	1%	0	
DIGIT 5 PROXIMAL PHALANX, RIGHT	0		0		1	1%	0	
DIGIT 5 MEDIAL PHALANX, RIGHT	132	98%	125	99%	112	98%	102	95%
NON-OSSIFIED								
DIGIT 4 MEDIAL PHALANX, RIGHT	0		0		1	1%	0	
DIGIT 5 MEDIAL PHALANX, RIGHT	2	1%	0		2	2%	3	3%
ADDITIONAL OSSIFICATION								
HUMERUS RIGHT	39	29%	50	40% #	66	58% ##	46	43% #
LEFT HIND LIMB								
INCOMPLETELY OSSIFIED								
TALUS LEFT	0		0		1	1%	0	
TOE 3 MEDIAL PHALANX, LEFT	1	1%	1	1%	0		0	
TOE 4 MEDIAL PHALANX, LEFT	126	93%	123	98%	106	93%	104	97%
NON-OSSIFIED								
TOE 4 MEDIAL PHALANX, LEFT	0		0		1	1%	0	
ADDITIONAL OSSIFICATION								
FEMUR LEFT	35	26%	48	38% #	46	40% #	27	25%
TIBIA LEFT	4	3%	1	1%	4	4%	1	1%
RIGHT HIND LIMB								
INCOMPLETELY OSSIFIED								
TALUS RIGHT	0		0		1	1%	0	
TOE 3 MEDIAL PHALANX, RIGHT	1	1%	0		0		0	
TOE 4 MEDIAL PHALANX, RIGHT	122	90%	117	93%	104	91%	102	95%
NON-OSSIFIED								
TOE 4 MEDIAL PHALANX, RIGHT	0		0		1	1%	0	
ADDITIONAL OSSIFICATION								
FEMUR RIGHT	30	22%	40	32%	40	35% #	28	26%
TIBIA RIGHT	4	3%	1	1%	2	2%	1	1%

9.3 Prenatal and Postnatal Development

Study title: **NETUPITANT: Pre- and Post-Natal Development in the Han Wistar Rat by Oral Administration**

Study no.:	NETU-10-23 or GEC0001
Study report location:	(b) (4)
Conducting laboratory and location:	
Date of study initiation:	June 25, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Netupitant, Batch #:29003531, 99.6%

Key Study Findings:

Netupitant was administered to pregnant females from Day 6 after mating to Day 20 of lactation by oral gavage. Bodyweight gain of F0 and F1 generations was reduced in the middle and high dose groups as compared to the respective control groups. F1 males from the high dose group showed a 2-day delay in completion of balano-preputial separation when compared with the controls. Attainment of the air righting reflex was slightly delayed in the middle and high dose groups as compared to the control group. Survival, behavioral development and reproductive performance of the F1 generation and survival and growth of F2 offspring up to Day 14 of age were unaffected. None of the observed effects in the offspring (F1 generation) are considered to be adverse, based on the small magnitude of these changes. Therefore, the NOAEL for pre- and post-natal development was 30 mg/kg/day.

Methods:

Doses:	0, 3, 10 and 30 mg/kg/day
Frequency of dosing:	daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	Thixotrope as aqueous suspensions
Species/Strain:	Harlan Han Wistar strain rats
Number/Sex/Group:	22/females/group
Satellite groups:	none
Study design:	Netupitant was administered to F0 females from Day 6 after mating to Day 20 of lactation.
Deviation from study protocol:	No deviation occurred which adversely affected the quality of the study.

Observations and Results

F_0 Dams

Survival:	One female in the low dose group was sacrificed in moribund condition.
Clinical signs:	No treatment related clinical signs
Body weight:	Bodyweight gain was reduced in the middle and high dose groups, primarily during the first day of treatment (Day 6 of gestation) to Day 14 of gestation.
Feed consumption:	Food consumption was reduced in the treatment groups.
Uterine content:	There were no treatment-related changes (see sponsor's tables below).
Necropsy observation:	There were no treatment-related changes.
Toxicokinetics:	Not done
Dosing Solution Analysis:	The mean concentrations of Netupitant in the dosing formulations were within 85 to 110% of the nominal concentrations.
Other:	Gestation length and gestation index were not altered by treatment with Netupitant. The results are summarized in the following sponsor's tables.

TABLE 11

Gestation length and gestation index - group values (F0)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group	Number of pregnant animals		Gestation length (days) 22	22.5	23	23.5	Number of live litters born	Gestation index (%)
Statistical test:					Lt			
1	22	N	4	8	9	1	22	100
		(%)	(18)	(36)	(41)	(5)		
2	22	N	4	10	8	0	22	100
		(%)	(18)	(45)	(36)			
3	21	N	10	7	4	0	21	100
		(%)	(48)	(33)	(19)			
4	21	N	7	8	5	1	21	100
		(%)	(33)	(38)	(24)	(5)		

TABLE 12

Litter size - group mean values (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group		Implantations	Total litter size	Live litter size on Day						
			Day	Before cull		After cull				
			1	1	4	4	7	11	14	18	21
Statistical test:		Wi	Wi	Sh	Wi						
1	Mean	11.7	10.9	10.9	10.9	7.8	7.8	7.8	7.8	7.8	7.8
	SD	2.4	2.4	2.4	2.4	0.9	0.9	0.9	0.9	0.9	0.9
	N	22	22	22	22	22	22	22	22	22	22
2	Mean	11.4	10.6	10.5	10.5	7.7	7.7	7.7	7.7	7.7	7.7
	SD	2.0	2.6	2.6	2.6	0.8	0.8	0.8	0.8	0.8	0.8
	N	21	21	21	21	21	21	21	21	21	21
3	Mean	11.4	10.6	10.4	10.3	7.5	7.5	7.5	7.5	7.5	7.5
	SD	2.3	2.6	2.6	2.7	1.5	1.5	1.5	1.5	1.5	1.5
	N	20	20	20	20	20	20	20	20	20	20
4	Mean	12.2	11.0	10.9	10.8	8.0	8.0	8.0	8.0	8.0	8.0
	SD	1.5	1.3	1.2	1.3	0.0	0.0	0.0	0.0	0.0	0.0
	N	21	21	21	21	21	21	21	21	21	21

TABLE 13

Offspring survival indices - group mean values (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group		Post implantation survival index (%)	Live birth index (%)	Viability index (%)	Lactation index (%) on Day 21
Statistical test:		Sh	Fe	Fe	
1	Mean	92.5	100.0	100.0	99.4
	N	22	22	22	22
2	Mean	92.0	99.3	100.0	100.0
	N	21	21	21	21
3	Mean	92.3	98.3	98.3	100.0
	N	20	20	20	20
4	Mean	90.4	98.8	99.0	100.0
	N	21	21	21	21

$$\text{Post-implantation survival index (\%)} = \frac{\text{Total number of offspring born}}{\text{Total number of uterine implantation sites}} \times 100$$

$$\text{Live birth index (\%)} = \frac{\text{Number of live offspring on Day 1 after littering}}{\text{Total number of offspring born}} \times 100$$

$$\text{Viability index (\%)} = \frac{\text{Number of live offspring on Day 4 before culling}}{\text{Number live offspring on Day 1 after littering}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{Number of live offspring on Day 14 or 21 after littering}}{\text{Number live offspring on Day 4 (after culling)}} \times 100$$

TABLE 14

Sex ratio - group mean values (F1)

Group	: 1	2	3	4
Compound	: Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	: 0	3	10	30

Group		Total on Day 1†			Live (before cull) on Day 1			Live (before cull) on Day 4			Live (after cull) on Day 4			Live (after cull) on Day 21		
		M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
Statistical test:				Wa			Wa			Wa						
1	Mean	5.0	5.9	45.4	5.0	5.9	45.4	5.0	5.9	45.4	3.6	4.2	46.6	3.6	4.1	46.9
	SD	2.2	1.9	15.7	2.2	1.9	15.7	2.2	1.9	15.7	0.9	1.0	10.3	0.9	1.0	10.6
	N	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
2	Mean	5.6	4.9	52.9	5.6	4.9	52.9	5.6	4.9	52.9	3.9	3.8	50.8	3.9	3.8	50.8
	SD	2.1	1.8	12.9	2.1	1.8	12.9	2.1	1.8	12.9	0.7	0.6	7.3	0.7	0.6	7.3
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
3	Mean	5.4	5.0	48.8	5.4	5.0	48.9	5.4	4.9	49.5	3.7	3.9	46.7	3.7	3.9	46.7
	SD	2.4	1.5	18.5	2.4	1.4	18.3	2.4	1.5	17.8	1.2	0.7	13.6	1.2	0.7	13.6
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
4	Mean	5.9	5.0	54.1	5.8	5.0	53.8	5.8	5.0	53.9	4.2	3.8	52.4	4.2	3.8	52.4
	SD	1.9	2.1	17.5	1.9	2.1	17.9	1.9	2.1	18.0	1.0	1.0	12.3	1.0	1.0	12.3
	N	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21

† May include offspring that died prior to the designated Day 1 of age. Unsexed offspring missing prior to Day 1 are not accounted for.

F_1 Generation

Survival: Offspring survival was not affected.

Clinical signs: There were no treatment-related changes.

Bodyweight: Bodyweights in the middle and high dose groups (male and female) were significantly lower on postnatal Days 1 to 28 (see table 15 below).

Feed consumption: Food consumption was lower in the middle and high dose group as compared to the control.

Physical development: The selected F1 female animals showed no delay in vaginal opening.

F1 males from the high dose group showed a 2-day delay in completion of balano-preputial separation as

compared with the controls.

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Neurological assessment: Attainment of the air righting reflex was slightly delayed in the middle and high dose groups, as compared to the control group (see sponsor's table 17 below). Age at attainment of the surface righting reflex and the number of offspring showing satisfactory pupil and startle responses, motor activity, accelerating rotarod performance, learning ability and memory were unaffected by maternal treatment.

Reproduction: There were no treatment-related changes in mating and fertility parameters.

Other: Litter size (F2 generation), offspring survival and sex ratio were not altered by maternal treatment of F0 females.

The results were presented in the sponsor's tables 31-37 below.

TABLE 15

Bodyweight - group mean values (g) for offspring (F1)

Group	: 1	2	3	4
Compound	: Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	: 0	3	10	30

Group / Sex		Day of age (before cull) 1	4	Day of age (after cull) 4	7	11	14	18	21	25	28
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1M	Mean	6.4	9.6	9.5	15.0	23.8	30.8	39.1	47.7	63.7	79.7
	SD	0.6	1.0	1.0	1.3	2.1	2.6	3.0	4.1	6.5	7.5
	N	22	22	22	22	22	22	22	22	20	20
2M	Mean	6.4	9.6	9.6	15.1	23.8	30.6	38.9	48.2	64.7	81.3
	SD	0.6	1.3	1.2	1.6	2.1	2.3	2.8	3.7	5.3	5.7
	N	21	21	21	21	21	21	21	21	20	20
3M	Mean	6.0*	8.9*	8.9*	14.1*	22.4*	29.4	37.3	45.1*	61.2	77.4
	SD	0.6	0.9	0.9	1.4	2.3	2.8	3.3	4.1	5.6	6.8
	N	19	19	19	19	19	19	19	19	19	19
4M	Mean	5.9**	8.2**	8.1**	12.6**	20.1**	26.3**	33.9**	41.4**	55.8**	71.3**
	SD	0.5	0.8	0.8	1.3	2.1	2.4	2.8	3.9	5.1	6.3
	N	21	21	21	21	21	21	21	21	19	19

TABLE 15 - continued

Bodyweight - group mean values (g) for offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group / Sex		Day of age (before cull) 1	4	4	7	Day of age (after cull) 11	14	18	21	25	28
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1F	Mean	6.2	9.3	9.3	14.8	23.5	30.4	38.4	46.9	61.0	75.5
	SD	0.7	1.2	1.2	1.7	2.6	3.1	3.6	4.7	6.6	8.0
	N	22	22	22	22	22	22	22	22	20	20
2F	Mean	6.2	9.3	9.4	14.8	23.6	30.2	38.1	46.9	60.9	74.8
	SD	0.6	1.1	1.2	1.7	2.0	2.3	2.8	3.7	4.3	5.1
	N	21	21	21	21	21	21	21	21	20	20
3F	Mean	5.7*	8.7	8.7	13.7*	22.0*	28.8	36.3*	43.8*	57.7	71.6
	SD	0.6	1.1	1.1	1.4	2.2	2.7	3.1	3.6	5.7	6.4
	N	20	20	20	20	20	20	20	20	20	20
4F	Mean	5.6**	8.0**	8.0**	12.3**	19.7**	25.8**	33.3**	40.5**	55.1**	69.2**
	SD	0.4	0.8	0.8	1.2	2.0	2.3	2.5	3.3	5.3	5.6
	N	21	21	21	21	21	21	21	21	19	19

TABLE 17

Pre-weaning examinations - group mean values for offspring (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group		Surface righting Day of age	Air righting Day of age		Pupil reflex (% pass)	Startle response (% pass)
Statistical test:		Wi	Wi			
1	Mean	3.4	17.0	Number of animals tested	171	171
	SD	0.64	0.48	Number of animals failed	0	0
	N	22	22	% of animals passed	100.0	100.0
2	Mean	3.6	17.1	Number of animals tested	161	161
	SD	0.65	0.60	Number of animals failed	0	0
	N	21	21	% of animals passed	100.0	100.0
3	Mean	3.6	17.4*	Number of animals tested	150	150
	SD	0.67	0.55	Number of animals failed	0	0
	N	20	20	% of animals passed	100.0	100.0
4	Mean	3.6	17.3*	Number of animals tested	168	168
	SD	0.53	0.46	Number of animals failed	0	0
	N	21	21	% of animals passed	100.0	100.0

TABLE 31

Motor activity - group mean scores (beam breaks) (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group /Sex	Number of animals	Beam level	Time (minutes) 6	12	18	24	30	36	42	48	54	60	Total
Statistical test:			Wi	Wi	Wi	Wi	Wi	Sh	Wi	Wi	Wi	Wi	Wi
1M	20	High	29.5	27.2	10.4	6.2	7.2	2.5	1.3	3.5	6.2	4.4	98.3
		SD	16.7	20.0	14.5	8.7	11.9	3.7	4.1	10.6	12.7	9.2	56.5
2M	20	High	49.2	32.6	13.0	9.8	5.7	3.7	3.8	3.8	7.1	4.2	132.7
		SD	28.5	21.1	14.1	12.4	13.4	7.8	6.8	6.9	15.3	9.7	64.4
3M	20	High	47.7	24.7	18.7	7.0	10.0	8.6	1.9	3.6	3.2	4.6	129.8
		SD	28.4	19.9	18.9	9.4	14.1	12.7	6.3	11.4	6.9	10.8	78.1
4M	20	High	39.0	26.0	15.5	5.8	6.0	4.0	3.4	6.5	8.5	4.9	119.4
		SD	25.2	15.6	13.8	8.6	9.8	6.7	6.3	14.3	12.0	8.4	69.3
Statistical test:			Wi	Wi	Wi	Wi	Wi	Wi	Sh	Wi	Wi	Wi	Wi
1M	20	Low	116.2	76.2	39.7	24.7	21.6	19.0	8.9	15.2	21.3	20.9	363.4
		SD	38.1	40.3	47.3	27.1	29.0	29.9	19.7	30.9	28.9	35.7	181.6
2M	20	Low	146.3	79.3	52.2	42.9	18.0	22.0	16.2	24.8	25.9	21.9	449.3
		SD	52.5	22.4	28.5	39.5	22.6	31.9	21.2	29.1	41.8	33.5	148.4
3M	20	Low	129.9	73.1	56.8	40.1	36.2	27.2	5.9	18.3	18.0	15.9	421.0
		SD	46.2	36.2	30.2	36.9	36.4	31.5	9.9	40.9	27.3	23.9	186.4
4M	20	Low	129.0	77.9	56.8	39.3	33.8	23.0	29.1*	25.9	31.4	23.9	469.8*
		SD	31.3	35.4	25.6	30.5	35.7	26.9	42.0	27.8	27.6	24.2	108.3

TABLE 31 - continued

Motor activity - group mean scores (beam breaks) (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group /Sex	Number of animals	Beam level	Time (minutes) 6	12	18	24	30	36	42	48	54	60	Total
Statistical test:			Wi	Wi	Wi	Wi	Wi	Sh	Fe	Wi	Sh	Wi	Wi
1F	20	High	37.2	31.4	18.3	5.9	7.5	3.6	4.6	7.4	4.5	8.8	128.8
		SD	18.8	17.2	17.2	10.4	13.6	7.1	9.8	12.7	15.4	14.7	68.7
2F	20	High	42.8	28.5	15.2	7.4	4.5	4.2	2.2	6.4	2.0	3.0	115.8
		SD	22.1	20.6	15.2	9.2	8.7	7.5	5.8	15.6	5.4	9.8	54.0
3F	20	High	39.9	28.1	17.4	12.8	8.3	8.1	2.7	5.8	7.8	6.4	137.2
		SD	20.7	14.9	14.2	13.3	16.1	14.5	6.7	17.1	11.7	10.9	85.4
4F	20	High	33.2	24.6	11.4	7.9	9.5	4.8	3.8	3.0	7.7	7.3	113.1
		SD	21.3	20.8	13.9	11.7	12.2	9.8	10.1	8.5	13.1	17.2	77.5
Statistical test:			Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi	Sh	Wi	Wi
1F	20	Low	132.6	82.2	51.8	27.1	25.0	16.4	24.7	27.3	12.9	30.9	430.7
		SD	49.2	36.5	40.1	33.3	32.2	22.6	34.9	37.6	25.5	38.1	149.0
2F	20	Low	129.2	77.4	55.5	36.5	21.6	19.6	16.9	18.9	11.0	13.5	399.9
		SD	47.8	33.7	30.5	33.4	28.8	29.2	26.4	35.4	19.2	30.9	125.1
3F	20	Low	142.3	76.8	62.5	41.5	22.6	22.1	18.1	28.5	34.9	24.3	473.4
		SD	38.6	26.9	38.3	27.9	26.7	31.5	30.9	37.2	44.6	32.4	188.6
4F	20	Low	116.3	71.3	46.1	37.0	42.7	18.9	19.8	16.0	33.7	33.5	435.1
		SD	30.8	33.1	36.0	38.3	34.6	30.2	30.8	31.1	47.5	36.3	210.3

TABLE 32

Accelerating rotarod - group mean times (seconds) (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

		Males		Females	
Group		Number of animals	Maximum time^	Number of animals	Maximum time^
Statistical test:			Wi		Wi
1	Mean	20	259	20	275
	SD		39		33
2	Mean	20	235	20	250*
	SD		37		42
3	Mean	20	240	20	235**
	SD		43		46
4	Mean	20	247	20	247**
	SD		43		31

^ Maximum time achieved during three trials (test terminated if 300 seconds achieved)

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TABLE 33

Morris maze performance - group mean values (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group /Sex	Number of animals		Day 1 T	Day 1 F	Day 1 S	Day 2 T	Day 2 F	Day 2 S	Day 3 T	Day 3 F	Day 3 S	Day 4 T	Day 4 F	Day 4 S
Statistical test:			Wi	Sh	Wi	Wi	Sh	Wi	IWi	Fe	Wi	Wi	Fe	Wi
1M	20	Mean	55.0	0.9	18.4	38.3	0.6	13.0	23.7	0.2	8.9	14.8	0.1	6.5
		SD	18.5	1.0	4.4	20.5	0.9	4.6	12.7	0.4	3.9	7.9	0.2	2.8
		%		55.0			40.0			15.0			5.0	
2M	20	Mean	59.7	1.3	20.9	33.2	0.5	11.2	25.4	0.3	8.9	12.4	0.0	5.6
		SD	22.0	1.0	8.7	21.8	0.8	4.5	20.7	0.6	5.3	6.6	0.0	2.4
		%		70.0			30.0			20.0			0.0	
3M	20	Mean	54.1	1.0	20.2	29.7	0.4	10.7	17.7	0.0	6.8	16.3	0.0	6.5
		SD	20.9	1.0	8.4	14.0	0.5	3.8	7.3	0.0	2.7	10.3	0.0	3.9
		%		65.0			40.0			0.0			0.0	
4M	20	Mean	59.1	1.4	20.9	44.9	0.8	14.8	29.6	0.3	9.8	14.7	0.0	6.2
		SD	18.3	0.7	6.7	23.1	1.0	5.6	18.8	0.5	5.6	8.6	0.0	3.1
		%		95.0			50.0			30.0			0.0	

T Trial time (seconds - mean of 3 trials)
F Number of failed trials (90 seconds)
S Sector entries (mean of 3 trials)
% Percentage of animals with at least 1 failed trial (90 seconds)

TABLE 33 - continued

Morris maze performance - group mean values (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group /Sex	Number of animals		Day 1 T	Day 1 F	Day 1 S	Day 2 T	Day 2 F	Day 2 S	Day 3 T	Day 3 F	Day 3 S	Day 4 T	Day 4 F	Day 4 S
Statistical test:			Wi	Sh	Wi	Wi	Sh	Wi	Wi	Fe	Wi	Wi	Fe	Wi
1F	20	Mean	58.0	1.3	18.8	36.0	0.4	12.6	18.6	0.0	7.3	17.8	0.1	7.3
		SD	20.9	0.8	6.4	16.8	0.7	5.1	10.5	0.0	3.1	10.5	0.3	3.8
		%		85.0			30.0			0.0			10.0	
2F	20	Mean	43.9	0.7*	15.2	36.2	0.6	10.6	25.5	0.2	7.9	17.9	0.0	6.7
		SD	16.6	0.8	5.0	24.1	0.8	5.5	16.3	0.5	3.9	7.0	0.0	2.4
		%		45.0			40.0			15.0			0.0	
3F	20	Mean	44.8	0.7*	16.6	33.4	0.4	12.3	27.0	0.1	9.6	17.6	0.1	6.7
		SD	18.7	0.9	6.6	15.7	0.6	4.5	13.3	0.3	4.3	11.2	0.2	3.5
		%		50.0			35.0			10.0			5.0	
4F	20	Mean	53.2	0.8*	19.9	34.6	0.5	12.0	26.1	0.2	8.9	16.9	0.1	6.7
		SD	19.0	0.8	5.7	22.1	0.8	6.2	15.3	0.5	4.7	9.6	0.2	3.1
		%		55.0			25.0			15.0			5.0	

T Trial time (seconds - mean of 3 trials)
F Number of failed trials (90 seconds)
S Sector entries (mean of 3 trials)
% Percentage of animals with at least 1 failed trial (90 seconds)

TABLE 34

Balano-preputial separation and vaginal opening - group mean age and bodyweight at attainment (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group		Time of completion for balano preputial separation Day of age	Bodyweight (g) at balano preputial separation	Time of completion for vaginal opening Day of age	Bodyweight (g) at vaginal opening
Statistical test:		Wi	lWi	Wi	Wi
1	Mean	46	193	33	102
	SD	1.7	18.5	2.4	13.1
	N	20	20	20	20
2	Mean	46	196	33	100
	SD	2.2	11.8	2.5	12.5
	N	20	20	20	20
3	Mean	47	195	34	100
	SD	3.1	23.9	2.3	8.7
	N	20	20	20	20
4	Mean	48**	189	33	92*
	SD	3.3	27.3	2.4	12.0
	N	20	20	20	20

TABLE 35

Pre-coital interval - group values (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group	Number of animals		Pre-coital interval (days) 1-4	5-8	9-12	13-15
1	20	N	19	0	0	1
		(%)	(95)			(5)
2	20	N	20	0	0	0
		(%)	(100)			
3	20	N	20	0	0	0
		(%)	(100)			
4	20	N	18	1	0	1
		(%)	(90)	(5)		(5)

TABLE 36

Mating performance and fertility - group values (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group and sex	Number paired	Number mating	Number achieving pregnancy	Percentage mating	Conception rate (%)	Fertility index (%)
1M	20	20	18	100	90	90
2M	20	20	20	100	100	100
3M	20	20	20	100	100	100
4M	20	20	19	100	95	95
1F	20	20	18	100	90	90
2F	20	20	20	100	100	100
3F	20	20	20	100	100	100
4F	20	20	19	100	95	95

TABLE 37

Gestation length and gestation index - group values (F1)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group	Number of pregnant animals		Gestation length (days) 22	22.5	23	23.5	Number of live litters born	Gestation index (%)
Statistical test:					Lt			
1	18	N	3	3	11	1	18	100
		(%)	(17)	(17)	(61)	(6)		
2	20A	N	6	4	9	0	19	95
		(%)	(32)	(21)	(47)			
3	20A	N	3	3	11	2	18	90
		(%)	(16)	(16)	(58)	(11)		
4	19	N	7	6	6	0	19	100
		(%)	(37)	(32)	(32)			

A Percentage distribution of gestation lengths calculated from 19 animals - one pregnant female failed to litter

F_2 Generation

Survival: There were no effects.
Bodyweight: Bodyweights on post-natal day 1 to 14 were unaffected.
External evaluation: There were no treatment related effects.
Litter parameters: F2 litter size at birth, offspring survival, and sex ratio were not affected.

The results were presented in the sponsor's tables below.

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TABLE 38

Litter size - group mean values (F2)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group		Implantations	Total litter size Day 1	Live litter size on Day Before cull 1	Before cull 4	After cull 4	After cull 7	After cull 11	After cull 14
Statistical test:		Wi	Wi	Wi	Wi				
1	Mean	11.5	10.8	10.6	10.5	7.4	7.4	7.4	7.4
	SD	3.1	3.5	3.5	3.5	1.4	1.4	1.4	1.4
	N	18	18	18	18	18	18	18	18
2	Mean	12.8	11.8	11.8	11.8	7.5	7.5	7.5	7.5
	SD	3.9	3.8	3.8	3.8	1.4	1.4	1.4	1.4
	N	19	19	19	19	19	19	19	19
3	Mean	11.3	10.1	10.1	10.1	7.6	7.6	7.6	7.6
	SD	2.8	2.7	2.6	2.6	1.0	1.0	1.0	1.0
	N	18	18	18	18	18	18	18	18
4	Mean	13.1	11.6	11.4	11.3	7.9	7.9	7.9	7.9
	SD	2.2	2.3	2.4	2.4	0.5	0.5	0.5	0.5
	N	19	19	19	19	19	19	19	19

TABLE 39

Offspring survival indices - group mean values (F2)

Group	:	1	2	3	4
Compound	:	Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	:	0	3	10	30

Group		Post implantation survival index (%)	Live birth index (%)	Viability index (%)	Lactation index (%) on Day 14
Statistical test:		Wi	Fe	Fe	
1	Mean	92.1	97.3	99.6	100.0
	N	18	18	18	18
2	Mean	92.6	100.0*	100.0	100.0
	N	19	19	19	19
3	Mean	89.2	99.5	100.0	100.0
	N	18	18	18	18
4	Mean	89.5	97.7	99.1	100.0
	N	19	19	19	19

$$\text{Post-implantation survival index (\%)} = \frac{\text{Total number of offspring born}}{\text{Total number of uterine implantation sites}} \times 100$$

$$\text{Live birth index (\%)} = \frac{\text{Number of live offspring on Day 1 after littering}}{\text{Total number of offspring born}} \times 100$$

$$\text{Viability index (\%)} = \frac{\text{Number of live offspring on Day 4 before culling}}{\text{Number live offspring on Day 1 after littering}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{Number of live offspring on Day 14 or 21 after littering}}{\text{Number live offspring on Day 4 (after culling)}} \times 100$$

TABLE 40

Sex ratio - group mean values (F2)

Group	: 1	2	3	4
Compound	: Control	Netupitant	Netupitant	Netupitant
Dose (mg/kg/day)	: 0	3	10	30

Group		Total on Day 1†			Live (before cull) on Day 1			Live (before cull) on Day 4			Live (after cull) on Day 4			Live (after cull) on Day 14		
		M	F	%M	M	F	%M	M	F	%M	M	F	%M	M	F	%M
Statistical test:				Wa			Wa			Wa						
1	Mean	5.2	5.4	51.6	5.2	5.4	51.5	5.1	5.4	51.2	3.7	3.7	51.6	3.7	3.7	51.6
	SD	2.2	2.7	19.8	2.1	2.7	19.6	2.1	2.7	20.1	0.8	1.3	16.4	0.8	1.3	16.4
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
2	Mean	5.5	6.3	46.7	5.5	6.3	46.7	5.5	6.3	46.7	3.5	3.9	47.1	3.5	3.9	47.1
	SD	2.7	2.9	16.6	2.7	2.9	16.6	2.7	2.9	16.6	1.0	1.0	9.2	1.0	1.0	9.2
	N	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
3	Mean	4.6	5.4	45.6	4.6	5.4	45.6	4.6	5.4	45.6	3.6	4.0	47.1	3.6	4.0	47.1
	SD	1.9	1.9	13.1	1.9	1.9	13.1	1.9	1.9	13.1	0.8	0.8	8.3	0.8	0.8	8.3
	N	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
4	Mean	5.6	5.8	48.4	5.6	5.8	48.4	5.5	5.8	48.1	3.9	4.0	49.1	3.9	4.0	49.1
	SD	2.2	2.0	14.9	2.2	2.0	14.9	2.0	2.0	14.5	0.8	0.7	9.2	0.8	0.7	9.2
	N	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19

† May include offspring that died prior to the designated Day 1 of age. Unsexed offspring missing prior to Day 1 are not accounted for.

10 Special Toxicology Studies

3-Day IV local tolerance study in rabbits (# 1007324)

Netupitant was given intravenously in the marginal ear vein of rabbits at 0 (vehicle control), 1, 3, and 10 mg/kg/day for 3 days. The vehicle was 5% glucose solution. Local reactions including swollen and bluish ear were noted in all treatment groups, but were more severe at the high dose.

In vitro hemolysis and plasma precipitation and turbidity study using rat blood (#1008464)

The intravenous formulation of netupitant had a slight hemolytic effect on rat blood at concentrations up to 500 µg/ml. The formulation was negative in plasma turbidity and plasma precipitation tests with drug concentrations up to 500 µg/ml.

In vitro hemolysis and plasma precipitation and turbidity study using human blood (# 1006117)

The intravenous formulation of netupitant produced hemolysis in human blood at concentrations ≥ 250 µg/ml. The formulation was also positive in the plasma turbidity and plasma precipitation tests at drug concentrations ≥ 250 µg/ml. The results were summarized in the following tables (taken from the sponsor's report).

Table 1 Ro 67-3189/000 (GFE 0785): *In-Vitro* hemolysis test with human heparinated blood

Conc. of test solution (%) in 5% Glucose	100	80	60	50	40	25	12.5	6.25	3.12	1.56
Conc. of Ro 67-3189/000 (µg/mL) in the assay	500	400	300	250	200	125	62.5	31.2	15.6	7.8
Hemolysis (%)	50	37	10	1,2	0.3	0.2	0.2	0.2	0.2	0.1

Table 2 Ro 67-3189/000 (GFE 0785): *In-Vitro* plasma precipitation test with human plasma

Dilution of Ro 67-3189/000 in 5% Glucose	Undiluted	1:2	1:4	1:8
Conc. of Ro 67-3189/000 (µg/ml) in the assay volume (1 mL)	500	250	125	62.5
Plasma precipitation	Mild	mild	None	none

Phototoxicity study in mouse fibroblasts (# 1003848)

The potential phototoxicity of netupitant was studied *in vitro* using the 3T3 fibroblast neutral red uptake assay. Murine fibroblasts were incubated with 0.75-96.0 µg/ml

netupitant in the presence of UVA exposure, followed by incubation in the dark for one hour. Under these conditions, netupitant was considered to be non-phototoxic.

Antigenicity study (# 1007385)

Netupitant was examined in male guinea pigs for active systemic and passive anaphylaxis. In the active systemic anaphylaxis test, 5 male guinea pigs were actively sensitized three times (once every other week) by subcutaneous injection of 6 mg netupitant (alone or in combination with guinea pig serum albumin) in emulsions including Freund's adjuvant. These animals were then challenged by intravenous injection of 1 mg netupitant (alone or in combination with ovalbumin). In the passive cutaneous anaphylaxis test, 2 male guinea pigs were passively sensitized by subcutaneous injection of sera from actively sensitized guinea pigs. These animals were then challenged by intravenous injection of 1 mg netupitant (alone or in combination with ovalbumin). No signs of antigenicity were observed in these tests.

In vitro phospholipidosis study in bovine corneal fibroblasts (# 1009769)

To evaluate drug-induced phospholipid accumulation in cytoplasm, bovine corneal fibroblasts were cultured and treated with collagenase. The cells were then exposed to netupitant, M1, M2, or M3 at concentrations of 2.5-20 μM in the culture medium for 72 hours. Accumulated phospholipids were identified as punctuate, black intra-cytoplasmic grains or inclusions, as detected using light microscopy. The results indicated that netupitant, M1, M2 and M3 induced phospholipid accumulation in the cytoplasm at concentrations of 2.5 μM or higher.

11 Integrated Summary and Safety Evaluation

The standard antiemetic drug regimen for prevention of CINV (chemotherapy-induced nausea and vomiting) includes an NK_1 receptor antagonist, a 5-HT_3 receptor antagonist, and a corticosteroid. AKYNZEO capsule is a fixed-dose combination of two active drug substances, netupitant and palonosetron HCl. Netupitant is an NK_1 receptor antagonist, and palonosetron is a 5-HT_3 receptor antagonist. Palonosetron hydrochloride is an approved drug that is available in two formulations, Aloxi® Injection (approved in 2003) and Aloxi® Capsules (approved in 2008). Aloxi® Injection is indicated for prevention of CINV and prevention of PONV (postoperative nausea and vomiting). Aloxi® Capsule is indicated for prevention of CINV only. The approved IV dose is 0.25 mg and the approved oral dose is 0.5 mg. Netupitant and palonosetron produce antiemetic effects through direct action on separate neuro-pathways (i.e. antagonism of NK_1 receptors and 5-HT_3 receptors, respectively). These drugs represent two pharmacologic classes in the standard regimen for prevention of CINV, and show a similar pharmacokinetic profile in terms of extended plasma half-life. These factors provided the rationale for development of AKYNZEO as a fixed-dose combination for use in the prevention of CINV. The sponsor seeks market approval of AKYNZEO for prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of highly

emetogenic cancer chemotherapy and moderately emetogenic cancer chemotherapy in adults.

The results of *in vitro* cardiovascular pharmacology studies indicated that netupitant and its metabolites (M1, M2, or M3) inhibited the hERG channel potassium K^+ currents in CHO cells with IC_{50} values of 0.76, 0.84, 43, and 4.4 µM, respectively. The results of *in vivo* cardiovascular pharmacology studies indicated that oral doses of netupitant at doses up to 50 mg/kg/day for 5 days had no significant effects on cardiovascular parameters in beagle dogs. Oral administration of netupitant at 50 mg/kg/day for 14 days produced a decrease in heart rate (15-19%) and prolongation of QT interval by ~5-8%, as compared to the control in conscious beagle dogs. Combination of oral palonosetron (10 mg/kg) and netupitant (50 mg/kg) given for 15 days prolonged the corrected QT interval by 15-23% as compared to pretreatment values in conscious telemetered dogs.

Netupitant is orally absorbed with oral bioavailability of 37-100% in rats, dogs, and monkeys. Netupitant has a long terminal half-life of 8-18 hours in rats and monkeys and 34-56 hours in dogs following an oral dose. Netupitant exhibits high protein binding (>99%) in plasma in rats, dogs, and humans. Three major metabolites, an N-demethylation product (M1), an N-oxidation product (M2), and M3 were identified in rats, dogs, and humans. Each of these metabolites exhibits high affinity for the recombinant human NK_1 receptor, similar to the parent compound. Biliary elimination was the major route of excretion accounting for ~86-90% of the dose being recovered in feces following oral and IV doses in rats and dogs.

Netupitant alone was tested in oral toxicity studies for up to 26 weeks in rats and 9 months in dogs. In the 13-week study in rats, netupitant was tested orally at 0, 3, 10 and 30 mg/kg/day for 13 weeks. Treatment with netupitant decreased the terminal body weight gain by 12% in males and 19% in females in the high-dose group. Slight change of terminal body weight gain was noted in the mid-dose group. Netutipant produced phospholipidosis in a dose-dependent manner. Phospholipidosis was not considered to be a dose limiting toxicity. Minimal necrosis was noted in the liver in four high-dose females (no incidence in other groups). Based on the effects on body weight and histopathological changes in the liver, the maximum tolerated dose (MTD) is estimated to be 20 mg/kg/day for females. The decrease in terminal body weight gain (12%) at 30 mg/kg/day in males suggests that the MTD is about 30 mg/kg/day in males, since no other dose limiting toxicity occurred at this dose.

In the 26-week study in rats, netupitant was tested orally at 0, 1, 3, or 10 mg/kg/day. Treatment with 10 mg/kg/day resulted in periacinar hepatocytic hypertrophy, thyroid follicular epithelial hypertrophy (males), thymic atrophy, increased aggregations of alveolar macrophages, and syncytial macrophages in mandibular and mesenteric lymph nodes and spleen. The NOAEL is considered to be 3 mg/kg/day based on the microscopic findings, although the high dose of 10 mg/kg/day was well tolerated.

In the 13-week study in dogs, netupitant was tested orally at 0, 1, 3, and 10 mg/kg/day. Administration of 10 mg/kg/day resulted in vacuolated macrophages or vacuolated tingible body macrophages in the lymphoid tissue (mesenteric or mandibular lymph nodes, Peyer's patches or colonic lymphoid tissue), suggesting a drug-induced phospholipidosis. Since the microscopic lesions are not considered to be adverse, the NOAEL is 10 mg/kg/day.

In the 9-month study in dogs, netupitant was tested orally at 0, 1, 3, and 10 mg/kg/day. Treatment with netupitant at 10 mg/kg/day slightly increased the QT_c and PQ intervals (males only), and produced minimal periacinar hepatocytic hypertrophy (males only). The NOAEL was 10 mg/kg/day.

The results from 4-week oral toxicity studies with palonosetron (RS-25259-197), submitted in NDA 21,372, are described below.

In the 4-week oral toxicity study in rats, RS-25259-197 was administered at 6, 18, 60, and 180 mg/kg/day. No treatment-related mortality was observed, but a mild decrease in hemoglobin, other hematology parameters, and liver enzymes were reported in animals in the 60 and 180 mg/kg/day groups. The identified target organs of toxicity in males were liver (increased weights, hepatocellular swelling and glycogen deposition along with decreased liver enzymes) and testes (reduced weights and histological correlates of degeneration/necrosis of the seminiferous epithelium and immature spermatogenic cells in the epididymis). In females, thymus (reduced weight, gross findings of small thymus and histological findings of thymic lymphoid atrophy) was the identified target organ of toxicity. The dose of 18 mg/kg/day was the no effect dose.

In the 4-week oral toxicity study in dogs, RS-25259-197 was administered at doses of 2, 6, and 20 mg/kg/day. Only transient clinical signs of salivation were observed in the 6 and 20 mg/kg/day groups. Reductions in absolute and relative weights of testes were observed at the 20 mg/kg dose, but there were no gross or histological correlates. No target organs of toxicity were identified in this study and the high dose of 20 mg/kg/day was considered as the no effect dose.

13-week oral toxicity studies with the combination of palonosetron and netupitant were performed in rats and dogs. In the 13-week oral toxicity study in rats, netupitant was administered at 1, 3, and 10 mg/kg/day in combination with palonosetron at 2, 6, and 18 mg/kg/day, respectively, by oral gavage. Additional groups were treated orally with 10 mg/kg/day netupitant or 18 mg/kg/day palonosetron. Slight increases in absolute and relative liver weights were noted in the high-dose combination group, the netupitant only group, and the palonosetron only group (up to 120% of the mean control weight). Histopathological examination revealed adrenal zona fasciculata hypertrophy, hepatocytic hypertrophy, and syncytial macrophages in the mesenteric lymph nodes, mainly in the high-dose combination and netupitant only groups. These effects are not considered to be adverse. Therefore, the NOAEL was 10 mg/kg/day netupitant + 18 mg/kg/day palonosetron.

In the 13-week oral toxicity study in dogs, netupitant was administered at 1, 3, and 10 mg/kg/day combined with palonosetron at 3, 5, and 10 mg/kg/day, respectively, by oral gavage for 13 weeks. Slight decreases in body weight gain and food consumption were noted in the high-dose group. The ST-interval, uncorrected QT-interval, and corrected QT intervals according to Van de Water and Fridericia were slightly prolonged in the high-dose females. No treatment-related histopathological changes were noted. The NOAEL was 3 mg/kg/day netupitant + 5 mg/kg/day palonosetron.

Palonosetron and netupitant have been tested separately and in combination in animals. Combination of netupitant and palonosetron did not produce additional toxicity as compared with either compound given alone. The tolerated doses and/or NOAELs of netupitant in the combination toxicity studies were consistent with the findings from the toxicity studies with netupitant alone.

Netupitant was negative in the Ames test, mouse lymphoma cell mutation assay, and rat micronucleus test.

Long-term studies in animals to evaluate carcinogenic potential of netupitant are not needed for support of the proposed indication, as recommended by the Executive Carcinogenicity Assessment Committee. Therefore, no carcinogenicity studies with netupitant were conducted. However, the Committee stated that a carcinogenicity evaluation may be needed to support other indications (see Executive CAC meeting minutes from July 1, 2008 in Appendix).

In the segment I fertility and early embryonic development study in rats, netupitant was given by oral gavage at doses of 3, 10, and 30 mg/kg/day to male and females rats starting at two weeks prior to mating, throughout a two-week mating period, and through gestation day 7 in females. Dosing of males continued until necropsy of partnered females, at least until gestation day 14. Treatment with netupitant at 30 mg/kg/day decreased body weight gain and food consumption. The number of corpora lutea, implantation sites, and fetuses per dam were slightly but statistically lower in the high dose group, relative to the control group. The number of corpora lutea in the high-dose group was within normal limits for this strain of rat. Netupitant did not clearly produce adverse effects on fertility or early embryonic development.

In the segment II embryo-fetal development study in rats, pregnant rats were treated with netupitant at 0 (vehicle), 3, 10 and 30 mg/kg/day during gestation days 6-17. Netupitant at 30 mg/kg/day decreased body weight gain and food consumption. Netupitant did not adversely affect embryo-fetal development and was not teratogenic. The maternal NOAEL was 10 mg/kg/day, based on the decrease (31%) in body weight gain in the 30 mg/kg/day females.

In the segment II embryo-fetal development study in rabbits, pregnant rabbits were treated with netupitant by oral gavage at 0 (vehicle), 3, 10 and 30 mg/kg/day during gestation days 6-18. A loss of 70 g in bodyweight occurred in the high-dose females during gestation days 6 to 19, as compared to a body weight gain of 88 g in the control

females. Three females had abortions (1 in the middle-dose group and 2 in the high-dose group). One female given 30 mg/kg/day had total litter death. Fetal body weights at 30 mg/kg were decreased by 8% in males, as compared to the control males. Limb and paw anomalies (position anomalies) were observed with higher incidence in the middle and high-dose groups. The combined incidence of all findings was 1/135, 1/126, 3/114, and 9/107 among fetuses, and 1/22, 1/22, 3/20, and 6/17 among litters, in the control, low, middle, and high-dose groups, respectively. An increased number of minimally/partially fused sternebrae was noted at 10 and 30 mg/kg/day, as compared to the control group. Based on the increased incidence of malformations (limb and paw anomalies, minimally/partially fused sternebrae) at 10 and 30 mg/kg/day, the NOAEL for embryo-fetal development is considered to be 3 mg/kg/day. The NOAEL for maternal toxicity was 10 mg/kg/day, based on the loss of bodyweight in the 30 mg/kg/day group during gestation days 6-19.

A consult request related to the embryo-fetal development study in rabbits was sent to the Reproductive and Developmental Toxicology Subcommittee (RDTS) on March 18, 2014. The committee concluded that the limb and paw anomalies, and minimally/ partially fused sternebrae were treatment-related and evidence of developmental toxicity.

In the prenatal and postnatal development study in rats, netupitant was administered by oral gavage to pregnant females from Day 6 after mating to Day 20 of lactation. Bodyweight gain of F0 and F1 generations was reduced in the middle and high dose groups as compared to the respective control groups. F1 males from the high dose group showed a 2-day delay in completion of balano-preputial separation when compared with the controls. Attainment of the air righting reflex was slightly delayed in the middle and high dose groups as compared to the control group. Survival, behavioral development, and reproductive performance of the F1 generation, and survival and growth of F2 offspring up to Day 14 of age were unaffected. None of the observed effects in the offspring (F1 generation) are considered to be adverse, based on the small magnitude of these changes. Therefore, the NOAEL for pre- and post-natal development was 30 mg/kg/day.

To evaluate the safety of the sponsor's pediatric study plan, an 8-week oral toxicity study of netupitant alone in juvenile rats is needed. This study should include evaluation of developmental parameters, neurobehavioral effects, and fertility. The proposed pediatric single-dose PK study (Study #1) using an oral liquid netupitant formulation in combination with Aloxi Injection given orally may be conducted before completion of the juvenile animal studies. However, the definitive juvenile rat toxicity study will be needed to support the pediatric clinical efficacy study in patients age 0 to < 17 years (Study #2). If the sponsor cannot develop an oral liquid formulation of netupitant alone that is suitable for use in patients ≤ 6 years old, the definitive juvenile rat toxicity study will still be needed to support an efficacy study in older pediatric patients (age > 6 to 11 years) using a solid oral dosage form. Please see the Late Cycle Meeting Background Package for additional recommendations related to the juvenile animal study program.

The table below summarizes the animal to human AUC multiples for netupitant, calculated from the animal AUC values associated with the NOAEL. Although the multiples from the general toxicity studies in rats and dogs ranged from 0.4 to 1.8, the mild toxicity that occurred in these studies does not raise a concern for the expected plasma exposure in humans at the recommended dose. Furthermore, given that AKYNZEO will be used intermittently (i.e. administered once with each cycle of chemotherapy), there is minimal concern about the absence of toxicity data obtained with higher systemic exposures to netupitant in repeat-dose studies. It is noted that dose selection for the 13-week and 9-month dog studies was based on 7-day and 4-week oral toxicity studies in dogs, which showed weight loss and aggregates of foamy or vacuolated macrophages in lymphoid tissue and intestinal mucosa, suggestive of phospholipidosis. These effects occurred at 15 mg/kg/day and higher.

Toxicity Study	Species	NOAEL (mg/kg/day)	Exposure (AUC) at NOAEL (ng•hr/mL)	Multiples of MRHD[a]
13-week oral	Rat	M: 10 F: 10	M: 14,000 F: 26,600	M: 1 F: 1.8
26-week oral	Rat	M: 3 F: 3	M: 6510 F: 17,200	M: 0.5 F: 1.2
13-week oral	Dog	M: 10[b] F: 10[b]	M: 11,000 F: 6470	M: 0.8 F: 0.4
9-month oral	Dog	M: 10[b] F: 10[b]	M: 20,500 F: 12,400	M: 1.4 F: 0.9
Segment 2 Reproductive	Rat	F: 30[b]	52,600	3.7
	Rabbit	F: 3	816	0.06

a: Human exposure at MRHD (300 mg): AUC = 14,401 ng•hr/mL
b: Highest dose tested

The plasma metabolite profile in humans differs substantially from that of rats and dogs. In humans, plasma exposure to the unchanged drug is predominant, with an AUC of at least 3 times that of the major metabolites M1, M2, or M3. In contrast, the AUC for M1 in the rat and dog toxicity studies either exceeded or was similar to the AUC for unchanged drug. The proportional exposure to M1 in rats and dogs was generally increased with dose.

In summary, netupitant alone was tested orally for up to 26-weeks in rats and 9-months in dogs. Treatment with netupitant induced phospholipidosis in both rats and dogs. The clinical significance of phospholipidosis in these studies is not clear. Incubation of cultured bovine corneal fibroblasts with netupitant, M1, M2 or M3 also produced phospholipidosis. Oral toxicity studies of 13-weeks duration with the combination of netupitant and palonosetron were performed in rats and dogs. The combination of netupitant and palonosetron did not produce additional toxicity as compared to either compound given alone. Netupitant was negative in the Ames test, mouse lymphoma cell mutation assay, and rat micronucleus test. Based on the findings in the segment II embryo-fetal development study with netupitant in rabbits, AKYNZEO is

developmentally toxic and should be given a Pregnancy Category C. From a nonclinical standpoint, this NDA should be approved for the proposed indication.

In addition, an oral toxicity study with netupitant alone of at least 8 weeks duration in juvenile rats is needed to support the proposed pediatric clinical efficacy study in patients age 0 to < 17 years. The juvenile rat study should include evaluation of developmental parameters, neurobehavioral effects, and fertility. The sponsor should submit the juvenile rat study protocol for review and evaluation prior to initiation of this study. The sponsor's proposed timeline for the pediatric study plan should be adjusted according to these recommendations.

Reviewer Signature _____

Ke Zhang, Ph.D.
Pharmacologist
Division of Gastroenterology and Inborn Errors Products

Supervisor Signature_____

David B. Joseph, Ph.D.
Lead Pharmacologist
Division of Gastroenterology and Inborn Errors Products

cc:
Orig NDA 205,718
DGIEP
DGIEP/PM
DGIEP/D. Joseph
DGIEP/K. Zhang
DGIEP/R. He

R/D Init.: D. Joseph 6/17/14

12 Appendix/Attachments

Executive CAC
Date of Meeting: July 1, 2008

Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Anne Pilaro, Ph.D., DBOP, Alternate Member
Sushanta Chakder, Ph.D., DGP, Acting Supervisory Pharmacologist
Ke Zhang, Ph.D., DGP, Presenting Reviewer

Author of Draft: Ke Zhang, Ph.D.

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

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (April 2006).

IND # 73,493
Drug Name: Palonosetron HCl and Netupitant (RO0673189), oral combination
Sponsor: HelsinnHealthcare SA
Lugano, Switzerland

Background: Netupitant is a NK_1 receptor antagonist and palonosetron is a 5-HT_3 receptor antagonist. It was reported that combination of a NK_1 receptor antagonist and a 5-HT_3 receptor antagonist is more effective in the control of acute and delayed emesis as compared to 5-HT_3 receptor antagonist alone. Palonosetron (Aloxi) is an approved drug for prevention of chemotherapy-induced nausea and vomiting. The combination of Netupitant and palonosetron is being developed as a single oral dose for prevention of chemotherapy-induced acute and delayed nausea and vomiting.

Netupitant was negative in the Ames tests, in the in vitro mouse lymphoma cell mutation assay, and in the in vivo rat micronucleus test.

In the current submission, the sponsor submitted a study protocol for a 2-year carcinogenicity study with netupitant in rats and a final report of a 13-week oral toxicity study with netupitant in rats.

Rat Carcinogenicity Study Protocol and Dose Selection:

In the 13-week oral toxicity study, rats were treated with netupitant at 0, 3, 10, and 30 mg/kg/day. Treatment with netupitant decreased the terminal body weight gain by 12% in males and 19% in females in the high dose group. A slight change in the terminal body weight gain was noted in the mid dose group (10 mg/kg/day). Treatment with netutipant induced phospholipidosis in a dose dependent manner. Minimal necrosis was noted in the liver in four high dose females (none in other groups). Based on the effects on the body weight, the MTD is estimated to be between doses of 10 and 30 mg/kg/day. The decreasing terminal body weight gain at the high dose of 30 mg/kg/day in males (12%) suggests that the MTD is about 30 mg/kg/day. There was no other dose limiting toxicity found in males at this dose. Therefore, the dose of 30 mg/kg/day is considered as MTD for males.

Executive CAC Recommendations and Conclusions:

The Committee noted that this indication would not normally trigger the need for a carcinogenicity study; however, a carcinogenicity evaluation may be needed to support other indications.

The Committee recommended doses of 0 (water gavage), 0 (vehicle), 2, 6, and 20 mg/kg/day for females and 0 (water gavage), 0 (vehicle), 3, 10, and 30 mg/kg/day for males, by oral gavage, based on MTD (decreased body weight gain).

The Committee recommended that the sponsor use both untreated (water gavage) and vehicle controls.

Hematology and clinical chemistry are generally not needed for 2-year carcinogenicity studies. Main study animals should not be bled during the study.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, DGP
/Grewal, DGP
/Chakder, DGP
/Zhang, DGP
/ASeifried, OND IO

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

/s/

KE ZHANG
06/18/2014

DAVID B JOSEPH
06/19/2014
I concur.

Comments on N205718 netupitant and palanosetron, Akynzeo

From: A. Jacobs, AD

Date: 6/18/14

1. I concur that there are no pharm/tox approval issues.

2. I concur with the pregnancy category of C

3. I have conveyed other comments to the reviewer, and they will be addressed as appropriate.

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

/s/

ABIGAIL C JACOBS
06/19/2014