CENTER FOR DRUG EVALUATION AND RESEARCH

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

NDA 205-718
Office of Clinical Pharmacology Review_PART2

INDIVIDUAL STUDY RESULTS and REVIEWER's COMMENTS

Study NETU-09-21: Oral ADME Study

Title of the Study
An Open Label, Single Dose Study in Healthy Male Subjects Designed to Assess the Mass Balance Recovery, Pharmacokinetics, Metabolite Profile and Metabolite Identification of 300 mg [^{14}C]-Netupitant

Methodology
This was a single dose study in 6 healthy male subjects. For all subjects, the mean total radioactivity recovered by 336 h was approximately 70%; therefore, subjects were required to collect feces samples for a 24 h period at home (456 to 480 h, Days 20 to 21), and both feces and urine samples for an additional 24 h period in the clinic (672 to 696 h, Days 29 to 30) to better characterize the terminal excretion of the total radioactivity. Blood, urine and feces samples were collected throughout the study for the analysis of total radioactivity, netupitant and metabolites M1, M2 and M3, characterization of metabolites and [^{14}C]-netupitant binding to plasma proteins.

PK Results
After oral administration of [^{14}C]-netupitant to healthy male subjects, netupitant was rapidly absorbed. Individual peak plasma netupitant concentrations, ranging from 99.2 to 517 ng/mL, were observed at 2 to 5.5 h post-dose (T_{max}). The actual dose of netupitant received ranged from approximately 187 to 264 mg.

The total drug-related material in plasma was higher than that of whole blood, as few subjects had detectable radioactivity levels measurable in whole blood. Mean plasma netupitant/plasma radioactivity ratios ranged from 0.13 to 0.49 over 96 h post-dose. The ratios were time dependent with values decreasing gradually beyond 24 h post-dose, indicating that the drug is being rapidly metabolized.

Exposure (AUC_{0-t}) and C_{max} geometric mean values for netupitant were approximately 34% and 41% of the exposure and C_{max} geometric mean values for total radioactivity; this difference is attributed to metabolite species. However, it should be noted that a full comparison of the netupitant and plasma radioactivity data cannot be made because of the difference between the lower limit of detection of total radioactivity and the limit of quantification of netupitant; this difference resulted in netupitant concentrations that were measurable at later sampling time points compared to total radioactivity, potentially skewing these comparisons. Further analysis of the plasma samples for the metabolites M1, M2, and M3 indicated that, on average, exposure to these metabolites was equivalent to 29%, 14%, and 33%, respectively, of the systemic exposure to netupitant; thus, these results confirm that M1, M2 and M3 are all major metabolites of

netupitant and account for >10% of parent drug-related exposure. Exposure to the additional metabolite M4, based on C_{max}, accounts for approximately 7% of parent drug exposure.

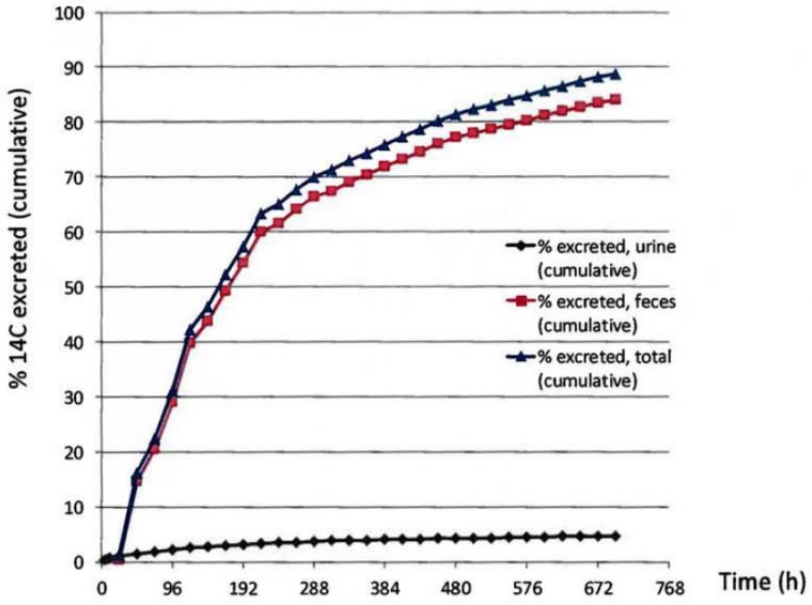
Approximately half the administered dose of radioactivity was recovered within 120 h of dosing.

Based on the total radioactivity recovered in all samples, including the additional collection periods, total radioactivity from the urine accounted for 3.95% (range 2.2% to 4.6%) of the dose and total radioactivity from the feces accounted for 70.7% (range 62.1% to 75.2%) of the dose at 696 h post-dose. These data indicate that the hepatic/biliary route, rather than renal clearance, is the major elimination route for drug- related entities.

Reviewer's comment: This recovery over 696 h post-dose is underestimated due to missing samples.

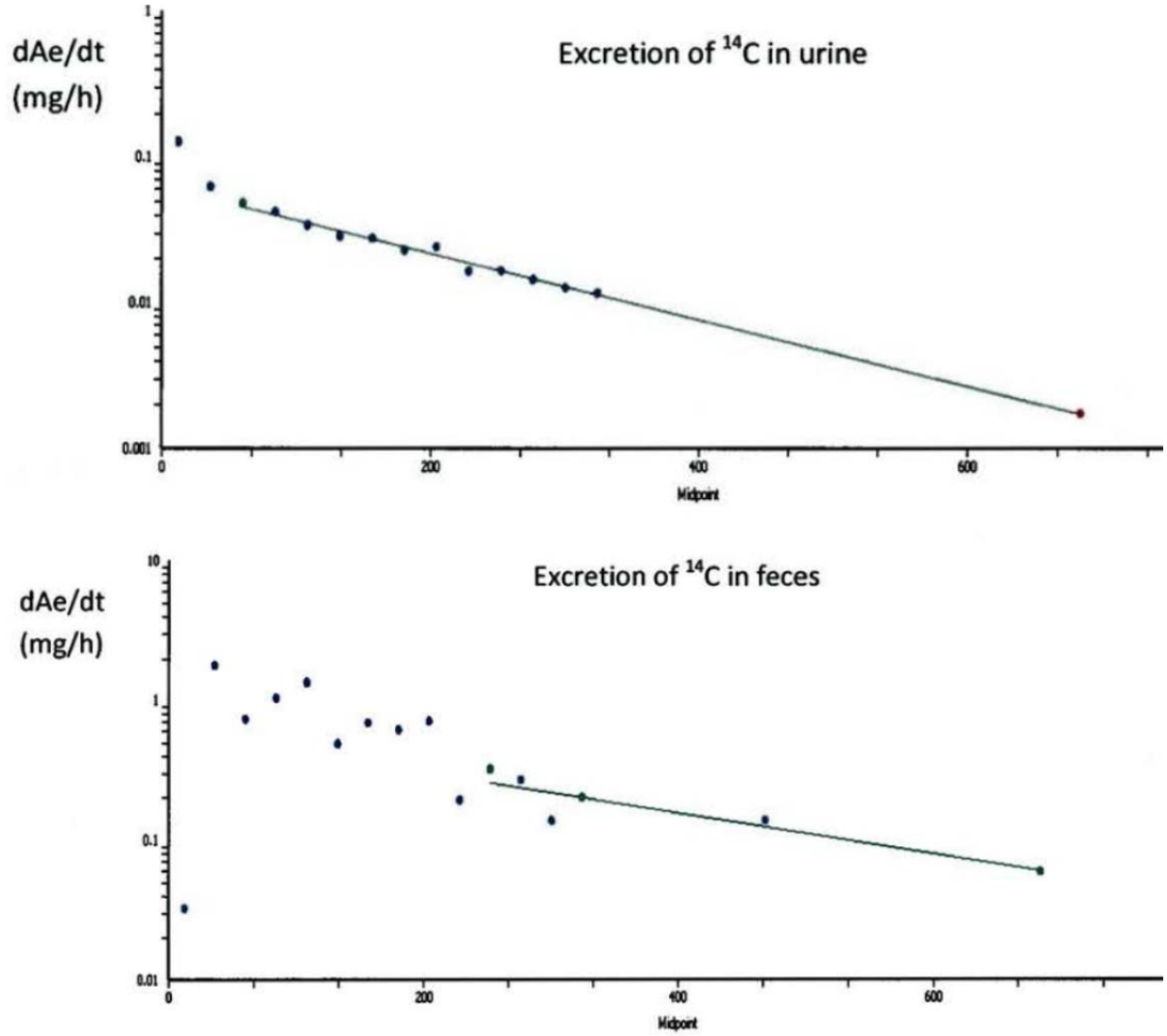
Subsequently including the extrapolated values for the periods 336 to 456 h and 480 to 672 h, the total drug-related material to have been excreted by 696 h post-dose via the feces and the urine was estimated to be 86.49% and 4.75%, respectively. Based on this the time estimated to reach 80%, 90% and 95% of excretion was 465 h, 665 h, and 866h, respectively.

Figure 1. Recover pattern of radioactivity after administration of [^{14}C] netupitant*



- Submitted in an amendment dated 5/22/14
- Values during the time from 336 to 456 h and from480-672 h were obtained by extrapolation taking the mean value of recoveries estimated in the collection intervals just prior to and just after the missing collection period as below.

Figure 2: Radioactivity excretion rates as a function of the mid-time of the sample collection intervals (A) urine data, (B) fecal data



Metabolite Identification Results

Netupitant was shown to undergo extensive metabolism, forming both phase I and phase II metabolites. Phase I metabolites observed included those formed through N- demethylation (mono and bis), mono and di-hydroxylation, N-oxidation, desaturation, N- formylation, oxidation and reduction to a keto group, and oxidation to an acid (including oxidation of the toluene methyl group to an acid). Intermediate metabolites in the 1- methylpiperazine

degradation pathway to the further oxidised 6-amino-pyridinyl derivatives were also observed. Phase II metabolites included those formed by glucuronidation and conjugation to a hexose (C6 sugar) group. A glucuronic acid conjugate of the acid ½ molecule of netupitant was also observed in urine.

Safety
One subject reported a total of 5 AEs during the study, of which 4 events (abdominal pain, diarrhea, dyspepsia and nausea) were considered IMP-related. All events were mild in severity and had resolved by the end of the study. There were no clinically significant findings in clinical laboratory assessments, vital signs parameters, ECG measurements or physical examinations.

Reviewer's comments: The actual administered dose was 200 mg although the dose of 300 was to be administered. This study shows that upon oral absorption netupitant is distributed extensively to the tissues, slowly released and metabolized over a long-period of time.

Study NP16603: Single Ascending Doses of Netupitant

Title of the Study

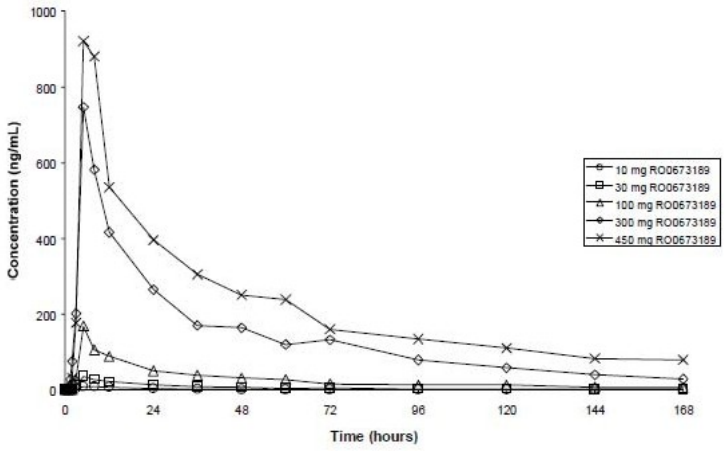
Double-blind, placebo controlled single ascending oral dose study of RO0673189 (netupitant) in healthy volunteers

Methodology
This was a single center, randomized double-blind, placebo-controlled single ascending dose study conducted in healthy males. Five dose levels of netupitant were investigated:
10, 30, 100, 300 and 450 mg. For each dose group, six subjects were randomly assigned to netupitant (4 subjects) or placebo (2 subjects).

Blood samples for pharmacokinetic analysis were collected pre-dose and at 15 and 45 min and 1, 1.5, 2, 3, 5, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144 and 168 h post-dose. Blood and urine samples for laboratory safety tests were collected at screening, pre-dose and at 24, 72 and 168 h post-dose.

PK Results
Following a lag time of up to 3 h, netupitant was absorbed in a first order fashion, with maximum plasma concentrations being reached at approximately 5 h post-dose. The terminal $t_{1/2}$ was estimated to be 30 to 60 h. The mean plasma concentration-time profiles for each dose group are shown below (Figure 1).

Figure 1 Mean Netupitant Plasma Concentration versus Time Curves



For doses up to 300 mg, there was a statistically significant over-proportional increase with dose in C_{max}, AUC_{last} and AUC_{0-}, for netupitant. Dose-proportionality was observed between the 300 mg and 450 mg doses, with ratios being close to one.

The 3 metabolites detected in animal studies, metabolites RO0681133 (M1), RO0713001 (M2) and RO0731519 (M3) were measurable with maximum metabolite plasma concentrations reaching one tenth to one fifth of parent levels, and AUC values of between one twentieth and one third of parent.

Reviewer's comment: In this study additional blood samples were taken pre-dose and 24 h post- dose for exploratory EM analysis of lamellar inclusion bodies, as a screen for phospholipidosis. This evaluation is considered only exploratory.

Study NP16601: Multiple Ascending Doses of Netupitant

Title of the Study

A Double-Blind, Randomized, Placebo-Controlled Evaluation of the Clinical Pharmacology of RO0673189 (Netupitant) Following Multiple Oral Dosing to Healthy Young And Elderly Volunteers

Methodology
Subjects fasted overnight for approximately 10 hours prior to each dose. They then received a standard breakfast which was to be consumed within 30 minutes and the study medication was administered within 5 minutes of completing the breakfast.

PK blood samples were taken at 15, 30 and 45 minutes and 1, 1.5, 2, 3, 5, 8, 12 and 24 h after dosing on Day 1 and 7. Additional samples were taken after the final dose at 36, 48, 60, 72, 96, 120, 144 and 168 h post-last dose.

Originally, the effect of age on the pharmacokinetics of netupitant was also planned to be investigated in this trial but the elderly portion of the study was discontinued and only the ascending dose portion of the study results in young healthy volunteers are presented.

PK Results
The PK data showed an increase in netupitant exposure of approximately 3-fold after 7 days of dosing consistently with the long $t_{1/2}$ of the compound. Mean maximum plasma concentrations and $AUC_{(0\text{-}23.5)}$ values recorded on Days 1 and 7 of dosing with 100, 300 or 450 mg of netupitant are shown in Table 1. Exposure to netupitant showed a slightly greater than proportional increase with dose. Low levels of the major metabolite RO068133 (M1) were detected, with levels not exceeding 30% of the parent.

Table 1 Mean Exposures on Day 1 and Day 7 following Daily Dosing with Netupitant for Seven Days

Dose	Day 1 (n=8)		Day 7 (n=8)	
	C_{max} (ng/mL)	$AUC_{(0\text{-}23.5)}$ (h.ng/mL)	C_{max} (ng/mL)	$AUC_{(0\text{-}23.5)}$ (h.ng/mL)
100mg	111 (23.1)	1360 (21.6)	269 (19.4)	4160 (24.0)
300mg	599 (38.0)	6400 (26.5)	1060 (19.0)	17100 (16.6)
450mg	720 (35.4)	9670 (34.9)	1790 (43.1)	28800(45.1)

Values are arithmetic means and coefficient of variation (CV%)

Safety
Daily doses of up to 450 mg of RO0673189 for 7 days were well tolerated in this study. The most frequent adverse event was headache. The majority of adverse events were of mild intensity and most were considered unrelated or remotely related to trial treatment. There were

no deaths or serious adverse events during the study, and no subject was withdrawn as a result of an adverse event.

Reviewer's comments:

There was a greater than dose-proportional increase in the systemic exposure as the dose increases from 100 mg to 300 mg after single and multiple doses while the dose-proportional increase in the systemic exposure was observed as the dose increases from 300 mg to 450 mg. These results suggest that doses higher than 300 mg the mechanisms that may limit the oral bioavailability are saturated.

This study provides safety information, although limited at the high systemic exposure. The mean Cmax for netupitant after multiple doses of 450 mg netupitant was about 3-fold higher than the mean Cmax after single dose administration of 300 mg netupitant. The mean AUC for netupitant after multiple doses of 450 mg netupitant was > 5-fold higher than the mean AUC observed in other PK studies.

PK in Special Populations

Study NP16600: Effects of Food and Age on Netupitant

Title of the Study

Evaluation of the Effects of Food and Age on the Pharmacokinetics of RO0673189 (Netupitant) in Healthy Volunteers

Methodology

The food effect portion of the study was an open-label, randomized cross-over study, where 12 healthy volunteers (aged 18 to 45 years) received a single oral dose of 300 mg of netupitant on 2 occasions, once under fasted conditions and once under fed conditions, with a 2-week (minimum) wash-out period between treatments.

The age effect portion of the study was a double-blind, randomized study, where 6 healthy elderly volunteers (aged 65 to 85 years) received a single oral dose of either 100 mg netupitant (4 subjects) or placebo (2 subjects).

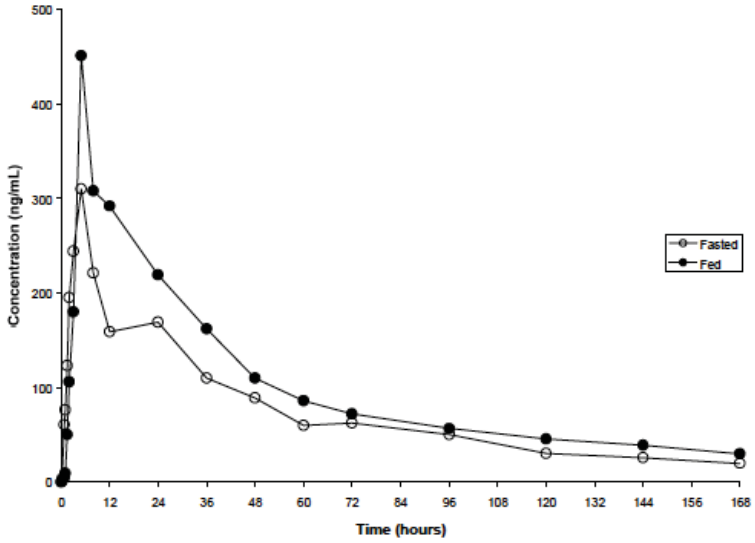
Subjects were fasted overnight (except for water) for at least 10 hours. Subjects in the fed treatment phase of the food effect part of the study and all subjects in the age effect study received a standardized breakfast prior to dosing. Subjects in the fasting treatment phase of the food effect study were administered the study medication after the overnight fast.

Blood samples for PK analysis of netupitant and its 3 main metabolites were collected pre-dose, and at 15 and 45 min and 1, 1.5, 2, 3, 5, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144 and 168 h post-dose.

PK Results

Netupitant was absorbed in a first-order fashion following oral administration. The mean plasma concentration-time profiles for fed and fasted administration are shown below (Figure 2). The peak plasma concentration and AUC, increased by between 69% (C_{max}) and 47% (AUC_{last}) on average on administration with breakfast compared to fasted administration. The bioavailability estimates and 95% confidence intervals (CIs) for treatment under fed conditions relative to fasted were 153% [122, 192] for $AUC_{0-\infty}$, 147% [117, 185] for AUC_{last} and 169% [102, 279] for C_{max}. The effect of food on the bioavailability of netupitant was highly variable between subjects, ranging from no effect to a greater than 3-fold increase. Exposure to the metabolites M1, M2 and M3 was also increased by between 5% (C_{max}, M2) and 57% ($AUC_{0-\infty}$, M1), on average, on administration with food.

Figure 2 Mean Netupitant Plasma Concentration Profiles Following Fasted and Fed Conditions in Healthy Volunteers



A single dose of 100 mg administered after food to elderly subjects (aged 66 to 70 years) resulted in similar exposure to that observed following administration of the same dose to younger volunteers (aged 23 to 44 years) in the single ascending dose study (Study NP16603). Given the wide inter-subject variability seen with this compound, there was
no significant difference between elderly subjects (in this study) and younger subjects in mean (min-max) C_{max} [elderly subjects: 136 (92 – 185) ng/mL; younger subjects (Study NP16603): 185 (144 – 224) ng/mL] or in AUC_{0-}, [4009 (3167 – 5046) h.ng/mL; younger subjects 4795 (3413 – 6284) h.ng/mL] for netupitant. These results suggest that age is unlikely to have any significant effect of the PK of netupitant. However, the number of subjects investigated was small (N=4).

Reviewer's comment:
In a definitive study with AKYNZEO, no significant food effect was observed (NETU-10-20). The effect of age on PK of netupitant is considered could not be adequately evaluated in this study due to the small number of study and the dose difference i.e.100 mg versus the proposed dose of 300 mg.

Study NETU-10-12: Effect of Food and Age on Netupitant/Palonosetron FDC Formulation

Title of Study
An Open-Label Trial to Investigate the Effect of Food and Age on the Pharmacokinetics of a Single Dose Administration of Oral Netupitant and Palonosetron (300 mg/0.5 mg) in Healthy Subjects and In Healthy Elderly Subjects

Methodology
For the investigation of the effect of food, a crossover was performed in 24 healthy male and female subjects, where drug administration in the fed state was compared to drug administration in the fasted state.

The effect of age was evaluated in a parallel group of **12** healthy elderly male and female subjects under fasted state and was compared to PK in all younger subjects from the crossover portion under fasted state.

In 1 period, the subjects received the investigational product in fasted state, and in the other period, the same subjects received the investigational product in the fed state. The parallel group of elderly subjects underwent 1 treatment period of 12 days with a single administration of the investigational product on Day 1. The subjects received the investigational product in the fasted state only.

In each treatment period, blood samples for PK analysis of netupitant and its metabolites M1, M2, and M3 were collected until 240 h after administration and blood samples for palonosetron were collected until 192 h after administration.

PK Results

Food effect. Under fed condition, the Cmax and AUC was 15-17% higher than under fasted condition. (Table 1)

Table 1 Mean ± SD Netupitant Pharmacokinetic Parameters after Oral Dose Administration of Netupitant/Palonosetron FDC (300 mg/0.5 mg) in Fed (T) and Fasted Conditions (R) and Results of Analysis of Variance

Parameter	T	R	PE%*	90%CI
C_{max} [µg/L]	649.8±141.6	596.4±233.0	117.74	100.65 - 137.74
AUC_{0-} [h·µg/L]	22391±8650	20039±8396	115.96	104.54 - 128.62
AUC_{0-tz} [h·µg/L]	19406±4919	17150±6122	117.88	106.66 - 130.27

Values are arithmetic means ±SD; *Point estimate (PE): ratio of geometric means (T/R)
CI: confidence interval, SD: standard deviation
T: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fed state (Test)

R: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fasted state (Reference)

For palonosetron, no relevant differences were observed between the fasted and fed condition (Table 2).

Table 2 Mean ± SD Palonosetron Pharmacokinetic Parameters after Oral Dose Administration of Netupitant/Palonosetron FDC (300 mg/0.5 mg) in Fed (T) and Fasted Conditions (R) and Results of Analysis of Variance

Parameter	T	R	PE%*	90%CI
C_{max} [ng/L]	767.9±159.2	785.6±223.5	99.00	93.05 - 105.33
$AUC_{0-\infty}$ [h·ng/L]	33199±6945	33645±8974	99.99	95.41 - 104.79
AUC_{0-tz} [h·ng/L]	29760±6539	30371±8416	99.29	94.51 - 104.30

Values are arithmetic means ±SD; * Point estimate (PE): ratio of geometric means (T/R)
CI: confidence interval, SD: standard deviation
T: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fed state (Test)
R: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fasted state (Reference)

Age effect.
In healthy elderly subjects, C_{max} and $AUC_{0-\infty}$ was 36% and 25% higher, respectively than in young adults (Table 3).

Table 3 Mean ± SD Netupitant Pharmacokinetic Parameters after Oral Dose Administration of Netupitant/Palonosetron FDC (300 mg/0.5 mg) in Fasted Conditions in Elderly Subjects (R+) and in Adult Subjects (R) and Results of Analysis of Variance

Parameter	R	R+	PE%*	90%CI
C_{max} [µg/L]	596.4±233.0	880.8±479.2	136.36	95.87 - 193.96
$AUC_{0-\infty}$ [h·µg/L]	20039±8396	24739±9390	124.91	95.29 - 163.75
AUC_{0-tz} [h·µg/L]	17150±6122	19604±6747	113.42	87.66 - 146.75

Values are arithmetic means ±SD; *Point estimate (PE): ratio of geometric means (R+/R)
CI: confidence interval, SD: standard deviation
R+: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fasted state to elderly subjects R: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fasted state to younger adults (Reference)

Comparison of the primary pharmacokinetic parameters C_{max} and $AUC_{0-\infty}$ of palonosetron showed a 10% higher mean Cmax in adult subjects, (90% CI from 95.96% to 127.11%) and a 37% higher mean AUC (90% CI from 117.44% to 159.56%) in elderly subjects compared to adult subjects. (Table 4).

Table 4 Mean ± SD Palonosetron Pharmacokinetic Parameters after Oral Dose Administration of Netupitant/Palonosetron FDC (300 mg/0.5 mg) in Fasted Conditions in Elderly Subjects (R+) and in Adult Subjects (R) and Results of Analysis of Variance

Parameter	R	R+	PE%*	90%CI
C_{max} [ng/L]	785.6±223.5	851.2±146.3	110.44	95.96 - 127.11
$AUC_{0-\infty}$ [h·ng/L]	33645±8974	45047±7903	136.89	117.44 - 159.56
AUC_{0-tz} [h·ng/L]	30371±8416	39577±6617	133.81	114.28 - 156.68

Values are arithmetic means ±SD; *Point estimate (PE): ratio of geometric means (R+/R)
CI: confidence interval, SD: standard deviation
R+: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fasted state to elderly subjects
R: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fasted state to younger adults (Reference)

Conclusions

Food-effect.
A high fat, high-caloric breakfast led to a delay in absorption of netupitant with an increase in exposure of about 16% for $AUC_{0-\infty}$ and about 18% for AUC_{0-tz} and C_{max}; however, the increase in netupitant exposure to this degree is considered clinically insignificant.

For palonosetron, the exposure was not affected by food. Based on the slight increase in netupitant exposure following food administration and the lack of effect of food on palonosetron concentrations, the FDC can be administered without regard to food.

Age effect. The effect of age on the pharmacokinetic parameters of netupitant and palonosetron was compared between 22 healthy adult subjects (22 and 45 years old) and 2 healthy elderly subjects (66 and 79 years old).

The exposure to netupitant and palonosetron was higher in elderly subjects with an increase of about 25% and 37% for $AUC_{0-\infty}$, about 13% and 34% for AUC_{0-tz}, and about 36% and 10% for C_{max}. An increase in exposure to both netupitant and palonosetron is not expected to be clinically relevant; therefore, no dosage adjustment is indicated for elderly subjects.

Reviewer's comments: The study results and conclusions are acceptable. In the tQT study, a single dose combination of 600 mg netupitant and 0.5 mg palonosetron was well tolerated.

Study NETU-10-10: Patients with Hepatic Impairment

Title of the Study

Pharmacokinetics of a Single Dose of Netupitant and Palonosetron Fixed-Dose Combination Capsules in Patients with Different Stages of Hepatic Impairment Based on Liver Cirrhosis Classified By Child-Pugh Score in Comparison to Healthy Volunteers

Methodology

This study was conducted in a single center according to an open label, 1-period, non-randomized study design. A maximum of 48 subjects were planned to be enrolled: 24 subjects (8 per group) with hepatic impairment classified by Child-Pugh scoring system as mild (Child-Pugh 5-6), moderate (Child Pugh 7-9) and severe (Child Pugh 10-15) and 24 healthy subjects (8 per group) matched to the subjects with hepatic impairment by age, weight and gender.

A single dose of netupitant/palonosetron FDC (300 mg/0.5 mg) was administered on Day 1 after an overnight fast of at least 10 hours. Blood samples for PK of netupitant and its metabolites M1, M2 and M3 were collected from pre-dose through 240 hours post-dose and blood samples for palonosetron PK were collected from pre-dose through 192 hours post-dose.

Subjects were discharged on Day 5 after blood sampling and returned on Days 7, 9 and 11 for PK sampling and safety measurements. Final check was performed on Day 11.

PK Results

In subjects with mild hepatic impairment, exposure to netupitant was slightly higher compared to matching healthy subjects with an increase of 11% for C_{max}, 28% for $AUC_{0\text{-}tz}$, and 19% for $AUC_{0\infty}$. The mean coefficient of variation of C_{max} was 65.7% in the group of subjects with mild hepatic impairment and 22.7% in the group of matching healthy subjects. The observed increase in exposure of netupitant was not statistically significant.

The formation of metabolite M1 was slightly delayed in mild hepatic impaired subjects compared to the matched healthy cohort (median T_{max} of 10 h versus 8 h). Mild hepatic impairment did not, however, have an impact on the time of appearance of the other netupitant metabolites M2 and M3 in plasma. In mild hepatic impaired subjects, exposure to metabolite M1 was reduced, and exposure to metabolite M2 was increased. For metabolite M3, a reduced maximum concentration and an increased total exposure was observed.

In subjects with mild hepatic impairment, maximum concentrations of palonosetron were slightly higher compared to matching healthy subjects with an increase of 14% for C_{max}. The increase was not statistically significant. Total exposure was significantly higher in mild hepatic impaired subjects compared to matching healthy subjects with an increase of 35% for $AUC_{0\text{-}tz}$ and 33% for $AUC_{0\text{-}\infty}$, the respective 90% CIs were 109% to 169% for $AUC_{0\text{-}tz}$, and 107% to 167% for $AUC_{0\text{-}\infty}$. (Table 1 and Table 2)

Table 1 Overview of the Pharmacokinetic Characteristics of Netupitant and Results of Statistical Analysis - Mild Hepatic Impairment

	Mild Hepatic Impairment[1] N=8	Normal Hepatic Function[1] N=8	Point Estimate Mild / Normal Ratio [%]	Lower Limit of 90% CI[2] [%]	Upper Limit of 90% CI[2] %]
C_{max}	.0±304.9 (65.7)	344.9±78.3 (22.7)	111.12	69.57	177.50
AUC_{0-tz}	87±8683 (52.0)	12486±5294 (42.4)	128.43	86.44	190.82
$AUC_{0-\infty}$	68±11824 (54.8)	58±21398 (101.6)	119.14	70.87	200.29

1 Values are arithmetic mean±standard deviation (coefficient of variation %)
2 Pre-specified no-effect limit for the confidence interval (CI): 80% to 125%

Table 2 Overview of the Pharmacokinetic Characteristics of Palonosetron and Results of Statistical Analysis- Mild Hepatic Impairment

	Mild Hepatic Impairment1 N=8	Normal Hepatic Function1 N=8	Point Estimate Mild / Normal Ratio [%]	Lower Limit of 90% CI [%]	Upper Limit of 90% CI %]
C_{max}	.1±270.4 (34.0)	673.5±137.2 (20.4)	113.86	92.30	140.46
AUC_{0-tz}	65±11669 (32.4)	26787±9273 (34.6)	135.45	108.66	168.84
$AUC_{0-\infty}$	63±12739 (31.3)	30627±9923 (32.4)	133.48	106.82	166.79

1 Values are arithmetic mean±standard deviation (coefficient of variation %)

In subjects with moderate hepatic impairment, exposure to netupitant was significantly higher compared to matching healthy subjects with an increase of 70% for C_{max}, 88% for AUC_{0-tz}, and 143% for $AUC_{0-\infty}$, the respective 90% CIs were 106% to 271% for C_{max}, 127% to 280% for AUC_{0-tz}, and 145% to 409% for $AUC_{0-\infty}$. The netupitant exposure parameters exhibited high variability in subjects with moderate hepatic impairment and moderate variability in matching healthy subjects (Table 3).

In moderate hepatic impaired subjects, the formation of metabolite M1 was delayed (median T_{max} of 10 h vs. 7 h), and the formation of metabolites M2 and M3 was accelerated (median T_{max}: 3 h vs. 5 h and 6 h vs. 12 h, respectively) compared to matching healthy subjects. In subjects with moderate hepatic impairment, maximum concentration and exposure (C_{max}, AUC_{0-tz}) to metabolite M1 was reduced, whereas for metabolite M2 and M3, C_{max} and AUC_{0-tz} were increased.

In subjects with moderate hepatic impairment, maximum concentration of palonosetron was similar to that of matching healthy subjects. Total exposure was significantly higher in moderate hepatic impaired subjects compared to matching healthy subjects with an increase of 60% for AUC_{0-tz} and 62% for AUC_{0-} , the respective 90% CIs were 129% to 200% for AUC_{0-tz}, and 129% to 202% for $AUC_{0-\infty}$. (Table 4)

Table 3 Overview of the Pharmacokinetic Characteristics of Netupitant and Results of Statistical Analysis- Moderate Hepatic Impairment

	Moderate Hepatic Impairment[1] N=8	Normal Hepatic Function N=8	Point Estimate Moderate / Normal Ratio [%]	Lower Limit of 90% CI[2][%]	Upper Limit of 90% CI[2] %]
C_{max}	.9±304.3 (68.9)	239.0±100.0 (41.8)	169.93	106.38	271.43
AUC_{0-tz}	88±9794 (53.0)	83±2896 (31.5)	188.32	126.75	279.81
AUC_{0-}	81±15495 (55.2)	10312±2881 (27.9)	243.15	144.64	408.77

1 Values are arithmetic mean±standard deviation (coefficient of variation %)
2 Pre-specified no-effect limit for the confidence interval (CI): 80% to 125%

Table 4 Overview of the Pharmacokinetic Characteristics of Palonosetron and Results of Statistical Analysis- Moderate Hepatic Impairment

	Moderate Hepatic Impairment[1] N=8	Normal Hepatic Function N=8	Point Estimate Moderate / Normal Ratio [%]	Lower Limit of 90% CI[%]	Upper Limit of 90% CI %]
C_{max}	7.6±124.0 (17.8)	712.6±163.2 (22.9)	98.59	79.91	121.62
AUC_{0-tz}	81±12066 (27.2)	35±12401 (43.3)	160.18	128.50	199.67
AUC_{0-}	48±15030 (29.0)	53±13230 (40.4)	161.80	129.48	202.18

1 Values are arithmetic mean±standard deviation (coefficient of variation %)

In subjects with severe hepatic impairment, maximum concentration and exposure to netupitant were higher compared to matching healthy subjects with an increase of 81% for C_{max}, 101% for AUC_{0-tz}, and 144% for AUC_{0-}.

The observed increase in exposure of netupitant was not statistically significant. It should be noted, however, that the small sample size and variability in the data may preclude a definitive

conclusion on these data (Table 5).

In severely hepatic impaired subjects, the formation of metabolite M2 was slightly accelerated compared to matching healthy subjects (2 and 3 h vs. 4.5 h). An impact of severe hepatic impairment on the formation of metabolites M1 and M3 could not be observed due to the high variability of data and the small sample size. No clear trend was observed for the effect of hepatic impairment on the exposure of metabolite M1, M2, and M3 due to the high variability of data and the small sample size.

In subjects with severe hepatic impairment, maximum concentration and exposure to palonosetron were lower compared to matching healthy subjects with a decrease of 31% for $C_{max,}$ 9% for $AUC_{0\text{-}tz,}$ and 18% for $AUC_{0\text{-}\infty}$. The decrease in exposure of palonosetron was not statistically significant. It should be noted, however, that the small sample size and variability in the data may preclude a definitive conclusion on these data (Table 6).

Table 5 Overview of the Pharmacokinetic Characteristics of Netupitant and Results of Statistical Analysis- Severe Hepatic Impairment

	Severe Hepatic Impairment[1] N=8	Normal Hepatic Function[1] N=8	Point Estimate Severe / Normal Ratio [%]	Lower Limit of 90% CI[2] [%]	Upper Limit of 90% CI[2] %]
C_{max}	469.6-1336	266.1-720.4	180.90	70.90	461.55
$AUC_{0\text{-}tz}$	21179-44845	10953-21506	200.80	90.95	443.28
$AUC_{0\text{-}\infty}$	26857-70952	13176-24180	244.57	86.54	691.18

1 Values are arithmetic mean±standard deviation (coefficient of variation %)
2 Pre-specified no-effect limit for the confidence interval (CI): 80% to 125%

Table 6 Overview of the Pharmacokinetic Characteristics of Palonosetron and Results of Statistical Analysis- Severe Hepatic Impairment

	Severe Hepatic Impairment[1] N=8	Normal Hepatic Function[1] N=8	Point Estimate Severe / Normal Ratio [%]	Lower Limit of 90% CI[2] [%]	Upper Limit of 90% CI[2] %]
C_{max}	399.1-1019	829.6-1030	68.97	45.32	104.97
$AUC_{0\text{-}tz}$	28142-65203	29423-75525	90.87	58.48	141.19
$AUC_{0\text{-}\infty}$	32029-69539	34116-96841	82.11	52.58	128.20

1 Values are arithmetic mean±standard deviation (coefficient of variation %)
2 Pre-specified no-effect limit for the confidence interval (CI): 80% to 125%

Reviewer's comments

The sponsor calculated the ratio of PK parameters between subjects with hepatic impairment and matching control group i.e. one group for mild hepatic impairment and another group for moderate hepatic impairment. Upon review of the data, the demographic information such as age and gender was similar across control groups to different degree of hepatic impairment while the PK parameters for netupitant showed differences among controls due to the variability among control groups. This variability between control groups confounded the evaluation of the effect of hepatic impairment on the PK of netupitant. Therefore PK parameters from patients with hepatic impairment were compared to the pooled control group. One healthy subject had a substantially high AUC for netupitant, that was similar to the highest AUC observed in a patient with severe hepatic impairment. The AUC was not considered reliable due to ~75% extrapolation for AUCi and excluded from the control group.

The mean AUC of netupitant was 58% and 101% higher in patients with mild and moderate hepatic impairment, respectively than in healthy subjects. The Cmax of netupitant was about 30% higher in patients with mild and moderate hepatic impairment. Only two patients with severe hepatic impairment provided PK data. In one patient with severe hepatic impairment, Cmax and AUC of netupitant were about 2- and 6-fold higher, respectively while Cmax and AUC of palonosetron were about 2-fold higher than the mean for control group.

The 2-fold higher AUC for netupitant was within the 2-fold higher AUC at 600 mg netupitant compared to that of 300 mg netupitant in the tQT study.

Table 7 Geometric mean and ratio of PK parameters for netupitant in subjects with hepatic impairment and healthy subjects

Hepatic impairment	Normal1 N=18	Mild (n=8)	Moderate (n=8)	Severe (n=2)	Mean ratio[%] mild/ normal	Mean ratio[%] moderate/ normal
Cmax	288	374	377	469.6, 1336	130	131
AUC0-∞	11712	18475	24282	26857, 70952	158	207

PK blood samples for netupitant were collected up to 240 hours post-dose

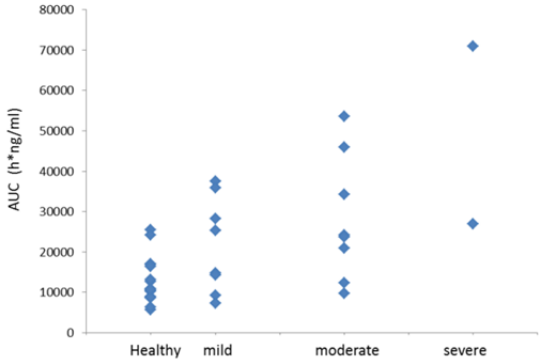
Table 8 Geometric mean of PK parameters for palonosetron in subjects with hepatic impairment and healthy subjects

Hepatic impairment	Normal1 N=18	Mild (n=8)	Moderate (n=8)	Severe (n=2)	Mean ratio[%] mild/ normal	Mean ratio[%] moderate / normal
Cmax	511	753	687	399, 1018.5	147	135
AUC0-∞	32210	38913	49819	32028, 69538	135	155

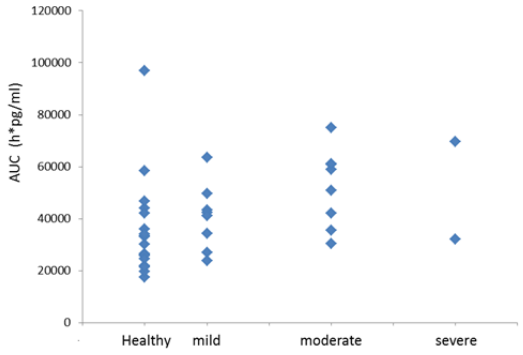
PK blood samples for palonosetron were collected up to 192 hours post-dose.

Figure 1 Individual AUCinf for (A) netupitant and (B) palonosetron in patients with hepatic impairment and in healthy subjects

(A) netupitant



(B) palonosetron



Drug-Drug Interaction Studies

Study NP16599: Netupitant with Midazolam and Erythromycin

Title of the Study

Impact of RO0673189 (Netupitant) on the Pharmacokinetics of Midazolam and Erythromycin, Two CYP3A4 Substrates, in Healthy Volunteers

Objectives

This study was designed to assess the impact of netupitant on the PK of midazolam and erythromycin, and the impact of these agents on netupitant.

Methodology

In every period, subjects fasted overnight for approximately 10 hours and received a standard breakfast prior to drug administration (days 1, 22 and 43). Study medication was administered with approximately 200 mL of water within 5 minutes of completing breakfast.

For subjects taking erythromycin or midazolam alone (period I for groups A and C, and period II for groups B and D), blood samples for pharmacokinetic analysis were taken pre-dose and at 15, 30 and 45 min and 1,1.5, 2, 3, 4, 6, 8, 12, 24, 36 and 48 h post-dose.

For subjects taking netupitant alone and netupitant in combination with erythromycin or midazolam (period I for groups B and D, period II for groups A and C and period III for all subjects), blood samples for pharmacokinetic analysis were taken as above with additional samples at 72, 96, 120, 144 and 168 h post-dose.

PK Results

The systemic exposure to the CYP3A4 substrate midazolam was significantly increased then taken in combination with netupitant compared to administration of midazolam alone, with C_{max} increasing by approximately 40% and $AUC_{0-\infty}$ by approximately 144% (Table 1).

Similar results were seen for erythromycin, with exposure as judged by C_{max} and $AUC_{0-\infty}$ approximately 30% higher following administration with netupitant compared to erythromycin taken alone. The mean (± SD) exposure parameters C_{max} and $AUC_{0-\infty}$ for each of the treatments are shown in Table 2.

Table 1 Mean (± SD) Pharmacokinetic Parameters Showing Exposure to Netupitant and Erythromycin Taken Alone and in Combination

Parameter	Midazolam			
	Midazolam alone	With netupitant	PE%*	90%CI
Cmax [µg/L]	29.1± 13.9	40.6± 20.2	136	116-159
AUC0-∞ [h·µg/L]	122± 47.2	298± 162	226	189-270
	Erythromycin			
	Erythromycin alone	With netupitant	PE%*	90%CI
Cmax [ng/L]	766± 780	985± 656	192	102-363
AUC0-∞ [h·ng/L]	2240± 1730	2890± 1720	156	80.4-302

*Point estimate (PE): ratio of geometric means (T/R) and 90% confidence interval (CI)

7.5 mg oral midazolam (tablet) (reference)
7.5 mg oral midazolam + netupitant 300mg (2 capsules of 150 mg each) (test)

500 mg oral erythromycin (tablet) (reference)
500 mg oral erythromycin +netupitant 300mg (2 capsules of 150 mg each) (test)

Reviewer's comments:
These results indicate that netupitant is a moderate inhibitor of CYP3A4 in vivo.

Study NETU-06-27: Netupitant with Palonosetron

Title of Study

Evaluation of Pharmacokinetic Interaction between Netupitant (450 mg, PO) and Palonosetron (0.75 mg, PO): a Randomized 3-way Crossover Study in Healthy Males and Females

Methodology

This was a randomized, open-label, single-dose, 3-period crossover study investigating 3 treatments:

Treatment A: oral netupitant 450 mg administered as single dose

Treatment B: oral palonosetron 0.75 mg and oral netupitant 450 mg administered simultaneously

Treatment C: oral palonosetron 0.75 mg administered as single dose

A total of 18 subjects (9 males and 9 females) were included in the study and randomized to treatment sequence. Each subject was to receive 1 of the 3 treatments during each of the 3 treatment periods.

The subjects fasted overnight (for approximately 10 hours) before dose administration on Day 1 in each treatment period. Fasting continued for 4 hours after dose administration. Water was allowed during fasting, except for 1 hour before and after dose administration.

In addition, during Treatment A only (netupitant single dose), fractional urine collection was performed. The subjects were discharged after collection of the 24 hour post-dose PK sample(s). The subjects then returned to the investigational site for PK blood sampling and delivery of collected urine every 24 hours until 240 hours post-dose (Day 11). There was a minimum wash-out of 14 days between Day 1 of any 2 consecutive treatment periods.

PK Results

Netupitant PK. The exposure to netupitant, in terms of C_{max} and AUC, was similar after administration of netupitant alone and in combination with palonosetron to healthy male and female volunteers. In addition, the 90% confidence intervals for the treatment geometric mean ratios of C_{max} and AUC for netupitant were contained within the equivalence range of 80-125%. Median T_{max}, reflecting rate of exposure, and median apparent $T_{½,z}$ of netupitant were not affected by palonosetron.

The extent of exposure to M3, which is pharmacologically equipotent to netupitant, was about 33% of the exposure to netupitant in terms of $AUC_{0-\tau}$, while it was 32% and 12% for M1 and M2, respectively. The pharmacokinetic parameters of the netupitant metabolites M1, M2 and M3 were similar after administration of netupitant alone and in combination with palonosetron. There were

neither any consistent nor relevant gender effects for M1, M2 or M3. Overall, palonosetron had no relevant impact on the pharmacokinetics of netupitant metabolites.

A very low fraction of the oral dose of netupitant was excreted unchanged into urine (mean fe was 0.03%).

There were no consistent indications of any gender effects for the pharmacokinetics of netupitant.

Palonosetron PK. The exposure to palonosetron, in terms of C_{max} and AUC, was similar after administration of palonosetron alone and in combination with netupitant to healthy male and female volunteers.
In general, the ratios indicate that the exposure to palonosetron was slightly higher in subjects treated with combination therapy compared to palonosetron alone, but not clinically relevant according to bioequivalence standards. The pharmacokinetic parameters obtained for palonosetron in this study (e.g. mean apparent $T_{1/2,z}$ and median T_{max}), were comparable to those obtained in previous oral single dose studies.

Consistent with other studies for palonosetron, mean C_{max} and mean AUC of palonosetron were 35-65% higher and median apparent T½,z was 15-47% longer in female subjects (35 hours after palonosetron alone and 47 hours after palonosetron + netupitant) compared to male subjects (30 hours after palonosetron alone and 32 hours after palonosetron + netupitant).

The extent of exposure to palonosteron metabolites M4 and M9, which have negligible pharmacologic activity, was 9 and 6%, respectively, of the exposure to palonosetron in terms of $AUC_{0-\infty}$. Overall, the pharmacokinetics of M4 were similar after administration of palonosetron alone and in combination with netupitant. For M9, mean C_{max} was 28% higher after the concomitant administration of palonosetron and netupitant. There were no apparent gender effects for M4 whereas mean AUC_{0-t} of M9 was 58-64% higher and median apparent $T_{1/2z}$ was 52-194% longer in females compared to males.

Table 1 Summary of Netupitant and Palonosetron Pharmacokinetic Parameters by Treatment

Treatment	Netupitant					Palonosetron				
	C_{max} (µg/L)		$AUC_{0-\infty}$ (h*µg/L)		$T_{1/2}$ (h)	C_{max} (ng/L)		$AUC_{0-\infty}$ (h*ng/L)		$T_{1/2}$ (h)
	Mean (SD)	Geo. Mean (CV%)	Mean (SD)	Geo. Mean (CV%)	Median	Mean (SD)	Geo. Mean (CV%)	Mean (SD)	Geo. Mean (CV%)	Median
Netu450 mg (N=18)	650.2 (257.8)	575.1 (39.6)	25927 (10156)	24000 (39.2)	71.81	-	-	-	-	-

Palo 0.75 mg +Netu 450 mg (N=18)	659.7 (325.7)	560.0 (49.4)	26241 (13219)	23182 (50.4)	78.31	1863.1 (487.1)	1799.9 (26.1)	77254 (25402)	72596 (32.9)	36.91
Palo 0.75 mg (N=17)	-	-	-	-	-	1638.4 (415.5)	1587.2 (25.4)	70813 (20415)	67593 (28.8)	34.73

Reviewer's comment
The results of this study indicated that no relevant pharmacokinetic interaction is expected when 300 mg netupitant and 0.5 mg palonosetron are administered as a combination therapy. The fraction of an oral dose of netupitant excreted unchanged in urine was very low (less than 1%). While it is unknown if the outpatient based collection of urine samples may have affected the results, the observed negligible excretion to urine is consistent with the known elimination pathway and the findings from the ADME study.

Study NETU-06-07: Netupitant with Oral Dexamethasone

Title of Study

Evaluation of Pharmacokinetic Interaction Between Three Doses of Oral Netupitant and Oral Dexamethasone Regimen: a Randomized Three Period Crossover Study in Healthy Males and Females

Methodology

This was a randomized, open, 3-period crossover study utilizing an incomplete Latin Square design. A total of 30 male and female subjects were to be randomized to 1 of the 3 treatment sequences ABC, BDA or CAD, corresponding to the following treatments:

Treatment A: dexamethasone regimen alone (20 mg on Day 1, followed by 8 mg b.i.d. [every 12 hours] from Day 2 to Day 4)

Treatment B: dexamethasone regimen (20 mg on Day 1, followed by 8 mg b.i.d. [every 12 hours] from Day 2 to Day 4) plus oral netupitant 100 mg on Day 1 only.

Treatment C: dexamethasone regimen (20 mg on Day 1, followed by 8 mg b.i.d. [every 12 hours] from Day 2 to Day 4) plus oral netupitant 300 mg on Day 1 only.

Treatment D: dexamethasone regimen (20 mg on Day 1, followed by 8 mg b.i.d. [every 12 hours] from Day 2 to Day 4) plus oral netupitant 450 mg on Day 1 only.

Ten subjects (5 males and 5 females) were to be randomized to each treatment sequence. In each treatment period the subjects fasted overnight (for approximately 10 hours before dose administration) and up to 4 hours after dose administration on Day 1.

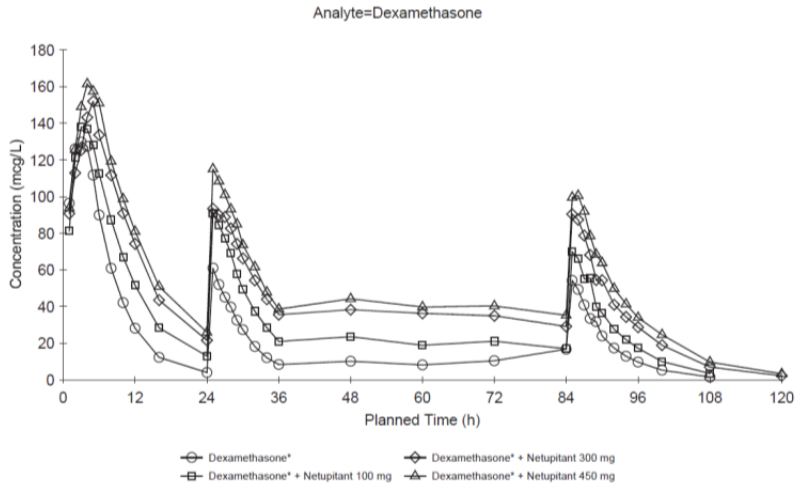
Repeated PK blood sampling (determination of netupitant and dexamethasone) was performed

up to the 120 hour post-dose. There was a wash-out of no less than 14 days between Day 1 of 2 consecutive treatment periods.

PK Results

Dexamethasone. Co-administration of netupitant significantly increased the exposure to dexamethasone in a dose- and time-dependent manner. The mean plasma concentrations of dexamethasone when coadministered with netupitant are shown in Figure 1.

Figure 1 Arithmetic Mean Plasma Concentration of Dexamethasone versus Planned Time, by Treatment



* Dexamethasone 20 mg on Day 1, followed by 8 mg b.i.d. (every 12 hours) from Day 2 to Day 4

Pharmacokinetic parameters for dexamethasone alone and after 100, 300 and 450 mg of netupitant are shown below in Table 1. The AUC_{0-24} (Day 1) of dexamethasone increased 1.5, 1.7 and 1.8-fold with co-administration of 100, 300 and 450 mg netupitant, respectively. The AUC_{24-36} (Day 2) and of dexamethasone increased 2.1, 2.4 and 2.6-fold and AUC_{84-108} and $AUC_{84-\infty}$ (Day 4) increased 1.7, 2.4 and 2.7-fold, with coadministration of 100, 300 and 450 mg netupitant, respectively.

Dexamethasone C_{max} on Day 1 was only slightly affected by co-administration of netupitant (1.1–1.2-fold increase during co- administration with 100–450 mg netupitant) while C_{max} on Day 2 and Day 4 was increased approximately 1.7-fold in subjects administered netupitant.

Dexamethasone C_{min} on Days 2–4 was increased approximately 2.8, 4.3 and 4.6-fold with coadministration of 100, 300 and 450 mg netupitant, respectively. The $T_{½,z}$ of dexamethasone was increased by 1.9–3.2 hours on Day 1 and by 2.0–2.4 hours on Day 4.

There was no relevant change in T_{max} for dexamethasone when administered in

combination with netupitant. There was no relevant gender effect for AUC or C_{min} but C_{max} was slightly higher in female subjects.

Table 1 Summary of the Pharmacokinetic Parameters for Dexamethasone

Parameter	Dexamethasone Alone (N=22)	Dexamethasone + Netu 100 mg (N=15)	Dexamethasone + Netu 300 mg (N=13)	Dexamethasone + Netu 450 mg (N=16)
AUC_{0-24} [h* µg/L]	1089 (352)	1444 (320)	1782 (369)	1984 (430)
AUC_{24-36} [h*µg/L]	330 (126)	600 (90)	760 (174)	871 (159)
AUC_{84-108} [h*µg/L]	364 (157)	558 (137)	836 (221)	1005 (252)
$AUC_{84-\infty}$ [h*µg/L]	390 (174)	599 (166)	913 (251)	1119 (308)
$C_{max\ (0-24h)}$ (µg/L)	156.5 (38.6)	161.2 (32.0)	169.9 (26.9)	190.4 (35.5)
$C_{max\ (24-36h)}$ (µg/L)	62.7 (19.6)	94.9 (16.4)	100.3 (26.1)	118.4 (27.4)
$C_{max\ (84-108h)}$ (µg/L)	58.2 (18.6)	80.7 (29.0)	96.2 (26.0)	110.0 (29.8)
$C_{min\ (24-36h)}$ (µg/L)	8.4 (6.7)	21.0 (6.1)	35.7 (10.3)	38.7 (8.9)
$C_{min\ (36-48h)}$ (µg/L)	10.2 (7.1)	23.6 (7.0)	38.3 (10.8)	44.3 (10.6)
$C_{min\ (48-60h)}$ (µg/L)	8.2 (6.8)	18.9 (6.4)	36.3 (11.4)	39.8 (12.5)
$C_{min\ (60-72h)}$ (µg/L)	10.5 (7.1)	21.1 (5.3)	35.0 (10.0)	40.4 (11.7)
$C_{min\ (72-84h)}$ (µg/L)	16.8 (41.3)	17.0 (7.0)	29.2 (9.9)	35.3 (11.9)
$T_{max\ (0-24h)}$ [h]	3.00 (1.00 ; 5.00)	4.00 (2.00 ; 6.00)	4.00 (1.00 ; 5.08)	4.00 (1.00 ; 6.00)
$T_{max\ (24-36h)}$ [h]	1.00 (0.98 ; 4.00)	2.00 (0.98 ; 3.00)	1.97 (0.97 ; 5.00)	1.01 (0.98 ; 5.00)
$T_{max\ (84-108h)}$ [h]	1.01 (1.00 ; 5.00)	1.02 (1.00 ; 6.00)	2.00 (1.00 ; 3.00)	1.50 (0.98 ; 3.00)
$T_{½,z\ (Day\ 1)}$ [h]	3.66 (2.67 ; 6.99)	5.50 (4.12 ; 7.72)	6.52 (4.70 ; 7.72)	7.50 (5.47 ; 8.31)
$T_{½,z\ (Day\ 4)}$ [h]	4.42 (3.21 ; 7.30)	4.73 (3.81 ; 7.98)	6.45 (4.29 ; 7.63)	6.83 (5.19 ; 9.66)

Mean and SD are shown, except for T_{max} and $T_{½,z}$ where median and range are shown.

Netupitant. The extent of exposure to netupitant increased in a higher than proportional manner in the studied dose range of 100 to 450 mg. The dose normalized AUC_{0-t} and $AUC_{0-\infty}$ were increased higher than proportionally by 34 and 38%, respectively, after 450 mg netupitant. The increase in C_{max} was approximately dose-proportional. PK parameters for netupitant were comparable to results obtained in previous studies: the PK profile of netupitant was not significantly altered in the presence of dexamethasone. There were no indications of any gender effect on the PK parameters for netupitant.

Table 2. Summary of PK parameters for netupitant

Parameter	Dexamethasone + Netu 100 mg (N=15)	Dexamethasone + Netu 300 mg (N=13)	Dexamethasone + Netu 450 mg (N=16)
AUC_{0-24} [h*mcg/L]	1947 (563)	6846 (2099)	10730 (4521)
AUC_{0-t} [h*mcg/L]	3143 (841)	12350 (3566)	21334 (7007)
AUC_{0-inf} [h*mcg/L]	3464 (924)	13967 (4311)	25881 (9965)
C_{max} [mcg/L]	221.4 (61.3)	671.5 (242.4)	1183.5 (640.8)
t_{max} (h)	4.00 (3.00 ; 5.50)	4.50 (3.00 ; 5.50)	4.50 (4.00 ; 12.00)
$t_{½,z}$ (h)	42.85 (33.78 ; 91.40)	47.03 (32.76 ; 60.64)	56.90 (22.48 ; 105.95)

Mean and SD are shown, except for t_{max} and $t_{½,z}$ where median and range are shown.

Reviewer's comments

The mechanism of this drug interaction is most likely due to inhibition of CYP3A4.
Reduction of the dexamethasone dose is therefore recommended when netupitant is co-administered. It was noted that the inhibitory effect was significant on Day 4 after single dose administration of netupitant and the extent of inhibition was similar between on Day2 and Day4 while the plasma concentrations for netupitant were decreased suggesting potential contribution of metabolites. Analyses based on [I]/Ki values over time suggested that the sum of [I]/Ki would be decreased to below 0.1 on Day 6 after single dose administration of 300 mg netupitant.

Study NETU-07-01: Netupitant with Oral Digoxin

Title of Study

Evaluation of Pharmacokinetic Interaction Between Netupitant (450 mg PO, Single Dose) and Digoxin (0.25 mg PO, Daily): An Open-Label, One-Way Study in Healthy Males and Females

Methodology

This was an open-label study in a total of 16 healthy subjects (8 males and 8 females). Each subject received a loading dose of 3 x 0.5 mg digoxin on Day 1, followed by a daily oral dose of 0.25 mg digoxin for 11 consecutive days and 450 mg netupitant on Day 8.

Subjects were admitted to the Clinical Unit twice: on Days 1 to 2 for safety reasons during digoxin loading phase and on Days 5 to 9 (4 overnight stays) for pharmacokinetic blood sampling.

PK Results

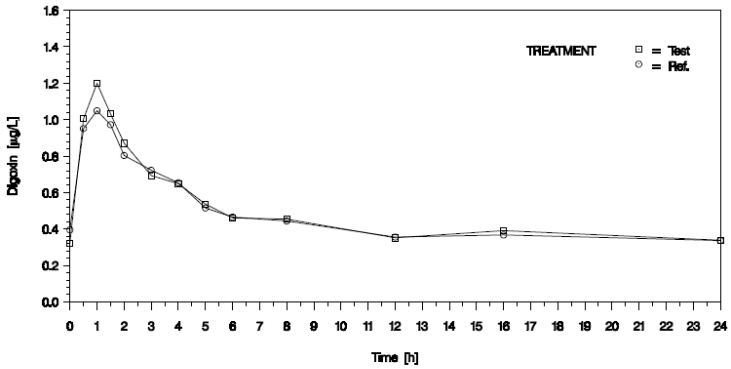
In this study, no influence on the extent of exposure of digoxin at steady-state after co-administration of netupitant was observed (Table 1)

Table 1 Point Estimates of Digoxin PK Parameters

Pharmacokinetic Parameter	Point Estimate Test/Ref.	90% Confidence Interval
$AUC_{(0\text{-}24h,ss)}$	104.13	95.86 - 113.11
$C_{max,ss}$	108.97	90.30 - 131.49
$C_{min,ss}$	96.65	88.84 - 105.14

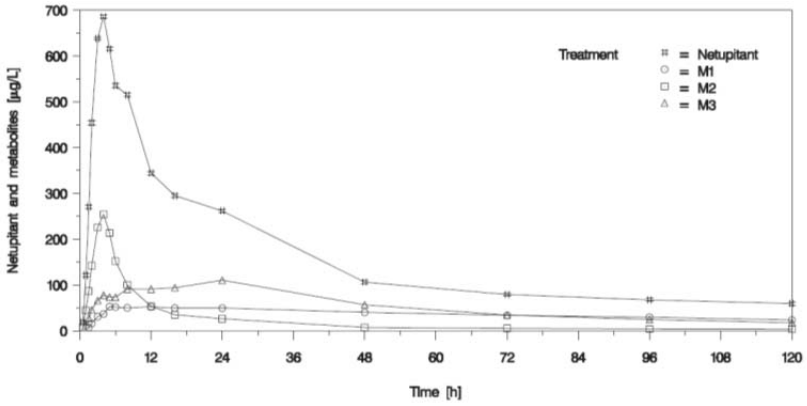
Mean minimum concentrations of digoxin during the Day 6-8 study period did not fluctuate, indicating that digoxin was at steady state. After netupitant administration, on Days 8-12, mean minimum concentrations appeared stable, also confirming that there was no effect of netupitant on digoxin concentrations. The excretion of digoxin in urine was 55% without netupitant and 57% after netupitant co-administration, indicating the insignificant effects of netupitant on the P-gp mediated urinary excretion of digoxin.

Figure 1 Arithmetic mean concentrations-time profile of digoxin (mcg/L) in plasma



Test: Digoxin plus netupitant (Day 8; N=16)
Ref.: Digoxin alone (Day 6; N=16)
Source: see Appendix 14, Figure 14.2.1.1

Figure 2 Arithmetic mean concentration-time profile of netupitant, M1, M2, and M3 (mcg/L) administered with digoxin (n=16)



Source: see Appendix 14, Table 14.2.6

There were no gender differences observed regarding extent of exposure to digoxin with or without netupitant. The digoxin pharmacokinetic parameters generated in this study are consistent with those described in published literature. Pharmacokinetic parameters generated

in this study for netupitant and its metabolites were also consistent with previous data generated in the netupitant development program.

Digoxin was used in this study as a probe drug to assess the effect of netupitant on P-glycoprotein. If netupitant were to inhibit P-glycoprotein, the extent of digoxin bioavailability, as measured by systemic exposure (AUC), would be increased. This effect is typically quite dramatic when interactions are seen, with an example of the well-known interaction between itraconazole and digoxin producing a 68% increase in digoxin AUC. No influence on the extent of exposure of digoxin after coadministration of netupitant was observed.

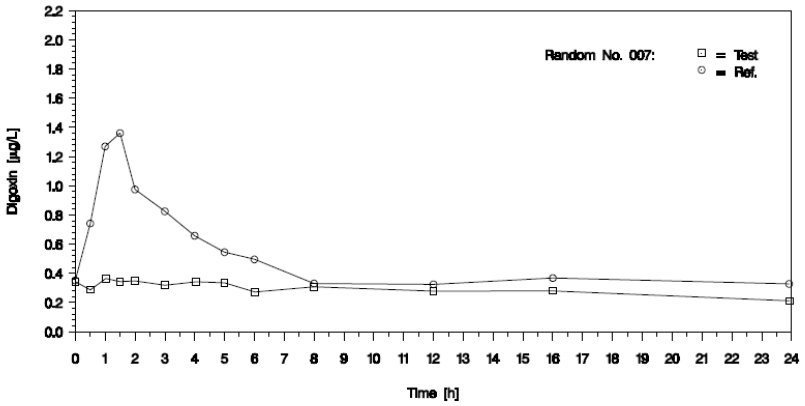
Table 2 Summary of Digoxin PK Parameters

Pharmacokinetic Parameter	**Statistic**	**Digoxin Alone N=16**	**Digoxin with Netupitant N=16**
AUC(0-24h,ss) [h^{s}µg/L]	Mean (SD)	10.96 (2.39)	11.37 (2.38)
	Geo.Mean (Geo.SD)	10.69 (1.27)	11.13 (1.24)
	Median (min - max)	10.63 (5.72-15.01)	11.35 (6.80-16.43)
C_{max},ss [µg/L]	Mean (SD)	1.129 (0.334)	1.239 (0.285)
	Geo.Mean (Geo.SD)	1.092 (1.29)	1.190 (1.40)
	Median (min - max)	1.003 (0.848 - 2.057)	1.280 (0.365 - 1.700)
T_{max},ss [h]	Median (min - max)	1.00 (0.50 - 1.50)	1.00 (0.50 - 1.50)

Reviewer's comments

One subject (Subject 007) did not have typical digoxin concentration-time profile in the period with netupitant co-administration. The plasma concentration of digoxin was above LLOQ but near (0.2 mcg/L) throughout the PK sampling period. It is unclear if the dosing of digoxin was done properly. As such the reviewer recalculated after excluding PK parameters from subject 007. The geometric mean ratio for Cmax and AUC excluding subject 007 was 117.9 and 107.6, respectively.

Figure 3 Concentrations-time profile of digoxin (mcg/L) in Subject 007



TEST = DIGOXIN WITH NETUPITANT
REF. = DIGOXIN ALONE

In this study the median Tmax of netupitant was 4 hours and the plasma concentration of netupitant when the maximum absorption of digoxin occurred. The inhibitory effects of itraconazole on P-gp in the kidney resulted in an increased in the systemic exposure and half-life of digoxin when itraconazole was administered an hour before digoxin administration. The median Tmax for itraconazole is 3-4 hours.

Study NETU-10-08: Netupitant with Oral Contraceptives

Title of Study
An Open-Label, Randomized, Two-Way, Crossover Trial to Evaluate the Effect of a Single Dose Administration of Oral Netupitant and Palonosetron (300 mg/0.5 mg) on the Pharmacokinetics of Ethinylestradiol and Levonorgestrel after Single Dose Oral Administration in Healthy Female Subjects

Methodology
This single-center study was conducted according to an open, randomized, two way, cross-over design in 24 healthy female subjects. The treatments were administered on Day 1 of each period according to the assigned treatment sequence. The Reference treatment (oral contraceptive alone) was administered in one period and the Test treatment (co-administration of oral contraceptive and FDC of netupitant and palonosetron) was administered in the other period. Administrations were separated by a washout phase of 28 days.

Blood samples for pharmacokinetics of ethinylestradiol and levonorgestrel were collected in both periods until 96 h after administration of the Test or Reference treatment. Blood samples for pharmacokinetics of netupitant (and its metabolites) and palonosetron were collected only in the period where the Test treatment was administered. The last blood sample for palonosetron and netupitant was collected 192 h and 240 h after administration of the Test treatment, respectively.

PK Results
Effect on PK of ethinylestradiol

The extent of absorption of ethinylestradiol based on AUC_{0-t} and $AUC_{0-\infty}$ was 16% and 12% higher, respectively, after intake of the oral contraceptive together with netupitant and palonosetron compared to intake of the oral contraceptive alone.

The C_{max} of ethinylestradiol was not significantly different after administration of the oral contraceptive together with netupitant and palonosetron compared to administration of the oral contraceptive alone. The point estimate of the Test/Reference ratio for C_{max} was 105.09% (90% CIs: 98.33%; 112.32%) and AUC_{0-}, (102.80%; 122.22%).

Table 1 Mean Exposure Parameters for Ethinylestradiol after Oral Administration of Microgynon® with and without Netupitant/Palonosetron (300 mg/0.5 mg)

Parameter	T	R	PE*	90%CI
C_{max} [pg/mL]	120.6±28.3	115.6±30.9	105.09	98.33 - 112.32
$AUC_{0-\infty}$ [h•pg/mL]	1224±428.7	1091±400.9	112.09	102.80 - 122.22
AUC_{0-tz} [h•pg/mL]	1071±397.0	928.3±383.2	116.05	106.21 - 126.79

Values are arithmetic means ±SD; SD: standard deviation. *Point estimate (PE): ratio of geometric means
CI: confidence interval, SD: standard deviation
R: two tablets each containing 30 µg ethinylestradiol and 150 µg levonorgestrel (Microgynon®) (Reference)
T: two tablets each containing 30 µg ethinylestradiol and 150 µg levonorgestrel (Microgynon®) and one capsule containing netupitant and palonosetron 300 mg/0.5 mg (Test)

Effect on PK of levonorgestrel. The extent of absorption of levonorgestrel based on AUC_{0-t} and $AUC_{0-\infty}$was 46% and 40% higher, respectively, after administration of the oral contraceptive together with netupitant and palonosetron compared to administration of the oral contraceptive alone.

Mean C_{max} of levonorgestrel was not significantly different after administration of the oral contraceptive together with netupitant and palonosetron compared to administration of the oral contraceptive alone.

Table 2 Mean Exposure Parameters for Levonorgestrel after Oral Dose Administration of Microgynon® with and without Netupitant/Palonosetron (300 mg/0.5 mg)

Parameter	T	R	PE*	90%CI
C_{max} [pg/mL]	8.11±2.93	8.23±2.79	98.06	92.53 - 103.92
AUC_{0-}, [h•ng/mL]	87.4±54.1	60.0±37.0	146.21	129.38 - 165.22
AUC_{0t-z} [h•ng/mL]	113.1±63.5	80.4±42.4	139.55	123.55 - 157.61

Values are arithmetic means ±SD; SD: standard deviation. *Point estimate (PE): ratio of geometric means
CI: confidence interval, SD: standard deviation
R: two tablets each containing 30 µg ethinylestradiol and 150 µg levonorgestrel (Microgynon®) (Reference)

T: two tablets each containing 30 µg ethinylestradiol and 150 µg levonorgestrel (Microgynon®) and one capsule containing netupitant and palonosetron 300 mg/0.5 mg (Test)

PK of netupitant and palonosetron.
The effect of the oral contraceptive (ethinylestradiol and levonorgestrel) on the pharmacokinetics of netupitant, its metabolites M1, M2, and M3, and palonosetron was not investigated in this study. However, no marked effects on rate and extent of absorption of netupitant, its metabolites M1, M2, and M3, and palonosetron were observed when compared to the pharmacokinetic parameters shown in previous clinical studies.

Reviewer's comments: The study results are acceptable.

Study NETU-10-11: Netupitant/Palonosetron FDC with Ketoconazole and Rifampicin

Title of Study
An Open-Label, Randomized, Two-Groups, Two-Way, Cross-Over Trial to Evaluate a Possible Influence of Oral Ketoconazole, a CYP3A4 Inhibitor and Oral Rifampicin, a CYP3A4 Inducer, on the Pharmacokinetics of Netupitant and Palonosetron After Single Dose Administration as Fixed Dose Combination (300 mg/0.5 mg).

Methodology
This single-center study was conducted according to an open-label, randomized, two- group, two-way cross-over design in 36 healthy male and female subjects to evaluate the influence of oral ketoconazole (Group 1, N=18 subjects) and oral rifampicin (Group 2, N=18 subjects) on the pharmacokinetics of netupitant and palonosetron. Each of the two groups underwent a screening phase lasting a maximum of 21 days, a treatment phase with two treatment periods separated by a washout of 28 days between the two FDC administrations and a final check (within 3 to 8 days after the end of the second period).

A single dose of netupitant/palonosetron FDC was administered alone in one period and either together with the CYP3A4 inhibitor ketoconazole (Group 1) or with the CYP3A4 inducer rifampicin (Group 2) in the other period. In both groups and both periods, blood samples for pharmacokinetics of netupitant and its metabolites were collected until 240 h after administration of the netupitant/palonosetron FDC and blood samples for palonosetron were collected until 192 h after administration of the FDC.

PK Results

Assessment of ketoconazole effect
Administration of the CYP3A4 inhibitor ketoconazole with netupitant/palonosetron FDC increased the exposure of netupitant and resulted in an $AUC_{0\text{-}tz}$ of 1.8 fold, $AUC_{0\text{-}\infty}$ of 2.4 fold, and C_{max} of 1.3 fold when compared to the administration of netupitant/palonosetron FDC alone. Coadministation with ketoconazole did not affect the pharmacokinetics of palonosetron.

The primary pharmacokinetic parameters C_{max} and $AUC_{0-\infty}$ of netupitant showed a higher maximum plasma concentration and a higher overall plasma exposure after intake of netupitant/palonosetron FDC with ketoconazole (T1) than after intake of netupitant/palonosetron FDC alone (R1) (Table 1).

Table 1 Mean ± SD Netupitant Pharmacokinetic Parameters after Oral Administration of Netupitant/Palonosetron (300 mg/0.5 mg) with and without Ketoconazole (400 mg q.d.)

Parameter	T1	R1	PE%*	90%CI
C_{max} [µg/L]	650.2±217.6	546.0±241.0	125.42	101.27 - 155.33
$AUC_{0-\infty}$ [h·µg/L]	43459±16911	17971±5618	239.88	205.60 - 279.89
AUC_{0-tz} [h·µg/L]	28494±7703	16072±5132	180.42	159.51 - 204.06

Values are arithmetic means ±standard deviation (SD); *Point estimate (PE): ratio of geometric means (T1/R1) and 90% confidence interval (CI)
T1: One capsule of netupitant/palonosetron (300 mg/0.5 mg) in combination with 400 mg q.d. (2 tablets of 200 mg) ketoconazole (Test 1)
R1: One capsule of netupitant/palonosetron (300 mg/0.5 mg) (Reference)

The primary pharmacokinetic parameters C_{max} and $AUC_{0-\infty}$ of palonosetron showed a higher maximum plasma concentration and a higher overall plasma exposure after intake of netupitant/palonosetron FDC with ketoconazole (T1) compared to intake of netupitant and palonosetron FDC alone (R1)(Table 2).

Table 2 Mean±SD Palonosetron Pharmacokinetic Parameters after Oral Administration of Netupitant/Palonosetron (300 mg/0.5 mg) with and without Ketoconazole (400 mg q.d.)

Parameter	T1	R1	PE%*	90%CI
C_{max} [ng/L]	898.7±220.1	775.3±185.0	115.35	109.62 - 121.37
$AUC_{0-\infty}$ [h·ng/L]	40910±9261	37524±9577	110.09	105.43 - 114.96
AUC_{0-tz} [h·ng/L]	36899±8667	32564±7459	113.41	108.26 - 118.80

Values are arithmetic means ±standard deviation (SD); *Point estimate: ratio of geometric means (T1/R1) and 90% confidence interval (CI)
T1: One capsule of netupitant/palonosetron (300 mg/0.5 mg) in combination with 400 mg q.d. (2 tablets of 200 mg) ketoconazole (Test 1)
R1: One capsule of netupitant/palonosetron (300 mg/0.5 mg) (Reference)

The formation of M1 and M3 was delayed when netupitant was co-administered with ketoconazole compared to intake of netupitant/palonosetron FDC alone (median T_{max} of about 96 vs.12 h for M1 and 24 vs. 12 h for M3). Concomitant ketoconazole did not appear to have an impact on the time of the appearance of M2 in plasma. Mean maximum plasma concentration (C_{max}) and overall plasma exposure (AUC_{0-tz}) for all three metabolites M1, M2, and M3 were

lower under co-administration with ketoconazole. Mean metabolite to parent ratios were 24.9%, 6.4%, and 15.1% for T1 compared to 30.3%, 12.1%, and 28.1% for R1.

Assessment of rifampicin effect

Administration of the CYP3A4 inducer rifampicin with netupitant/palonosetron FDC alone decreased the exposure of netupitant and resulted in an $AUC_{0\text{-}tz}$ of 5.5 fold, $AUC_{0\text{-}\infty}$ of 5.9 fold, and C_{max} of 2.6 fold when the administration of the netupitant/palonosetron FDC alone was compared to the administration of netupitant/palonosetron FDC with the CYP3A4 inducer rifampicin. Coadministration of rifampicin resulted in a 15-20% decrease in palonosetron exposure.

The primary PK parameters C_{max} and $AUC_{0\text{-}\infty}$ of netupitant showed a lower maximum plasma concentration and a lower overall plasma exposure after intake of netupitant/palonosetron FDC with rifampicin (T2) than after intake of netupitant and palonosetron FDC alone (R2) (Table 3).

Table 3 Mean±SD Netupitant Pharmacokinetic Parameters after Oral Administration of Netupitant/Palonosetron (300 mg/0.5 mg) with and without Rifampicin (600 mg q.d.) and Results of Analysis of Variance

Parameter	T2	R2	PE%*	90%CI
C_{max} [µg/L]	225.6±156.3	498.1±225.6	37.90	28.81 - 49.86
$AUC_{0\text{-}\infty}$ [h·µg/L]	3463±2790	16944±5915	16.92	12.70 - 22.55
$AUC_{0\text{-}tz}$ [h·µg/L]	3362±2766	15210±4977	18.05	13.56 - 24.01

Values are arithmetic means±standard deviation (SD); *Point estimate (PE): ratio of geometric means (T2/R2) and 90% confidence interval (CI)

T2: One capsule of netupitant/palonosetron (300 mg/0.5 mg) in combination with 600 mg q.d. (1 tablet of 600 mg) rifampicin (Test 2)

R2: One capsule of netupitant/palonosetron (300 mg/0.5 mg) (Reference)

The primary pharmacokinetic parameters C_{max} and $AUC_{0\text{-}\infty}$ and of palonosetron showed a lower maximum plasma concentration and a lower ove rall plasma exposure after intake of netupitant/palonosetron FDC with rifampicin (T2) than after intake of netupitant and palonosetron FDC alone (R2) (Table 4).

Table 4 Mean±SD Palonosetron Pharmacokinetic Parameters after Oral Administration of Netupitant/Palonosetron (300 mg/0.5 mg) with and without Rifampicin (600 mg q.d.) and Results of Analysis of Variance

Parameter	T2	R2	PE%*	90%CI
C_{max} [ng/L]	654.5±138.4	772.2±206.0	85.44	81.11 - 90.01
$AUC_{0\text{-}\infty}$ [h·ng/L]	28354±7851	35714±13467	81.03	76.96 - 85.32
$AUC_{0\text{-}tz}$ [h·ng/L]	25557±7679	32371±13055	80.64	76.43 - 85.09

Values are arithmetic means ±standard deviation (SD); *Point estimate (PE): ratio of geometric means (T2/R2) and 90% confidence interval (CI)
T2: One capsule of netupitant/palonosetron (300 mg/0.5 mg) in combination with 600 mg q.d. (1 tablet of 600 mg) rifampicin (Test 2)
R2: One capsule of netupitant/palonosetron (300 mg/0.5 mg) (Reference)

After intake of netupitant/palonosetron FDC with rifampicin, the formation of metabolites was accelerated compared to intake of netupitant/palonosetron FDC alone (median T_{max} of about 6 vs. 12 h for M1, 4 vs. 5 h for M2, 8 vs. 10 h for M3). For metabolites M1 and M3, mean maximum plasma concentration (C_{max}) and overall plasma exposure ($AUC_{0-\infty}$) was slightly lower after co-administration with rifampicin. Mean metabolite to parent ratios for M1 and M3 were 34.9% and 45.5% for T2 compared to 29.3% and 26.8% for R2. For M2, mean maximum plasma concentration and overall plasma exposure were higher after co-administration with rifampicin. Mean metabolite to parent ratios for M2 were 176.9% for T2 compared to 11.0% for R2.

Reviewer's comments: In vitro netupitant was metabolized mainly by CYP3A4 and to a lesser degree by CYP2C9 and CYP2D6. The significant effects of rifampin on the netupitant systemic exposure can reduce the efficacy of netupitant and the combination therapy. Although the dose-response relationship among the combinations with netupitant 100 mg, 200 mg and 300 mg was not evident for the delayed and overall phase. In the acute phase, the proportion of patients with CR was numerically higher with 300 mg netupitant (98%) than with lower doses of netupitant (92%). Nevertheless the overall CR rate in the acute phase was > 90% with combinations, the potential reduction of efficacy due to a decrease in the systemic exposure to netupitant would make the combination therapy without additional benefit over the monotherapy.

Study NETU-10-09: Netupitant/Palonosetron FDC with Chemotherapy

Title of Study
A Single Dose, Open-Label, Randomized, Two Period, Cross-Over, Drug-Drug Interaction Study of Oral Palonosetron and Netupitant Fixed Dose Combination on the PK of Three Chemotherapeutics (Docetaxel, Etoposide, Cyclophosphamide) Metabolized by CYP3A4 in Cancer Patients

Methodology
This multicenter, single dose, randomized, open label, 2 period, cross-over pharmacokinetic (PK) study was designed to evaluate the effects of oral netupitant administered as fixed dose combination with palonosetron on the PK profile of 3 different chemotherapeutic agents (docetaxel, etoposide, and cyclophosphamide) given to cancer patients.

Forty-eight (48) male and female patients • 18 years with histologically and / or cytologically confirmed malignant diseases and scheduled to receive at least 2 courses of 1 of the 3 chemotherapy agents and considered eligible to participate in this study, were planned to be enrolled into 1 of the 3 groups of 16 patients according to the chemotherapy regimen they were scheduled to receive.

Within each group, all patients were to receive 1 of the 3 chemotherapeutic agents (docetaxel, etoposide or cyclophosphamide) for 2 consecutive cycles (hereafter referred to as treatment periods 1 and 2); Day 1 of the 2 treatment periods was separated by at least 3 weeks.
The patients received a single oral dose of netupitant/palonosetron fixed dose combination (FDC; test Investigational Medicinal Product [IMP]) during either the first or the second treatment period. The antiemetic treatment for the alternate period was standardized, and all patients were given oral palonosetron 0.5 mg (Aloxi®, reference IMP). The treatment order was randomized within each group of patients receiving the same chemotherapeutic agent.

The patients received the test IMP on Day 1 of the test period (1 of the 2 treatment periods), 1 h before the start of their intravenous chemotherapy. During the alternate period, oral palonosetron (Aloxi®) was given 1 h before the start of chemotherapy for prevention of nausea and vomiting. Dexamethasone as antiemetic premedication was allowed provided it was given at both study periods. Rescue medication was allowed during the study if needed, according to the Investigator's opinion, (e.g. prochlorperazine, thiethylperazine, metoclopramide, etc.). However, drugs that undergo CYP3A4-mediated metabolism, or are CYP3A4 inhibitors or inducers were to be avoided.

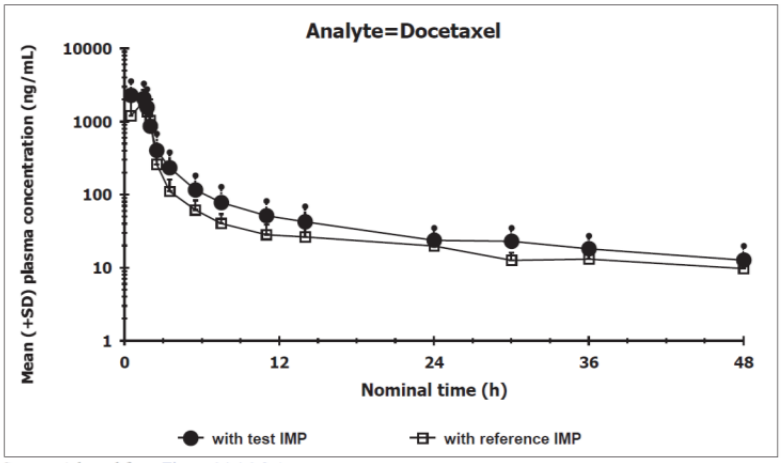
The PK profile of the 3 chemotherapeutic agents given with netupitant/palonosetron FDC (test IMP) was compared to the profile following a regimen of oral Aloxi® (0.5 mg palonosetron, reference IMP) alone. In addition, the safety profile and tolerability of the oral netupitant/palonosetron FDC, given together with an intravenous course of any of the 3 chemotherapy agents, was evaluated in this population of cancer patients. Furthermore, the PK of netupitant, netupitant metabolites and palonosetron in this population was explored.

PK Results

Docetaxel *(N=8, PK set N=7).* The mean plasma concentration curve obtained for docetaxel co-administered with netupitant/palonosetron FDC was overall similar in shape to that obtained for docetaxel with palonosetron alone. However, the mean docetaxel concentration curve in the netupitant/palonosetron FDC period was slightly higher than in the palonosetron alone period, primarily for the first few samples taken during and shortly after completion of the intravenous (**IV**) infusion. In particular, the concentration curves for 6 of the 7 patients who completed both treatment periods were slightly higher in the test than in the reference period, and the 7th patient had virtually the same concentrations in both periods. Exposure in the test period was approximately 37% higher for AUC_{0-t} and 50% for C_{max} than the exposure in the reference period.

The estimated mean ratios for the test to reference PK parameter values (expressed as % of the reference) were 135% and 149% for AUC_{0-t} and C_{max}, respectively; this suggests that there may be a drug-drug interaction between IV docetaxel and netupitant in the form of FDC with palonosetron. Also, the upper limits of the 90% CIs were >125.0% for AUC_{0-t} and C_{max}.

Figure 2 Mean Docetaxel Plasma Concentrations Combining the Curves for the 2 Treatment Periods, Log-linear Scales – PK Population Excluding Site 93 Patients



Source: Adapted from Figure 14.4.1.2.A

Reference IMP = palonosetron (N=7); test IMP = netupitant/palonosetron FDC (N=8).

Table 15 Evaluation of an Interaction between Docetaxel and Netupitant: ANOVA Results – PK Set

Chemo-therapeutic agent	Ln-transformed parameter	Least square means (SE) Chemo with FDC (Test)	Least square means (SE) Chemo with palonosetron (Reference)	Estimated Mean Ratio Test to Reference (%)	90% CI Lower limit	90% CI Upper limit
Docetaxel	$AUC_{0-\infty}$	8.63 (.[a])	8.42 (.[a])	124.36	-[a]	-[a]
	AUC_{0-t}	8.57 (0.11)	8.27 (0.11)	135.16	98.90	184.71
	C_{max}	8.03 (0.11)	7.64 (0.11)	149.01	108.50	204.66

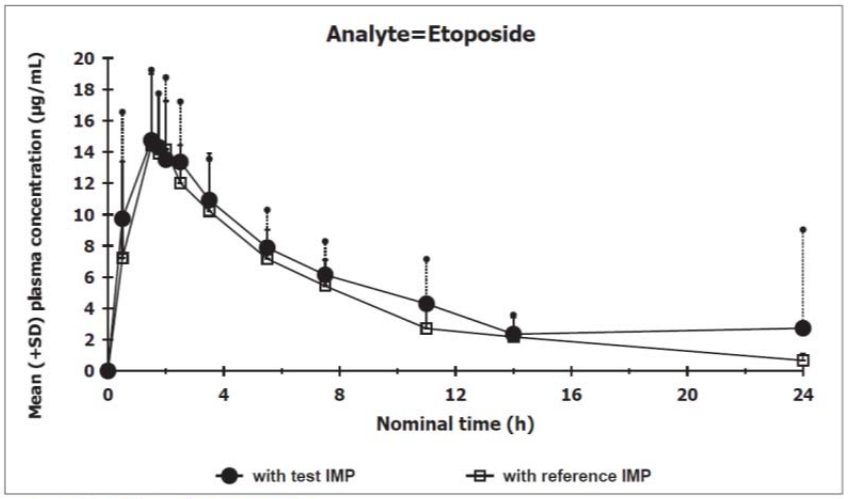
Source: Table 14.2.5

SE = Standard error; FDC = netupitant/palonosetron fixed dose combination; CI = Confidence interval.

[a] Because n=2, the SE and the 90% CI could not be calculated.

Etoposide *(N=12, PK set N=11).* The mean concentration curve obtained for etoposide co-administered with netupitant/palonosetron FDC was overall similar in shape to that obtained for etoposide with palonosetron alone. However, the mean etoposide curve in the netupitant/palonosetron FDC co-administration period was slightly higher than in the palonosetron alone period, which was visible from the first post-dose sample onward. The exposure in terms of AUC_{0-t} in the FDC period was approximately 21% higher than that in the reference period, while C_{max} and $AUC_{0-\infty}$ values were similar for both treatment periods.

Figure 3 Mean Etoposide Plasma Concentrations Combining the Curves for the 2 Treatment Periods, Linear Scales– PK Population Excluding Site 93 Patients



Source: Adapted from Figure 14.4.1.1.B

Reference IMP = palonosetron (N=12); test IMP = netupitant/palonosetron FDC (N=12).

Table 18 Evaluation of an Interaction between Etoposide and Netupitant: ANOVA Results – PK Set

Chemo-therapeutic agent	Ln-transformed parameter	Least square means (SE) Chemo with FDC (Test)	Least square means (SE) Chemo with palonosetron (Reference)	Estimated Mean Ratio Test to Reference (%)	90% CI Lower limit	90% CI Upper limit
Etoposide	$AUC_{0-\infty}$	4.64 (0.05)	4.61 (0.05)	103.45	89.87	119.08
	AUC_{0-t}	4.78 (0.08)	4.53 (0.08)	128.00	105.28	155.62
	C_{max}	2.93 (0.05)	2.83 (0.05)	110.20	95.99	126.53
	$AUC_{0-\infty}$ [a]	4.74 (0.09)	4.51 (0.09)	125.22	98.53	159.13

Source: Table 14.2.5

SE = Standard error; FDC = netupitant/palonosetron fixed dose combination; CI = Confidence interval.

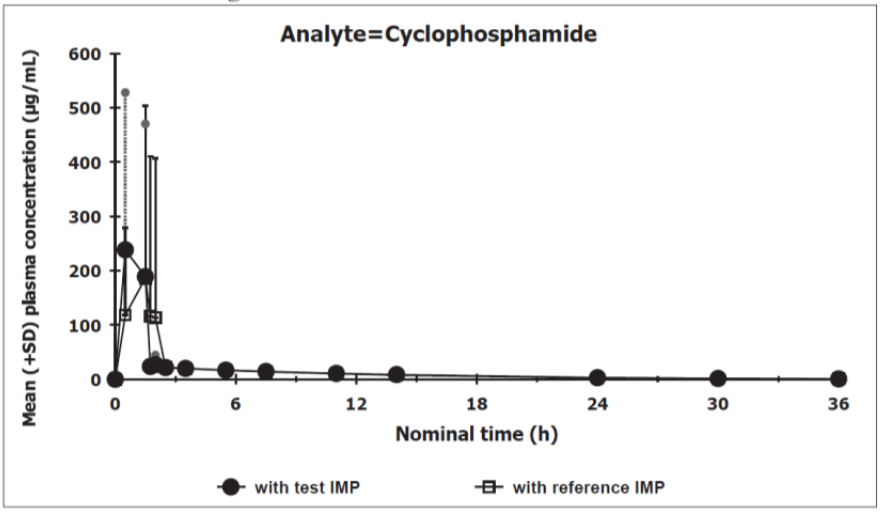
[a] Result including $AUC_{0-\infty}$ values for Patients 91008 and 96002.

Cyclophosphamide (N=10, PK set N=10). The mean concentration curve obtained for cyclophosphamide co-administered with netupitant/palonosetron FDC was overall similar in shape to that obtained for cyclophosphamide with palonosetron alone. There was high variability between the patients, even during the infusion phase. In 7 of 10 patients, the plots of individual data revealed a lower cyclophosphamide C_{max} in the netupitant/palonosetron FDC period than in the palonosetron alone period. In 3 patients, C_{max} values were much higher in the netupitant/palonosetron FDC period than in the palonosetron alone period. For cyclophosphamide AUC_{0-t}, 4 patients had higher values after palonosetron co-administration alone and 5 had higher values after FDC while 1 patient had the same values in both periods. The resulting very high individual test to reference ratios influenced the estimated mean ratio to such an extent that the data suggest a slight increase in PK parameters: cyclophosphamide exposure, in terms of mean C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, was 8%, 14%, and 14% higher, respectively, in the netupitant/palonosetron FDC co-administration period than after

palonosetron.

The estimated mean ratios for the test to reference parameter values (expressed as % of the reference) were between 119% and 127%; this suggests that there may be a minimal drug-drug interaction between IV cyclophosphamide and netupitant administered in the form of FDC with palonosetron. However, the large differences between results for the 2 treatments in individual patients did not result in consistent differences between the curves for the test and reference treatments.

Figure 5 Mean Cyclophosphamide Plasma Concentrations Combining the Curves for the 2 Treatment Periods, Linear Scales – PK Population Excluding Site 93 Patients[a]



Source: Adapted from Figure 14.4.1.1.C

[a] In the Cyclophosphamide group no patients were enrolled at Site 93.

Reference IMP = palonosetron (N=10); test IMP = netupitant/palonosetron FDC (N=10).

Table 21 Evaluation of an Interaction between Cyclophosphamide and Netupitant: ANOVA Results – PK Set

		Least square means (SE)		Estimated	90% CI	
Chemo-therapeutic agent	Ln-transformed parameter	Chemo with FDC (Test)	Chemo with palonosetron (Reference)	Mean Ratio Test to Reference (%)	Lower limit	Upper limit
Cyclo-phosphamide	$AUC_{0-\infty}$	6.06 (0.15)	5.88 (0.15)	120.12	81.58	176.89
	AUC_{0-t}	6.05 (0.15)	5.87 (0.15)	119.52	81.03	176.28
	C_{max}	4.93 (0.44)	4.69 (0.44)	127.36	40.17	403.75

Source: Table 14.2.5

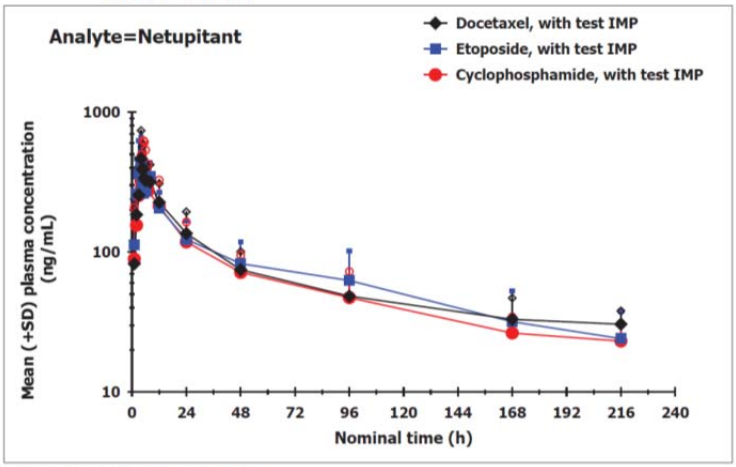
SE = Standard error; FDC = netupitant/palonosetron fixed dose combination; CI = Confidence interval

Netupitant. Overall, geometric mean PK parameters of netupitant exposure when the netupitant/palonosetron FDC was co-administered with any chemotherapy were approximately 440 ng/mL for C_{max}, approximately 14 h*µg/mL for AUC_{0-t} and approximately 16 h*µg/mL for $AUC_{0-\infty}$. These values are in agreement with previous studies in healthy subjects.

The mean netupitant concentrations obtained in each of the 3 chemotherapy groups after netupitant/palonosetron FDC co-administration were not essentially different, and elimination occurred at approximately the same rate. Netupitant was quantifiable in the last sample at 9 days post-dose in >50% of the patients.

Netupitant C_{max} was observed approximately 4h (median t_{max}) after the netupitant/palonosetron FDC intake, irrespective of the chemotherapeutic agent. Netupitant $AUC_{0-\infty}$ values could often not be derived with sufficient accuracy as the very long half-life of netupitant resulted in large extrapolations, despite sampling to 9 days after dose intake. The variability (geometric CV%) for C_{max} was overall high (50% to 70%) and moderate for the other parameters. The mean $t_{1/2z}$ values, ranging between approximately 70 h and 90 h, were not essentially different across the 3 treatment groups. GeoMean CL/F values were 18 to 19 L/h across the 3 chemotherapy groups.

Figure 8 Mean Netupitant Plasma Concentrations Combining the Curves for th 3 Chemotherapy Groups, Log-linear Scales – PK Population Excluding Site 93 Patients



Source: Adapted from Figure 14.4.1.4.1
Test IMP = netupitant/palonosetron FDC.
Docetaxel: N=8, Etoposide: N=12, Cyclophosphamide N=10.

Netupitant metabolite M1. Overall exposure to netupitant metabolite M1 relative to netupitant was approximately 8% for C_{max} and between approximately 30% (docetaxel and etoposide group) and 35% (cyclophosphamide group) for AUC_{0-t}. These values are in agreement with previous studies in healthy subjects.

Netupitant M1 C_{max} was observed approximately 7 h (median t_{max}) after netupitant/palonosetron FDC intake in the cyclophosphamide group, and at 12 h and 18 h post-dose in the docetaxel and etoposide groups, respectively. Exposure to netupitant metabolite M1 in terms of GeoMean C_{max} and AUC_{0-t} was similar across the chemotherapy groups. $AUC_{0-\infty}$ values for netupitant M1 for the docetaxel group could not be calculated as derived $t_{1/2z}$ values were not sufficiently accurate.

The variability for C_{max} and AUC_{0-t} (geometric CV%) was overall moderate. The mean $t_{1/2z}$ values were similar for the etoposide group (82 h) and cyclophosphamide group (91 h).

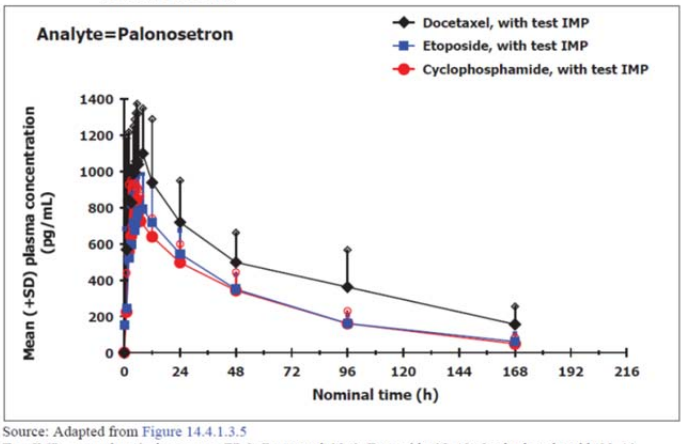
Netupitant metabolite M2. Overall, exposure to netupitant metabolite M2 relative to netupitant in terms of C_{max} ranged from approximately 45% in the etoposide (N=12) and cyclophosphamide (N=10) groups to 70% in the docetaxel group (N=8). The relative exposure to M2 in terms of AUC_{0-t} ranged from 20% in the etoposide and cyclophosphamide groups to 30% in the docetaxel group. These values are in agreement with previous studies in healthy subjects.

Netupitant M2 C_{max} was observed approximately 4 h (median t_{max}) after netupitant/palonosetron FDC intake irrespective of the chemotherapeutic agent. Exposure to netupitant metabolite M2 in terms of GeoMean C_{max} and AUC_{0-t} values was approximately 50% to 60% higher in the docetaxel group than in the etoposide and cyclophosphamide groups. $AUC_{0-\infty}$ values were calculated for only half of the patients or less, as the derived elimination rates were often not reliable. The terminal elimination took place at mean concentrations of 10 to 20 ng/mL and the contribution of this phase to the overall exposure was limited despite the slow elimination with individual $t_{1/2z}$ values >80 h. The variability for C_{max} and AUC_{0-t} (geometric CV%) was overall moderate to high. The $t_{1/2z}$ values across the etoposide and cyclophosphamide groups were not essentially different (mean and median values of approximately 60 h).

Netupitant metabolite M3. Exposure to netupitant metabolite M3 relative to netupitant was approximately 14% for C_{max} and approximately 34% for AUC_{0-t}. These values are in agreement with previous studies in healthy subjects.

Netupitant M3 C_{max} was observed 12 h (median t_{max}) after netupitant/palonosetron FDC capsule intake, irrespective of the chemotherapeutic agent. Exposure to netupitant metabolite M3 in terms of GeoMean C_{max} and AUC_{0-t}, and $AUC_{0-\infty}$ as available, was similar across the 3 chemotherapy groups. The variability for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ (geometric CV%) was overall moderate. The $t_{1/2z}$ values for netupitant M3 were calculated for most patients in each chemotherapy group. Although the terminal elimination took place at low mean concentrations, the contribution of this phase to the overall exposure exceeded 20% in several patients due to the slow elimination, with individual $t_{1/2z}$ values >100 h. The $t_{1/2z}$ values across the chemotherapy groups were not essentially different (mean and median values of approximately 65 h to 80 h).

Figure 17 Mean Palonosetron Plasma Concentrations Combining the Curves for the 3 Chemotherapy Groups, Linear Scales – PK Population Excluding Site 93 Patients



Source: Adapted from Figure 14.4.1.3.5
Test IMP = netupitant/palonosetron FDC; Docetaxel: N=8, Etoposide: N=12, Cyclophosphamide N=10.

Palonosetron. Overall, geometric mean PK parameters of palonosetron exposure when netupitant/palonosetron FDC was co-administered with any chemotherapy were approximately 900 pg/mL for C_{max}, approximately 50000 h*pg/mL for AUC_{0-t} and approximately 57000 h*pg/mL for $AUC_{0-\infty}$. These values are in agreement with previous studies in healthy subjects. Palonosetron C_{max} was observed approximately 5 h (median t_{max}) after the netupitant/palonosetron FDC capsule intake, irrespective of the chemotherapeutic agent. Exposure to palonosetron in terms of GeoMean C_{max} and $AUC_{0-\infty}$ values was approximately 30% (C_{max}) to 65% ($AUC_{0-\infty}$) higher in the docetaxel group than in the etoposide and cyclophosphamide groups. The variability for these parameters (geometric CV%) was moderate overall. The mean $t_{1/2z}$ values were similar in the etoposide and cyclophosphamide groups, but appeared to be approximately 20 h longer in the docetaxel group. The GeoMean CL/F value was lower in the docetaxel group than in the etoposide and cyclophosphamide groups. Results shown in the docetaxel group, however, need to be interpreted with caution because of the small sample size (N=8).

Reviewer's comments

According to the population PK analyses in phase 3 trial, the PK of netupitant and palonosetron in cancer patients was similar with in healthy subjects. Please see the Pharmacometrics review by Dr. Jingyu Yu for more details. Underlying reason for apparent high systemic exposure to palonosetron in the docetaxel is unclear.

Study NP16602: Apomorphine Challenge in Healthy Volunteers

Title of Study
Randomized, Double-Blind, Placebo-Controlled Evaluation of the Anti-Emetic Effect of RO0673189 (Netupitant) Following Apomorphine Challenge in Healthy Volunteers

Methodology
Study NP16602 was a randomised, double-blind, placebo-controlled study in which 32 healthy subjects were assigned to 4 dosing groups of subjects each. Within each group, 6 subjects received a single dose of netupitant and two subjects received placebo. Eligible subjects were fasted overnight and received a standardized breakfast prior to dosing with netupitant or placebo. All subjects then received a subcutaneous injection of 50µg/kg apomorphine (an emetogen) at between 8 and 24 hours post-dose. Doses and timing of the apomorphine challenge for each group are shown in Table 1.

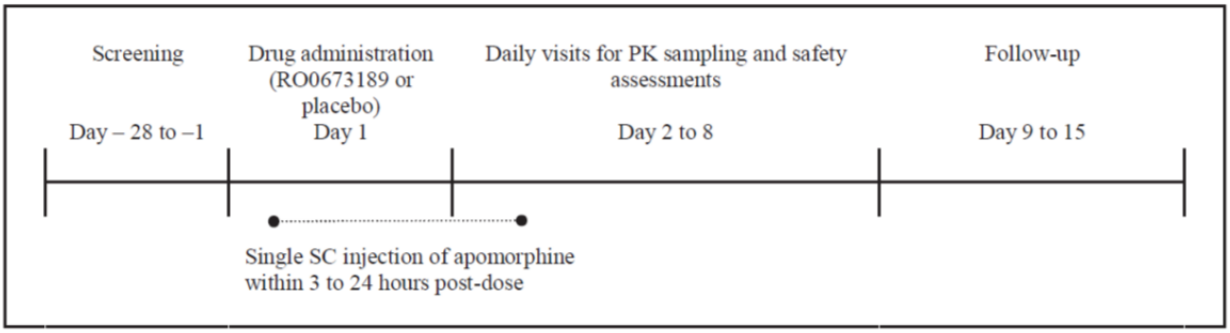


Table 1 Dose Groups and Timing of Apomorphine Challenge

Netupitant Dose Group	Interval Between Netupitant Dose and Apomorphine Injection
100 mg (I)	24 h
100 mg (II)	8 h
300 mg	12 h
450 mg	12 h

Emesis occurring during the 90-minute period following apomorphine injection was evaluated by the degree of nausea, measured at 10 minute intervals using a visual analogue scale, the occurrence of vomiting and the total number of vomits and retches.

Blood samples for pharmacokinetic analysis were collected pre-dose and at 15 and 45 min and 1, 1.5, 2, 3, 5, 8, 12, 24, 36, 48, 60, 72, 96, 120, 144 and 168 h post-dose.

PD Results

Analysis of the results by plasma netupitant concentration at the time of apomorphine challenge showed a decrease in the incidence of vomiting with increasing netupitant levels. No subject in the highest concentration group (> 300 ng/mL, corresponding to 1 subject taking 300 mg and 5 subjects taking 450 mg) experienced vomiting, a statistically significant result compared to placebo (p = 0.010).

Subjects with lower netupitant concentrations also experienced less vomiting than the placebo group. In the three groups with lower netupitant concentrations, 50% of subjects experienced no vomiting (N=18), compared with 25% of subjects vomiting-free in the placebo group (N=8). In total, 15 out of the 24 subjects who received netupitant experienced no vomiting, with six subjects having fewer than 6 vomiting episodes. Only 2 of the 8 subjects receiving placebo experienced less than 6 vomiting episodes.

Retching was reduced in subjects treated with active drug, but no trend was observed between concentration groups. The results were skewed by one subject in the highest concentration group, who experienced a very high number of retches. Nausea tended to increase with netupitant concentration, with the exception of the lowest concentration ≤ 50 ng/mL, which recorded the lowest levels.

Table 2
Summary of Vomiting Episodes and Area under the Nausea VAS

RO0673189 Concentration	0 ng/mL (N=8)	≤ 50 ng/mL (N=6)	51 – 100 ng/mL (N=6)	101-300 ng/mL (N=6)	> 300 ng/mL (N=6)
Vomiting Episodes					
Mean	10.3	2.7	4.2	3.5	0.0
Range	0.0 – 27.0	0.0 – 10.0	0.0 – 18.0	0.0 – 13.0	0.0 – 0.0
AUC of Nausea VAS					
Mean	2207.9	1469.8	3089.8	3480.2	4117.8
Range	170 - 5435	10 - 3399	1184 - 5164	1552 - 5840	2030 - 6527

PK Results

Netupitant was absorbed in a first order fashion, reaching maximum plasma concentrations at approximately 5 h post-dose. The $t_{1/2}$ was estimated to be approximately 50 h.
These results suggest that netupitant provides better control for emesis compared with placebo following apomorphine challenge. A concentration-effect relationship was demonstrated with complete control of vomiting at plasma concentrations of > 300 ng/mL. However, there was a trend towards an increase in nausea with increasing plasma concentrations. The study medication was well tolerated by all subjects in this study.

Reviewer's comments: This study is exploratory only to guide the dose selection for a phase 2 trial.

Study NETU-06-08: PET Study in Healthy Volunteers

Title of the Study
A Positron Emission Tomography (PET) Study to Assess the Degree of Neurokinin-1 (NK_1) Receptor Occupancy in the Human Brain After Single Doses of Netupitant in Healthy Male Subjects Using ^{11}CGR205171 As Tracer

Methodology
This was a single dose, randomized, open-label, PET study investigating the degree of occupancy of NK_1 receptors in the human brain after single oral doses of netupitant in healthy male subjects. The study consisted of a screening visit, a baseline PET visit, a treatment period with up to 5 post dose PET scans and a follow-up visit. The screening assessments were performed within 28 days before dose administration, the baseline PET visit was performed within 7 days before dose administration and the follow-up visit was performed 14 ±2 days after dose administration.

At the baseline PET visit, eligibility criteria were re-checked and subjects still considered eligible were randomized and subjected to a baseline PET scan. On Day 1, subjects were admitted to the investigational site and a single dose of netupitant (100, 300 or 450 mg) was administered. Blood samples for determination of netupitant plasma concentrations were collected regularly for up to 97 hours after dose administration. PET scans were performed 6, 24, 48, 72 and 96 hours after dose administration. The subjects were discharged from the investigational site after the 24 hour post-dose PET scan and then returned for additional PET scans and PK blood sampling.

Results
Median T_{max} ranged from 5.56 to 5.74 hours indicating that the PET scans between 6 and 7 hours post dose were performed close to C_{max}.

This study showed that netupitant showed that netupitant binds to NK_1 receptor antagonist in the human brain with an ability to block NK_1 receptors. The anticipated high NK_1-RO (90% or higher) close to expected C_{max} (6 hours post dose) was achieved for occipital cortex and frontal cortex for all investigated doses as well as for striatum (for 300 and 450 mg netupitant) and anterior cingulate (for 100 and 450 mg netupitant).

All doses showed a blockade of the NK_1 receptors and for most regions the NK_1-RO declined slowly until 96 hours post dose in a dose-dependent fashion. In the 100 mg dose group, 4 of 6 regions still had a mean NK_1-RO over 70% at 96 hours post dose. In the highest dose group (450 mg), 5 of 6 regions had a mean NK_1- RO near to 80% or higher at 96 hours post dose. A comparison of the results for the dose groups (100 mg, 300 mg and 450 mg) showed a general but low increase in NK_1-ROs with increasing dose.

Table 1. NK1 receptor occupancy (%) in striatum and frontal cortex at 6, 24, 48, 72 and 96 hours after administration of netupitant by dose

Table 11 NK_1 receptor occupancy (%) in **striatum** at 6, 24, 48, 72 and 96 hours after administration of netupitant, by dose - PD analysis set

		Netupitant 100 mg N=2	Netupitant 300 mg N=2	Netupitant 450 mg N=2
NK_1 receptor occupancy (%) at 6 hours	n	2	2	2
	Mean	84.0	92.5	98.0
	SD	1.4	7.8	2.8
	CV%	2	8	3
NK_1 receptor occupancy (%) at 24 hours	n	2	2	2
	Mean	76.0	86.5	88.5
	SD	2.8	6.4	2.1
	CV%	4	7	2
NK_1 receptor occupancy (%) at 48 hours	n	2	2	2
	Mean	65.5	85.0	94.5
	SD	7.8	5.7	4.9
	CV%	12	7	5
NK_1 receptor occupancy (%) at 72 hours	n	2	2	2
	Mean	64.0	78.0	90.0
	SD	0.0	2.8	8.5
	CV%	0	4	9
NK_1 receptor occupancy (%) at 96 hours	n	2	2	2
	Mean	48.5	76.0	82.5
	SD	7.8	1.4	7.8
	CV%	16	2	9

N=Number of subjects in the specific group
n=Number of subjects with data available

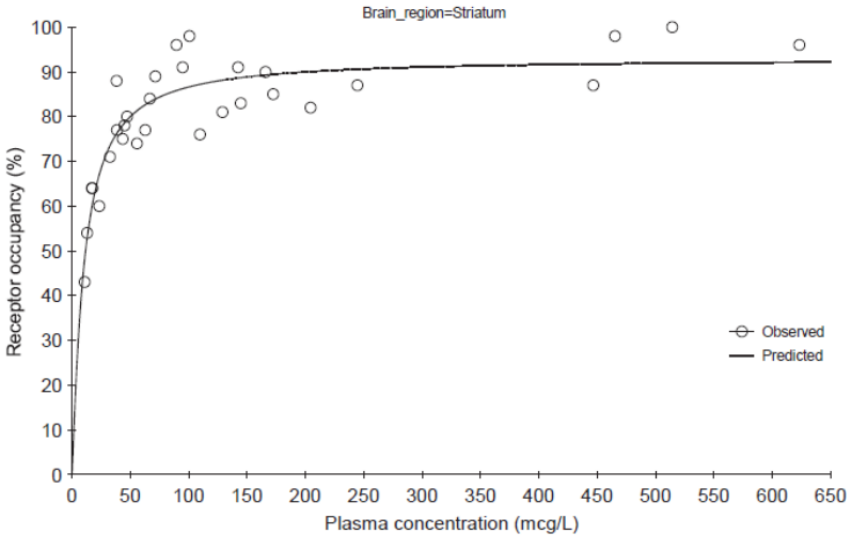
Table 13 NK_1 receptor occupancy (%) in **frontal cortex** at 6, 24, 48, 72 and 96 hours after administration of netupitant, by dose - PD analysis set

		Netupitant 100 mg N=2	Netupitant 300 mg N=2	Netupitant 450 mg N=2
NK_1 receptor occupancy (%) at 6 hours	n	2	2	2
	Mean	90.0	93.5	95.0
	SD	4.2	6.4	7.1
	CV%	5	7	7
NK_1 receptor occupancy (%) at 24 hours	n	2	2	2
	Mean	90.5	86.0	93.0
	SD	0.7	7.1	7.1
	CV%	1	8	8
NK_1 receptor occupancy (%) at 48 hours	n	2	2	2
	Mean	83.0	87.5	95.5
	SD	0.0	6.4	6.4
	CV%	0	7	7
NK_1 receptor occupancy (%) at 72 hours	n	2	2	2
	Mean	82.5	90.0	94.5
	SD	2.1	4.2	0.7
	CV%	3	5	1
NK_1 receptor occupancy (%) at 96 hours	n	2	2	2
	Mean	79.5	89.5	85.5
	SD	2.1	4.9	2.1
	CV%	3	6	2

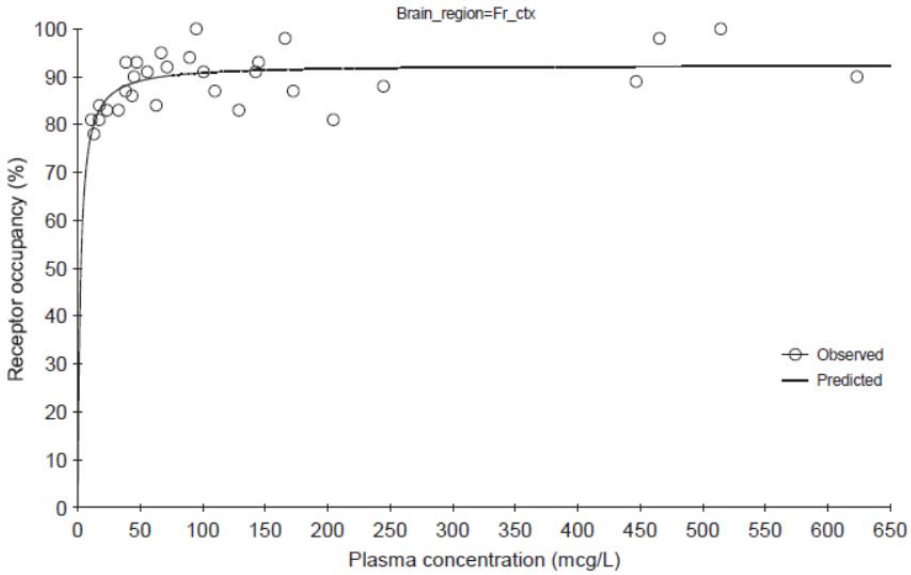
N=Number of subjects in the specific group
n=Number of subjects with data available

Figure 1 NK_1-ROs- Netupitant concentrations in (A) Striatum and (B) Frontal cortex

(A) Striatum



(B) Frontal cortex



Summary

The anticipated high RO (90% or higher) close to expected C_{max} (6 hours after dosing) was achieved after one single dose of netupitant for occipital cortex (100–450 mg), frontal cortex

(100–450 mg), striatum (300 and 450 mg) and anterior cingulate (100 and 450 mg). Therefore it is expected that a minimum single oral dose between 100 and 300 mg netupitant would be necessary to provide an NK_1-RO of 90% close to C_{max} in the majority of the outlined brain regions. This was supported by the analysis of the PK/PD relationship in striatum.

All doses showed a relatively long duration of blockade of the NK_1 receptors with moderate to high NK_1-RO for all investigated brain regions at 96 hours post dose. There was a dose dependent decline in NK_1-ROs over time with a slightly faster decline in the lowest dose group. For the highest dose of netupitant a mean NK_1-RO higher than 90% was achieved in striatum, frontal cortex and anterior cingulate until 72 hours and in occipital cortex even up to 96 hours.

Reviewer's comments:
NK1 receptors are widely distributed throughout the brain. Per the study report, the delineated regions of interest (ROIs) were selected to reflect changes in NK1-RO in the whole brain rather than the target region for the emesis control because of the ill-defined mechanism of emesis.

Based on this study and the apomorphine-challenge study, netupitant doses 100 mg, 200 mg, and 300 mg were selected for the phase 2 trial.

Study NETU-11-01: Single Ascending Dose PK Study for intravenous netupitant in Healthy Volunteers

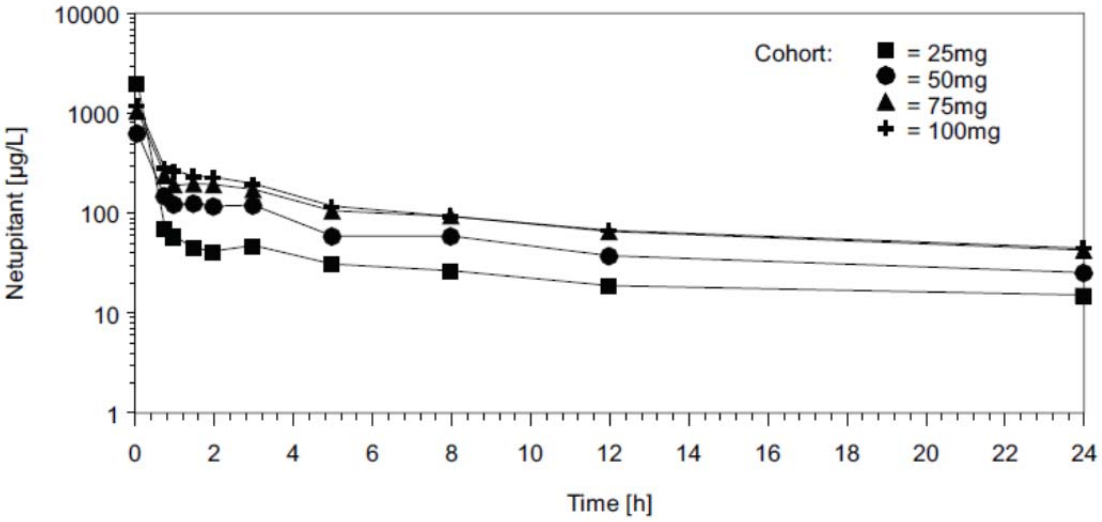
Title of the Study

A Single Ascending Dose Study To Assess the Safety and Pharmacokinetics of Intravenously Administered Netupitant in Healthy Volunteers. A double-blind, placebo-controlled, unbalanced, phase I study.

PK Results

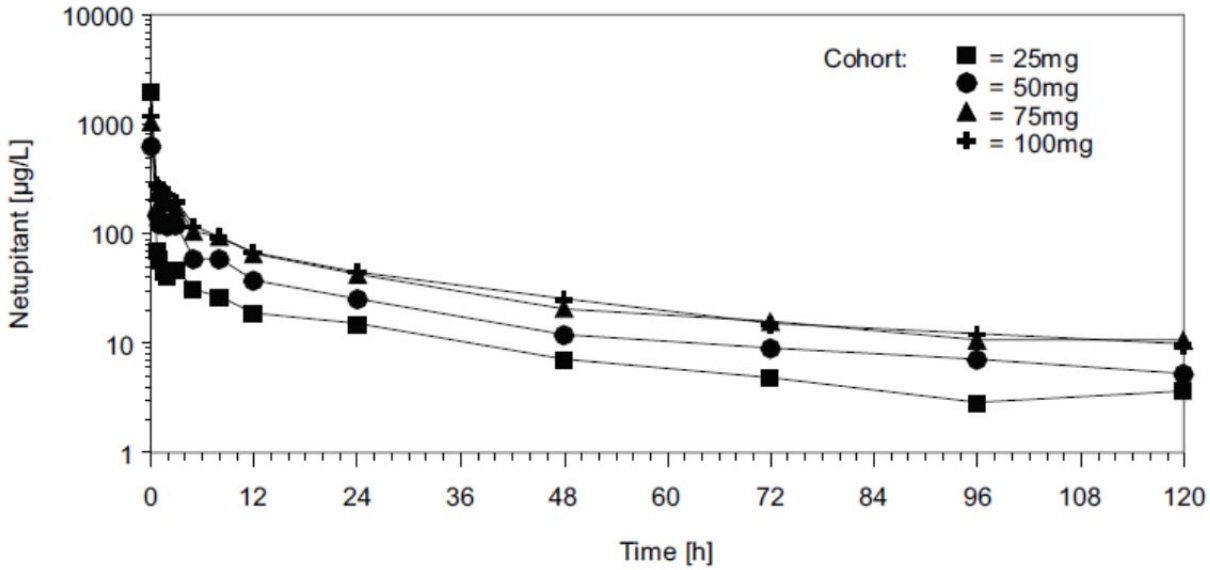
The PK of netupitant and its metabolites M1, M2 and M3 were investigated after IV administration of 4 single ascending doses of netupitant (25 mg, 50 mg, 75 mg, and 100 mg).

Figure 1 Arithmetic mean concentrations of netupitant over (A)24 hours and (B)120 hours after start of infusion of netupitant 25, 50, 75, and 100 mg



(A)

(B)



The netupitant plasma concentrations declined rapidly after end of infusion of the netupitant 25 mg dose and less than 10% of the initial netupitant concentration measured at the end of infusion was detected at 0.75 hours after start of infusion.

The administration of the subsequent dose levels (i.e., netupitant 50 mg, 75 mg, and 100 mg) was performed with an infusion duration of 30 minutes to avoid episodes of very high netupitant plasma concentrations. The prolonged infusion duration resulted in reduced netupitant peak concentrations at the end of infusion with lower variability of data (CVs of about 37%, 23%, and 32% for the netupitant 50 mg, 75 mg and 100 mg doses, respectively). An increase of peak netupitant plasma concentrations with ascending netupitant doses infused over 30 minutes was observed.

An increase of mean systemic netupitant exposure (AUC0-last) was also seen with ascending netupitant doses. The variability of AUC0-last was low with slightly higher variability for the 25 mg dose cohort (CV of about 19%) than for the other dose cohorts (CV of about 12% and 13%). The extent of exposure of all tested IV netupitant doses was lower than the exposure obtained after oral administration of netupitant 300 mg (i.e., the mean values of AUC0-tlast were <12,000 h*μg/L).

After IV infusion of netupitant, the mean volume of distribution was high (about 493 L to 1524 L) and increased with ascending netupitant doses.

The elimination of netupitant was slow with a long terminal elimination half-life (mean of 27 hours to 78 hours). The mean total clearance ranged between 12.7 L/h and 18.9 L/h.

Netupitant metabolites M1, M2, and M3 were detected in plasma after IV administration of all tested dose levels. Netupitant was rapidly metabolized to metabolite M2 with the first quantifiable concentrations measured already at the end of infusion for all tested dose levels and a median Tmax of about 3 hours. For metabolite M3, the first quantifiable concentrations for the highest dose level of netupitant 100 mg were also observed at the end of infusion. For the lower dose levels, quantifiable concentrations were observed within the first 1.5 hours after start of infusion (a.s.i.). Median M3 Tmax was about 24 hours for all dose levels except for the highest dose level of 100 mg where the median Tmax was about 18 hours. For metabolite M1, first quantifiable concentrations were measured 1 hour a.s.i for the highest dose level of netupitant 100 mg. For all other dose levels, quantifiable concentrations were observed within the first 12 hours a.s.i. Median Tmax was about 24 hours for the 25 mg and 50 mg dose level and about 18 hours for the 75 mg and 100 mg dose levels. With the only exception of Cmax of netupitant after the faster infusion rate applied with the 25 mg dose, all mean exposure parameters

(AUC0-tlast, AUC0-inf, and Cmax) of netupitant and metabolites M1, M2, and M3 increased with the IV netupitant doses of 25 mg, 50 mg, 75 mg, and 100 mg.

Figure 2. Dose-normalized AUC by dose

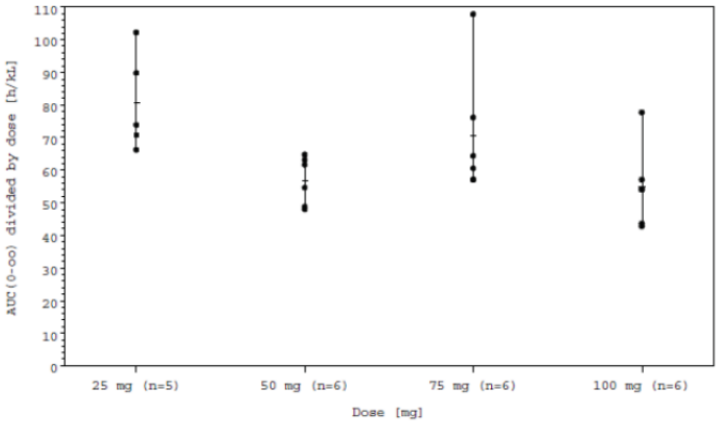


Table 1 Pharmacokinetic parameters of netupitant after infusion of netupitant 25, 50, 75, and 100 mg

Characteristic of netupitant	Statistics	Netupitant 25 mg (N=6)*	Netupitant 50 mg (N=6)	Netupitant 75 mg (N=6)	Netupitant 100 mg (N=6)
C_{max} [µg/L]	n	5	6	6	6
	Mean	2009.5	634.5	1058.6	1203.4
	SD	1120.91	237.36	246.18	386.52
	CV [%]	55.78	37.41	23.26	32.12
	Geo. mean	1778.9	594.1	1035.1	1146.4
	Geo. SD	1.73	1.51	1.26	1.42
	Median	1358.8	651.3	1013.9	1209.9
	Minimum	1026	327	766	632
	Maximum	3548	948	1412	1752
T_{last} [h]	n	5	6	6	6
	Mean	110.40	120.02	119.76	120.00
	SD	13.15	0.03	0.58	0.00
	CV [%]	11.91	0.02	0.48	0.00
	Geo. mean	N.C.	N.C.	N.C.	N.C.
	Geo. SD	N.C.	N.C.	N.C.	N.C.
	Median	120.00	120.00	120.00	120.00
	Minimum	96.00	120.00	118.58	120.00
	Maximum	120.02	120.07	120.00	120.00
T_{max} [h]	n	5	6	6	6
	Mean	0.07	0.50	0.50	0.50
	SD	0.00	0.00	0.01	0.00
	CV [%]	0.00	0.00	1.62	0.00
	Geo. mean	N.C.	N.C.	N.C.	N.C.
	Geo. SD	N.C.	N.C.	N.C.	N.C.
	Median	0.07	0.50	0.50	0.50
	Minimum	0.07	0.50	0.50	0.50
	Maximum	0.07	0.50	0.52	0.50

Characteristic of netupitant	Statistics	Netupitant 25 mg (N=6)*	Netupitant 50 mg (N=6)	Netupitant 75 mg (N=6)	Netupitant 100 mg (N=6)
$AUC_{(0\text{-last})}$ [h*µg/L]	n	5	6	6	6
	Mean	1884.2	2498.2	4180.0	4575.9
	SD	365.67	299.32	478.83	601.41
	CV [%]	19.41	11.98	11.46	13.14
	Geo. mean	1857.9	2483.8	4158.5	4542.6
	Geo. SD	1.20	1.12	1.12	1.14
	Median	1664.6	2371.9	3957.7	4647.0
	Minimum	1601	2191	3751	3759
	Maximum	2443	2933	5021	5435
$AUC_{(0\text{-inf})}$ [h*µg/L]	n	5	6	6	6
	Mean	2015.6	2843.8	5291.4	5492.4
	SD	374.88	370.72	1469.85	1267.18
	CV [%]	18.60	13.04	27.78	23.07
	Geo. mean	1989.0	2823.3	5148.8	5381.5
	Geo. SD	1.20	1.14	1.28	1.24
	Median	1848.1	2909.5	4687.5	5411.7
	Minimum	1658	2400	4279	4277
	Maximum	2556	3241	8090	7777
$\%AUC_{(last\text{-}\infty)}$ [%]	n	5	6	6	6
	Mean	6.6	11.6	17.4	15.2
	SD	3.07	8.64	16.56	8.30
	CV [%]	46.89	74.25	94.97	54.51
	Geo.Mean	6.0	9.7	13.6	13.3
	Geo.SD	1.59	1.85	1.99	1.81
	Median	5.9	8.9	12.4	14.1
	Minimum	3	4	7	5
	Maximum	11	29	51	30
$t_{1/2\lambda z}$ [h]	n	5	6	6	6
	Mean	27.06	43.66	77.77	61.01
	SD	5.34	32.19	103.92	39.47
	CV [%]	19.72	73.74	133.62	64.69
	Geo. mean	26.58	37.45	49.41	52.91
	Geo. SD	1.25	1.73	2.44	1.75
	Median	28.88	33.07	38.96	48.50
	Minimum	18.40	24.32	26.77	27.16
	Maximum	32.69	108.59	289.37	136.29
V_d [L]	n	5	6	6	6
	Mean	492.9	1076.7	1339.4	1523.6
	SD	116.56	671.20	1262.37	635.28
	CV [%]	23.65	62.34	94.25	41.70
	Geo. mean	482.0	956.8	1038.3	1418.5
	Geo. SD	1.27	1.64	2.03	1.51
	Median	463.7	877.2	933.0	1465.8
	Minimum	373	555	507	916
	Maximum	638	2417	3870	2528

Safety
The netupitant infusion was locally not well tolerated except for the lowest dose of netupitant 25 mg. Overall 4 of the 24 subjects administered with IV netupitant developed an infusion site thrombosis (2 subjects at the highest dose level of 100 mg and 1 subject each at the 50 mg and 75 mg dose levels). These events were considered to have a possible (50 mg and 75 mg) or probable (100 mg) relation to the IMP. The dose escalation process was stopped after administration of 100 mg netupitant due to safety reasons. **This IV netupitant formulation will not be further studied in humans due to the poor local tolerability profile observed in this study**.

Reviewer's comments: The absolute oral bioavailability was not studied. In a cross-study comparison of PK parameters after single oral administration of 100 mg netupitant, the total clearance was lower after IV administration. The CL and CL/F were comparable.

Table 2. Mean (%CV) PK Parameters for netupitant after single dose administration of oral or intravenous Netupitant at 100 mg in healthy subjects

PK parameters Mean (CV [%])	Oral Netupitant[1] (N=4)	I.V. Netupitant[2] (N=6)
Cmax [µg/L]	168 (15.9)	1203.4 (32.12)
AUC(0-last) [h•µg/L]	4359 (26.1)	4575.9 (13.14)
AUC(0-inf) [h•µg/L]	4795 (27.3)	5492.4 (23.07)
$T_{1/2}$ (h)	54.2 (33.1)	61 (65)
CL (L/h) CL/F	22.1 (27.6)	18.9 (20.5)
Vd (L) Vd/F	1713 (40.3)	1523.6 (41.7)

[1]Study RO16603: PK sampling up to 168 h post-dose

[2]NETU-11-01: intravenous infusion over 15 min; infusion site thrombosis occurred in 2 patients; PK sampling up to 120 h after start of infusion

Table 3 Mean (%CV) PK Parameters for metabolites of netupitant after single dose administration of oral or intravenous Netupitant at 100 mg in healthy subjects

PK parameters Mean (CV [%])	Oral Netupitant[1] (N=4)	I.V. Netupitant[2] (N=6)
M1: Demethyl metabolite		
Cmax [µg/L]	14.2 (22.9)	9.1 (16.5)
AUC(0-last) [h•µg/L]	978 (26.2)	744 (130)
AUC(0-inf) [h•µg/L]	1170 (15.6)	1224 (20.8)
M2: N-oxide metabolite		
Cmax-M2 [µg/L]	34.9 (21.3)	39.6 (34.5)
AUC(0-last) [h•µg/L]	279 (46.8)	419.7 (34.6)
AUC(0-inf) [h•µg/L]	500 (23.2)	494 (34.4)
M3: OH-methyl metabolite		
Cmax [µg/L]	25.2 (24.2)	23.5 (24.9)
AUC(0-last) [h•µg/L]	1300 (33.9)	1464 (34.7)
AUC(0-inf) [h•µg/L]	1570 (27.6)	1712 (33.7)

[1]Study RO16603: PK sampling up to 168 h post-dose

[2]NETU-11-01: PK sampling up to 120 h after start of infusion

In –Vitro Studies:

Title: NK1 Receptor Antagonist Ro 67-3189: In Vitro Plasma Protein Binding and Blood/Plasma Partitioning in Man and Various Animal Species.

Report No: 1006047

Specific Aims: To determine the *in vitro* protein binding of Ro 67-3189 in plasma of different species and to assess the blood/plasma partitioning.

Study Date: 03/1999-05/1999

Test site: F. Hoffmann-La Roche Ltd., Basle, Switzerland, Dept. PRNS

Sponsor: F. Hoffmann-La Roche, Ltd.

Study Design:

Test Item: Ro 67-3189 (NK1 receptor antagonist)

Tested Concentration: 9-1300 ng/mL in human plasma

Plasma reference:

Table 1 Biochemistry values in plasma pools from the various species

Species	n	total protein g/L	albumin g/L	AGP g/L
man	18	68	46	0.69
Dog	4	53	29	not measured
Ra	>20	61	32	not measured
gerbil rats	>6	46	29	not measured

For in vitro blood/plasma partitioning, blood was obtained from one male healthy volunteer, two dogs and four rats.

Study Method:

Binding Study:

The protein binding was evaluated by equilibrium dialysis at 37°C and pH 7.4 after addition of ^{14}C- or ^{3}H-labeled drug to plasma. Binding to isolated human serum albumin (HSA), human α1-acid glycoprotein (AGP), and to diluted bovine fetal serum (BFS) was assessed in addition.

The time required to reach equilibrium was investigated for ^{14}C-Ro 67-3189/003 at drug concentration of 2.6 µg/mL and pH of 7.4, and it was determined to be approximately 5 hrs. For all the subsequent binding studies with ^{3}H-labeled Ro 67-3189/004 to determine the plasma protein binding in the various species, the dialysis time was set to 5.0 hours. 600 µL of blank plasma was added when sample were removed from the buffer solution from the dialysis cells to minimize the loss of substance due to non-specific adsorption to the material. The resulting dilution of the buffer samples was determined by weigh.

The pH dependency of the protein binding was also determined between final pH values 7.0 and 7.8 by using 0.133 M Søerensen phosphate buffers.

Blood/Plasma Partitioning:

Freshly drawn blood was centrifuged to generate a small erythrocyte free plasma layer, and equilibrated at 37°C for 30 min. The erythrocyte-free plasma layer were then spiked with ^{14}C-Ro 67-3189/003 where the drug concentrations ranged from 10 to 14000 ng/mL, and were immediately mixed at 37°C.

After 30 min of incubation at 37°C, aliquots were removed, centrifuged and the drug concentrations were measured in plasma and whole blood by liquid scintillation counting. Selected rests of spiked blood samples were let to stay at 21° (RT) for 30 min to measure the influence of the temperature on the partitioning. The hematocrit (H) was determined in the freshly drawn blood using a hematocrit centrifuge and hematocrit reader.

The reversibility of the partitioning was measured at the end of the incubation by re-suspending the erythrocytes in fresh blank plasma for 30 min at 37°C.

Bioanalytical Method:

Concentrations of ^{14}C-Ro 67-3189/003 and ^{3}H-Ro 67-3189/004 were determined in duplicate in buffer, plasma, protein solutions and whole blood (triplicate) by liquid scintillation counting. The limit of quantification in the buffer samples was 0.5 ng/mL for ^{14}C-Ro 67-3189, and 0.0015 ng/mL for ^{3}H-Ro 67-3189,

Data Analysis:

Protein Binding:

The approximate time for protein binding to achieve equilibrium was calculated by fitting the % free drug versus time (t) data to the equation:

$$\%free = \%free_{ss} \cdot (1-e^{-kt})$$

where $\%free_{ss}$ is the percent free at equilibrium, and k is a first order rate constant for the equilibration. The time to achieve equilibrium is taken as 5 times the equilibrium half-life (t1/2 = 0.693/k).

The free fraction fu, was calculated as the ratio between the concentration found in the buffer dialysate (C_B) and the concentration in the corresponding plasma or protein solution (C_{Pe}) dialysate at the end of the dialysis:

$$fu = C_B/C_{Pe}$$

The free fraction was corrected for osmotic fluid volume shifts (which is considered to be relevant when plasma is dialyzed for time periods longer than 4 h) according to the equation given by Boudinot and modified by Lohmann:

$$fu = C_B \cdot V_{Pi} / [C_{Pe} \cdot V_{Pe} - C_B \cdot (V_{Pe} - V_{Pi}]$$

where V_{Pi} and V_{Pe} are the initial and final plasma volume, respectively.

Blood/Plasma Partitioning

The blood/plasma concentration ratio (λ) was calculated from:

$$\lambda = C_W/C_P = (H \cdot C_E/C_P) + (1-H)$$

where C_W, C_P and C_E are the drug concentration in whole blood, plasma, and erythrocytes, respectively, and H the hematocrit value.

The fraction f_E of drug in erythrocytes was calculated from:

$$f_E = Q_E/Q_W = (\lambda+H-1)/\lambda$$

where Q_E is the amount of drug in the erythrocyte compartment and Q_W the amount of drug in whole blood.

Note that if no drug is bound the red blood cells, C_E/C_P tends to zero, and λ will simplify to:

$$\lambda = 1 - H$$

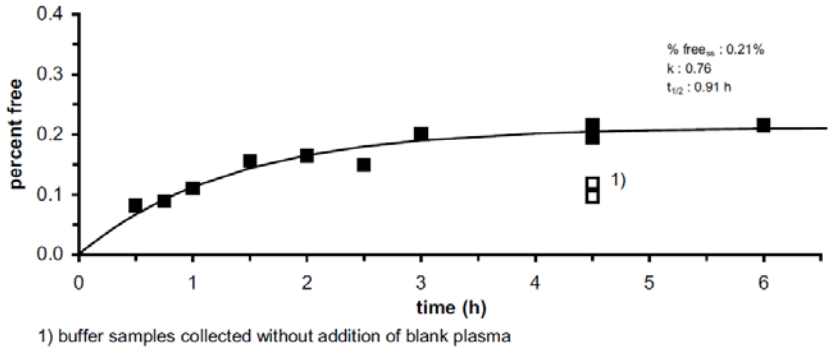
Results:

Protein Binding in Human Plasma

Equilibrium Kinetic:

The time required to reach equilibrium in human plasma was determined with ^{14}C-Ro 67-3189/003 at a concentration of 2600 ng/mL and found to be about 5 hours.

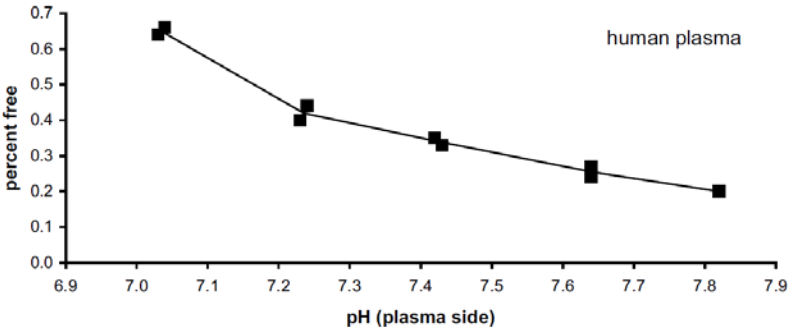
Figure 1 Ro 67-3189: Percent free as function of time (human plasma)



All the values were corrected for fluid volume shift Determined in human plasma with ^{14}C Ro 67-3189/003 at pH 7 4 and 2 6 µg/mL

Influence of pH:

Figure 2 Ro 67-3189: Influence of the pH on the binding



Determined in human plasma at a concentration of 82 ng/mL; The time of dialysis was 5h; all the values were corrected for fluid volume shift

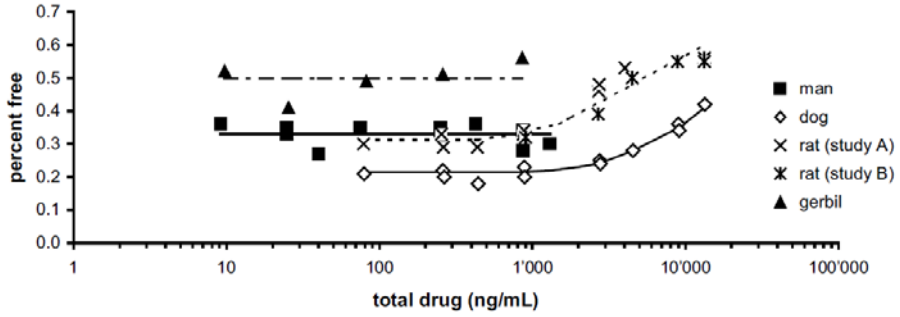
Influence of Concentration:

The protein binding in human plasma was constant (99.7% binding) over the whole concentration range tested (9-1300 ng/mL).

Table 2: ^{3}H-Ro 67-3189: In vitro binding to human plasma (pH 7.41)

concentration of Ro 67-3189 (ng/mL) plasma	buffer	% free 2)	% bound 2)	correction factor 3)	EtOH (%)
9.13	0.0376	0.36	99.64	1.14	0.5
24.7	0.0936	0.33	99.67	1.15	0.5
24.7	0.0958	0.35	99.65	1.11	0.5
39.9	0.121	0.27	99.73	1.11	0.5
74.9	0.298	0.35	99.65	1.14	0.5
249	0.927	0.34	99.66	1.09	0.5
251	0.987	0.35	99.65	1.11	0.5
425	1.69	0.36	99.64	1.11	0.5
786	4.03	0.45	99.55	1.13	**1.5**
797	4.16	0.46	99.54	1.13	**1.5**
867	3.27	0.34	99.66	1.12	0.5
865	2.67	0.28	99.72	1.11	0.5
1'300	4.41	0.30	99.70	1.13	0.5
MEAN 5)		**0.33**	99.67		
SD		0.032			

Figure 3 **Ro 67-3189: *In vitro* plasma protein binding in man and various animal species**



Binding to Isolated Human Plasma Proteins:

The relative contribution of albumin (HSA) and α1-acid glycoprotein (AGP) to the overall plasma binding in man was determined at drug concentrations ranging from 20 to 2600 ng/mL, both proteins separately and/or in combination at physiological concentrations of 41 g/L (HSA) and 0.6 and 1.8 g/L (AGP).

Binding of Ro 67-3189 to HAS was constant (fu=2.9%) within the concentration range tested (20 to 2'290 ng/mL) while binding to AGP was concentration dependent.

Table 3: ^{3}H-Ro 67-3189: In vitro binding to isolated human plasma Proteins

matrix 1)	concentration of ^{3}H-Ro 67-3189 protein side ng/mL	buffer ng/mL	protein side µM	**% free**	% bound
HSA : 41 g/L	20.3	0.591	0.035	2.9	97.1
	66.1	1.81	0.11	2.7	97.3
	214	6.45	0.37	3.0	97.0
	732	21.5	1.3	2.9	97.1
	2'290	69.6	4.0	3.0	97.0

	MEAN			**2.9**	97.1
	SD				0.1
AGP : 0.62 g/L	17.6	0.676	0.030	3.8	96.2
	52.6	2.88	0.091	5.5	94.5
	200	10.4	0.35	5.2	94.8
	636	46.3	1.1	7.3	92.7
	1'810	190	3.1	11	89
	MEAN			n.c.	n.c.
	SD				
HSA : 41 g/L + AGP : 0.62 g/L	22.8	0.312	0.039	1.4	98.6
	73.2	1.07	0.13	1.5	98.5
	247	3.33	0.43	1.4	98.6
	808	12.8	1.4	1.6	98.4
	2'420	39.3	4.2	1.6	98.4
	MEAN			**1.5**	98.5
	SD				0.12
HSA : 41 g/L + AGP : 1.8 g/L	25.3	0.150	0.044	0.59	99.41
	76.8	0.446	0.13	0.58	99.42
	255	1.66	0.44	0.65	99.35
	856	4.91	1.5	0.57	99.43
	2'580	17.6	4.5	0.68	99.32
	MEAN			**0.61**	99.39
	SD				0.05
FBS [2] : 10%	59.6	6.13	0.10	10	90
	197	21.7	0.34	11	89
	621	76.6	1.1	12	88
	1'790	314	3.1	18	82
	4'930	1'110	8.5	23	77
	MEAN			n.c.	n.c.
	SD				

1) measured concentration 2) foetal bovine serum n.c. not calculated

Blood/Plasma Partition

The mean blood/plasma concentration ratio (λ) in human was 0.69 at 37°C and 21°C. Partitioning was independent of the drug concentration (concentration range tested: 52-994 ng/mL). The fraction of drug in erythrocytes (f_E) was 13%. The partitioning was reversible.

Table 4: ^{14}C-Ro 67-3189: In vitro blood/plasma concentration ratio (λ)

at 37°C						**at 21°C**				
MAN	concentration of Ro 67-3189 ng/mL blood	plasma	λ	H	f_E	concentration of Ro 67-3189 ng/mL blood	plasma	λ	H	f_E
Distribution	52.0	75.1	**0.69**	0.40	0.13	51.8	74.5	**0.70**	0.40	0.14
	100	147	**0.68**	0.40	0.11	300	446	**0.67**	0.40	0.11
	308	448	**0.69**	0.40	0.13	985	1'430	**0.69**	0.40	0.13
	610	895	**0.68**	0.40	0.12					
	994	1'420	**0.70**	0.40	0.14					
MEN			**0.69**	0.40	0.13			**0.69**	0.40	0.12
SD			0.01		0.01			0.01		0.01
Reversibility 1)	0.201	0.320	**0.63**	***0.45***	0.12	not measured				

1) the hematocrite value was increased to 0.45 for the reversibility study because of the lack of blank plasma

Reviewer's Comment:

1. *This review only focused on human data although animal data were also included in the study report.*
2. *Ro 67-3189 was found to be highly protein bound (>99%) in human plasma with 0.33% of mean percentage of free drug. The protein binding in human plasma was concentration independent up to 1300 ng/mL.*
3. *The blood/plasma concentration ratio (λ) in human was 0.69 and it was independent of the drug concentration up to 1 μg/mL. The fraction of drug in erythrocytes (f_E) was about 13% in man.*
4. *The tested concentration of 9-1300 ng/mL of Ro67-3189 is acceptable as they approximately cover the expected C_{max} values in human subjects at the clinical dose (300 mg) where the observed Cmax = 550-880 ng/mL.*

Title: RO0681133, RO0713001 and RO0731519, Metabolites of NK1 Receptor Antagonist RO0673189: In Vitro Plasma Protein Binding and Blood/Plasma Partitioning in Man, Dog and Rat.

Report No: 1010388
Specific Aims: To determine the *in vitro* binding of the major metabolites of RO0673189, namely RO0681133, RO0713001 and RO0731519, to plasma proteins in man, dog and rat, and to assess the partitioning between blood and plasma.

Study Date: 10/2001-06/2003
Test site: F. Hoffmann-La Roche Ltd., Basle, Switzerland, Dept. PRNS

Sponsor: F. Hoffmann-La Roche, Ltd.

Study Design:
Test Item: 3 Metabolites of RO067-3189 : ^{14}C-RO0681133 (desmethyl derivative, M1), ^{14}C-RO0713001 (N-oxide derivative, M2) and ^{14}C-RO0731519 (OH-methyl derivative, M3)

Plasma reference:
The plasma pools for protein binding studies were obtained from healthy adult volunteers (n=11, male and female). For in vitro blood/plasma partitioning, blood was obtained from a single healthy male Volunteer.

Study Method:

Binding Study:

The protein binding of RO0681133, RO0713001 and RO0731519, three major metabolites of the NK1 receptor antagonist RO0673189 was evaluated by equilibrium dialysis at 37^{o}C and pH 7.4 after addition of ^{14}C-labeled compounds to human plasma. The time required to reach equilibrium was investigated by conducting dialysis for different time period (0.5 h - 5.5 h). pH was measured at the end of dialysis. 600 µL of blank plasma was added when sample were removed from the buffer solution from the dialysis cells to minimize the loss of substance due to non-specific adsorption to the material. The resulting dilution of the buffer samples was determined by weigh.

Blood/Plasma Partitioning:

Freshly drawn blood was centrifuged to generate a small erythrocyte free plasma layer, and equilibrated at room temperature (21^{o}C) and 37°C. The erythrocyte-free plasma layer were then spiked with aliquots of metabolites where the drug concentrations in blood ranged from 70 to 5900 ng/mL depending on the metabolites and species, and were immediately mixed at desired constant temperature. After 30 min of incubation, aliquots were removed, centrifuged and the drug concentrations were measured in plasma and whole blood by liquid scintillation counting. The hematocrit (H) was determined in the freshly drawn blood using a hematocrit centrifuge and hematocrit reader.

The reversibility of the partitioning was measured at the end of the incubation by re-suspending the erythrocytes in fresh blank plasma for 30 min at the same temperature.

Bioanalytical Method:
Concentrations of ^{14}C-labelled metabolites in buffer (single or duplicate) plasma (duplicate) and whole blood (triplicate) were determined by liquid scintillation counting. The limit of quantification (LOQ) was 10 ng/mL in blood and 0.3 ng/mL in plasma and buffer.

Data Analysis:

Protein Binding:

The approximate time for protein binding to achieve equilibrium was calculated by fitting the % free drug versus time (t) data to the equation:

$$\%free = \%free_{ss} \cdot (1-e^{-kt})$$

where $\%free_{ss}$ is the percent free at equilibrium, and k is a first order rate constant for the equilibration. The time to achieve equilibrium is taken as 5 times the equilibrium half-life (t1/2 = 0.693/k).

The free fraction fu, was calculated as the ratio between the concentration found in the buffer dialysate (C_B) and the concentration in the corresponding plasma or protein solution (C_{Pe}) dialysate at the end of the dialysis:

$$fu = C_B/C_{Pe}$$

The free fraction was corrected for osmotic fluid volume shift (which is considered to be relevant when plasma is dialyzed for time periods longer than 4 h) according to the equation given by Boudinot and modified by Lohmann:

$$fu = C_B \cdot V_{Pi} / [C_{Pe} \cdot V_{Pe} - C_B \cdot (V_{Pe}-V_{Pi}]$$

where V_{Pi} and V_{Pe} are the initial and final plasma volume, respectively.

Blood/Plasma Partitioning

The blood/plasma concentration ratio (λ) was calculated from:

$$\lambda = C_W/C_P = (H \cdot C_E/C_P) + (1-H)$$

where C_W, C_P and C_E are the drug concentration in whole blood, plasma, and erythrocytes, respectively, and H the hematocrit value.

The fraction f_E of drug in erythrocytes was calculated from:

$$f_E = Q_E/Q_W = (\lambda+H-1)/\lambda$$

where Q_E is the amount of drug in the erythrocyte compartment and Q_W the amount of drug in whole blood.

Results:

Table 1: Comparison of in vitro Plasma Protein Binding in Man of Parent Drug RO0673189 and its Metabolites RO0681133, RO0713001 and RO0731519

	free fraction (expressed as percentage)			
	RO0673189 [1)]	**RO0681133**	**RO0713001**	**RO0731519**
mean [2)] **MAN**	**0.33** up to 1'300 ng/mL no	**0.91** up to 2'500 ng/mL no	**2.3** up to 2'500 ng/mL no	**0.88** up to 2'000 ng/mL no

1) Research Report No 1006047 (b) (4)
2) Mean value calculated within the linear range of binding
3) max: value obtained at the highest concentration tested
no : no influence of the concentration over the whole concentration range tested

Table 2: Comparison of in vitro Blood/Plasma Partitioning in Man of Parent Drug RO0673189 and its Metabolites RO0681133, RO0713001 and RO0731519

	blood/plasma concentration ratio (λ)			
	RO0673189 [1)]	**RO0681133**	**RO0713001**	**RO0731519**

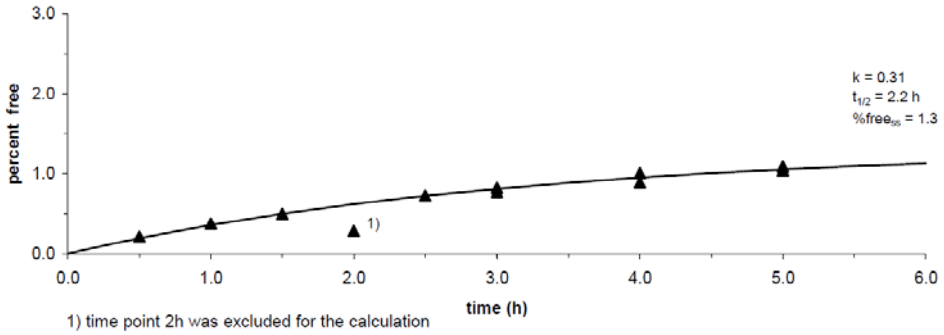
MAN mean [2)]	**0.69** up to 1'000 ng/mL no	**1.1** up to 2'500 ng/mL no	**0.69** up to 2'500 ng/mL no	**0.61** up to 2'300 ng/mL no

1) Research Report No 1006047 (b) (4)
2) Mean value calculated within the linear range of partitioning
3) max: value obtained at the highest concentration tested
no : no influence of the concentration over the whole concentration range tested

RO068113:

The time required to reach equilibrium in human plasma was determined with ^{14}C-RO0 681133 at a concentration of 2300 ng/mL and found to be about 11 hours. However, dialysis time for subsequent protein binding study was set to 5.5 hr

Figure 1 RO0681133 : Percent Free as Function of Time



All the values were corrected for fluid volume shift. Determined in human plasma with ^{14}C RO0681133 at pH 7.37 and 2.3 µg/mL

The protein binding of ^{14}C-RO0 681133 in human plasma was nearly constant (99.1% binding) over the whole concentration range tested (121-2550 ng/mL).

Table 3: ^{14}C-RO0 681133: In vitro binding to human plasma

concentration of RO0681133 [1)] (ng/mL) plasma	buffer	% free [2)]	% bound [2)]	FVS correction factor	pH [4)]
121	1.28	0.93	99.07	1.14	7.45
375	4.07	0.97	99.03	1.12	nm
1'130	10.2	0.81	99.19	1.12	nm
2'550	25.8	0.90	99.10	1.12	nm
2'540	26.7	0.94	99.06	1.12	7.44
MEAN		**0.91**	99.09	1.12	**7.45**
SD		0.06		0.01	0.01

nm : not measured

The mean blood/plasma concentration ratio (λ) in human was 1.1 at 37°C and 21°C. Partitioning was independent of the tested drug concentration (124 - 2460ng/mL). The partitioning was reversible.

Table 4: ^{14}C-RO0681133: In vitro blood/plasma concentration ratio (λ) in Man

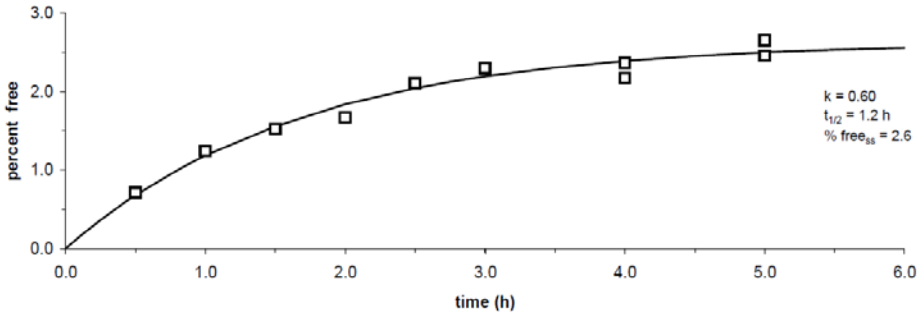
	at 37°C			at 21°C		
	concentration of RO0681133 (ng/mL) blood	plasma	λ	concentration of RO0681133 (ng/mL) blood	plasma	λ
Distribution	124	114	**1.09**			
	338	312	**1.08**	336	317	**1.06**
	1'140	1'040	**1.10**			
	2'460	2'190	**1.12**	2'450	2'240	**1.09**
	MEAN		**1.10**	**MEAN**		**1.08**
	SD		0.02	SD		0.02
Reversibility [4)]	1'210	1'060	**1.14**	not measured		

1) The hematocrite value was 0.38 (man)
4) The erythrocytes were resuspended in fresh blank plasma for 30 min at 37 C

RO0713001

The time required to reach equilibrium in human plasma with ^{14}C-RO0 713001 at a concentration of 2300 ng/mL was approximately 5 hours. The dialysis time was set to 5.5 hours to determine the plasma protein binding in the various species.

Figure 2 RO0713001 : Percent Free as Function of Time



All the values were corrected for fluid volume shift. Determined in human plasma with ^{14}C RO0713001 at pH 7.37 and 2.3 µg/mL

The protein binding of ^{14}C-RO0713001 in human plasma was nearly constant (97.7% binding) over the whole concentration range tested (115-2500 ng/mL).

Table 5: ^{14}C-RO0713001: In vitro binding to human plasma

concentration of RO0713001 [1)] (ng/mL) plasma	buffer	% free [2)]	% bound [2)]	FVS correction factor	pH [4)]
115	2.82	2.2	97.8	1.12	7.43
347	8.72	2.3	97.7	1.11	nm
1'120	29.8	2.4	97.6	1.11	nm
2'480	63.8	2.3	97.7	1.12	nm
2'500	70.3	2.5	97.5	1.11	7.44
MEAN		**2.3**	97.7	1.11	**7.44**
SD		0.1		0.01	0.01

The mean blood/plasma concentration ratio (λ) in human was 0.69 at 37°C and 21°C. Partitioning was

independent of the tested drug concentration (74-2500 ng/mL). The partitioning was reversible.

Table 6: ^{14}C-RO0713001: In vitro blood/plasma concentration ratio (λ) in Man

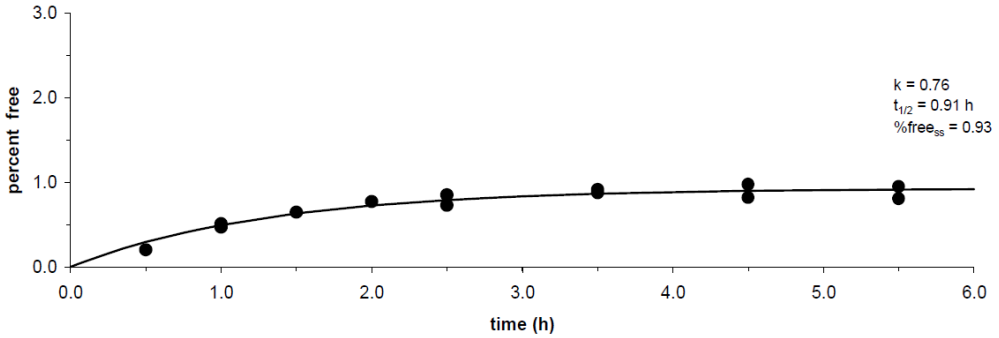
	at 37°C			at 21°C		
	concentration of RO0713001 (ng/mL) blood	plasma	λ	concentration of RO0713001 (ng/mL) blood	plasma	λ
Distribution	73.8	107	**0.69**			
	337	489	**0.69**	338	489	**0.69**
	1'190	1'740	**0.68**			
	2'500	3'630	**0.69**	2'490	3'650	**0.68**
	MEAN		**0.69**	**MEAN**		**0.69**
	SD		< 0.01	SD		0.01
Reversibility [3)]	388	561	**0.69**	not measured		

1) The hematocrite value was 0.38 (man), 3) The erythrocytes were resuspended in fresh blank plasma for 30 min at 37 C

RO0731519

The time to reach equilibrium in human plasma with ^{14}C-RO0731519 at a concentration of 2200 ng/mL was approximately 4.5 hours. The dialysis time was set to 5.5 hours to determine the plasma protein binding in the various species.

Figure 3 RO0731519 : Percent Free as Function of Time



All the values were corrected for fluid volume shift. Determined in human plasma with ^{14}C RO0731519 at pH 7.39 and 2.2 µg/mL

The protein binding of ^{14}C-RO0 731519 in human plasma was mostly constant (99.1% binding) over the whole concentration range tested (114-2070 ng/mL).

Table 7: ^{14}C-RO0 731519: In vitro binding to human plasma

concentration of RO0731519 [1)] (ng/mL) plasma	buffer	% free [2)]	% bound [2)]	FVS correction factor	pH [4)]
114	1.10	0.85	99.15	1.13	7.40
317	3.04	0.87	99.13	1.10	7.38
309	3.28	0.94	99.06	1.13	nm
1'000	9.97	0.88	99.12	1.14	7.39
2'070	21.2	0.95	99.05	1.08	7.39
1'980	18.0	0.81	99.19	1.13	nm
MEAN		**0.88**	99.12	1.12	**7.39**
SD		0.05		0.02	0.01

The mean blood/plasma concentration ratio (λ) in human was about 0.62 and was independent of temperature (37°C, 31°C and 21°C). Partitioning was independent of the tested drug concentration (118-2310 ng/mL) at31°C. The partitioning was reversible.

Table 8: ^{14}C-RO0731519: In vitro blood/plasma concentration ratio (λ) in Man

	at 37°C			at 31°C [2]			at 21°C		
	concentration of RO0731519 (ng/mL)		λ	concentra ion of RO0731519 (ng/mL)		λ	concentration of RO0731519 (ng/mL)		λ
	blood	plasma		blood	plasma		blood	plasma	
Distribution	352	566	**0.62**	118	196	**0.60**	336	551	**0.61**
				335	552	**0.61**			
				1'120	1'830	**0.61**	2'480	3'960	**0.63**
				2'310	3'620	**0.64**			
	MEAN		**0.62**	**MEAN**		**0.61**	**MEAN**		**0.62**
	SD			SD		0.02	SD		0.01
Reversibility [4]	not measured			393	624	**0.63**	not measured		

1) The hematocrite value was 0 43 (man) 2) The study was conducted at 31°C due to a technical problem affecting the temperature regulation
4) The erythrocytes were resuspended in fresh blank plasma for 30 min at 37°C

Reviewer's Comment:

1. *This review only focused on human data although animal (rat and dog) data were also included in the study report.*
2. *The protein binding was 99.1% for RO0681133, 97.7% for RO0713001 and 99.1% for RO0731519 in human plasma. The protein binding was concentration independent over a concentration range which exceeds the maximum plasma concentrations expected in man.*
3. *The blood/plasma concentration ratio (λ) in human was 1.1 for RO0681133, 0.69 for RO0713001 and 0.61 for RO0731519. The ratio was independent of the drug concentration range which exceeds the maximum plasma concentrations expected in man.*
4. *The tested concentration for RO0681133 (121-2540 ng/mL) is acceptable as it covers the expected Cmax in human subject at the clinical dose of 300 mg where the Cmax of RO0681133 was 40-50 ng/ml.*
5. *The tested concentration for RO0713001 (74-2500 ng/mL) is acceptable as it covers the expected Cmax in human subject at the clinical dose of 300 mg where the Cmax of RO0713001 was 100-350 ng/mL.*
6. *The tested concentration for RO0731519 (115-2000 ng/mL) is acceptable as it covers the expected Cmax in human subject at the clinical dose of 300 mg where the Cmax of RO0731519 was 50-90 ng/mL.*

Title: In vitro metabolism of the NK1 receptor antagonist RO0673189: I. Kinetic parameters and Metabolites formed in incubations of liver microsomes, recombinant cytochromes and hepatocytes of different species, including man.

Report No: 1003832

Specific Aims: The aim of this study was to evaluate the major metabolites formed during the elimination of RO0673189 in rats, dog, marmoset and man

Study Date: 08/1998-05/2001

Test Site: F. Hoffmann-La Roche Ltd., Basle, Switzerland,

Sponsor: F. Hoffmann-La Roche, Ltd.

Study Design:

Test Item: [^{14}C]- RO0673189 (MW: 578.6 g/mol)

Study Method:
The major metabolic steps of RO0673189 have been studied in several *in vitro* incubation systems including human hepatocytes, liver microsome and microsomes containing recombinant enzymes.

Hepatocytes:
Hepatocytes from human liver tissue were prepared from hepatic surgical resections. Freshly prepared hepatocytes were seeded in collagen coated six-well plates at the density of 1.5 x10^6 cells (for human). When cell culture was ready, they were incubated with10 µM of test compounds for 24 hours.

Liver Microsomes:
Human liver microsomes were prepared from frozen human liver tissue (pooled tissue of 10 human livers, obtained from hepatic surgical resections). 10 µM of test compound was incubated with human liver microsome (100 µg protein/assays) for 20 minutes at 37°C in presence of NADPH. The reaction was terminated by the addition of 500 µl acetonitrile, centrifuged for 10 min at 15'000 g and the supernatant was analyzed by HPLC.

Table 1: Characteristics of the microsomal preparations used:

Code	Enzyme system	Protein (mg/ml)	P450 (nmol/mg protein)	Preparation Date*
HLM	Microsomes from a pool of 10 human livers	28.16	0.418	20.08.1998

Recombinant Enzymes:
The enzymes were expressed in E. coli and isolated as a membrane fraction. The radiolabeled test compound RO0673189 at a concentration of 5 µM (1 µM for CYP2C9) was incubated with four of the major human CYP450 isoenzymes (CYP3A4, CYP2C9, 2C19 and 2D6) at 100 to 600 pmol CYP450/ml and the incubations were initiated by the addition of NADPH (1 mM). After incubated for 30 min to 1 hour at 37°C, the reaction was terminated by the addition of 500 µl acetonitrile. After centrifugation for 10 min at 15'000 g and the supernatant was analyzed by HPLC.

Table 2: Characteristics of the recombinant human CYP450 enzyme preparations used:

Code	Enzyme system	Protein (mg/ml)	P450 (nmol/mg protein)	Preparation Date*
rhCYP3A4	Rec. human CYP450 3A4	5.04	0.703	10.03.2000
rhCYP2C9	Rec. human CYP450 2C9	19.16	0.519	25.05.1999
rhCYP2C19	Rec. human CYP450 2C19	15.10	0.520	30.10.1998
rhCYP2D6	Rec. human CYP450 2D6	15.58	0.655	05.05.1999

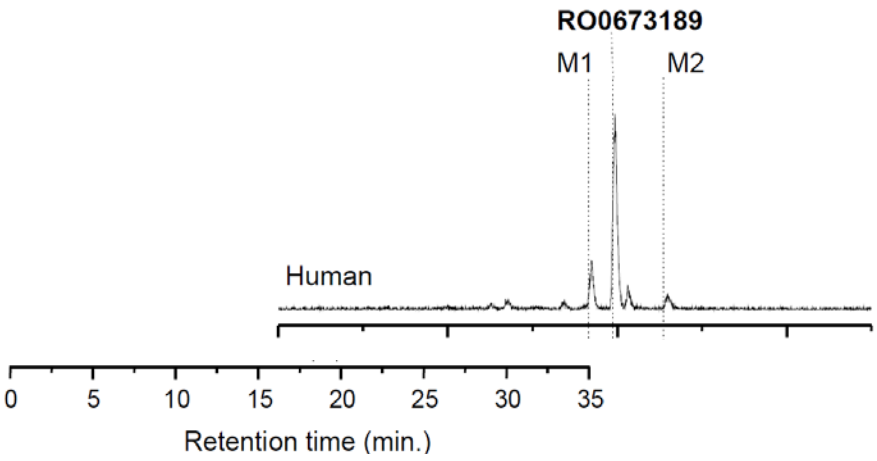
* all preparations were performed at Roche Basel at the dates indicated and stored frozen in aliquots at –80°C.

Characterization of metabolites:
The metabolites of the radiolabeled RO0673189-003 (580.6 g/mol) formed by human liver microsomes were analyzed by LC-MS and compared with the three synthesised metabolites RO0681133, RO0713001, and RO0731519.

Bioanalytical Method: Samples were analyzed with HPLC and LC-MS.

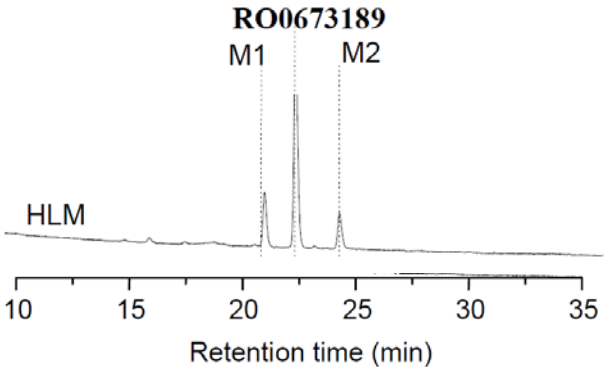
Results:
Hepatocytes:
Incubation of 10 µM of RO0673189 in human hepatocytes for 24 hours had resulted two metabolites (M1 and M2).

Figure 1: Metabolite profiles obtained by incubation (24 hours) of Ro RO0673189-003 (10 µM) with Human hepatocytes.



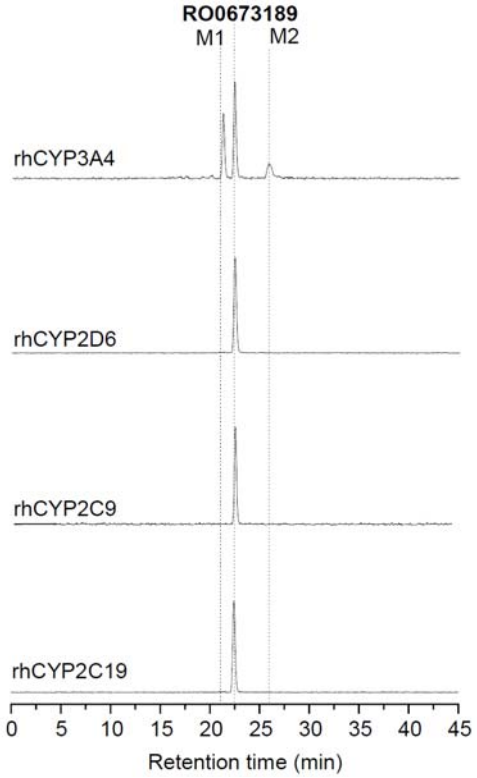
Liver Microsomes:
Incubation of 10 µM of RO0673189 in human liver microsome for 20 minutes had also resulted two metabolites (M1 and M2).

Figure 2: Metabolite profiles obtained by incubation of RO0673189 (10 µM) with human (20 min) liver microsomes (100µg protein/ml).



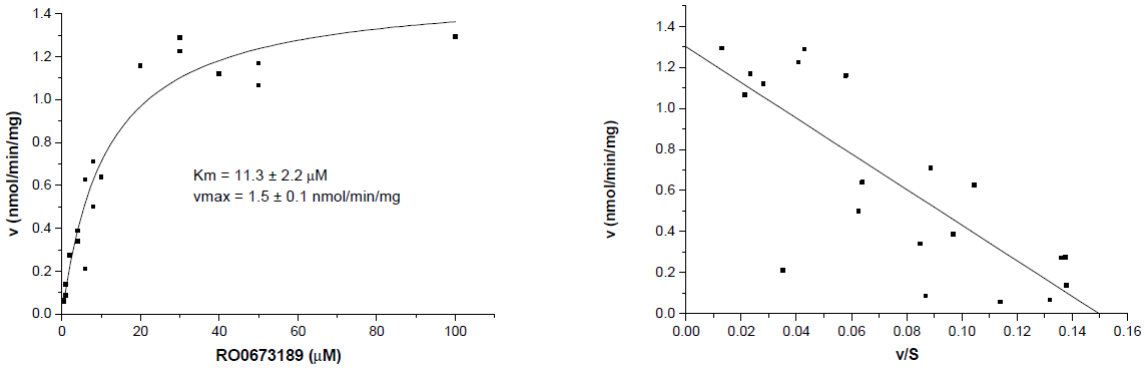
Recombinant Enzymes:
The contribution of different microsomal CYP450 enzymes to the metabolism of RO0673189 was studied by utilizing recombinant human enzymes. Based on the Figure 3, it appears that CYP2C9, 2C19 and 2D6 do not catalyst the formation of any metabolite of RO0673189 while CYP3A4 appears to metabolize RO0673189 to the same metabolites (M1 and M2) that were observed when RO0673189 was incubated with human liver microsomes and hepatocytes.

Figure 3: Incubation (30 min - 1 hour) of Ro RO0673189-003 (1 - 5 µM) with recombinant CYP450 from E. coli membranes (100 – 500 pmol CYP450/ml)



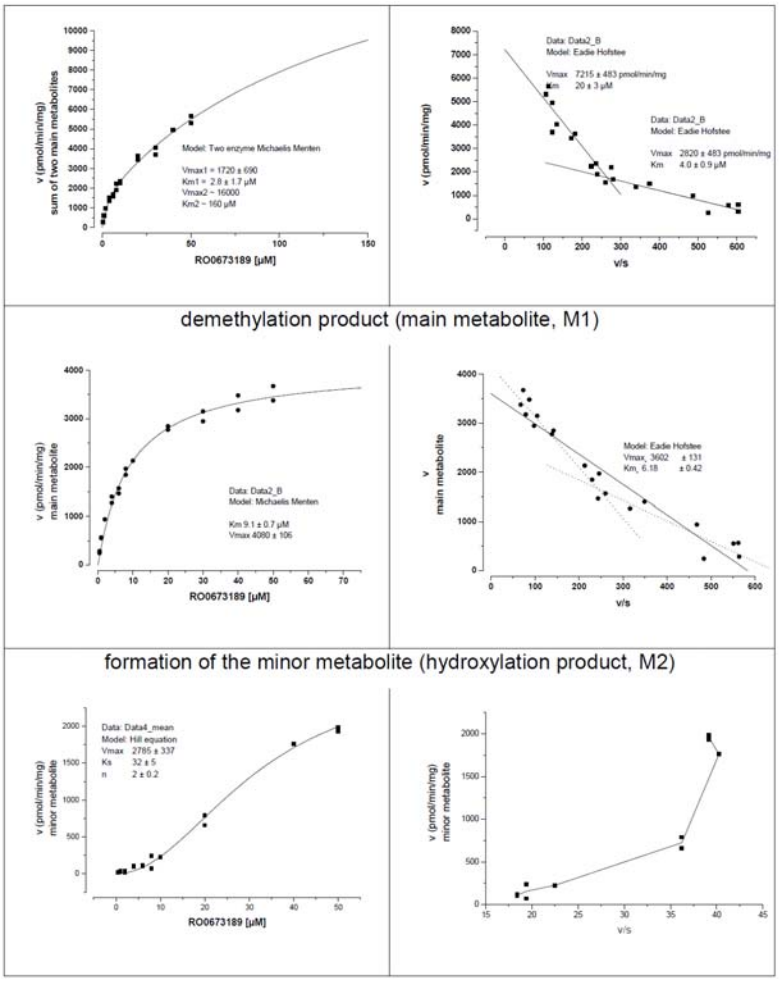
Kinetic Studies:
The overall metabolism of the radiolabeled RO0673189 was studied in human, liver microsomes and membranes containing rhCYP3A4, and the initial velocity of the disappearance of RO0673189 (0.1 to 100 µM) was determined under linear product formation.

Figure 4: Determination of the enzyme kinetic parameters of the formation of the metabolites of RO0673189 in human liver microsomes (100 µg/ml)



For rhCYP3A4 incubations kinetic parameters were determined for the formation of the metabolites separately

Figure 5: Determination of the enzyme kinetic parameters of the formation of the two main metabolites of RO0673189 catalyzed by rec. human CYP3A4 expressed in E. coli membranes (20 nmol P450/assay).



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Characterization of Metabolites**:**
Based on the similarity in retention time in HPLC analysis and results of MS analysis, RO0681133 fitted with one of the major metabolites, the N-demethylation product marked as M1 while RO0713001 fitted with the second major metabolite, a N-oxidation product, marked as M2. The minor metabolite M3 was found to be identical with RO0731519, showing a hydroxylation of the toloyl-methyl group of the molecule.

Figure 6: Analysis of minor and major metabolites obtained after incubation (30 min) of Ro RO0673189-003 (10 µM) with Human Liver Microsomes (250 µg protein) and comparison with synthetic reference Compounds

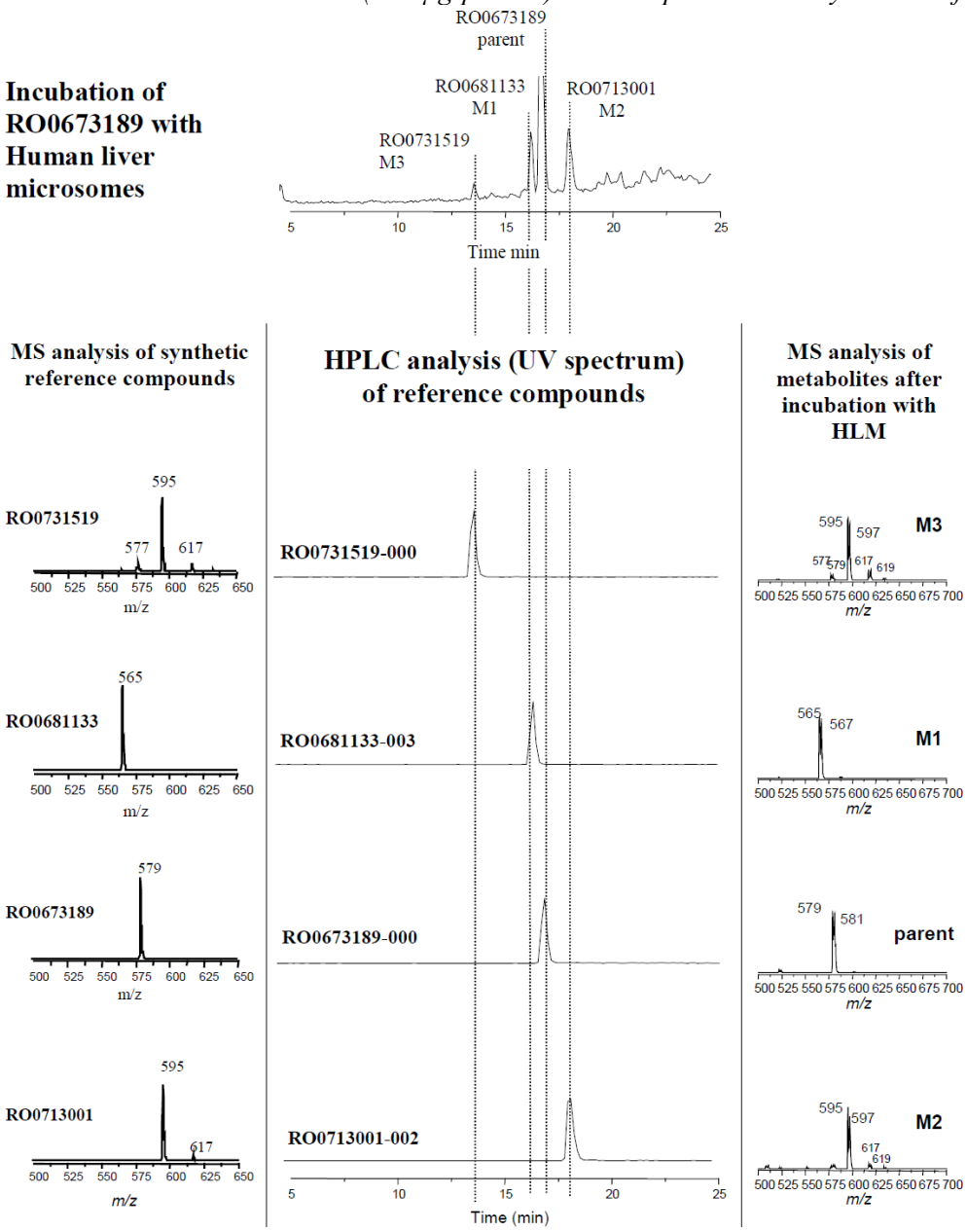
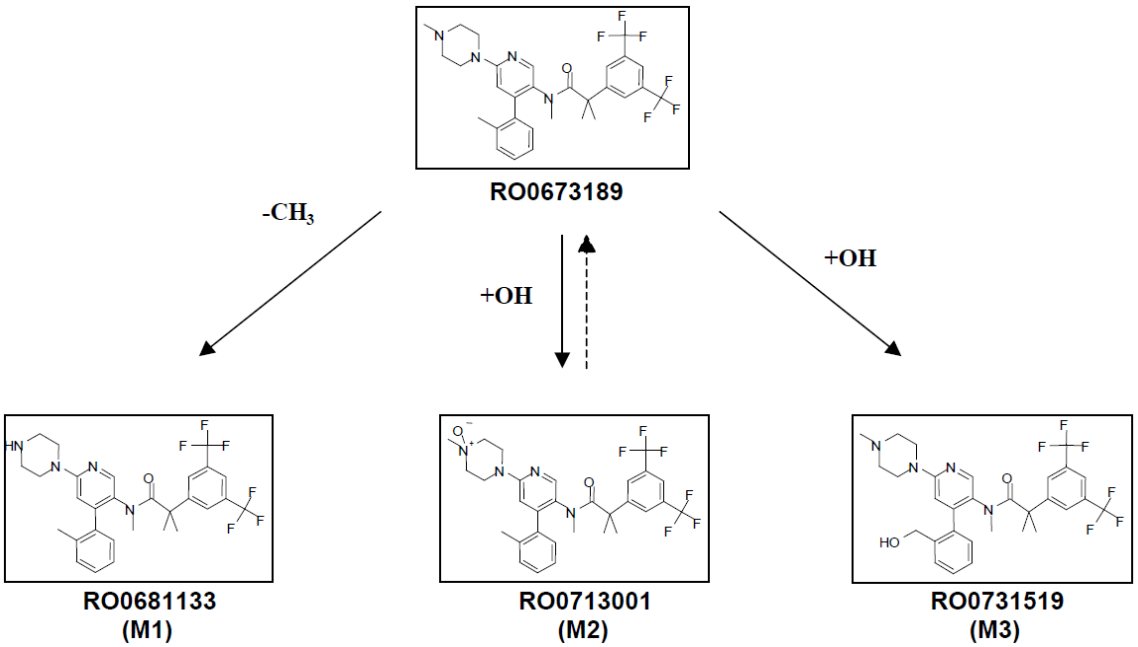


Figure 7: Proposed metabolic pathway of RO0673189



Reviewer's Comment:

1. *This review only focused on the metabolic stability of RO0673189 in human although data for several animal species were also included in this study report.*
2. *It is not clear if the test systems (hepatocytes, liver microsome) were not properly validated in terms of various CYP enzymes prior to the use.*
3. *The sponsor did not provide detailed information about the hepatocyte (e.g. how many subjects, pooled vs. individual).*
4. *The experiment conditions did not include proper controls (both positive and negative) during the incubation.*
5. *The sponsor did not evaluate the potential of CYP1A2, 2B6 and 2C8 to metabolize RO067318.*
6. *The results of kinetic studies are difficult to interpret as the experimental conditions (e.g., duration of incubation, kinetic measurement of formation of metabolite vs. disappearance of parent) were not clear in the study report.*
7. *The concentration of 10 µM in hepatocytes and liver microsome and 1-5 µM in recombinant enzymes are acceptable as they approximately represent the expected C_{max} and 10 times C_{max} in human subject where the Cmax at the clinical dose was 550-880 ng/mL (≈ 1-.5 µM).*

Title: Netupitant: Reaction Phenotyping with Human Liver Microsomes and Human CYP1A2, CYP2B6 and CYP2C8 cDNA expressed enzymes.

Report No: NETU-13-21

Specific Aims: The purpose of this study was to identify in vitro the major drug metabolizing enzymes in human that were responsible for the metabolism of netupitant.

Study Date: 07/08/2013 - 07/23/2013

Test Site: (b) (4)

Sponsor: Helsinn Healthcare, Switzerland.

Study Design:
Test Item: Netupitant (MW: 578.6 g/mol)

Test System: Commercially available Human Liver Microsomes (pool 50 donors mix gender) and Human CYP1A2, CYP2B6 and CYP2C8 cDNA expressed enzymes were used.

Study Method:
Test compound netupitant at the concentration of 10 µM was incubated with Human Liver Microsomes (0.8 mg/ml) in the presence and in the absence of different selective CYP isoforrn inhibitors and with recombinant CYP I A2, 2B6 and 2C8 (20 pmol P450) in Dulbecco's buffer, pH 7.4, for 60 minutes at 37°C in duplicates. Metabolism was started by the addition of NADPH (final concentration 1 mM) after 5 min pre-incubation time in 96 well plates. Aliquots of the incubation mixture were taken at time 0, and after 5, 10, 30 and 60 minutes incubation, the metabolism was stopped by the addition of an equal volume of acetonitrile containing deuterated standards of netupitant and its metabolites Ml, M2 and M3; samples were centrifuged and the supernatant was analyzed by LC-MS/MS to investigate both the disappearance of parent compound Netupitant and the formation of the metabolites Ml, M2 and M3.

The inhibitors used for each CYP isoform were: 100 µM Furafylline (for CYPI A2), 100 µM Ticlopidine (CYP2B6), 100 µM Trimetoprim (CYP2C8), 100 µM Sulfaphenazole (CYP2C9), 100 µM Nootkatone (CYP2C 19), 100 µM Quinidine (CYP2D6), 1 µM Ketoconazole (CYP3A4) and 1000 µM Aminobenzotriazole (generic CYPs inhibitor).

Controls:
Specific probe substrates for each enzyme were incubated as positive controls to check the metabolic activity of the test systems used (Human Liver Microsomes and cDNA expressed enzymes): Tacrine (CYP1A2), Bupropion (CYP2B6), Paclitaxel (CYP2C8), Diclofenac (CYP2C9), S-Mephenytoin (CYP2Cl 9), Dextromethorphan (CYP2D6) and Midazolam (CYP3A4).

Bioanalytical Method:
The incubation samples were analyzed by LC-MS/MS to assess the formation of metabolites M l, M2 and M3 from the parent compound Netupitant

Calculation:
Intrinsic clearance (CLint) was calculated using the half-life approach where the half-life and CLint were determined from the concentration remaining at the different sampling points. By plotting the natural logarithmic (LN) value of the concentration of the compound remaining against the time, the slope was calculated by linear regression analysis and converted into the half-life (T112) and Clint expressed as

μL/min/mg protein for human liver microsomes or μL/min/pmol P450 for cDNA expressed enzymes:

$$T_{1/2} = LN(2)/\text{-slope}$$

$$CL_{int} = LN(2)/T_{1/2} \text{ / mg protein or pmolP450}$$

Results:

Based on study results of inhibition study in human microsome, it appear the metabolism of netupitant to M1, M2 and M3 is mainly mediated by CYP3A4 and lesser extent by CYY2C9 and CYP2D6. CYP1A2, CYP2B6, CYP2C8 and CYP2C19 do not appear to contribute to the netupitant metabolism. Study in CYPIA2, 2B6 and 2C8 cDNA expressed enzymes further confirms that these enzymes do not contribute to netupitant metabolism.

Table 1 Netupitant disappearance in HLM and cDNA CYPs expressed systems in the presence and in the absence of CYPs inhibitors.

Netupitant						
Matrix	Inhibitor	$T_{1/2}$ (min)	$T_{1/2}$ Mean	Cl_{int} (μL/min/mg)	Cl_{int} Mean	Comment
HLM	--	23.31		37.18		
		30.83	27.07	28.10	32.64	
HLM	Furafylline (CYP1A2)	25.38		34.13		
		22.81	24.10	37.98	36.06	
HLM	Ticlopidine (CYP2B6)	29.07		29.81		
		26.91	27.99	32.20	31.00	
HLM	Trimetoprim (CYP2C8)	17.34		49.98		
		18.69	18.02	46.35	48.16	
HLM	Sulfaphenazole (CYP2C9)	164.35		5.27		
		203.09	183.72	4.27	4.77	
HLM	Nootkatone (CYP2C19)	11.91		72.78		
		18.92	15.41	45.78	59.28	
HLM	Quinidine (CYP2D6)	59.13		14.65		
		65.49	62.31	13.23	13.94	
HLM	Ketoconazole (CYP3A4)	424.65		2.04		
		696.18	560.42	1.24	1.64	
CYP1A2 Supersomes	--	167.05		0.21*		
		248.69	207.87	0.14*	0.17*	Non Metabolic Decline
CYP2B6 Supersomes	--	83.64		0.41*		
		155.85	119.74	0.22*	0.32*	Non Metabolic Decline
CYP2C8 Supersomes	--	56.31		0.62*		
		66.29	61.30	0.52*	0.57*	Non Metabolic Decline
CYP1A2 Supersomes	Furafylline (CYP1A2)	99.36		0.35		
		166.29	132.83	0.21	0.28*	Non Metabolic Decline
CYP2B6 Supersomes	Ticlopidine (CYP2B6)	103.80		0.33*		
		72.74	88.27	0.48*	0.41*	Non Metabolic Decline
CYP2C8 Supersomes	Trimetoprim (CYP2C8)	32.77		1.06*		
		21.11	26.94	1.64*	1.35*	Non Metabolic Decline
HLM	Amonobenzotriazole	165.60		5.23		
		267.61	216.60	3.24	4.23	

* Clint expressed as μL/min/pmolP450

Figure 1 Netupitant Metabolites Formation Rate in HLM system.

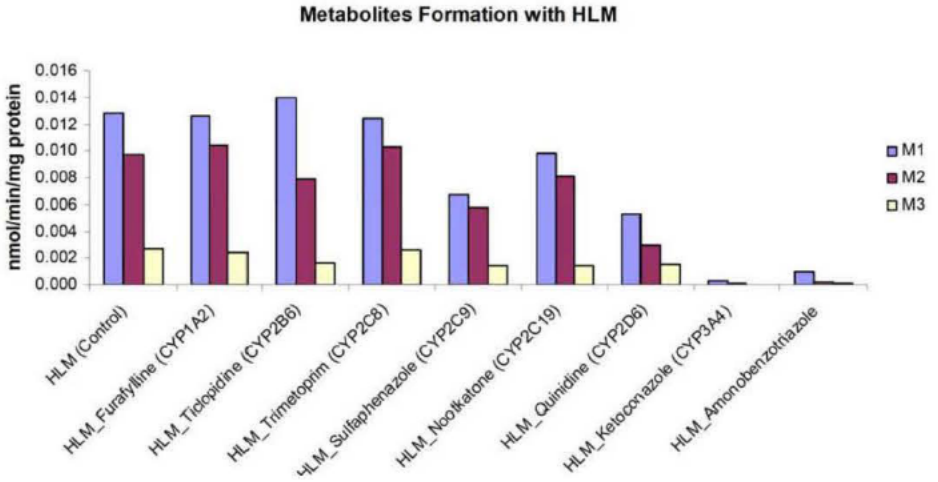


Table 2 Netupitant metabolite M1, M2 and M3 formation rate in HLM and cDNA expressed systems in the presence and in the absence of CYPs inhibitors.

Matrix	Inhibitor	M1 Formation Rate (nmol/min/mg)	Mean	% M1 Formation vs Control	M2 Formation Rate (nmol/min/mg)	Mean	% M2 Formation vs Control	M3 Formation Rate (nmol/min/mg)	Mean	% M3 Formation vs Control
HLM (Control)	--	0.018			0.012			0.0036		
		0.008	0.013	*100*	0.007	0.010	*100*	0.0017	0.003	*100*
HLM	Furafylline (CYP1A2)	0.017			0.014			0.0033		
		0.008	0.013	98.9	0.007	0.010	107.1	0.0016	0.002	91.3
HLM	Ticlopidine (CYP2B6)	0.019			0.011			0.0021		
		0.009	0.014	109.0	0.005	0.008	81.7	0.0011	0.002	59.9
HLM	Trimetoprim (CYP2C8)	0.017			0.013			0.0035		
		0.008	0.012	97.5	0.007	0.010	106.1	0.0018	0.003	99.4
HLM	Sulfaphenazole (CYP2C9)	0.009			0.008			0.0019		
		0.005	0.007	52.5	0.004	0.006	60.1	0.0010	0.001	53.4
HLM	Nootkatone (CYP2C19)	0.011			0.009			0.0016		
		0.009	0.010	76.9	0.007	0.008	83.1	0.0013	0.001	55.5
HLM	Quinidine (CYP2D6)	0.007			0.004			0.0022		
		0.003	0.005	41.1	0.002	0.003	31.1	0.0010	0.002	59.5
HLM	Ketoconazole (CYP3A4)	0.0004			0.0001			0.000029		
		0.0002	0.00028	2.2	0.0001	0.000075	0.8	0.000004	0.000017	0.6
CYP1A2 Supersomes*	--	< 0.0001			< 0.0001			< 0.0001		
		< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001	
CYP2B6 Supersomes*	--	< 0.0001			< 0.0001			< 0.0001		
		< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001	
CYP2C8 upersomes*	--	< 0.0001			< 0.0001			< 0.0001		
		< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001	
CYP1A2 Supersomes*	Furafylline (CYP1A2)	< 0.0001			< 0.0001			< 0.0001		
		< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001	
CYP2B6 Supersomes*	Ticlopidine (CYP2B6)	n.d.			n.d.			n.d.		
		< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001	
CYP2C8 Supersomes*	Trimetoprim (CYP2C8)	< 0.0001			< 0.0001			< 0.0001		
		< 0.0001	< 0.0001		< 0.0001	< 0.0001		< 0.0001	< 0.0001	
HLM	Amonobenzotriazole	0.00133			0.00002			0.000106		
		0.0005	0.001	7.3	0.00043	0.000	2.3	0.000030	0.000068	2.6

* Metabolite Formation Rate Data are expressed as nmol/min/pmolP450

Table 3 Positive control reference of CYPs activity.

Matrix	Substrate	$T_{1/2}$ (min)	Cl_{int} (µL/min/mg protein)	Metabolite Formation Rate (nmol/min/mg protein)
HLM	Tacrine	91.88	10.89	917.68
HLM	Bupropione	66.26	18.02	1558.75
HLM	Paclitaxel	61.74	19.38	570.62
HLM	Diclofenac	7.05	124.3	1849.38
HLM	S-Mephenytoin	135.26	7.22	18.63
HLM	Dextrometorphan	35.92	24.27	5218.16
HLM	Midazolam	6.10	143.8	42812.50
CYP1A2	Tacrine	12.93	2.684*	3390.60#
CYP2B6	Bupropione	128.81	0.434*	146.75#
CYP2C8	Paclitaxel	25.83	2.686*	32.11#

* Clint expressed as µL/min/pmolP450

Data are expressed as nmol/min/pmolP450

Reviewer's Comment:

1. *The choices for reference inhibitors were appropriate.*
2. *The choices of model substrates as positive controls were appropriate. The reported activities of the CYS enzymes (Clint) of these microsomes were within the historical range that was observed.*
3. *The concentration of 10 µM in liver microsome and in recombinant enzymes are acceptable as they approximately represent the expected C_{max} and 10 times C_{max} in human subject where the Cmax at the clinical dose was 550-880 ng/mL (≈ 1-1.5 µM).*
4. *Based on this study result, it appear the metabolism of netupitant to M1, M2 and M3 is mainly mediated by CYP3A4 and lesser extent by CYY2C9 and CYP2D6.*

Title: In Vitro Metabolism Of The NK1 Receptor Antagonist RO0673189: II. Drug-Drug Interaction Studies with RO0673189 and Major Metabolites (RO0681133 and RO0713001), Involving Major Human Cytochrome P450 Isoenzymes

Report No: 1003907

Specific Aims: To evaluate the in vitro inhibition potential of RO0673189 for the major human cytochrome P450 isoenzymes CYP1A2, 2C9, 2C19, 2D6 and 3A4 utilizing human liver microsomes and isoform selective substrates.

Study Date: 08/1998-03/2002

Test Site: F. Hoffmann-La Roche Ltd. Basel Switzerland

Sponsor: Hoffmann-La Roche, Ltd

Study Design:

Test Item:
RO0673189 (netupitant) : MW = 578.6 g/mol)
RO0681133 (M1): MW= 567 g/mol
RO0713001 (M2): 597 g/mol

Test Concentration:
RO0673189 (netupitant): 0, 0.5, 1, 10 and 100 µM
RO0681133 (M1) and RO0713001 (M2): 0-30 µM

Liver microsome:
Microsomes were prepared from frozen human liver tissue, pooled tissue of 10 human livers, obtained from hepatic surgical resections.

Study Method:
Human liver microsomal protein was incubated with RO0673189 (0, 0.5, 1, 10, and 100□M) and the corresponding selective model substrates at 37°C in the presence of NADPH generating system for specified duration of incubation time. No pre-incubation was carried out. The enzymatic reactions were terminated by addition of methanol or acetonitrile.

The inhibition potential of metabolites of RO0673189, namely RO0681133 and RO0713001 were evaluated for CYP3A4 enzyme only. The human liver microsome (bought from (b) (4) pool of 10 livers) was incubated with model substrate 20 µM testosterone and test compounds RO0681133 (M1) and RO0713001 (M2) at 0-30 µM for 20 minutes in presence of NADPH at 37oC. The enzymatic reaction was termination of addition of methanol.

Table 1: Experimental Conditions to evaluate the inhibitory potential of CYP isoforms

CYP Isoforms	CYP model substrate Concentrations	Metabolite of CYP substrate	HLM Protein Amount	Incubation Time
CYP1A2	tacrine 25 µM	1-hydroxy tacrine	0.5 mg/ml	12 min

CYP2C9	diclofenac 2-50 μM	4-hydroxy diclofenac	0.1 mg/ml	5 min
CYP2C19	S-mephenytoin 32.8 μM	4-hydroxymephenytoin	1 mg/ml	30 min
CYP2D6	bufuralol 40 μM	1- hydroxy bufuralol	1 mg/ml	30 min
CYP3A4	midazolam 2-50 μM	1 hydroxymidazolam	0.1 mg/ml	10 min
CYP3A4	testosterone 5- 20 μM	6-β-hydroxytestosterone	0.075 mg/ml	20 min
CYP3A4	nifedipine 20 μM	Oxidized nifedipine	0.2 mg/ml	10 min
CYP3A4	simvastatin 3 μM		0.02 mg/ml	5 min

Bioanalytical Method: HPLC method.

Data analysis: Not provided.

Results:
RO0673189, at concentration 0-100 μM, did not inhibit enzymes CYP1A2, 2C19 and 2D6 (IC50 >100 μM as demonstrated in Figure 1, 4 and 5, respectively.

RO0673189 had shown some inhibition toward CYP2C9 with approximate IC50 value of 22.6 μM (Figure 2). Further studies with different concentration of model substrate had shown that RO0673189 inhibition of CYP2C9 is through competitive inhibition with Ki value of 25 μM as shown in the Dixon Plot (Figure 3).

The inhibitory potential of RO0673189 for the CYP3A4 enzyme was evaluated with four different model CYP3A4 substrates, testosterone, midazolam, nifedipine and simvastatin. All of the model CYP3A4 substrate had demonstrated that RO0673189 is an inhibitor of CYP3A4 with IC50 value of 1.7-12 μM (Figure 6, 7, 10 and 11). Further studies with different concentrations of testosterone and midazolam had demonstrated that the CYP3A4 inhibition is a competitive inhibition as shown in Dixon plots with Ki value of 1.1 μM with testosterone (Figure 8) and 2.2 μM with Midazolam (Figure 9).

The inhibition potential of RO0673189 metabolites, namely RO0681133 (M1) and RO0713001 (M2) were evaluated for CYP3A4 enzyme only with testosterone as the model substrate. RO0681133 (M1) appears to be an inhibitor of CYP3A4 with IC50 value of 1.2 μM (Figure 12). Due to solubility issue, RO0713001 (M2) was tested up to 1 μM, and notable inhibition was observed even at 1 μM (Figure 13).

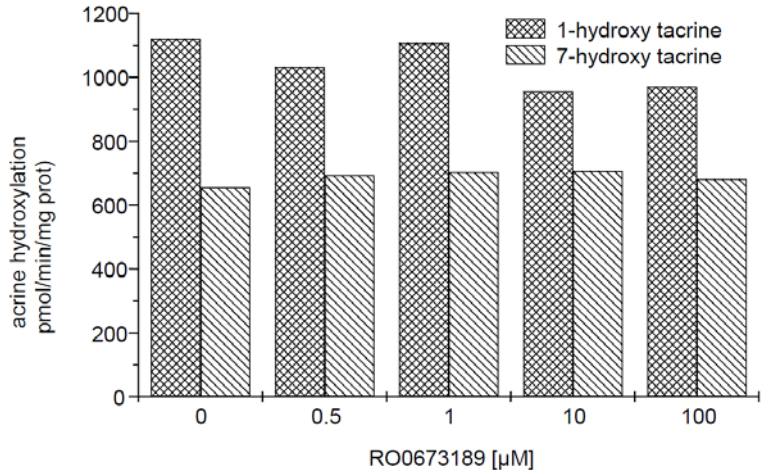
Table 2: Inhibition of CYP Enzyme by RO0673189 and its metabolites

CYP450 isoenzme	**CYP1A2**	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4**	**CYP3A4**	**CYP3A4**	**CYP3A4**
Substrate used	Tacrine	Diclofenac	Mephenytoin	Bufuralol	Midazolam	Testosterone	Nifedipine	Simvastatin
Substrate conc (uM)	25	5	32.8	40	5	20	20	3
HLM conc. (mg/ml)	0.5	0.1	1	1	0.1	0.075	0.2	0.02
IC50 (uM)								
RO0673189	>>100	22.6 ± 3 18.0 ± 6	>100	>>100	5.9 ± 1.0	1.7 ± 0.2	12.0 ± 0.5	10.5 ± 0.8
RO0681133 (M1)						1.2 ± 0.5		
RO0713001 (M2)						> 1 μM		

Table 3: Inhibition of CYP450 metabolism; apparent Ki (µM) of netupitant

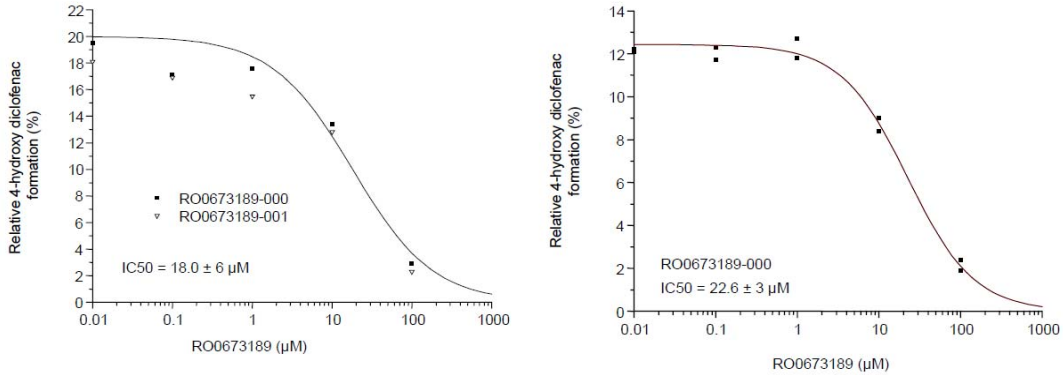
CYP450 isoenzme	**CYP2C9**	**CYP3A4**	**CYP3A4**
Substrate used	Diclofenac	Testosterone	Midazolam
Substrate conc.(uM)	2, 5, 10, 50	5, 10, 20	2, 5, 10, 50
HLM protein conc.(mg/ml)	0.1	0.075	1
Inhibitor conc. (uM)	0, 0.1, 0.5, 1, 5, 10	0, 0.2, 0.5, 1	0, 0.1, 0.5, 1, 5, 10
apparent Ki (uM)	25.0 ± 7.4	1.1 ± 0.2	2.2 ± 0.6
Inhibition mechanism	competitive	competitive	competitive

Figure 1: Inhibition potential of RO0673189 on tacrine hydroxylation, a reaction specific for CYP1A2.



Tacrine (25 µM) was incubated with human liver microsomes (500 µg protein /ml) for 20 minutes with the inhibitor (n = 2).

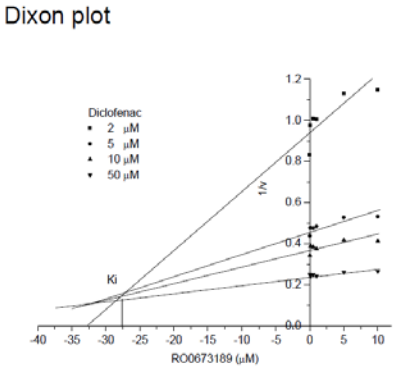
Figure 2: Inhibition potential of RO0673189 on diclofenac 4'- hydroxylation, a reaction specific for CYP2C9



Diclofenac (5 µM) was incubated with human liver microsomes (100 µg/ml) for 5 minutes with the inhibitor (n = 2). Results of different experiments.

Figure 3: Interaction of RO0673189 with cytochrome P450 2C9, measured by the isoenzyme-specific

hydroxylation of diclofenac in human liver microsomes. Dixon plot and nonlinear fitting.

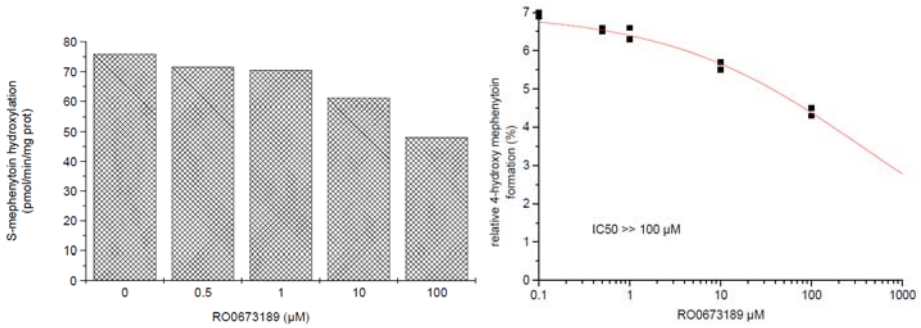


Non-linear fitting (Grafit)

competitive inhibition

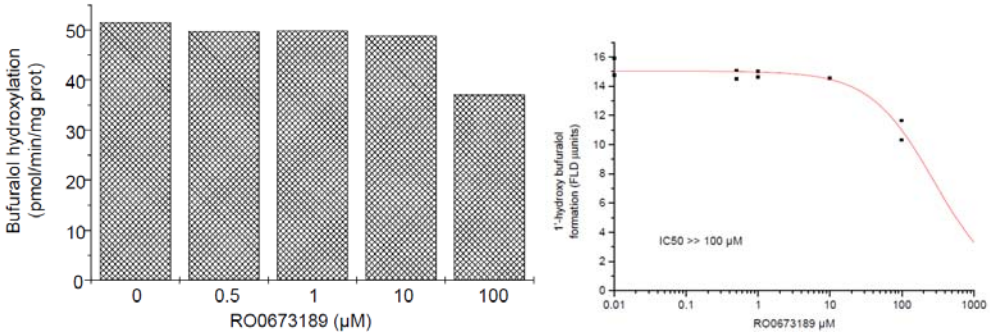
Vmax: 4.47 ± 0.08 nmol/min/mg (diclofenac)
app Km: 5.99 ± 0.34 µM (diclofenac)
app Ki: 25.0 ± 7.4 µM (Ro 67-3189)

Figure 4: Inhibition potential of RO0673189 on S-mephenytoin hydroxylation, a reaction specific for CYP2C19.



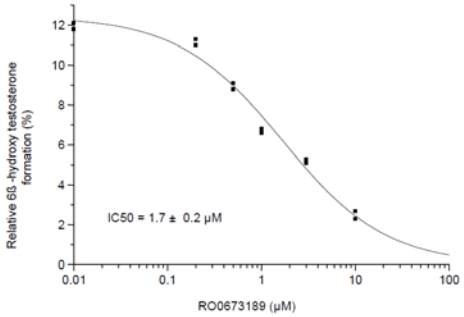
S-mephenytoin (32.8 µM) was incubated with human liver microsomes (1 mg/ml) for 30 minutes with the inhibitors indicated (n = 2).

Figure 5: Inhibition potential of RO0673189 on bufuralol 1'- hydroxylation, a reaction specific for CYP2D6.



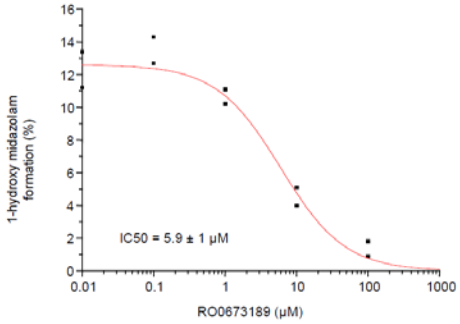
Bufuralol (40 µM) was incubated with human liver microsomes (1 mg/ml) for 30 minutes with the inhibitor (n = 2).

Figure 6: Inhibition potential of RO0673189 on CYP3A4, measured by testosterone 6-beta hydroxylation.



Testosterone (20 µM) was incubated with human liver microsomes (75 µg/ml) for 20 minutes with the inhibitor indicated (n = 2).

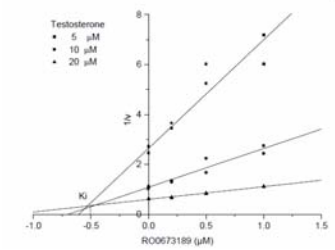
Figure 7: Inhibition potential of RO0673189 on CYP3A4, measured by midazolam 1'- hydroxylation.



Midazolam (5 µM) was incubated with human liver microsomes (100 µg/ml) for 10 minutes with the inhibitor (n = 2).

Figure 8: Interaction of RO0673189 with cytochrome P450 3A4, measured by the isoenzyme-specific 6β-hydroxylation of testosterone in human liver microsomes. Dixon plot and non-linear fitting

Dixon plot

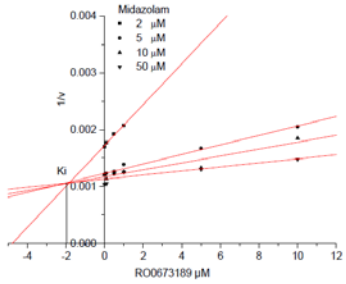


Non-linear fitting (Grafit)

competitive inhibition
Vmax: 5075 nmol/min/mg (testosterone)
app Km: 60790 µM (testosterone)
app Ki: 1.1 ± 0.2 µM (RO0673189)

Figure 9: Interaction of RO0673189 with cytochrome P450 3A4, measured by the isoenzyme-specific 1-hydroxylation of midazolam in human liver microsomes. Dixon plot and nonlinear fitting.

Dixon plot

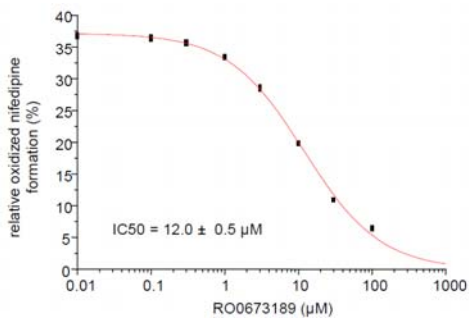


Non-linear fitting (Grafit)

competitive inhibition

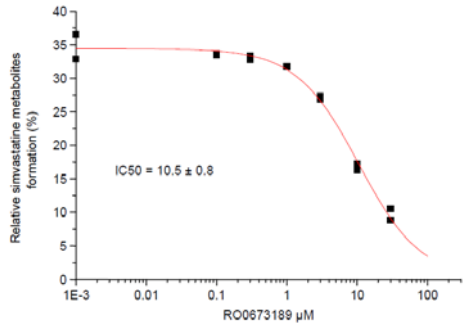
Vmax: 0.89 ± 0.03 nmol/min/mg (midazolam)
app Km: 0.88 ± 0.19 µM (midazolam)
app Ki: 2.2 ± 0.6 µM (RO0673189)

Figure 10: Inhibition potential of RO0673189 on nifedipine oxidation, a reaction specific for CYP3A4.



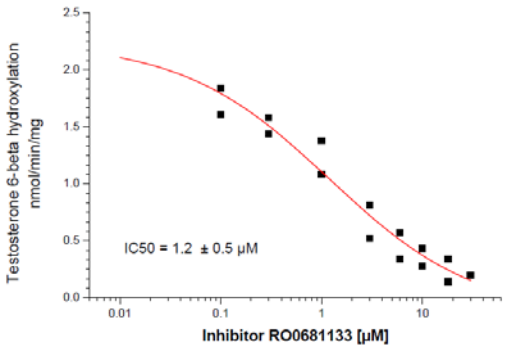
Nifedipine (20 µM) was incubated with human liver microsomes (0.2 mg/ml) for 10 min. with the inhibitor.

Figure 11: Inhibition potential of RO0673189 on CYP3A4, measured by metabolization of simvastatin.



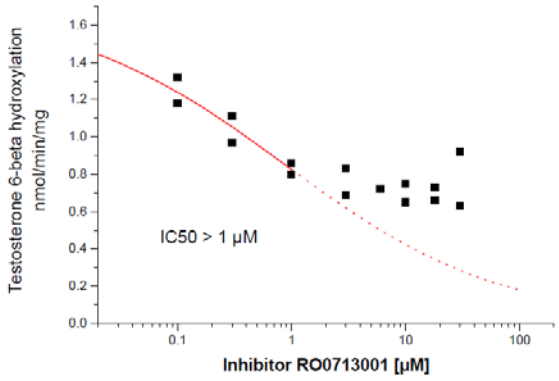
Simvastatin (3 µM) was incubated with human liver microsomes (20 µg/ml) for 5 min. with the inhibitor.

Figure 12: Inhibition potential of RO0681133 on CYP3A4, measured by testosterone 6-beta hydroxylation.



Testosterone (20 µM) was incubated with human liver microsomes (50 µg/ml) for 20 minutes with the inhibitor indicated (n = 2).

Figure 13: Inhibition potential of RO0713001 on CYP3A4, measured by testosterone 6-beta hydroxylation.



Testosterone (20 µM) was incubated with human liver microsomes (50 µg/ml) for 20 minutes with the inhibitor indicated (n = 2).

Reviewer's Comment:

1. *The study did not include positive control (known inhibitors) to evaluate the validity of the test system (human liver microsome) regarding CYP enzymes (optional). Nonetheless, the activity of CYP enzymes toward model substrates in absence of netupitant as inhibitor was within the historical data observed.*
2. *The concentration of RO0673189 at 0- 100 µM are acceptable as they approximately cover the C_{max} and 10 times C_{max} values to be expected in human subjects or patients taking this drug at the clinical dose of 300 mg.*
    a. *Observed Cmax = 550-880 ng/mL (≈ 1-1.5 µM)*
3. *Concentration of metabolites (M1 and M2) at 0-30 µM are acceptable as they approximately cover the C_{max} and 10 times C_{max} values for the corresponding metabolites to be expected in human subjects or patients taking this drug at the clinical dose of 300 mg:*
    a. *Observed Cmax for M1 (RO0681133) is 40-50 ng/ml (0.07-0.09 µM)*
    b. *Observed Cmax for M2 (RO0713001) is 100-350 ng/mL (0.17-0.58 µM).*
4. *The choices of CYP-specific model substrates and their concentrations to evaluate the inhibitory potential on each CYP isoforms were acceptable as covering the range around each Km value.*
5. *RO0673189 is not considered to be an inhibitor of CYP1A2, CYP2C19, and CYP2D6, as it did not produce a significant inhibitory effect on these CYP enzymes.*
6. *RO0673189 is shown to be an inhibitor of CYP2C9 with Ki of 25 µM. Since [I]/Ki= 1.5 µM /25 µM = 0.06<0.1, clinical in - vivo interaction with CYP2C9 is less likely.*
7. *RO0673189 is shown to be an inhibitor of CYP3A4 with Ki of 1.1-2.2 µM, and a follow-up <u>in-vivo evaluation is recommended</u> for the following reasons:*
    a. *Systemic exposure: Cmax/Ki = 1.5 µM /1.1 µM = 1.4>1*
    b. *Gut exposure: $[I]_{gut}$/Ki= 2074 µM / 1.1 µM = 1885>>>10 where $[I]_{gut}$ = dose/250 ml = 300 mg/250ml= 1.2 g/L.*
8. *The sponsor did not evaluate time-dependent inhibition potential with pre-incubation.*
9. *The sponsor did not evaluate the inhibition potential of RO0673189 for CYP2B6 and CYP2C8.*
10. *The sponsor only evaluated the inhibition potential of metabolites (M1 and M2) for CYP3A4, not other enzymes.*

Title: Netupitant, M1, M2, and M3: Determination of the potential inhibition (IC_{50}) of CYP1A2, CYP3A4, CYP286, CYP2C8, CYP2C9, CYP2C19, CYP2D6

Report No: NETU-13-20

Specific Aims: To determine the potential inhibitory effect of Netupitant towards CYP2C8 and CYP286 and of its three major metabolites Ml, M2, M3 towards the major human liver CYP enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2Cl 9, CYP2D6, and CYP3A4), using human liver microsomes.

Study Date: 07/2013

Test Site: (b) (4)

Sponsor: Hoffmann-La Roche, Ltd

Study Design:

Test Item: Netupitant and its metabolites M1, M2 and M3

Test Concentration: 0.3, l, 3, 10, 30 and 100 μM

Liver microsome:
Pooled human liver microsomes (from 50 individuals) used in this study were purchased from (b) (4) In Vitro Technologies. The microsomes were characterized by the supplier in respect to its CYP enzyme activities.

Study Method:
Human liver microsomal protein was incubated with test compound and the corresponding selective model substrates at 37°C in the presence of NADPH generating system for specified duration of incubation time. No pre-incubation was carried out. The enzymatic reactions were terminated by addition of ice-cold acetonitrile.

For M1, M2 and M3 inhibition was evaluated towards the following CYP450 isoforms:
CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2Cl9, CYP2D6, and CYP3A4 (two substrates) using six test concentrations (e.g. 0.3, 1, 3, 10, 30 and 100 μM).

For Netupitant inhibition was evaluated towards CYP2B6 and CYP2C8 using six relevant test concentrations (e.g. 0.3, 1, 3, 10, 30 and 100 μM).

The quantity of CYP specific substrate metabolites produced during the incubation with and without the compound was evaluated in triplicate. In addition positive controls were included into each experiment. All incubations were performed under linear conditions with respect to time, protein concentration and amount of product formed.

Table 1: Experimental Conditions to evaluate the inhibitory potential of CYP isoforms

CYP Isoforms	CYP model substrate Concentrations	HLM Protein Amount	Incubation Time	Positive Control
CYP1A2	Tacrine 6 µM	0.1 mg/ml	10 min	α-naphthoflavone 0.01, 0.1, 0.3 µM
CYP2B6	Bupropion 43 µM	0.1 mg/mL	20 min	Ticlopidine 0.1, 1, 3 µM
CYP2C8	Paclitaxel 14 µM	0.1 mg/mL	20 min	Quercetine 0.6, 6, 18 µM
CYP2C9	Diclofenac 7 µM	0.1 mg/ml	10 min	Sulfaphenazole 0.1 , 1, 3 µM
CYP2C19	S-mephenytoin 71 µM	0.2 mg/ml	40 min	Ticlopidine 0.1 , l, 3 µM
CYP2D6	Dextromethorphan 5 µM	0.1 mg/ml	10 min	Quinidine 0.01, 0.1, 0.3 µM
CYP3A4	midazolam 2.1 µM	0.1 mg/ml	10 min	Ketoconazole 0.0l, 0.1, 0.3 µM
CYP3A4	testosterone 43 µM	0.1 mg/ml	20 min	Ketoconazole 0.0l, 0.1, 0.3 µM

Bioanalytical Method: LC-MS/MS

Data analysis:

IC50 were calculated with a non-linear regression (sigmoidal dose-response curve):

Y =Bottom + (Top-Bottom)/(1+ 10^((LogEC50-X)))

where X is the Logarithm of concentration and Y the % of activity remaining.

The percentage of CYP mediated enzyme activity remaining in the presence of a certain compound X is given by:

$$\frac{\%\text{ metabolized in presence of compound X}}{\%\text{ metabolized in absence of compound X (control)}} \times 100 = \%\text{ activity remaining}$$

For the prediction of likelihood of an *in vivo* inhibition the ratio of estimated intrinsic clearance values in absence and presence of an inhibitor (R) was calculated as follow:

R = l+ [I]/Ki

Where [I] is the Cmax value measured in plasma after single administration in human at the therapeutic dose of 300 mg of netupitant

Results:

- Nutpitant showed weak inhibition toward both CYP2B6 and CYP2C8 with IC50 values of 33.39 µM and 50.4 µM, respectively. However, since Cmax/Ki are <0.1, in vivo relevance of this interaction is unlikely.
- M1 showed inhibition toward CY 2B6, 2C8, 2D6, 3A4, and weak inhibition toward CYP 1A2, 2C9, 2C19. However, since Cmax/Ki >0.1 for only CYP3A4, an in vivo study is recommended

for CYP3A4. The study had already conducted in vivo DDI study with netupitant concomitantly administered with CYP3A4 substrate midazolam.

- M2 and M3 showed weak inhibition toward all evaluated CYP enzymes. Since Cmax/Ki<0.1, no in vivo follow up study is needed.

Netupitant batch GMP0449							
P450 Enzyme	**Enzyme Reaction**	**IC_{50} (μM)**	**95%C.I.**	**R^2**	**Ki**	**I/Ki**	**R**
CYP2B6	Bupropion hydroxylation	32.39	27.22 to 38.55	0.9	16.20	0.08	1.08
CYP2C8	Paclitaxel 6-hydroxylation	50.43	39.36 to 64.60	0.9	25.22	0.05	1.05

M1 batch 14-NETU.i13/2/1							
P450 Enzyme	**Enzyme Reaction**	**IC_{50} (μM)**	**95%C.I.**	**R^2**	**Ki**	**I/Ki**	**R**
CYP1A2	Tacrine-1-hydroxylation	39.39	23.43 to 66.22	0.9	19.70	0.01	1.01
CYP2B6	Bupropion hydroxylation	4.89	2.87 to 8.33	0.9	2.44	0.04	1.04
CYP2C8	Paclitaxel 6-hydroxylation	4.74	4.09 to 5.50	1.0	2.37	0.04	1.04
CYP2C9	Diclofenac 4'-hydroxylation	26.41	18.45 to 37.80	0.8	13.21	0.01	1.01
CYP2C19	S-mephenytoin 4'-hydroxylation	33.26	23.86 to 46.38	0.8	16.63	0.01	1.01
CYP2D6	Dextromethorphan O-demethylation	8.54	4.84 to 15.08	0.9	4.27	0.02	1.02
CYP3A4 midazolam	Midazolam 1'-hydroxylation	0.51	0.44 to 0.59	1.0	0.25	0.42	1.42
CYP3A4 testosterone	Testosterone 6b-hydroxylation	0.70	0.64 to 0.77	1.0	0.35	0.30	1.30

M2 batch 14-NETU.i12/2/1							
		IC_{50} (μM)	95%C.I.	R^2	Ki	I/Ki	R
CYP1A2	Tacrine-1-hydroxylation	> 100	NA	NA	NA	NA	NA
CYP2B6	Bupropion hydroxylation	23.72	16.03 to 35.10	0.8	11.86	0.03	1.03
CYP2C8	Paclitaxel 6-hydroxylation	> 100	NA	NA	NA	NA	NA
CYP2C9	Diclofenac 4'-hydroxylation	> 100	NA	NA	NA	NA	NA
CYP2C19	S-mephenytoin 4'-hydroxylation	57.45	41.08 to 80.34	0.8	28.73	0.01	1.01
CYP2D6	Dextromethorphan O-demethylation	58.12	41.10 to 82.18	0.8	29.06	0.01	1.01
CYP3A4 midazolam	Midazolam 1'-hydroxylation	38.84	32.20 to 46.85	0.9	19.42	0.02	1.02
CYP3A4 testosterone	Testosterone 6β-hydroxylation	39.04	25.49 to 59.78	0.9	19.52	0.02	1.02

M3 batch 14-NETU.i22/62/1							
		IC_{50} (μM)	95%C.I.	R^2	Ki	I/Ki	R
CYP1A2	Tacrine-1-hydroxylation	> 100	NA	NA	NA	NA	NA
CYP2B6	Bupropion hydroxylation	23.62	15.94 to 35.01	0.8	11.81	0.01	1.01
CYP2C8	Paclitaxel 6-hydroxylation	26.95	21.07 to 34.48	0.9	13.48	0.01	1.01
CYP2C9	Diclofenac 4'-hydroxylation	> 100	NA	NA	NA	NA	NA
CYP2C19	S-mephenytoin 4'-hydroxylation	77.03	53.66 to 110.6	0.8	38.52	0.004	1.00
CYP2D6	Dextromethorphan O-demethylation	74.97	51.88 to 108.3	0.9	37.49	0.004	1.00
CYP3A4 midazolam	Midazolam 1'-hydroxylation	10.95	8.80 to 13.63	0.9	5.48	0.03	1.03
CYP3A4 testosterone	Testosterone 6β-hydroxylation	9.45	6.22 to 14.36	0.8	4.72	0.03	1.03

Table 9. Positive Controls: IC_{50} (μM) Summary

P450 Enzyme	Positive control	IC50 (μM)	95%C.I.	R2
CYP1A2	α-naphthoflavone	0.004	0.002 to 0.006	1.0
CYP2B6	Ticlopidine	0.10	0.07 to 0.14	1.0
CYP2C8	Quercetin	2.52	2.15 to 2.96	1.0
CYP2C9	Sulfaphenazole	0.23	0.16 to 0.33	1.0
CYP2C19	Ticlopidine	0.54	0.41 to 0.72	1.0
CYP2D6	Quinidine	0.02	0.01 to 0.03	1.0
CYP3A4 midazolam	Ketoconazole	0.03	0.03 to 0.04	1.0
CYP3A4 testosterone	Ketoconazole	0.02	0.01 to 0.02	1.0

Reviewer's Comment:

1. *The concentration of 0.3- 100 μM are acceptable as they approximately cover the C_{max} and 10 times C_{max} values of netupitant and its metabolites to be expected in human subjects or patients taking this drug at the clinical dose of 300 mg.*
    a. *Observed Cmax for netupitant = 550-880 ng/mL (≈ 1-1.5 μM)*
    b. *Observed Cmax for M1 is 40-50 ng/ml (0.07-0.09 μM)*
    c. *Observed Cmax for M2 is 100-350 ng/mL (0.17-0.58 μM).*
    d. *Observed Cmax for M3 is 50-90 ng/mL (0.08-0.144 μM)*
2. *The choices of CYP-specific model and their concentrations to evaluate the inhibitory potential on each CYP isoforms were acceptable as covering the range around each Km value.*
3. *Choices of model inhibitors as positive controls were acceptable.*
4. *The sponsor did not evaluate time-dependent inhibition potential with pre-incubation.*
5. *Netupitant showed weak inhibition toward both CYP2B6 and CYP2C8 with IC50 values of 33.39 μM and 50.4 μM, respectively. However, since Cmax/Ki are <0.1, no follow-up in vivo study is recommended.*
6. *M1 showed inhibition toward CYP 2B6, 2C8, 2D6, 3A4, and weak inhibition toward CYP 1A2, 2C9, 2C19. However, since Cmax/Ki >0.1 for only CYP3A4, an in vivo study is recommended for CYP3A4. The sponsor had already conducted in vivo DDI study with netupitant concomitantly administered with CYP3A4 substrate midazolam.*
7. *M2 and M3 showed weak inhibition toward all evaluated CYP enzymes. Since Cmax/Ki<0.1, no in vivo follow up study is needed.*

		IC_{50} (μM)	Ki	Cmax Range (μM)		Cmax/Ki Range	
Netupitant	CYP2B6	32.39	16.2	1	1.5	0.062	0.093
	CYP2C8	50.43	25.22	1	1.5	0.040	0.059
M1	CYP1A2	39.39	19.7	0.07	0.09	0.004	0.005
	CYP2B6	4.89	2.44	0.07	0.09	0.029	0.037
	CYP2C8	4.7	2.37	0.07	0.09	0.030	0.038
	CYP2C9	26.4	13.21	0.07	0.09	0.005	0.007
	CYP2C19	33.26	16.63	0.07	0.09	0.004	0.005
	CYP2D6	8.54	4.27	0.07	0.09	0.016	0.021
	CYP3A4	0.51	0.25	0.07	0.09	0.280	0.360
	CYP3A4	0.7	0.35	0.07	0.09	0.200	0.257
M2	CYP1A2	>100	NA	0.17	0.58	NA	NA
	CYP2B6	23.72	11.86	0.17	0.58	0.014	0.049
	CYP2C8	>100	NA	0.17	0.58	NA	NA
	CYP2C9	>100	NA	0.17	0.58	NA	NA
	CYP2C19	57.45	28.73	0.17	0.58	0.006	0.020
	CYP2D6	58.12	29.06	0.17	0.58	0.006	0.020
	CYP3A4	38.84	19.42	0.17	0.58	0.009	0.030
	CYP3A4	39.04	19.52	0.17	0.58	0.009	0.030
M3	CYP1A2	>100	NA	0.08	0.144	NA	NA
	CYP2B6	23.62	11.81	0.08	0.144	0.007	0.012
	CYP2C8	26.95	13.48	0.08	0.144	0.006	0.011
	CYP2C9	>100	NA	0.08	0.144	NA	NA
	CYP2C19	77.03	38.52	0.08	0.144	0.002	0.004
	CYP2D6	74.97	37.49	0.08	0.144	0.002	0.004
	CYP3A4	10.95	5.48	0.08	0.144	0.015	0.026
	CYP3A4	9.45	4.72	0.08	0.144	0.017	0.031

Title: In Vitro Evaluation of the Possible Induction Of CYP1A2, CYP 2C9, CYP 2C19 and CYP 3A4 By Netupitant, M1, M2 And M3 In Long-Term Monolayer Cultures Of Freshly Isolated Human Hepatocytes

Report No: NETU-10-27

Specific Aims: The objective of this study was to determine the possible in vitro induction of the cytochrome P450 (CYP450) enzymes 1A2, 2C9, 2C19 and 3A4 by Netupitant and its metabolites M1, M2 and M3 in human hepatocytes.

Study Date: 05/2010-08-2010

Test Site (b) (4)

Sponsor: Helsinn Healthcare SA, Switzerland

Study Design:

Test Item:
Netupitant (MW = 578.6 g/mol) and its metabolites M1, M2 and M3

Tested Concentrations:
Netupitant: 0.2, 2 and 20 μM,
M1, M2 and M3: 0.02, 0.2 and 2 μM

Hepatocytes Preparation:
Long-term monolayer cultures of freshly isolated human hepatocytes from 3 different donors were used for each enzyme evaluation. The hepatocytes were plated by the supplier (b) (4) in 24 well plates coated with collagen I at a density of 0.38 million hepatocytes per well.

Human hepatocytes from six individuals were used.

- First donor (for 1A2 and 3A4): male, 61 years, HEP220460
- Second donor (for 1A2 and 3A4): male, 68 years, HEP220470
- Third donor (for 1A2 and 3A4): female, 75 years, HEP220474

- First donor (for 2C9+2C19): male, 66 years, HEP220473
- Second donor (for 2C9 and 2C19): male, 70 years, HEP220477
- Third donor (for 2C9 and 2C19): male, 62 years, HEP220486

The hepatocytes were characterized by the supplier in respect to various phase I (CYP1A2, CYP3A4/5, CYP2B6, CYP2D6, CYP2C19) and phase II (glucuronidation and sulfation) enzyme activities. In all six hepatocytes donor preparations used in the study, evaluated enzyme activities were within the historical range.

Validation of Test System:
The in vitro CYP induction study with human hepatocytes was considered acceptable if the following criteria were met:

- Phenacetin (substrate for CYP1A2), tolbutamide (substrate for CYP2C9), S-mephenytoin (substrate for CYP2C19) and midazolam (substrate for CYP3A4) are metabolised in the vehicle control incubations for not more than 30%.
- The positive control inducers should result in a >2-fold increase in enzyme activity of standard substrates as compared to the vehicle control (based on metabolite peak area).

Controls:

- Known inducers, omeprazole for CYP1A1 and rifampicin for CYP2C9, CYP2C19 and CYP 3A4/5, were included as positive controls:
- The study also had a vehicle control that did not contain any substrate and another untreated control as the negative control.

Study Method:

Hepatocytes from three different donors were incubated with 0.2, 2 and 20 µM Netupitant or 0.02, 0.2 and 2 µM M1, M2 and M3 or positive control inducers omeprazole or rifampicin for 72 hours at 37°C. The exposure medium was refreshed every 24 hours. Incubations were carried out in duplicate. In addition, two wells were left untreated to determine the basal CYP1A2, CYP2AC9, CYP2C19 and CYP3A4 activity of the hepatocytes.

At the end of the 72 hours of incubation period, the activity of target enzyme CYP1A2, CYP2C9, CYP2C19 and CYP3A4 was assessed by incubating the hepatocyte with model substrate for each target enzyme and measuring the appearance rate of their respective metabolites.

Table 1: Overview of substrates and inducers

CYP isoenzyme	Species	Model Substrate	Metabolite	Known Inducer (positive control)
1A2	Human	Phenacetin (60 µM)	acetaminophen	Omeprazole (50 µM)
2C9	Human	Tolbutamide (30 µM)	4-hydroxytolbutamide	Rifampicin (30 µM)
2C19	Human	S-mephenytoin (50 µM)	4'-hydroxymephenytoin	Rifampicin (10 µM)
3A4	Human	Midazolam (4 µM)	1'-hydroxymidazolam	Rifampicin (50 µM)

Bioanalytical Method:

The disappearance of model substrate and appearance of metabolites were determined with LC-PDA-MS method.

Data Analysis:

Enzyme induction of the positive control was calculated using the following equation

$$Fold.Induction = \frac{Mean.Peak.area(MPA)_{positve\ control}}{Mean.Peak.Area(MPA)_{vehicle}}$$

The induction of the test substance was calculated as percentage of positive control using the following equation:

$$\%Positive \cdot Control = \frac{MPA(test \cdot sample) - MPA(negative \cdot control)}{MPA(positive \cdot control) - MPA(negative \cdot control)} \times 100\%$$

in which MPA represents the mean peak area of the metabolite in the corresponding incubation conditions.

A test substance is considered an inducer, if:

- It produces a change in enzyme activity that is equal to or greater than 40% of the positive control
- The induction is reproducible in hepatocytes from different donors

Results:
Netupitant, M1, M2 and M3 did not induce CYP1A2, CY2C9, CYP2C19 and CY3A4/5 enzyme activities when hepatocytes from three different human donors were treated with Netupitant up to 20 uM and M1, M2 and M3 up to 2 uM concentration after 72 hours of incubation based on induction threshold of 40% of the positive control.

The test conditions were appropriate for measuring the target enzyme CYP1A2, CYP2C9, CYP2C19 and CYP3A4 activities as there were more than 2-fold induction in presence of positive controls (known inducer) and model substrate for each target enzyme were not metabolized in the vehicle control incubation for more than 30%.

In the hepatocytes treated with Netupitant at a concentration of 20 µM, the cells were detached after the exposure period in one patch or no substrate metabolite was formed in another batch indicating is that Netupitant was likely cytotoxic at 20 µM.

Table 2: CYP induction for the 3 donors after substrate incubation

CYP1A2 induction									
Test Substance	**Concentration**	**Fold induction[3] (Compared to vehicle control)**				**Percentage of control inducer**			
	(µM)	Donor 1	Donor 2	Donor 3	Induction	Donor 1	Donor 2	Donor 3	Induction
Vehicle control[1]		0.9	1.3	1.1		0	0	0	
Omeprazole[1]	50	13.8	19.9	11.7	Yes	100	100	100	Yes
Netupitant[2]	0.2	0.8	0.8	0.7	No	< 0	< 0	< 0	No
Netupitant[2]	2	0.9	1.0	0.6	No	< 0	< 0	< 0	No
Netupitant[2]	20	0.1	0.1	0.0	No	< 0	< 0	< 0	No
M1[2]	0.02	0.8	1.1	1.1	No	< 0	0.5	1.0	No
M1[2]	0.2	0.8	1.0	0.8	No	< 0	< 0	< 0	No
M1[2]	2	0.7	0.9	0.7	No	< 0	< 0	< 0	No
Vehicle control[1]		1.2	1.2	1.3		0.0	0.0	0.0	
Omeprazole[1]	50	9.7	19.6	9.1	Yes	100	100	100	Yes
M2[2]	0.02	1.1	1.0	0.9	No	1.4	0.1	< 0	No
M2[2]	0.2	1.0	1.0	0.7	No	< 0	0.1	< 0	No
M2[2]	2	1.0	1.0	0.6	No	< 0	0.1	< 0	No
M3[2]	0.02	1.1	1.1	1.0	No	1.1	0.7	0.4	No
M3[2]	0.2	1.0	1.1	0.8	No	0.0	0.5	< 0	No
M3[2]	2	0.9	0.8	0.5	No	< 0	< 0	< 0	No

CYP 2C9 induction									
Test Substance	**Concentration**	**Fold induction[3] (Compared to vehicle control)**				**Percentage of control inducer**			
	(µM)	Donor 1	Donor 2	Donor 3	Induction	Donor 1	Donor 2	Donor 3	Induction
Vehicle control[1]		1.2	1.4	0.8		0	0	0	
Rifampicin[1]	30	2.2	2.3	3.6	Yes	100	100	100	Yes
Netupitant[2]	0.2	0.6	0.7	0.9	No	<0	<0	<0	No
Netupitant[2]	2	0.5	0.8	1.3	No	<0	<0	12.1	No
Netupitant[2]	20	0.0	0.1	0.0	No	<0	<0	<0	No
M1[2]	0.02	0.5	1.0	1.2	No	<0	<0	6.2	No
M1[2]	0.2	0.4	0.9	1.0	No	<0	<0	<0	No
M1[2]	2	0.5	0.8	1.3	No	<0	<0	10.3	No
Vehicle control[1]		1.0	1.2	1.0		0	0	0	
Rifampicin[1]	30	2.5	2.4	2.7	Yes	100	100	100	Yes
M2[2]	0.02	1.1	1.0	0.9	No	8.4	3.2	<0	No
M2[2]	0.2	0.9	0.9	1.0	No	<0	<0	0.2	No
M2[2]	2	1.0	1.1	1.0	No	1.3	4.0	<0	No
M3[2]	0.02	0.8	1.0	1.0	No	<0	<0	<0	No
M3[2]	0.2	0.7	1.0	0.8	No	<0	1.0	<0	No
M3[2]	2	0.8	0.8	1.0	No	<0	<0	<0	No

CYP 2C19 induction									
Test Substance	Concentration	Fold induction[3] (Compared to vehicle control)				Percentage of control inducer			
	(µM)	Donor 1	Donor 2	Donor 3	Induction	Donor 1	Donor 2	Donor 3	Induction
Vehicle control[1]		1.1	1.2	0.9		0	0	0	
Rifampicin[1]	10	5.6	5.9	7.5	Yes	100	100	100	Yes
Netupitant[2]	0.2	1.2	1.0	1.2	No	5.1	0.9	3.0	No
Netupitant[2]	2	2.3	2.7	2.3	Yes	28.4	34.5	19.8	No
Netupitant[2]	20	0.0	0.0	0.0	No	<0	<0	<0	No
M1[2]	0.02	1.1	1.4	1.0	No	2.3	8.7	0.3	No
M1[2]	0.2	1.2	1.5	1.0	No	5.0	9.8	0.0	No
M1[2]	2	2.3	1.9	2.3	No	29.3	18.2	20.3	No
Vehicle control[1]		1.1	1.1	0.8		0	0	0	
Rifampicin[1]	10	4.7	5.7	6.9	Yes	100	100	100	Yes
M2[2]	0.02	1.1	0.8	1.0	No	2.4	<0	0.7	No
M2[2]	0.2	1.3	1.3	1.0	No	8.5	5.7	<0	No
M2[2]	2	2.3	2.8	1.9	Yes	34.0	37.7	16.0	No
M3[2]	0.02	1.1	1.3	0.9	No	1.7	5.4	<0	No
M3[2]	0.2	1.0	1.0	1.0	No	1.2	0.0	<0	No
M3[2]	2	1.2	0.6	0.9	No	4.7	<0	<0	No

CYP 3A4 induction									
Test Substance	Concentration	Fold induction[3] (Compared to vehicle control)				Percentage of control inducer			
	(µM)	Donor 1	Donor 2	Donor 3	Induction	Donor 1	Donor 2	Donor 3	Induction
Vehicle control[1]		1.4	1.0	1.2		0	0	0	
Rifampicin[1]	50	4.5	2.2	2.8	Yes	100	100	100	Yes
Netupitant[2]	0.2	0.5	0.6	0.6	No	< 0	< 0	< 0	No
Netupitant[2]	2	0.2	0.2	0.2	No	< 0	< 0	< 0	No
Netupitant[2]	20	0.0	0.0	0.0	No	< 0	< 0	< 0	No
M1[2]	0.02	0.7	0.9	0.9	No	< 0	< 0	< 0	No
M1[2]	0.2	0.3	0.4	0.5	No	< 0	< 0	< 0	No
M1[2]	2	0.1	0.1	0.2	No	< 0	< 0	< 0	No
Vehicle control[1]		1.6	1.0	1.1		0	0	0	
Rifampicin[1]	50	4.2	2.1	2.4	Yes	100	100	100	Yes
M2[2]	0.02	0.9	1.1	1.0	No	< 0	13.4	< 0	No
M2[2]	0.2	0.9	0.9	0.9	No	< 0	< 0	< 0	No
M2[2]	2	0.3	0.3	0.6	No	< 0	< 0	< 0	No
M3[2]	0.02	1.2	1.2	0.9	No	6.6	19.9	< 0	No
M3[2]	0.2	0.4	0.8	0.4	No	< 0	< 0	< 0	No
M3[2]	2	0.1	0.2	0.1	No	< 0	< 0	< 0	No

[1] Vehicle and inducer controls are the average of duplicate incubations per donor.
[2] Test substance incubations are the average of triplicate incubations per donor.
[3] Fold induction is the average of the metabolite peak area of the test substance incubation divided by the metabolite peak area of the vehicle control incubation, except for the fold induction of vehicle control which is the average of the metabolite peak area of the test substance incubation divided by the metabolite peak area of the medium control incubation.

Reviewer's Comment:

1. *The concentration of* Netupitant *at 0.2- 20 µM are acceptable as they approximately cover the C_{max} and 10 times C_{max} values to be expected in human subjects or patients taking this drug at the clinical dose of 300 mg.*
    a. *Observed Cmax = 550-880 ng/mL (≈ 1-1.5 µM)*
2. *Concentration of metabolites (M1, M2 and M3) at 0.02-2 µM are acceptable as they approximately cover the C_{max} values of metabolites to be expected in human subjects or patients taking this drug at the clinical dose of 300 mg:*
    a. *Observed Cmax for M1 is 40-50 ng/ml (0.07-0.09 µM)*
    b. *Observed Cmax for M2 is 100-350 ng/mL (0.17-0.58 µM).*
    c. *Observed Cmax for M3 is 50-90 ng/mL (0.08-0.144 µM).*
3. *The choice for positive controls (model inducers) are acceptable*

4. *The choices of CYP-specific model substrate to evaluate the CYP enzyme activities were acceptable.*
5. *Netupitant up to 20 μM and M1, M2 and M3 up to 2 μM are not considered to be inducers of CYP1A2, CY2C9, CYP2C19 and CY3A4/5 enzyme as it did not produce a change that is equal to or greater than 40% of the positive control.*
6. *The sponsor did not evaluate the potential of Netupitant and its metabolites M1, M2 and M3 to be an inducer of CYP2B6.*

APPEARS THIS WAY ON ORIGINAL

Title: Interaction Studies of Netupitant with Human Pgp / MDR1 (ABCB1)

Report No: NETU-06-13

Specific Aims: To evaluate the interaction of Netupitant with the ABC efflux transporter: human MDR1 (Pgp/ABCB1).

Study Date: 11/2006

Test Site: (b) (4)

Sponsor: Helsinn Healthcare SA, Switzerland

Test Item: Netupitant: (MW = 578.6 g/mol)

The sponsor evaluated the interaction of netupitant with P-gp transporter in 3 different assay methods, ATPase assay, Calcein Assay and bidirectional transporter assay on monolayer. Since Calcein assay did not provide any additional new information compared to bidirectional transport assay, it was not reviewed in detail.

ATPase Assays Activation and inhibition Assay:
Assay system: Membrane vesicles isolated from Sf9 insect cells overexpressing human MDR1 transporter

Effect of Netupitant on MDR1-ATPase activation was measured in the presence of increasing concentrations of Netupitant (0.14, 0.41, 1.23, 3.70, 11.11, 33.33, 100 and 300 μM). Each concentration was tested in duplicate.

Inhibitory effect of the netupitant on verapamil (40 μM) or digoxin (100 μM)-induced MDR1-ATPase activity was measured in the presence of the activator (varapamil or digoxin) and increasing concentrations of the netupitant.

> In the ATPase assay the amount of phosphate generated from the cleavage of ATP by the transporter is measured. If a test compound is a substrate of the given transporter, it will dose-dependently increase the amount of phosphate generated in the system. If the activation type assay shows stimulation of ATPase activity with increasing drug concentration, then the test drug is likely to be a transported substrate. Inhibition type ATPase assay can reveal the interaction with the transporter, without distinguishing substrate and inhibitor.

Figure 1. Activation and inhibition of MDR1 transporter by Netupitant measured in the ATPase assay (verapamil induced)

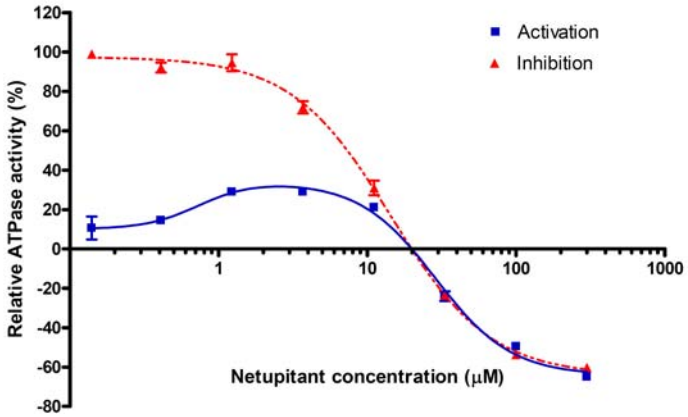


Table 1. Activation and inhibition of MDR1 transporter measured in the ATPase assay

Activation assay	EC50 [μM]	0.37
	Maximal Efficacy	29 %
Inhibition assay	IC50 [μM]	7.2
	Maximal Efficacy	100 %

In the activation assay of standard MDR1 ATPase assay, netupitant shows 29 % maximal activation compared to verapamil (100%). In the standard inhibition assay, netupitant shows 100% inhibition of verapamil induced ATPase activity.

Figure 2. Activation and inhibition of MDR1 transporter by Netupitant measured in the nonstandard (DDI model) ATPase assay

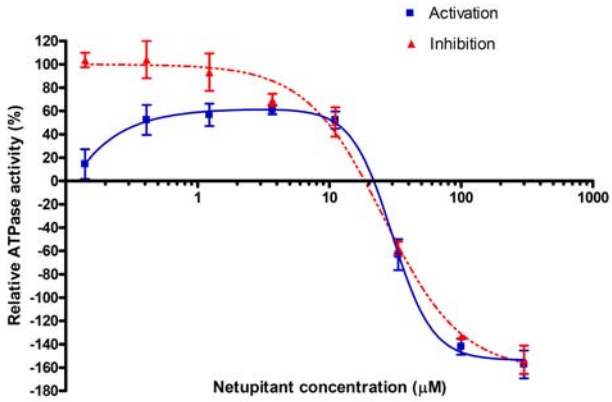


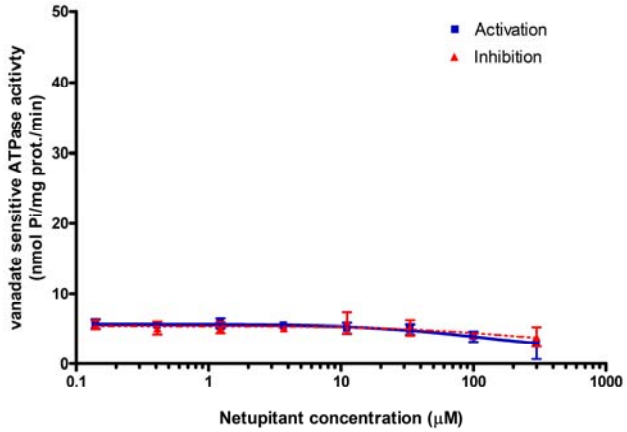
Table 2. Activation and inhibition of MDR1 transporter measured in the ATPase assay

Activation assay	EC50 [μM]	0.18
	Maximal Efficacy	61 %
Inhibition assay	IC50 [μM]	6.8
	Maximal Efficacy	100 %

In this, non-standard (DDI) activation assay Netupitant shows 61 % maximal activation compared to digoxin (100%) in MDR1 ATPase assay. In the inhibition assay, netupitant shows 100% inhibition of

digoxin induced ATPase activity.

Figure 3. Activation and inhibition of def MRP(negative control) transporter by Netupitant measured in the ATPase assay



In this assay, netupitant shows no transporter specific interaction on control membrane to validate the test system.

Reviewer's Comment:

Based on the ATPase activation assay, netupitant may be a substrate of P-gp. However, further studies are needed for confirmation.

Caco-2 Monolayer:

Bidirectional (A-B and B-A) permeability of ^{3}H-digixin was evaluated in the presence of increasing concentration of netupitant (0.2, 1 5 µM) on Caco-2 cell line (on 24-well plate) at 37^{o}C after 2 hours of incubation in duplicate. The paracellular permeability of the monolayer was assessed using ^{14}C-mannitol (Papp(A/B) = 2.13x10^{-6} cm/s). 60 µM Verapamil (known P-gp inhibitor) was included as the positive control.

Figure 4. Apparent permeability (Papp) of ^{3}H-digoxin in the apical-to-basolateral (A-B) and basolateral-to-apical (B-A) direction in the presence of different concentrations of Netupitant

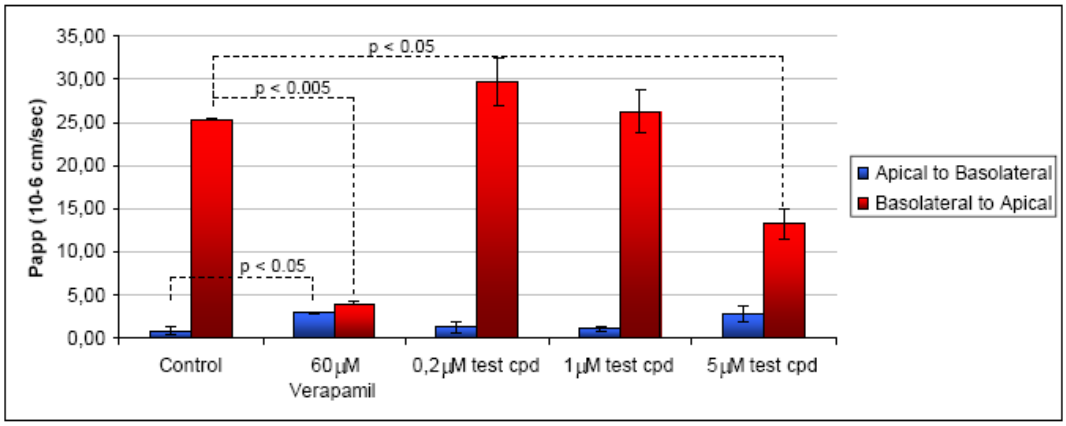


Table -3: Apparent permeability (Papp) of ^{3}H-digoxin in the apical-to-basolateral (A-B) and

basolateral-to-apical (B-A) direction in the presence of different concentrations of Netupitant

	Apical to Basolateral Papp (10^{-6} cm/sec)	Basolateral to Apical Papp (10^{-6} cm/sec)	Efflux Ratio
Control	0.87	25.32	29
60 uM Verapamil	2.98	4.07	1.4
Netupitant (0.2 µM)	1.25	29.73	23.8
Netupitant (1 µM)	1.07	26.23	24.5
Netupitant (5 µM)	2.8	13.24	4.7

Reviewer's Comment:

1. *The sponsor only evaluated potential of netupitant being an inhibitor of P-gp in Caco-2 cell monolayer in this study. The sponsor did not evaluate the potential of netupitant being a substrate of P-gp transporter in Caco-2 cell monolayer (bidirectional transport assay) with net flux ratio information or evaluate the permeability of netupitant in the presence of potent P-gp inhibitor to predict the in vivo relevance of this interaction.*
2. *The study system appears to be reasonable as the mannitol permeability was within the expected range, and the study had appropriate model substrate (digoxin) and appropriate positive control (Verapamil).*
3. *The concentration of netupitant at 0.2, 1, 5 µM are acceptable as they approximately cover the C_{max} value expected in human subjects or patients taking this drug at the clinical dose of 300 mg. Observed Cmax = 550-880 ng/mL (≈ 1-1.5 µM)*
4. *Based on the result of this study, netupitant seems to inhibit P-gp at concentration dependent manner. However, IC50 value was not determined in this study. Netupitant's potential inhibition interaction with P-gp in vivo at clinical dose cannot be ruled out. The sponsor did conduct a follow up in-vivo drug-drug interaction study with digoxin concomitantly administered with netupitant.*
5. *Netupitant's potential to induce P-gp transporter does not need to be evaluated since it has already been shown that netupitant and its metabolites do not induce CYP3A4 in in-vitro study NETU-10-27.*

Title: In vitro Interaction Studies of Netupitant and its three metabolites (M1, M2 and M3) with human BCRP, BSEP, MRP2 and MDR1 Efflux Transporters and with human OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2 Uptake Transporters

Report No: NETU-12-81

Specific Aims The purpose of this study was to provide data on the interaction of Netupitant, M1, M2 and M3 with the human ABC (efflux) transporters: BCRP (ABCG2/MXR), BSEP (ABCB11/sPgp), MRP2 (ABCC2) and MDR1 (ABCB1/P-gp) and the human uptake transporters: OATP1B1 (OATP2, OATP-C), OATP1B3 (OATP8), OAT1, OAT3, OCT1 and OCT2.

Study Date: 01/2013-06/2013

Test Site: (b) (4)

Sponsor: Helsinn Healthcare SA, Switzerland

Test Item: Netupitant and its metabolites (M1, M2, and M3)

Study Method:

1. Vesicular Transport Inhibition Assays for Efflux Transporter

The sponsor had used vesicular transport inhibition assay to evaluate the inhibition potential of efflux transporter by netupitant and its metabolites. Vesicular transport assays were performed with inside-out membrane vesicles prepared from cells overexpressing human ABC transporters on 96-well plates. The netupitant, M1, M2, and M3 (at 0.01, 0.04, 0.12, 0.37, 1.11, 3.33, 10 and 30 μM) were incubated with membrane vesicle preparations (total protein: 50 μg/well or 25 μg/well in case of BCRP) and the probe substrate in triplicates. Incubations were carried out in the presence of ATP or AMP to distinguish between transporter-mediated uptake and passive diffusion into the vesicles. At the end of the incubation period, the amount of probe substrate trapped in the vesicles was quantified by liquid scintillation counting.

Table 1. Vesicular transport assay parameters

Transporter	Probe substrate	Reference inhibitor
human BCRP (ABCG2)	E3S (1 μM)	Ko134 (1 μM)
human BSEP (ABCB11, sP-gp)	Taurocholate (2 μM)	cyclosporin A (20 μM)
human MRP2 (ABCC2)	E217βG (50 μM)	Benzbromarone (100 μM)
human MDR1 (ABCB1/P-gp)	NMQ (2 μM)	Verapamil (100 μM)

Controls:

- Incubation with AMP was included for background activity values for all data points.
- Incubation without testing compounded (solvent only) was included to provide 100% activity values.
- A reference inhibitor was included to serve as positive control for inhibition.
- Membrane vesicle preparations from parental cells or Sf9 cells expressing defective transporters or beta-gal provided negative controls for function.

Calculation:

For all wells, the amount of the translocated probe substrate was determined in cpm. Relative activities were calculated with the following equation:

$$Activity\ \% = \frac{A-B}{C-D} \times 100$$

Legend:

A: amount of translocated substrate in the presence of TA and ATP
B: amount of translocated substrate in the presence of TA and AMP
C: amount of translocated substrate in the presence of solvent and ATP
D: amount of translocated substrate in the presence of solvent and AMP

2. Uptake Transporters Inhibition and Substrate Assays

Uptake transporters were evaluated using CHO cells or FlpIn293 cells stably expressing the respective uptake transporters.

Table 2. Parameters of uptake transporter assays

Transporter	Incubation Time (inhibition)	Probe substrate	Reference inhibitor	Negative Control Cell Line
human OATP1B1	10	E3S (0.1 µM)	Cerivastatin (100 µM)	Parental CHO
human OATP1B3	10	Fluo-3 (10 µM)	Fluvastatin (30 µM)	Parental CHO
human OAT1	3	PAH (1.33 µM)	Benzbromarone (200 µM)	Parental CHO
human OAT3	3	E3S (1 µM)	Probenecid (200 µM)	Mock transfected HEK293
human OCT1	20	Metformin (3.63 µM)	Verapamil (100 µM)	Parental CHO
human OCT2	10	Metformin (3.63 µM)	Verapamil (100 µM)	Parental CHO

Inhibition Assessment:

Inhibition potential of netupitant, M1, M2, and M3 were evaluated by incubating netupitant, M1, M2, and M3 at 0.01, 0.04, 0.12, 0.37, 1.11, 3.33, 10 and 30 µM concentrations with cells stably expressing the uptake transporter and the probe substrates on 96-well plate at 37 ± 1 °C in pH 7.4 buffer in triplicates. After the incubation, the cells were washed twice with buffer and lysed with 0.1 M NaOH (1 mM $CaCl_2$ in 5% SDS in case of OATP1B3). Fluo-3 transport (OATP1B3) was determined by measuring fluorescence using 485 nm and 520 nm as the excitation and emission wavelengths, respectively. Radiolabelled probe substrate transport was determined with liquid scintillation counting.

Controls:

1. Uptake transport in parental cells (non-transfected) provided background activity values for all data points.
2. Incubation without test compound (solvent only) provided 100% activity values.
3. A reference inhibitor served as positive control for inhibition.

Calculation of relative activities:

The amount of translocated probe substrate was determined for each well in cpm or RFU or nM. Relative activities were calculated from the equation:

$$Activity\ \% = \frac{A-B}{C-D} \times 100$$

Legend:

A: amount of translocated substrate in the presence of TA in transfected cells
B: amount of translocated substrate in the presence of TA in parental cells
C: amount of translocated substrate in the presence of solvent in transfected cells

D: amount of translocated substrate in the presence of solvent in parental cells

Substrate Assessment:
The cellular uptake of netupitant, M1, M2, and M3 into cells was determined by incubating netupitant, M1, M2, or M3 at 1 and 10 µM concentrations with cells overexpressing the uptake transporter and control cells on 24-well plates at 37 ± 1 °C in pH 7.3 buffer for 2 and 20 min. After the incubation, the reactions were quenched by removing uptake buffer and the washing the cells twice. Cells were lysed by adding MetOH:H_2O (3:1) and incubated for 10 minutes at 37 ± 1 °C. The amount of TA in the cell lysates was determined by LC/MS. The amount of protein in each well was quantified using the BCA kit for protein determination.

Calculation of fold activation value
The fold activation value was defined as the ratio of uptake of TA or probe substrate into transfected and parental cells:

$$\text{Fold activation} = UPT_{TRP} / UPT_{Parental}$$

Legend:
UPT_{TRP}: accumulated amount of TA or probe substrate in transfected cells normalized by protein content [pmol/mg protein]
$UPT_{Parental}$: accumulated amount of TA or probe substrate in parental cells normalized by protein content [pmol/mg protein]

3. MDCKII Monolayer for MDR1 and BCRP Substrate and Inhibition Assays

The monolayer assays were performed using parental and MDR1 or BCRP transfected MDCKII cell monolayers cultured on the 24-well Transwell inserts.

Substrate Assessment (bidirectional transport):
Bidirectional transport through monolayers was determined by incubating of netupitant, M1, M2, and M3 (3, 10 and 30 µM) with parental and MDR1/BCRP transfected MDCKII cell monolayers (seeded on 24-well Transwell inserts) at 37 ± 1 °C. After the incubation, aliquots (100 µl) were taken from the receptor chambers to determine the amount of translocated TA. Samples were taken from the donor chambers before and after incubation to determine the initial concentration (C_0) and recovery (R) of the test compound. Amount of netupitant, M1, M2, and M3 was determined by LC/MS.
The digoxin/prazosin efflux ratio was determined as a positive control for MDR1/BCRP function. As a follow-up, bidirectional transport of M2 in parental and MDR1 transfected MDCKII cells was determined in the presence and absence of the MDR1 inhibitor PSC833 to confirm the specificity of the transport in MDCKII-MDR1 cells.

Table 3: Monolayer assay parameters; MDCKII-MDR1/MDCKII-BCRP and MDCKII parental cells bidirectional permeability measurements

Monolayer assay type	Compound	Direction	Concentration	Incubation Time (min)
MDCKII, MDCKII-MDR1	M1	A-B/B-A	3, 10 and 30 µM	0, 15, 30, 60 and 120
	M2	A-B/B-A	3, 10 and 30 µM	0, 15, 30, 60 and 120
	M3	A-B/B-A	3, 10 and 30 µM	0, 15, 30, 60 and 120
	Lucifer yellow	A-B/B-A	40 µg/ml	120
	antipyrine	A-B	50 µM	30
	digoxin	A-B/B-A	5 µM	120

MDCKII, MDCKII-BCRP	Netupitant	A-B/B-A	3, 10 and 30 µM	0, 15, 30, 60 and 120
	M1	A-B/B-A	3, 10 and 30 µM	0, 15, 30, 60
	M2	A-B/B-A	3, 10 and 30 µM	0, 15, 30, 60 and 120
	M3	A-B/B-A	3, 10 and 30 µM	0, 15, 30, 60 and 120
	Lucifer yellow	A-B/B-A	40 µg/ml	120
	antipyrine	A-B	50 µM	30
	prazosin	A-B/B-A	1 µM	60
MDCKII, MDCKII-BCRP	M2	A-B/B-A-B	30 µM	120
	M2 + PSC833	A-B/B-A	30 + 10 µM	120
	Digoxin	A-B/B-A	5 µM	120
	Digoxin+PSC833	A-B/B-A	5 + 10 µM	120
	Lucifer yellow	A-BB	40 µg/ml	120
	antipyrine	A-B	50 µM	30

Inhibition Assessment:

Bidirectional transport of model substrates (digoxin/prazosin) for MDR1 and BCRP in parental and MDR1/BCRP transfected MDCKII cells was determined in the presence and absence of Netupitant, M1, M2 and M3 (10 and 30 µM) or the reference inhibitor PSC833 or Ko134. The reference inhibitor (10 µM PSC833 or 1 µM Ko134) or the test compound, 30 µM TA, was added to both apical and basolateral chambers of the wells. After incubation at 37 ± 1 °C, aliquots (100 µl) were taken from the receptor chambers to determine the amount of translocated digoxin/prazosin via liquid scintillation. The donor compartments were sampled before and after incubation to determine the initial concentration (C_0) and recovery (R) of digoxin/prazosin.

Table 4: Treatment groups; MDCKII-MDR1/BCRP and parental cell permeability measurements

Monolayer assay type	Model Substrate	Direction	Inhibitor	Incubation Time (min)
MDCKII, MDCKII-MDR1	Digoxin (5 µM)	A-B/B-A	NA	120
	Digoxin (5 µM)	A-B/B-A	M1, M2 and M3: 10 and 30 µM	120
	Digoxin (5 µM)	A-B/B-A	PSC833 (10 µM)	120
	Lucifer yellow (40 µg/ml)	A-B	NA	120
	Antipyrine (50 µM)	A-B	NA	30
MDCKII, MDCKII-BCRP	Prazosin (1 µM)	A-B/B-A	NA	60
	Prazosin (1 µM)	A-B/B-A	Netupitant, M1, M2 and M3: 10 and 30 µM	60
	Prazosin (1 µM)	A-B/B-A	Ko134 (1 µM)	60
	Lucifer yellow (40 µg/ml)	A-B	NA	120
	Antipyrine (50 µM)	A-B	NA	30

Controls:

1. The transepithelial electric resistance (TEER) was determined for each well prior to the experiment to confirm the confluency of the monolayers to the experiment. Values above 150 Ω/cm2 were accepted.

2. Monolayer integrity markers included the measurement of low (Lucifer yellow) and high (antipyrine) permeability compounds in each experiment. Values were accepted below 2 × 10-6 cm/s for LY and above 50 × 10-6 cm/s for antipyrine.
3. The efflux ratio of digoxin (positive control for MDR1-mediated active efflux) was accepted when above 3 for the digoxin control. The efflux ratio of digoxin in the presence of the reference inhibitor PSC833 (10 μM) was reduced to 1 ± 0.5.
4. The efflux ratio of prazosin (positive control for BCRP-mediated active efflux) accepted when above 3 for the prazosin control. The efflux ratio of prazosin in the presence of the reference inhibitor Ko134 (1 μM) was reduced to 1 ± 0.5.

Calculation:
The following equation was used for apparent permeability coefficient (P_{app}):

$$P_{app} = \frac{dQ}{dT} \times \frac{1}{A \times C_0}$$

Legend:
dQ: amount of transported test drug
dT: incubation time
A: surface of porous membrane in cm2 (standard: 0.7)
C_0: initial concentration of the compound in the donor compartment

Efflux ratio (ER) = $Papp_{B-A}$ / $Papp_{A-B}$

For MDCKII-MDR1/BCRP cells, efflux ratios were calculated as ER_T/ER_P where (ER_T) and (ER_P) are the efflux ratios for the transfected and the parental cells (used for negative controls), respectively.

Recovery (R) was calculated according to the following formula to allow for estimation of metabolism and/or non-specific binding:

$$R(\%) = \frac{Q_{apical} + Q_{basolateral}}{Q_0} \cdot 100\%$$

Legend:
Q_{Apical}: amount of test drug detected in the apical chamber in pmol
$Q_{Basolateral}$: amount of test drug detected in basolateral chamber in pmol
Q_0: amount of test drug detected at t = 0 in pmol

Bioanalysis:
- Digoxin/prazosin samples were analyzed with liquid scintillation counting.
- Lucifer yellow samples were analyzed by measuring fluorescence, with excitation at 430 nm and emission at 520 nm.
- Antipyrine and TA (netupitant, M1, M2, and M3) were analyzed with LC-MS system and HPLC-MS system.

Results:

1. Vesicular Transport Inhibition Assays for Efflux Transporter

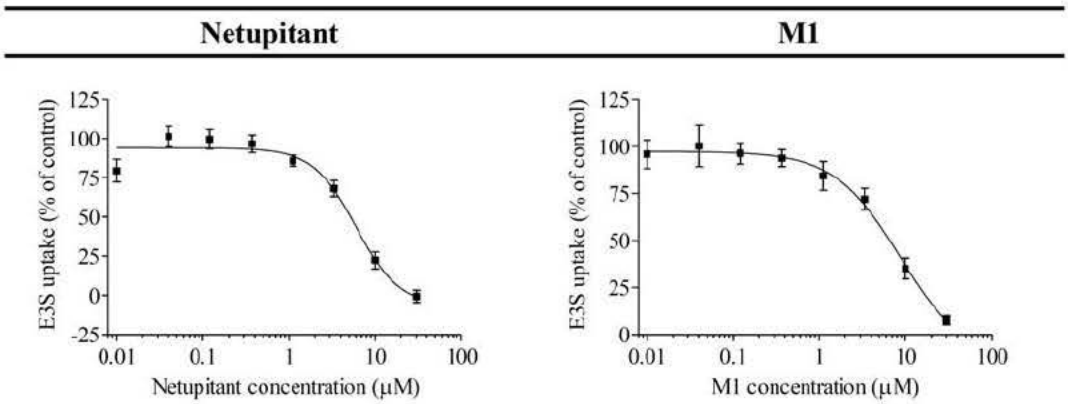


Figure 1. **Inhibition of BCRP-mediated E3S transport by Netupitant and M1 in the vesicular transport inhibition assay**

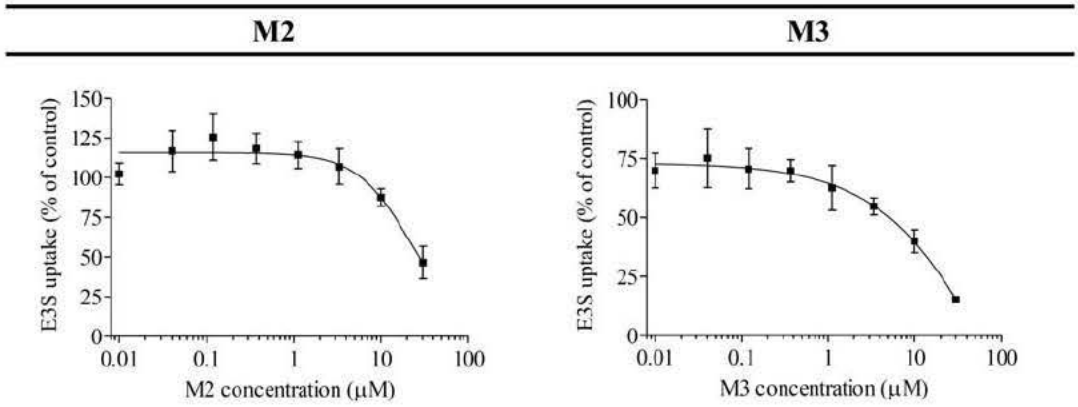


Figure 2. **Inhibition of BCRP-mediated E3S transport by M2 and M3 in the vesicular transport inhibition assay**

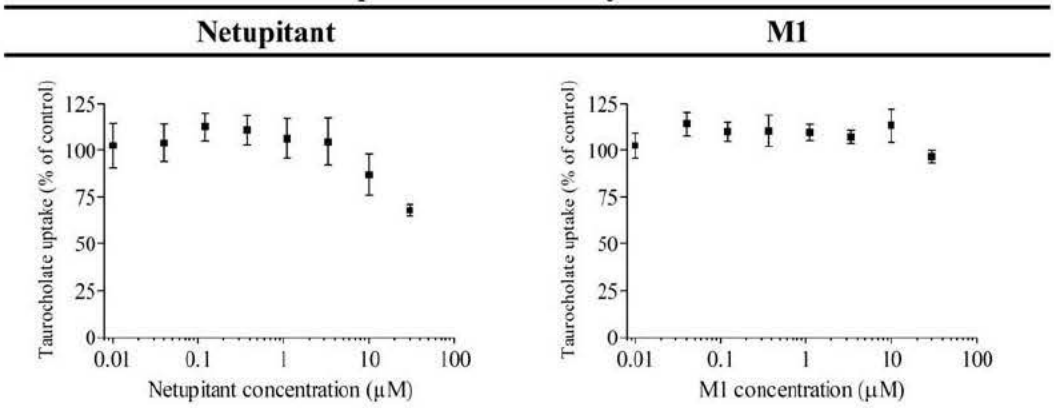


Figure 3. **Inhibition of BSEP-mediated taurocholate transport by Netupitant and M1 in the vesicular transport inhibition assay**

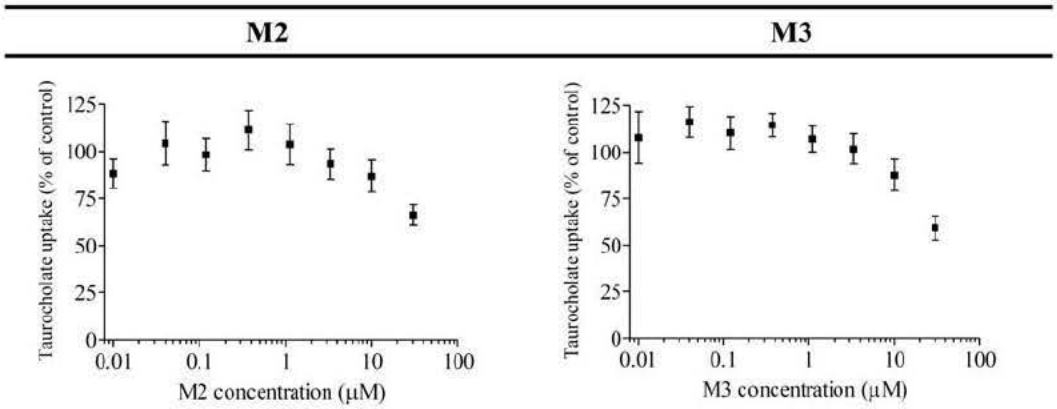


Figure 4. **Inhibition of BSEP-mediated taurocholate transport by M2 and M3 in the vesicular transport inhibition assay**

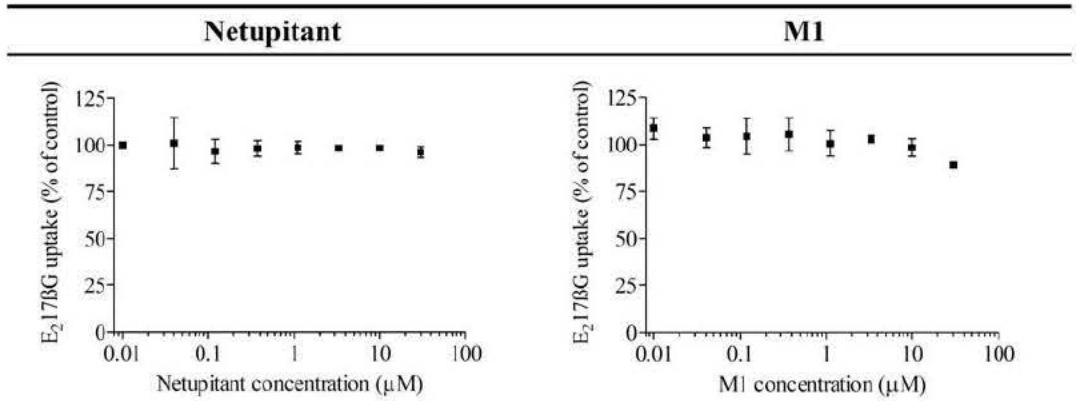


Figure 5. **Inhibition of MRP2-mediated $E_2$17ßG transport by Netupitant and M1 in the vesicular transport inhibition assay**

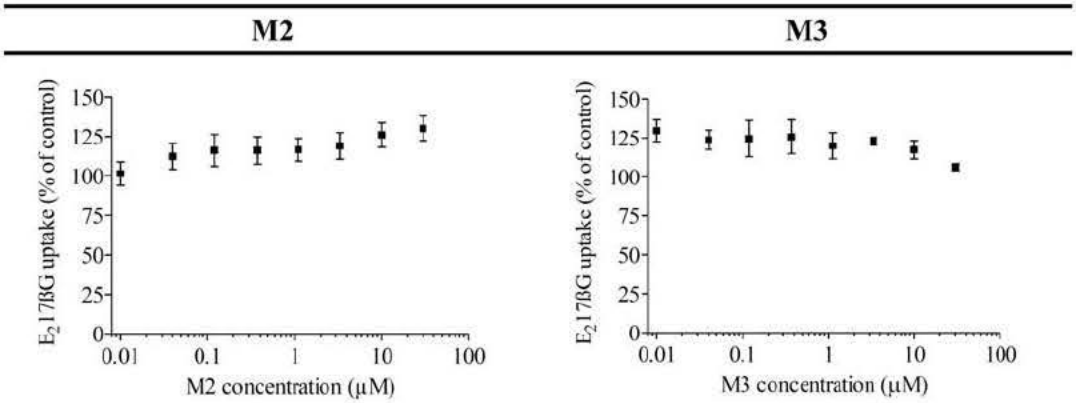


Figure 6. **Inhibition of MRP2-mediated $E_2$17ßG transport by M2 and M3 in the vesicular transport inhibition assay**

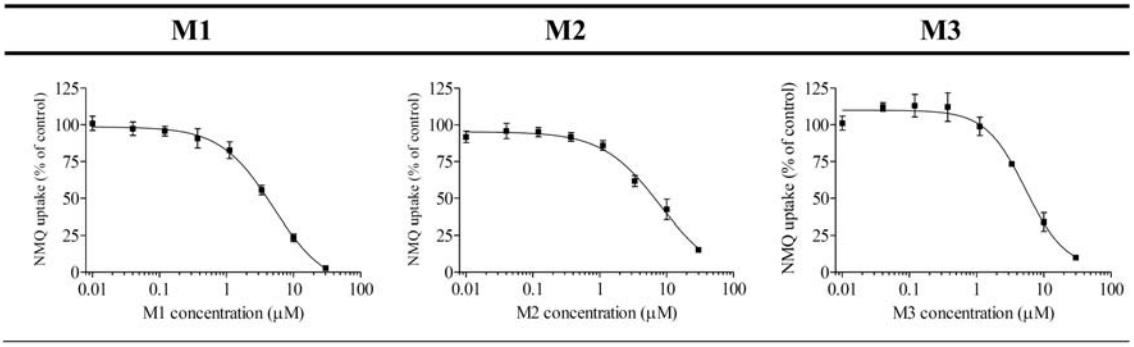


Figure 7. Inhibition of MDR1-mediated NMQ transport by M1, M2 and M3 in the vesicular transport inhibition assay

Table 5. Calculated Reaction Parameters from vesicular transport inhibition assays

Test article	Vesicular transport inhibition	IC_{50} (μM)	maximum inhibition (% of control)
Netupitant	BCRP	6.00	100%
M1		8.64	92%
M2		22.6	53%
M3		10.6	85%
Netupitant	BSEP	NA	32%
M1		ND	no interaction up to 30 μM
M2		NA	34%
M3		NA	41%
Netupitant	MRP2	ND	no interaction up to 30 μM
M1		ND	no interaction up to 30 μM
M2		ND	no interaction up to 30 μM
M3		ND	no interaction up to 30 μM
M1	MDR1	4.95	97%
M2		8.00	85%
M3		5.35	90%

ND: IC_{50} could not be determined as no interaction was observed within the concentration range tested
NA: Not applicable

Reviewer's Comments:

- *Netupitant, M1, M2 and M3 do not inhibit MRP2 up to 30 μM concentration and thus IC50 values were not determined for MRP2 transporter.*
- *Netupitant, M2 and M3 slightly inhibit BSEP while M1 does not show any inhibition toward BSEP up to 30 μM concentration. Therefore, IC50 values could not be determined for BSEP transporter.*
- *Netupitnat, M1, M2 and M3 inhibit BCRP in concentration dependent manner. Since total Cmax/IC50 are less for 0.1 for M1, M2 and M3, further studies are not needed for the metabolites. However, since total Cmax/IC50 is greater than 0.1 for parent drug netupitant, a follow up in vivo study may be recommended.*

- *Netupitnat: Cmax/IC50= (1-1.5µM)/6 µM = (0.167-0.25)>0.1*
- *M1: Cmax/IC50=(0.07-0.09 µM)/8.6 µM =(0.008-0.01)<0.1*
- *M2: Cmax/IC50= (0.17-0.58 µM)/22.6 µM =(0.0075-0.026) <0.1*
- *M3: Cmax /IC50 = (0.08-0.144 µM)/10.6 µM = (0.0075-0.013) <0.1*

- *M1, M2 and M3 inhibit inhibits MDR1in concentration dependent manner. However, since Cmax/IC50 <0.1 for all metabolites, not further studies are needed. However, inhibition potential of netupitant for MDR1 transporter was not evaluated in this experiment.*
  - *M1: Cmax/IC50=(0.07-0.09 µM)/4.95 µM =(0.014-0.018)<0.1*
  - *M2: Cmax/IC50= (0.17-0.58 µM)/8.0 µM =(0.02125-0.0725) <0.1*
  - *M3: Cmax /IC50 =(0.08-0.144 µM)/5.35 µM = (0.014-0.027) <0.1*
- *The reference inhibitors (positive controls) for all evaluated efflux transporters had adequate level of inhibition to confirm the function of the transporters in the applied vesicles.*
- *Negative controls with membrane vesicle prepared from parental cell had very minimum transport of model substrate into the vesicle.*

2. Uptake Transporters Inhibition and Substrate Assays

Inhibition Assessment:

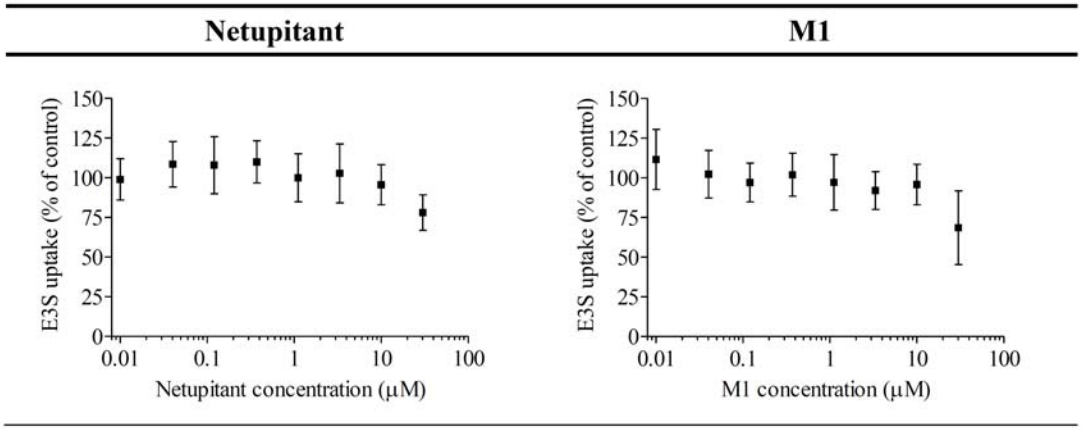


Figure 20. Modulation of OATP1B1-mediated E3S transport by Netupitant and M1 in the uptake transporter inhibition assay

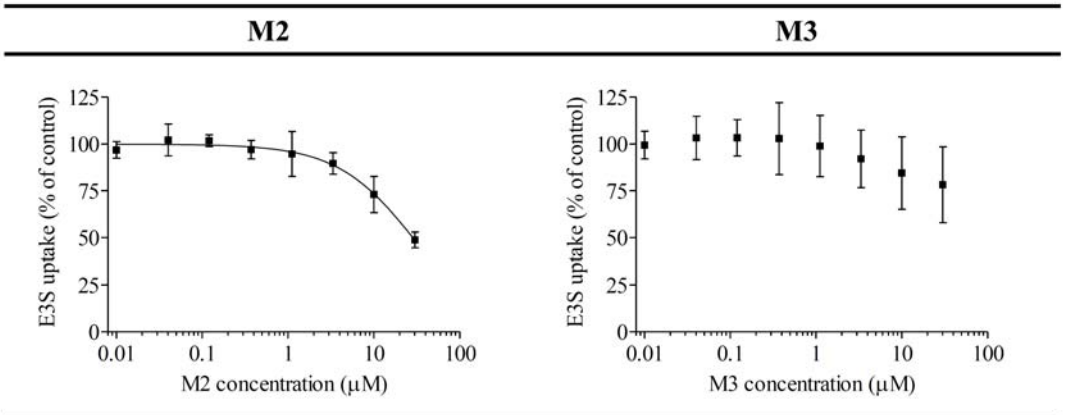


Figure 21. Modulation of OATP1B1-mediated E3S transport by M2 and M3 in the uptake transporter inhibition assay

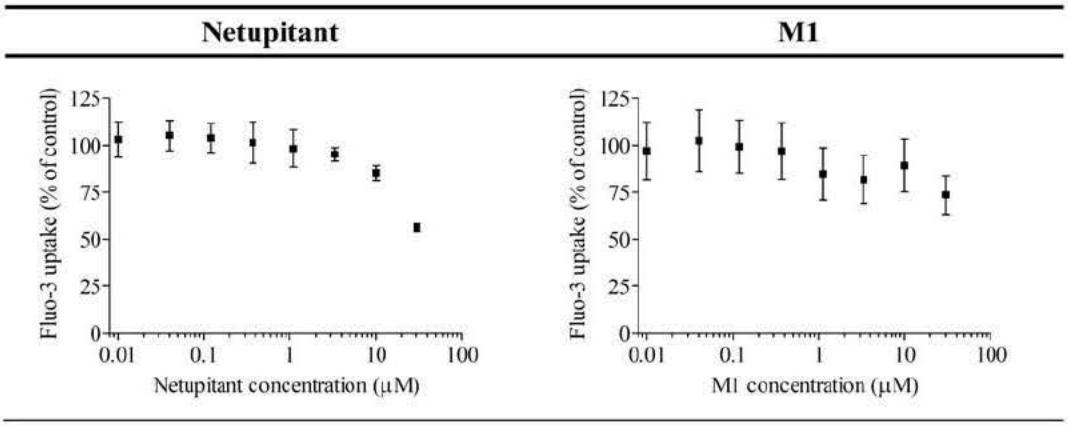


Figure 22. **Modulation of OATP1B3-mediated Fluo-3 transport by Netupitant and M1 in the uptake transporter inhibition assay**

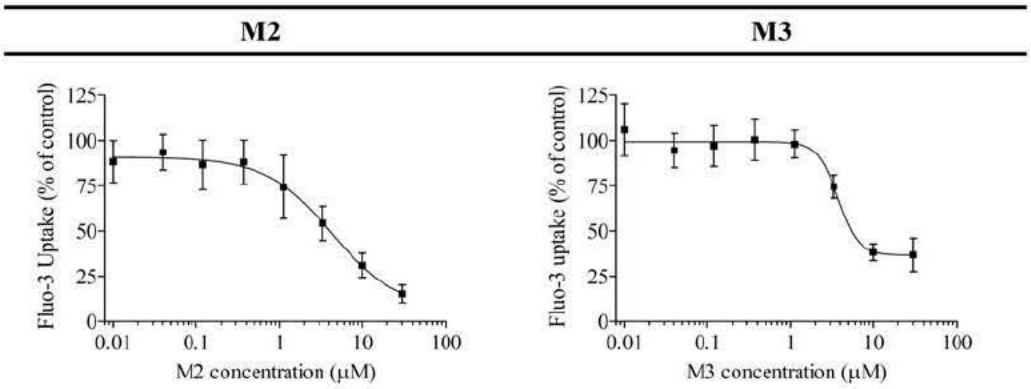


Figure 23. **Modulation of OATP1B3-mediated Fluo-3 transport by M2 and M3 in the uptake transporter inhibition assay**

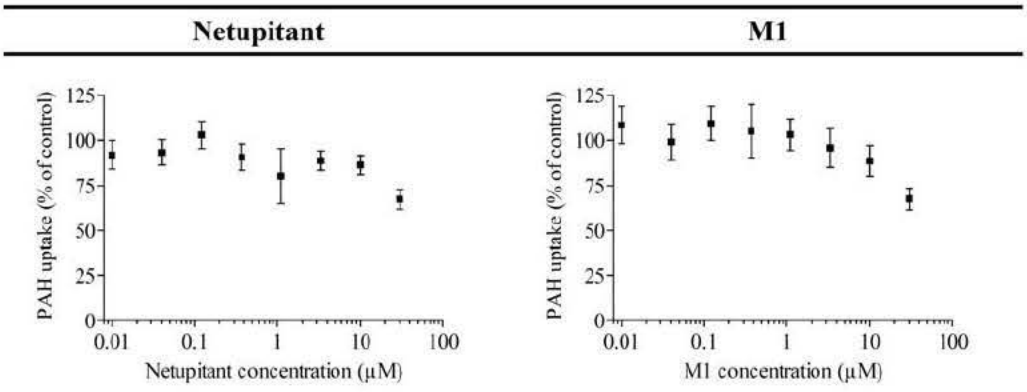


Figure 24. **Modulation of OAT1-mediated PAH transport by Netupitant and M1 in the uptake transporter inhibition assay**

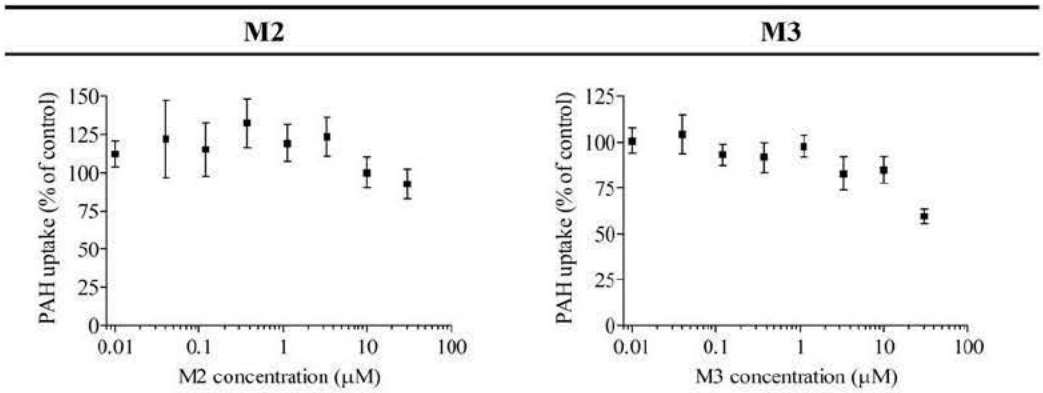


Figure 25. Modulation of OAT1-mediated PAH transport by M2 and M3 in the uptake transporter inhibition assay

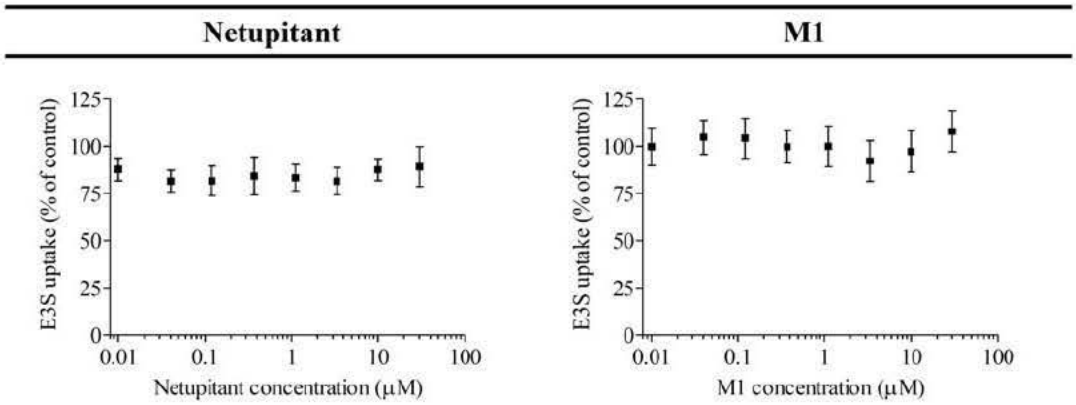


Figure 26. Modulation of OAT3-mediated E3S transport by Netupitant and M1 in the uptake transporter inhibition assay

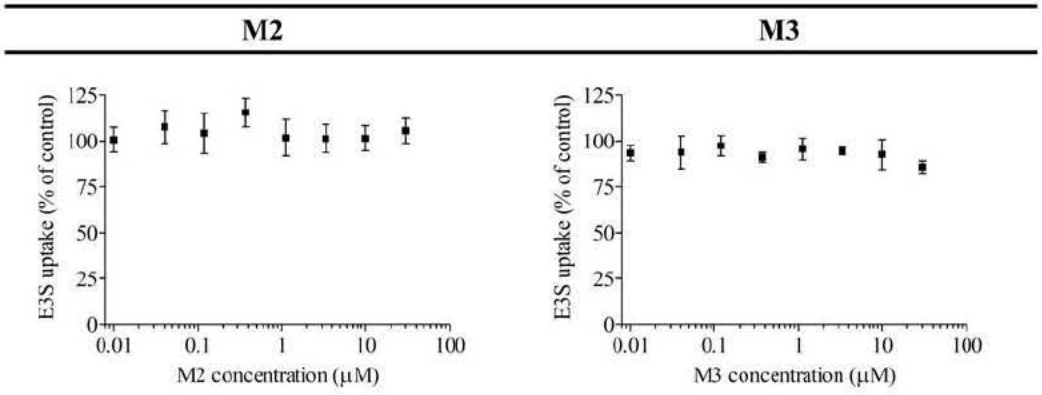


Figure 27. Modulation of OAT3-mediated E3S transport by M2 and M3 in the uptake transporter inhibition assay

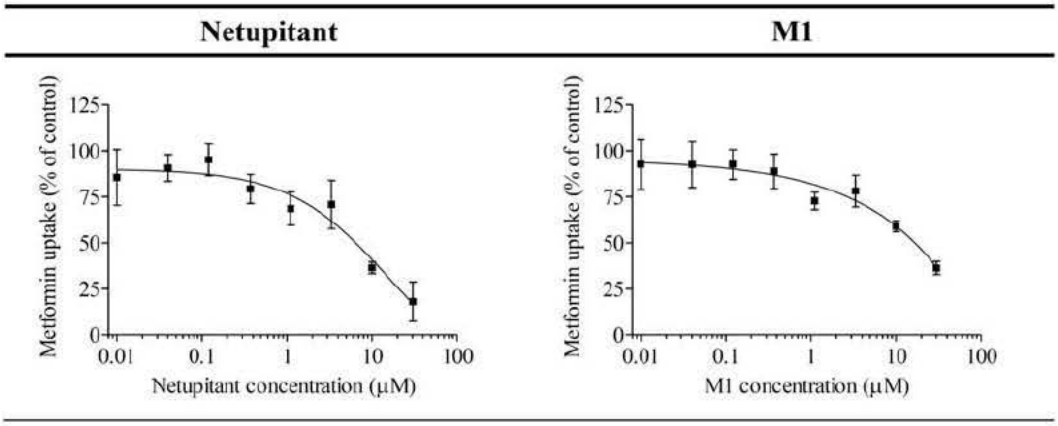


Figure 28. Modulation of OCT1-mediated metformin transport by Netupitant and M1 in the uptake transporter inhibition assay

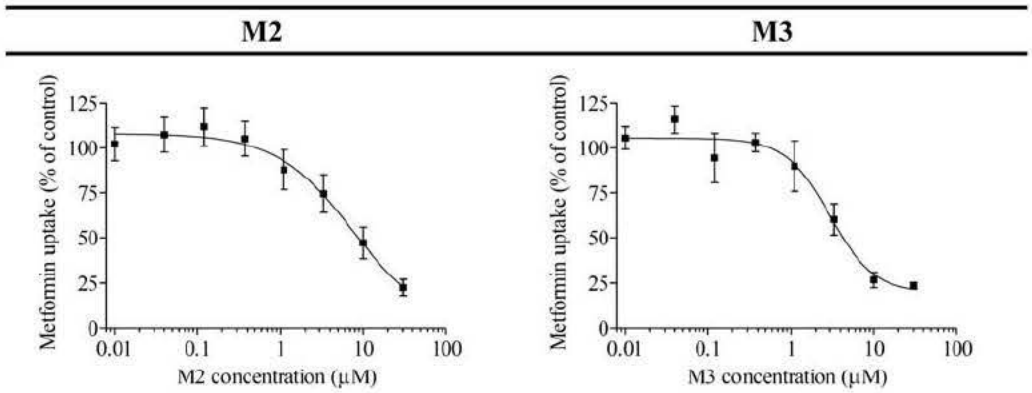


Figure 29. Modulation of OCT1-mediated metformin transport by M2 and M3 in the uptake transporter inhibition assay

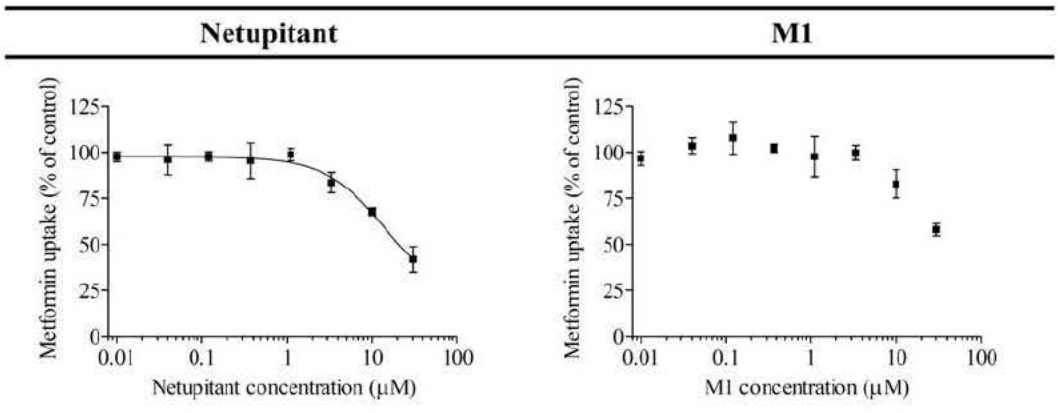


Figure 30. Modulation of OCT2-mediated metformin transport by Netupitant and M1 in the uptake transporter inhibition assay

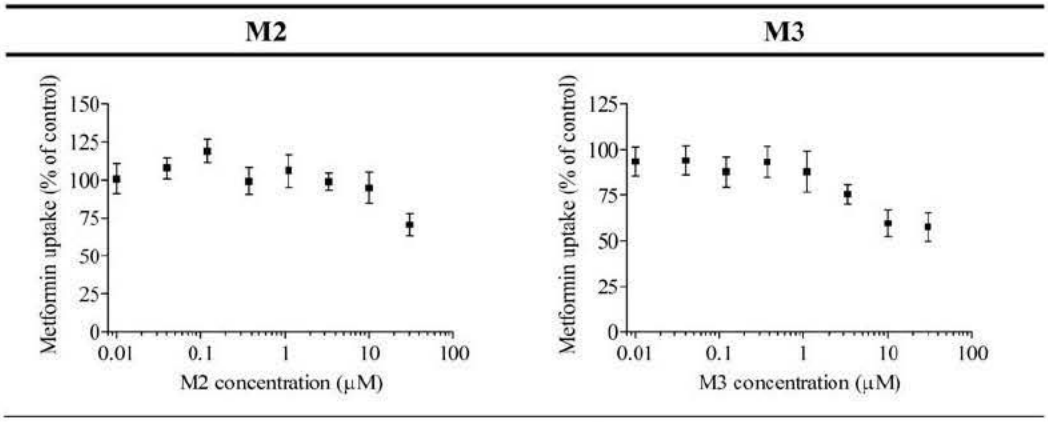


Figure 31. Modulation of OCT2-mediated metformin transport by M2 and M3 in the uptake transporter inhibition assay

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Table 22. Calculated reaction parameters from uptake transporter inhibition assays

Test article	Uptake transporter inhibition	IC_{50} (µM)	maximum inhibition (% of control)
Netupitant	OATP1B1	NA	22%
M1		NA	31%
M2		≥30.0	51%
M3		NA	22%
Netupitant	OATP1B3	NA	44%
M1		NA	26%
M2		4.3	85%
M3		9.6	63%
Netupitant	OAT1	NA	33%
M1		NA	32%
M2		ND	no interaction up to 30 µM
M3		NA	40%
Netupitant	OAT3	ND	no interaction up to 30 µM
M1		ND	no interaction up to 30 µM
M2		ND	no interaction up to 30 µM
M3		ND	no interaction up to 30 µM
Netupitant	OCT1	7.9	82%
M1		19.0	64%
M2		7.4	77%
M3		4.4	76%
Netupitant	OCT2	22.3	58%
M1		NA	42%
M2		NA	29%
M3		NA	43%

ND: IC_{50} could not be determined as no interaction was observed within the concentration range tested
NA: Not applicable

Reviewer's Comments:

- *OATP1B1: Netupitant, M1 and M3 showed weak inhibition toward OATP1B1, and thus, IC50 values could not be estimated. However, M2 did show some inhibition toward OATP1B1 with IC50 of >30 µM. Since total Cmax/IC50 = 0.58 µM /30 µM =0.02 < 0.1, a follow-up in-vivo study is not needed.*
- *OATP1B3: Netupitant and M1 showed weak inhibition toward OATP1B3 and IC50 value would not be estimated up to 30 µM. M2 and M3 inhibited OATP1B3 with IC50 values of 4.3 and 9.6 µM. Since Cmax/IC50 = 0.144 µM /9.6 µM = 0.015<0.1 for M3, an in-vivo study for to evaluate the inhibition potential of M3 toward OATP1B3 is not needed. Although total Cmax/IC50 = 0.58 µM /4.3 µM = 0.13 >0.1 for M2, R-value = 1+ (fu x I in,max/IC50) = 1.08 <1.25 and thus, in vivo study is not needed.*
- *OAT1: Netupitant, M1, M2 and M3 do not appear to inhibit OAT1 significantly up to 30 µM concentration and thus, IC50 value could not be determined.*
- *OAT3: Netupitant, M1, M2 and M3 do not inhibit OAT3.*

- *OCT1: Netupitant, M1, M2 and M3 all appear to inhibit OCT1 in concentration dependent manner. For netupitant, although Cmax/IC50 =0.19 for OCT1, it is not substantially larger than 0.1. Since Cmax/IC50 <0.1 for OCT2, and OCT1 and OCT2 have overlapping substrate specificities, we do not anticipate a significant in-vivo OCT1 interaction for netupitant.*
    - *Netupitant: Cmax/IC50 = (1-1.5 µM) /7.9 µM = (0.13-0..19) >0.1*
    - *M1: Cmax/IC50 = (0.07-0.09 µM) /19 µM = (0.0037-0.0047)<0.1*
    - *M2: Cmax/IC50 = (0.17-0.58 µM)/7.4 µM =(0.023-0.078)<0.1*
    - *M3 Cmax/IC50 = (0.08-0.144 µM) /4.4 µM =(0.018-0.033) <0.1*
- *OCT2: Netupitant appears to inhibit OCT2 in concentration dependent manner with IC50 value of 22.3 µM while M1, M2 and M3 did not show significant inhibition toward OCT2. Since Cmax/IC50 = (1-1.5 µM) /22.3 µM = (0.045-0.07) <0.1, in-vivo follow up study is not needed.*
- *The reference inhibitors (positive controls) for all evaluated uptake transporters had adequate level of inhibition to validate the test system to confirm the function of the transporters.*

Substrate Assessment:

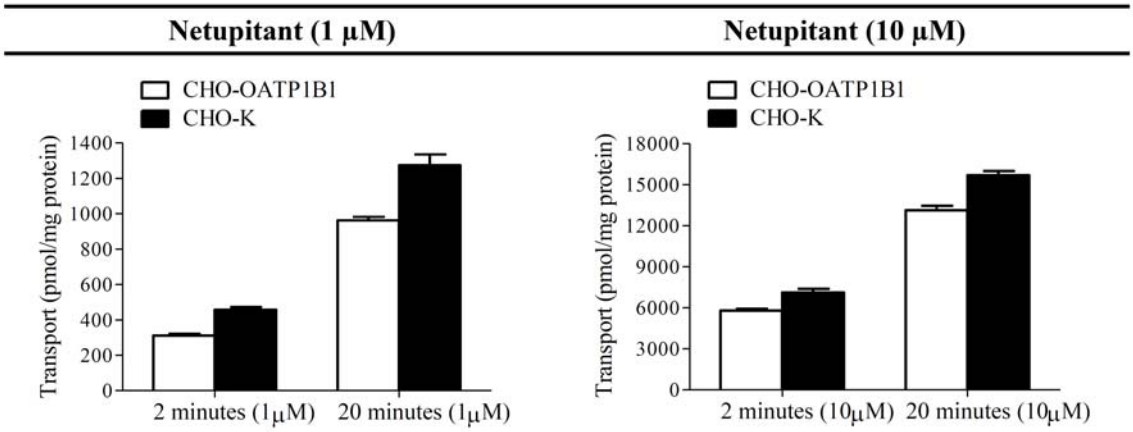

Figure 32. Accumulation of Netupitant in OATP1B1 expressing and control cells in the uptake transporter substrate feasibility assay

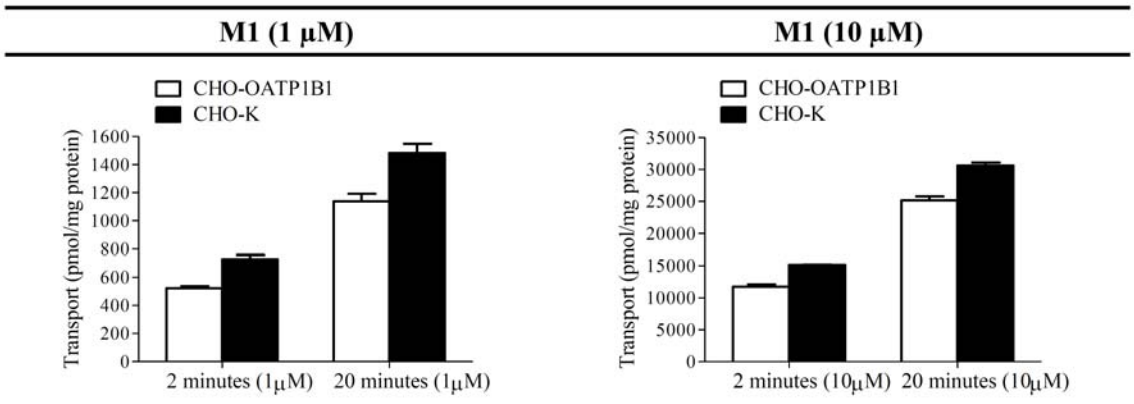

Figure 33. Accumulation of M1 in OATP1B1 expressing and control cells in the uptake transporter substrate feasibility assay

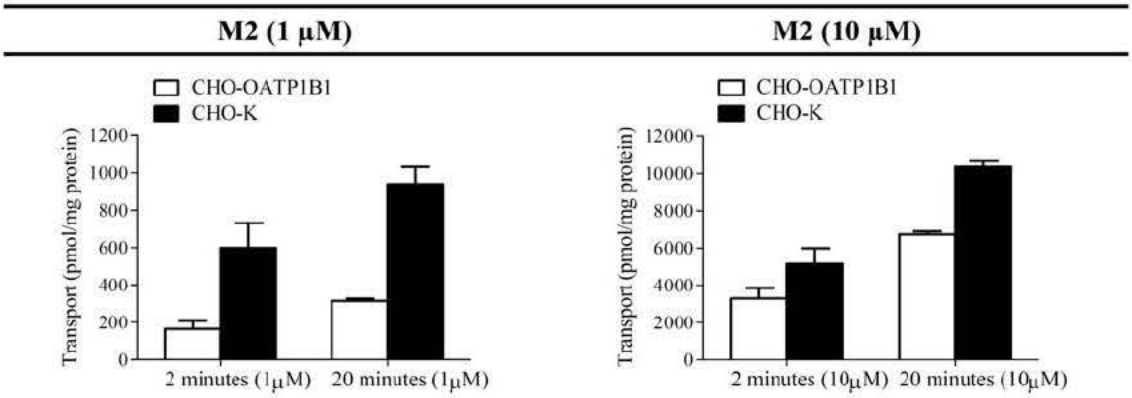

Figure 34. Accumulation of M2 in OATP1B1 expressing and control cells in the uptake transporter substrate feasibility assay

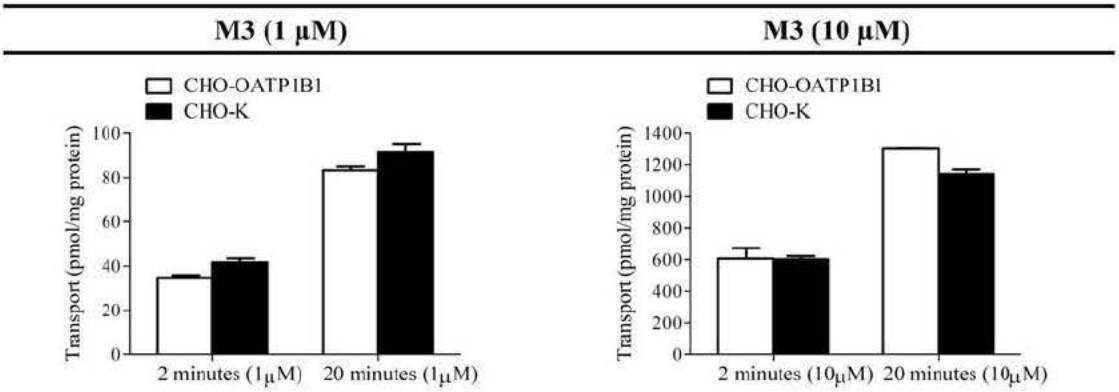

Figure 35. Accumulation of M3 in OATP1B1 expressing and control cells in the uptake transporter substrate feasibility assay

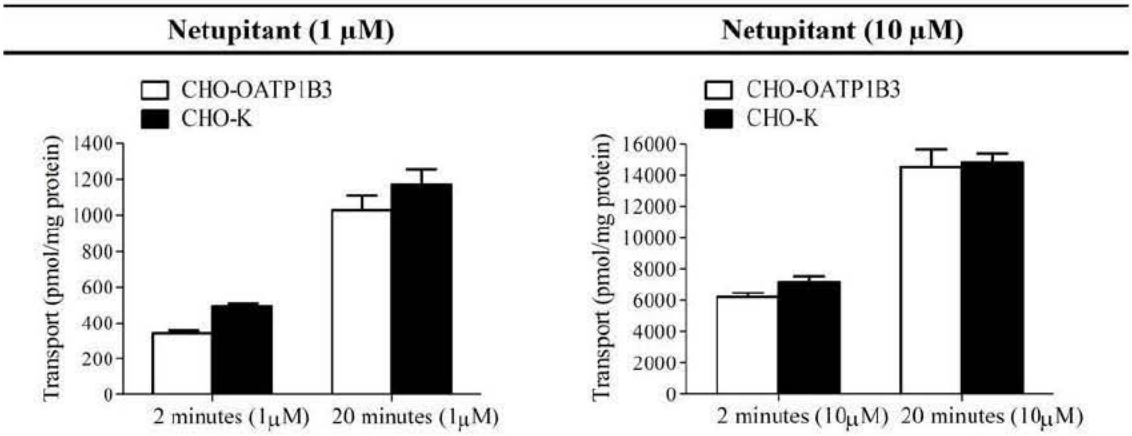

Figure 36. Accumulation of Netupitant in OATP1B3 expressing and control cells in the uptake transporter substrate feasibility assay

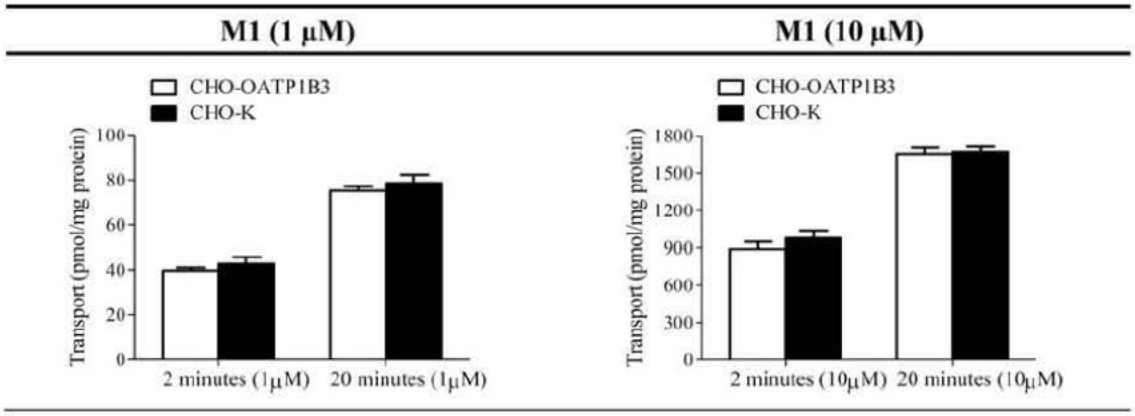

Figure 37. Accumulation of M1 in OATP1B3 expressing and control cells in the uptake transporter substrate feasibility assay

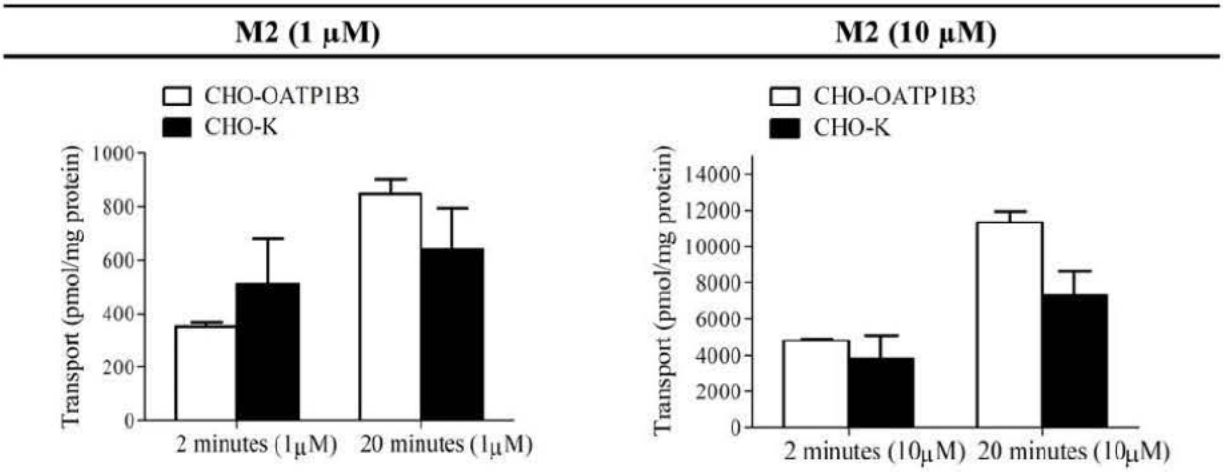

Figure 38. Accumulation of M2 in OATP1B3 expressing and control cells in the uptake transporter substrate feasibility assay

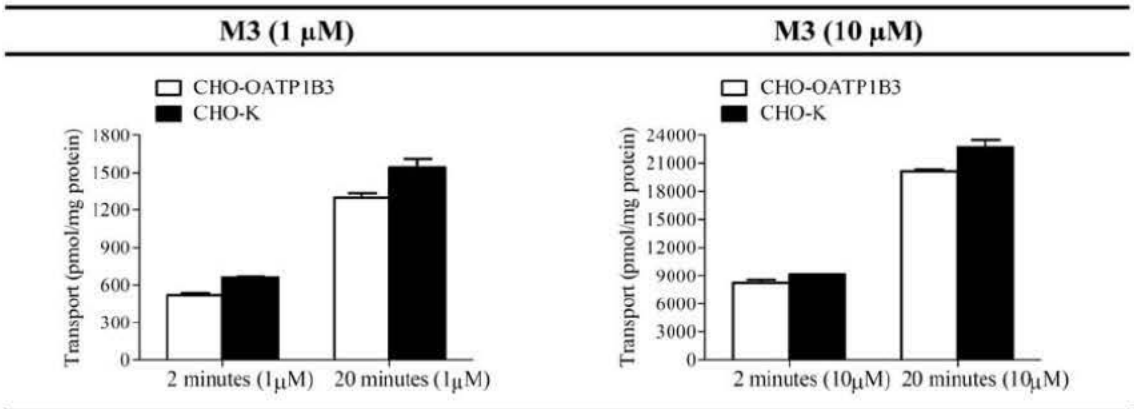

Figure 39. Accumulation of M3 in OATP1B3 expressing and control cells in the uptake transporter substrate feasibility assay

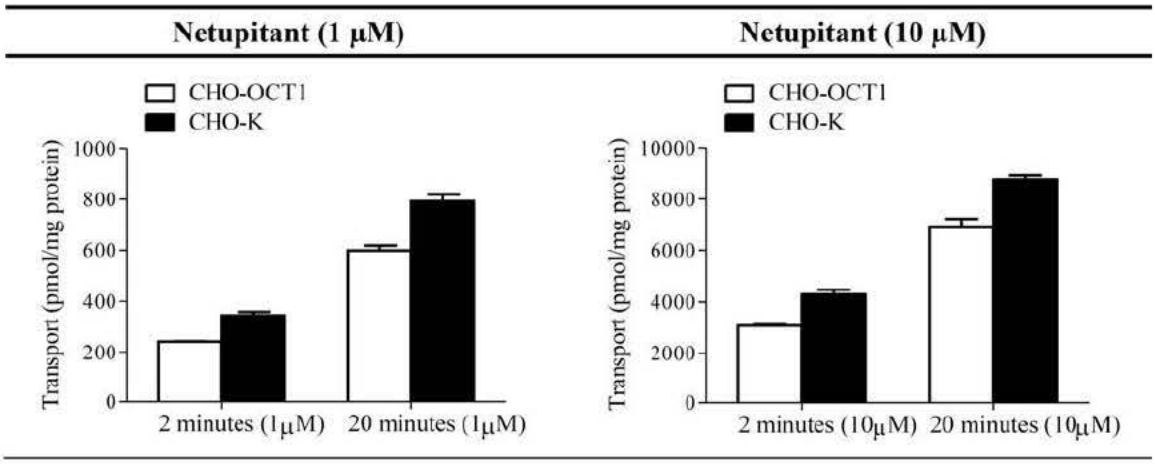

Figure 40. Accumulation of Netupitant in OCT1 expressing and control cells in the uptake transporter substrate feasibility assay

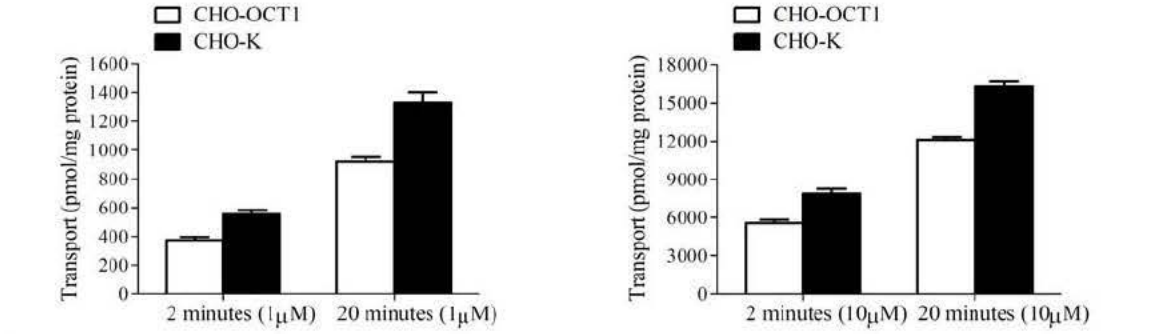

Figure 41. Accumulation of M1 in OCT1 expressing and control cells in the uptake transporter substrate feasibility assay

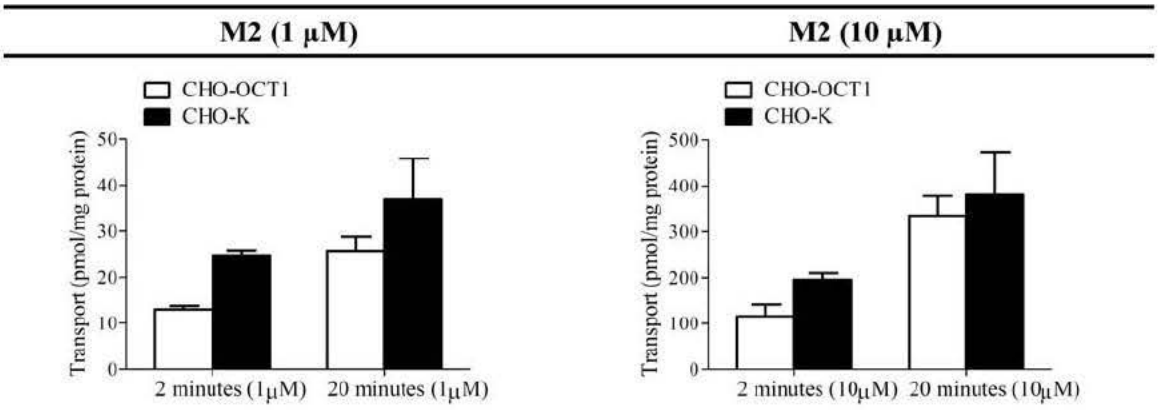

Figure 42. Accumulation of M2 in OCT1 expressing and control cells in the uptake transporter substrate feasibility assay

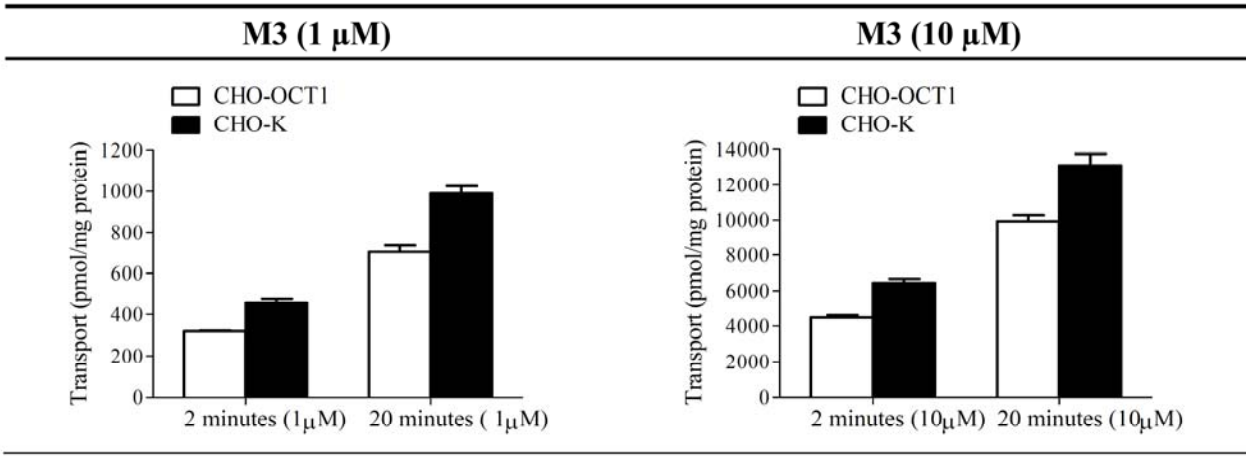

Figure 43. Accumulation of M3 in OCT1 expressing and control cells in the uptake transporter substrate feasibility assay

Table 23. Calculated reaction parameters from the uptake substrate experiments

Transporter	Test article	Fold at 1 μM / 2 min	Fold at 1 μM / 20 min	Fold at 10 μM / 2 min	Fold at 10 μM / 20 min
OATP1B1	Netupitant	0.68	0.76	0.81	0.84
	M1	0.72	0.77	0.77	0.82
	M2	0.28	0.33	0.64	0.65
	M3	0.70	0.76	0.84	0.95
OATP1B3	Netupitant	0.69	0.88	0.87	0.98
	M1	0.85	0.88	0.84	0.91
	M2	0.69	1.33	1.26	1.55
	M3	0.79	0.84	0.89	0.89
OCT1	Netupitant	0.70	0.76	0.71	0.79
	M1	0.68	0.69	0.71	0.75
	M2	0.51	0.68	0.57	0.85
	M3	0.70	0.71	0.69	0.76

Reviewer's Comments:

- *As Netupitant and its metabolites are primarily eliminated through hepatobiliary route, the sponsor choice to evaluate the potential of Netupitant and its metabolites being substrate for OATP1B1 and OATP1B3 was appropriate.*
- *As none of the test compound showed ≥2 fold increase in uptake in transfected cells compared to parental cell, netupitant, M1, M2 and M3 do not appear to be substrates for OATP1B1, OATP1B3 and OCT1.*
- *Regarding the positive controls for these transporter, fold increase in uptake of model substrate (positive controls) in transfected cells compared to parental cell were not reported. However, the sponsor evaluated the uptake of model substrates in absence and presence of model inhibitor of for these specific transporters to validate the test system. Uptake of these model substrates were substantially inhibited in the presence of model inhibitor based on the raw data.*

3. MDCKII Monolayer for MDR1 and BCRP Substrate and Inhibition Assays

Substrate Assessment (bidirectional transport):

Calculated reaction parameters from MDCKII-MDR1 studies

Test article	Assay	ER		Net ER
		MDCKII	MDCKII-MDR1	MDCKII-MDR1 / MDCKII
M1	bidirectional permeability of M1	3 µM: 0.17 ± 0.64	3 µM: 0.13 ± 0.59	0.74 ± 0.87
		10 µM: 0.13 ± 0.58	10 µM: 0.25 ± 0.28	1.86 ± 0.64
		30 µM: 15.07 ± 0.32	30 µM: 2.37 ± 0.31	0.16 ± 0.44
M2	bidirectional permeability of M2	3 µM: 15.31 ± 0.13	3 µM: 90.88 ± 0.18	5.94 ± 0.22
		10 µM: 0.36 ± 0.28	10 µM: 96.84 ± 0.15	269.52 ± 0.32
		30 µM: 0.45 ± 0.19	30 µM: 2.76 ± 0.33	6.17 ± 0.38
M2+PSC833	bidirectional permeability of M2 in the presence of PSC833	digoxin: 4.16± 0.57	digoxin: 16.62 ± 3.66	4.00 ± 1.03
		+PSC833: 0.98 ± 0.05	+PSC833: 1.26 ± 0.03	1.28 ± 0.07
		M2 (30 µM): 2.42 ± 0.45	M2 (30 µM): 2.61 ± 1.24	1.08 ± 0.55
		+ PSC833: 0.79 ± 0.15	+ PSC833: 1.01 ± 0.17	1.29 ± 0.33
M3	bidirectional permeability of M3	3 µM: 5.92 ± 0.73	3 µM: 0.14 ± 0.44	0.02 ± 0.85
		10 µM: 0.25 ± 0.27	10 µM:0.31 ± 0.36	1.27 ± 0.45
		30 µM: 0.42 ± 0.12	30 µM:0.40 ± 0.12	0.96 ± 0.17

Calculated Reaction Parameters From MDCKII-BCRP Studies

Test article	Assay	ER		Net ER
		MDCKII	MDCKII-BCRP	MDCKII-BCRP/ MDCKII
Netupitant	bidirectional permeability of Netupitant	3 µM:0.00 ± 0.41	3 µM:0.01 ± 0.38	1.50 ± 0.56
		10 µM:0.01 ± 0.42	10 µM: 0.03 ± 0.29	2.75 ± 0.51
		30 µM:0.10 ± 0.35	30 µM:1.02 ± 0.21	10.6 ± 0.41
Netupitant		10 µM: 0.27 ± 0.25	10 µM: 0.11 ± 0.30	0.40 ± 0.39
M1	bidirectional permeability of M1	3 µM: 0.00 ± 0.49	3 µM:0.01 ± 0.40	18.59 ± 0.63
		10 µM:0.11 ± 0.39	10 µM: 0.15 ± 0.39	1.32 ± 0.55
		30 µM: 4.18 ± 0.30	30 µM: 3.49 ± 0.34	0.83 ± 0.45
M1		10 µM: 0.57 ± 0.30	10 µM: 1.11 ± 0.30	1.94 ± 0.43
M2	bidirectional permeability of M2	3 µM: 0.13 ± 0.44	3 µM:0.02 ± 0.26	0.15 ± 0.51
		10 µM:0.31 ± 0.26	10 µM: 0.11 ± 0.18	0.36 ± 0.31
		30 µM: 0.34 ± 0.20	30 µM: 1.12 ± 0.04	3.31 ± 0.2
M2		10 µM: 5.95 ± 0.08	10 µM: 0.25 ± 0.21	0.04 ± 0.22
M3	bidirectional permeability of M3	3 µM:0.04 ± 0.38	3 µM: 0.05 ± 0.38	1.11 ± 0.53
		10 µM:0.09 ± 0.37	10 µM:0.22 ± 0.29	2.31 ± 0.47
		30 µM: 0.48 ± 0.17	30 µM: 0.33 ± 0.11	0.69 ± 0.2
M2		10 µM: 1.31 ± 0.10	10 µM: 0.81 ± 0.09	0.62 ± 0.13

Reviewer's Comments:

- *The study system was appropriately validated with positive controls. Based on the raw data, positive control digixon and prazosin as model substrate for MDR1 and BCRP had net flux ratio > 2 in all experiments, and net flux ratio of these model substrates were substantially reduced in the presence of model inhibitor for these transporter (PSC833 and Ko134 were used as model inhibitors for MDR1 and BCRP, respectively).*
- *The sponsor did not evaluate the potential of netupitant being a substrate of MDR1 (P-gp).*
- *As the net flux ratio for M1 and M3 were below 2 at all concentrations, M1 and M3 are not substrate of MDR1.*
- *The net flux ratio of M2 for MDR1 was > 2 at all tested concentration. The sponsor further evaluated the potential for M2 being a substrate for MDR1 in presence of MDR1 inhibitor. Efflux of M2 in was further reduced in presence of MDR inhibitor suggesting that M2 is a substrate for MDR.*
- *Netupitant, M1, M2 and M3, are not substrates of BCRP transporter*
    - *Although the net flux ratio when corrected for parental cell are > 2 for under certain conditions, it appears that it was due to very low flux ratio in parental cells. Based on efflux ratio in BCRP transfected cells alone, none of the tested compounds are substrates of BCRP transporter as efflux ratio for all of them were less than 2 in BCRP transfected cells.*
    - *Repeated experiments at 10 µM reconfirmed that Netupitant, M1, M2 and M3, are not substrates of BCRP transporter as both efflux ratio in transfected cells alone and net efflux ratio when corrected for parental cells are <2 for all tested compounds.*

Inhibition Assessment:

Calculated reaction parameters from MDCKII-MDR1 studies

Test article	Assay	ER		Net ER
		MDCKII	MDCKII-MDR1	MDCKII-MDR1 / MDCKII

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Digoxin + M1	bidirectional permeability of digoxin with M1	digoxin:1.54 ± 0.15	digoxin:24.13 ± 4.46	15.7 ± 4.46
		+ PSC833:0.97 ± 0.08	+ PSC833:1.43 ±0.20	1.47 ± 0.22
		+ M1 (30 µM): 1.01 ± 0.06	+ M1 (30 µM): 1.12 ± 0.11	1.11 ± 0.13
		digoxin: 1.86 ± 0.48	digoxin: 31.63 ± 2.66	17.04 ± 4.67
		+ PSC833: 0.98 ± 0.03	+ PSC833:1.28 ± 0.02	1.30 ± 0.05
		+ M1 (10 µM): 1.25 ± 0.06	+ M1 (10 µM): 9.21 ± 0.67	7.38 ± 0.66
Digoxin + M2	bidirectional permeability of digoxin with M2	digoxin: 1.47 ± 0.13	digoxin: 40.21 ± 5.46	27.45 ± 5.46
		+ PSC833: 0.98 ± 0.10	+ PSC833: 1.30 ± 0.15	1.32 ± 0.18
		+ M2 (30 µM): 1.2 ± 0.12	+ M2 (30 µM): 28.6 ± 2.53	23.88 ± 2.54
		digoxin: 1.86 ± 0.48	digoxin: 31.63 ± 2.66	17.04 ± 4.67
		+ PSC833: 0.98 ± 0.03	+ PSC833: 1.28 ± 0.02	1.30 ± 0.05
		+ M2 (10 µM): 1.45 ± 0.11	+ M2 (10 µM): 28.68 ± 2.81	19.8 ± 2.45
Digoxin + M3	bidirectional permeability of digoxin with M3	digoxin: 1.54 ± 0.15	digoxin:24.13 ± 4.46	15.7 ± 4.46
		+ PSC833: 0.97 ± 0.08	+ PSC833:1.43 ± 0.20	1.47 ± 0.22
		+ M3 (30 µM): 1.00 ± 0.06	+ M3 (30 µM): 2.13 ± 0.32	2.12 ± 0.32
		digoxin: 1.86 ± 0.48	digoxin:31.63 ± 2.66	17.04 ± 4.67
		+ PSC833: 0.98 ± 0.03	+ PSC833: 1.28 ± 0.02	1.30 ± 0.05
		+ M3 (10 µM): 1.16 ± 0.06	+ M3 (10 µM): 8.44 ± 1.82	7.28 ± 1.62

Calculated reaction parameters from MDCKII-BCRP studies

Test article	Assay	ER		Net ER
		MDCKII	MDCKII-BCRP	MDCKII-BCRP/ MDCKII
Prazosin + Netupitant	bidirectional permeability of prazosin with Netupitant	prazosin: 0.91 ± 0.09	prazosin:17.35 ± 0.83	18.97 ± 0.84
		+Ko134:0.87 ± 0.20	+Ko134: 0.91 ± 0.08	1.04 ± 0.22
		+ Netupitant (30 µM): 0.91 ± 0.01	+ Netupitant (30 µM): 6.25 ± 0.30	6.86 ± 0.30
		prazosin: 0.97 ± 0.08	prazosin: 14.28 ± 1.18	14.76 ± 1.68
		+Ko134: 0.90 ± 0.04	+Ko134:1.31 ± 0.13	1.46 ± 0.16
		+ Netupitant (10 µM): 0.86 ± 0.08	+ Netupitant (10 µM): 17.56 ± 1.40	20.35 ± 2.51
Prazos + M1	bidirectional permeability of	prazosin: 0.91 ± 0.09	prazosin:17.35 ± 0.83	18.97 ± 0.84
		+Ko134: 0.87 ± 0.20	+Ko134:0.91 ± 0.08	1.04 ± 0.22

	prazosin with M1	+ M1 (30 µM): 0.96 ± 0.03	+ M1 (30 µM): 1.72 ± 0.10	1.80 ± 0.10
		prazosin: 0.97 ± 0.08	prazosin: 14.28 ± 1.18	14.76 ± 1.68
		+Ko134: 0.90 ± 0.04	+Ko134: 1.31 ± 0.13	1.46 ± 0.16
		+ M1 (10 µM): 0.94 ± 0.09	+ M1 (10 µM): 12.54 ± 1.67	13.29 ± 2.19
Prazosin + M2	bidirectional permeability of prazosin with M2	prazosin: 0.91 ± 0.09	prazosin: 17.35 ± 0.83	18.97 ± 0.84
		+Ko134: 0.87 ± 0.20	+Ko134: 0.91 ± 0.08	1.04 ± 0.22
		+ M2 (30 µM): 0.86 ± 0.04	+ M2 (30 µM): 20.99 ± 4.40	24.34 ± 4.40
		prazosin: 0.97 ± 0.08	prazosin: 14.28 ± 1.18	14.76 ± 1.68
		+Ko134: 0.90 ± 0.04	+Ko134: 1.31 ± 0.13	1.46 ± 0.16
		+ M2 (10 µM): 0.88 ± 0.06	+ M2 (10 µM): 18.25 ± 1.5	20.63 ± 2.20
Prazosin + M3	bidirectional permeability of prazosin with M3	prazosin: 0.91 ± 0.09	prazosin: 17.35 ± 0.83	18.97 ± 0.84
		+Ko134: 0.87 ± 0.20	+Ko134: 0.91 ± 0.08	1.04 ± 0.22
		+ M3 (30 µM): 0.93 ± 0.04	+ M3 (30 µM): 3.77 ± 0.59	4.08 ± 0.59
		prazosin: 0.97 ± 0.08	prazosin: 14.28 ± 1.18	14.76 ± 1.68
		+Ko134: 0.90 ± 0.04	+Ko134: 1.31 ± 0.13	1.46 ± 0.16
		+ M3 (10 µM): 0.87 ± 0.14	+ M3 (10 µM): 15.15 ± 1.62	17.47 ± 3.35

Reviewer's Comments:

- *The study system was appropriately validated with positive controls with model inhibitor and substrates. Based on the raw data, model substrates digixon and prazosin for MDR1 and BCRP had net flux ratio > 2 in all experiments, and net flux ratio of these model substrates were substantially reduced in the presence of model inhibitor for these transporter (PSC833 and Ko134 were used as model inhibitors for MDR1 and BCRP, respectively).*
- *The sponsor did not evaluate the potential of netupitant being an inhibitor of MDR1 (P-gp) in this study.*
- *While M2 did not inhibit MDR1 and BCRP at both 10 µM and 30 µM, M1 and M3 inhibited MDR1 in concentration dependent manner. However, IC50 values were not determined in this monolayer cell system. Based on rough estimate of IC50 around 10 µM or based on the IC50 values from the vesicular system, an in-vivo study is not needed for M1 and dM3.*
- *Netupitant, M1 and M3 inhibited BCRP in concentration dependent manner where no inhibitions were observed at 10 µM and inhibition was observed at 30 µM. However, IC50 values were not determined in this monolayer cell system. Since no significant P-gp inhibitory effect of netupitant was observed with Digixin in in-vivo where 5 µM netupitant have inhibited P-gp transporter in vitro, we do not anticipate a significant BCRP inhibitory effect of netupitan in vivo since netupitnat at 10 µM did not inhibit BCRP transporter in vitro.*

Overall Reviewer's Comment:

1. *The tested concentration of Netupitant, M1, M2 and M3 up to 30 µM was acceptable as they approximately cover the C_{max} and 10 times C_{max} values to be expected in human subjects or patients taking this drug at the clinical dose of 300 mg.*
    - *Observed Cmax of Netupitant is 550-880 ng/mL (≈ 1-1.5 µM)*
    - *Observed Cmax for M1 is 40-50 ng/ml (0.07-0.09 µM)*
    - *Observed Cmax for M2 is 100-350 ng/mL (0.17-0.58 µM).*
    - *Observed Cmax for M3 is 50-90 ng/mL (0.08-0.0.144 µM).*

2. *Although the sponsor did not evaluate the potential of netupitant to inhibit MDR1 (P-gp) in both vesicular transport system and monolayer system in this study, the sponsor did conducted an in-vivo drug-drug interaction study with digoxin administered concomitantly with netupitant to evaluate the inhibition potential of P-gp by netupitant.*

3. *The sponsor did not evaluate the potential of netupitant being a substrate or inhibitor of P-gp transporter in this study.*

APPEARS THIS WAY ON ORIGINAL

Title: Determination Of The Permeability Of [^{14}C]-Netupitant Using the Parallel Artificial Membrane Permeability Assay (PAMPA)

Report No: 494368-NETU-10-26

Specific Aims: The aim of this study was to determine the permeability of [^{14}C]-Netupitant using the parallel artificial membrane permeability assay (PAMPA).

Study Date: 07/07/2010 – 07/15/2010

Test Site: (b) (4)

Sponsor: Helsinn Healthcare SA, Switzerland

Study Design:

Test Item: [^{14}C] Netupitant

Tested Concentrations: 0.1, 0.5, 2, 10 and 50 μM

Test System: BD GentestTM Pre-Coated PAMPA plate system: A 96-well microtiter plate assembled with a 96 well filter plate containing an artificial lipid membrane barrier mimicking the intestinal epithelium was used.

Controls: As controls, the reference compounds [^{3}H]-propranolol (high permeability) and sulfasalazine (low permeability) were included in the assay at one concentration in triplicate.

Study Method:
The permeability of [^{14}C]-netupitant was determined at five different concentrations (0.1, 0.5, 2, 10 and 50 μM) in triplicate in PAMPA. [^{14}C]-Netupitant, [^{3}H]-propranolol or sulfasalazine were applied at the donor site of the pre-coated filter plate wells. The filter plate was coupled with the receiver plate and the assembly was incubated at room temperature for 5 hours. At the end of the incubation, the plates were separated and 150 μL solution from each well of the filter plate and the receiver plate exposed to [^{14}C]-Netupitant or [^{3}H]-propranolol was transferred to scintillation vials and analyzed using liquid scintillation counting. Solutions containing sulfasalazine were transferred to a UV-transparent 96-well plate and analyzed using a spectrophotometer at 360 nm.

Data Analysis:
The permeability of a compound (in cm/s) and the mass retention (in %) were calculated using the following formula:

$$P_e = \frac{-\ln [1-C_A(t) / C_{equilibrium}]}{A\,(1/V_D + 1/V_A)\,t}$$

Mass retention:

$$R = 1 - [C_D(t)V_D + C_A(t)V_A] / (C_0V_D)$$

Where:
P_e = estimated permeability
C_0 = initial compound concentration in donor well (mM)
$C_D(t)$ = compound concentration in donor well at time t (mM)

$C_A(t)$ = compound concentration in acceptor well at time t (mM)
V_D = donor well volume (= 0.3 ml)
V_A = acceptor well volume (= 0.2 ml)
$C_{equilibrium} = [C_D(t)V_D + C_A(t)V_A] / (V_D + V_A)$
A = filter area = 0.3 cm^2
t = incubation time = 18000 s (= 5 hr)

Acceptance Criteria:
The PAMPA assay was considered acceptable if it meets the following criteria:

- The permeability of sulfasalazine should be below (b) (4)
- The permeability of propranolol should be above (b) (4)

Evaluation

- If P_e (b) (4) a compound is considered to have low permeability
- If P_e nm/s) a compound is considered to have medium permeability
- If P_e (b) (4) a compound is considered to have high permeability.

Results:
Sulfasalazine and Propranolol had acceptable level of low and high permeabilities validating the PAMPA system. Based on the mass retention, while sulfasalazine has low non-specific binding to the filter, propranolol has approximately 70% non-specific binding to the surface of the plate or is trapped inside the artificial membrane.

Table-1: Permeability of sulfasalazine (exposure concentration: 100 µM)

Sample	OD360 receptor	OD360 donor	$C_{equilibrium}$ uM	P_e cm/s	P_e nm/s	Mass Retention %
Sulfasalazine A11	0.099	0.935	56	3.46E-07	3.5	4.8
Sulfasalazine B11	0.101	0.892	54	4.58E-07	4.8	9.5
Sulfasalazine C11	0.099	0.928	56	3.49E-07	3.9	5.6
			Average	3.84E-07	3.8	6.6

Table-2: Permeability of propranolol (exposure concentration: 213 nM)

Based on the measured and theoretical concentration of netupitant on the donor side, it appears that netupitant did not dissolve well in the buffer used in the experiment. Therefore, the actual concentration of netupitant that is exposed at the donor side is much lower than what is stated theoretically.

Table-3: [^{14}C]-Netupitant concentrations in the initial spiked samples

Spike	Theoretical Concentration (Bq/mL)	Measured Concentration (Bq/mL)	Recovery (%)
0.1 µM Netupitant	46.4	25.3	54
0.5 µM Netupitant	232	30.6	13
2 µM Netupitant	928	85.4	9
10 µM Netupitant	4640	2104	45
50 µM Netupitant	23200	13134	57

Permeability at theoretical concentrations of 0.1, 0.5 and 2 µM were not determined since the concentrations of [^{14}C]- Netupitant in the receptor compartment were below the limit of detection (which is 25 dpm).

At theoretical concentrations of 10 and 50 µM, the permeability of [^{14}C]-Netupitant was determined to be $1.1x10^{-6}$ cm/s (= 10.6 nm/s) and $2.5x10^{-6}$ cm/s (24.6 nm/s), respectively. According to the sponsor's criteria, netupitnant's permeability would correspond to medium to high permeability. At both 10 and 50 uM concentration, netupitant had about 65-70% non-specific binding or is trapped inside the artificial membrane.

Table -4: Permeability of [14C]-Netupitant

Sample	DPM receptor	DPM donor	$C_{equilibrium}$ Bq/ml	P_e cm/s	P_e nm/s	Mass Retention %
Netupitant A1	< LOD	< LOD	n.d.	n.d.	n.d.	n.d.
Netupitant B1	< LOD	46.00	n.d.	n.d.	n.d.	n.d.
Netupitant C1	< LOD	< LOD	n.d.	n.d.	n.d.	n.d.
Concentration Netupitant: 0.1 µM			**Average**	n.d.	n.d.	n.d.
Netupitant A2	< LOD	180	n.d.	n.d.	n.d.	n.d.
Netupitant B2	< LOD	205	n.d.	n.d.	n.d.	n.d.
Netupitant C2	< LOD	57	n.d.	n.d.	n.d.	n.d.
Concentration Netupitant: 0.5 µM			**Average**	n.d.	n.d.	n.d.
Netupitant A3	< LOD	787	n.d.	n.d.	n.d.	n.d.
Netupitant B3	< LOD	1089	n.d.	n.d.	n.d.	n.d.
Netupitant C3	< LOD	534	n.d.	n.d.	n.d.	n.d.
Concentration Netupitant: 2 µM			**Average**	n.d.	n.d.	n.d.
Netupitant A4	222	6678	455	1.24E-06	12.4	64.0
Netupitant B4	157	5999	407	9.73E-07	9.7	67.8
Netupitant C4	143	5461	370	9.71E-07	9.7	70.7
Concentration Netupitant: 10 µM			**Average**	1.06E-06	10.6	67.5
Netupitant A5	2632	39208	2731	2.52E-06	25.2	65.3
Netupitant B5	2323	38881	2695	2.24E-06	22.4	65.8
Netupitant C5	2363	33694	2351	2.63E-06	26.3	70.2
Concentration Netupitant: 50 µM			**Average**	2.46E-06	24.6	67.1

< LOD: below limit of detection (25 dpm), n.d.: not determined, values below LOD.

Title: Determination Of Passive Diffusion Of [^{14}C]Netupitant Using Bi-Directional Assay In Caco-2 Cells

Report No: 494369-NETU-10-25

Specific Aims: The aim of this study was to determine the passive diffusion and the apparent permeability of [^{14}C]Netupitant using Caco-2 cells.

Study Date: 06/28/2010 – 08/16/2010

Test Site: (b) (4)

Sponsor: Helsinn Healthcare SA, Switzerland

Study Design:

Test Item: [^{14}C] Netupitant

Tested Concentrations: 1, 10 and 100 μM

Test System: Caco-2 cells plated in a 24-transwell plate

Controls: As controls of monolayer integrity, the reference compounds [^{3}H]-propranolol (high permeability) and [^{3}H]-mannitol (low permeability) were included in the assay. Additionally, the integrity of the Caco-2 cell monolayer was checked by measuring the transepithelial electrical resistance (TEER) (>1000 Ohm.cyou m^2).

Study Method: Permeability of [^{14}C]Netupitant at three concentrations (1, 10, and 100 μM) were evaluated from apical side to the basolateral side (A→B) and from the basolateral side to the apical side (B→A) on Caco-2 cells in triplicate wells and was repeated on two different days.
Before the start of the experiments, the medium on the apical and basolateral side was refreshed and cells were incubated for 30 minutes. The experiment was started by adding transport buffer containing a test substance or a vehicle to the apical and/or the basolateral compartment. The apical compartments were filled with 300 μL of transport buffer and the basolateral compartments were filled with 900 μL of transport buffer. After 30, 60, and 120 minutes of incubation (at 37.0 ± 1.0°C and 5.0 ± 0.5% CO2), a 50 μL sample was drawn from the receiver compartment, which was immediately replaced with transport buffer. The samples were analyzed using liquid scintillation counting (LSC). At the end of the experiment, samples from both the apical and basolateral compartments were analyzed to determine the recovery.

Data Analysis: The apparent permeability (Papp) of test items across the monolayer was calculated as follows:

$$Papp = (Vr/C_0)(1/S)(dC/dt)$$

Where
Papp is apparent permeability,
Vr is the volume of medium in the receiver chamber,
C_0 is the concentration of the test drug in the donor chamber,
S is the surface area of monolayer,

dC/dt is the linear slope of the drug concentration in the receptor chamber with time after correcting for dilution.

The sponsor states that as netupitant showed high non-specific binding (table-3), the apparent permeability for netupitant was calculated using the final donor concentration at the termination of the incubation instead of C0 to avoid an underestimation of the permeability.

Acceptance Criteria:

The bi-directional transport assay with Caco-2 cells was considered acceptable if it meets the following criteria:

- The TEER value should be (b) (4) Ωcm2 above background value (transwell without cells).
- Permeability values of mannitol were below (b) (4) cm/s (<50 nm/s).
- The propranolol:mannitol permeability ratio was (b) (4)

Evaluation

- If P (b) (4) compound is considered to have low permeability
- If P_e (b) (4) compound is considered to have medium permeability
- If P_e (b) (4) a compound is considered to have high permeability.

Results:

Mannitol and Propranolol had acceptable level of permeability validating the Caco-2 monolayer cell system. Mannitol permeability was below 50 nm/s in both directions and propranolol/mannitol permeability ratio was > 5 for all experiment.

Table-1: Permeability of Mannitol and Propranolol

	Mannitol		Propranolol		
Experiment	$P_{A/B}$ (nm/s)	$P_{B/A}$ (nm/s)	$P_{A/B}$ (nm/s)	$P_{B/A}$ (nm/s)	$(P_{B/A})prop/(P_{B/A})man$
1	0.7	3.0	263	388	130
2	7.3	10.7	262	471	44.0

Based on the measured and theoretical concentration of netupitant on the donor side, the actual concentration of netupitant that is exposed at the donor side is much lower than what is stated theoretically at 1 and 10 µM concentrations. The sponsor states that the lower concentration measured in buffer can be explained by the poor aqueous solubility of netupitant.

Table-2: [^{14}C]-Netupitant concentrations in the initial spiked buffer samples

Experiment	Contents	Theoretical [^{14}C]Netupitant concentration (DPM)	Measured concentration (DPM)	Recovery (%)
1	1 µM [^{14}C]Netupitant in buffer	640	337	52.5
	10 µM [^{14}C]Netupitant in buffer	6405	3507	54.8
	100 µM [^{14}C]Netupitant in buffer	64050	66371	104
2	1 µM [^{14}C]Netupitant in buffer	644	294	45.5
	10 µM [^{14}C]Netupitant in buffer	6675	3683	55.2
	100 µM [^{14}C]Netupitant in buffer	63600	71792	113

The sponsor stated that because netupitant showed high non-specific binding (with low recovery % in table 3), the apparent permeability was calculated using the final donor concentration at the end of the

incubation and the apparent permeability was determined with the actual measured concentrations for the calculations (table 4).
The permeability of [^{14}C]Netupitant from the A-B side was above 200 nm/s and the permeability from the B-A was above 20 nm/s. According to the sponsor's criteria, [^{14}C]Netupitant would be considered to have medium to high permeability under the conditions used in this study.

Table-3: Measured [^{14}C]Netupitant concentrations in the initial spiked buffer, donor and receptor samples

Experiment	Contents spike	Measured concentration in spike (DPM)	[^{14}C]Netupitant concentration in donor compartment (DPM)	[^{14}C]Netupitant concentration in receptor Compartment (DPM)	Recovery (%)
1 A → B [1)]	1 µM [^{14}C]Netupitant in buffer	337	47.4 31.9 32.7	0.0 5.2 1.4	14 14 11
	10 µM [^{14}C]Netupitant in buffer	3507	533 576 569	43.8 42.6 35.0	19 20 19
	100 µM [^{14}C]Netupitant in buffer	66371	6892 7016 7174	807[3)] 594 536	14 13 13
1 B → A [2)]	1 µM [^{14}C]Netupitant in buffer	337	134 141 131	8.3 15.3 11.3	41 43 40
	10 µM [^{14}C]Netupitant in buffer	3507	2310 2426 2393	179 160 188	68 71 70
	100 µM [^{14}C]Netupitant in buffer	66371	26010 26340 27570	704 843 1040	40 40 42
2 A → B [1)]	1 µM [^{14}C]Netupitant in buffer	294	75.3 67.5 77.4	16.3 16.6 16.3	42 40 43
	10 µM [^{14}C]Netupitant in buffer	3683	662 695 701	52.3 56.1 50.6	22 23 23
	100 µM [^{14}C]Netupitant in buffer	71792	9323 10380 9073	924 917 935	17 18 17
2 B → A [2)]	1 µM [^{14}C]Neutpitant in buffer	294	164 179 170	63.2 76.0 82.2	63 70 67
	10 µM [^{14}C]Netupitant in buffer	3683	2361 2399 2220	180 203 205	36 37 34
	100 µM [^{14}C]Netupitant in buffer	71792	24074 24385 22747	1779 2942 931[3)]	34 35 32

[1)] Apical to basolateral transport; donor compartment = apical side, receptor compartment = basolateral side
[2)] Basolateral to apical transport; donor compartment = basolateral side, receptor compartment = apical side
[3)] Outlier, due to an analytical error

Table-4: Permeability of [^{14}C]Netupitant at the initial concentrations C_o of 1, 10, and 100 µM

Experiment	C_0 (µM)	$P_{A/B}$ (nm/s)	$P_{B/A}$ (nm/s)
1	1	n.a.	126
	10	336	102
	100	342	50.9

2	1	853	666
	10	292	127
	100	439	165

n a : not applicable Could not be determined since no detectable [^{14}C]Netupitant was present in receiver compartment)

Reviewer's Comment:

- *The tested concentration for this permeability study was 1, 10 and 100 µM. The recommended concentration of drug for permeability studies are 0.01, 0.1, and 1 times the highest dose strength dissolved in 250 ml which would correspond to approximately 20, 200 and 200 µM (the proposed dose is (300 mg/250 mL) / (578.6g/mol)= 2074µM.*
- *This study is not adequate to categorize the drug for BCS classification as the suitability of this method was not evaluated with sufficient number of model drugs.*

  Generally, to demonstrate suitability of a permeability method intended for application of the BCS, a rank-order relationship between test permeability values and the extent of drug absorption data should be established using a sufficient number of model drugs (20 models drugs for in vitro cell culture methods) to allow precise differentiation between drug substances of low and high intestinal permeability attributes.
- *Expression of P-gp was not characterized in the Caco-2 cell monolayer system with a model substrate.*
- *Passive permeability cannot be assumed for netupitant for following reasons:*
  - *Netupitant does not have linear PK as Netupitant systemic exposure increased more than dose-proportional manner with dose increase from 100 mg to 300 mg.*
  - *in-vitro permeability of netupitant changes with initial concentration of drug*
  - *The rate of transport from apical-to-basolateral is different than the rate of transport from basolateral-to-apical direction for netupitant*
- *Due to solubility issue, the actual concentration in donor compartment is different that the theoretical concentration.*
- *Netupitant showed high non-specific binding (30-90%).*
- *The apparent permeability was calculated using the final donor concentration at the end of the incubation instead of initial donor concentration.*
- *Overall, the result of this study is difficult to interpret for above reasons.*

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07/07/2014

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07/07/2014

ADDENDUM to CLINICAL PHARMACOLOGY REVIEW

NDA	**205-718**	
Submission Date(s)	September 29, 2013	
Brand Name	**Akynzeo®**	
Generic Name	Netupitant and palonosetron	
Reviewer	Insook Kim, Ph.D., Dilara Jappar, Ph.D.	
Team Leader	Sue-Chih Lee, Ph.D.	
PM Reviewer	Jingyu "Jerry" Yu, Ph.D.	
PM Team Leader	Nitin Mehrotra, Ph.D.	
OCP Division	Division of Clinical Pharmacology 3 Division of Pharmacometrics	
OND Division	Division of Gastroenterology and Inborn Errors Products	
Sponsor	Helsinn	
Relevant IND(s)	73,493	
Submission Type	Original	NME
Formulation; Strengths;	Fixed dose combination of 300 mg netupitant and 0.5 mg palonosetron in oral capsule Each capsule contains three 100 mg tablets of netupitant and 0.5 mg soft-gel capsule of palonosetron	
Proposed indication	• Prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy[1] • Prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy[1]	
Dosing Regimen	One AKYNZEO capsule administered approximately one hour prior to the start of chemotherapy AKYNZEO can be taken with or without food	

1 Executive Summary

This is an addendum to the clinical pharmacology review of NDA 205-718 dated May 30, 2014 to discuss the Post-Marketing Study Recommendations. The application is submitted in support of an approval of AKYNZEO®, a fixed dose combination of 0.5 mg palonosetron, a 5-HT3 receptor antagonist and 300 mg netupitant, a NK1 receptor antagonist for prevention of chemotherapy induced nausea and vomiting (CINV). One AKYNZEO capsule contains one

capsule of 0.5 mg palonosetron and three tablets of 100 mg netupitant. Palonosetron, one of two active moieties of AKYNZEO®, has been approved for the prevention of CINV as an intravenous injection and oral capsule. On the other hand, netupitant a new molecular entity has not been approved for any indications. The sponsor intends to market netupitant only as a combination product but not as a single component product.

1.1 Post-Marketing Studies

We recommend following post-marketing studies to improve the labeling of AKYNZEO pending its approval.

- In vivo drug interaction study to evaluate the duration of inhibitory effects of AKYNZEO on CYP3A4 enzyme activity beyond 4 days after single dose administration of AKYNZEO.

  Rationale: Co-administration of a single dose of netupitant increased the exposure to dexamethasone, a substrate of CYP3A4 by 1.7-fold on Day 1 and up to 2.4-fold on Day 2 and Day 4. The potential inhibitory effect of netupitant on CYP3A4 was not studied beyond Day 4. Given AKYNZEO will be used in patients who require multiple medications for underlying disease treatment as well as supportive care, a study is necessary to provide adequate information for use of AKYNZEO with concomitant medications that are CYP3A4 substrates.

- In-vitro study to evaluate the potential of netupitant being a substrate for P-gp transporter in bi-directional transport assay system

  Rationale: The potential of netupitant being a substrate for P-gp in ATPase activation assay suggested that netupitant is likely a substrate for P-gp. However, information is lacking whether netupitant is a substrate for P-gp on bi-directional transport assay system, which is considered a confirmatory study.

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BIOPHARMACEUTICS REVIEW
Office of New Drug Quality Assessment

NDA	205-718
Submission Date	September 27, 2013
Brand Name	Akynzeo™
Generic Name	Netupitant / Palonosetron
Formulation; Strength(s)	300 mg/0.5 mg capsule
Indication	Treatment of nausea and vomiting associated with cancer chemotherapy
Applicant	Helsinn Healthcare S.A. Limited
Reviewer	Assadollah Noory, Ph.D.
Team Leader	Tapash Ghosh, Ph.D.
Acting Supervisor	Richard Lostritto, Ph.D.
OPQ Division	ONDQA-Biopharmaceutics
OND Division	DGIEP
Stamp Dates	September 27, 2013; January 17, 2014; March 27, 2014

EXECUTIVE SUMMARY

Under the provisions of 505(b)(1), Helsinn Healthcare S.A. submitted for approval of their Akynzeo™ netupitant/palonosetron (300 mg/0.5 mg) capsule, for the prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately and highly emetogenic cancer chemotherapy. This biopharmaceutics review involves review of two bioequivalence (BE) studies that provided a bridge between the late Phase 1 formulation, Phase 3 formulation, and the to-be-marketed formulation. In addition, dissolution method development and validation reports as well as proposed regulatory dissolution acceptance criteria were also reviewed.

BE Studies: The application for netupitant/palonosetron (300 mg /0.5 mg) capsule includes two bioequivalence study reports. Study NETU-09-07 provides a bridge between a late Phase 1 formulation with a Phase 3 formulation and Study NETU-11-02 provides a bridge between two Phase 3 formulations from two manufacturing facilities (to-be-marketed formulation). The studies were randomized, open-label, balanced, two-treatment, two-sequence, four-period, single-dose, replicate crossover under fasting conditions.

Study NETU-09-07

Forty-seven subjects completed this balanced, randomized, open-label, two-treatment, two-sequence, four-period, single-dose, replicate crossover bioequivalence study under fasting conditions study. Plasma PK parameter estimates, point estimates as ratio of test over reference expressed as percent, and the 90% confidence intervals are shown in the following table.

Table 1: Summary Statistics

PK-Parameter	Test	Reference	Point Estimate (%)	90% Confidence Interval
Netupitant				
C_{max} (ng/mL)	434.12 ± 242.09	431.76 ± 260.33	106.92	92.91 – 123.04
AUC_{0-t} (ng/mL×h)	12321.44 ± 5209.58	12058.30 ± 5609.79	105.92	96.24 – 116.58
$AUC_{0-\infty}$ (ng/mL×h)	14401.61 ± 7307.75	14375.41 ± 7335.11	101.57	91.17 – 113.16
Palonosetron				
C_{max} (ng/mL)	1.53 ± 0.39	1.53 ± 42	100.18	97.15 – 103.31
AUC_{0-t} (ng/mL×h)	52.19 ± 95	52.05 ± 17.50	100.19	97.10 – 103.38
$AUC_{0-\infty}$ (ng/mL×h)	56.71 ± 18.59	57.10 ± 34	99.37	96.54 – 102.28

The 90% confidence limits for netupitant and palonosetron are within 80% to 125% for AUC and C_{max} indicating that the late Phase 1 and Phase 3 formulations are bioequivalent under fasting conditions.

Study NETU-11-02

Eighty-two subjects completed this randomized, open-label, two-treatment, two-sequence, four-period, single-dose, replicate crossover bioequivalence study under fasting conditions. Plasma PK parameter estimates, point estimates as ratio of test over reference expressed as percent, and the 90% confidence intervals are presented in the following table.

Table 2: Summary Statistics

PK-Parameter	Test	Reference	Point Estimate (%)	90% Confidence Interval
Netupitany				
C_{max} (ng/mL)	453.96 ± 238.01	486.83 ± 268.02	92.72	86.41 – 99.50
AUC_{0-t} (ng/mL×h)	12736.28 ± 4892.81	13627.64 ± 5745.25	93.93	89.35- 98.74
$AUC_{0-\infty}$ (ng/mL×h)	13862.50 ± 5761.88	15031.75 ± 6858.15	92.62	87.34 – 98.22
Palonosetron				
C_{max} (ng/mL)	1.27 ± 0.33	1.24 ± 0.31	102.36	100.38 – 104.37
AUC_{0-t} (ng/mL×h)	44.68 ± 12.41	44.32 ± 13.07	101.11	99.32 – 102.94
$AUC_{0-\infty}$ (ng/mL×h)	48.17 ± 12.70	47.59 ± 13.40	101.08	99.23 – 102.96

The 90% confidence limits for netupitant and palonosetron are within 80% to 125% for AUC and C_{max} indicating that the formulation manufactured in Ireland is bioequivalent to formulation manufactured (b) (4) under fasting conditions.

In summary, Study NETU-09-07 demonstrated that late Phase 1 formulation manufactured in Catalent Philadelphia is bioequivalent to the Phase 3 formulation manufactured in Catalent Philadelphia, and study NETU-11-02 demonstrated that the Phase 3/to-be-marketed formulation manufactured in Ireland is bioequivalent to the Phase 3 formulation manufactured in (b) (4)

Dissolution:

The following table shows the dissolution methods and acceptance criteria proposed by the Sponsor for both the intermediate and the finished fixed dose combination products.

Drug Name	Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume	Acceptance Criteria
Palonosetron	Capsule/Combination Capsule	(b) (4)				
Netupitant	Tablet/Combination Capsule					
(b) (4)						

In the mid-cycle communication with the Sponsor, the Agency recommended the following dissolution acceptance criteria for both the intermediate and the finished products.

Active component	USP Apparatus	Speed (rpm)	Dissolution Medium	Medium Volume	Acceptance Criteria
Palonosetron	(b) (4)				
Netupitant					

In response to the mid-cycle communication the Sponsor proposed the following acceptance criteria for the intermediate products.

	NDA proposed specification for dissolution	FDA request	Helsinn Healthcare response
Intermediate Palonosetron Softgel	(b) (4)		
Intermediate Netupitant Tablet			

With respect to the finished product, the sponsor proposed the following acceptance criteria until the evaluation of additional dissolution data from five new batches (shown below); the Sponsor will then revisit the netupitant acceptance criteria for their FDC capsule.

	NDA proposed specification for Palonosetron dissolution	FDA request	Helsinn Healthcare response
Netupitant-Palonosetron combination capsule	[redacted] (b) (4)		
Netupitant-Palonosetron combination capsule	[redacted]		

The following table summarizes the dissolution acceptance criteria for palonosetron and netupitant intermediate products and the finished fixed-dose combination product.

Drug Name	Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume	Acceptance Criteria
Intermediate Products						
Palonosetron	Capsule/Combination Capsule	USP Paddle	75 rpm	0.01 N HCL	**500 mL**	(b) (4) **in 30 minutes**
Netupitant	Tablet/Combination Capsule	USP Paddle	100 rpm	0.07M Phosphate buffer pH 6.8 containing 1% sodium SDS	**900 mL**	(b) (4) **in 45 minutes**
Finished Product						
Palonosetron	Capsule/Combination Capsule	USP Paddle	75 rpm	0.01 N HCL	**500 mL**	(b) (4) **in 30 minutes**
Netupitant	Tablet/Combination Capsule	USP Paddle	100 rpm	0.07M Phosphate buffer pH 6.8 containing 1% sodium SDS	**900 mL**	(b) (4) **in 60 minutes**

In essence, the sponsor did not propose any change for palonosetron either for intermediate or for the FDC finished product. However, for netupitant, they accepted the Agency's proposal for the intermediate product but for the FDC finished product, they still wanted (b) (4) in 60 minutes until they gather more information. The proposal is acceptable by the Agency.

Recommendation:

Based on the ONDQA-Biopharmaceutics review, NDA 205-718 is recommended for approval. The sponsor agreed to submit dissolution data as a post approval supplement (PAS) from first five batches following approval of the product and will revisit the netupitant dissolution acceptance criteria in the final fixed dose combination product.

Signature 06/11/2014
Assadollah Noory, Ph.D.
Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

Signature 06/11/2014
Tapash Ghosh, Ph.D.
Team Leader
Office of New Drug Quality Assessment

BACKGROUND

Netupitant-Palonosetron combination fixed-dose combination capsules are composed of the following:

- Three (3) intermediate 100 mg netupitant tablets;
- One (1) intermediate palonosetron softgel capsule containing (b) (4) 0.50 mg of palonosetron (0.56 mg of palonosetron hydrochloride);
- One (1) size 0 hard gelatin capsule consisting of a white body with black imprint "HE1" and a caramel cap.

Thus the dosage delivered by one capsule of the drug product is 300 mg netupitant and 0.5 mg palonosetron. The design intent was to develop an oral fixed dose combination to allow administration of two drug substances in a single dosage form prior to each chemotherapy cycle.

The 100 mg netupitant tablet and the 0.50 mg palonosetron softgel are produced as intermediate drug products. They are referred to by the applicant as Intermediate Netupitant Tablet and Intermediate Palonosetron Softgel. It should be mentioned that 0.50 mg palonosetron softgel capsule was always used as the approved product (NDA 22233), manufactured jointly by both Catalent Pharma Solutions, USA and Helsinn Birex Pharmaceuticals, Ireland for Helsinn Healthcare SA, Switzerland.

Palonosetron hydrochloride injectable (Aloxi®) was approved on July 25, 2003 for the prevention of chemotherapy-induced nausea and vomiting, but netupitant is a new molecular entity (NME).

The empirical formula of netupitant is $C_{35}H_{32}F_6N_4O$ with a molecular weight of 578.6. Netupitant is a white to off-white powder; it is very slightly soluble in water; (b) (4); soluble in isopropanol, ethanol, and (b) (4) Netupitant is classified as a BCS Class 2, poorly soluble and highly permeable based on the Biopharmaceutics Classification System.

The empirical formula of palonosetron hydrochloride is $C_{19}H_{24}N_2O$•HCl with a molecular weight of 332.9. Palonosetron hydrochloride is a white to off-white crystalline powder; it is freely soluble in water; soluble in propylene glycol; (b) (4) slightly soluble in ethanol, and (b) (4) Palonosetron hydrochloride is classified as a BCS Class 1, highly soluble and highly permeable based on the Biopharmaceutics Classification System.

Composition of the Fixed-Dose Netupitant-Palonosetron Combination Capsule is given in the following Table:

Ingredient	Reference	Function	%w/w	Quantity (mg)
Intermediate Netupitant Tablet				
Netupitant	Internal	Active ingredient	(b) (4)	
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			
Purified water	USP/Ph. Eur			
Silicon dioxide/ (b) (4)	NF/Ph. Eur			
Sodium stearyl fumarate	NF/Ph. Eur			
Magnesium stearate (b) (4)	NF/Ph. Eur			
Total	---	---		
Intermediate Palonosetron Softgel				
Palonosetron HCl	Internal	Active ingredient	(b) (4)	
(b) (4)	Ph. Eur.	(b) (4)		
Glycerin (b) (4)	USP/Ph. Eur			
Polyglyceryl oleate (b) (4)	Internal			
Purified water	USP/Ph. Eur			
Butylated hydroxyanisole (BHA)	NF/Ph. Eur			
(b) (4)	NF/Ph. Eur.			
Theoretical Fill Weight	---	---		
Gelatin Capsule Shell. (b) (4)				
Gelatin (b) (4) NF/Ph. Eur	Internal	(b) (4)		---
(b) (4)	Internal			---
	USP/Ph. Eur			---
	USP/Ph. Eur			---
Ingredients Used Durin (b) (4)				
(b) (4)	NF/Ph. Eur	(b) (4)		---
	Internal			---
	Internal			---
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)				

BIOANALYTICAL: The concentrations of netupitant and palonosetron in human plasma were determined by using liquid chromatography mass spectrometry (HPLC/MS/MS) methods. Validation of the bioanalytical methods performance used for the determination of concentrations of netupitant and palonosetron in plasma are presented in the following table.

Analytical Parameters	Netupitant	Palonosetron
Analytical Range	2.00 to 500.00 ng/mL	45.00 to 1500.00 Pg/mL
Between-batch Precision (%)	2.92 to 3.62	2.2% to 5.6%
Between-batch Accuracy (%)	0.41 to 1.92	0.0% to 1.8%
Within-batch Precision (%)	1.45 to 4.88	1.1% to 7.2%
Within-batch Accuracy (%)	-1.58 to 3.56	0.1% to 3.3%
Recovery (%)	62.0	95.6
Freeze-thaw Stability LQC (three cycles) (%)	6.28	-1.7
Freeze-thaw Stability HQC (three cycles) (%)	0.74	2.1
Freezer Stability LQC (%)	5.50*	-11.48**
Freezer Stability HQC (%)	-0.43*	-14.90**
*: 34 Months at -70°C	** : 22 Months at -20°C	

The bioanalytical method is acceptable for the determination of concentrations of netupitant and palonosetron from the plasma samples.

Bioequivalence study NETU-09-07. This study was conducted to bridge Phase II and Phase III studies, using an extemporaneous combination of Netupitant 300 mg (two Netupitant Capsules, 150 mg) plus Palonosetron 0.50 mg softgel versus Netupitant- Palonosetron Combination Capsules (300 mg/0.50 mg). Dissolution data for both netupitant and palonosetron was provided. The data indicated that the formulations were bioequivalent and that the Phase II and Phase III formulations were bridged.

6.1. Study NETU-09-07

Title:

Bioequivalence study of a new netupitant/palonosetron fixed dose combination (300 mg/0.50 mg) versus extemporaneous combination of netupitant 300 mg and palonosetron 0.50 mg after single dose administration to healthy male and female volunteers

Principal Investigator:

Milko Radicioni, MD
Cross Research S.A., Phase I Unit - Via F.A. Giorgioli 14
CH-6864 Arzo, Switzerland

Study Start Date: July 22, 2009	Study End Date: January 27, 2010

Treatments:

Test:

Netupitant/palonosetron fixed dose combination, 300 mg/0.50 mg hard gel capsules, Catalent Philadelphia, USA. Batch release N. 29702-005 (bulk batch # A09148); Expiration January 2010.

References:

Netupitant 2 x 150 mg capsules, [redacted] (b) (4), batch release N. 29702-005 (bulk batch # N0700277); Expiration January 2010 plus Palonosetron 0.50 mg softgel capsules, Catalent Pharma Solutions, USA, batch release N. 29702-005 (bulk batch # 07-JM-309); Expiration January 2010.

Objective: To assess the bioequivalence of netupitant and palonosetron of a fixed dose combination (300 mg/0.50 mg hard gel capsules, Catalent Philadelphia, USA) versus netupitant 2 x 150 mg capsules [redacted] (b) (4) plus palonosetron 0.50 mg softgel capsules (Catalent Pharma Solutions, USA) in healthy male and female subjects under fasting conditions and to monitor the safety and tolerability of test and reference products following a single dose administration.

Study Design: The study was a balanced, randomized, open-label, two-treatment, three-sequence, four-period, single-dose, replicate crossover bioequivalence study under fasting conditions. The study medications were administered with 180 mL of mineral water. The wash-out period was 28 days.

Study Population: Forty-seven of fifty subjects enrolled completed the study. Three subjects withdrew their consent. The following table contains subjects' demographics.

Table 5: Study Subjects

Subjects Demographics	
Gender	24 Male; 26 Female
Age (yr)	32.3 ± 6.1 (19-45)
Weight (kg)	68.7 ± 12.6 (51.0-91.6)
Height (cm)	170.3 ± 9.7 (154–195)
BMI (kg/m^2)	23.5 ± 2.4 (19.2-28.8)
Race	41 White, 7 Hispanic, 2 Mestizo
Note: Data presented as mean ± SD (Range)	

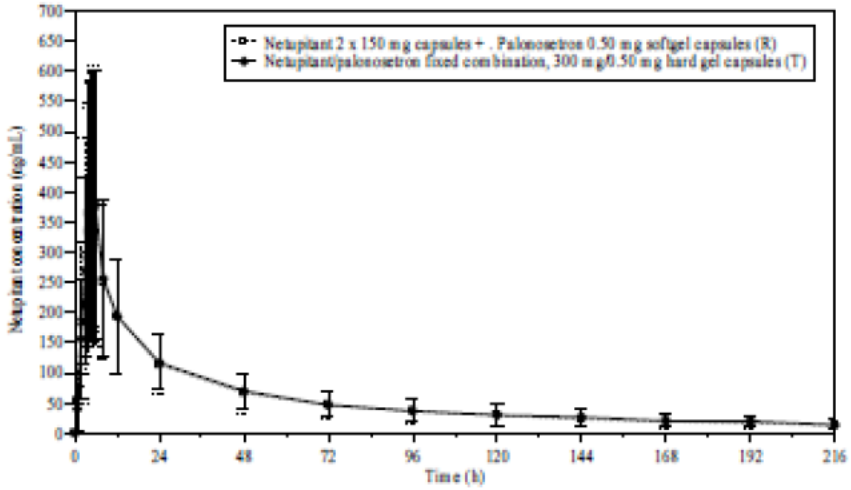
Sample Collection for Pharmacokinetic Measurements:
Blood samples were collected at the following specified times during each period for the determination of concentrations of netupitant and palonosetron in plasma: prior to dosing (zero hour) and at 1, 2, 3, 4, 4.5, 5, 5.5, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192 and 216 hours post dosing.

Pharmacokinetic and Statistical Analysis: The pharmacokinetic parameters were determined using WinNonLin® version 5.2 for netupitant and palonosetron, shown in the following table.

Table 6: Summary of Pharmacokinetic Parameters, Mean ± SD, (N=47)

PK-Parameter	Test	Reference
Netupitant		
C_{max} (ng/mL)	434.12±242.09	431.76±260.33
T_{max} (hr)*	5.00 (2.00-12.00)	5.00 (2.00-12.00)
AUC_{0-t} (ng•h/mL)	12321.44±5209.58	12058.30±5609.79
AUC_{inf} (ng•h/mL)	14401.61±7307.75	14375.41±7335.11
$T_{1/2}$ (hr)	95.62±58.84	98.69±51.53
Palonosetron		
C_{max} (ng/mL)	1.53±0.39	1.53±0.42
T_{max} (hr)*	5.00 (1.00-12.00)	4.50 (1.00-12.00)
AUC_{0-t} (ng•h/mL)	52.19±17.95	52.05±17.50
AUC_{inf} (ng•h/mL)	56.71±18.59	57.10±18.34
$T_{1/2}$ (hr)	44.15±15.16	43.28±14.31
* - Mean (Range)		

The plasma concentration time profiles for netupitant are shown in the following figure.



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The plasma concentration time profiles for palonosetron are shown in the following figure.

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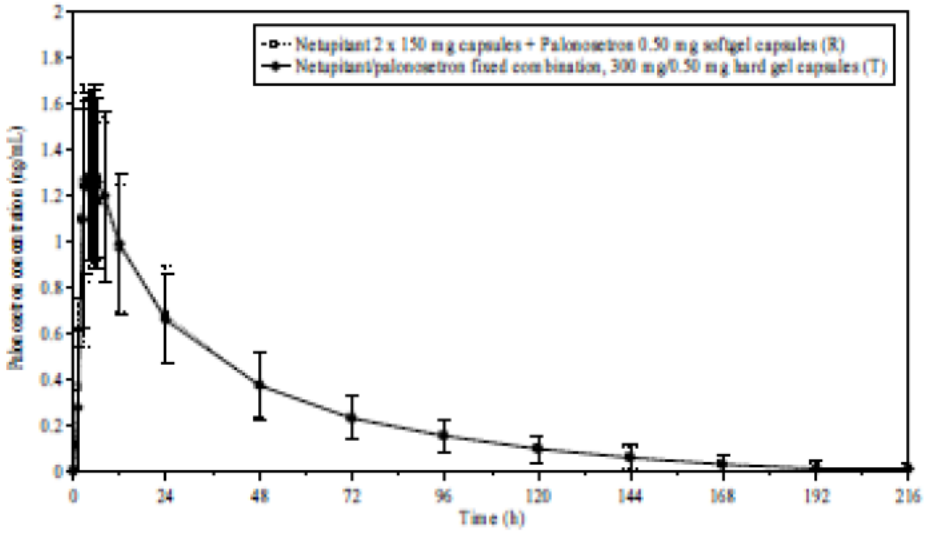

SAS® software for Windows release 9.2 was used for the statistical analysis of this bioequivalence study. The ProcMIXED procedure was used with treatment as the random effect. The results are shown in the following table.

Table7: Summary Statistics

Netupitant		**Geometric mean ratio**	
Treatment comparison	**Parameter**	**PE%**	**90% CI**
Test vs. Reference	C_{max}	106.92%	92.91 – 123.04%
	AUC_{0-t}	105.92%	96.24 – 116.58%
	$AUC_{0-\infty}$	101.57%	91.17 – 113.16%

Palonosetron		**Geometric mean ratio**	
Treatment comparison	**Parameter**	**PE%**	**90% CI**
Test vs. Reference	C_{max}	100.18%	97.15 - 103.31%
	AUC_{0-t}	100.19%	97.10 - 103.38%
	$AUC_{0-\infty}$	99.37%	96.54 - 102.28%

A statistical reanalysis performed by this reviewer using SAS version 9.3 confirmed that netupitant/palonosetron fixed combination, 300 mg/0.50 mg hard gel capsules, Catalent Philadelphia, USA has similar bioavailability to netupitant 2 x 150 mg capsules plus one 0.50 mg softgel palonosetron capsule, (b) (4) under fasting conditions.

Conclusion:
The 90% confidence limits for netupitant and palonosetron are within 80% - 125% for AUC and C_{max} indicating that netupitant/palonosetron fixed combination, 300 mg/0.50 mg hard gel capsules, Catalent Philadelphia, USA is bioequivalent to netupitant 2 x 150 mg capsules plus one 0.50 mg softgel palonosetron capsule, (b) (4) under fasting conditions.

6.2. Study NETU-11-02

Bioequivalence Study NETU-11-02. This study was conducted to bridge manufacturing sites of the Netupitant-Palonosetron Combination Capsule used during development [redacted] to the commercial site, HBP (Damastown, Mulhuddart-Dublin 15, Ireland).

Title: Bioequivalence study of the netupitant/palonosetron fixed dose combination product by Helsinn Birex Pharmaceuticals (Ireland) versus the netupitant/palonosetron fixed dose combination product by [redacted] after a single dose administration to healthy male and female volunteers.

Principal Investigator: Milko Radicioni, MD
Cross Research S.A., Phase I Unit - Via F.A. Giorgioli 14
CH-6864 Arzo, Switzerland

Study Start Date: May 5, 2011	Study End Date: October 30, 2011

Treatments:

Test:

Netupitant/palonosetron fixed dose combination, 300 mg/0.50 mg hard gel capsule, Batch N 31000861. Expiration November 2012, Helsinn Birex Pharmaceuticals Ltd., ***Ireland***.

References:

Netupitant/palonosetron fixed dose combination, 300 mg/0.50 mg hard gel capsule, Batch N 0901640. Expiration December 2011, [redacted]

Objective: To assess the bioequivalence between two different production batches at two different manufacturing sites of netupitant/palonosetron 300 mg/0.50 mg fixed dose combination capsules, [(test) manufactured by Helsinn Birex Pharmaceutical Ltd., Ireland, batch 31000861 and (reference) manufactured by [redacted] batch N0901640] and to assess the safety and tolerability of test and reference after single dose administration under fasting conditions.

Study Design: The study was a randomized, open-label, two-treatment, two-sequence, four-period, single-dose, replicate crossover bioequivalence study under fasting conditions. The study medications were administered with 180 mL of mineral water. The wash-out period was 28 days.

Study Population: Eighty-two (82) of eighty-eight (88) subjects enrolled completed the study. Four subjects withdrew their consent, one subject showed positive test for barbiturate, and one subject showed positive test for pregnancy. The following table contains subjects' demographics.

Subjects Demographics	
Gender	69 Male; 19 Female
Age (yr)	33.6 ± 8.0 (19 – 50)
Weight (kg)	73.6 ± 11.8 (51 – 103)
Height (cm)	173.6 ± 8.9 (150 – 201)
BMI (kg/m^2)	24.3 ± 2.4 (19.3 – 29.0)
Race	80 white; 3 black, 2 hispanic; 3 others
Note: Data presented as mean ± SD (Range)	

Sample Collection for Pharmacokinetic Measurements: Blood samples were collected at the following specified times during each period for the determination of concentrations of netupitant and palonosetron in plasma: prior to dosing (zero hour) and at 1, 2, 3, 4, 4.5, 5, 5.5, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192 and 216 hours post dosing.

Pharmacokinetic and Statistical Analysis: The pharmacokinetic parameter determined using WinNonLin® version 5.2 for netupitant and palonosetron are shown in the following table.

Table 9: Summary of Plasma Pharmacokinetic Parameters, Mean ± SD

PK-Parameter	Test	Reference
Netupitant		
C_{max} (ng/mL)	453.96±238.01	486.83±268.02
T_{max} (hr)	5.00 (2.00-24.00)	5.25 (2.00-8.00)
AUC_{0-t} (ng•h/mL)	12736.28±4892.81	13627.64±5745.2
AUC_{inf} (ng•h/mL)	13862.50±5761.88 *	15031.75±6858.15 *
$T_{1/2}$ (hr)	76.62±28.83 *	77.78±29.99 **
Palonosetron		
C_{max} (pg/mL)	1270.50±327.47	1240.62±309.75
T_{max} (hr)	3.00 (2.00-12.00)	3.00 (2.00-8.00)
AUC_{0-t} (pg•h/mL)	44679.60±12408.91	44323.39±13067.24
AUC_{inf} (pg•h/mL)	48165.20±12696.09 *	47592.96±13403.20 **
$T_{1/2}$ (hr)	37.22±10.82 *	38.16±11.76 **
T_{max}: median (range) ; * N=147; ** N=146		

The plasma concentration time profiles for netupitant are shown in the following figure.

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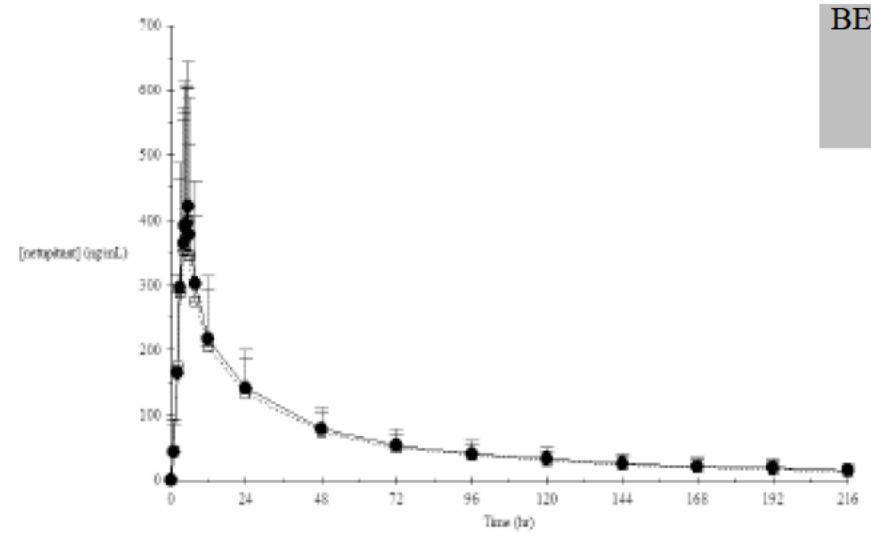
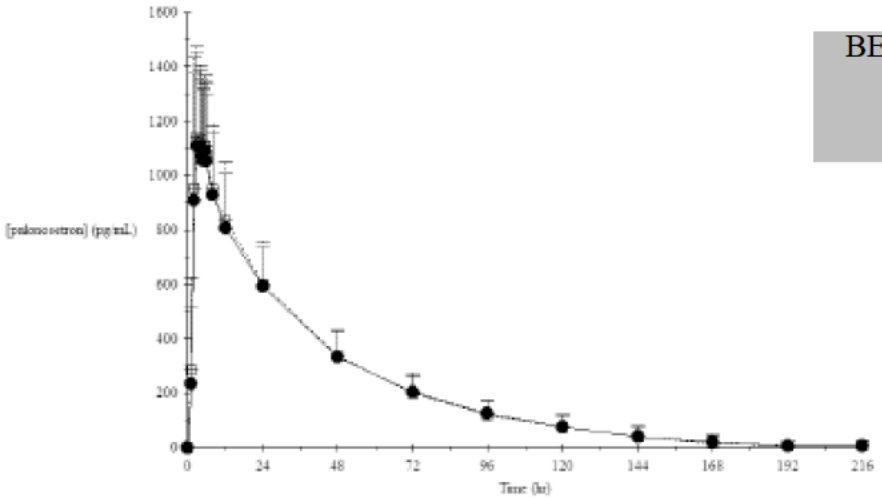


The plasma concentration time profiles for palonosetron are shown in the following figure.



SAS® software for Windows release 9.2 was used for the statistical analysis of this bioequivalence study. The ProcMIXED procedure was used with treatment as the random effect. The results are shown in the following table.

Table 10: Summary Statistics

Netupitant		**Geometric mean ratio**	
Treatment comparison	**Parameter**	**PE%**	**90%CI**
Test vs. Reference	**C_{max}**	**92.72**	**86.41 – 99.50**
	AUC_{0-t}	**93.93**	**89.35 – 98.74**
	$AUC_{0-\infty}$	**92.62**	**87.34 – 98.22**
Palonosetron		**Geometric mean ratio**	
Treatment comparison	**Parameter**	**PE%**	**90%CI**
Test vs. Reference	**C_{max}**	**102.36**	**100.38 – 104.37%**
	AUC_{0-t}	**101.11**	**99.32 – 102.94%**
	$AUC_{0-\infty}$	**101.08**	**99.23 – 102.96%**

A confirmatory statistical reanalysis was performed by this reviewer using SAS version 9.3. The ProcMIXED procedure was used with subject within the sequence as the random effect. A comparative result of 90% confidence limits reported by the sponsor and analyzed by this reviewer is shown in the following table.

		90 % confidence Limits	
Netupitant			
	Parameter	**Sponsor Reported**	**Reviewer Analysis**
	C_{max}	**86.41 – 99.50**	**85.21 – 100.90**
	AUC_{0-t}	**89.35 – 98.74**	**88.51 – 99.60**
	$AUC_{0-\infty}$	**87.34 – 98.22**	**87.11 – 100.00**
Palonosetron			
	Parameter	**Sponsor Reported**	**Reviewer Analysis**
	C_{max}	**100.38 – 104.37%**	**100.00 – 104.76**
	AUC_{0-t}	**99.32 – 102.94%**	**99.01 – 103.25**
	$AUC_{0-\infty}$	**99.23 – 102.96%**	**98.81 – 103.25**

The 90% confidence limits for netupitant and palonosetron are within 80% - 125% for AUC and C_{max} indicating that the formulation manufactured at Helsinn Birex Pharmaceuticals Ltd., Ireland is bioequivalent to the formulation manufactured at [redacted] (b) (4) under fasting conditions.

Conclusion: The 90% confidence limits for netupitant and palonosetron are within 80% - 125% for AUC and C_{max} indicating that the formulation manufactured at Helsinn Birex Pharmaceuticals Ltd., Ireland is bioequivalent to the formulation manufactured at [redacted] (b) (4) under fasting conditions.

Bridging Support with Dissolution data: Dissolution data were provided for:

Combination Capsule batch 30005284 (encapsulated by HBP using Intermediate Netupitant Tablet batch 30004380 (manufactured by HBP, Ireland) and Intermediate Palonosetron Softgel batch 10JM-164 (manufactured by Catalent)).

Netupitant-Palonosetron Combination Capsule batch N0901409 (encapsulated by [redacted] (b) (4) using Intermediate Netupitant Tablet batch N0901098 (manufactured by [redacted] (b) (4) and Intermediate Palonosetron Softgel batch 09JM-270 (manufactured by Catalent).

Calculations of f_2 were performed for these dissolution profiles. The f_2 value for the netupitant dissolution profiles was 52.4, indicating that they are similar. The f_2 value for the palonosetron dissolution profiles was 71.3, indicating that they are similar. The same variability for the combination capsule (final drug product) was observed. The data for netupitant is reproduced below. Data for the Mean of 12 tablets was used to calculate f_2.

The data indicated that the formulations were bioequivalent and that the formulations manufactured at the different facilities were bridged.

OSI inspection

The memorandum from OSI dated May 29, 2014 indicates that the inspection reports of clinical and analytical sites of study NETU-11-02 is satisfactory by the Agency.

DISSOLUTION

Sponsor developed an *in vitro* dissolution method for the netupitant in the netupitant-palonosetron combination capsules [redacted] (b) (4) (b) (4) (b) (4) The Sponsor adapted the dissolution method for palonosetron from the approved method for palonosetron capsule (NDA 22-233). The approved dissolution methodology for palonosetron is USP Apparatus 2 (paddle) at 75 rpm in 500 mL of 0.01N HCl dissolution medium at 37.0±0.5° C.

Netupitant Analytical Method

A UV spectrophotometric assay at (b) (4) was used to detect netupitant in dissolution medium. The analytical parameters are shown in the following table.

Analytical Parameters	Netupitant
Analytical Range Evaluated	0.0827831 -0.40432 mg/mL
Linearity (10, 25, 50, 75, 150 and 200%)	R^2=0.9996
Between Analyst Precision (%) Analyst 1-2	4.3%
Within Run Precision (%)	0.5%
Accuracy (%)	99.0 to 100.4%
Recovery (%)	99.4 to 102%
Solution Stability in medium at RT for 9 days	100.52%

Method Specificity: A placebo hard gelatin capsule containing one palonosetron HCI 0.5 mg softgel was analyzed to evaluate and confirm method specificity and possible interference. The placebo interference was (b) (4) An overlay scan of netupitant in dissolution medium, and the netupitant working reference standard are shown in the following figure.

Figure 1. Overlaid UV/Vis Spectra of Dissolution Medium (Blank), Netupitant Working Standard Solution, Placebo Sample Solution and Dissolution Accuracy Solutions for Over Encapsulated Palonosetron HCl Softgel, 0.5 mg/Netupitant Tablets, 300 mg Combination Product

The figure confirms that at (b) (4) there was no significant interference from palonosetron and/or any other material (s) on netupitant absorbance.

Dissolution Method Robustness for Intermediate Netupitant Tablet:

(b) (4)

The Sponsor demonstrated that their dissolution method is robust and it can be used as product release methodology. The selection of 0.07M Phosphate Buffer pH 6.8 + 1% SDS as the dissolution medium for netupitant is acceptable.

Palonosetron Analytical Method

A validated reverse phase HPLC procedure with a gradient mobile phase and UV detection at (b) (4) nm was used for determination of palonosetron concentrations. (b) (4)

The analytical parameters are shown in the following table.

Analytical Parameters	Palonosetron
Analytical Range Evaluated	0.25245 to 1.5147 mcg/mL
Linearity (10, 25, 50, 75, 150 and 200%	R=1.000
Between Analyst and HPLC Systems Precision (%)	0.2 to 1.4%
Within Run Precision (%)	0.2 to 0.4%
Accuracy (%)	99.5 to 100.6%
Recovery (%)	99.5 to 100.1%
Solution Stability in dissolution medium at 5° C for 5 days	98.9 to 99.9%

The analytical methods for determintion of concentration of netupitant and palonosetron are acceptable.

DISSOLUTION METHOD DEVELOPMENT

(b) (4)

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In response to the mid-cycle communication the Sponsor proposed the following acceptance criteria for the intermediate products.

	NDA proposed specification for dissolution	FDA request	Helsinn Healthcare response
Intermediate Palonosetron Softgel	(b) (4)		
Intermediate Netupitant Tablet			

With respect to the finished product, the sponsor proposed the following acceptance criteria for the finished product until the evaluation of additional dissolution data from five new batches and then they will revisit the netupitant acceptance criteria.

	NDA proposed specification for Palonosetron dissolution	FDA request	Helsinn Healthcare response
Netupitant-Palonosetron combination capsule	(b) (4)		

	NDA proposed specification for Netupitant dissolution	FDA request	Helsinn Healthcare response
Netupitant-Palonosetron combination capsule	(b) (4)		

In essence, the sponsor did not propose any change for palonosetron either for intermediate or for the FDC finished product. However, for netupitant, they accepted the Agency's proposal for the intermediate product but for the FDC finished product, they still wanted (b) (4) in 60 minutes until they gather more information. The proposal is acceptable by the Agency.

The following Table summarizes the dissolution acceptance criteria for palonosetron and netupitant intermediate products and the finished fixed-dose combination product.

Drug Name	Dosage Form	USP Apparatus	Speed (rpm)	Medium	Volume	Acceptance Criteria
Intermediate Products						
Palonosetron	Capsule/Combination Capsule	USP Paddle	75 rpm	0.01 N HCL	**500 mL**	(b) (4) **in 30 minutes**
Netupitant	Tablet/Combination Capsule	USP Paddle	100 rpm	0.07M Phosphate buffer pH 6.8 containing 1% sodium SDS	**900 mL**	(b) (4) **in 45 minutes**
Finished Product						
Palonosetron	Capsule/Combination Capsule	USP Paddle	75 rpm	0.01 N HCL	**500 mL**	(b) (4) **in 30 minutes**
Netupitant	Tablet/Combination Capsule	USP Paddle	100 rpm	0.07M Phosphate buffer pH 6.8 containing 1% sodium SDS	**900 mL**	(b) (4) **in 60 minutes**

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/s/

ASSADOLLAH NOORY
06/11/2014

TAPASH K GHOSH
06/11/2014

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA	**205-718**	
Submission Date(s)	9/27/13; 11/8/13; 12/16/14; 12/20/14; 1/9/14; 4/4/14; 4/30/14; 5/12/14; 5/14/14; 5/21/14; 5/22/14	
Brand Name	**Akynzeo®**	
Generic Name	Netupitant and palonosetron	
Reviewer	Insook Kim, Ph.D., Dilara Jappar, Ph.D.	
Team Leader	Sue-Chih Lee, Ph.D.	
PM Reviewer	Jingyu "Jerry" Yu, Ph.D.	
PM Team Leader	Nitin Mehrotra, Ph.D.	
OCP Division	Division of Clinical Pharmacology 3 Division of Pharmacometrics	
OND Division	Division of Gastroenterology and Inborn Errors Products	
Sponsor	Helsinn	
Relevant IND(s)	73,493	
Submission Type	Original	NME
Formulation; Strengths;	Fixed dose combination of 300 mg netupitant and 0.5 mg palonosetron in oral capsule Each capsule contains three 100 mg tablets of netupitant and 0.5 mg soft-gel capsule of palonosetron	
Proposed indication	• Prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy[1] • Prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy[1]	
Dosing Regimen	One AKYNZEO capsule administered approximately one hour prior to the start of chemotherapy AKYNZEO can be taken with or without food	

Table of Contents

1 Executive Summary

The application is submitted in support of an approval of AKYNZEO®, a fixed dose combination of 0.5 mg palonosetron, a 5-HT3 receptor antagonist and 300 mg netupitant, a NK1 receptor antagonist for prevention of chemotherapy induced nausea and vomiting (CINV). One AKYNZEO capsule contains one capsule of 0.5 mg palonosetron and three tablets of 100 mg netupitant. Palonosetron, one of two active moieties of AKYNZEO®, has been approved for the prevention of CINV as an intravenous injection and oral capsule. On the other hand, netupitant a new molecular entity has not been approved for any indications. The sponsor intends to market netupitant only as a combination product but not as a single component product.

1.1 Recommendations

The Office of Clinical Pharmacology found the submission acceptable from a clinical pharmacology standpoint provided a mutual agreement on labeling languages is reached.

1.2 Post-Marketing Studies

Post-marketing are currently under discussion. .

1.3 Summary of Clinical Pharmacology and Biopharmaceutics Findings

In support of AKYNZEO, 23 studies were conducted for PK or PD. In addition 15 in vitro studies were conducted. Pharmacokinetics of palonosetron and netupitant was studied in healthy subjects, cancer patients and patients with hepatic impairment after administration of AKZYNZEO. PK and PD of netupitant alone were also studied during the early development phase prior to combination with palonosetron. General clinical pharmacology of palonosetron mainly relies on the previous findings from the approval of Aloxi products. AKYNZEO is proposed to be available only as one strength i.e. 300 mg netupitant and 0.5 mg palonosetron and to be administered as a single dose at one hour prior to the initiation of chemotherapy. As such

except on study for multiple dose PK of netupitant, PK of netupitant and palonosetron was characterized after single dose administration.

Exposure (Dose)-Response Relationship

Efficacy

In a dose-finding study (NETU-07-07), the proportion of patients with complete response (CR)[2] was compared between palonosetron monotherapy at 0.5 mg and the combinations of 0.5 mg palonosetron with netupitant at three different doses i.e.100 mg, 200 mg, and 300 mg. The CR rate was evaluated during the 0-24 h (acute phase), 24-120 h (delayed phase) and 0-120 h (overall phase) after the administration of chemotherapeutics. The study was designed to show the difference between the combination therapy and palonosetron alone but not between doses. The concentration-response relationship was not studied because PK samples were not collected.

No evident dose-response relationship was observed among doses for the CR rate in the delayed and overall phases. Compared to palonosetron monotherapy, all three combinations of palonosetron and netupitant showed statistically significant difference in the proportion of patients with CR during the delayed and overall phases. On the other hand, only the combination with 300 mg netupitant showed statistically significant difference for the CR rate in the acute phase in comparison to palonosetron monotherapy. The combination with netupitant 300 mg showed a numerically higher CR rate for the acute phase CINV than lower doses.

Study NETU-07-07 is proposed to establish the contribution of netupitant component to the prevention of CINV in addition to palonosetron component and the clinical efficacy of the combination product for the prevention of CINV associated with HEC, cisplatin-based chemotherapy[3].

Based on the statistically significant difference in the prevention of CINV in the acute, delayed and overall phases compared to palonosetron monotherapy, the combination of 0.5 mg palonosetron and 300 mg netupitant was selected for phase 3 clinical trials. The combination of 0.5 mg palonosetron and 300 mg netupitant resulted in the significantly higher proportion of patients with CR over palonosetron alone with respect to the CR in the delayed phase, the acute and overall phases after the administration of an anthracycline and cyclophosphamide regimen for the treatment of a solid malignant tumor (NETU-08-18). Please see the statistics review for more details.

Safety:

Overall the most common adverse reactions (an incidence ≥2 %), assessed by investigators as treatment related, were headache and constipation. In the dose-finding study, the incidence of TEAEs was higher with combinations of 200 mg or 300 mg netupitant (NETU) (50% and 54%, respectively) than with 100 mg NETU (40%). The overall rate of TEAE was higher after the combination treatment with NETU 300 mg/PALO 0.5 mg compared to oral PALO 0.5 mg alone

[2] Defined as no emesis and no rescue medication

[3] http://www.cancer.gov/cancertopics/pdq/supportivecare/nausea/HealthProfessional/page5#Reference5.2

(70% vs. 61%, respectively) in the integrated safety analysis. For the detailed review of safety profile, please see the clinical review by Dr. Nancy Snow, Medical Officer of DGIEP.

Effects on QTc interval

To assess the potential effect of the combination therapy, a thorough QT study was conducted at doses up to 600 mg NETU in combination with 1.5 mg PALO in healthy subjects. No significant QTc interval prolongation was observed when single dose 600 mg NETU and 1.5 mg PALO was co-administered[4]. Consistently, the exposure-response relationship was not evident between ddQTcF and concentrations of NETU and its metabolites as well as concentrations of PALO and its metabolites. No significant effect of PALO on the QTc interval was consistent with the previous report of no effect of PALO on the QTc doses up to 2.25 mg after intravenous administration[5].

The supratherapeutic dose in this study provides the safety margin of 2 fold for netupitant and 3 fold for palonosetron. The supratherapeutic dose provided higher Cmax and similar AUC for NETU in patients with moderate hepatic impairment.

Pharmacokinetic/ Biopharmaceutics Properties

This review is mainly focused on netupitant while general PK characteristics of palonosetron were previously reviewed during the approval of single ingredient products[6].

AKYNZEO

After single dose administration of AKYNZEO in healthy subjects, the peak plasma concentrations for netupitant and palonosetron were reached in about 5 hours. Concomitant food did not significantly affect the systemic exposure to netupitant and palonosetron. In cancer patients, the rate and extent of absorption of netupitant and palonosetron were similar to those in healthy subjects.

No significant PK interactions between netupitant and palonosetron were observed.

Netupitant

Distribution

Population PK analysis indicates that the apparent central and peripheral volume of distribution (Vz/F) was estimated to be 486 L and 1170 L, respectively. Human plasma protein binding of netupitant is greater than 99.5% at drug concentration ranging from 10-1300 ng/ml and protein binding of its major metabolites (M1, M2 and M3) are greater than 97% at drug concentrations ranging from 100 to 2000 ng/mL.

Metabolism

In in vitro studies netupitant is metabolized mainly by CYP3A4 and by CYP2C9 and CYP2D6 to a lesser degree. Three major metabolites were identified desmethyl derivative, M1; N-oxide

[4] Study NETU-07-20. For more details, see the IRT-QT team reviews of the thorough QT study dated 1/19/2010 (IND 73,493 SDN 024) and 3/3/14 (NDA 205-718)
[5] Aloxi Package Insert
[6] Clinical pharmacology review of original NDA 21-371

derivative, M2; OH-methyl derivative, M3 in vivo and were all shown to bind to human substance P/neurokinin 1 (NK_1) receptor in vitro. Mean AUC for metabolites M1, M2, and M3 was 29%, 14% and 33% of netupitant, respectively.

Elimination

In cancer patients, the apparent median elimination half-life of netupitant was 88 hours and the estimated median systemic clearance was 20.5 L/h based on population PK analysis.

Upon oral administration of labeled netupitant, about 50% and 75% of the administered radioactive dose was recovered from the excreta (urine and feces) collected over 120 h and 336 h (2 weeks), respectively. Over 2 weeks the total of 3.95 % and 70.7 % of the radioactive dose was recovered in urine and feces, respectively. The mean fraction of oral dose of netupitant excreted unchanged in urine was less than 1 % suggesting renal clearance is not a significant elimination route for netupitant.

Specific populations

Currently no dosage adjustment for palonosetron is recommended by renal or hepatic impairment.

Age

In cancer patients population PK analysis indicated that age (within the range of 29 to 75 years old) did not influence the pharmacokinetics of netupitant or palonosetron.

Gender

The Cmax for netupitant was 35 % higher in females than in males but the AUC was similar between males and females. For palonosetron 25-35% higher AUC and Cmax were observed in female subjects than in male subjects consistently with the previous observation.

Hepatic Impairment

In patients with mild or moderate hepatic impairment, the mean Cmax for netupitant was about 30% higher and the mean AUC0-∞ was 56% and 107% higher, respectively than in healthy subjects. The Cmax for netupitant in two patients with severe hepatic impairment was 63% and 463% higher compared to the mean Cmax in healthy subjects. .

In patients with mild or moderate hepatic impairment, the mean Cmax for palonosetron was about 35-40% higher and the mean AUC0-∞ was 35% and 55% higher, respectively than in healthy subjects.

Renal Impairment

There was no dedicated PK study to evaluate the effect of renal impairment on PK of netupitant. On the other hand, no significant effect of CL_{CR} on PK of netupitant was noted in the population PK analysis while the effect of CL_{CR} was noted for palonosetron consistently with the current labeling for palonosetron. The pharmacokinetics has not been studied in subjects with end-stage renal disease for either palonosetron or netupitant.

In vitro studies for evaluation of drug interaction potential assessment

Based on the in-vitro studies, netupitant and M1 are inhibitors of CYP3A4. In addition, netupitant is an inhibitor of P-gp and BCRP transporters.

CYP inhibition:

In *in vitro* studies, netupitant and its metabolite M1 are inhibitors of CYP3A4. A follow-up *in vivo* study with CYP3A4 substrate midazolam was conducted.

Netupitant did not inhibit CYP1A2, CYP2C19, and CYP2D6 *in vitro*. *In vivo* drug interactions via inhibition of CYP2B6, 2C8 and 2C9 at the clinical dose of 300 mg are unlikely based on weak inhibition of toward these enzymes in *in vitro* studies.

M1 showed inhibition toward CYP2B6, 2C8, 2D6, and 3A4, and weak inhibition toward CYP 1A2, 2C9, 2C19. However, since Cmax/Ki >0.1 for only CYP3A4, *in vivo* drug interaction via M1 inhibition toward CYP enzyme is unlikely except for CYP3A4.

M2 and M3 showed weak inhibition toward all major CYP enzymes. Since Cmax/Ki<0.1 for all enzymes, *in vivo* drug interaction via M2 or M3 inhibition individually toward CYP enzyme is unlikely.

CYP induction:

Netupitant up to 20 μM and its metabolites (M1, M2 and M3) up to 2 μM are not inducers of CYP1A2, CYP2C9, CYP2C19 and CYP3A4. The sponsor did not evaluate the potential of netupitant and its metabolites to induce CYP2B6.

Transporters:
Netupitant is an inhibitor of P-gp and BCRP transporters based on *in vitro* studies. Potential of netupitant being a substrate for P-gp was not evaluated adequately. In addition, M2 is shown to be a substrate for P-gp.
Based on *in vitro* data, *in vivo* interaction of netupitant as a substrate for BCRP, OATP1B1, OATP1B3, and OCT1, or as an inhibitor of BSEP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2 is unlikely.
In addition, based on the *in vitro* data, *in vivo* interaction of three major metabolites, M1, M2 and M3 as substrates of BCRP, OATP1B1, OATP1B3, and OCT1, or as inhibitors of MDR1, BCRP, BSEP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2 are unlikely.

In vivo drug interactions
(A) Effect of other drugs on the PK of netupitant and palonosetron
CYP3A4 inhibitor: Co-administration of AKYNZEO with ketoconazole increased the mean Cmax and AUC of netupitant by 25% and 140%, respectively compared to those after administration of AKYNZEO without ketoconazole. Co-administration of ketoconazole increased the mean AUC and Cmax for palonosetron was about 10-15%. The labeling should include a cautionary statement about co-administration of AKYNZEO with strong CYP3A4 inhibitors when necessary.

CYP3A4 inducer: Co-administration of AKYNZEO with rifampicin decreased the mean Cmax and AUC of netupitant by 62%, and 82%, respectively compared to those after AKYNZEO alone. Co-administration of rifampicin decreased the mean Cmax and AUC of palonosetron by 15% and 20%, respectively. Use of AKYNZEO in patients who have been on CYP3A4 inducers at the time of AKYNZEO administration is not recommended to ensure the efficacy of combination therapy.

(B) Effect of netupitant or Akynzeo on PK of other drugs

Drugs that are CYP3A4 substrates

Netupitant component of AKYNZEO is a moderate CYP3A4 inhibitor and the increase in the systemic exposure to concomitant drugs that are CYP3A4 substrates was observed to a various degree when AKYNZEO or netupitant alone was co-administered. The significant inhibitory effect was shown for 4 days. While there is no study done beyond 4 days, the inhibitory effect on CYP3A4 is estimated to last at least for 6 days after single dose administration of AKYNZEO. Close monitoring for sign of adverse events for concomitant CYP3A4 substrates especially with narrow therapeutic window is recommended.

Midazolam: When netupitant 300 mg was co-administered with oral midazolam, the mean Cmax and AUC of midazolam was increased by 36% and 226%, respectively. Based on this result, netupitant is considered a moderate CYP3A4 inhibitor. The effect of netupitant on midazolam was studied only on the day of co-administration.

Dexamethasone: The potential effect of netupitant on PK of dexamethasone, a CYP3A4 substrate was studied. Palonosetron is not an inhibitor of CYP3A4; therefore, its effect was not studied. The coadministration of a single dose of netupitant (300 mg) with oral dexamethasone regimen (20 mg on Day 1, followed by 8 mg b.i.d. from Day 2 to Day 4) significantly increased the exposure to dexamethasone in a dose-dependent manner. When netupitant was co-administered on Day 1, the mean AUC of dexamethasone was increased by 1.7-fold on Day 1 and up to 2.4-fold on Day 2 and Day 4. The potential inhibitory effect of netupitant on CYP3A4 was not studied beyond Day 4. Therefore the sum of individual [I]/Ki[7] for netupitant and its metabolites was calculated. The mean [I]/Ki ranged 0.0134-0.167 (ranged 0.089-0.285) on Day 4 when the AUC of dexamethasone was still 2-fold higher than the control. The mean total [I]/Ki decreased to below 0.1 on Day 6 and was 0.093 (0.049- 0.210) at 140 h post-dose. This estimation suggests that drug interaction via CYP3A4 inhibition by netupitant is less likely on Day 6 but cannot be ruled out. Of note in this study the half-life of netupitant and the metabolite M1 of which the Ki for CYP3A4 was lower than that of netupitant, was estimated to be shorter than those observed in other studies suggesting a possibility of underestimation of the duration of inhibitory effects on CYP3A4.

Chemotherapeutics

[7] The sum of [I]/Ki values for netupitant, M1, M2, and M3 using observed plasma concentrations up to 120 h post-dose and extrapolated plasma concentrations beyond 120 h. Ki values were obtained from in-vitro inhibition studies.

The systemic exposure to intravenously given chemotherapeutic agents (docetaxel, etoposide,) that are metabolized by CYP3A4 was increased to a different degree (10-49%) when AKYNZEO was co-administered than when chemotherapeutic agents were coadministered with palonosetron alone in cancer patients.

When co-administered with AKYNZEO the mean Cmax and AUC of intravenously administered docetaxel were 49% and 35% higher, respectively. The systemic exposure to intravenously administered etoposide and cyclophosphamide was also increased when AKYNZEO was co-administered by 10-28%

Oral contraceptive
AKYNZEO, when given with a single oral dose of 60 μg ethinyl estradiol and 300 μg levonorgestrel increased the mean AUC of levonorgestrel by 46% while AKYNZEO had no significant effect on the mean AUC of ethinyl estradiol.

P-glycoprotein substrate
Digoxin
When netupitant 450 mg was concurrently administered with digoxin, the systemic exposure and urinary excretion of digoxin at steady-state was not significantly affected.

2 Question-Based Review

2.1 General Attributes of the drug

2.1.1 What pertinent regulatory background or history contributes to the current assessment of the clinical pharmacology and biopharmaceutics of this drug?

Netupitant is a NK1 receptor antagonist and a new molecular entity. It is proposed as an oral fixed dosage form in combination with palonosetron, a 5-HT3 receptor antagonist, for the prevention of chemotherapy-induced nausea and vomiting after moderately or highly emetogenic chemotherapy.
Netupitant is not approved for any indications. Currently aprepitant (EMEND®), NK1 receptor antagonist is commercially available for oral and intravenous administration for the CINV and PONV.

Palonosetron hydrochloride is available as an IV formulation (ALOXI®) marketed in the US since September 2003 for prevention of chemotherapy-induced nausea and vomiting (CINV) and post-operative nausea and vomiting (PONV). The oral formulation, soft gelatin capsule (0.50 mg) of palonosetron was approved in the US for CINV (2008) but has not been marketed in the US.

Aloxi Capsule is indicated for:

- Moderately emetogenic cancer chemotherapy -- prevention of acute nausea and vomiting associated with initial and repeat courses

Aloxi for injection is indicated for:

- Moderately emetogenic cancer chemotherapy -- prevention of acute and delayed nausea and vomiting associated with initial and repeat courses
- Highly emetogenic cancer chemotherapy -- prevention of acute nausea and vomiting associated with initial and repeat courses

The indication for PONV is not proposed in this application.

To conform to the combination rule, the sponsor conducted the efficacy trials in comparison to oral Aloxi. In addition, to establish the contribution of oral palonosetron to the prevention of nausea and vomiting induced by HEC, a non-inferiority trial in comparison to intravenous Aloxi was conducted. The sponsor does not propose HEC-CINV indication for oral Aloxi based on the non-inferiority trial.

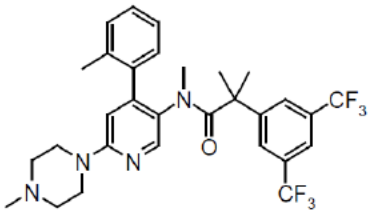
With recent re-classification of emetogenicity of anthracycline-cyclophosphamide regimen used in a clinical trial for MEC from MEC to HEC, the exact indication for regarding the prevention of nausea and vomiting by the degree of emetogenicity of chemotherapy is under discussion. The discussion on the indication is deferred to the clinical review.

2.1.2 What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Netupitant

Netupitant is white to off-white powder very slightly soluble in water. (b) (4)

Netupitant is not hygroscopic.

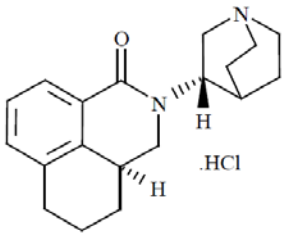


Molecular formula:	$C_{30}H_{32}F_6N_4O$	
Molecular weight	578.61	$g \cdot mol^{-1}$
Log D at pH (b) (4)	(b) (4)	
Log D at pH		
Log P		
pKa1		
pKa2		

Figure 1. Structure of Netupitant

Palonosetron

Palonosetron hydrochloride is a white to off-white crystalline powder. It is freely soluble in water. Palonosetron hydrochloride is essentially non-hygroscopic. Palonosetron hydrochloride drug substance is synthesized solely as the (b) (4) isomer.



Molecular formula:	$C_{19}H_{24}N_2O \cdot HCl$	
Molecular weight	332.87	$g \cdot mol^{-1}$
pKa	8.9	

Figure 2. Structure of palonosetron

Akynzeo®, a netupitant-palonosetron fixed-dose combination (FDC) capsule contains three 100 mg netupitant tablets and one palonosetron 0.5 mg softgel capsule. The palonosetron 0.5 mg softgel capsule in combination capsule is similar to the currently approved Aloxi capsule with the exceptions of (b) (4) and capsule size.

In the dose-finding study and the study establishes the contribution of netupitant and the efficacy of combination therapy for HEC induced nausea and vomiting, netupitant and palonosetron were also given as an extemporaneous combination. In this trial netupitant was formulated in a standard hard gelatin capsule, while palonosetron was the soft gelatin capsule currently approved. A bioequivalence study was conducted between the FDC and the extemporaneous combination.

2.1.3 What are the proposed mechanism(s) of action and therapeutic indication(s)?

Netupitant is a substance P/neurokinin 1 (NK1) receptor antagonist. Palonosetron is a 5-HT3 receptor antagonist.

Chemotherapeutic agents exert their emetic stimulus via processes that involve the release of serotonin and substance P and subsequent activation of the 5-HT3 and NK1 receptors.

The proposed indications are as below.

- prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy
- prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of moderately emetogenic cancer chemotherapy

The proposed indications have been previously granted. With updated classification of emetogenicity of certain chemotherapeutic regimens, the indications are currently being reconsidered.

2.1.4 What are the proposed dosage(s) and route(s) of administration?

Akynzeo® is a solid oral Netupitant/Palonosetron 300mg/0.50 mg fixed dose combination capsule (FDC), composed of one size 0 hard gelatin capsule.

Each capsule contains three intermediate netupitant tablets (3×100 mg netupitant tablets) and one intermediate palonosetron 0.5 mg softgel capsule.

The proposed dosage regimen is 1 hour before chemotherapy by oral administration without regard of food.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The clinical efficacy and safety of Akynzeo is supported by three clinical trials in cancer patients (NETU-07-07, NETU-08-18, NETU-10-29). In addition, an efficacy trial PALO-10-01 was conducted to establish the contribution of oral palonosetron to the efficacy in prevention of CINV associated with cisplatin-based highly emetogenic chemotherapy. In PALO-10-01, the efficacy of oral palonosetron was evaluated in comparison to intravenous palonosetron, which is approved for the prevention of acute CINV associated with HEC.

In clinical pharmacology program, pharmacokinetics of netupitant and its metabolites was extensively studied and 14 clinical pharmacology related in-vitro studies were conducted. Some clinical pharmacology studies were conducted for netupitant alone and some studies were conducted for the combination product. Because the proposed dosage regimen is a single dose per chemotherapy cycle, the clinical pharmacology studies for netupitant and for FDC were mostly conducted after a single dose administration except multiple dose PK for netupitant.

On the other hand the clinical pharmacology of palonosetron was mostly referenced to the previously conducted studies in support of oral and intravenous Aloxi.

For the list of studies, please see Appendix 1.

2.2.2 What is the basis for selecting the response endpoints and how are they measured in clinical pharmacology and clinical studies?

The proposed indication is to prevent chemotherapy induced nausea and vomiting (CINV).

Accordingly clinical efficacy was evaluated based on the proportion of patients with complete response (CR) (defined as no emesis, no rescue medication) in the 0-24 h (acute phase), the 25-120 h (delayed phase) and the 0-120 h (overall phase) post chemotherapy.

Other efficacy endpoints such as time to first emetic episode, time to first rescue medication, time to treatment failure (based on time to the first emetic episode or time to the first rescue medication, whichever occurs first) were also evaluated.

In two phase 1 pharmacodynamics studies, NK1-receptor occupancy in brain and the prevention of apomorphine-induced nausea and vomiting were explored for netupitant.

2.2.3 Are the active moieties in the plasma and urine appropriately identified and measured to assess pharmacokinetic parameters and exposure-response relationships?

Yes. Both netupitant and palonosetron were quantified in the plasma. Netupitant was also measured in urine. See Section 2.6 for more details.

2.2.4 Exposure-Response Evaluation

2.2.4.1 What are the characteristics of the exposure-response relationships for efficacy?

Clinical efficacy

The dose-response relationship to evaluate the contribution of netupitant at different dose levels to the CR rate in addition to palonosetron was explored in patients receiving cisplatin-based highly emetogenic chemotherapy (HEC) (NETU-07-07).

In Study NETU-08-18 in which the combination of 300 mg netupitant/0.5 mg palonosetron was studied in patients receiving anthracycline-cyclophosphamide regimen, PK samples were collected. However, a formal assessment of exposure-response relationship could not be made due the limited PK data collected in the clinical studies as only 117 patients out of 726 (~16%) in AKYNZEO arm in the study NETU-08-18.

In NETU-07-07, there was no significant dose-response relationship in the proportion of patients with CR in overall and delayed phase among three netupitant doses of 100 mg, 200 mg and 300 mg. PK samples were not collected so the concentration-response relationship was not studied.

In this study netupitant was co-administered with oral palonosetron at the approved dose of 0.5 mg as well as with oral dexamethasone (Dexa) as a standard of care. The dosage regimen for oral dexamethasone was reduced in combination treatment arms for the increase in systemic exposure to dexamethasone due to inhibition of metabolizing enzyme by netupitant. As such for Palo only treatment, oral Dexa was given at 20 mg on Day 1 and 8 mg twice daily on Days 2-4. For treatment arm with netupitant, oral Dexa dose was reduced to 12 mg on Day 1 and 8 mg once daily on Days 2-4. This study was designed to compare the complete response rate with the Palo

alone treatment so was not powered to show differences among the combinations with different netupitant doses. (Table 1and Figure 3)

The complete response rate over 120 hours after treatment with PALO+NETU ranged 87.4% and 89.6% and was similar among netupitant doses. All treatment with PALO+NETU showed statistically higher CR rate in overall phase and delayed phase while only combination with NETU 300 mg was statistically better than PALO alone for prevention of CINV in acute phase. Based on this study the combination of PALO 0.5 mg with NETU 300 mg was selected for subsequent efficacy trials. Study NETU-07-07 also established the contribution of netupitant component to the prevention of CINV in addition to Palo component and the clinical efficacy of the combination therapy for the prevention of CINV associated with HEC, cisplatin-based chemotherapy. The review of statistical analysis of NETU-07-07 is deferred to the biostatistics reviewer.

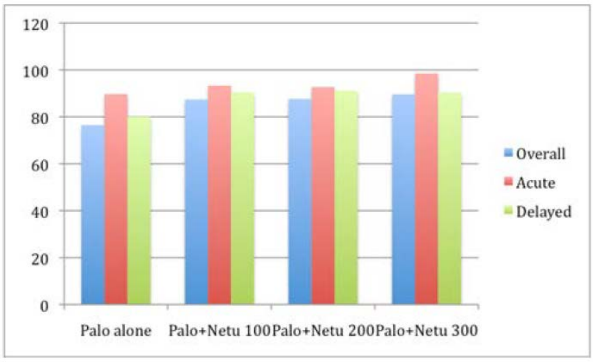
Table 1 Complete Response Rate (NETU-07-07)

	PALO alone (N=136)	PALO + NETU100 mg (N=135)	PALO + NETU200 mg (N=137)	PALO + NETU 300 mg (N=135)
Overall (0-120 hours)				
Number (%) of Patients	104 (76.5)	118 (87.4)	120 (87.6)	121 (89.6)
Difference from palonosetron alone (%) with 95% CI		10.9 (1.9, 20.0)	11.1 (2.1, 20.1)	13.2 (4.4, 21.9)
p-value[1]		0.018	0.017	0.004
Delayed phase (25-120 hours)				
Number (%) of patients	109 (80.1)	122 (90.4)	125 (91.2)	122 (90.4)
Difference from palonosetron alone (%), [95% CI]	-	10.2 [1.9, 18.6]	11.1 [2.9, 19.3]	10.2 [1.9, 18.6]
p-value[1]	-	0.018	0.010	0.018
Acute phase (0-24 hours)				
Number (%) of patients	122 (89.7)	126 (93.3)	127 (92.7)	133 (98.5)
Difference from palonosetron alone (%), [95% CI]	-	3.6 [-3.0, 10.2]	3.0 [-3.7, 9.7]	8.8 [3.3, 14.3]
p-value[1]	-	0.278	0.383	0.007

Source: NETU-07-07, Table 16 and 18

[1]p-value from logistic regression analysis including gender as covariate

Figure 3 Complete Response Rate in the Overall, Acute and Delayed Phase (NETU-07-07)



The time to first emetic episode was significantly longer for patients treated with netupitant compared to palonosetron alone. The Kaplan-Meier plot showed that the 300 mg dose appeared to have the largest effect, with the curves starting to diverge approximately 6-8 hours after chemotherapy (Figure 4). Curves show the higher efficacy of netupitant 300 mg from 24 through 44 hours compared to lower netupitant doses.

Figure 4 Time to first emetic episode

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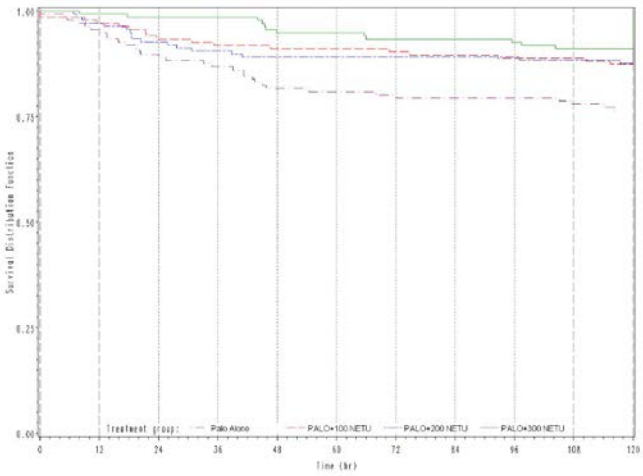


In a phase 3 trial, AKYNZEO demonstrated superiority to PALO monotherapy in prevention of delayed (primary efficacy endpoint), acute, and overall time periods (secondary efficacy endpoints) (Table 2).

Table 2 Complete Response delayed, acute and overall cycle 1 (NETU-08-18)

	NETU/PALO FDC (N=724)	PALO alone (N=725)
Delayed		
Responder, n (%)	557 (76.9)	504 (69.5)
Difference from palonosetron alone, %	7.4	
CMH OR (95% CI)	1.48 (1.16; 1.87)	
p-value[a]	0.001	
Acute		
Responder, n (%)	640 (88.4)	616 (85.0)
Difference from palonosetron alone, %	3.4	
CMH OR (95% CI)	1.37 (1.00; 1.87)	
p-value[a]	0.047	
Overall		
Responder, n (%)	538 (74.3)	483 (66.6)
Difference from palonosetron alone, %	7.7	
CMH OR (95% CI)	1.47 (1.17; 1.85)	
p-value[a]	0.001	

(a) p-value from CMH test, stratified by age class and region.

Pharmacodynamics Study

Doses of netupitant used in study NETU-07-07 (100 mg, 200 mg and 300 mg) were selected based on exploratory pharmacodynamics studies such as apomorphine challenge study and NK1 receptor occupancy study.

An initial study NP16602 using an apomorphine challenge model showed that administration of netupitant reduced the incidence of vomiting induced by apomorphine compared to placebo. Netupitant appeared to reduce the incidence of emetic episodes in a concentration dependent manner. No vomiting occurred when plasma concentrations were >300 ng/mL at the time of the challenge compared with 75% of the subjects receiving placebo (Table 3).

Table 3 Apomorphine challenge study: Summary of Vomiting episodes and Area under the Nausea VAS[8]

Netupitant Concentration	0 ng/mL (placebo) (N=8)	≤50 ng/mL (N=6)	51-100 ng/mL (N=6)	101-300 ng/mL (N=6)	>300 ng/mL (N=6)
Vomiting Episodes Mean	10.3	2.7	4.2	3.5	0.0
Range	0.0-27.0	0.0-10.0	0.0-18.0	0.0-13.0	0.0-0.0
AUC of Nausea VAS					
Mean	2207.9	1469.8	3089.8	3480.2	4117.8
Range	170-5435	10-3399	1184-5164	1552-5840	2030-6527
p-value		0.3852	0.3009	0.1398	0.0305

The extent of receptor occupancy (RO) by netupitant was measured by Positron Emission Tomography (PET) technology (NETU-06-08) after single dose administration of netupitant at 100 mg, 300 mg and 450 mg. In this study, the RO (90% or higher) close to the expected Cmax was achieved after a single dose of netupitant in the occipital cortex (100-450 mg), frontal cortex (100–450 mg), striatum (300 and 450 mg) and anterior cingulate (100 and 450 mg). The time- and concentration-dependent NK1-RO was apparent in Striatum region but it was not as apparent in other brain regions. In an exploratory PK/PD analysis using the Sigmoid Emax model, 225 ng/mL was estimated to correspond to an NK1-RO of 90% in stratum region. A comparison of the results for the dose groups (100 mg, 300 mg and 450 mg) showed a general but low increase in NK1-ROs with increasing dose. **(Table 4, Figure 5)**

Together the individual Cmax values in the receptor occupancy study and the mean Cmax observed in other single oral dose studies (from 92 to 168 ng/mL after 100 mg and from 335 to 747 ng/mL after 300 mg), a single oral dose between 100 and 300 mg of netupitant was suggested to be needed to reach an NK1-RO level in striatum of at least 90% close to the expected Cmax in the majority of the brain regions evaluated in the PET study.

Initially 200 mg netupitant was proposed to be the clinical dose so the tQT study was conducted for a combination with netupitant 200 mg and the 3-fold higher supratherapeutic dose. Later the the combination with 300 mg netupitant was selected for clinical trials and for marketing (NETU-07-07).

[8] The degree of nausea was recorded using a 100 mm visual analogue scale (VAS) ranging from 0 (no nausea) to 100 (severe nausea)

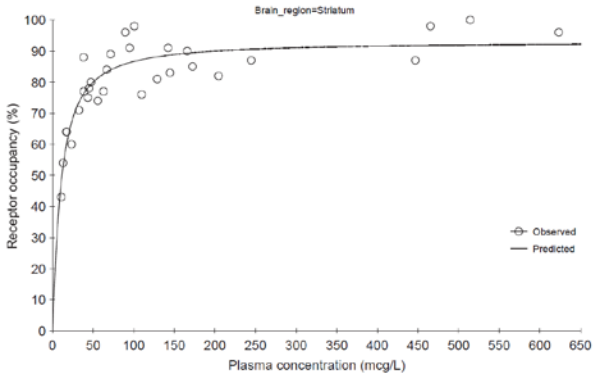
Table 4 Average Neurokinin-1 Receptor Occupancy (NK1-RO) in Striatum at 6, 24, 48, 72, and 96 Hours after Administration of a Single Dose of 100, 300, and 450 mg Netupitant

		Netupitant 100 mg N=2	Netupitant 300 mg N=2	Netupitant 450 mg N=2
NK_1 receptor occupancy (%) at 6 hours	n	2	2	2
	Mean	84.0	92.5	98.0
	SD	1.4	7.8	2.8
	CV%	2	8	3
NK_1 receptor occupancy (%) at 24 hours	n	2	2	2
	Mean	76.0	86.5	88.5
	SD	2.8	6.4	2.1
	CV%	4	7	2
NK_1 receptor occupancy (%) at 48 hours	n	2	2	2
	Mean	65.5	85.0	94.5
	SD	7.8	5.7	4.9
	CV%	12	7	5
NK_1 receptor occupancy (%) at 72 hours	n	2	2	2
	Mean	64.0	78.0	90.0
	SD	0.0	2.8	8.5
	CV%	0	4	9
NK_1 receptor occupancy (%) at 96 hours	n	2	2	2
	Mean	48.5	76.0	82.5
	SD	7.8	1.4	7.8
	CV%	16	2	9

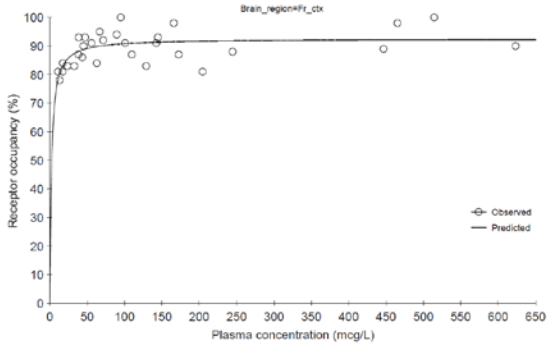
N=Number of subjects in the specific group
n=Number of subjects with data available

Figure 5 NK_1-RO-netupitant concentration in (A) Striatum and (B) Frontal cortex

(A) Striatum



(B) Frontal cortex



2.2.4.2 What are the characteristics of the exposure-response relationships for safety?

In Study NETU-07-07, the netupitant dose-dependent increase in TEAE was not evident. The incidence of TEAEs was generally similar among treatment groups (Table 5). The overall rate of TEAE was higher after the combination treatment with NETU 300 mg/PALO 0.5 mg compared to oral PALO 0.5 mg alone i.e. 70% vs. 61% (Table 6). For detailed review of safety profile, please see the clinical review.

Table 5 Summary of subjects with treatment emergent adverse events

MedDRA SOC Preferred Term	Palo Alone (N=136) n (%)	PALO+ 100 NETU (N=135) n (%)	PALO+ 200 NETU (N=138) n (%)	PALO + 300 NETU (N=136) n (%)
Any TEAE	68 (50.0%)	55 (40.7%)	71 (51.4%)	68 (50.0%)
TEAE related to study drugs	17 (12.5%)	18 (13.3%)	24 (17.4%)	21 (15.4%)
TEAE related to dexamethasone	15 (11.0%)	23 (17.0%)	21 (15.2%)	17 (12.5%)
Any related TEAE	27 (19.9%)	31 (23.0%)	38 (27.5%)	34 (25.0%)
Severe TEAE	7 (5.1%)	4 (3.0%)	8 (5.8%)	8 (5.9%)
Severe TEAE related to study drugs	2 (1.5%)	0 (0.0%)	3 (2.2%)	0 (0.0%)
Severe TEAE related to dexamethasone	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Any related severe TEAE	2 (1.5%)	0 (0.0%)	3 (2.2%)	0 (0.0%)

Table 6 Overview of Treatment-emergent Adverse Events combined – Cycle 1 (Phase 2/3 Cancer Patients)

	Netupitant–Palonosetron (mg)				Palonosetron (mg)		
	100/0.50 (N=135)	200/0.50 (N=138)	300/0.50 (N=1169)	Total (N=1442)	IV 0.25 (N=369)	Oral 0.50 (N=1231)	Total (N=1600)
Number (%) of patients with ≥ 1:							
TEAE	55 (40.7)	71 (51.4)	818 (70.0)	944 (65.5)	191 (51.8)	754 (61.3)	945 (59.1)
Drug-related TEAE	18 (13.3)	24 (17.4)	96 (8.2)	138 (9.6)	24 (6.5)	81 (6.6)	105 (6.6)
Serious TEAE	1 (0.7)	1 (0.7)	31 (2.7)	33 (2.3)	36 (9.8)	51 (4.1)	87 (5.4)
Drug-related serious TEAE	–	1 (0.7)	1 (0.1)	2 (0.1)	–	2 (0.2)	2 (0.1)
TEAE leading to death	1 (0.7)	–	7 (0.6)	8 (0.6)	12 (3.3)	8 (0.6)	20 (1.3)
TEAE leading to discontinuation	–	1 (0.7)	13 (1.1)	14 (1.0)	1 (0.3)	5 (0.4)	6 (0.4)
Drug-related TEAE leading to discontinuation	–	1 (0.7)	1 (0.1)	2 (0.1)	–	2 (0.2)	2 (0.1)

TEAE = treatment-emergent adverse event

Source: Modified from Module 5.3.5.3, ISS Tables 2.2.1.1 through Table 2.2.1.4

2.2.4.3 Does this drug prolong the QT or QTc interval?

No significant QTc interval prolongation was observed when a combination of 600 mg NETU and 1.5 mg PALO was administered to healthy subjects (NETU-07-20)[9] (Table 7, Figure 6).
No significant effect of PALO on QTc interval was previously shown at doses up to 2.25 mg after intravenous administration of PALO alone. Consistently there was no evident exposure-response relationship between ddQTcF and concentrations of netupitant and its metabolites, M1, M2, and M3 as well as concentrations of palonosetron and its metabolite M4 and M9 in the tQT study with doses up to netupitant 600 mg/palonosetron 1.5 mg. Therefore this study demonstrates the lack of effects on QTc interval by addition of NETU up to 600 mg.

At the time of the tQT study, 200 mg NETU/0.5 mg PALO was predicted to be a therapeutic dose so the 3-fold higher dose i.e. NETU 600 mg/PALO 1.5 mg was chosen as a supratherapeutic dose. The dose-proportional increase in the systemic exposure was observed for both netupitant and palonosetron. The supratherapeutic dose in this study provides the safety margin of 2 fold for the proposed dose for Akynzeo® i.e. NETU 300 mg/PALO 0.5 mg. The supratherapeutic dose cover the Cmax observed in patients with moderate hepatic impairment and in healthy subjects when ketoconazole, a strong CYP3A4 inhibitor was co-administered.

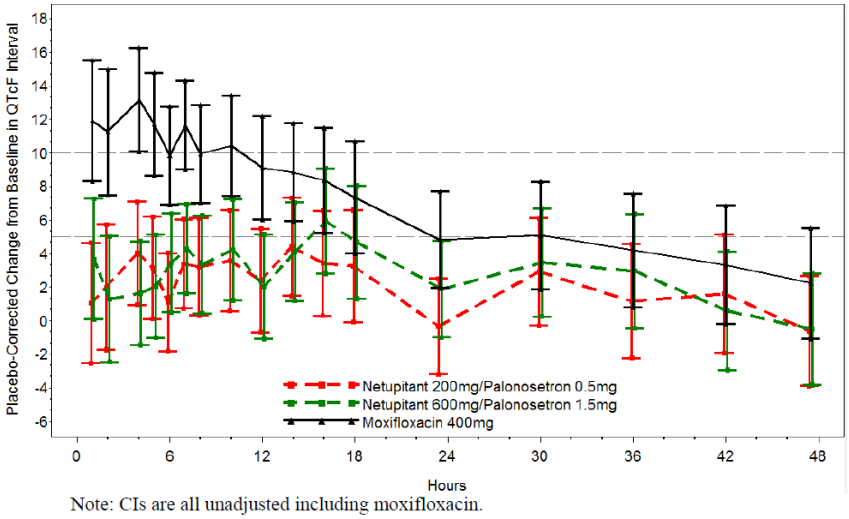
[9] Please see the IRT-QT team reviews of the thorough QT study dated 1/19/2010 (IND 73,493 SDN 024) and 3/3/14 (NDA 205-718) for more details.

Table 7 The Point Estimates and the 90% CIs Corresponding to the Largest Upper Bounds for netupitant/palonosetron (200 mg/0.50 mg and 600 mg/1.50 mg) and the Largest Lower Bound for Moxifloxacin (Analysis by FDA IRT-QT team)

Treatment	Time (hour)	ΔΔQTcF (ms)	90% CI (ms)
Netupitant/palonosetron (200 mg/0.50 mg)	14	4.4	(1.5, 7.3)
Netupitant/palonosetron (600 mg/1.50 mg)	16	5.9	(2.8, 9.1)
Moxifloxacin 400 mg*	4	13.2	(10.1, 16.3)

* Multiple endpoint adjustment was not applied. The largest lower bound after Bonferroni adjustment for 5 timepoints (1, 2, 4, 5, and 6 hours) was 9.2 ms.

Figure 6 Mean (90% CI) **dd**QTcF-time profile



Note: CIs are all unadjusted including moxifloxacin.

From IRT-QT review of tQT study (*1/20/2010), Page 24)*

2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

No. The dose-response relationship was not evident among the combination with netupitant 100 mg-300 mg and the CR rate greater than 85% for primary and key secondary endpoints at all doses. However, the proposed dose is supported by efficacy trials and NETU 300 mg/PALO 0.5 mg combination was the only combination that showed significantly higher CR rate than PALO alone for all primary and key secondary endpoints to support the efficacy of the combination and the contribution of netupitant to the prevention of CINV associated with cisplatin-based highly-emetogenic chemotherapy.

2.2.5 Pharmacokinetic Characteristics

2.2.5.1 What are the PK characteristics of netupitant and its major metabolite?

This review will mainly discuss the PK characteristics of netupitant which is a new molecular entity. The general PK characteristics for palonosetron were previously studied for the approval of oral and intravenous palonosetron.

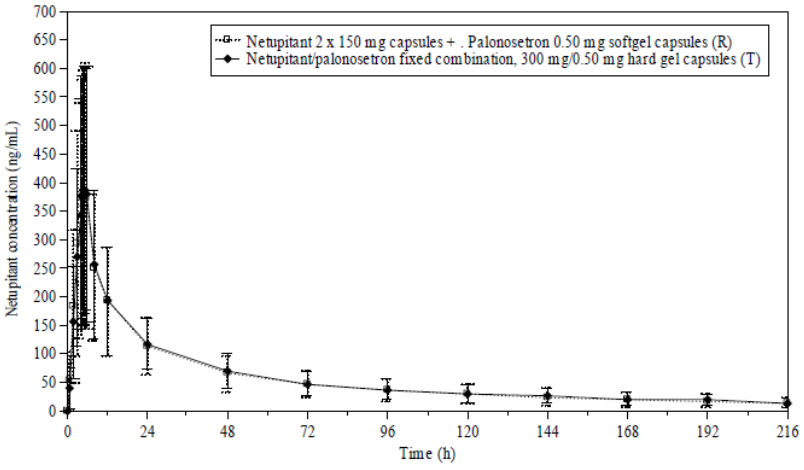
Netupitant

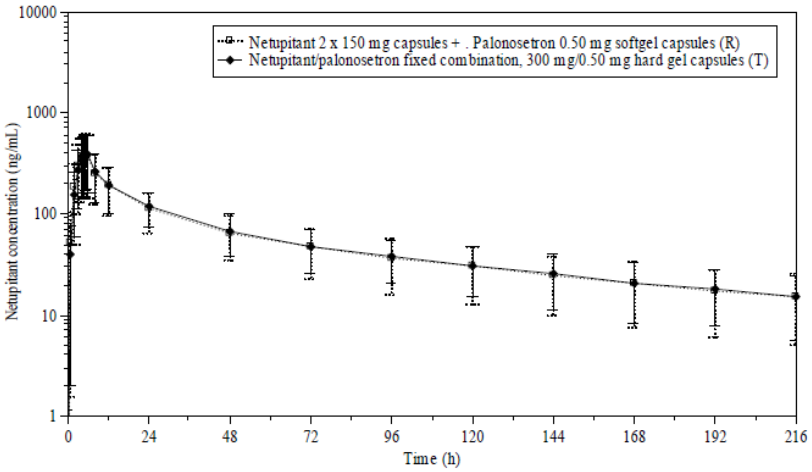
After oral administration, netupitant is absorbed slowly reaching peak in approximately 4-5 hours. Plasma concentrations were measurable between 0.25 and 3 hours and plasma concentrations followed a first order absorption process. Netupitant was eliminated from the body in a multi- exponential fashion, with an apparent mean terminal half-life ranging, on average, from 30 to 100 hours (for doses of 30 mg to 450 mg). Substantial distribution of netupitant into tissues was indicated by a large volume of distribution (Vz/F) ranged from approximately 850 to over 2000 L. The apparent oral plasma clearance (CL/F) ranged from approximately 10 to 35 L/h, indicating that the compound is slowly cleared from blood. The renal clearance of netupitant ranged 4.2-39 mL/h (Figure 7).

Netupitant undergoes extensive metabolism to form three major active metabolites in plasma. Metabolites M1 and M3 reached the maximum concentration much later (17-32 h on average) than netupitant and metabolite M2 (approximately 5 h). Metabolites M1, M2 and M3 accounted on average for 29%, 14%, and 33% of parent exposure, respectively, in terms of AUC0-t in the ADME study. A minor metabolite identified later during the development, M4 accounted for about 3% of parent drug exposure. All four metabolites were shown to bind to human NK1 receptor *in vitro*.

Figure 7 Mean (±SD) plasma netupitant concentration (ng/mL) vs. time profiles after single oral administration of AKYNZEO (T) and Netupitant and Palonosetron extemporaneous combination (R) used in the dose-finding study (NETU 09-07)

(A)





(B)

Palonosetron[10]

After single dose administration of AKYNZEO in healthy subjects, the peak plasma concentrations for palonosetron were reached in about 5 hours (Figure 8).

Distribution

Palonosetron has a volume of distribution of approximately 8.3 ± 2.5 L/kg. Approximately 62 % of palonosetron is bound to plasma proteins.

Metabolism

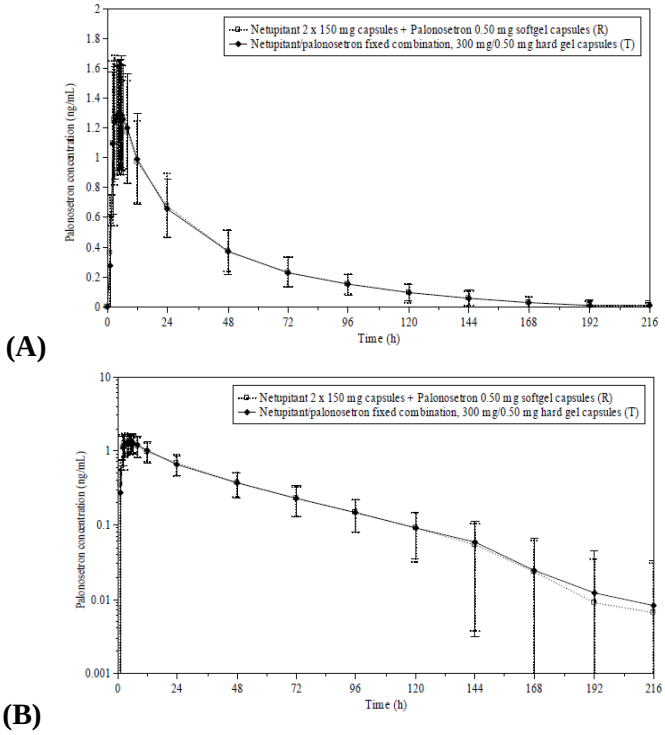
Palonosetron is eliminated by multiple routes with approximately 50 % metabolized to form two primary metabolites: N-oxide-palonosetron and 6-S-hydroxy-palonosetron. These metabolites each have less than 1 % of the 5-HT_3 receptor antagonist activity of palonosetron. *In vitro* metabolism studies have suggested that CYP2D6 and to a lesser extent, CYP3A4 and CYP1A2 are involved in the metabolism of palonosetron. However, clinical pharmacokinetic parameters are not significantly different between poor and extensive metabolizers of CYP2D6 substrates.

Elimination

(b) (4)

[10] Package Insert for Aloxi I.V.

Figure 8 Mean (±SD) plasma palonosetron concentration (ng/mL) vs. time profiles after single oral administration of AKYNZEO(T) and Netupitant and Palonosetron extemporaneous combination (R) (NETU 07-07)



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2.2.5.2 What are the single dose and multiple dose PK parameters?

Multiple dose PK was studied for netupitant only in this development program. After once daily dosing for 7 days, the systemic exposure gets about 3 fold higher than that after single dose as expected from a long half-life of netupitant. There was a greater than dose-proportional increase in the systemic exposure as the dose increases from 100 mg to 300 mg after single and multiple doses while the dose-proportional increase in the systemic exposure was observed as the dose increases from 300 mg to 450 mg (Table 8,Table 9, Table 10).

Table 8 Mean ± SD (%CV) Pharmacokinetic Parameters of netupitant and palonosetron after single dose administration of Akynzeo® in healthy subjects (NETU-09-07)

	Cmax (ng/ml)	Tmax (h)	AUCt (ng*h/ml)	AUCinf (ng*h/ml)	$T_{1/2}$ (h)
Netupitant (n=47)	434.1 ± 242.1 (55.7)	5 (2-12)	12321 ± 5209.6 (42)	14401.6 ± 7307.8 (50.7)	95.6 ± 58.8
Palonosetron	1.5 ± 0.4	5	51.2 ± 18	56.7 ± 18.6	44.2 ± 15.2

(n=47)	(26.7)	(1-12)	(35)	(32.8)	

Table 9 Mean ± SD (%CV) Pharmacokinetic Parameters of netupitant and its metabolites after single dose administration of Akynzeo® under fasting condition in healthy subjects (NETU-10-12)

	Cmax (ng/ml)	Tmax (h)	AUCt (ng*h/ml)	AUCinf (ng*h/ml)	$T_{1/2}$ (h)
Netupitant (n=22)	596.4±233.0 (39.1)	5 (4-8)	17150±6122 (35.7)	20039±8396 (41.9)	101.2±52.8 (52.2)
M1	43.7±12.4 (28.4)	12 (6-24)	4933±1452 (29.4)	5886±2235 (38.0)	82.2±37.0 (45.0)
M2	202.2±97.3 (48.1)	4.5 (3-5.5)	2076±929.1 (44.8)	2254±945.3 (41.9)	48.9±45.7 (93.4)
M3	81.8±37.9 (46.3)	12 (4.5-24)	5348±2323 (43.4)	5841±2654 (45.4)	65.6±29.1 (44.4)

Table 10 Mean (± SD) Pharmacokinetic Parameters of Netupitant after single and multiple dose administration in healthy subjects (NP16601)

Dose	Single dose (n=8)			Multiple doses (n=8)				
mg	Cmax (ng/ml)	Tmax (h)	$AUC_{0-23\ 5}$ (ng*h/ml)	Cmax (ng/ml)	Tmax (h)	$AUC_{0-23\ 5}$ (ng*h/ml)	AUCinf (ng*h/ml)	AF[1]
100	111 ± 25.6 (23)	4.7 ± 0.71 (15)	1360 ± 294 (22)	269 ± 52.2 (19)	4.5 ± 0.93 (21)	4160 ± 998 (24)	21400 (29.7)	3.06 (12)
300	599 ± 228 (38)	5.5 ± 2.83 (51)	6400 ± 1700 (26)	1060 ± 202 (19)	5.4 ± 2.94 (54)	17100 ± 2850 (17)	93800 (32.6)	2.74 (12)
450	720 ± 255 (35)	5.4 ± 1.06 (20)	9670 ± 3380 (35)	1790±770 (43)	7.1 ± 6.85 (96)	28800 ± 13000 (45)	139000 (46.4)	2.93 (14)

[1]Accumulation Factor

PK blood samples were collected up to 168 h post-last dose

2.2.5.3 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients? (NETU-10-09)

Overall, the exposure to netupitant and its metabolites and palonosetron in cancer patients, in terms of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values, were consistent with data reported in healthy subjects.

Netupitant

PK of netupitant was studied in cancer patients who are treated with moderate or highly emetogenic chemotherapy. In Study NETU-10-09, a single dose administration of combination

(Netu 300 mg and Palo 0.5 mg) with selected chemotherapy agents (docetaxel, etoposide, or cyclophosphamide) to evaluate the pharmacokinetics of netupitant, its main metabolites and palonosetron along with the PK of the chemotherapeutic agents

In a cross study comparison, PK parameters for netupitant in cancer patients were similar to that in healthy subjects (Table 11). The systemic exposure to netupitant in cancer patients were within the range observed in healthy subjects across multiple studies and seems to be independent of the chemotherapeutic regimen co-administered i.e. docetaxel, etoposide, or cyclophosphamide.

Consistently the population PK analysis showed that PK for netupitant in cancer patients was similar to that in healthy subjects.

Table 11 Pharmacokinetic Parameters (mean ± SD) of netupitant after single administration of combination (Netu+Palo) in cancer patients

		Cmax (ng/mL)	Tmax[3] (h)	AUCt (ng*h/mL)	AUCi (ng*h/mL)
Healthy subjects[1] (n=47)		434 ± 242 (56)	5 (2-15)	12321 ± 5210 (42)	14402 ± 7308 (51)
Patients with cancer[2]	NP+Doc (n=8)	486±48.9 (51)	4 (4-6)	14280 ± 4703 (33)	16130 ± 4955 (31) (n=2)
	NP+Eto (n=12)	519±263.2 (51)	4 (3-8)	15220 ± 5956 (39)	18160±8296 (46) (n=9)
	NP+Cyc (n=10)	477±231.3 (48)	4.24 (2.1-5)	13480 ± 3560 (26)	16440±4897 (30) (n=5)

[1]Study NETU-09-07: Following single dose administration of FDC *(Source: Listing 16.2.6.1.)*
[2] Study NETU-10-09: Following single dose administration of FDC
[3]Median (min-max)

In cancer patients, exposure to M1 and M3 relative to netupitant was similar to healthy subjects, accounting for 8-14% for Cmax and approximately 30-35% for AUC0-t (Table 12).

Table 12 Mean Exposure Data to Metabolites M1, M2, M3 after Administration of 300 mg Netupitant to Cancer Patients (NETU-10-09)

		M1			M2			M3		
Treatment:		**C_{max}**	**AUC_{0-t}**	**$AUC_{0-\infty}$**	**C_{max}**	**AUC_{0-t}**	**$AUC_{0-\infty}$**	**C_{max}**	**AUC_{0-t}**	**AUC_{0}**
FDC capsule with:		**ng/mL**	**h·ng/mL**	**ng/mL**	**h·ng/mL**	**ng/mL**	**h·ng/mL**	**ng/mL**	**h·ng/mL**	**ng/mL**
Docetaxel	n	8	8	0[a]	8	8	2	8	8	4

(N=8)	Mean	36	4356	NA	361	4746	8527	64	4915	5946
	CV (%)	32	41	NA	57	57	10	30	27	34
ratio M/P	**%**	**7**	**31**	**NA**	**74**	**29**	**53**	**13**	**34**	**37**
Etoposide	n	12	12	4	12	12	6	12	12	8
(N=12)	Mean	41	4579	4203	219	2785	3719	74	5038	5294
	CV (%)	34	34	44	55	42	41	44	31	32
ratio M/P		**8**	**30**	**23**	**42**	**15**	**20**	**14**	**33**	**29**
Cyclophosphamide	n	10	10	4	10	10	4	10	10	6
(N=10)	Mean	40	4705	5993	215	2594	3061	68	4530	5821
	CV (%)	32	25	18	28	28	30	58	37	33
ratio M/P		**8**	**35**	**36**	**45**	**16**	**19**	**14**	**34**	**35**

NOTE: Ratio between C_{max} and AUC of metabolites vs parent are also provided (M/P). For a better comparison among groups, AUC_{0-t} is also reported as in some cases, the description of the terminal phase was not completely reliable.

[a] not reliable results in this patient group. NA: not accountable. Digits were rounded.

No significant influence of chemotherapeutics (doxorubicin, epirubicin, fluorouracil) on the disposition of netupitant and palonosetron was noted by population PK analysis performed for the subset of patients (n=117) during a phase 3 trial (NETU-10-02).

Palonosetron

In cross-study comparison, mean AUC and mean Cmax for palonosetron tended to be lower in cancer patients than in healthy subjects. PK of palonosetron was generally similar between healthy subjects and cancer patients (Table X). PK of palonosetron in cancer patients was similar to the previous observation (Table 13, Table 14).

Table 13 Pharmacokinetic Parameters (mean±SD) of Palonosetron after single administration of combination (Netu+Palo) in cancer patients

		Cmax (ng/mL)	Tmax[3] (h)	AUCt (ng*h/mL)	AUCi (ng*h/mL)
Healthy subjects[1] (n=47)		1.53 ± 0.39 (26)	5 (1-12)	52.2 ± 18 (34)	56.7 ± 18.6 (32.78)
Patients with cancer[2]	NP+Doc (n=8)	1.16 ± 0.38 (33)	4.75 (1-12)	74.9 ± 31.8 (43)	85.6 ±4.4 (51)
	NP+Eto (n=12)	0.90 ± 0.35 (38)	5.5 (0-12)	43.8 ±11.7 (27)	49.3 ±12.8 (26)
	NP+Cyc (n=10)	0.85 ± 1.9 (22)	5 (2-7)	40.4 ± 13.7 (34)	48.1 ±15.6 (32)

[1]Study NETU-09-07: Following single dose FDC administration under fasting condition (Source: Listing 16.2.6.4.)

[2]Study NETU-10-09: Following single dose administration of FDC administration

[3]Median (min-max)

Table 14 From Aloxi capsule Package Insert

Table 2: Mean PK parameters[1] (± SD) of palonosetron after a single dose of 0.5 mg Aloxi Capsules in healthy subjects and cancer patients

PK Parameters	Healthy subjects (n=36)	Cancer patients (n=12)
C_{max} (ng/mL)	0.81 ± 0.17	0.93 ± 0.34
T_{max} (h)	5.1 ± 1.7	5.1 ± 5.9
AUC_{∞} (ng·h/mL)	38.2 ± 11.7	49.7 ±12.2
$t_{1/2}$ (h)	37 ± 12	48 ± 19

[1] a cross-study comparison

The mean Cmax and AUCinf of palonosetron was approximately 30% and 65% higher, respectively in the docetaxel group than in the etoposide and cyclophosphamide groups (Figure 9). The reason for apparently higher systemic exposure to palonosetron in patients who received docetaxel is unclear.

2. 2.5.4 What are the characteristics of netupitant absorption?

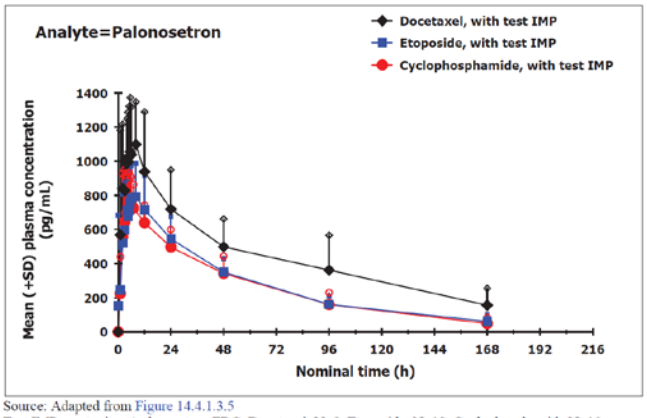
Measurable plasma netupitant concentrations were detected between 15 minutes and 3 hours after single dose oral studies. Plasma concentrations reached Cmax in approximately 5 hours.

The absolute bioavailability was not adequately studied.
Nevertheless, in a cross-study comparison, the total clearance and the volume of distribution were similar between oral and intravenous administration although somewhat higher after oral administration (Table 15). The exposure to metabolites was generally comparable with higher systemic exposure to M1 after oral than i.v. administration (Table 16). Of note there was greater than dose-proportional increase in the systemic exposure to netupitant from 100 mg to 300 mg in dose-ascending studies.

Due to the cross-study comparison and the small number of subjects support the PK data, a reliable conclusion cannot be drawn from this comparison.

Of note, after intravenous administration, infusion site thrombosis was noted in some subjects. According to the study report, the intravenous formulation for netupitant will not be further studied.

Figure 9 Mean Palonosetron Plasma Concentrations –time after administration of FDC with chemotherapy – PK Population Excluding Site 93 Patients



Source: Adapted from Figure 14.4.1.3.5
Test IMP = netupitant/palonosetron FDC; Docetaxel: N=8, Etoposide: N=12, Cyclophosphamide N=10.

Table 15 Mean (%CV) PK Parameters after single dose administration of oral or intravenous Netupitant at 100 mg in healthy subjects

PK parameters Mean (CV [%])	Oral Netupitant[1] (N=4)	I.V. Netupitant[2] (N=6)
Cmax [µg/L]	168 (15.9)	1203.4 (32.12)
AUC(0-last) [h•µg/L]	4359 (26.1)	4575.9 (13.14)
AUC(0-inf) [h•µg/L]	4795 (27.3)	5492.4 (23.07)
$T_{1/2}$ (h)	54.2 (33.1)	61 (65)
CL (L/h) CL/F	22.1 (27.6)	18.9 (20.5)
Vd (L) Vd/F	1713 (40.3)	1523.6 (41.7)

[1]Study RO16603: PK sampling up to 168 h post-dose
[2]NETU-11-01: intravenous infusion over 15 min; infusion site thrombosis occurred in 2 patients; PK sampling up to 120 h after start of infusion

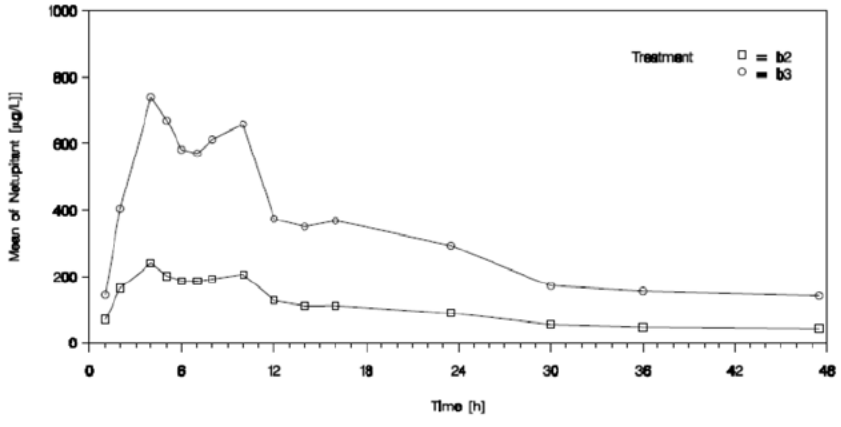
Table 16 Mean (%CV) PK Parameters for metabolites of netupitant after single dose administration of oral or intravenous Netupitant at 100 mg in healthy subjects

PK parameters Mean (CV [%])	Oral Netupitant[1] (N=4)	I.V. Netupitant[2] (N=6)
M1: Demethyl metabolite		
Cmax [µg/L]	14.2 (22.9)	9.1 (16.5)
AUC(0-last) [h•µg/L]	978 (26.2)	744 (130)
AUC(0-inf) [h•µg/L]	1170 (15.6)	1224 (20.8)
M2: N-oxide metabolite		
Cmax-M2 [µg/L]	34.9 (21.3)	39.6 (34.5)
AUC(0-last) [h•µg/L]	279 (46.8)	419.7 (34.6)
AUC(0-inf) [h•µg/L]	500 (23.2)	494 (34.4)
M3: OH-methyl metabolite		
Cmax [µg/L]	25.2 (24.2)	23.5 (24.9)
AUC(0-last) [h•µg/L]	1300 (33.9)	1464 (34.7)
AUC(0-inf) [h•µg/L]	1570 (27.6)	1712 (33.7)

[1]Study RO16603: PK sampling up to 168 h post-dose
[2]NETU-11-01: PK sampling up to 120 h after start of infusion

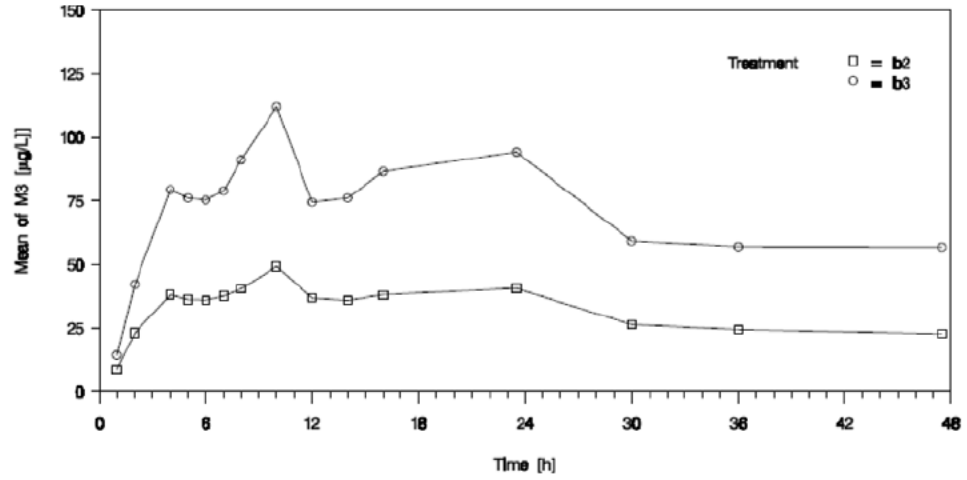
The possibility enterohepatic circulation of netupitant and metabolite M3 was suggested by multiple peaks in the plasma concentration-time profile (Figure 10, Figure 11).

Figure 10 Mean netupitant concentration-time profile (NETU 07-20)



b2: 200 mg NETUPITANT (50 mg + 150 mg) + 0.50 mg PALONOSETRON (1 x 0.50 mg)
b3: 600 mg NETUPITANT (4 x 150 mg) + 1.50 mg PALONOSETRON (3 x 0.50 mg)

Figure 11 Mean metabolite M3 concentration-time profile (NETU 07-20)



b2: 200 mg NETUPITANT (50 mg + 150 mg) + 0.50 mg PALONOSETRON (1 x 0.50 mg)
b3: 600 mg NETUPITANT (4 x 150 mg) + 1.50 mg PALONOSETRON (3 x 0.50 mg)

2.2.5.5 What are the characteristics of drug distribution?

Netupitant is extensively distributed into tissues and is highly bound to plasma protein (>99%). Similarly, metabolites M1, M2, M3 plasma proteins ranged between 97.7 to > 99%. In vitro Blood/Plasma ratios were 0.69 (netupitant and M2), 0.61 (M3) and 1.1 (M1) (Table 17)

The apparent volume of distribution after administration of 300 mg netupitant ranged, on average, from approximately 800 to more than 2000 L, suggesting an extensive distribution out of the circulation.

In cancer patients, the volume of distribution did not change. The volume of distribution in the central compartment (V2) and in the peripheral compartment (V3) in cancer patients were estimated through a population PK model. The final median V2 and V3 estimates accounted for 486 L and 1170 L, respectively. Inter- individual variability for V2 was moderate (43.5%).

The protein binding was evaluated by equilibrium dialysis at 37°C and pH 7.4 after addition of ^{14}C-labeled netupitant or its metabolites to human plasma. Human plasma protein binding of netupitant is greater than 99.5% at drug concentration ranging from 10-1300 ng/ml and protein binding of its major metabolites (M1, M2 and M3) are greater than 97% at drug concentrations ranging from 100 to 2000 ng/mL. The protein binding and blood/plasma ratio was concentration independent over a concentration range which exceeds the maximum plasma concentrations expected in man.

Table 17 Protein binding and blood/plasma ratio for netupitant and major metabolites

	Plasma protein Binding Mean ± SD (tested concentration range)	blood/plasma ratio (λ) Mean ± SD (tested concentration range)
Netupitant	99.67 ± 0.032 % (10-1300 ng/ml)	0.69 ± 0.01 (50- 1000 ng/ml)
M1 (RO0681133)	99.09 ± 0.06 (120-2540 ng/mL)	1.1 ± 0.02 (125-2460 ng/ml)
M2 (RO0713001)	97.7 ± 0.1 (115-2500 ng/ml)	0.69 ± 0.01 (74-2500 ng/ml)
M3 (RO0731519)	99.12 ± 0.05 (115-2000 ng/ml)	0.61 ± 0.02 (118-2310 ng/ml)

2.2.5.7 What are the characteristics of drug metabolism?

In vitro studies showed that the metabolism of netupitant to M1, M2 and M3 is mainly mediated by CYP3A4 and lesser extent by CYP2C9 and CYP2D6 (study NETU-13-21).

Netupitant was shown to undergo extensive metabolism, forming both phase 1 and phase II metabolites. After oral administration, more than 30 metabolites were identified in fecal samples and 13 metabolites in urine samples. Three metabolite, M1, M2, and M3 were detected in plasma and measured in pharmacokinetics studies for netupitant (Figure 10). In later development phase, an additional metabolite, M4 was identified. In humans extent of exposure (AUC) data indicated that M1 has the highest exposure relative to the parent (35%), followed by M3 (29%). M2 and M4 corresponded to the 13% and 3% of the parent, respectively. Mean C_{max} corresponded to 11-12% of parent netupitant for M1, 41-46% for M2, 15-16% for M3 and 6% for M4.

In vitro binding studies showed that M1, M2, M3, and M4 bind to the human NK_1 receptor. In vitro studies showed netupitant is metabolized mainly by CYP3A4 and to a lesser degree CYP2C9 and CYP2D6. In vitro CYP3A4 appears to metabolize netupitant to metabolites M1 and M2.

Figure 12 The proposed metabolic pathways for netupitant and chemical structure of netupitant metabolites identified in human plasma

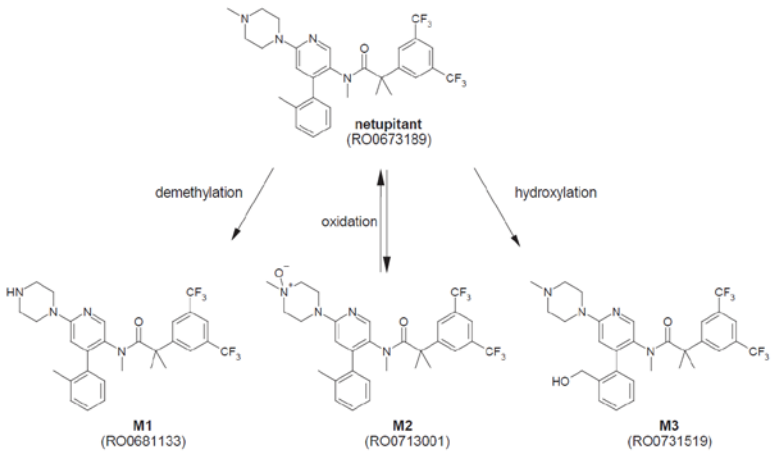
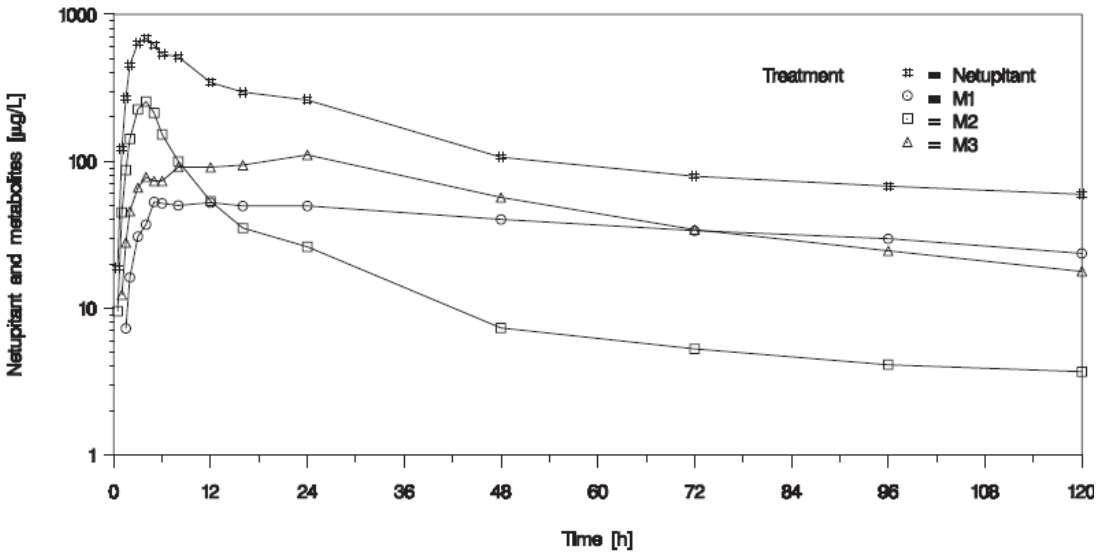


Figure 13 Mean plasma concentrations-time profile for netupitant and its metabolites (NETU-07-01)



* After single dose administration of 450 mg netupitant

2.2.5.8 What are the characteristics of drug excretion?

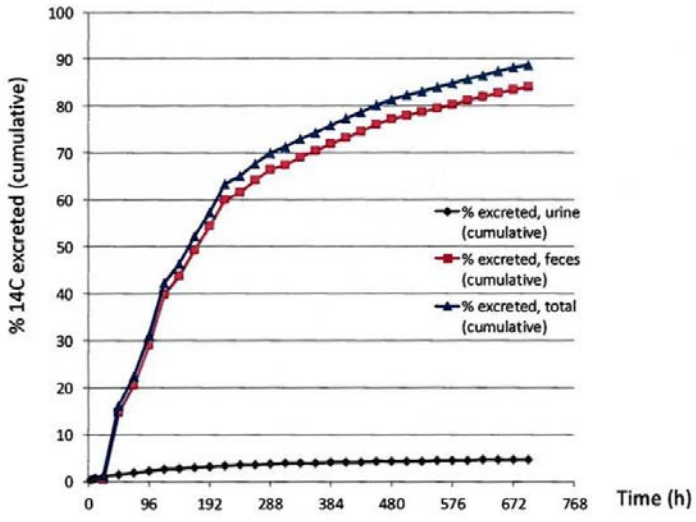
Following oral administration, netupitant is mainly excreted via feces over prolonged period of time. Netupitant and its metabolites are primarily eliminated through hepatobiliary route. A single oral dose of [14C]-Netupitant (187-264 mg) as oral suspension was administered to 6 healthy male subjects in an ADME study. Blood, urine and feces were collected up to 336 h post-dose (Day 15).

Approximately 50% of the administered radioactive dose was recovered within 120 h post-dose and an average of 73% of administered radioactivity was recovered within 336 h: 70% from the feces and 4% from the urine. Thirteen phase I and II metabolites (glucuronic acid derivatives) were identified in the urine within 192 h collection time and about 30 metabolites were identified in the feces. Netupitant and metabolites M1 and M3 were not detected in the urine collected over 336 h. Excretion in urine over 192 h post-dose was mainly represented by metabolites accounting for approximately 3% of the radioactive administered dose.

The contribution of renal excretion of unchanged netupitant to the total clearance is negligible. After single dose of 450 mg netupitant, unchanged netupitant was not detectable in most of urine sample collected over 120 hours from 18 healthy subjects. In 5 out of 18 subjects, netupitant was detectable in urine collected within 48 hours and the renal clearance of netupitant ranged 0.07-0.65 ml/min (4.2-39 ml/h) in those subjects. (NETU-06-27)

Since the recovery of the radioactivity was less than 90% at 336 h, subjects were required to collect feces samples for an additional period (456 to 480 h) at home, and both fecal and urine samples for an additional period (672 to 696 h) in the clinic.
Including the extrapolated values (based on the assumption that the excretion was proceeding at a steadily decreasing rate for the periods 336 to 456 h and 480 to 672 h), the total drug-related material excreted by 696 h post-dose via the feces was estimated to be 86.5%; a mean of 4.7% of drug-related material was estimated to have been excreted in the urine in the same time period (Figure 12).

Figure 14 Recovery of of Radioactive Components in urine and feces after Administration of a Single Oral Dose of [14C]-Netupitant*



*Values during the time from 336 to 456 h and from 480-672 h were obtained by extrapolation taking the mean value of recoveries estimated in the collection intervals just prior to and just after the missing collection period.

Biliary excretion of netupitant was observed in animals. Netupitant is excreted into bile in bile duct cannulated rat and dog. In rats, the biliary excretion accounted for 12 to 40% of the dose (4 and 2 mg/kg, oral and IV, respectively) after 96 h post dosing and less than 1% of the dose was found in urine. The unchanged netupitant accounted for 27 to 40% of the total biliary drug-related material and the main metabolites M1 (7-13%), M2 (5-12%), M3 (9-13%) and M8 (2.5-7%) were observed. (Report 1009719).

In bile cannulated dogs treated with a single oral (6 mg/kg) and intravenous (2 mg/kg) administration the biliary excretion after 72 hours accounted for 30-39% and 59% of the oral and intravenous dose, respectively. The unmetabolized netupitant accounted for 2 to 4% of the total biliary drug-related material while metabolites M2 accounted for 30-43%. (Report 1009870)

In some netupitant concentration-time profiles, a second peak was observed suggesting that netupitant may undergo the enterohepatic circulation.

2.2.5.9 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

The plasma exposure to netupitant increased with dose in a slightly supra-proportional fashion at lower doses 100 mg to 300 mg, but showed dose- proportionality at higher doses from 300 mg to 450 mg (Table 18).

Table 18 ***Mean (± SD) Pharmacokinetic Parameters of Netupitant after single and multiple dose administration in healthy subjects (NP16601)***

Dose	Single dose (n=8)		Multiple doses (n=8)		
mg	Cmax (ng/ml)	$AUC_{0\text{-}23\ 5}$ (ng*h/ml)	Cmax (ng/ml)	$AUC_{0\text{-}23\ 5}$ (ng*h/ml)	AUCinf (ng*h/ml)
100	111 (23)	1360 (22)	269 (19)	4160 (24)	21400 (29.7)
300	599 (38)	6400 (26)	1060 (19)	17100 (17)	93800 (32.6)
450	720 (35)	9670 (35)	1790 (43)	28800 (45)	139000 (46.4)

[1]Accumulation Factor

Similarly, when a single dose netupitant/palonosetron combination was administered at 200 mg/0.5 mg and 600 mg/1.5 mg combination, a dose-proportional increase in mean AUC and Cmax was observed for both netupitant and palonosetron (Table 19).

Table 19 Mean (± SD) PK parameters for netupitant and palonosetron after co-administration of NETU 600 mg and PALO 1.5 mg in healthy subjects

Dose (mg)	Netupitant (n=48)			Palonosetron (n=49)		
Netu/palo	Cmax (ng/ml)	Tmax[1] (h)	$AUC_{0-47\ 5}$ (ng*h/ml)	Cmax (ng/ml)	Tmax[1] (h)	$AUC_{0-47\ 5}$ (ng*h/ml)
200/0.5	253.9 ± 122	4.1 (2.1-10.2)	4587 ± 1939	849 ±248.8	6.2 (2.2-8.2)	23219 ±5905
600/1.5	816.2 ±456.6	5.2 (4.2-10.1)	14369 ±6720	2648 ±596.8	4.2 (1.2-14.2)	69178 ±13978

[1]Median (min-max)

2.2.5.10 Do the PK parameters change with time following chronic dosing?

AKYNZEO® is developed for a single dose administration at 1 hour prior to the initiation of chemotherapy.

The PK parameters did not significantly change after multiple doses. The median half-life and apparent clearance for netupitant was similar to that after single dose. The systemic exposure to netupitant exposure was approximately 3-fold higher compared to that after single dose in consistent with the long half-life. The degree of accumulation for metabolites M1 and M3 was greater than netupitant while lower degree of accumulation for metabolite M2 was noted compared to netupitant (Table 20).

2.2.5.1 What is the variability of PK parameters in volunteers and patients?

Moderate variability of PK parameters of netupitant was observed with % CV of 25-60% in healthy subjects across studies.

According to population PK analysis, CV% for clearance and the central volume of distribution was 65.4% and 43.5%, respectively in cancer patients showing similar degree of variability with those in healthy subjects. The CV% for other PK parameters was not estimated in the population PK analysis. There is no difference in pharmacokinetics for HV and patients.

Table 20 Arithmetic Mean (%CV) PK Parameters of Netupitant and Metabolites After Single and Daily Treatment for 7 Days (NP16601)

Dose	Day 1 (n=8)				Day 7 (n=8)				
	Cmax (ng/mL)	$AUC_{(0-23.5)}$ (h.ng/mL)	CL/F (L/h)	T1/2 (h)	Cmax (ng/mL)	$AUC_{(0-23.5)}$ (h.ng/mL)	CLss/F (L/h)	T1/2 (h)	Accumulation index
	Netupitant								
100mg	111 (23.1)	1360 (21.6)	NC	NC	269 (19.4)	4160 (24)	25.0 (26.5)	76.1 (22.9)	3.1 (12)
300mg	599 (38)	6400 (26.5)	NC	NC	1060 (19)	17100 (16.6)	17.8 (19.5)	82.5 (27.3)	2.7 (12.2)
450mg	720 (35.4)	9670 (34.9)	NC	NC	1790 (43.1)	28800 (45.1)	17.7 (37.2)	78.5 (26.8)	2.9 (0.41)
	Metabolite M1								
100mg	12.7 (17.6)	220 (37.1)	NC	NC	61.3 (22.5)	1280 (24)	NC	80.7 (23)	5.8 (1.1)
300mg	37.5 (29.2)	618 (22.5)	NC	NC	198 (14)	4230 (27.7)	NC	82.6 (26.4)	7.1 (1.6)
450mg	58.8 (23.4)	1040 (19.9)	NC	NC	328 (21.2)	6770 (23.3)	NC	104 (28.5)	6.5 (0.85)
	Metabolite M2								
100mg	25.3 (33.5)	220 (27.5)	NC	NC	27.2 (35.3)	308 (28.2)	NC	125 (83.7)	1.4 (8.5)
300mg	116 (26.6)	891 (29.9)	NC	NC	110 (16.4)	1240 (15.7)	NC	87.2 (43.6)	1.5 (26.8)
450mg	1783 (29.1)	1320 (24.7)	NC	NC	171 (29.0)	1850 (20.3)	NC	77 (29.7)	1.4 (12.1)
	Metabolite M3								
100mg	15.9 (11.3)	292 (12.6)	NC	NC	59.7 (22.3)	1210 (23.6)	NC	61.3 (20.9)	4.1 (19.8)
300mg	49.6 (14.4)	869 (13.4)	NC	NC	219 (20.8)	4360 (18.7)	NC	83.3 (31)	5.1 (25.3)
450mg	89.8 (36.5)	1560 (29.0)	NC	NC	360 (35.4)	7020 (26.4)	NC	80.6 (26.1)	4.5 (13.2)

2.3 Intrinsic Factors

2.3.1 What intrinsic factors influence PK and/or response and what is the impact of any differences in exposure on efficacy or safety responses?

The effects of age, gender, and hepatic impairment on the systemic exposure were evaluated in dedicated PK studies as well as in a subset of patients during phase 3 trial by a population PK approach.

Age

In Study NETU-10-12 the effect of age on the pharmacokinetic parameters of netupitant and palonosetron (FDC) was evaluated by comparing 22 healthy adult subjects aged between 22 and 45 years with 12 healthy elderly subjects, aged between 66 and 79 years. In elderly subjects, the AUC0-∞, AUC0-tz, and Cmax increased by 25%, 13%, and 36% for netupitant and by 37%, 34%,

and 10% for palonosetron, respectively compared to those in younger adults (Table 21, Table 22).

Table 21 Comparison of Systemic Exposure to netupitant between Young and Elderly subjects after single dose administration of AKYNZEO (NETU-10-12)

Parameter	Young Subjects (22-45 yr)		Elderly Subjects (66-79 yr)	
	N	Mean (SD)	N	Mean (SD)
C_{max} [ng/mL]	22	596.4 (233)	12	880.8 (479.2)
AUC_{0-t} [ng.h/mL]	22	17150 (6122)	12	19604 (6747)
$AUC_{0-\infty}$ [ng.h/mL]	22	20039 (8396)	12	24739 (9390)
Vz/F[L]	22	2851 (1633)	12	4101 (5406)
CL/F [L/h]	22	20.5 (10.8)	12	18.7 (12.5)
$T_{1/2}$ [h]	22	101.2 (52.8)	12	129.6 (72.7)

Table 22 Comparison of PK parameters for netupitant and palonosetron after AKYNZEO administration between Healthy Young and Elderly subjects

(A) Netupitant

Pharmacokinetic Parameter for	Point estimate [%]	90% Confidence Interval [%]
C_{max}	136.36	95.87 - 193.96
AUC_{0-tz}	113.42	87.66 - 146.75
$AUC_{0-\infty}$	124.91	95.29 - 163.75

(B) Palonosetron

Pharmacokinetic Parameter for	Point estimate [%]	90% Confidence interval [%]
C_{max}	110.44	95.96 - 127.11
AUC_{0-tz}	133.81	114.28 - 156.68
$AUC_{0-\infty}$	136.89	117.44 - 159.56

*Point estimate (PE): ratio of geometric means (Parameter for the elderly/Parameter for the young)

Gender

In an exploratory analysis of pooled data of phase 1 PK studies (112 males and 41 females), a trend of 35% higher Cmax for netupitant in female and comparable AUCt for netupitant between males and females was noted (Table 23). Similarly to the previous observation, palonosetron Cmax and AUCT was 30% and 36% higher in females, respectively than in males. About 30% higher Cmax for netupitant was noted than in males. There was no significant difference for AUC of netupitant by gender.

The safety of mildly increased Cmax to netupitant and palonosetron in females was studied in Study NETU-08-18 and NETU 10-29 in which 98% of patients were female patients with breast cancer. More male patients were included in NETU-07-07 (56.6% males vs. 43.4% females at 300 mg) while the overall number of male patients exposed to 300 mg netupitant is significantly low compared to the number of female patients.

Table 23 Comparison for mean systemic exposure to netupitant by gender with 300 mg netupitant in individual studies after administration

Parameter [Unit]	Female Subjects		Male Subjects		Source
	No. of PK profiles (n° of subjects)	Mean (SD)	No. of PK profiles (n° of subjects)	Mean (SD)	
Cmax [ng/mL]	48 (24)	472.5 (285.0)	46 (23)	394.0 (182)	NETU-09-07^
	34 (17)	608.0 (286.9)	130 (65)	413.7 (206.5)	NETU-11-02 T^
	34 (17)	659.2 (301)	130 (65)	441.8 (240.3)	NETU-11-02 R^
AUC0-t [ng/mL x h]	48 (24)	12075 (5561)	46 (23)	12579 (4864)	NETU-09-07^
	34 (17)	13221 (4516)	130 (65)	12609 (4995)	NETU-11-02 T^
	34 (17)	14703 (5147)	130 (65)	13346 (5878)	NETU-11-02 R^
T1/2 [hours]	48 (24)	113.7 (78.4)	46 (23)	79.7 (25.6)	NETU-09-07^
	34 (17)	95.7 (38.4)	130 (65)	72.5 (24.7)	NETU-11-02 T^
	34 (17)	92.6 (33.5)	130 (65)	74.3 (28.2)	NETU-11-02 R^

T = test, R= reference

Hepatic impairment

The effect of hepatic impairment on PK of netupitant and palonosetron was studied after administration of administration of AKYNZEO (NETU-10-10).

Currently no dosage adjustment is recommended for palonosetron by hepatic impairment based on the clinical experiences with higher doses while the mean AUC for palonosetron was 35% and 55% higher in patients with mild and moderate hepatic impairment, respectively compared to that in healthy subjects after single dose AKYNZEO administration (Table 24).

The increase in the systemic exposure to netupitant and palonosetron in patients with hepatic impairment was observed (Table 25, Figure 13).

The mean AUC of netupitant was 58% and 101% higher in patients with mild and moderate hepatic impairment, respectively than in healthy subjects. The Cmax of netupitant was about 30% higher in patients with mild and moderate hepatic impairment. Only two patients with severe hepatic impairment provided PK data. In one patient with severe hepatic impairment, Cmax and AUC of netupitant were about 2- and 6-fold higher, respectively while Cmax and AUC of palonosetron were about 2- higher than the mean for control group.

Table 24 Geometric mean and ratio of PK parameters for netupitant in subjects with hepatic impairment and healthy subjects

Hepatic impairment	Normal1 N=18	Mild (n=8)	Moderate (n=8)	Severe (n=2)	Mean ratio[%] mild/ normal	Mean ratio[%] moderate/ normal
Cmax	288	374	377	469.6, 1336	130	131
AUC0-∞	11712	18475	24282	26857, 70952	158	207

PK blood samples for netupitant were collected up to 240 hours post-dose

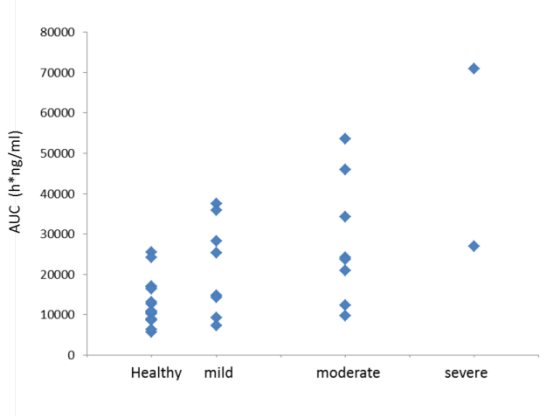
Table 25 Geometric mean of PK parameters for palonosetron in subjects with hepatic impairment and healthy subjects

Hepatic impairment	Normal1 N=18	Mild (n=8)	Moderate (n=8)	Severe (n=2)	Mean ratio[%] mild/ normal	Mean ratio[%] moderate / normal
Cmax	511	753	687	399, 1018.5	147	135
AUC0-∞	32210	38913	49819	32028, 69538	135	155

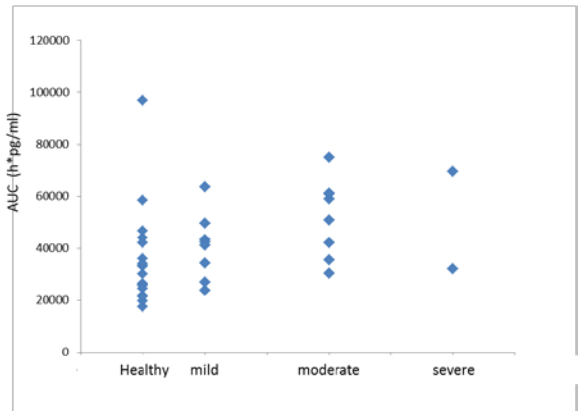
PK blood samples for palonosetron were collected up to192 hours post-dose.

Figure 15 Individual AUCinf for (A) netupitant and (B) palonosetron in patients with hepatic impairment and in healthy subjects

(A) netupitant



(B) palonosetron



Reviewer's comments:

The sponsor calculated the ratio of PK parameters between subjects with hepatic impairment and matching control group i.e. one group for mild hepatic impairment and another group for moderate hepatic impairment. Upon review of the data, the demographic information such as age and gender was similar across control groups to different degree of hepatic impairment while the PK parameters for netupitant showed differences among controls due to the variability across groups. This variability between control groups confounded the evaluation of the effect of hepatic impairment on the PK of netupitant. Therefore PK parameters from patients with hepatic impairment were compared to the pooled control group. One healthy subject had a substantially high AUC for netupitant, that was similar to the highest AUC observed in a patient with severe hepatic impairment. The AUC was not considered reliable due to ~75% extrapolation for AUCi and excluded from the control group.

Renal impairment

In previous study, mild to moderate renal impairment did not significantly affect palonosetron pharmacokinetic parameters.

The pharmacokinetics of neither palonosetron nor netupitant was studied in subjects with end-stage renal disease.

There was no dedicated PK study to evaluate effect of renal impairment on PK for netupitant. On the other hand, that no significant effects of mild and moderate renal impairment was noted in the population PK analysis. Please see the Pharmacometrics Review by Dr. Jingyu Yu for more details.

A minor contribution of renal clearance to total clearance for netupitant was shown by minimal urinary excretion of unchanged netupitant in urine. About 4 % of administered dose was excreted in urine over 366 h in the ADME study and negligible amount of unchanged netupitant (<1% of administered dose) was detectable in urine samples from a subset of subjects after 450 mg netupitant dose.

The incidence of TEAEs was analyzed by creatinine clearance in phase 3 trials. There was an increasing trend of incidence of TEAEs regardless of treatment as the creatinine clearance

decreased (Table 26). Nevertheless the significant difference in the number of patients by renal function hampers a definitive conclusion.

Briefly, per protocol patients with severe renal impairment or patients on dialysis were not included in the multi-cycle Phase 3 studies. The proportion of patients with normal renal function was 64.3% (1198/1862) while the proportion of patients with mild and moderate renal impairment was 31.1% (580/1862) and 4.3% (80/1862), respectively. Patients with moderate renal impairment tended to be older with mean age of 64.6 years compared to patients with normal and mild renal impairment with mean age of 51.9 and 58.5 years, respectively. Other demographic characteristics were similar among groups by renal function. The detailed review of safety by renal function is deferred to the clinical reviewer.

Table 26 Incidence of TEAE in multi-cycle Phase 3 trials by creatinine clearance

	Treatment		
Renal function	Netu/Palo (300/0.5)	Palo 0.5 mg	Aprepitant/palo
Normal (90 ml/min ≤ CLcr)	89.5% (n/N= 598/668)	88.4% (421/476)	92.6% (50/54)
Mild (60 ml/min ≤ CLcr < 90 ml/min)	91.5% (280/317)	88.1% (192/218)	88.9% (40/45)
Moderate (30 ml/min ≤ CLcr <60 ml/min)	93.3% (42/45)	93.3% (28/30)	100% (5/5)

Source: Table 7.2.1 in Integrated Summary of Safety

2.3.2 Based upon what is known about exposure-response relationships and their variability, and the groups studied, what dosage regimen adjustments, if any, are recommended for each of these groups?

Akynzeo® is proposed to be available as a fixed dose combination product consisted of 300 mg netupitant and 0.5 mg palonosetron. No other strengths will be available. As such the dosage adjustment for one component is not a feasible option. Currently no dosage adjustment is recommended for the approved palonosetron products based on organ impairment or other factors.

2.3.2.1 Elderly

No significant need for dosage adjustment for elderly patients.

2.3.2.2 Pediatrics

No studies were conducted in pediatric patients. A request for a (b) (4) for pediatric studies has been submitted with this application.

2.3.2.3 Gender

No need for dosage adjustment for gender.

2.3.2.4 What are the covariates affecting the PK of netupitant based on population PK analysis?

Please see the Pharmacometrics review by Dr. Jingyu Yu in Appendix for more detail.

Based on the population PK analysis, there appears to be no effect of race, age and body weight on PK of netupitant. The population PK analysis also suggested that there appears to be no statistically significant effect of mild and moderate renal impairment on the clearance of netupitant. This is expected since renal pathway is a minor route of elimination for netupitant.

The covariates for netupitant including body weight, BMI, age, race, smoking status, markers of cardiac, renal and liver function were evaluated for their potential influence on CL and volume of distribution (V2) in cancer patients in a population PK analysis performed during a comparative Phase 3 trial in 117 subjects in the FDC PK subgroup.

A two- compartment base model with first order absorption adequately described the observed PK data of netupitant. The median netupitant apparent clearance was estimated to be 20.9 L/h and the volume of distribution to the central compartment was estimated to be 419 L. Based on sponsor's analysis, none of the covariates had significant impact on the PK of netupitant based on population PK analysis.

However, it is worth noting that for some intrinsic and extrinsic factors, population PK analysis is either supportive or limited. For example, only 4 male subjects were included in the population PK analysis as the patients enrolled in phase 3 trial is predominantly female patients with breast cancer. Therefore the effect of gender on PK cannot be evaluated in the population PK analysis. The impact of the smoking status, chemotherapy (doxorubicin, epirubicin, fluorouracil) and rescue medications on the PK of netupitant in combination with palonosetron were evaluated by population PK analysis. None of those factors appears to significantly influence the disposition of netupitant and palonosetron. However, it should be noted that definitive conclusions regarding these factors cannot be made as the population PK analysis may lack power to detect the effect of these factors due to the study design and/or insufficient PK sampling.

2.3.2.5 Renal Impairment

Currently no dosage adjustment for palonosetron alone treatment in patients with renal impairment is not recommended. Given the minimal contribution of renal excretion to the total body clearance of netupitant and Akynzeo will be used as a single dose administration, the possibility of accumulation of netupitant in patients with renal impairment is minimal. However, because the PK and safety data is limited for netupitant in patients with moderate to severe renal impairment and patients on dialysis, Akynzeo should be used with caution in patients with severe renal impairment and with end-stage renal impairment.

2.3.2.6 Hepatic Impairment

Currently dosage adjustment for palonosetron by hepatic impairment is not recommended.

Because only limited information is available for use of netupitant in patients with severe hepatic impairment, Akynzeo should be used with caution in patients with severe hepatic impairment if the

co-administration deemed necessary.

Limited information is available for the systemic exposure for netupitant higher than the observed in patients with moderate renal impairment.

The observed Cmax and AUC for netupitant and palonosetron in patients with mild and moderate hepatic impairment is lower or similar within the exposure at 600 mg in the tQT study (n=47). On the other hand in a multiple dose PK study with 450 mg once daily for 7 days (n=8), the Cmax and AUC for netupitant was 2-fold and > 5-fold higher than those after single dose administration of 300 mg.

2.3.2.7 What pregnancy and lactation use information is there in the application?

PK were not evaluated in pregnant women or lactating females. Given the target patient populations are under chemotherapy and Akynzeo would be given as a single dose, it is unlikely that patients would be pregnant or lactating at the time of Akynzeo administration. Nevertheless because of the long half-life of netupitant and palonosetron, lactation should be avoided at least for a month after Akynzeo administration.

2.3.2.8. What other human factors are important to understanding the drug's efficacy and safety?

The patients enrolled in the clinical trials were stratified by gender as female gender is known to be more susceptible to emesis. The emetogenicity is considered to be dependent on chemotherapy regimen such as highly-emetogenic chemotherapy and moderately emetogenic chemotherapy. The categorization may be revised based on clinical observations.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors influence dose- exposure and what is the impact of any differences in exposure on response?

Coadministration of rifampin, a strong CYP3A4 inducer reduced the Cmax and AUC to netupitant component of AKYNZEO by 62% and 82%, respectively. In the dose-finding study, the combination of 100 mg netupitant or 200 mg netupitant with 0.5 mg palonosetron did not show significant difference from palonosetron monotherapy for the prevention of CINV during acute phase while statistically significant difference was demonstrated during delayed phase. The combination with 300 mg netupitant showed numerically higher CR rate than the combination with 200 mg netupitant during acute phase (98% vs. 92%) although the study was not designed to show the difference among doses.

2.4.2 Drug-Drug Interactions

2.4.2.1 Is there an *in vitro* basis to suspect *in vivo* drug-drug interactions?

In *in vitro* studies, netupitant is a substrate and an inhibitor of CYP3A4. The sponsor has conducted follow-up *in vivo* studies with a strong CYP3A4 inhibitor ketoconazole, a CYP3A4 inducer rifampicin and a CYP3A4 substrate midazolam.

In addition, netupitant is an inhibitor of P-gp and BCRP transporters. The sponsor conducted a follow-up *in vivo* study with P-gp substrate Digoxin and have shown that netupitant do not alter the exposure of digoxin significantly when administered concomitantly. However, netupitant's potential interaction with BCRP was not evaluated *in vivo*. Since no significant *in vivo* inhibitory effect of netupitant on BCRP transporter is anticipated based on *in vitro* data with weak inhibition toward BCRP and negative *in vivo* inhibition data with P-gp substrate digoxin, we do not request an additional *in vivo* study to evaluate the potential of netupitant to inhibit BCRP.

2.4.2.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?

The sponsor conducted two *in-vitro* studies to identify the enzyme responsible for netupitant metabolism (study 103832 and study NETU-13-21). It appear that the metabolism of netupitant to M1, M2 and M3 is mainly mediated by CYP3A4 and lesser extent by CYY2C9 and CYP2D6 (Table 27 and Figure 14).

As netupitant is primarily metabolized by CYP3A4, the sponsor had conducted follow-up *in vivo* drug-drug interaction studies with a strong CYP3A4 inhibitor ketoconazole and a CYP3A4 inducer rifampicin. Since both CYP2C9 and CYP2D6 have polymorphism, metabolism of netupitant could be influenced by genetics.

Study 103832:

In the first study (study 103832), 10 μM netupitant was incubated with human hepatocytes for 24 hours and with human liver microsomes (HLM) for 20 minutes, and the supernatant was analyzed with HPLC to characterize the metabolic pathway of netupitant. Following the incubation of netupitant, both human hepatocytes and liver microsomes had resulted two metabolites of netupitant, M1 and M2. However, both test systems, hepatocytes and liver microsome, were not properly validated in terms of various CYP enzymes (both phase 1 and phase 2) prior to the use or have proper controls (positive or negative) during the incubations. Therefore, it is difficult to interpret the data from this metabolism study in hepatocytes and liver microsomes.

The contribution of different microsomal CYP450 enzymes to the metabolism of netupitant (RO0673189) was studied by utilizing recombinant human enzymes. The enzymes were expressed in E. coli and isolated as a membrane fraction. The radiolabeled netupitant at 5 μM (1 μM for CYP2C9) was incubated with four of the major human CYP450 isoenzymes (CYP3A4, CYP2C9, 2C19 and 2D6) at 100 to 600 pmol CYP450/ml and the incubations were initiated by the addition of NADPH (1 mM). After the incubation for 30-60 min at 37°C, the reaction was terminated, and the supernatant was analyzed with HPLC. The results of this study suggested that CYP2C9, 2C19 and 2D6 do not catalyst the formation of any metabolite of netupitant while CYP3A4 appears to metabolize netupitant to the same metabolites (M1 and M2) that were observed when netupitant was incubated with human liver microsomes and hepatocytes. However, the sponsor did not evaluate the potential of CYP1A2, 2B6 and 2C8 to metabolize netupitant in this study.

Study NETU-13-21

In this second study, 10 μM netupitant was incubated with human liver microsomes (0.8 mg/ml) in the presence and absence of different selective CYP isoform inhibitors and with recombinant CYP 1A2, 2B6 and 2C8 (20 pmol P450) in pH 7.4 buffer for 60 minutes at 37°C in duplicates. Specific probe substrates for each enzyme were incubated as positive controls to validate the metabolic activities of the test systems used (human liver microsomes and cDNA expressed enzymes).

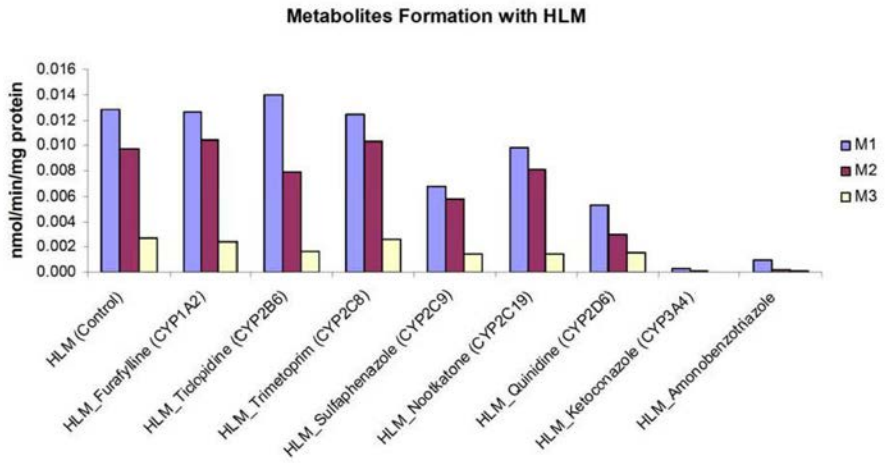
Based on the results of this inhibition study in human microsome, it appear the metabolism of netupitant to M1, M2 and M3 is mainly mediated by CYP3A4 and lesser extent by CYP2C9 and CYP2D6. In addition, CYP1A2, CYP2B6, CYP2C8 and CYP2C19 do not appear to contribute to the metabolism of netupitant. Study in CYPIA2, 2B6 and 2C8 cDNA expressed enzymes further confirms that these enzymes do not contribute to netupitant metabolism.

Table 27 Netupitant disappearance in Human Liver Microsomes and cDNA CYPs expressed systems in the presence and in the absence of CYP's inhibitors

Netupitant						
Matrix	Inhibitor	$T_{1/2}$ (min)	$T_{1/2}$ Mean	Cl_{int} (μL/min/mg)	Cl_{int} Mean	Comment
HLM	--	23.31	27.07	37.18	32.64	
		30.83		28.10		
HLM	Furafylline (CYP1A2)	25.38	24.10	34.13	36.06	
		22.81		37.98		
HLM	Ticlopidine (CYP2B6)	29.07	27.99	29.81	31.00	
		26.91		32.20		
HLM	Trimetoprim (CYP2C8)	17.34	18.02	49.98	48.16	
		18.69		46.35		
HLM	Sulfaphenazole (CYP2C9)	164.35	183.72	5.27	4.77	
		203.09		4.27		
HLM	Nootkatone (CYP2C19)	11.91	15.41	72.78	59.28	
		18.92		45.78		
HLM	Quinidine (CYP2D6)	59.13	62.31	14.65	13.94	
		65.49		13.23		
HLM	Ketoconazole (CYP3A4)	424.65	560.42	2.04	1.64	
		696.18		1.24		
CYP1A2 Supersomes	--	167.05	207.87	0.21*	0.17*	Non Metabolic Decline
		248.69		0.14*		
CYP2B6 Supersomes	--	83.64	119.74	0.41*	0.32*	Non Metabolic Decline
		155.85		0.22*		
CYP2C8 Supersomes	--	56.31	61.30	0.62*	0.57*	Non Metabolic Decline
		66.29		0.52*		
CYP1A2 Supersomes	Furafylline (CYP1A2)	99.36	132.83	0.35	0.28*	Non Metabolic Decline
		166.29		0.21		
CYP2B6 Supersomes	Ticlopidine (CYP2B6)	103.80	88.27	0.33*	0.41*	Non Metabolic Decline
		72.74		0.48*		
CYP2C8 Supersomes	Trimetoprim (CYP2C8)	32.77	26.94	1.06*	1.35*	Non Metabolic Decline
		21.11		1.64*		
HLM	Amonobenzotriazole	165.60	216.60	5.23	4.23	
		267.61		3.24		

* Clint expressed as μL/min/pmolP450

Figure 16 Netupitant metabolites formation rate in HLM system



2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?

Netupitant up to 20 μM and M1, M2 and M3 up to 2 μM are not considered to be inducers of CYP1A2, CY2C9, CYP2C19 and CY3A4/5 enzyme. The sponsor did not evaluate the potential of netupitant and its metabolites M1, M2 and M3 to induce CYP2B6.

Based on *in vitro* study, netupitant and its metabolite M1 are considered to be CYP3A4 inhibitors. The sponsor did conduct a follow up *in vivo* study with CYP3A4 substrate midazolam.

Netupitant did not inhibit CYP1A2, CYP2C19, and CYP2D6 *in vitro*. *In vivo* drug interactions via inhibition of CYP2B6, 2C8 and 2C9 at the clinical dose of 300 mg are unlikely based on weak inhibition of toward these enzymes in *in vitro* studies.

M1 showed inhibition toward CYP 2B6, 2C8, 2D6, 3A4, and weak inhibition toward CYP 1A2, 2C9, 2C19 in *in vitro* studies. However, since Cmax/Ki >0.1 for only CYP3A4, *in vivo* drug interaction via M1 inhibition toward CYP enzyme is unlikely except for CYP3A4.

M2 and M3 showed weak inhibition toward all major CYP enzymes. Since Cmax/Ki<0.1 for all enzymes, *in vivo* drug interaction via M2 and M3 inhibition toward CYP enzyme is unlikely.

Induction (Study NETU-10-27):
Hepatocytes from three different donors were incubated with 0.2, 2 and 20 μM netupitant or 0.02, 0.2 and 2 μM of M1, M2 and M3 or positive control inducers (omeprazole for CYP1A2 or rifampicin for CYP2C9, 2C19 and 3A4) for 72 hours at 37^{o}C in duplicates. The exposure medium was refreshed every 24 hours. In addition, two wells were left untreated to determine the basal CYP1A2, CYP2AC9, CYP2C19 and CYP3A4 activities of the hepatocytes as negative control. At the end of the 72 hours of incubation period, the activities of target enzymes CYP1A2, CYP2C9, CYP2C19 and CYP3A4 were assessed by incubating the hepatocytes with model substrates (Phenacetin for CYP1A2, tolbutamide for CYP2C9, S-mephenytoin for CYP2C19 and

midazolam for CYP3A4) for each target enzymes and measuring the appearance rate of their respective metabolites. Prior to the use, the hepatocytes were characterized by the supplier in respect to various phase I (CYP1A2, CYP3A4/5, CYP2B6, CYP2D6, CYP2C19) and phase II (glucuronidation and sulfation) enzyme activities.

Netupitant, M1, M2 and M3 did not induce CYP1A2, CY2C9, CYP2C19 and CY3A4/5 enzyme activities when hepatocytes from three different human donors were treated with netupitant up to 20 μM concentration and M1, M2 and M3 up to 2 μM concentrations after 72 hours of incubation based on induction threshold of 40% of the positive control.

Inhibition (Study 1003907):
Human liver microsomal (pooled from 10 human livers) protein was incubated with netupitant (0, 0.5, 1, 10, and 100 μM) and the corresponding selective model substrates at 37°C in the presence of NADPH generating system for a specified duration of incubation time. No pre-incubation was carried out to assess the time-dependent inhibition.
The enzymatic reactions were terminated by addition of methanol or acetonitrile. The inhibition potential of metabolites of netupitant (RO0673189), namely RO0681133 (M1) and RO0713001 (M2) were evaluated for CYP3A4 enzyme only. However, this study did not contain positive controls with known inhibitors to validate the test system regarding CYP enzymes activities. Nonetheless, the activity of CYP enzymes toward model substrates in the absence of netupitant as inhibitor was within the historical data observed.

Table 28 Inhibition of CYP Enzyme by netupitant and its metabolites

CYP450 isoenzyme	**CYP1A2**	**CYP2C9**	**CYP2C19**	**CYP2D6**	**CYP3A4**	**CYP3A4**	**CYP3A4**	**CYP3A4**
Substrate used	Tacrine	Diclofenac	Mephenytoin	Bufuralol	Midazolam	Testosterone	Nifedipine	Simvastatin
Substrate conc (μM)	25	5	32.8	40	5	20	20	3
HLM conc. (mg/ml)	0.5	0.1	1	1	0.1	0.075	0.2	0.02
Incubation Time (min)	12	5	30	30	10	20	10	5
IC50 (μM)								
RO0673189 (netupitant)	>>100	22.6 ± 3 18.0 ± 6	>100	>>100	5.9 ± 1.0	1.7 ± 0.2	12.0 ± 0.5	10.5 ± 0.8
RO0681133 (M1)						1.2 ± 0.5		
RO0713001 (M2)						> 1 μM		

Table 29 Inhibition of CYP450 metabolism by netupitant; apparent Ki

CYP450 isoenzyme	**CYP2C9**	**CYP3A4**	**CYP3A4**
Substrate used	Diclofenac	Testosterone	Midazolam
Substrate conc.(μM)	2, 5, 10, 50	5, 10, 20	2, 5, 10, 50
HLM protein conc.(mg/ml)	0.1	0.075	1
Inhibitor conc. (μM)	0, 0.1, 0.5, 1, 5, 10	0, 0.2, 0.5, 1	0, 0.1, 0.5, 1, 5, 10
apparent Ki (μM)	25.0 ± 7.4	1.1 ± 0.2	2.2 ± 0.6
Inhibition mechanism	competitive	competitive	competitive

Netupitant, at concentration 0-100 μM, did not inhibit enzymes CYP1A2, 2C19 and 2D6 (IC50 >100 μM).

Netupitant had shown weak inhibition toward CYP2C9 with approximate IC50 value of 22.6 ± 3 μM. Further studies with different concentration of model substrate had shown that netupitant's inhibition of CYP2C9 is through competitive inhibition with Ki value of 25 ± 7.4 μM. Since Cmax/Ki= 1.5 μM / 25 μM= 0.06<0.1, clinical *in vivo* relevance of this interaction with CYP2C9 is less likely.

The inhibitory potential of netupitant for the CYP3A4 enzyme was evaluated with four different model CYP3A4 substrates, testosterone, midazolam, nifedipine and simvastatin. All of the model CYP3A4 substrates had demonstrated that netupitant is an inhibitor of CYP3A4 with IC50 value of 1.7-12 μM. Further studies with different concentrations of testosterone and midazolam had demonstrated that the CYP3A4 inhibition is a competitive inhibition with Ki value of 1.1 μM with testosterone and 2.2 μM with midazolam.

The inhibition potential of netupitant metabolites, namely RO0681133 (M1) and RO0713001 (M2) were evaluated for CYP3A4 enzyme only with testosterone as the model substrate. RO0681133 (M1) appears to be an inhibitor of CYP3A4 with IC50 value of 1.2 μM. Due to solubility issue, RO0713001 (M2) was tested up to 1 μM, and notable inhibition was observed even at 1 μM.

A follow-up *in vivo* study to evaluate the potential of netupitant to inhibit CYP3A4 is recommended for the following reasons:

- Systemic exposure: Cmax/Ki = 1.5 μM /1.1μM = 1.4>0.1
- Gut exposure: $[I]_{gut}$/Ki= 2074 μM / 1.1 μM = 1885>>>10 where $[I]_{gut}$ = dose/250 ml = 300 mg/250ml= 1.2 g/L.

Inhibition (Study NETU-13-20):

Human liver microsomal (pooled from 50 human livers) protein was incubated with test compound (netupitant, M1, M2 and M3) at 0.3, 1, 3, 10, 30 and 100 μM and the corresponding selective model substrates at 37^{o}C in the presence of NADPH generating system for a specified duration of incubation time. No pre-incubation was carried out to assess the time-dependent inhibition(Table 30).

The enzymatic reactions were terminated by addition of ice-cold acetonitrile. The human liver microsomes was characterized in respect CYP enzyme activities prior to the use. In addition, this study included appropriate positive controls with model CYP inhibitors of each CYP isozymes.

Netupitant inhibition was evaluated towards CYP2B6 and CYP2C8. M1, M2 and M3 inhibition was evaluated towards the CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (two substrates).

Table 30 Experimental conditions for CYP inhibition studies

CYP Isoforms	CYP model substrate Concentrations	HLM Protein Amount	Incubation Time	Positive Control
CYP1A2	Tacrine 6 μM	0.1 mg/ml	10 min	α-naphthoflavone 0.01, 0.1, 0.3 μM
CYP2B6	Bupropion 43 μM	0.1 mg/mL	20 min	Ticlopidine 0.1, 1, 3 μM
CYP2C8	Paclitaxel 14 μM	0.1 mg/mL	20 min	Quercetine 0.6, 6, 18 μM
CYP2C9	Diclofenac 7 μM	0.1 mg/ml	10 min	Sulfaphenazole 0.1 , 1, 3 μM
CYP2C19	S-mephenytoin 71 μM	0.2 mg/ml	40 min	Ticlopidine 0.1 , l, 3 μM
CYP2D6	Dextromethorphan 5 μM	0.1 mg/ml	10 min	Quinidine 0.01, 0.1, 0.3 μM
CYP3A4	midazolam 2.1 μM	0.1 mg/ml	10 min	Ketoconazole 0.0l, 0.1, 0.3 μM
CYP3A4	testosterone 43 μM	0.1 mg/ml	20 min	Ketoconazole 0.0l, 0.1, 0.3 μM

Netupitant showed weak inhibition toward both CYP2B6 and CYP2C8 with IC50 values of 33.39 μM and 50.4 μM, respectively. However, since Cmax/Ki are <0.1, no follow-up *in vivo* study is recommended (table 31).

A metabolite, M1 showed inhibition toward CYP 2B6, 2C8, 2D6, 3A4, and weak inhibition toward CYP 1A2, 2C9, 2C19. However, since Cmax/Ki >0.1 for only CYP3A4, an *in vivo* study is recommended for CYP3A4. The sponsor had already conducted *in vivo* DDI study with netupitant concomitantly administered with CYP3A4 substrate midazolam.

Metabolites, M2 and M3 showed weak inhibition toward all evaluated CYP enzymes. Since Cmax/Ki<0.1, no *in vivo* follow up study is needed.

Table 31 [I]/Ki values of metabolites for CYP isoforms

		IC_{50} (μM)	Ki	Cmax Range (μM)		Cmax/Ki Range	
Netupitant	CYP2B6	32.39	16.2	1	1.5	0.062	0.093
	CYP2C8	50.43	25.22	1	1.5	0.040	0.059
M1	CYP1A2	39.39	19.7	0.07	0.09	0.004	0.005
	CYP2B6	4.89	2.44	0.07	0.09	0.029	0.037
	CYP2C8	4.7	2.37	0.07	0.09	0.030	0.038
	CYP2C9	26.4	13.21	0.07	0.09	0.005	0.007
	CYP2C19	33.26	16.63	0.07	0.09	0.004	0.005
	CYP2D6	8.54	4.27	0.07	0.09	0.016	0.021
	CYP3A4	0.51	0.25	0.07	0.09	0.280	0.360
	CYP3A4	0.7	0.35	0.07	0.09	0.200	0.257
M2	CYP1A2	>100	NA	0.17	0.58	NA	NA
	CYP2B6	23.72	11.86	0.17	0.58	0.014	0.049
	CYP2C8	>100	NA	0.17	0.58	NA	NA
	CYP2C9	>100	NA	0.17	0.58	NA	NA
	CYP2C19	57.45	28.73	0.17	0.58	0.006	0.020
	CYP2D6	58.12	29.06	0.17	0.58	0.006	0.020
	CYP3A4	38.84	19.42	0.17	0.58	0.009	0.030
	CYP3A4	39.04	19.52	0.17	0.58	0.009	0.030
M3	CYP1A2	>100	NA	0.08	0.144	NA	NA
	CYP2B6	23.62	11.81	0.08	0.144	0.007	0.012
	CYP2C8	26.95	13.48	0.08	0.144	0.006	0.011
	CYP2C9	>100	NA	0.08	0.144	NA	NA
	CYP2C19	77.03	38.52	0.08	0.144	0.002	0.004
	CYP2D6	74.97	37.49	0.08	0.144	0.002	0.004
	CYP3A4	10.95	5.48	0.08	0.144	0.015	0.026
	CYP3A4	9.45	4.72	0.08	0.144	0.017	0.031

2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?

Netupitant and its metabolites interaction with transporters were evaluated *in vitro* in Study NETU-06-13 (netupitant's interaction with P-gp) and study NETU-12-81.

Based on *in vitro* studies, parent drug netupitant is an inhibitor of P-gp and BCRP transporter. The sponsor had conducted a follow up *in vivo* study with digoxin to evaluate the P-gp inhibitory effect of netupitnat. Potential of netupitant being substrate of P-gp was not evaluated adequately. In addition, M2 is shown to be a substrate for P-gp. Based on *in vitro* data, *in vivo* interaction of netupitant as substrate for BCRP, OATP1B1, OATP1B3, and OCT1, or as inhibitor of BSEP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2 is unlikely.

In addition, based on the *in vitro* data, *in vivo* interaction of three major metabolites, M1, M2 and M3 as substrates of BCRP, OATP1B1, OATP1B3, and OCT1, or as inhibitors of MDR1, BCRP, BSEP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2 are unlikely.

Efflux Transporters:

Substrate:

The sponsor evaluated the potential of netupitant being a substrate for P-gp in ATPase activation assay, which suggested that netupitant may be a substrate for P-gp. However, the sponsor did not evaluate whether netupitant is substrate for P-gp on bi-directional transport assay system with net flux ratio information or evaluate the permeability of netupitant in the presence of potent P-gp inhibitor to predict the *in vivo* relevance of this interaction in this NDA application.

Potential of M1, M2 and M3 being substrate for P-gp and potential of netupitant, M1, M2 and M3 being substrate for BCRP was evaluated in MDR1 or BCRP transfected MDCKII monolayer cultured cells (table 32 and table 33). Bidirectional transport through monolayers was determined by incubating of test compound at 3, 10 and 30 µM concentration with parental and MDR1/BCRP transfected MDCKII cell monolayers at 37 ± 1 °C. After the incubation, aliquots of samples were taken at 0, 15, 30, 60, 12 minutes from the receptor chambers to determine the amount of translocated test compound. The digoxin (5 µM) / prazosin (1 µM) efflux ratio was determined as a positive control for MDR1/BCRP function. As a follow-up, bidirectional transport of M2 in parental and MDR1 transfected MDCKII cells was determined in the presence and absence of the MDR1 inhibitor PSC833 to confirm the specificity of the transport in MDCKII-MDR1 cells.

Table 32 Net Efflux Ratio from MDCKII-MDR1 studies for metabolites

Test article	Assay	ER		Net ER
		MDCKII	MDCKII-MDR1	MDCKII-MDR1 / MDCKII
M1	bidirectional permeability of M1	3 µM: 0.17 ± 0.64	3 µM: 0.13 ± 0.59	0.74 ± 0.87
		10 µM: 0.13 ± 0.58	10 µM: 0.25 ± 0.28	1.86 ± 0.64
		30 µM: 15.07 ± 0.32	30 µM: 2.37 ± 0.31	0.16 ± 0.44
M2	bidirectional permeability of M2	3 µM: 15.31 ± 0.13	3 µM: 90.88 ± 0.18	5.94 ± 0.22
		10 µM: 0.36 ± 0.28	10 µM: 96.84 ± 0.15	269.52 ± 0.32
		30 µM: 0.45 ± 0.19	30 µM: 2.76 ± 0.33	6.17 ± 0.38
M2+PSC833	bidirectional permeability of M2 in the presence of PSC833	digoxin: 4.16± 0.57	digoxin: 16.62 ± 3.66	4.00 ± 1.03
		+PSC833: 0.98 ± 0.05	+PSC833: 1.26 ± 0.03	1.28 ± 0.07
		M2 (30 µM): 2.42 ± 0.45	M2 (30 µM): 2.61 ± 1.24	1.08 ± 0.55
		+ PSC833: 0.79 ± 0.15	+ PSC833: 1.01 ± 0.17	1.29 ± 0.33
M3	bidirectional permeability of M3	3 µM: 5.92 ± 0.73	3 µM: 0.14 ± 0.44	0.02 ± 0.85
		10 µM: 0.25 ± 0.27	10 µM:0.31 ± 0.36	1.27 ± 0.45
		30 µM: 0.42 ± 0.12	30 µM:0.40 ± 0.12	0.96 ± 0.17

Table 33 Net Efflux Ratio From MDCKII-BCRP Studies for netupitant and its metabolites

Test article	Assay	ER		Net ER
		MDCKII	MDCKII-BCRP	MDCKII-BCRP/ MDCKII
Netupitant	bidirectional permeability of Netupitant	3 µM:0.00 ± 0.41	3 µM:0.01 ± 0.38	1.50 ± 0.56
		10 µM:0.01 ± 0.42	10 µM: 0.03 ± 0.29	2.75 ± 0.51
		30 µM:0.10 ± 0.35	30 µM:1.02 ± 0.21	10.6 ± 0.41
Netupitant		10 µM: 0.27 ± 0.25	10 µM: 0.11 ± 0.30	0.40 ± 0.39
M1	bidirectional permeability of M1	3 µM: 0.00 ± 0.49	3 µM:0.01 ± 0.40	18.59 ± 0.63
		10 µM:0.11 ± 0.39	10 µM: 0.15 ± 0.39	1.32 ± 0.55
		30 µM: 4.18 ± 0.30	30 µM: 3.49 ± 0.34	0.83 ± 0.45
M1		10 µM: 0.57 ± 0.30	10 µM: 1.11 ± 0.30	1.94 ± 0.43
M2	bidirectional permeability of M2	3 µM: 0.13 ± 0.44	3 µM:0.02 ± 0.26	0.15 ± 0.51
		10 µM:0.31 ± 0.26	10 µM: 0.11 ± 0.18	0.36 ± 0.31
		30 µM: 0.34 ± 0.20	30 µM: 1.12 ± 0.04	3.31 ± 0.2
M2		10 µM: 5.95 ± 0.08	10 µM: 0.25 ± 0.21	0.04 ± 0.22
M3	bidirectional permeability of M3	3 µM:0.04 ± 0.38	3 µM: 0.05 ± 0.38	1.11 ± 0.53
		10 µM:0.09 ± 0.37	10 µM:0.22 ± 0.29	2.31 ± 0.47
		30 µM: 0.48 ± 0.17	30 µM: 0.33 ± 0.11	0.69 ± 0.2
M2		10 µM: 1.31 ± 0.10	10 µM: 0.81 ± 0.09	0.62 ± 0.13

As the net flux ratio for M1 and M3 were below 2 at all concentrations, M1 and M3 are not substrate of MDR1. The net flux ratio of M2 for MDR1 was > 2 at all tested concentration. The sponsor further evaluated the potential for M2 being a substrate for MDR1 in presence of MDR1 inhibitor. Efflux of M2 in was further reduced in presence of MDR inhibitor suggesting that M2 is a substrate for MDR.

Netupitant, M1, M2 and M3, are not substrates of BCRP transporter. Although the net flux ratio when corrected for parental cell are > 2 for under certain conditions, it appears that it was due to very low flux ratio in parental cells. Based on efflux ratio in BCRP transfected cells alone, none of the tested compounds are substrates of BCRP transporter as efflux ratio for all of them were less than 2 in BCRP transfected cell. Repeated experiments at 10 µM reconfirmed that netupitant, M1, M2 and M3, are not substrates of BCRP transporter as both efflux ratio in transfected cells alone and net efflux ratio when corrected for parental cells are <2 for all tested compounds.

Inhibition:

P-gp on Caco-2 monolayer cells:

The sponsor evaluated the interaction of netupitant with P-gp transporter in 3 different assay methods, ATPase assay, Calcein Assay and bidirectional transporter assay on monolayer (Study

NETU-06-13). Since as bidirectional permeability assay on monolayers is currently regarded as the definitive assay for identifying P-gp substrates and inhibitors, this review only focused on the data from the bidirectional permeability assay on monolayers.

Potential of netupitant being an inhibitor of P-gp was evaluated in Caco-2 cell where the bidirectional (A-B and B-A) permeability of a model P-gp substrate ^{3}H-digixin was evaluated in the presence of increasing concentration of netupitant (0.2, 1 5 µM) on Caco-2 cell line (on 24-well plate) at 37°C after 2 hours of incubation in duplicate. The paracellular permeability of the monolayer was assessed using ^{14}C-mannitol (Papp(A/B) = 2.13x10^{-6} cm/s). 60 µM Verapamil (known P-gp inhibitor) was included as the positive control (Table 34).

Table 34 Apparent permeability (Papp) of ^{3}H-digoxin in the apical-to-basolateral (A-B) and basolateral-to-apical (B-A) direction in the presence of different concentrations of Netupitant

Inhibitors	Apical to Basolateral P_{app} (10^{-6}cm/sec)	Basolateral to Apical P_{app} (10^{-6}cm/sec)	Efflux Ratio
Control	0.87	25.32	29
60 µM Verapamil	2.98	4.07	1.4
Netupitant (0.2 µM)	1.25	29.73	23.8
Netupitant (1 µM)	1.07	26.23	24.5
Netupitant (5 µM)	2.8	13.24	4.7

Based on the result of this study, netupitant seems to inhibit P-gp at concentration dependent manner. However, IC50 value was not determined in this study. Therefore, relevance of this *in vitro* inhibitor interaction in *in vivo* cannot be predicted. Thus, netupitant's potential to inhibit P-gp *in vivo* at clinical dose cannot be ruled out. The sponsor did conduct a follow up *in vivo* drug-drug interaction study with digoxin (a model P-gp substrate).

MDR1, BCRP, BSEP and MRP2 on Vesicular Transport:

The sponsor had used vesicular transport inhibition assay to evaluate the inhibition potential of efflux transporters MDR1, BCRP, BSEP and MRP2 by netupitant and its metabolites. Vesicular transport assays were performed with inside-out membrane vesicles prepared from cells overexpressing human ABC transporters on 96-well plates. The netupitant, M1, M2, and M3 (at 0.01, 0.04, 0.12, 0.37, 1.11, 3.33, 10 and 30 µM) were incubated with membrane vesicle preparations (total protein: 50 µg/well or 25 µg/well in case of BCRP) and the probe substrate in triplicates. Incubations were carried out in the presence of ATP or AMP to distinguish between transporter-mediated uptake and passive diffusion into the vesicles. Reference inhibitors for each efflux transporters were included to serve as positive controls for inhibition (Table 35, Table 36)

Table 35 Vesicular transport assay parameters

Transporter	Probe substrate	Reference inhibitor
human BCRP (ABCG2)	E3S (1 μM)	Ko134 (1 μM)
human BSEP (ABCB11, sP-gp)	Taurocholate (2 μM)	cyclosporine A (20 μM)
human MRP2 (ABCC2)	E217βG (50 μM)	Benzbromarone (100 μM)
human MDR1 (ABCB1/P-gp)	NMQ (2 μM)	Verapamil (100 μM)

Table 36 Efflux Transporters inhibition from vesicular transport inhibition assays

Test article	Vesicular transport inhibition	IC_{50} (μM)	maximum inhibition (% of control)
Netupitant	BCRP	6.00	100%
M1		8.64	92%
M2		22.6	53%
M3		10.6	85%
Netupitant	BSEP	NA	32%
M1		ND	no interaction up to 30 μM
M2		NA	34%
M3		NA	41%
Netupitant	MRP2	ND	no interaction up to 30 μM
M1		ND	no interaction up to 30 μM
M2		ND	no interaction up to 30 μM
M3		ND	no interaction up to 30 μM
M1	MDR1	4.95	97%
M2		8.00	85%
M3		5.35	90%

ND: IC_{50} could not be determined as no interaction was observed within the concentration range tested
NA: Not applicable

- Netupitant, M1, M2 and M3 do not inhibit MRP2 up to 30 μM concentration and thus IC50 values were not determined for MRP2 transporter.
- Netupitant, M2 and M3 slightly inhibited BSEP while M1 did not show any inhibition toward BSEP up to 30 μM concentration. Therefore, IC50 values could not be determined for BSEP transporter.
- Netupitant, M1, M2 and M3 inhibit BCRP in concentration dependent manner.
    - Netupitant: Cmax/IC50= (1-1.5 μM)/6 μM = (0.167-0.25)>0.1
    - M1: Cmax/IC50=(0.07-0.09 μM)/8.6μM =(0.008-0.01)<0.1
    - M2: Cmax/IC50= (0.17-0.58 μM)/22.6 μM=(0.0075-0.026) <0.1
    - M3: Cmax /IC50 = (0.08-0.144 μM)/10.6 μM= (0.0075-0.013) <0.1

- M1, M2 and M3 inhibit inhibits MDR1in concentration dependent manner.
    - Netupitant: was not evaluated in this study.
    - M1: Cmax/IC50=(0.07-0.09 μM)/4.95 =(0.014-0.018)<0.1
    - M2: Cmax/IC50= (0.17-0.58 μM)/8.0 =(0.02125-0.0725)<0.1
    - M3: Cmax /IC50 =(0.08-0.144 μM)/5.35 = (0.014-0.027) <0.1

MDR1 and BCRP on MDCKII Transfected Monolayer Cell:

Inhibition potential of M1, M2 and M3 for MDR1 transporter and inhibition potential of netupitant, M1, M2 and M3 for BCRP transporter were evaluated in MDR1 or BCRP transfected MDCKII monolayer cultured cells. Bidirectional transport of model substrates for MDR1 (digoxin at 5 μM) and BCRP (prazosin 1uM) were determined in the presence and absence of netupitant, M1, M2 and M3 (10 and 30 μM) at 37 ± 1 °C after 60 minutes and 120 minutes incubation for prazosin and digoxin, respectively. The reference inhibitors PSC833 (10 μM) for MDR1 and Ko134 (1 μM) for BCRP were also included as positive controls (Table 37, Table 38).

Table 37 Inhibition of MDR1 transporter by metabolites of netupitant from MDCKII-MDR1 studies

Test article	Assay	ER		Net ER
		MDCKII	MDCKII-MDR1	MDCKII-MDR1 / MDCKII
Digoxin + M1	bidirectional permeability of digoxin with M1	digoxin:1.54 ± 0.15	digoxin:24.13 ± 4.46	15.7 ± 4.46
		+ PSC833:0.97 ± 0.08	+ PSC833:1.43 ±0.20	1.47 ± 0.22
		+ M1 (30 μM): 1.01 ± 0.06	+ M1 (30 μM): 1.12 ± 0.11	1.11 ± 0.13
		digoxin: 1.86 ± 0.48	digoxin: 31.63 ± 2.66	17.04 ± 4.67
		+ PSC833: 0.98 ± 0.03	+ PSC833:1.28 ± 0.02	1.30 ± 0.05
		+ M1 (10 μM): 1.25 ± 0.06	+ M1 (10 μM): 9.21 ± 0.67	7.38 ± 0.66
Digoxin + M2	bidirectional permeability of digoxin with M2	digoxin: 1.47 ± 0.13	digoxin: 40.21 ± 5.46	27.45 ± 5.46
		+ PSC833: 0.98 ± 0.10	+ PSC833: 1.30 ± 0.15	1.32 ± 0.18
		+ M2 (30 μM): 1.2 ± 0.12	+ M2 (30 μM): 28.6 ± 2.53	23.88 ± 2.54
		digoxin: 1.86 ± 0.48	digoxin: 31.63 ± 2.66	17.04 ± 4.67
		+ PSC833: 0.98 ± 0.03	+ PSC833: 1.28 ± 0.02	1.30 ± 0.05
		+ M2 (10 μM): 1.45 ± 0.11	+ M2 (10 μM): 28.68 ± 2.81	19.8 ± 2.45
Digoxin + M3	bidirectional permeability of digoxin with M3	digoxin: 1.54 ± 0.15	digoxin:24.13 ± 4.46	15.7 ± 4.46
		+ PSC833: 0.97 ± 0.08	+ PSC833:1.43 ± 0.20	1.47 ± 0.22
		+ M3 (30 μM): 1.00 ± 0.06	+ M3 (30 μM): 2.13 ± 0.32	2.12 ± 0.32
		digoxin: 1.86 ± 0.48	digoxin:31.63 ± 2.66	17.04 ± 4.67
		+ PSC833: 0.98 ± 0.03	+ PSC833: 1.28 ± 0.02	1.30 ± 0.05
		+ M3 (10 μM): 1.16 ± 0.06	+ M3 (10 μM): 8.44 ± 1.82	7.28 ± 1.62

Net ER (net efflux ratio)

Table 38 Inhibition of BCRP transporter by netupitant and its metabolites from MDCKII-BCRP studies

Test article	Assay	ER		Net ER
		MDCKII	MDCKII-BCRP	MDCKII-BCRP/ MDCKII
Prazosin + Netupitant	bidirectional permeability of prazosin with Netupitant	prazosin: 0.91 ± 0.09	prazosin:17.35 ± 0.83	18.97 ± 0.84
		+Ko134:0.87 ± 0.20	+Ko134: 0.91 ± 0.08	1.04 ± 0.22
		+ Netupitant (30 µM): 0.91 ± 0.01	+ Netupitant (30 µM): 6.25 ± 0.30	6.86 ± 0.30
		prazosin: 0.97 ± 0.08	prazosin: 14.28 ± 1.18	14.76 ± 1.68
		+Ko134: 0.90 ± 0.04	+Ko134:1.31 ± 0.13	1.46 ± 0.16
		+ Netupitant (10 µM): 0.86 ± 0.08	+ Netupitant (10 µM): 17.56 ± 1.40	20.35 ± 2.51
Prazos + M1	bidirectional permeability of prazosin with M1	prazosin: 0.91 ± 0.09	prazosin:17.35 ± 0.83	18.97 ± 0.84
		+Ko134: 0.87 ± 0.20	+Ko134:0.91 ± 0.08	1.04 ± 0.22
		+ M1 (30 µM): 0.96 ± 0.03	+ M1 (30 µM): 1.72 ± 0.10	1.80 ± 0.10
		prazosin: 0.97 ± 0.08	prazosin: 14.28 ± 1.18	14.76 ± 1.68
		+Ko134: 0.90 ± 0.04	+Ko134: 1.31 ± 0.13	1.46 ± 0.16
		+ M1 (10 µM): 0.94 ± 0.09	+ M1 (10 µM): 12.54 ± 1.67	13.29 ± 2.19
Prazosin + M2	bidirectional permeability of prazosin with M2	prazosin: 0.91 ± 0.09	prazosin: 17.35 ± 0.83	18.97 ± 0.84
		+Ko134: 0.87 ± 0.20	+Ko134: 0.91 ± 0.08	1.04 ± 0.22
		+ M2 (30 µM): 0.86 ± 0.04	+ M2 (30 µM): 20.99 ± 4.40	24.34 ± 4.40
		prazosin: 0.97 ± 0.08	prazosin: 14.28 ± 1.18	14.76 ± 1.68
		+Ko134: 0.90 ± 0.04	+Ko134: 1.31 ± 0.13	1.46 ± 0.16
		+ M2 (10 µM): 0.88 ± 0.06	+ M2 (10 µM): 18.25 ± 1.5	20.63 ± 2.20
Prazosin + M3	bidirectional permeability of prazosin with M3	prazosin: 0.91 ± 0.09	prazosin: 17.35 ± 0.83	18.97 ± 0.84
		+Ko134: 0.87 ± 0.20	+Ko134: 0.91 ± 0.08	1.04 ± 0.22
		+ M3 (30 µM): 0.93 ± 0.04	+ M3 (30 µM): 3.77 ± 0.59	4.08 ± 0.59
		prazosin: 0.97 ± 0.08	prazosin: 14.28 ± 1.18	14.76 ± 1.68
		+Ko134: 0.90 ± 0.04	+Ko134: 1.31 ± 0.13	1.46 ± 0.16
		+ M3 (10 µM): 0.87 ± 0.14	+ M3 (10 µM): 15.15 ± 1.62	17.47 ± 3.35

- M2 did not inhibit MDR1 and BCRP at both 10 µM and 30 µM, which is contrary to the vesicular transport inhibition assay result where M2 inhibited both MDR1 and BCRP in concentration dependent manner. Since bi-directional assay in monolayer is considered to be more reliable assay than the vesicular system, M2 is not considered as an inhibitor of MDR1 and BCRP.

- M1 and M3 inhibited MDR1 in concentration dependent manner. However, IC50 values were not determined in this monolayer cell system. Based on rough estimate of IC50

around 10 μM or based on the IC50 values from the vesicular system, an *in vivo* study is not needed for M1 and M3.

- Netupitant, M1 and M3 inhibited BCRP in concentration dependent manner where no inhibitions were observed at 10 μM and inhibition was observed at 30 μM. However, IC50 values were not determined in this monolayer cell system. Since no significant P-gp inhibitory effect of netupitant was observed with Digixin in in-vivo where 5 μM netupitant have inhibited P-gp transporter *in vitro,* we do not anticipate a significant BCRP inhibitory effect of netupitan *in vivo* since netupitnat at 10 μM did not inhibit BCRP transporter *in vitro*.

Uptake Transporters:
Uptake transporters were evaluated using CHO cells or FlpIn293 cells stably expressing the respective uptake transporters (Table 39).

Table 39 Experimental conditions for uptake transport assay

Transporter	Incubation Time (inhibition)	Probe Model substrate	Reference model inhibitor	Negative Control Cell Line
human OATP1B1	10	E3S (0.1 μM)	Cerivastatin (100 μM)	Parental CHO
human OATP1B3	10	Fluo-3 (10 μM)	Fluvastatin (30 μM)	Parental CHO
human OAT1	3	PAH (1.33 μM)	Benzbromarone (200 μM)	Parental CHO
human OAT3	3	E3S (1 μM)	Probenecid (200 μM)	Mock transfected HEK293
human OCT1	20	Metformin (3.63 μM)	Verapamil (100 μM)	Parental CHO
human OCT2	10	Metformin (3.63 μM)	Verapamil (100 μM)	Parental CHO

Substrate:
As netupitant and its metabolites are primarily eliminated through hepatobiliary route, the sponsor have evaluated the potential of netupitant and its metabolites M1, M2, and M3 being substrate for uptake transporters OATP1B1, OATP1B3 and OCT1 in CHO cells or FlpIn293 cells stably expressing the respective uptake transporters. The cellular uptake of netupitant, M1, M2, and M3 into cells was determined by incubating them at 1 and 10 μM concentrations with cells overexpressing the uptake transporters and control cells on 24-well plates at 37 ± 1 °C in pH 7.3 buffer for 2 and 20 minutes.

In the positive controls, the sponsor did not evaluate the fold increase in uptake of model substrates (positive controls) in transfected cells compared to parental cell. However, the uptake of model substrates in absence and presence of model inhibitors of for these specific transporters were evaluated to validate the test system. Uptake of these model substrates were substantially inhibited in the presence of model inhibitors.

Table 40 Fold increase in uptake in transfected cells compared to parental cell for uptake transporters for netupitant and its metabolites

Transporter	Test article	Fold at 1 µM / 2 min	Fold at 1 µM / 20 min	Fold at 10 µM / 2 min	Fold at 10 µM / 20 min
OATP1B1	Netupitant	0.68	0.76	0.81	0.84
	M1	0.72	0.77	0.77	0.82
	M2	0.28	0.33	0.64	0.65
	M3	0.70	0.76	0.84	0.95
OATP1B3	Netupitant	0.69	0.88	0.87	0.98
	M1	0.85	0.88	0.84	0.91
	M2	0.69	1.33	1.26	1.55
	M3	0.79	0.84	0.89	0.89
OCT1	Netupitant	0.70	0.76	0.71	0.79
	M1	0.68	0.69	0.71	0.75
	M2	0.51	0.68	0.57	0.85
	M3	0.70	0.71	0.69	0.76

None of the test compound, netupitant, M1, M2 and M3 showed ≥ 2 fold increase in uptake in transfected cells compared to parental cell suggesting that netupitant, M1, M2 and M3 are not substrates for OATP1B1, OATP1B3 and OCT1 (Table 40).

Inhibition:

Potential of netupitant, M1, M2, and M3 being an inhibitor of OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2 were evaluated by incubating them at 0.01, 0.04, 0.12, 0.37, 1.11, 3.33, 10 and 30 µM concentrations with cells stably expressing the those uptake transporters and the probe substrates on 96-well plate at 37 ± 1 °C in pH 7.4 buffer in triplicates. A reference inhibitor served as positive control for inhibition (Table 41).

- OATP1B1: Netupitant, M1 and M3 showed weak inhibition toward OATP1B1, and thus, IC50 values could not be estimated. However, M2 did show some inhibition toward OATP1B1 with IC50 of >30 µM. Since total Cmax/IC50 = 0.58 µM / 30 µM = 0.02 < 0.1, a follow-up *in vivo* study is not needed.

- OATP1B3: Netupitant and M1 showed weak inhibition toward OATP1B3 and IC50 values could not be estimated up to 30 µM. M2 and M3 inhibited OATP1B3 with IC50 values of 4.3 and 9.6 µM. Since Cmax/IC50 = 0.144 µM /9.6 µM = 0.015<0.1 for M3, an *in vivo* follow up study for to evaluate the inhibition potential of M3 toward OATP1B3 is not needed. Although total Cmax/IC50 = 0.58 µM /4.3 µM = 0.13 >0.1 for M2, R-value = 1+ (fu x I in,max/IC50) = 1.08 <1.25 and thus, *in vivo* study is not needed

- OAT1: Netupitant, M1, M2 and M3 do not appear to inhibit OAT1 significantly up to 30 µM concentration and thus, IC50 values could not be determined.

Table 41 Inhibition of uptake transporter by netupitant and its metabolites

Test article	Uptake transporter inhibition	IC_{50} (μM)	maximum inhibition (% of control)
Netupitant	OATP1B1	NA	22%
M1		NA	31%
M2		≥30.0	51%
M3		NA	22%
Netupitant	OATP1B3	NA	44%
M1		NA	26%
M2		4.3	85%
M3		9.6	63%
Netupitant	OAT1	NA	33%
M1		NA	32%
M2		ND	no interaction up to 30 μM
M3		NA	40%
Netupitant	OAT3	ND	no interaction up to 30 μM
M1		ND	no interaction up to 30 μM
M2		ND	no interaction up to 30 μM
M3		ND	no interaction up to 30 μM
Netupitant	OCT1	7.9	82%
M1		19.0	64%
M2		7.4	77%
M3		4.4	76%
Netupitant	OCT2	22.3	58%
M1		NA	42%
M2		NA	29%
M3		NA	43%

ND: IC_{50} could not be determined as no interaction was observed within the concentration range tested
NA: Not applicable

- OAT3: Netupitant, M1, M2 and M3 do not inhibit OAT3.

- OCT2: Netupitant appears to inhibit OCT2 in concentration dependent manner with IC50 value of 22.3 μM while M1, M2 and M3 did not show significant inhibition toward OCT2. Since Cmax/IC50 = (1-1.5 μM)/22.3 μM = (0.045-0.07) < 0.1, *in vivo* follow up study is not needed.

- OCT1: Netupitant, M1, M2 and M3 all appear to inhibit OCT1 in concentration dependent manner.
    - Netupitant: Cmax/IC50 = (1-1.5 μM) /7.9 μM = (0.13-0.19) >0.1
    - M1: Cmax/IC50 = 0.07-0.09 μM /19 μM = (0.0037-0.0047)<0.1
    - M2: Cmax/IC50 = (0.17-0.58 μM)/7.4 μM = (0.023-0.078)<0.1
    - M3 Cmax/IC50 = 0.08-0.144 μM /4.4 μM =(0.018-0.033) <0.1

For netupitant, although Cmax/IC50 =0.19 for OCT1, it is not substantially larger than 0.1. Since Cmax/IC50 <0.1 for OCT2, and OCT1 and OCT2 have overlapping substrate specificities, we do not anticipate a significant in-vivo OCT1 interaction for netupitant.

Induction:
Potential of netupitant and its metabolites to induce transporters were not evaluated in this NDA submission. Potential of netupitant and its metabolites to induce P-gp transporter do not need to be evaluated since it has already been shown that netupitant and its metabolites do not induce CYP3A4 in *in vitro* study NETU-10-27.

2.4.2.5 Are there other metabolic/transporter pathways that may be important?

The sponsor did not explore the following potential metabolic/transporter that may be important:

- The potential of netupiant being a substrate for P-gp was not evaluated.
- The potential of netupitant and its metabolites M1, M2 and M3 to induce CYP2B6 were not explored.

2.4.2.6 Does the label specify co-administration of another drug and, if so, has the interaction potential between these drugs been evaluated?

As a combination product, netupitant and palonosetron are to be co-administered. In addition, the efficacy of AKYNZEO was evaluated as a combination therapy with dexamethasone.

Interaction between netupitant and palonosetron
There was no significant PK drug interaction between netupitant and palonosetron.
Concomitant administration of a single dose netupitant 450 mg and a single dose palonosetron 0.75 mg did not significantly affect the PK of each other (Table 42).
These results are consistent with *in vitro* study results for different major metabolic enzyme i.e.CYP3A4 and CYP2D6 for netupitant and palonosetron, respectively and lack of significant inhibitory effects on CYP3A4 by palonosetron and CYP2D6 by netupitant.

The proposed clinical dose for the combination product is netupitant 300 mg and palonosetron 0.5 mg and no significant PK interaction is expected based on these results. These results also indicate that the contribution of palonosetron to the efficacy in the combination with netupitant is expected to be similar with that of palonosetron alone treatment.

Table 42 PK Parameters during the 3 Treatment Periods, with Netupitant Alone (450 mg), with Netupitant in Combination with Palonosetron (450/0.75 mg) and with Palonosetron Alone (0.75mg)

Treatment	Netupitant					Palonosetron				
	Cmax (µg/L)		AUC0-∞ (h*µg/L)		T1/2 (h)	Cmax (ng/L)		AUC0-∞ (h*ng/L)		T1/2 (h)
	Mean (SD)	Geo. Mean (CV%)	Mean (SD)	Geo. Mean (CV%)	Median	Mean (SD)	Geo. Mean (CV%)	Mean (SD)	Geo. Mean (CV%)	Median
Netu 450 mg (N=18)	650.2 (257.8)	575.1 (39.6)	25927 (10156)	24000 (39.2)	71.81	-	-	-	-	-
Palo 0.75 mg +Netu 450 mg (N=18)	659.7 (325.7)	560.0 (49.4)	26241 (13219)	23182 (50.4)	78.31	1863.1 (487.1)	1799.9 (26.1)	77254 (25402)	72596 (32.9)	36.91
Palo 0.75 mg (N=17)	-	-	-	-	-	1638.4 (415.5)	1587.2 (25.4)	70813 (20415)	67593 (28.8)	34.73

Interaction with dexamethasone

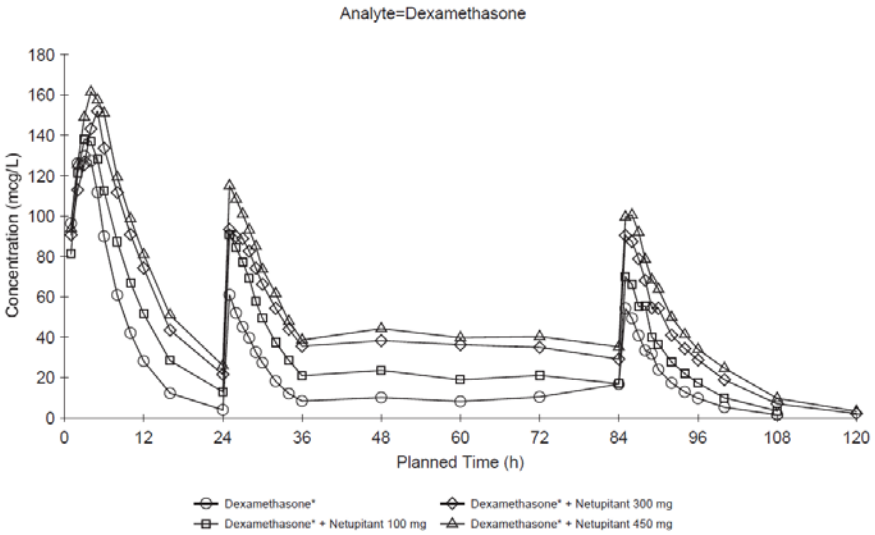
For CINV associated with highly emetogenic chemotherapy, dexamethasone (Dexa) is typically used as a multi-day regimen of 20 mg on Day 1 and 8 mg BID on Days 2-4 for adults. For CINV associated with moderately emetogenic chemotherapy, dexamethasone is typically used as a single dose of 20 mg on Day 1 only for adults.

The effect of netupitant on dexamethasone PK was studied by co-administering netupitant on Day 1 with dexamethasone multi-day regimen (20 mg on Day 1, followed by 8 mg b.i.d. on Days 2-4). The effect of netupitant was studied at doses of 100 mg, 300 mg, and 450 mg and PK of dexamethasone was evaluated on Days 1, 2 and 4.

The systemic exposure to dexamethasone was increased in a netupitant dose-dependent manner (Figure 15). The mean AUC0-24 was 1.5, 1.7, and 1.8-fold higher with co-administration of 100, 300, and 450 mg, respectively compared to that without netupitant. Notably the inhibitory effect of netupitant on CYP3A4 lasted till 4 days after single dose administration of 300 mg netupitant indicated by the 2.4-fold higher AUC84-∞ to dexamethasone. After co-administration of 300 mg netupitant Dexamethasone C_{max} was increased: up to 1.2-fold increase on Day 1, and 1.7 fold increase on Day 2 and Day 4. The Cmin was about 3-4 fold higher over 4 days with concomitant netupitant 300 mg (Table 43,Table 44).

Combined with this study results and no effects of palonosetron on CYP3A4 *in vitro*, the doses for dexamethasone regimen with netupitant and palonosetron was reduced to 12 mg from 20 mg on Day 1 and to 8 mg QD to 8 mg BID on Days 2-4 compared to the dexamethasone regimen for palonosetron alone treatment in Phase 2 and 3 trials.

Figure 17 Mean plasma concentration-time profile for dexamethasone by netupitant dose



* Dexamethasone 20 mg on Day 1, followed by 8 mg b.i.d. (every 12 hours) from Day 2 to Day 4

Table 43 Mean (SD) Pharmacokinetic Parameters for Dexamethasone after Administration with single dose Netupitant on Day 1

Day	Parameter	Dexamethasone alone (N=22)	Dexamethasone + Netu 100 mg (N=15)	Dexamethasone + Netu 300 mg (N=13)	Dexamethasone + Netu 450 mg (N=16)
Day 1	Cmax (µg/L)	156.5 (38.6)	161.2 (32.0)	169.9 (26.9)	190.4 (35.5)
	Tmax[1] [h]	3 (1, 5)	4 (2, 6)	4 (1, 5)	4 (1, 6)
	AUC0-24 [h* µg/L]	1089 (352)	1444 (320)	1782 (369)	1984 (430)
Day 2	Cmax (24-36h) (µg/L)	62.7 (19.6)	94.9 (16.4)	100.3 (26.1)	118.4 (27.4)
	Tmax[1] (24-36h) [h]	1 (1, 4)	2 (1, 3)	2 (1, 5)	1 (1, 5)
	AUC24-36 [h*µg/L]	330 (126)	600 (90)	760 (174)	871 (159)
Day 4	Cmax (84-108h) (µg/L)	58.2 (18.6)	80.7 (29.0)	96.2 (26.0)	110.0 (29.8)
	Tmax [1] (84-108h) [h]	1 (1, 5)	1 (1, 6)	2 (1, 3)	1.5 (1, 3)
	AUC84-108 [h*µg/L]	364 (157)	558 (137)	836 (221)	1005 (252)
	AUC84-∞ [h*µg/L]	390 (174)	599 (166)	913 (251)	1119 (308)

[1] T_{max} : median (min, max)

Per the Agency's request, the sponsor estimated [I]/Ki for CYP3A4 inhibition by netupitant and its metabolites, mainly M1 beyond Day 4[11].

[11] Response to the Information Request dated May 21, 2014

PK samples for netupitant and its metabolites were collected up to 120 h and plasma concentrations beyond the last observed concentrations were extrapolated[12]. In this study the median half-lives for netupitant and metabolite M1 were of 47 h and 68 h and was estimated relatively shorter than those in other studies. For example the mean half-life of 70-100 h was estimated for netupitant and M1 in other studies.

Table 44 Mean Ratio PK parameters for Dexamethasone with and without concomitant 300 mg netupitant

Day	Pharmacokinetic parameter	Mean Ratio* (%)	90% Confidence Interval* (%)	
			Lower	Upper
Day 1	Cmax (0-24h)	111.0	102.3	120.5
	AUC0-24	171.6	156.7	188.0
Day 2	Cmax (24-36h)	166.3	150.3	184.1
	AUC24-36	243.0	217.7	271.3
Day 4	Cmax (84-108h)	174.9	155.5	196.8
	AUC84-108	238.2	220.7	257.1
	AUC84-inf	243.2	225.7	262.1

To assess the potential inhibitory effects on CYP3A4 over time, [I]/Ki values were computed for netupitant and these metabolites individually then added to calculate the total [I]/Ki at given time point. The mean total [I]/Ki ranged 0.0134-0.167 (ranged 0.089-0.285) on Day 4 when the AUC of dexamethasone was still 2-fold higher than the control. The mean total [I]/Ki decreased to below 0.1 on Day 6 and was 0.093 (0.049- 0.210) at 140 h post-dose. This estimation suggests that drug interaction via CYP3A4 inhibition by netupitant is less likely on Day 6 but cannot be ruled out (Table 45, Figure 16).

Table 45 Mean ± SD of [I]/Ki (min-max) for netupitant and its metabolites

Time(h) post-dose	Netupitant	M1	M2*	M3*	Total
84	0.054 ± 0.018 (0.03-0.089)	0.106 ± 0.035 (0.060-0.180)	0	0.007 ± 0.003 (0.003-0.013)	0.167 ± 0.053 (0.097-0.283)
120	0.038 ± 0.015 (0.020-0.080)	0.074 ± 0.031 (0.041-0.157)	0	0.004 ± 0.002 (0.002-0.010)	0.117 ± 0.048 (0.064-0.248)
140	0.029	0.0613	0	0.003	0.093 ± 0.042 (0.049-**0.210**)

*In vitro studies indicated that M2 and M3 are not inhibitors of CYP3A4

[12] Extrapolations were made using the equation $[I]_t = [I]_{t\,last} \cdot e^{-\frac{\ln 2}{t_{1/2,z}} \cdot (t - t\,last)}$

Figure 18 Mean [I]/Ki values-time profile (A) for netupitant and its metabolites M1, M2 and M3 after administration of netupitant 300 mg plus dexamethasone , (B) the sum of [I]/Ki

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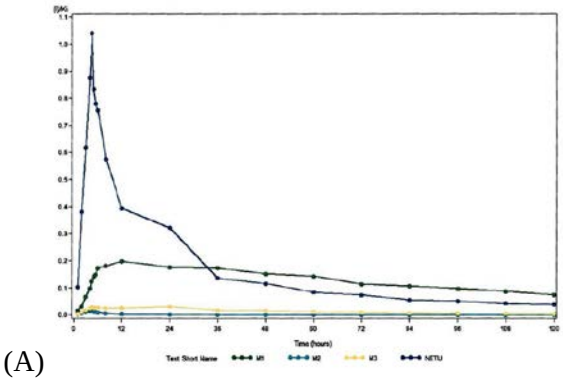
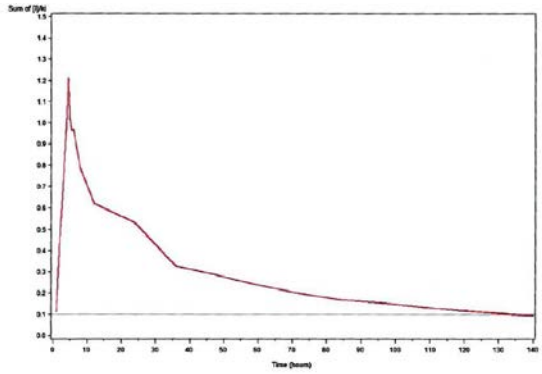


(A)



(B)x

Interaction with chemotherapy

Potential interactions between netupitant/palonosetron combination and chemotherapeutics were studied with docetaxel, etoposide, and cyclophosphamide in cancer patients. Within each group, all patients received an intravenous (IV) administration of 1 of the 3 chemotherapeutic agents (docetaxel, etoposide or cyclophosphamide) for 2 consecutive cycles; Day 1 of the 2 treatment periods was separated by at least 3 weeks. The patients received a single oral dose of AKYNZEO during either the first or the second treatment period and oral palonosetron 0.5 mg (Aloxi®, reference IMP) in the alternate period.

Docetaxel and etoposide[13] are metabolized primarily by CYP3A4, and cyclophosphamide is metabolized by multiple CYP enzymes including CYP3A4. In Study 08-08, the clinical efficacy of FDA was studied in patients who received anthracycline and cyclophosphamide based chemotherapy. The dosage of chemotherapeutic agents varied among patients: docetaxel, 75 to 100 mg/m^2; etoposide, 35 to 100 mg/m^2; cyclophosphamide, 500 to 1000 mg/m^2 ; however, was consistent between two treatment periods (Table 46,Table 47).

Docetaxel

[13] Kawashiro et al. (1998) A study on the metabolism of etoposide and possible interactions with antitumor or supporting agents by human liver microsomes, JPET 286(3):1294

With netupitant/palonosetron combination, docetaxel exposure was approximately 37% higher for AUC_{0-t} and 50% for C_{max} than the exposure only with palonosetron.

Etoposide

The AUC_{0-t} in the FDC period was approximately 21% higher than that in the reference period, while C_{max} and $AUC_{0-\infty}$values were similar for both treatment periods.

Cyclophosphamide

When co-administered with FDC, the mean AUC and Cmax for cyclophosphamide were 19% and 27% higher, respectively compared to those after co-administration with palo alone. This suggests that there may be a minimal drug-drug interaction between IV cyclophosphamide and netupitant administered in the form of FDC with palonosetron.

Table 46 Docetaxel, Etoposide and Cyclophosphamide Plasma Exposure in Co-administration either with Netupitant /Palonosetron (FDC, test) or Palonosetron Alone (Reference)*

	Cmax ng/ml	AUC0-t h*ng/ml	AUC0-∞ h*ng/ml	Cmax mg/ml	AUC0-t h*mg/ml	AUC0-∞ h*mg/ml	Cmax mg/ml	AUC0-t h*mg/ml	AUC0-∞ h*mg/ml
	Docetaxel with FDC			Etoposide with FDC			Cyclophosphamide with FDC		
n	8	8	6	12	12	9	10	10	10
Mean	3119	5610	5063	18.4	122	111	307	526	533
SD	625	2093	1827	3.5	47.9	21.4	324	408	417
CV (%)	20.1	37.3	36.1	19.1	39.2	19.2	105	77.7	78.2
	Docetaxel with Palonosetron			Etoposide with Palonosetron			Cyclophosphamide with Palonosetron		
n	7	7	3	12	12	10	10	10	10
Mean	2093	3941	4398	17.73	99.3	111	285	476	481
SD	616	1019	1368	2.601	26.5	26.7	334	415	421
CV (%)	29.5	25.8	31.1	14.7	26.7	23.9	117	87.3	87.7

*PK samples were collected up to 24, 36, 48 hr post-dose for etoposide, cyclophosphamide, and docetaxel, respectively.

Table 47 Mean ratio of PK parameters for chemotherapy after co-administration with FDC or oral Aloxi

Chemo-therapeutic agent	PK parameter	Least square means (SE)		Estimated Mean Ratio Test to Reference (%)	90% CI	
		Chemo with FDC (Test)	Chemo with Palonosetron (Reference)		Lower limit	Upper limit
Docetaxel	AUC0-∞	8.63*	8.42*	124.36	-	-
	AUC0-t	8.57 (0.11)	8.27 (0.11)	135.16	98.90	184.71
	Cmax	8.03 (0.11)	7.64 (0.11)	149.01	108.50	204.66
Etoposide	AUC0-∞	4.64 (0.05)	4.61 (0.05)	103.45	89.87	119.08
	AUC0-t	4.78 (0.08)	4.53 (0.08)	128.00	105.28	155.62
	Cmax	2.93 (0.05)	2.83 (0.05)	110.20	95.99	126.53
	AUC0-∞	4.74 (0.09)	4.51 (0.09)	125.22	98.53	159.13
Cyclo-phosphatide	AUC0-∞	6.06 (0.15)	5.88 (0.15)	120.12	81.58	176.89
	AUC0-t	6.05 (0.15)	5.87 (0.15)	119.52	81.03	176.28
	Cmax	4.93 (0.44)	4.69 (0.44)	127.36	40.17	403.75

*Because n=2, the SE and the 90% CI could not be calculated

SE = Standard error; FDC = netupitant/palonosetron fixed dose combination; CI = Confidence interval

CYP3A4 substrates

Midazolam and erythromycin (NP16599)

When administered concomitantly with oral netupitant 300 mg, the exposure of CYP3A4 substrate was increased (Cmax and AUC were approximately 92 and 56% higher for erythromycin while Cmax and AUC were 36% and 126% higher for midazolam) (Table 48). Co-administration with midazolam and erythromycin did not affect the exposure to netupitant. Based on about 2 fold increase in midazolam's systemic exposure, netupitant at 300 mg is considered as a moderate inhibitor of CYP3A4 in vivo.

Oral contraceptive: ethinylestradiol and levonorgestrel

The potential effects of FDC on PK of an oral contraceptive (Mycrocynon®: a fixed dose combination of 0.03 mg ethinylestradiol and 0.15 mg levonorgestrel) were studied after a single dose administration of OC with and without AKYNZEO in healthy female subjects (n=24). For bioanalytical assay, two tablets of Mycrocynon® were administered although the approved dose is one tablet of Mycrocynon®.

When given with AKYNZEO the mean Cmax and AUC0-∞ of
Ethinylestradiol was 5% and 16% higher, respectively. For the levonorgesterel component, there was no effect of the FDC on levonorgestrel Cmax, while exposure parameters (AUC0-∞ and AUClast) were increased by approximately 40% (Table 49).

Table 48 Erythromycin and Midazolam Exposure (Means) with and without Netupitant (300 mg)

Parameter	Midazolam			
	Midazolam alone	With netupitant	PE%*	90%CI
Cmax [µg/L]	29.1± 13.9	40.6± 20.2	136	116-159
AUC0-∞ [h·µg/L]	122± 47.2	298± 162	226	189-270
	Erythromycin			
	Erythromycin alone	With netupitant	PE%*	90%CI
Cmax [ng/L]	766± 780	985± 656	192	102-363
AUC0-∞ [h·ng/L]	2240± 1730	2890± 1720	156	80.4-302

*Point estimate (PE): ratio of geometric means (T/R) and 90% confidence interval (CI)

7.5 mg oral midazolam (tablet) (reference)

7.5 mg oral midazolam + netupitant 300mg (2 capsules of 150 mg each) (test)

500 mg oral erythromycin (tablet) (reference)

500 mg oral erythromycin +netupitant 300mg (2 capsules of 150 mg each) (test)

Table 49 PK Parameters (mean ±SD) for Ethinylestradiol and Levonorgestrel after Oral Administration of Microgynon® with and without AKYNZEO

Parameter	**Ethinylestradiol**			
	With FDC (T)	**Without FDC (R)**	**PE***	**90%CI**
Cmax [pg/mL]	120.6±28.3	115.6±30.9	105.09	98.33 - 112.32
AUC0t-z [h•pg/mL]	1071±397.0	928.3±383.2	116.05	106.21 - 126.79
AUC0-∞ [h•pg/mL]	1224±428.7	1091±400.9	112.09	102.80 - 122.22
	Levonorgestrel			
Cmax [ng/mL]	8.11±2.93	8.23±2.79	98.06	92.53 - 103.92
AUC0t-z [h•ng/mL]	87.4±54.1	60.0±37.0	146.21	129.38 - 165.22
AUC0-∞ [h•ng/mL]	113.1±63.5	80.4±42.4	139.55	123.55 - 157.61

*Point estimate (PE): ratio of geometric means (T/R) and 90% confidence interval (CI)

R: two tablets each containing 30 µg ethinylestradiol and 150 µg levonorgestrel (Microgynon®) (Reference)

T: two tablets each containing 30 µg ethinylestradiol and 150 µg levonorgestrel (Microgynon®) and one capsule containing netupitant and palonosetron 300 mg/0.5 mg (Test)

2.4.2.7 Are there any in vivo drug-drug interaction studies that indicate the exposure is different when drugs are co-administered?

Drug Interaction Studies

With strong CYP3A4 inhibitor

Ketoconazole

Single dose AKYNZEO was administered with ketoconazole following once daily administration of 400 mg ketoconazole for 12 days and the PK of netupitant and palonosetron were compared to that after administration of AKYNZEO alone (NETU-10-11).

Netupitant

Co-administration with ketoconazole, a strong CYP3A4 inhibitor with AKYNZEO increased mean Cmax by 1.3-fold and mean AUC by 2.4-fold when compared to the administration of AKYNZEO alone. Mean Cmax and AUC were lower for all three metabolites after co-administration of ketoconazole (Table 50,Table 51).

Metabolites of netupitant

M1

Concomitant ketoconazole delayed the median Tmax for M1 from 12 h to 96 h. The average metabolite to parent ratio for M1 based on AUCinf was 24.9% with ketoconazole and 30.3% without ketoconazole.

M2

Concomitant ketoconazole did not affect the median Tmax for M2 (Tmax was 5.5 h for both treatments). The average metabolite to parent ratio for M2 based on AUCinf was 6.37% with ketoconazole and 12.1% without ketoconazole.

M3

With ketoconazole the median Tmax for M3 was delayed from 12 h to 24 h. The average metabolite to parent ratio for M3 based on AUCinf was 15.1% with ketoconazole compared with 28.1% without ketoconazole.

Palonosetron

Concomitant ketoconazole did not affect the pharmacokinetics of palonosetron.

Table 50 Mean (±SD) PK Parameters after Oral Administration of Netupitant/Palonosetron (300 mg/0.5 mg) with and without Ketoconazole (400 mg q.d.) (NETU-10-11)

Parameter	T With Keto (n=18)	R Without Keto (n=18)	PE%*	90%CI
	Netupitant			
Cmax [µg/L]	650.2±217.6	546.0±241.0	125.42	101.27 - 155.33
AUC0-tz [h·µg/L]	28494±7703	16072±5132	180.42	159.51 - 204.06
AUC0-∞[h·µg/L]	43459±16911	17971±5618	**239.88**	205.60 - 279.89
	Palonosteron			
Cmax [ng/L]	898.7±220.1	775.3±185.0	115.35	109.62 - 121.37
AUC0-tz [h·ng/L]	36899±8667	32564±7459	113.41	108.26 - 118.80
AUC0-∞ [h·ng/L]	40910±9261	37524±9577	110.09	105.43 - 114.96

*Point estimate (PE): ratio of geometric means (T1/R1) and 90% confidence interval (CI)

T: One capsule of netupitant/palonosetron (300 mg/0.5 mg) in combination with 400 mg q.d. (2 tablets of 200 mg) ketoconazole (Test 1) :

R: One capsule of netupitant/palonosetron (300 mg/0.5 mg) (Reference)

Table 51 Mean (±SD) PK parameters of metabolites of netupitant with and without ketoconazole

Metabolite	M1		M2		M3	
Ketoconazole	With	Without	With	Without	With	Without
Cmax [µg/L]	21.2±7.44 (35.0)	39.2±10.2 (26.1)	34.7±17.7 (50.9)	195.1±92.5 (47.4)	26.8±10.0 (37.4)	74.5±26.3 (35.3)
AUC0-tz [h·µg/L]	3767±1193 (31.7)	4552±1167 (25.6)	1936±1131 (58.4)	1987±963.7 (48.5)	3609±1610 (44.6)	4530±1433 (31.6)
AUC0-∞ [h·µg/L]	9847±4435 (45.0)[a]	5307±1310 (24.7)	2613±1604 (61.4)[b]	2161±1053 (48.7)	6055±2369 (39.1)[c]	4851±1446 (29.8)

Source: In-Text Tables 11.4-3, 11.4-5, 11.4-7 in Study Report of NETU-10-11

[a]n=9; [b]n=17; [c]n=16

Strong CYP3A4 inducer

Rifampicin

Single dose FDC was administered with rifampicin following once daily administration of 600 mg rifampicin for 17 days and PK of netupitant and palonosetron were compared to that after administration of FDC alone (NETU-10-11).

Co-administration of rifampicin, a strong CYP3A4 inducer rifampicin with netupitant/palonosetron FDC decreased the mean Cmax and AUC0-∞ by 2.6, and 5.9 fold, respectively. Co-administration of rifampicin decreased the mean AUC for palonosetron by 20% (Table 52).

Table 52 Pharmacokinetic Parameters (Mean±SD) after Administration of AKYNZEO with and without Rifampicin (600 mg q.d.) (NETU-10-11)

Parameter	T With Rif	R Without Rif	PE%*	90%CI
	Netupitant			
Cmax [µg/L]	225.6±156.3	498.1±225.6	37.90	28.81 - 49.86
AUC0-tz [h·µg/L]	3362±2766	15210±4977	18.05	13.56 - 24.01
AUC0-∞ [h·µg/L]	3463±2790	16944±5915	16.92	12.70 - 22.55
	Palonosteron			
Cmax [ng/L]	654.5±138.4	772.2±206.0	85.44	81.11 - 90.01
AUC0-tz [h·ng/L]	25557±7679	32371±13055	80.64	76.43 - 85.09
AUC0-∞[h·ng/L]	28354±7851	35714±13467	81.03	76.96 - 85.32

*Point estimate (PE): ratio of geometric means (T/R) and 90% confidence interval (CI)

T: One capsule of netupitant/palonosetron (300 mg/0.5 mg) in combination with 600 mg q.d. (1 tablet of 600 mg) rifampicin (Test):

R: One capsule of netupitant/palonosetron (300 mg/0.5 mg) (Reference)

Metabolites of netupitant

Concomitant administration with rifampicin increased the systemic exposure to M2 but decreased the systemic exposure to M1 and M3 suggesting that M2 is the major metabolite formed by CYP3A4 while M1 and M3 are further metabolized by CYP3A4 or other enzymes inducible by rifampicin (Table 53).

Table 53 Mean (±SD; CV) PK parameters of metabolites of netupitant with and without Rifampicin

Metabolite	M1		M2		M3	
Rifampicin	With	Without	With	Without	With	Without
Cmax [µg/L]	28.4±13.3 (46.9)	40.0±10.8 (26.9)	938.8±454.3 (48.4)	174.4±66.0 (37.8)	62.4±42.8 (68.6)	66.4±17.9 (26.9)
AUC0-∞ [h·µg/L]	1151±511.6 (44.4)	4944±1721 (34.8)	5406±3141 (58.1)	1854±787.3 (42.5)	1767±1366 (77.3)	4491±1901 (42.3)
AUC0-tz [h·µg/L]	943.4±541.3 (57.4)	4266±1273 (29.9)	5379±3132 (58.2)	1733±750.8 (43.3)	1478±1174 (79.5)	4217±1698 (40.3)

Source: In-Text Tables 11.4-4, 11.4-6, 11.4-8 in Study Report of NETU-10-11
[a] n=16; [b] n=18; [c] n=11

P-glycoprotein
Digoxin

In vitro studies showed that netupitant interacts with P-glycoprotein (P-gp) resulting in a concentration-dependent modulation of digoxin transport. Therefore, an in vivo drug interaction study with digoxin, a P-gp substrate was conducted to assess the effects of netupitant on the pharmacokinetics of digoxin at steady-state in healthy volunteers (n=16; NETU-07-01).

Digoxin was administered once daily 0.25 mg digoxin for 11 consecutive days [Days 2-12] following loading dose of 0.75 mg digoxin on Day 1 and a single dose 450 mg netupitant was administered on Day 8. The systemic exposure to digoxin on Day 6 and Day 8 was compared.

The PK and urinary excretion of digoxin was similar in the presence and absence of netupitant (Table 54, Table 55)

This study results indicate that when netupitant was co-administered with digoxin simultaneously, it does not significantly affect the PK and overall absorption of digoxin at steady-state.

Table 54 Mean PK parameters for Digoxin in the Absence and Presence of Netupitant (NETU-07-01)

PK parameter	**Digoxin alone N=16**	**Digoxin with Netupitant N=16**	PE (90% CI)
AUC(0-24h,ss) [h*µg/L]	10.9 (2.4)	11.4 (2.4)	104.13 (95.86 - 113.11)
Cmax,ss [µg/L]	1.13 (0.3)	1.24 (0.3)	108.97 (90.30 - 131.49)
Tmax,ss [h]	1 (0.5 - 1.5)	1.03 (0.5 - 1.5)	n/a

Tmax: Median (min - max)

Table 55 Mean (SD) urinary excretion of digoxin (N=16)

Pharmacokinetic parameter [Unit]	Visit (treatment)	Male (N=8)	Female (N=8)	Total (N=16)
$Ae_{0\text{-}24h}$ [µg]	DAY 6 (digoxin alone)	138.2 (44.30)	136.4 (39.1)	137.3 (40.4)
	DAY 8 (digoxin + netupitant)	145.2 (39.4)	140.2 (29.5)	142.7 (33.7)
$Ae_{0\text{-}24h}$ [% of dose]	DAY 6 (digoxin alone)	55.29 (17.72)	54.57 (15.64)	54.93 (16.15)
	DAY 8 (digoxin + netupitant)	58.09 (15.75)	56.07 (11.81)	57.08 (13.49)

Source: Table 14.2.6

Reviewer's comments:
This study demonstrated that no significant effects of netupitant on digoxin absorption and urinary excretion when netupitant and digoxin were concurrently administered. In this study, the median Tmax for digoxin was 1 h when netupitant concentration is about 80% lower than the mean Cmax (mean Cmax for netupitant was 755 mcg/L and mean concentration at 1 hour was 121 mcg/L) and the median Tmax for netupitant was 4 h.

No significant effects on the urinary excretion of digoxin suggest that the effect of netupitant on the P-gp on the kidney was not significant.

2.4.2.9 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

The efficacy of AKYNZEO is based on the indirect pharmacodynamics drug-drug interactions. The binding of serotonin and NK1 released upon administration of emetogenic chemotherapy is proposed to be blocked by AKYNZEO.

2.4.2.10 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions or protein binding?

Single dose netupitant could increase the systemic exposure to dexamethasone administered by 2 fold on 3 days after single dose administration. The inhibitory effect was not studied beyond 4 days after administration of netupitant while estimated to last for at least 6 days based on [I]/Ki values.

2.4.3 What issues related to dose, dosing regimens or administration are unresolved, and represent significant omissions?

None.

2.5 General Biopharmaceutics

2.5.1 What are the solubility and the permeability of netupitant?

The permeability of netupitant was determined Parallel Artificial Membrane Permeation Assay (PAMPA) and CACO-2 cell line.

With the PAMPA model, the permeability of netupitant was determined to be 1.1 x 10^{-6} cm/s (10.6 nm/s) and 2.5 x 10-^{6} cm/s (24.6 nm/s) at concentrations of 10 and 50 μM, respectively (NETU-10-26). As controls, the reference compounds [^{3}H]-propranolol (high permeability) and sulfasalazine (low permeability) were included in the assay. However, netupitant had very high, about 65-70% non-specific binding in this study. In addition, it appears that netupitant did not dissolve well in the buffer used in the experiment, and therefore, the actual concentration of netupitant that is exposed at the donor side is much lower than what is stated theoretically.

With Caco-2 model, permeability of [^{14}C]Netupitant at three concentrations (1, 10, and 100 uM) were evaluated from apical side to the basolateral side (A→B) and from the basolateral side to the apical side (B→A) on Caco-2 cells in triplicate wells and was repeated on two different days. As controls of monolayer integrity, the reference compounds [^{3}H]-propranolol (high permeability) and [^{3}H]-mannitol (low permeability) were included in the assay. However, in this study, netupitant had very high, about 30-90% non-specific binding. In addition, it appears that netupitant did not dissolve well in the buffer used in the experiment, and therefore, the actual concentration of netupitant that is exposed at the donor side is much lower than what is stated theoretically. Furthermore, the apparent permeability was calculated using the final donor concentration at the end of the incubation instead of initial donor concentration.

Table 56 ***Permeability of [^{14}C]Netupitant at the initial concentrations C_o of 1, 10, and 100 μM***

Experiment	C0 (μM)	$P_{A/B}$ (nm/s)	$P_{B/A}$ (nm/s)
1	1	n.a.	126
	10	336	102
	100	342	50.9
2	1	853	666
	10	292	127
	100	439	165

n.a.: not applicable. Could not be determined since no detectable [^{14}C] Netupitant was present in receiver compartment

Both of these permeability studies are hard to interpret as both studies had very high non-specific binding and actual concentration in donor side is significantly different than the theoretical concentration. In addition, the suitability of Caco-2 cell method was not evaluated with sufficient number of model drugs, and the expression of P-gp transporter on Caco-2 cell was not characterized with a model substrate.

2.5.2 What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial?

The review of bioequivalence study is deferred to the biopharmaceutics review in the ONDQA.

The to-be-marketed formulation was used in phase 3 trials but the sponsor proposes to change the manufacturing site for marketing. In addition, extemporaneous combinations of netupitant and Aloxi was used for the phase 2 dose-finding study which establishes the contribution of netupitant to the combination product as well as the efficacy of the combination for the prevention of CINV associated with cisplatin-based chemotherapy (HEC).

Two pivotal BE studies were conducted to bridge the manufacturing site change and between the extemporaneous formulation and the to-be-marketed formulation. One study was to bridge the Phase 2 formulation (extemporaneous combination of capsules containing netupitant (b) (4) plus Aloxi® softgel administered simultaneously) and the Phase 3 formulation (FDC containing three 100 mg netupitant intermediate tablets and one 0.5 mg palonosetron softgel) in study NETU-09-07. The palonosetron softgel in the FDC is different from the approved Aloxi oral softgel for the size of capsule (b) (4) (Table 57).
Bioequivalence was established between the phase 2 formulation and the phase 3 formulation and between two FDCs with the same formulations manufactured at 2 different manufacturing sites: HBP (test formulation, Phase 3/proposed commercial material) and (b) (4) (reference formulation, Phase 3 material). Both FDCs contained Intermediate netupitant tablets and a palonosetron softgel (NETU-11-02) (Table 58).

Table 57 Bioequivalence between extemporaneous combination used in phase 2 trial and FDC formulation used in phase 3 trial (NETU-09-07)

Netupitant		
Parameter	**Point Estimate %**	**90% CI**
Cmxax	106.92%	92.91 – 123.04%
AUC0-t	105.92%	96.24 – 116.58%
AUC0-∞	101.57%	91.17 – 113.16%
Palonosetron		
Parameter	**PE%**	**90% CI**
Cmax	100.18%	97.15 - 103.31%
AUC0-t	100.19%	97.10 - 103.38%
AUC0-∞	99.37%	96.54 - 102.28%
Test: FDC formulation (300 mg netupitant/0.5 mg palonosetron capsules) Reference: extemporaneous combination 2x150mg netupitant capsules+0.5 softgel palonosetron		

Table 58 Bioequivalence between FDC formulations manufactured at different sites (NETU-11-02)

Netupitant Geometric mean ratio		
Parameter	**PE%**	**90% CI**
Cmax	92.72%	86.41 – 99.50%
AUC0-t	93.93%	89.35 – 98.74%
AUC0-f	92.62%	87.34 – 98.22%
Palonosetron		
Parameter	**PE%**	**90% CI**
Cmax	102.36%	100.38 - 104.37%
AUC0-t	101.11%	99.32 – 102.94%
AUC0-f	101.08%	99.23 – 102.96%
Test: FDC (Helsinn Birex manufacturer) Reference: FDC (b) (4) manufacturer)		

2.5.3 What is the effect of food on the bioavailability (BA) of the drug from the dosage form?

About 17% increase in systemic exposure to netupitant by a high fat meal was observed while a high fat meal did not affect the PK of palonosetron.

NETU-10-12: The food effect study was conducted after administration of a single dose of FDC in 24 healthy male and female subjects. On Day 1 of each treatment period, a single oral dose of the FDC of 300 mg netupitant and 0.5 mg palonosetron was administered. The drug was administered 30 min after start of a high fat meal[14] following 10 hour overnight fast.

In this study the high fat, high-caloric breakfast led to a delay in absorption of netupitant. There was an increase in systemic exposure of about 16% for $AUC_{0-\infty}$ and about 18% for AUC_{0-tz} and C_{max} (Table 59,Table 60). For palonosetron, the systemic exposure was not significantly affected by a high fat meal (Table 61).

[14] The content of the high-fat, high-caloric breakfast followed the recommendations given in the FDA guidance "Food Effect Bioavailability and Fed Bioequivalence Studies". The breakfast contained 150 protein calories, 250 carbohydrate calories and 500 to 600 fat calories resulting in a total caloric content of about 945 kcal.

Table 59 Comparison of Netupitant C_{max} and Exposure Values between Fasted and Fed Healthy Subjects

Food	N	Cmax (ng/mL) mean (%CV)	AUC0-∞ (ng*h/mL) mean (%CV)
Fasted	22	596.4 (39.1)	20039 (41.9)
Fed	22	649.8 (21.8)	22391(38.6)

Table 60 Effect of food on netupitant PK (n=22)

Pharmacokinetic Parameter for Netupitant	ANOVA CV [%]	Ratio	Point estimate [%]	90% Confidence interval [%]
AUC_{0-tz} [h·µg/L]	19.41	T/R	117.88	106.66 - 130.27
$AUC_{0-\infty}$ [h·µg/L]	20.13	T/R	115.96	104.54 - 128.62
C_{max} [µg/L]	30.87	T/R	117.74	100.65 - 137.74

T: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fed state (Test)
R: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fasted state (Reference)
ANOVA = Analysis of variance, CV = coefficient of variation, *Point estimate (PE): ratio of geometric means (T/R)
Source: Table 14.2.6.1

Table 61 Effect of food on palonosetron PK (n=22)

Pharmacokinetic Parameter for Palonosetron	ANOVA CV [%]	Ratio	Point estimate [%]	90% Confidence interval [%]
AUC_{0-tz} [h·ng/L]	9.50	T/R	99.29	94.51 - 104.30
$AUC_{0-\infty}$ [h·ng/L]	9.04	T/R	99.99	95.41 - 104.79
C_{max} [ng/L]	11.96	T/R	99.00	93.05 - 105.33

T: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fed state (Test)
R: one capsule of 300 mg netupitant and 0.5 mg palonosetron in fasted state (Reference)
ANOVA = Analysis of variance, CV = coefficient of variation, *Point estimate (PE): ratio of geometric means (T/R)
Source: Table 14.2.6.2

2.6 Analytical Section

2.6.1 How the active moieties are identified and measured in the plasma/urine in the clinical pharmacology and biopharmaceutics studies?

Akynzeo® contains two active ingredients, netupitant and palonosetron. The concentrations of netupitant and palonosetron in human plasma were determined using validated liquid chromatography mass spectrometry (HPLC/MS/MS) methods.

Netupitant and its metabolites were quantified by validated LC/MS and LC/MS/MS methods, using stable label internal standards (IS) for each analyte. Partial validations were conducted throughout the development program to account for changes in assay method including extraction method, the change to a 96 well-plate format and selectivity in presence of palonosetron.

Bioanalytical assay method for palonosetron was based on the previously established method used for studies supporting the approval of Aloxi. The method was further validated to account for the change to a 96 well-plate format and selectivity in presence of netupitant.

Bioanalytical assay for co-administered medications

During the development of the combination program, several other drugs were also analyzed in clinical trials. Corresponding assays were developed and validated with acceptable accuracy and precision.

Midazolam (NP16599)
Midazolam was isolated from plasma by liquid/liquid extraction and determined by LC-MS/MS. The limits of quantification were 0.100 ng/mL for all assay batches. The inter-day precision of the assay was below 3.8% (CV). The inter- day accuracy of the assay was better than 95.3%.

Erythromycin (NP16599)
Erythromycin was isolated from plasma by liquid/liquid extraction and determined by LC-MS/MS. The limit of quantification was 20.0 ng/mL for all assay batches. The inter-day precision of the assay was below 7.2% (CV). The inter-day accuracy of the assay was between 93.6% and 108.1%.

Dexamethasone (NETU-06-07)
Dexamethasone was isolated from plasma through liquid/liquid extraction and measured by LC-MS/MS. The original method by which the LLOQ was determined to be 1.06 µg/L (precision=8.77%, accuracy =-3.03%) was partially validated further to determine LLOQ at the concentration of 1.004 µg/L (n=6, precision = 13.45%; accuracy = -4.28%). The total precision for dexamethasone in human plasma was in the range from 6.02% (at 95.393 µg/L) to 6.98% (at 2.687 µg/L). The accuracy for dexamethasone was better than 8%. The presence of netupitant did not disturb the recovery. The acceptance criteria (precision and accuracy <15%) were met for all analytes.

Digoxin (NETU-07-01)
Digoxin was measured in plasma and urine using liquid/liquid extraction from plasma and a validated LC/MS/MS method. The inter-day precision for digoxin in human plasma was in the range from 5.27% (at 3.9 mg/L) to 9.65% (at 2.522 mg/L). The inter-day accuracy for digoxin was better than -4%. The inter-day precision for digoxin in human urine was in the range from 6.53% (38.997 mg/L) to 8.77% (25.220 mg/L). The accuracy for digoxin in urine was better than 4%.

Ethinylestradiol/levonorgestrel (NETU-10-08)

For the analysis of ethinylestradiol and levonorgestrel in plasma, liquid/liquid extraction and a validated LC-MS/MS method was used. The LLOQ was 5.111 pg/mL for ethinylestradiol and 0.492 ng/mL for levonorgestrel. Calibration ranges were 5.1-230 pg/mL for ethinylestradiol and 0.492 – 22.123 ng/mL for levonorgestrel. Precision was better than 10% and 5 for ethinylestradiol and levonorgestrel respectively and accuracy was better than 5% and 3% for ethinylestradiol and levonorgestrel, respectively.

Docetaxel (NETU-10-09)

Docetaxel was measured in human plasma using solid-liquid extraction and a validated LC/MS/MS assay. The calibration range was 1-500 ng/mL and the LLOQ was 1 ng/mL. Inter-assay accuracy (in the QC range) ranged from 1.6% to 5.2% and inter-day precision ranged from 3.1% to 4.9%. At the LLOQ, inter-day accuracy was 3.9%, and inter-day precision was 5.7%.

Etoposide (NETU-10-09)

Etoposide was measured in human plasma using a validated LC- MS/MS assay, after protein precipitation. The inter-day accuracy (in the QC range) ranged from 1.8% to 5.7% and inter-day precision ranged from 3.2% to 5.0%. At the LLOQ, inter-day accuracy was 1.5%, and inter-day precision was 7.2%.

Cyclophosphamide (NETU-10-09)

Cyclophosphamide was measured in human plasma LC/MS/MS assay, after protein precipitation. The calibration range was 0.10 -50.0 ng/mL and the LLOQ was 100 ng/mL. The inter-day accuracy (in the QC range) ranged from -1.5% to 5.2% and inter-day precision ranged from 1.3% to 2.1%. At the LLOQ, inter-day accuracy was -0.7%, and inter-day precision was 6.2%.

2.6.2 Which metabolites have been selected for analysis and why?

NETUPITANT

Three oxidative metabolites (M1, M2, and M3) were isolated from an in vitro incubation of netupitant with recombinant human CYP3A4. A fourth metabolite was identified during ADME study (NETU-09-21). In vitro all the metabolites showed binding affinity to human NK1 receptor.

The exposure to metabolites M1, M2, and M3 resulted >10% of the parent drug exposure, in term of AUC0-t., although only M3 resulted >10% of the total radioactivity exposure. M4 was not observed in previous preclinical study, but identified later, during human mass balance study, and quantified in study NETU-11-23 accounting 3% of the parent drug exposure, in term of AUC0-t. Although active, this metabolite was; therefore, considered of negligible clinical relevance.

PALONOSETRON

Bioanalytical methods for palonosetron and its metabolites quantitation developed during clinical development of netupitant/palonosetron FDC, were based on previously validated methods for palonosetron during clinical development as single agent. The lowest limit of quantitation was 50 pg/mL for palonosetron and M4, and 10 or 50 pg/mL for M9, depending on the study.

2.6.3 What is the range of the standard curve? What are the lower and upper limits of quantification (LLOQ/ULOQ)? What is the accuracy, precision and selectivity at these limits?

Validation of the bioanalytical methods performance used for the determination of concentrations of netupitant and palonosetron in plasma are presented in Table 62 and Table 63.

Table 62 Bioanalytical Method Validation

Analytical Parameters	Netupitant	Palonosetron
Analytical Range	2 to 500 ng/mL	45 to 1500 pg/mL
Between-batch Precision (%)	2.92 to 3.62	2.2% to 5.6%
Between-batch Accuracy (%)	0.41 to 1.92	0.0% to 1.8%
Within-batch Precision (%)	1.45 to 4.88	1.1% to 7.2%
Within-batch Accuracy (%)	-1.58 to 3.56	0.1% to 3.3%
Recovery (%)	62.0	95.6
Freeze-thaw Stability LQC (three cycles) (%)	6.28	-1.7
Freeze-thaw Stability HQC (three cycles) (%)	0.74	2.1
Freezer Stability LQC (34 months, -70°C) (%)	5.50*	-11.48**
Freezer Stability HQC (34 months, -70°C) (%)	-0.43*	-14.90**
*- 34 Months at -70°C	** - 22 Months at -20°C	

The bioanalytical method is acceptable for the determination of concentrations of netupitant and palonosetron from the plasma samples.

In all the methods, calibration curve fitting was obtained by least-square linear regression analysis of weighted analyte concentration (1/X2) versus peak area of the analyte/IS (Y).

The approach followed was to re-assay approximately 5-10% of the entire PK study samples both for netupitant and/or metabolites and palonosetron. Two samples of each concentration time profile were re-analyzed: one sample around C_{max} and another sample with a concentration > LLOQ. At least 67% of all re-analyzed incurred samples had not to deviate by more than ±20% of their original concentration.

The methods developed for netupitant and metabolites, as well palonosetron, metabolites, and other co- administered drugs, were selective enough to generate reliable data. Indeed, the interference between the analytes and the other drugs, or endogenous substances, was within the acceptance criteria established (defined as 20% of the LLOQ analyte response or <5% of the IS response) in all the experiments conducted.

The precision and accuracy of netupitant and metabolites, as well as palonosetron and metabolites, in the presence of other drugs, and vice versa, was not affected and proved to be within the 15% acceptance criteria established. The precision and accuracy of netupitant and

metabolites, as well as palonosetron in the presence of 5% of lysed blood or up to 50% of a standard hyperlipidemic matrix, was within the 15% acceptance criteria established.

Table 63 Bioanalytical Method Validation for netupitant and its metabolites in plasma and urine

Validation Study Code	Matrix	Analyte	Linearity range (ng/mL) (LLOQ-ULOQ)	Inter-day Precision	Inter-day accuracy
				CV% (QC range)	% RE (QC range)
073/06-05.NT	urine	N	0.1-20 mg/L	8.09-9.31	-2.54-4.69
		M1	0.1-20 mg/L	5.45-10.25	0.23-7.92
		M2	0.1-20 mg/L	6.10-12.99	-5.09-6.39
		M3	0.1-20 mg/L	6.18-8.28	-1.99-2.20
078/08-05.NT	plasma	N	2-1000	2.61-3.43	0.91-5.45
		M1	2-1000	2.39-4.98	4.17-8.49
		M2	2-1000	2.4-7.96	7.55-13.57
		M3	2-1000	2.2-3.91	5.90-10.65
047/11-052.NP	plasma	N	2-500	2.92-3.62	0.41-1.92
		M1	2-500	3.07-3.18	-4.08 ~ -2.18
		M2	2-500	3.06-3.70	-2.99 ~ -5.09
		M3	2-500	2.39-2.85	-2.52 ~ -0.97
244/11-05.NT	plasma	M2	2-500	1.5-6.5	-2.8- 1.3
		M4	2-500	1.9-6.1	0.0-2.4

3 Major Labeling Recommendations

1) Add a statement “Avoid use in patients who are already on CYP3A4 inducers” in Section 7.
2) Add a statement about the duration of CYP3A4 inhibitory effects after single dose administration of AKYNZEO in Section 7.
3) Add the subheading of “Drug Interactions” and “Specific Population” in Section 12.3 and move detailed PK study results from Sections 7 and 8.
4) Detailed labeling recommendations will be conveyed to the sponsor during the labeling negotiation.

4 Appendices

4.1 Table of Clinical Pharmacology Studies

BA/BE study

Study #	Study	Objective and Design	Dose and Dosage Form	Subjects Characteristic (range and mean±SD)
BP-17408	An exploratory relative BA study	Randomized, open-label, three-way crossover to evaluate bioavailability of two different formulations (SDS and SE capsules), and to evaluate the SE formulation with food.	Single PO Netupitant 450 mg sodium dodecyl sulfate (SDS) capsule formulation and netupitant 450 mg sucrose ester (SE) capsule formulation	18 HVs (12M, 6F) Age : 24-59 yrs
NETU-11-23	Comparative bioavailability study	Comparison between three formulations with different dissolutions profiles. Randomized, open label, 3-treatment, 6- sequence, 3-period crossover study.	Single PO FDC 300 mg/0.5 mg capsule with standard dissolution, FDC 300 mg/0.5 mg capsule with slow dissolution, and extemporaneous netupitant 300 mg suspension plus palonosetron 0.50 mg softgel	24 HVs (24 M) Age :18-47 yrs
NETU-08-12	Pilot bioequivalence study	BE of different formulations (final FDC and extemporaneous) Randomized, open label, single-dose, 2 period, two-sequence crossover, pilot study	Single PO FDC 300 mg/0.5 mg capsules vs. Netupitant 2 x 150 mg capsules plus Palonosetron 0.5 mg softgel given as extemporaneous combination	8 (8M) healthy subjects (19-45 yrs)
NETU-09-07	BE study	BE of different formulations (final FDC and extemporaneous) Randomized, open label, single-dose, 2 period, two-sequence crossover, pilot study.	Single PO FDC 300 mg/0.5 mg capsules vs. Netupitant 2 x 150 mg capsules plus Palonosetron 0.5 mg softgel given as extemporaneous combination	50 HVs (26F, 24M) PK population: 47 subjects (23M; 24F) 19-45 yrs.
NETU-11-02	BE study .	Bioequivalence study between FDC capsules with same formulation produced by two different manufacturers: Helsinn Birex Pharmaceuticals (Ireland)(Planned Commercial Product) and (b) (4) (Phase 3 and late phase 1 FDC).	Single PO FDC 300 mg /0.5 mg manufactured by (b) (4) vs. FDC 300 mg /0.5 mg manufactured by HBP	88 HVs (19F, 69M) 82 completed. PK population for netupitant: 82 subjects (65M; 17F) PK population for palonosetron: 79 subjects (63 M; 16F)

Studies with Netupitant alone (1)

Study #	Study	Objective and Design	Dose and Dosage Form	Subjects Characteristic (range and mean±SD)
NETU-09-21	Mass balance study	ADME Study Mass balance and metabolic profile	Single PO [^{14}C]-Netupitant oral suspension 300 mg (nominal dose) 2.22 MBq (60 µCi)	6 HVs (5 completed) (6M, 0F) 32-56 yrs
NP16599	Impact of netupitant on the pharmacokinetics of midazolam and erythromycin,	PK/safety drug- interaction trial with erythromycin and midazolam	Single PO Netupitant 300 mg Midazolam 7.5mg Erythromycin 500mg	20 HVs (20M, 0F) age 20-32 20 subjects received active drug
NP16600	Evaluation of the effects of food and age on the PK of netupitant	Food and Age effect trial of netupitant:	Single PO Food Effect: netupitant 300 mg (2x150 mg caps)	Food effect: 12 HVs (12M, 0F) ,age 23-44(Y) Age effect: 6 HVs (6M, 0F) age 66-72 (E) 16 received active drug, 2 received placebo
NP16601	Multiple ascending dose PK study in healthy young and elderly volunteers	Randomized, double-blind, placebo-controlled, multiple ascending dose in two parts (young and elderly subjects); PK/safety in healthy young and elderly volunteers	Multiple PO Placebo, netupitant 100, 300, 450 mg	33 HVs (33M, 0F) 30 HVs age 21-44 yrs (Y); 3 HVs 65-68 (E) 26 subjects received active drug; 1 placebo and 1elderly dropped out.
NP16602	Apomorphine Challenge PK/PD study:	Randomized, double- blind, placebo controlled four- group single ascending dose	Single PO Placebo Netupitant 100 mg Netupitant 300 mg Netupitant 450 mg	32 HVs (30M, 2F) age 18-45 24 received active drug (23M, 1F)
NP16603	Single ascending dose PK study of netupitant	Randomized, double-blind, placebo- controlled single-ascending dose PK/safety in healthy volunteers	Single PO Placebo Netupitant 10 mg Netupitant 30 mg Netupitant100 mg Netupitant 300 mg Netupitant 450 mg	30 HVs (30M, 0F) age 19-43 20 received active drug-10 placebo

Studies with Netupitant alone (2)

Study #	Study	Objective and Design	Dose and Dosage Form	Subjects Characteristic (range and mean±SD)
NETU-06-07	PK Interaction between Netupitant and Oral Dexamethasone Regimen:	Randomized, open, 3-period crossover study (incomplete Latin Square design)	Treatment A: Dexamethasone (20 mg on Day 1, 8 mg bid from Day 2 to Day 4) Treatment B: Dexamethasone (as in group A) plus Netupitant 100 mg on Day 1 Treatment C: Dexamethasone (as in group A) plus Netupitant 300 mg on Day 1 Treatment D: Dexamethasone (as in group A) plus Netupitant 450 mg on Day1.	26 HVs (15M, 11F) 25 subjects (14 M, 11 F) age 18-42 were treated and included in at least one population set.
NETU-06-08	Receptor occupancy study using PET	Single-dose, randomized, open-label PET study investigating the degree of occupancy of NK1 receptors in the human brain after single oral doses in HVs Support dose selection for Phase 2 trials	Single PO Netupitant 100 mg Netupitant 300 mg Netupitant 450 mg	6 HVs completed (6M, 0F) 2 M per each dose level Age 20-25 yrs
NETU-07-01	PK interaction between netupitant and digoxin	PK/safety drug-interaction trial with Digoxin:	Single PO Netupitant 450 mg on Day 8 Digoxin 0.25 mg loading dose 3x0.5 mg on Day1, followed by 0.25 mg for 11 consecutive Days	16 HVs (8M, 8F) 19-45 yrs

Studies with netupitant and palonosetron combination (1)

Study #	Study	Objective and Design	Dose and Dosage Form	Subjects Characteristic (range and mean±SD)
NETU-06-06	Drug Interaction between netupitant and Palonosetron.	Randomized, open-label, single dose, 3 period study to evaluate the PK interaction between netupitant and palonosetron in healthy volunteers	Single PO Netupitant 450 mg Netupitant 450 mg + Palonosetron 0.75 mg Palonosetron 0.75 mg	18 HVs (9M, 9F) 18-36 yrs
NETU-07-20	Thorough QT study:	Randomized double- blind (except moxifloxacin), double-dummy, parallel group placebo and open-label positive controlled study to investigate possible ECG effects of netupitant and palonosetron	Single PO Placebo netupitant 200 mg + palonosetron 0.5 mg netupitant 600 mg + palonosetron 1.50 mg moxifloxacin 400 mg	200 (106M, 94F) (196 completed) Age :19-45 yrs
NETU-10-02	Population PK study	Population PK study NETU-10-02, Subgroup from NETU-08-18 Phase 3 study	Single PO Group 1 – oral netupitant/palono setron (300 mg/0.50 mg) FDC + dexamethasone 12 mg on Day 1. Group 2 – oral palonosetron 0.50 mg (Aloxi) and oral dexamethasone 20 mg on Day 1. Group 1 only netupitant and palonosetron measurements were included in the analysis.	Netupitant analysis: 117 (4M, 113F), 55 yrs* (29-75); Race: 101 Caucasian, 16 Asian. BW: 71* (34 – 125) kg BMI: 27.43* (14.72-41.74) kg/m2 Palonosetron analysis: 118 (5M, 113F) 55 yrs* (29-75); Race: 102 Caucasian, 16 Asian. BW: 71*(34-125) kg BMI: 27.6* (14.72-41.74) kg/m2 *median
NETU-10-08	Drug-Interaction trial with oral contraceptives:	Open, randomized, two-way crossoer trial to evaluate the effect of FDC on the PK of ethinylestradiol and levonorgestrel in healthy female subjects	Single PO FDC 300 mg / 0.5mg + two tablets of Microgynon® (30 µg ethinylestradiol + 150 µg levonorgestrel per tablet) vs. Two tablets of, Microgynon®	24 HVs (0M, 24F) 19-40 yrs

Studies with netupitant and palonosetron combination (2)

Study #	Study	Objective and Design	Dose and Dosage Form	Subjects Characteristic (range and mean±SD)
NETU-10-09	DDI between FDC and chemo (docetaxel,etoposide, cyclophosphamide)	Single-dose, open- label, two period Randomized, crossover drug interaction study of the oral netupitant/palonosetron FDC on the PK of docetaxel, etoposide or cyclophosphamide in cancer patients.	Single PO Netupitant/Palonosetron FDC 300 mg / 0.5mg + Docetaxel/Etoposide/ Cyclophosphamide vs. Palonosetron 0.5mg + Docetaxel/Etoposide/ Cyclophosphamide	Cancer patients Docetaxel Group: 8 cancer patients (7M, 1F; 50-81 yrs) Etoposide Group: 12 cancer patients (11M, 1F; 22-73 yrs) Cyclophosphamide Group: 10 cancer patients (1M, 9F; 33-69 yrs)
NETU-10-10	Hepatic impairment	Single center, open label, one period PK study in patients with different stages of hepatic impairment	Single PO FDC 300 mg/ 0.5 mg	In total:36 Patients (26 men, 10 women) (39-71 years old) 8 pts. with mild hepatic Impairment 8 pts. with moderate hepatic Impairment 2 pts. with severe hepatic impairment
NETU-10-11	drug- interaction study with ketoconazole and rifampicin:	Open, randomized two-group, two-way crossover study to evaluate the effect of concomitant administration of ketoconazole or rifampicin, on the PK of netupitant and palonosetron	Single PO FDC 300 mg 0.5 mg plus Ketoconazole 400 mg x 12 consecutive days or Rifampicin 600 mg x 17 consecutive days	36 HVs (21M, 15F) PK population: N=35 Ketoconazole group: 17 subjects (6F; 11M; 33-55 yrs,) Rifampicin group: 18 subjects (8F; 10M; 32-55 yrs)
NETU-10-12	Food and Age effect trial with FDC:	Open, randomized, two-way, cross-over study to investigate the effect of food (comparison fasted and fed condition) with one parallel group of elderly subjects to investigate the effect of age (comparison elderly versus younger subjects in the fasted group).	Single PO FDC 300 mg/0.5 mg	36 HVs (22M, 14F) PK population in cross-over part: 22 HVs (22-45 years) PK population in the parallel part: 12 HVs (66-79 years).
NEPA-13-11	Gender effects on PK using pooled PK data	Post-hoc analysis report to evaluate the effect of gender on in-vivo palonosetron and netupitant pharmacokinetic profiles-	Information in study NETU-09-07, NETU-11-02 and NETU-11-23	Pooled analysis in 153 HVs 18-50y: 112 M; 41 F Netupitant: 330 pooled PK profiles in M; 116 pooled PK profiles in F Palonosetron: 298 pooled PK profiles in M; 112 pooled PK profiles in F

4.2 Pharmacometric Review

OFFICE OF CLINICAL PHARMACOLOGY: PHARMACOMETRIC REVIEW

1 SUMMARY OF FINDINGS

1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 What are the covariates affecting the PK of netupitant based on population PK analysis?

No statistically significant covariates were identified in population PK analysis. A two-compartment base model with first order absorption adequately described the observed PK data of netupitant. The median netupitant apparent clearance was estimated to be 20.9 L/h and the volume of distribution was estimated to be 419 L. Based on sponsor's analysis, none of the covariates had significant impact on the PK of netupitant based on population PK analysis (Table 1). However, it is worth noting that for some intrinsic and extrinsic factors, dedicated studies were available and therefore population PK analysis is supportive. For gender, only 4 male subjects were included in the population PK analysis, therefore the effect of gender on PK should be derived primarily based on the dedicated studies. For hepatic impairment, a dedicated study was conducted to evaluate the effect of hepatic impairment on PK. Based on the population PK analysis, there appears to be no effect of race and body weight on PK of netupitant. In addition there was no dedicated renal impairment study conducted for netupitant. Based on population PK analysis, there appears to be no statistically significant effect of mild and moderate renal impairment on the clearance of netupitant. This is expected since renal pathway is a minor route of elimination for netupitant. For drug-drug interactions, the data from dedicated clinical pharmacological studies were available to evaluate the effect of drug interactions on netupitant PK. The impact of the smoking status, chemotherapy (doxorubicin, epirubicin, fluorouracil) and rescue medications on the PK of netupitant in combination with palonosetron were evaluated by population PK analysis. None of those factors appears to significantly influence the disposition of netupitant and palonosetron. However, it should be noted that definitive conclusions regarding these factors cannot be made as the population PK analysis may lack power to detect the effect of these factors due to the study design and/or insufficient PK sampling.

Table 1. Demographics and baseline characteristics in population PK analysis for netupitant (n=117)

Covariate	N=117
Continuous Variables	Median (range)
Age (years)	55 (29-75)
Body mass index (kg/m^2)	27.43 (14.72-41.74)
Body weight (kg)	71 (34 – 125)
Baseline ALT (IU/L)	18 (6 – 105)
Baseline AST (IU/L)	19 (9 – 90)
Baseline alkaline phosphatase (IU/L)	70 (20-1192)
Baseline total bilirubin (µmol/L)	6 (1 – 18)
Baseline albumin (g/dL)	44 (29-52)
Baseline creatinine clearance (mL/min)	100 (31 – 150)
Baseline neutrophil count (10^9/L)	4 (1.5 – 9.4)
Categorical Variables	Count (%)
Sex (females/males)	113 (96.6%)/ 4 (4.3%)
Race (Caucasian/Asian)	101 (86.3%) /16 (13.7%)
Tobacco usage (smoker/non-smoker/ex-smoker)	16 (13.7%)/ 95 (81.2%)/ 6 (5.1%)
ECOG performance status (0/1/2)	75 (64.1%)/41 (35%)/1 (0.9%)
Chemotherapeutic regimen (Cyclophosphamide and Doxorubicin/ Cyclophosphamide and Epirubicin)	74 (63.2%)/43 (36.8%)
Taking cyclophosphamide	117 (100%)
Taking doxorubicin	74 (63.2%)
Taking epirubicin	49 (41.9%)
Taking fluorouracil	44 (37.6%)
Not taking rescue medication*, acute phase	110 (94.0%)
Not taking rescue medication*, delayed phase	102 (87.2%)
Not taking rescue medication*, overall phase	102 (87.2%)
*Among patients taking rescue, all but one took metoclopramide	

Source: Sponsor's data analysis report for study NETU-10-02, Page 37

1.1.2 What are the exposure-response relationship for efficacy and safety?

A formal assessment of exposure-response relationship could not be made due the limited PK data collected in the clinical studies as only 117 patients out of 726 (~16%) in FDC arm in the study NETU-08-18 had PK samples.

1.2 Recommendations

The application is acceptable from pharmacometrics perspective. Following are the recommendations:

- No dose adjustment required based on race and body weight
- No dose adjustment required based on age (29-75 years)
- No dose adjustment required for mild or moderate renal impairment

1.3 Label Statements

See section 3 in clinical pharmacology review.

4.3 IRT-QT team review (For detailed review, please see the original review dated 1/20/2010)

Interdisciplinary Review Team for QT Studies Consultation:

Thorough QT Study Review

IND or NDA	IND 73493
Generic Name	Netupitant/Palonosetron
Sponsor	Helsinn Healthcare SA
Indication	Prevention of Chemotherapy-Induced Nausea and Vomiting
Dosage Form	Fixed-Dose Combination Capsule
Drug Class	Antiemetic
Therapeutic Dosing Regimens	Netupitant 200 mg + Palonosetron 0.50 mg
Duration of Therapeutic Use	Acute
Maximum Tolerated Dose	Not determined
Submission Number and Date	SDN 024 (09 November 2009)
Review Division	Division of Gastroenterology Products (HFD-180)

1 SUMMARY

1.1 QT INTERDISCIPLINARY REVIEW COMMENTS

The following comments should be conveyed to the sponsor:

1. The moxifloxacin profile is not exactly what we expected. The rising phase is missing. The maximum moxifloxacin induced ΔΔQTcF effect appears almost at the first available time point, which is 1 hr after dose. We want to understand what happened before hour 1. Please extract data for moxifloxacin, placebo at 15 minute, 30 minute post-dose and the corresponding baseline for us to evaluate.
2. Monitoring LVEF and troponins in the phase 3 trials should be considered.
3. Since netupitant belongs to the same drug class as Casapirtant which was withdrawn from the market due to cardiotoxicity (hypotension, bradycardia, QT prolongation and troponin elevation in clinical studies along with myocardial necrosis and phopholipidosis in non-clinical studies) a separate review of the cardiac safety pharmacology and toxicology studies by the DCRP pharmacologist Dr. Muriel Saulnier was requested. Her recommendations are as follows:
    - The adverse effects on QT at higher dosages and the tissue phospholipidosis are of concern since they appear to be a class -related effect. We recommend that sponsor examines for phospholipidosis (that was linked to QT prolongation) in the heart of rats and dogs in the chronic toxicity studies using electron microscopy.

1.2 OVERALL SUMMARY OF FINDINGS

The sponsor used QTcI as their primary correction method. Based on our evaluation for different correction methods, we believe QTcF corrects RR more sufficiently than QTcI in this study; therefore, FDA analysis results based on QTcF are suggested for the labeling.

No significant QTcF prolongation effect of netupitant/palonosetron (therapeutic dose 200 mg/0.50 mg and supratherapeutic dose 600 mg/1.50 mg) was detected in this TQT study. The largest upper bounds of the 2-sided 90% CI for the mean difference between two netupitant/palonosetron dose and placebo groups were below 10 ms, the threshold for regulatory concern as described in ICH E14 guidelines. The largest lower bound of the two-sided 90% CI for the ΔΔQTcF for moxifloxacin was greater than 5 ms; however, the moxifloxacin profile is missing a rising phase (Figure 9); therefore, we would like to evaluate moxifloxacin induced ΔΔQTcF effect at 15 minutes and 30 minutes post-dose as well.

In this double-blind, randomized, parallel-group study, 200 healthy subjects received 200 mg netupitant/0.50 mg palonosetron, 600 mg netupitant/1.50 mg palonosetron, placebo, and a single dose of moxifloxacin 400 mg. Overall summary of findings is presented in Table 1.

Table 1: The Point Estimates and the 90% CIs Corresponding to the Largest Upper Bounds for netupitant/palonosetron (200 mg/0.50 mg and 600 mg/1.50 mg) and the Largest Lower Bound for Moxifloxacin (FDA Analysis)

Treatment	Time (hour)	ΔΔQTcF (ms)	90% CI (ms)
Netupitant/palonosetron (200 mg/0.50 mg)	14	4.4	(1.5, 7.3)
Netupitant/palonosetron (600 mg/1.50 mg)	16	5.9	(2.8, 9.1)
Moxifloxacin 400 mg*	4	13.2	(10.1, 16.3)

* Multiple endpoint adjustment was not applied. The largest lower bound after Bonferroni adjustment for 5 timepoints (1, 2, 4, 5, and 6 hours) was 9.2 ms.

The supratherapeutic dose (netupitant 600 mg/palonosetron 1.50 mg) produces mean netupitant and palonosetron C_{max} values 3.2-fold higher than the mean C_{max} for the therapeutic dose (netupitant 200 mg/palonosetron 0.50 mg). The expected high clinical exposure scenario for netupitant is unknown at this time. Hepatic impairment may decrease netupitant's clearance as hepatic metabolism is the route of metabolism. However, exposure data in patients with hepatic impairment is not available. The accumulation of netupitant is 2.76-3.06 after 7 days dosing, so the 600 mg supratherapeutic dose in this study may not cover high clinical exposure with chronic dosing and hepatic impairment. Intrinsic and extrinsic factors have not been shown to significantly increase palonosetron exposure, so the 1.50 mg supratherapeutic dose is expected to cover the high clinical exposure scenario.

4.3 OCP Filing Form

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	205-718	Brand Name	Akynzeo
OCP Division (I, II, III, IV, V)	DCP3	Generic Name	Netupitant/palonosetron
Medical Division	DGIEP	Drug Class	Anti-emetics
OCP Reviewer	Insook Kim, Ph.D. Dilara Jappar, Ph.D.	Indication(s)	Prevention of acute and delayed CINV with initial and repeat courses of highly emetogenic chemotherapy and moderately emetogenic chemotherapy
OCP Team Leader	Sue-Chih Lee, Ph.D.	Dosage Form	Fixed dose combination capsule
Pharmacometrics Reviewer	Jingyu "Jerry" Yu, Ph.D.	Dosing Regimen	60 min prior to initiation of chemotherapy
Pharmacometrics Team Leader	Nitin Mehrotra, Ph.D.	Route of Administration	Oral
Date of Submission	9/27/2013	Sponsor	Helsinn
Estimated Due Date of OCP Review	5/30/2014	Priority Classification	S
Medical Division Due Date	5/30/2014		
PDUFA Due Date	9/26/2014		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x	12		Two active ingredients in plasma and urine Drugs used in DDI studies
I. Clinical Pharmacology				
Mass balance:	x	1		NETU-09-21 (netupitant)
Isozyme characterization:	x	1		#1003832
Blood/plasma ratio:	x	2		#1006047 (parent drug) #1010388 (metabolites)
Plasma protein binding:	x		Same study as B/P ratio	#1006047 (parent drug) #1010388 (metabolites)
Pharmacokinetics (e.g., Phase I) -	x			
Healthy Volunteers-				
single dose:	x	1		NP16603
multiple dose:	x	1		NP16601
Patients-				
single dose:	x	1		NETU-10-09 (PK from a DDI study)
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:	x			
fasting / non-fasting multiple dose:	x			

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Drug-drug interaction studies -				
In-vivo effects on primary drug:	x	1		NETU-10-11 (ketoconazole and rifampicin on FDC)
In-vivo effects of primary drug:	x	7		NP16599 (netupitant on midazolam and erythromycin) NETU-06-06 (netupitant on palonosetron) NETU-06-27 (netupitant on palonosetron) NETU-07-01 (netupitant on digoxin) NETU-06-07 (netupitant on dexamethasone) NETU-10-08 (FDC on oral contraceptive) NETU-10-09 (FDC on chemotherapy in patients)
In-vitro:	x	9		
Subpopulation studies -				
ethnicity:				
gender:	x	1		NEPA-13-11 pooled analysis of 3 studies to evaluate gender PK
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:	x	1		NETU-10-10
PD -				
Phase 2:	x	1		NETU-07-07
Phase 3:	x	3		NETU-08-18 PALO-10-01 NETU-10-29
PK/PD -				
Phase 1 and/or 2, proof of concept:	x	3		NP16602 (apomorphine challenge study) NETU-06-08 (PET study) NETU-07-20 (TQT)
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:	x	1		NETU-10-02 (population PK from Phase 3 trial)
II. Biopharmaceutics				
Absolute bioavailability				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:	x	2		Formulation development BP-17408 NETU-11-23
Bioequivalence studies -				
traditional design; single / multi dose:	x	3		NETU-08-12 (pilot BE) NETU-09-07 (BE for palo and between phase 2 formulation and phase 3 formulation) NETU-11-02 (BE for manufacturing site change)
replicate design; single / multi dose:				
Food-drug interaction studies	x	2		NP166600 (netupitant) NETU-10-12 (FDC)
Bio-waiver request based on BCS				
BCS class	x			
Dissolution study to evaluate alcohol induced dose-dumping				

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric Study Plan	x	1		Requesting (b) (4)
Literature References				
Total Number of Studies		54		

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	x			
2	Has the applicant provided metabolism and drug-drug interaction information?	x			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	x			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	x			
5	Has a rationale for dose selection been submitted?	x			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	x			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	x			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	x			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	x			
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	x			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?			x	Fixed dose combination product

File name: 5_Clinical Pharmacology and Biopharmaceutics Filing Form/Checklist for NDA_BLA or Supplement 090808

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	PSP to request a (b) (4)
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	x			
	General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?	x			

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?
____Yes____

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

- Please provide the assay validation for cardiac troponin levels (cTnI). If such information is already submitted, please guide the reviewer to the location of the information.

- For Study NETU-07-20, please extract data for moxifloxacin, placebo at 15 minute, 30 minute post-dose and the corresponding baseline for us to evaluate. Upon the review of the thorough QT study, IRT-QT review team found that the moxifloxacin profile was missing the rising phase. The maximum moxifloxacin induced ddQTcF effect appeared almost at the first available time point, which was 1 hr after dose. Therefore we request additional analysis to understand what happened before hour 1.

Insook Kim, Ph.D.	10/29/13
Reviewing Clinical Pharmacologist	Date
Sue-Chih Lee, Ph.D.	10/29/13
Team Leader/Supervisor	Date

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

/s/

INSOOK KIM
05/30/2014

DILARA JAPPAR
05/30/2014

JINGYU YU
05/30/2014

NITIN MEHROTRA
05/30/2014

SUE CHIH H LEE
05/30/2014