CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

207145Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology/Biopharmaceutics Review

PRODUCT (Generic Name):	Safinamide (NW-1015)
PRODUCT (Brand Name):	XADAGO
NDA:	207145
DOSAGE FORM:	Tablets
DOSAGE STRENGTHS:	50 mg and 100 mg
INDICATION:	Add-on therapy for the treatment of patients with Parkinson's disease
NDA TYPE:	Standard
SUBMISSION DATE:	Dec 29, 2014
SPONSOR:	Newron Pharmaceuticals
PRIMARY REVIEWER:	Hristina Dimova, Ph.D.
REVIEWER (*in vitro* studies):	Jagan Parepally, Ph.D.
TEAM LEADER:	Angela Men, M.D, Ph.D.
PHARMACOMETRICS REVIEWER:	Atul Bhattaram, Ph.D.
PHARMACOMETRICS TEAM LEADER:	Kevin Krudys, Ph.D.
OCPB DIVISION:	DCP-I
OND DIVISION:	HFD-120

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1.0 EXECUTIVE SUMMARY

Safinamide is a new chemical entity whose mechanism of action includes voltage gated sodium channel blockade, inhibition of release of glutamate, and selective, reversible inhibition of Monoamine Oxidase B (MAO-B), thus producing both non-dopaminergic and dopaminergic pharmacological effects.

The sponsor seeks approval for safinamide as adjunct therapy for the treatment of subjects with idiopathic Parkinson's disease (PD) as add-on to:
• a single DA-agonist at a stable dose in early-stage (b) (4) PD patients and
• (b) (4) levodopa (LD) alone or in combination with other PD medications in mid- to late-stage (b) (4) patients
*Note: Safinamide will not be recommended for early PD

Safinamide is formulated for oral administration as an immediate-release tablet (50 mg and 100 mg).

The clinical program for safinamide included 37 trials: 20 Phase I, 8 Phase II, and 9 Phase III trials. A total of 3184 subjects were enrolled in these trials: 607 subjects were enrolled in non-therapeutic trials, and 2519 subjects with PD were enrolled in therapeutic trials. In addition, 41 *in vitro* studies have been submitted in the NDA, addressing the absorption, distribution, biotransformation and drug-drug interaction (DDI) potential of safinamide.

The efficacy of safinamide as add-on treatment in mid-to late-stage PD (LSPD) patients with motor fluctuations currently receiving LD alone or in combination with other PD medications was evaluated in two double-blind, placebo-controlled studies: SETTLE (Study 27919; 50-100 mg/day; 24 weeks), and Study 016/018 (50 and 100 mg/day; 2-year, double-blind, placebo-controlled study). Statistically significant improvements in ON Time and OFF Time, as well as motor symptoms (UPDRS III), without any increases in troublesome dyskinesia were demonstrated in both studies; in addition, the effect on ON Time was maintained at the end of the 24-month double-blind treatment period for both safinamide doses as compared to placebo. Patients should start on the lowest effective dose of 50 mg and can have their dose increased to 100 mg/day based on therapeutic need. No dose adjustment of safinamide is proposed on the basis of gender, race, ethnicity, or renal function. In patients with moderate hepatic impairment, safinamide dose should not exceed 50 mg. Concomitant use of safinamide with other monoamine oxidase inhibitors is contraindicated.

Three placebo-controlled studies have been conducted in early-stage PD (ESPD) patients on a DA-agonist (Studies 009, 015 and MOTION), however no clinical benefit has beed demonstrated in ESPD patients and safinamide will not be recommended for early PD. Please refer to the review by Dr Len Kapcala (Medical Officer, DNP) for more details.

1.1 RECOMMENDATION

The Office of Clinical Pharmacology (OCP/DCP I) has reviewed the clinical Pharmacology and Biopharmaceutics sections of NDA 207145. The submission is acceptable from a Clinical Pharmacology and Biopharmaceutics point of view provided the sponsor agrees with the Agency's labeling recommendations.

Post-Marketing Commitments:

1. A clinical study to characterize drug-drug interaction of safinamide and BCRP substrates in healthy volunteers

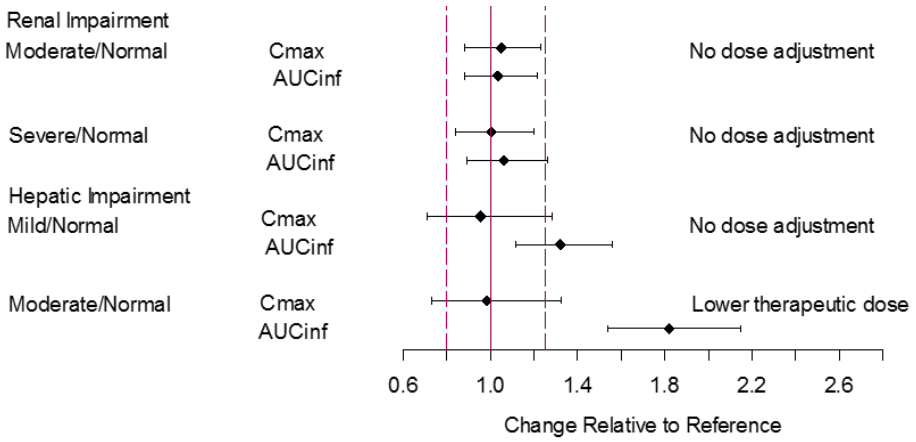
NW-1689 is a major metabolite of safinamide found in plasma at the concentration of approximately 160% of parent compound, safinamide. NW-1689 inhibited BCRP with an IC_{50} of 3.7 ± 0.5 μM. The average maximal plasma concentration of Safinamide was approximately 4 μM in Parkinson's disease patients treated with the highest dose of 100 mg/day. Based on this information from in vitro evaluation, there is a need for further in vivo drug interaction study at post-approval stage.
Substrates of BCRP include methotrexate, mitoxantrone, imatinib, irrinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan.

1.2 OVERALL SUMMARY OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FINDINGS

The findings from overall clinical pharmacology and biopharmaceutics section are as follows:
The efficacy of safinamide as add-on treatment in mid-to late-stage PD (LSPD) patients with motor fluctuations currently receiving LD alone or in combination with other PD medications was evaluated in two double-blind, placebo-controlled studies: SETTLE (Study 27919; 50-100 mg/day; 24 weeks), and Study 016/018 (50 and 100 mg/day; 2-year, double-blind, placebo-controlled study). The primary efficacy parameter was the change from baseline in 'ON Time without troublesome dyskinesia'. Secondary efficacy parameters included OFF Time, UPDRS II and III (Unified Parkinson's Disease Rating Scale – sections II and III), and CGI-C (Clinical Global Impression of Change). Both the SETTLE and 016/018 studies indicated significant superiority of safinamide, compared to placebo, at the target doses of 50 and 100 mg/day for the primary and selected secondary efficacy variables; in addition, the effect on ON Time was maintained at the end of the 24-month double-blind treatment period for both safinamide doses as compared to placebo.
Three placebo-controlled studies have been conducted in early-stage patients on a DA-agonist (Studies 009, 015 and MOTION), however no clinical benefit has beed demonstrated in ESPD patients and safinamide will not be recommended for early PD. Please refer to the review by Dr Len Kapcala (Medical Officer, DNP) for more details. There were additional clinical pharmacology related issues with Trial 015 (Please refer to Sect. 2.5.2 of this QBR and the Clinical Pharmacology Individual Study review for more details).

<u>General Pharmacokinetics (ADME characteristics) of safinamide</u>

Absorption: Safinamide is classified as low solubility, highly permeability drug substance (BCS Class 2). Safinamide solubility is highly pH dependant: high solubility at low pH, low solubility at pH ≥6.8.
Safinamide T_{max} is 1.8-2.8 hours under fasting conditions, 0.75 h delay with food. Absolute bioavailability is high (95%), indicating that safinamide is almost completely absorbed after oral administration, and first pass metabolism is negligible.
Distribution: Safinamide volume of distribution (V_{ss}) is approximately 165 L, indicating extensive extravascular distribution. Safinamide is not highly protein bound (however its major metabolite NW-1689 is highly protein bound >99.5% binding).
Metabolism: Safinamide is almost exclusively eliminated via metabolism by non-microsomal enzymes (cytosolic amidases/MAO-A and ALDH), CYP3A4 iso-enzymes play only a minor role in safinamide overall biotransformation. About 7% of the drug is eliminated unchanged, mainly in urine. There are three major metabolites; none is expected to contribute to the pharmacological activity of the drug. NW-1689 is the main circulating metabolite in human plasma, exceeding the exposure of the parent (161% of parent). NW-1689 AG and NW-1153 account for about 16-18% and 10-11% of the parent drug exposure, respectively.
Elimination: Safinamide total clearance was determined to be 4.6 L/h (low clearance drug). Terminal half-life is 20-26 h. The primary route is through the kidney (76% of the safinamide dose recovered in the urine, primarily in the form of metabolites).
Single dose and multiple dose pharmacokinetics (PK):
Safinamide PK has been characterized in healthy men and women over a single-dose range of 50 mg to 10.0 mg/kg (~700mg) and multiple-dose range of 1.25 mg/kg/day (~88 mg/day) to 350 mg/day. Additionally, the PK of safinamide has been assessed in clinical trials of PD patients following administration of multiple doses of 50- 200 mg/day and patients with epilepsy (up to 350 mg/day).
Dose proportionality:
The increase in safinamide exposure (AUC and C_{max}) is dose proportional in the range of doses studied (see above). The PK of safinamide are time-independent.
Pharmacokinetics in patients:
No difference in the PK of safinamide was observed between healthy subjects and PD patients.
Intrinsic Factors:
No dose adjustment of safinamide is proposed on the basis of gender, race, ethnicity, or renal function. In patients with moderate hepatic impairment, safinamide dose should not exceed 50 mg. Safinamide has not been studied in patients with severe hepatic impairment and is not recommended for this patient population.



DDI:
Concomitant use of safinamide with other monoamine oxidase inhibitors is contraindicated.
In Vitro Studies: *In vitro* metabolism studies indicated that there is no meaningful inhibition or induction of Cytochrome P450 (CYP) based enzymes by safinamide and its major metabolites at concentrations that are relevant for dosing. Safinamide or its major metabolites at clinically relevant concentrations are not inhibitors of MAO-A, levodopa decarboxylase or ALDH enzymes.
Safinamide is not a substrate of P-gp. Safinamide and its metabolites did not inhibit P-gp, or other transporters OCT2, OATP1B1, OATP1B3, BSEP, OAT1/3/4 or BCRP (except BCRP inhibition by NW-1689) at clinically relevant concentrations.
In Vivo Studies: Dedicated drug-drug interactions studies performed with ketoconazole, levadopa and CYP1A2 and CYP3A4 substrates (caffeine and midazolam), did not demonstrate any clinically significant effects on the PK profile of safinamide, or on the PK profile of co-administered LD or CYP1A2 and CYP3A4 substrates.
In addition, dietary tyramine restriction is not required with recommended doses of safinamide based on the results of three trials conducted to assess the pressor effect of tyramine during safinamide administration.

Biopharmaceutics:
BCS Class:
Safinamide can be considered a Biopharmaceutical Classification System (BCS) Class II drug (low solubility, high permeability). The drug substance is highly soluble at pH 1.2 and 4.5, but low soluble at pH 6.8 and 7.5. Absolute bioavailability is high (95%).
Bioequivalence (BE):
An *in vivo* BE study bridged the to-be-marketed (TBM) tablets to clinical trial tablets used for mid-late stage of PD.
Food Effect:
Safinamide can be administered without regards to meals or meal types. A slight delay in t_{max} was observed in the fed state relative to the fasted condition, there was no effect on safinamide $AUC_{0-\infty}$ and C_{max}.

2.0 QUESTION BASED REVIEW

2.1 GENERAL ATTRIBUTES

2.1.1 Drug/Drug Product Information:

Dosage Form/Strengths: immediate-release tablet (50 mg and 100 mg)

Indication: treatment of patients with Parkinson's disease (PD) as add-on therapy to a stable dose of a levodopa product alone or with other PD medications

Pharmacologic Class: dopaminergic and non-dopaminergic mechanism of action that includes voltage-gated sodium channel blockade, inhibition of release of glutamate, and selective, reversible inhibition of Monoamine Oxidase B (MAO-B).

Chemical Name: (S)-(+)-2-[4-(3-fluorobenzyl) oxybenzyl] aminopropanamide methanesulfonate

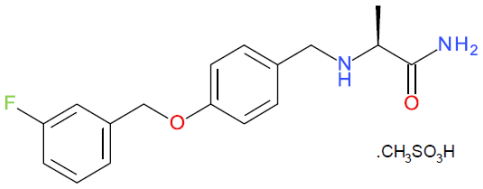
International Non-Proprietary Name: Safinamide

United States Adopted Name (USAN): Safinamide mesylate

Molecular formula: $C_{17}H_{19}FN_2O_2 \cdot CH_4O_3S$

Molecular mass: 398.45 g/mol as methanesulfonate salt; 302.34 g/mol as free base

Chemical structure:



Physical Characteristics: Safinamide mesylate is a white to off-white crystalline powder. Safinamide mesylate is freely soluble in water, methanol and dimethyl sulphoxide. Safinamide mesylate is sparingly soluble in ethanol and is practically insoluble in ethylacetate. In aqueous buffers that span a pH range of 1.2 to 7.5, safinamide mesylate is highly soluble at pH 1.2 and 4.5, but shows low solubility (< 0.4 mg/mL) at pH 6.8 and 7.5.

Formulation: immediate release tablet with a (b) (4) coating for oral administration.

The qualitative composition of the two tablet strengths (50 mg & 100 mg) is the same (see table in Sect. 2.5.2), and the composition of the two strengths is quantitatively proportional.
The tablets also contain the following inactive ingredients: microcrystalline cellulose, crospovidone, colloidal silicon dioxide, magnesium stearate, hypromellose, polyethylene glycol 6000, iron oxide (red), potassium aluminum silicate, and titanium dioxide.

2.1.2 Mechanism of action and therapeutic indication:

Safinamide is a reversible and selective MAO-B inhibitor (IC_{50} 79nM in human brain mitochondrial fraction and 9.3 nM in human platelet-rich plasma; > 1000-fold selective over MAO-A). Through this mechanism, safinamide produces an enhancement of the dopaminergic transmission in the brain, restoring the dopaminergic function that is impaired in PD. Increase in striatal dopamine (DA) levels and beneficial pro-dopaminergic behavioural motor effects have been demonstrated in preclinical PD models.
Safinamide also controls neuronal excitability by blocking voltage-gated sodium channels (VGSC) in a state-dependent manner (with IC_{50} values in the range of 1.6 - 4.9 μM for the different Na+ channel subtypes), and at higher concentrations, it inhibits calcium channels. The expected physiological effect is the modulation of the hyper-active neurons and the consequent regulation of the neurotransmitter release. In preclinical studies safinamide has reduced glutamate release induced by aberrant Na+ channel activity without affecting basal glutamate levels. This non-dopaminergic mechanism provides the potential for reducing L-dopa induced dyskinesia and excitotoxicity, as demonstrated in preclinical models of dyskinetic MPTP-lesioned primates and in rodent models of neuroprotection.
Safinamide additionally binds dopamine and serotonin transporters (DAT and SERT) with IC_{50} of 8.8 μM and 5.6 μM, respectively, leading to dopamine and serotonin uptake inhibition in brain synaptosomes with IC_{50} of 12.5 and 21 μM, respectively.

2.1.3 Proposed dosages and route of administration:
The sponsor proposes safinamide to be administered at the same doses and according to the same schedule (50 or 100 mg/day) in patients with Early Stage Parkinson's Disease (ESPD) on a single dopamine agonist, and in patients with Late Stage Parkinson's Disease patients (LSPD). The recommended dosing regimen is once daily, (b) (4) with or without food.
Note: However, no clinical benefit has beed demonstrated in ESPD patients and safinamide will not be recommended in ESPD by the Agency.
Overall, the clinical data support starting therapy with 50mg/day, which has shown a clinically relevant benefit, as judged by responder analyses, and ON time, but has a lower magnitude of effect than the 100mg/day dose. Patients should start on the lowest effective dose of 50mg and can have their dose increased to 100 mg/day based on therapeutic need. The dosing frequency i.e. once daily is supported by the long lasting duration of the MAO-B activity inhibition (a 75% and 69% inhibition was still seen 24h after safinamide 600μg/kg and 300 μg/kg dosing, Study (b) (4)-NW/PAR-254-00) and the elimination half-life of safinamide (20-26 hours).

2.2 GENERAL CLINICAL PHARMACOLOGY

2.2.1 What are the clinical studies used to support dosing or claims and what are their design features?

The studies (clinical pharmacology or clinical) that support dosing or claims/indication, are presented in the table below.
In addition, a population PK and PK/PD analysis described the pharmacokinetics of safinamide in PD patients utilizing data from three studies (Study 016, 27918 (MOTION) and 27919 (SETTLE)) and described the effect of safinamide treatment on ON Time utilizing data from two studies (016 and 27919).

Summary of Design Features of Pharmacology and Clinical Studies supporting dosing or claims

Report Number	Study design/ description	Treatment regimen/ duration	Study pop (HV/P) Gender (M/F) Age, Ethnic Origin	Salient Findings/Clinical Endpoints§	Pharmacokinetic characteristics*	Supports dosing
Clinical Pharmacology						
Study IPAS-PNU-194-99: Single and Multiple Dose Study in Healthy Volunteers	Double-blind, PL-controlled, randomized single ascending dose study	2.5, 5.0, 10.0mg/kg and PL Five single doses (separated by 7 day washout)	8 subjects HV Males 18-45 Caucasian	MAO-B inhibition was >90% at all doses	Pharmacokinetics was linear over the investigated dose range.	Lower doses were subsequently tested for effectiveness.
Study IPAS-NW/SS-215-99: Single and Multiple Dose Study in Healthy Volunteers	Open-label, one-way, single-and multiple dose design	Single doses of 25, 50, 75 and 150µg/kg (for MAO-B activity evaluation) Repeated administration of 2.5 and 5.0 mg/kg for 7 days (for PK evaluation)	16 subjects HV Males 18-45 Caucasian	Samples from both Study IPAS-NW/SS-215-99: and Study IPAS-NW/LD-231-00: were collected for MAO-B activity evaluation (n subjects = 4 for doses 12 and 150 µg/kg, n subjects =5 for doses 50 and 75µg/kg) Maximal MAO-B	Pharmacokinetics was linear both with single dose and repeated dose regimen. Terminal half-life was 21-24 h; steady-state was reached within 5 to 6 days.	75% MAO-B inhibition (dose of approx. 10mg/day) achieved 4hrs post dose: approx 50% inhibition at 24hrs post dose (trough) Duration of MAO-B inhibition supported once daily dosing
Study IPAS-NW/LD-231-00: Single and multiple dose in Healthy Volunteers	Open-label, one-way, design	Single doses of 25, 50, 75 and 150ug/kg (for MAO-B activity evaluation)Repeated administration of 1.25mg/kg for 7 days (for PK evaluation)	8 subjects HV Males 18-45 Caucasian	inhibition in platelets was 75% at the 150µg/kg dose, 4h post dose (corresponding to 10mg/day). 24h after 150µg/kg dosing MAO-B activity was still significantly inhibited (48.5%) .	PK was linear both with single doses 25 to 150ug/kg and multiple doses of 1.25mg/kg/day, and accumulation was as expected based on the half-life and on dosing. Steady state was reached within 5 to 6 days.	75% MAO-B inhibition achieved 4hrs post dose: approx 50% inhibition at 24hrs post dose (trough) Duration of MAO-B inhibition supported once daily dosing
Study IPAS-NW/PAR-254-00: Single Dose PK and PD Study in Healthy Volunteers Aged 55 -65	OL, three treatments, six sequence, three periods, placebo-controlled, randomized cross-over study	300ug/kg, 600ug/kg and placebo Three single doses separated by 14 days wash-out	6 subjects HV Males 55-65 Caucasian	A long-lasting effect on platelet MAO-B activity was observed, with an almost complete inhibition in the time range 1-12 h at both doses (maximal peak inhibition 86 and 93% at 300 and 600 µg/kg, respectively)	PK parameters in males aged 55-65 years were similar to those found in young volunteers in previous studies.	The 300 and 600µg/kg corresponding to doses of 20 and 40mg/day (or 0.5mg/kg/day), were associated with almost complete inhibition of MAO-B. Duration of MAO-B inhibition, (75% and 69% inhibition even after 24h post dosing 600µg/kg and 300 µg/kg) supported once daily dosing The 0.5mg/kg/day was subsequently used in the clinical study 009.

Clinical Studies						
NW-1015/009/II/2001	DB, PL controlled, dose finding study, add-on to DA, 3 parallel groups	Saf 0.5 mg/kg/day Saf 1.0 mg/kg/day PL Once a day 12 weeks	Total 172 subjects Saf 0.5 mg/kg/day 57 sub Saf 1.0 mg/kg/day 57 sub PL 58 sub M/F 111/61 Age 64 (39-74)	Primary endpoint: UPDRS III (% of pts with at least 30% improvement (reduction) compared with baseline, $p<0.05$ for 1.0mg/kg/day	No PK data was collected in this study	Supports indication: Early PD, (b) (4) Add-on to Dopamine Agonist. This study indicated that the dose of 1.0mg/kg was significantly superior to placebo in the ITT population (all patients randomized), as well as in the DA monotherapy population (excludes pts not on DA). Additionally, significant difference was also seen for 0.5 and 1.0mg/kg for mean change, although the difference was smaller in the 0.5mg/kg group. Benefit was noted at the lower dose of 0.5mg/kg/day (median dose approx 40 mg/day), however, significantly superior efficacy was noted at the higher dose of 1.0mg/kg/day (median dose approx 80 mg/day). Although the dose of approx 80mg/day dose was efficacious, the MTD was not defined. Higher doses (up to 150-200mg/day) were subsequently tested in study 015/017; two dose ranges of 50-100mg/day (target dose 100mg) and 150-200mg/day (target dose of 200mg) were tested.
NW-1015/012/II/2001	OL ascending dose in add on to levodopa or DA-agonists	-week titration period (Saf 100, 150, 200 mg/day titrated each 2 weeks) followed by highest dose maintenance up to week 102	25 Pts were enrolled Saf 100 - 200 mg/day + L-dopa (n=11; age 65.9; M/F 6/5) Saf 100 - 200 mg/day + single DA agonist (n=14; age 51.6; M/F 11/3)	Platelet MAO-B activity was measured 24 hours after 100 mg, 150,mg, 200mg safinamide administration at week 2, 4 and 6, respectively, resulting in 96%, 96%, 97% inhibition	Pharmacokinetic data showed linearity of plasma concentrations with increasing dose of safinamde after the administration of 100, 150 and 200 mg/day	No analysis for a dose/time response could be performed because inhibition was already near maximal at the lowest dose (100 mg) at week 2 and persisted for the duration of the trial. Preliminary data of efficacy at 200mg/day doses supported the evaluation of the 200mg dose in the phase III trial 015/017
NW-1015/015/III/2003	DB, PL controlled, add-on to DA, 3 parallel groups	Saf 50-100 mg/day (target dose 100mg) Saf 150-200 mg/day (target dose 200mg) PL Once a day 24 weeks	270 Saf 50-100 mg/day (target dose 100mg) 90 Saf 150-200 mg/day (target dose 200mg) 90 PL 90 M/F 169/101 Age 58.3 (30-82)	Primary endpoint: UPDRS III (mean change from baseline), $p<0.05$ for 50-100mg/day	The plasma concentrations observed showed a dose-dependent increase of exposure when comparing the two dose groups at steady state (visit 4 to 8). The data indicated time-independent PK, and accumulation was as expected based on the half-life and on dosing. PK data from this study were used in the Population PK/PD report	Efficay data indicated benefit of safinamide 50-100mg on motor symptoms (in Early PD, (b) (4) as an add-on to Dopamine Agonist). The overall rate of premature discontinuation was high (21.3%) in the 150- 200mg group compared with placebo and 50-100mg (both 10%). The higher dose did not show any additional benefit over the 50-100mg/day dose.
NW-1015/018/III/2006	Extension of Study 016; DB, PL-controlled, 3 parallel groups	Saf 50 mg/day Saf 100 mg/day PL Once a day 18 months	554 Saf 50 mg/day 189 Saf 100 mg/day 180 PL 175 M/F 384/143 Age 60.3 (34-80)	Primary endpoint, Change in DRS during 'ON' time ITT NS. Sub-population with Baseline DRS>4, $p<0.05$	No PK samples were collected	Supports indication: Mid-to-late PD patients, (b) (4) Add-on to L-dopa Efficay data supports benefit on ON time and other secoudary measures at 2 years especially for the 100mg/day dose
EMR 27919 SETTLE	DB, PL-controlled, add-on to L-Dopa, 2 parallel groups	Saf 50-100 mg/day (target dose 100mg) PL Once a day 24 weeks	549 / 478 Saf 50-100 mg/day (target dose 100mg) 274 PL 275 M/F 334/215 Age 62.0 (30-80)	Primary endpoint, Daily 'ON' time, change from baseline, $p<0.05$ for 50-100mg/day	PK samples were collected but not analysed	Supports indication: Mid-to-late PD patients, (b) (4) Add-on to L-dopa. Significant benefit noted for both primary and secondary measures. Efficay data confirmed significant benefit of 100mg/day.

NW-1015/017/III /2003	Extension of Study 015; DB, PL-controlled, 3 parallel groups	Saf 50-100 mg/day (target dose 100mg) Saf 150-200 mg/day (target dose 200mg) PL Once a day 48 weeks	227 Saf 50-100 mg/day (target dose 100mg) 80 Saf 150-200 mg/day (target dose 200mg) 69 PL 78 M/F 144/83 Age 59.1 (30-82)	Primary endpoint: Time to intervention [Landmark analysis: pooled safinamide NS; post hoc analysis 240-540 days], $p<0.05$ for 50-100mg/day group	The plasma concentrations observed increased dose-dependently.	Supports indication: Early PD (b) (4) Add-on to Dopamine Agonist
EMR 27918 MOTION		Saf 50 mg/day Saf 100 mg/day PL Once a day 24 weeks	679 Saf 50 mg/day 227 Saf 100 mg/day 227 PL 225 M/F 425/254 Age 62.0 (30-80)	Primary endpoint, UPDRS III change from baseline ITT population, n=679; p=0.07 for 100mg/day DA monotherapy (excluding 13 pts who were major protocol violators who were not on a stable DA (therapy) sub-population, n=666, $p<0.05$ for 100mg/day	PK samples were collected but not analysed	Supports indication: Early PD. (b) (4) Add-on to Dopamine Agonist Fixed doses of 50 and 100mg were evaluated in this study. The 100mg/day but not the 50mg/day showed a significant benefit on motor symptoms.
NW-1015/016/III/2006	DB, PL controlled, add-on to L-Dopa, 3 parallel groups	Saf 50 mg/day Saf 100 mg/day PL Once a day 24 weeks	669 Saf 50 mg/day 223 Saf 100 mg/day 224 PL 223 M/F 480/189 Age 59.8 (34-80)	Primary endpoint, Daily 'ON' time without troublesome dyskinesia, change from baseline, met $p<0.05$ for both 50 and 100mg/day	Concentration data from this study were used in the Population PK/PD report	Supports indication: Mid-to-late PD patients. (b) (4) Add-on to L-dopa Efficacy. Both doses well tolerated. Significant benefit noted for both primary and secondary measures at both 50 and 100mg/day.

2.2.2 What are the clinical endpoints and how are they measured in clinical pharmacology and clinical studies?

The rationale for the response endpoints in clinical studies is provided in the table below.

Study ID	Design Control Type	Study & Ctrl Drugs Dose & Regimen	Study Objective	# subs by arm entered / compl.	Dur.	Diagnosis Inclusion Criteria	Primary Endpoint(s)	Rationale for clinical endpoint
NW-1015/009/II/ 2001	DB, placebo controlled, dose finding study, add-on to DA, 3 parallel groups	Saf 0.5 mg/kg/day Saf 1.0 mg/kg/day Placebo Once a day	Change in motor symptoms and overall efficacy Safety	172 / 150 Saf 0.5 mg/kg/day 57/52 Saf 1.0 mg/kg/day 57/49 Placebo 58/49	12 weeks	Early PD, non-fluctuators	UPDRS III responders (≥ 30% improvement)	The UPDRS section III, motor symptoms, is considered the most important scale in measuring symptoms in early PD. This endpoint was selected based on published data demonstrating response to therapy in patients with early PD (Goetz et al, 2003). Experts in PD, 2003; Schrag, et al, 2006; Hauser, et al, 2011, Shulman, et al, 2010) recommend that results of new trials should be based on Minimal Clinically Important Change (MCIC). Approximately 2-3 points on the UPDRS III (in early patients). On average, this corresponds to less than 30% of the mean baseline score. This endpoint is routinely used as a primary measure in PD trials.
NW-1015/015/III/2003	DB, placebo controlled, add-on to DA, 3 parallel groups	Saf 50-100 mg/day (target dose 100mg) Saf 150-200 mg/day (target dose 200mg) Placebo Once a day	Change in motor symptoms and overall efficacy Safety	270 / 232 Saf 50-100 mg/day (target dose 100mg) 90/81 Saf 150-200 mg/day (target dose 200mg) 90/70 Placebo 90/81	24 weeks	Early PD, non-fluctuators	UPDRS III change from baseline	A change from baseline in UPDRS III primary endpoint was chosen in line with regulatory guidelines (FDA). It has been used in other approved Parkinson's Disease medications such as rasagiline, and ropinirole.

NW-1015/017/III /2003	Extension of Study 015; DB, placebo-controlled, 3 parallel groups	Saf 50-100 mg/day (target dose 100mg) Saf 150-200 mg/day (target dose 200mg) Placebo Once a day	Time to intervention and overall efficacy Safety	227 / 187 Saf 50-100 mg/day (target dose 100mg) 80/64 Saf 150-200 mg/day (target dose 200mg) 69/61 Placebo 78/62	48 weeks	Early PD, non-fluctuators	Time to intervention [Landmark analysis: 240-540 days]	The time to intervention endpoint was based on different studies; a 5 year study for ropinorole (Rascol et al 2006), as well as for pramipexole (Holloway et al, 2004) and carbergoline (DA vs L-dopa) (Bracco et al 2004).
EMR 27918 MOTION	DB, placebo-controlled, add-on to DA, 3 parallel groups	Saf 50 mg/day Saf 100 mg/day Placebo Once a day	Change in motor symptoms and overall efficacy Safety	679 / 610 Saf 50 mg/day 227/199 Saf 100 mg/day 227/210 Placebo 225/201	24 weeks	Early PD, non-fluctuators	UPDRS III change from baseline	A change from baseline in UPDRS III primary endpoint was chosen in line with regulatory practice. It has been used in other approved parkinson's disease medications such as pramipexole, rasagiline, and ropiniorole.
NW-1015/016/III/2006	DB, placebo controlled, add-on to L-Dopa, 3 parallel groups	Saf 50 mg/day Saf 100 mg/day Placebo Once a day	Change in motor fluctuations and overall efficacy Safety	669 /594 Saf 50 mg/day 223/202 Saf 100 mg/day 224/195 Placebo 223/197	24 weeks	Mid-to-late PD patients, fluctuators	Daily 'ON' time, change from baseline	This endpoint is based on prior precedence of FDA approval of COMTAN. All recent approvals are based on OFF and ON time in late stage PD patients. This endpoint was also agreed upon with the FDA at the End of PhII meeting and is line with current guidelines.
NW-1015/018/III/2006	Extension of Study 016; DB, placebo-controlled, 3 parallel groups	Saf 50 mg/day Saf 100 mg/day Placebo Once a day	Change in dyskinesia/ motor fluctuations and overall efficacy Safety	554 / 440 Saf 50 mg/day 189/148 Saf 100 mg/day 180/150 Placebo 175/142	18 months	Mid-to-late PD patients, fluctuators from study 016	Change in DRS during 'ON' time	The Dyskinesia Rating Scale is a validated, widely used scale developed to measure dyskineis in PD patients (Goetz et al, 1994). No drug has yet been approved to treat dyskinesia, so no precedence is available. Add-on treatment with amantadine showed significant benefit in reducing dyskinesia (although for a limited time), using the DRS (Thomas et al, 2004).
EMR 27919 SETTLE	DB, placebo-controlled, add-on to L-Dopa, 2 parallel groups	Saf 50-100 mg/day (target dose 100mg) Placebo Once a day	Change in motor fluctuations and overall efficacy Safety	549 / 478 Saf 50-100 mg/day (target dose 100mg) 274/241 Placebo 275/237	24 weeks	Mid-to-late PD patients, fluctuators	Daily 'ON' time, change from baseline	Same as for study 016

2.2.3 What are the characteristics of exposure/effectiveness relationships?

The sponsor conducted exposure-efficacy analysis to describe the relationship of safinamide plasma concentrations (average concentration over 24-hr dosing interval) and ON TIME values using the data obtained from Study 016 and Study 27919 (SETTLE) only. The On Time value was the sum of On Time plus On Time with minor dyskinesia during 18 hr of recording in a patient diary (at least 2 and up to 5 recording days) prior to a scheduled visit. The sponsor concluded no difference in the magnitude of the safinamide treatment effect between the 50 and 100 mg dose.

Independent analysis was not conducted by the reviewer because no specific questions about the dose were identified by the review team that would require additional learning from exposure-efficacy analysis.

2.2.4 What are the characteristics of exposure-safety relationships?

The sponsor did not conduct analysis on exposure-safety relationship. Independent analysis was not conducted by the reviewer because no specific questions were identified by the review team that would require additional learning from exposure-safety analysis.

2.2.5 Are the proposed dosage regimens adequately supported by the clinical trials and consistent with the dose-response relationship?

Yes. Clinical trials in mid- to late-stage fluctuating patients evaluated the efficacy and safety of a dose range of 50-100 mg/day of safinamide. According to sponsor, these doses were selected based on the results of Studies 009, in which the 1.0 mg/kg/day (~80 mg/day) group was significantly superior to placebo, while the 0.5 mg/kg/day (~40 mg/day) group was minimally effective in some analyses, indicating the need to evaluate higher doses, and 012, in which doses up to and including 200 mg/day were well tolerated during short-term use but had to be reduced after six weeks for the majority of subjects due to adverse events (AEs). The data suggested that higher doses, eg, 150 and 200 mg/day, were associated with greater efficacy. Study 015 showed that a dose range of 50-100 mg/day of safinamide was efficacious in treating motor symptoms, compared to placebo, as add-on therapy in PD subjects receiving a stable dose of a single dopamine agonist; a higher dose range (150-200 mg/day) did not show an incremental benefit, but it was associated with an increased incidence of premature discontinuations and dropouts due to AEs. Please refer to the review by Dr Len Kapcala (Medical Officer, DNP) for more details.

2.2.6 Does Safinamide prolong QT or QTc interval?

No. A randomized, double-blind, placebo-controlled, multiple-dose parallel groups study with an open-label, single-dose positive control (moxifloxacin 400 mg) was conducted to evaluate the potential effects of a 100 mg dose and a supratherapeutic dose (350 mg) of safinamide on ventricular repolarization by quantitative analysis of corrected QT intervals in healthy subjects.
Interdisciplinary Review Team Summary: No significant QTc prolongation effect of safinamide (100 mg and 350 mg) was detected in this TQT study. The largest upper bounds of the 2-sided 90% CI for the mean difference between safinamide (100 mg and 350 mg) and placebo 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, and the moxifloxacin profile over time was adequately described, indicating that assay sensitivity was established.
The supratherapeutic dose (350 mg) produces mean C_{max} 3.9-fold the mean C_{max} for the therapeutic dose (100 mg). These concentrations are above those for the sponsor's predicted worst case scenario (low body weight patient with hepatic impairment) and show that at these concentrations there are no detectable prolongations of the QT interval. The data suggests a concentration dependent shortening of QT interval.

2.2.7 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters?

Yes. Plasma concentrations of safinamide and the major human circulating inactive metabolites NW-1153 and NW-1689, were determined using validated liquid chromatography-tandem mass spectrometric detection (LC-MS/MS) methods. A method report was also generated for each clinical study.

Details pertaining to assay methodology, assay validation, acceptance criteria, and stability are provided in Section 2.6 and the individual study reviews.

Several drug-drug interaction studies were conducted with medications that were coadministered with safinamide. Information regarding the analytical methods used to support the analysis of co-administered compounds can be found in the individual study reviews (EMR701165_027, 28780, EMR701165-026, and 28558).

2.2.8 What are the general ADME characterstics of safinamide?

Absorption:

Safinamide absorption is rapid after single and multiple oral dosing, reaching Tmax in the range of 1.8 to 2.8 hours after dosing under fasting conditions.
The absolute bioavailability of a 50 mg safinamide film-coated tablet in fasted condition was determined in healthy subjects in study EMR701165-022. The PK results after single oral administration of 50 mg safinamide film-coated tablets in fasted condition and an IV infusion of a 25 ml solution containing 50 mg safinamide in fasted condition are summarized in the table below.

PK Parameters of Safinamide Plasma Concentrations after Single Oral Administration of 50 mg Safinamide Tablets in Fasted Condition and after IV Administration of 50 mg Safinamide (N=14)

PK Parameter	Treatment A (Fasted)	Treatment C* (IV Infusion)
C_{max} [ng/mL]	322.3 (17.9) 227.0 – 421.0	411.6 (18.8) 305.0 – 591.1
t_{max} [h]	2.5 2.0 – 5.0	0.5 0.25 – 5.0
AUC_{0-t} [ng/mL*h]	9811 (25.4) 6584 – 16737	10340 (20.8) 7173 – 13815
$AUC_{0-\infty}$ [ng/mL*h]	10205 (26) 6779 – 17012	10785 (22.0) 7314 – 14801
$t_{1/2}$ [h]	26.2 (19.2) 20.0 – 40.8	26.0 (11.3) 20.7 – 30.7
CL/f [L/h]	4.9 (26.0) 2.9 – 7.4	4.6 (22.0) 3.4 – 6.8
V_z/f [L]	185.0 (17.7) 142.9 – 260.5	174.1 (15.6) 146.6 – 222.7
F [%]	94.98 (9.25) 83.93 – 119.33	–

Estimates for Ratios of Geometric Means with 90% Confidence Intervals for Primary PK Parameters of Safinamide in Plasma - Oral/IV

Parameter	Treatment	Least Squares Means	Ratio T/R and 90% confidence interval [%]
$AUC_{0-\infty}$ (h*(ng/mL))	Tablet fasted (T)	10205.28	94.62 (90.09 - 99.39)
	Solution i.v. (R)	10785.15	

Absolute bioavailability was high (95%), indicating that safinamide is almost completely absorbed after oral administration, and first pass metabolism is negligible, see table below (metabolite levels after single dose oral safinamide administration were similar to that after IV administration).

PK Parameters of Safinamide Metabolites NW-1689, NW-1689 AG, and NW-1153 after Single Oral Administration of 50 mg Safinamide Tablets in Fasted Condition and After IV Administration of 50 mg Safinamide (N=14)

PK Parameter	Treatment A (Fasted)	Treatment C* (IV Infusion)
NW-1689		
C_{max} [ng/mL]	223.9 (27.3) 168.0 – 343.0	214.5 (31.3) 144.0 – 392.0
t_{max} [h]	12.0 8.0 – 24.0	18.0 8.0 – 24.0
AUC_{0-t} [ng/mL•h]	11744 (40.5) 7849 – 24246	11150 (52.0) 6352 – 27631
$AUC_{0-\infty}$ [ng/mL•h]	12296 (39.5) 8263 – 24924	11721 (49.6) 6721 – 28447
$t_{1/2}$ [h]	31.1 (26.9) 22.9 – 48.1	32.9 (20.5) 24.9 – 45.0
NW-1689 AG		
C_{max} [ng/mL]	54.5 (32.3) 37.7 – 132.0	45.4 (41.1) 18.9 – 90.9
t_{max} [h]	12.0 6.0 – 24.0	10.0 4.0 – 48.0
AUC_{0-t} [ng/mL•h]	1790 (35.5) 1121 – 3894	1386 (79.7) 177 – 2881
$AUC_{0-\infty}$ [ng/mL•h]	1890 (35.1) 1289 – 3904	1440 (79.8) 181 – 2902
$t_{1/2}$ [h]	27.3 (24.4) 18.2 – 41.5	23.7 (37.8) 12.9 – 39.9
NW-1153		
C_{max} [ng/mL]	37.2 (19.9) 25.1 – 49.2	27.0 (15.3) 21.2 – 35.6
t_{max} [h]	1.5 1.0 – 8.0	8.0 2.5 – 12.0
AUC_{0-t} [ng/mL•h]	853 (14.4) 688 - 1174	794 (17.7) 506 - 1019
$t_{1/2}$ [h]	20.5 (23.7) 12.9 – 31.9	22.5 (23.8) 14.8 – 31.0

* In Treatment C, dose adjusted C_{max} and AUC are displayed. Source: EMR701165-022 Table 14.4.2.1

Distribution:

The volume of distribution (Vss) is approximately 165 L, which is 2.5-fold of body volume, indicating extensive extravascular distribution of safinamide.

Plasma Protein Binding:

Plasma protein binding of safinamide and its metabolites NW-1153, NW-1689 and NW-1689 AG, was investigated *in vitro* in human plasma as well as purified human plasma proteins using equilibrium dialysis or ultrafiltration. Human serum albumin (HSA) was found to be the main protein responsible for plasma binding of safinamide and its metabolites. The concentration range investigated (0.5-50 µM) covered the *in vivo*

plasma concentrations in the clinical settings. Extent of plasma protein binding was generally independent of concentration. The bound fraction for safinamide, NW-1153, NW-1689 and NW-1689AG was 89%, 71%, >99.5% and 97% respectively.

Whole Blood Distribution:

The whole blood distribution of safinamide and its major metabolites NW-1153 and NW-1689 was investigated at 2.5 μM and 100 μM and found to be concentration-independent. Safinamide and NW-1153 were about equally distributed between blood cells and plasma with distribution coefficient $K_{BC/P}$ varying between 0.4 and 0.6. NW-1689 was found to be in plasma to a large extent since it is highly bound to plasma proteins. No specific uptake by red blood cells was observed for safinamide or its other main metabolites.

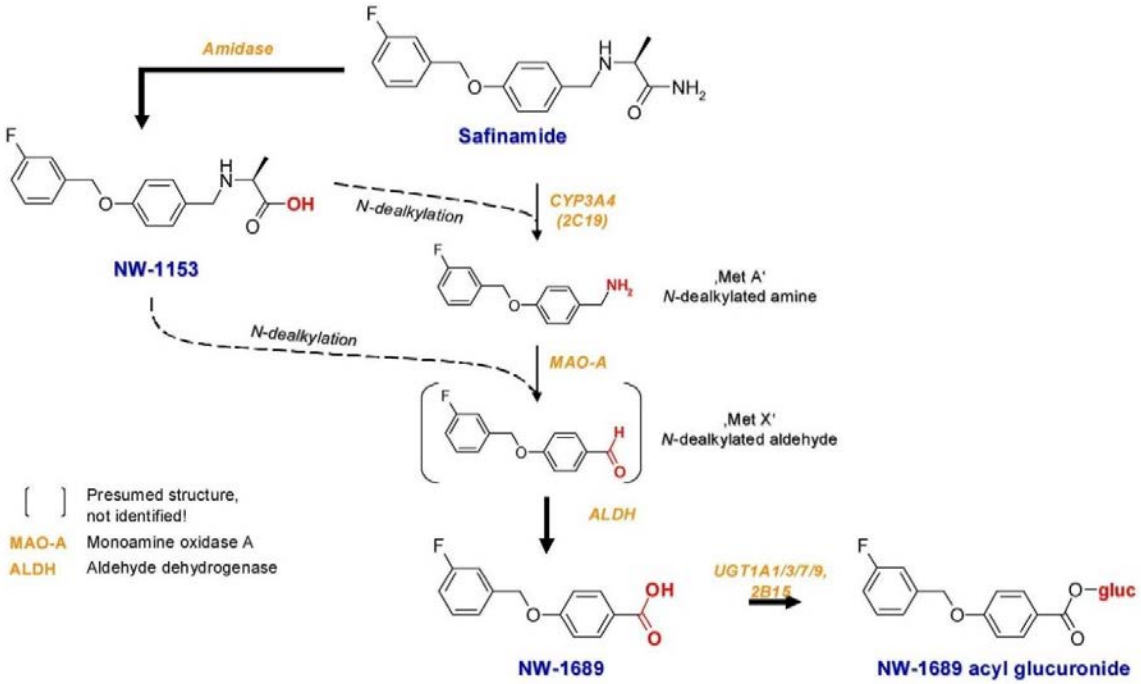
Metabolism:

Safinamide is metabolized to N-dealkylated amine ('Met A'), which accumulated in human hepatocytes upon inhibition of MAO-A. In human liver microsomes (HLM), the N-dealkylation step is catalyzed by CYP3A4 since safinamide clearance in HLM was inhibited by ketoconazole up to 90%. However, in human hepatocytes, CYP3A4 inhibition did not result in any meaningful reduction of safinamide clearance indicating that non-microsomal enzymes (cytosolic amidases/MAO-A) catalyze safinamide metabolism much more efficiently than CYP3A4.

The formation of NW-1153 is catalyzed by non-specific cytosolic amidases. Human fatty acid amide hydrolase (FAAH) was identified as a potential enzyme that contributes to the metabolic pathway of safinamide to NW-1153. NW-1153 was not observed in HLM incubations with safinamide, but only in hepatocytes. NW-1153 and N-dealkylated amine are converted to NW-1689. Because of the absence of aldehyde dehydrogenase or oxidase in human liver microsomes, NW-1689 was not formed. Met X is formed by MAO reactions. *In vitro* studies using four ALDH iso-enzymes have shown that all enzymes were catalytically active towards 'Met X' oxidation. Further, NW-1689 acyl glucuronide (NW-1689 AG) is formed from NW-1689 mediated by 5 different UGT1A isoenzymes, 1A1, 1A3, 1A7 and 1A9 along with UTG2B15.

In summary, *in vitro* studies suggest that safinamide is metabolized to the major extent by MOA-A and cytosolic amidases, and only very minimally by CYP3A4. An *in vivo* drug-drug interaction study using ketoconazole as an inhibitor was conducted and demonstrated that CYP3A4 does not play a relevant role in safinamide's metabolism. ALDH isoenzymes and UGT1A isoenzymes along with UTG2B15 are involved in the final steps of the metabolic pathway as represented in the figure below. Drug-drug interaction potential due to inhibition of a single ALDH or UGT isoenzyme is low since several isoenzymes are involved in safinamide metabolism.

Safinamide Metabolic Pathways



In the human mass balance study (CRO-02-33), the main radioactive component in plasma was parent safinamide, the AUC_{0-24} accounted for ~30% of the total radioactivity AUC_{0-24} (AUC_{TR}). The main circulating metabolite was identified as NW-1689, accounting for ~30% of AUC_{TR}. Two minor metabolites, NW-1199 and NW-1153 were identified, accounting for ~2 and ~1% of AUC_{TR}, respectively.
In human clinical studies, in which plasma samples were stabilized to prevent glucuronide hydrolysis, NW-1689 AG was found to represent 10-20% of the AUC of parent compound (28778, 28559, EMR 701165-025).
At steady state, NW-1689 was the main compound in plasma, exceeding the exposure of the parent drug after about 10 hours following dosing (see table below). NW-1689 AG and NW-1153 accounted for about 16-18% and 10-11% of the parent drug exposure. The half-lives of the metabolites are comparable to that of safinamide, i.e. 19.5 h, 25.3 h and 26.5 h for NW-1153, NW-1689 and NW-1689 AG, respectively.

Pharmacokinetic Parameters of Metabolites after Single and Multiple Doses of 100 mg Safinamide

Single dose (N=14)[a]					
Metabolite	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0-\infty}$ (ng/mL*h)	Metabolic ratio*	$t_{1/2}$ (h)
NW-1153	73.4 57.6 – 110	1.0 0.5 – 6.0	1813 1346 – 2372	0.11	19.5 15.8 – 24.9
NW-1689	472 296.0 – 781	10.0 4.0 – 12.0	21660 13949 – 42750	1.61	25.3 20.7 – 37.7
NW-1689 AG	104 68.6 -168	12.0 2.0 -36.0	4089 2182 -7150	0.18	26.5 18.4 -46.1
Multiple dose (N=61)[b]					
	C_{max} (ng/mL)	t_{max} (h)	AUC_{τ} (ng/mL*h)	Metabolic ratio*	$t_{1/2}$ (h)
NW-1153	121 64.1 – 166	1.3 1.1 – 8.0	2000 1167 – 2660	0.10	NA
NW-1689	1149 (481 – 6170)	8.0 2.1 – 24.0	23274 9727 – 138730	1.66	NA
NW-1689 AG	298 (89.2 – 738)	8.0 0.0 – 12.0	3787 473 – 10711	0.16	NA

For Cmax, AUC: Geomean and range (min-max), For tmax: median and range (min – max)
*, Metabolic ratios have been corrected for molecular weight
a Data from 28778, b: Data from Trial 28559

AUC_{∞} and AUC_{τ} at steady state were comparable for the respective metabolites showing that the predicted accumulation of the metabolites at steady-state was consistent with the data from the single dose. The metabolic ratio remained unchanged after steady state, compared to single dose administration.
Conclusion: Three circulating human metabolites (i.e. NW-1153, NW-1689 and NW-1689 acyl glucuronide (AG)) have been identified. These three metabolites have been shown to be pharmacologically inactive.

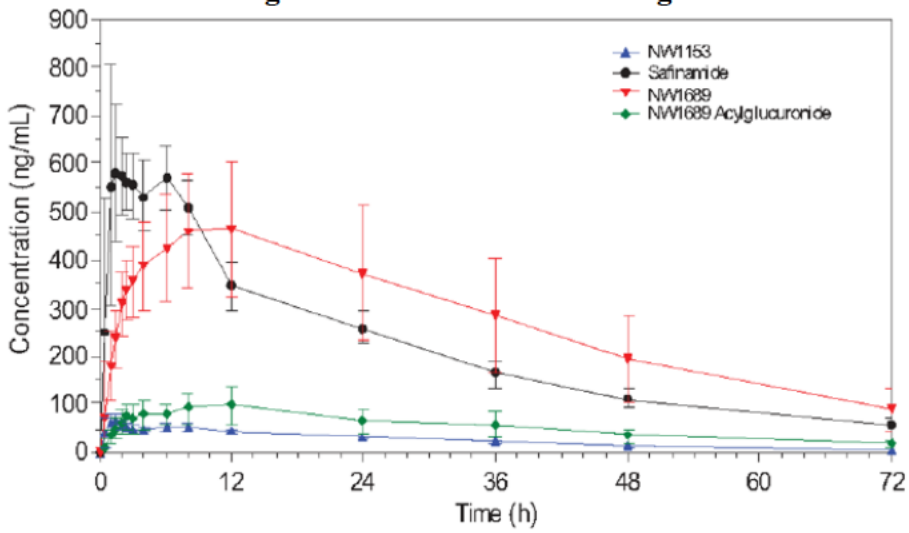
Elimination:

Total clearance was determined to be 4.6 L/hours, classifying safinamide as a low clearance drug. Safinamide terminal half-life is 20 to 26 hours, and steady-state is reached within 5 to 6 days.
Following almost complete metabolic transformation (only ~5% of the administered dose was found unchanged in urine within 48 hours), drug-related radioactivity was largely excreted in urine (76%) and only to a low extent in feces (1.5%) after 192 hours. The terminal elimination half-life of total radioactivity was ~80 hours.
The sum of metabolites NW-1153, NW-1689 and NW-1689 AG excreted in urine was approximately 39% (Study 28778). In urine, the main metabolites were NW-1153 (14-22% of dose) and NW-1689 AG (11-20% of dose), NW-1689 was detected only in trace amounts. In addition, a few minor metabolites (O-debenzylated safinamide (NW-1199), glycine-conjugated NW-1689 and traces of monohydroxylated safinamide derivatives were found in urine (CRO-02-33, 28778, 28696, EMR 701165-025).
The half-lives of the major metabolites are 19.5 h, 25.3 h and 26.5 h for NW-1153, NW-1689 and NW-1689 AG, respectively.

2.2.9 What are the basic pharmacokinetic parameters of safinamide after single and multiple doses?

The plasma concentration time profiles of parent safinamide and of metabolites are displayed in the figure below.
Pharmacokinetic parameters of safinamide after single and multiple (7 days) doses are shown in the table below.

Mean Safinamide and Metabolite Plasma Concentration Time Profiles (SD) After a Single Dose of Safinamide 100mg



Pharmacokinetic Parameters of Safinamide after Single and Multiple (7 days) Doses (Fasting condition)

Single Dose					
Parameter	**50 mg[1] (N=14)**	**100 mg[2] (N=29)**	**2.5 mg/kg3 (~175mg) (N=5-8)**	**5.0 mg/kg3 (~350mg) (N=8)**	**10.0 mg/kg3 (~700mg) (N=8)**
Cmax (ng/mL)	322 227-421	646 396-889	1201 796-1799	2927 2429-3243	6170 4312-9233
AUC* (ng/mL*h)	10205 6779-17012	19245 10177-27574	31799 23652-46107	76917 64437-101405	162244 110797-220323
t½ (h)	26.2 20.0-40.8	23.4 19.2-32.0	20.3 14.8-24.3	20.0 16.4-24.1	23.0 15.7-29.8
tmax (h)	2.5 2.0-5.0	2.0 1.5 -6.0	2.0 0.7-6.0	1.0 0.7-6.0	2.0 0.7-6.0
Multiple Dose					
Parameter	**1.25 mg/kg/d[4] (~88 mg/d) (N=8)**	**100 mg/d[5] (N=61)**	**2.5 mg/kg/d[6] (~175 mg/d) (N=8)**	**5.0 mg/kg/d[6] (~350 mg/d) (N=8)**	**350 mg/d[5] (N=58)**
Cmax (ng/mL)	1039 830-1265	1234 703-2120	2148 1787-2411	4431 3573-6564	4887 2920-7550
AUC* (ng/mL*h)	17023 13653-20932	19811 11733-33108	33742 25840-42499	68286 57225-97691	77257 48503-132378
t½ (h)	23.8 20.0-29.8	n.d.	23.9 20.8-29.4	20.8 16.4-27.2	n.d.
tmax (h)	3.0 1.0-4.0	2.1 1.1-8.0	2.0 0.7-6.0	1.5 1.0-6.0	2.1 1.1-6.0

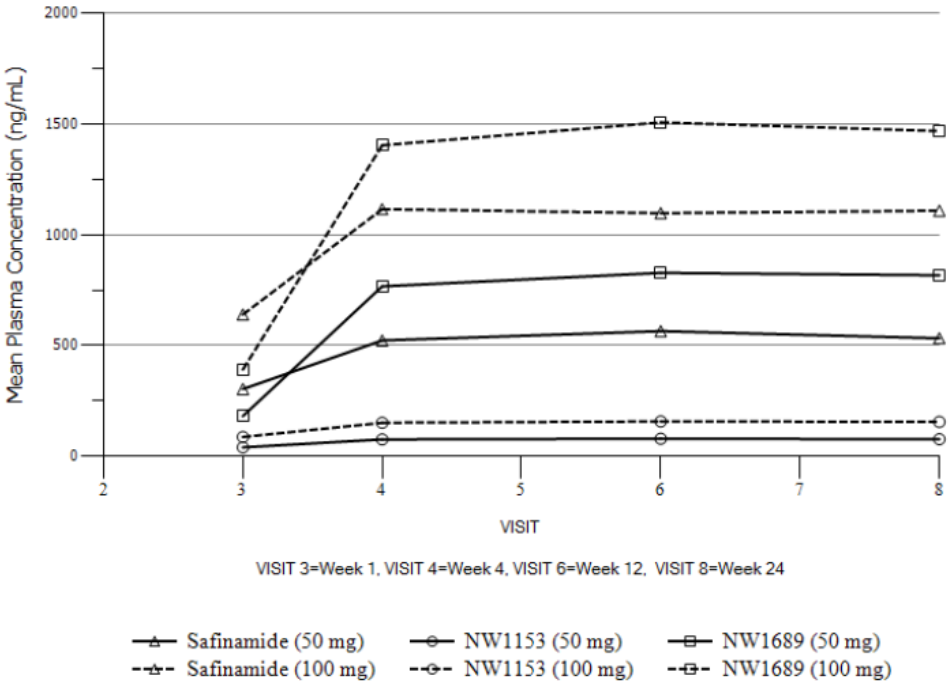
Cmax, AUC: Geomean and range (min-max); tmax: Median and range (min-max); *, AUC$_{\infty}$ for s.d.; AUCτ for m.d. 1 EMR701165_022; 2, EMR701165_021; 3, (b) (4) NU-194-99; 4, (b) (4) NW/LD-231-00; 5 (b) (4) 28559; 6 (b) (4) NW/SS-215-99

2.2.10 Do the pharmacokinetic parameters change with time following chronic dosing?

No. Pharmacokinetics of safinamide following multiple-dose administration were consistent with those observed after single-dose administration, indicating time-independent pharmacokinetics.

In the phase III, double-blind, placebo-controlled trial NW-1015/016/III/2006, two fixed doses of safinamide (50 and 100 mg) were compared to placebo, as add-on therapy to L-dopa in patients with idiopathic PD. Patients were randomized to receive the 50 mg, or the 100 mg/day starting from Day 1 (no titration). Plasma samples for determination of safinamide and two metabolites, NW-1153 and NW-1689, were obtained at Visit 3 (2 samples: pre-dose and 5h post dose), Visit 4, 6 and 8 (one sample scheduled between 1 and 8 hours post-dose). The Week 1 data was the first PK sample collected on the first day of dosing. As seen in the figure below, after accumulation at Week 4 (compared to the post-baseline Day 1 value), safinamide and metabolites' exposure remained the same up to Week 24. The ratio NW-1689/safinamide was approximately 1.4 and that of NW-1153/safinamide was approximately 0.1, indicating stable metabolic ratio across visits. In addition, safinamide and metabolite concentration were approximately doubled in the 100 mg/day dose group compared to the 50 mg/day dose group.

Mean Concentration Profiles of Safinamide and its Metabolites Observed in Study 016



2.2.11 What is the variability in the PK data?

At steady state safinamide PK parameters demonstrate low inter-subject and low intra-subject variability, see tables below.

Sample of Individual Data of Safinamide Plasma Concentrations (ng/mL) after Safinamide 100mg – Intra subject variability in healthy subjects

				Day 3	Day 4	Day 5	Day 6	Day 7
Treatment 100mg Safinamide	**RNN**	**SUBJ**	**Gender**	**48**	**72**	**96**	**120**	**144**
	242	17	Male	401	496	479	470	542
	248	33	Male	341	391	465	474	497
	249	47	Male	389	376	420	429	480
	255	72	Male	383	443	473	484	512
	257	81	Male	471	580	607	574	756
	261	107	Male	354	382	433	406	435
	267	167	Male	407	474	505	527	610
	272	192	Male	356	428	451	378	459
	103	9	Female	306	344	333	338	323
	108	61	Female	391	510	547	582	619
	110	58	Female	431	451	519	453	564
	114	92	Female	392	514	576	569	705
	117	116	Female	390	423	494	490	559

Variability in the PK Parameters of Safinamide in Plasma in Study EMR701165-021 [Geomean, (geoCV%) and range, n=29]

Parameter	T (Test Treatment) RC tablet	R (Reference Treatment) DC tablet
C_{max} (ng/mL)	**646** (17.8) 396 – 889	**685** (19.3) 461 – 1080
t_{max} * (h)	**2.0** 1.5 – 6.0	**2.0** 1.0 – 6.0
AUC_{0-t} (ng/mL*h)	**18113** (20.1) 9785 – 24263	**18583** (19.6) 10801 – 28661
$AUC_{0-\infty}$ (ng/mL*h)	**19245** (21.3) 10177 – 27574	**19715** (20.7) 11305 – 30035
$t_{1/2}$ (h)	**23.4** (14.1) 19.2 – 32.0	**23.1** (15.2) 16.9 – 34.8
CL/f (L/h)	**5.20** (21.3) 3.63– 9.83	**5.07** (20.7) 3.33 – 8.85
V_z/f (L)	**175.7** (18.6) 129.3 – 290.0	**169.2** (21.9) 101.4 – 271.5

Similar, the inter-individual variability in CL/F and Vd/F, estimated via popPK, was also relatively low (CV=22.5% for CL/F and CV=18.1% for Vd/F).

2.2.12 How do the pharmacokinetics of the drug in healthy volunteers compare to that in patients?

No dedicated plasma sampling has been performed in PD patient studies to characterize the full pharmacokinetic profile. In studies 016, 27918 (MOTION) and 27919 (SETTLE),

the relationship between the dose of safinamide and safinamide PK parameters was evaluated using a sparse-sampling, population approach (POP PK PD Study Report 016_27918_27919). The range of plasma concentrations in patients were in agreement with these in healthy subjects (see below), suggesting that there is no difference in the pharmacokinetics of safinamide between healthy subjects and PD patients.

Summary of Observed Safinamide Concentrations (ng/mL) for Study 27918 (MOTION), by Dose and Visit/Week

Study=27918 (MOTION)			Dose			
			50 mg		100 mg	
Visit	Week	Statistics	N	Value	N	Value
7	18	Median	195	562	203	1200
7	18	5th Percentile	195	298	203	679
7	18	25th Percentile	195	446	203	992
7	18	75th Percentile	195	714	203	1410
7	18	95th Percentile	195	956	203	1840
8	24	Median	179	575	185	1160
8	24	5th Percentile	179	240	185	513
8	24	25th Percentile	179	446	185	930
8	24	75th Percentile	179	718	185	1470
8	24	95th Percentile	179	911	185	1892

Pharmacokinetic Parameters of Safinamide after Multiple (7 days) Doses in Healthy Subjects ((b) (4) 28559; (b) (4) NW/ SS-215-99)

Parameter	1.25 mg/kg/d[4] (~88 mg/d) (N=8)	100 mg/d[5] (N=61)	2.5 mg/kg/d[6] (~175 mg/d) (N=8)	5.0 mg/kg/d[6] (~350 mg/d) (N=8)	350 mg/d[5] (N=58)
Cmax (ng/mL)	1039 830-1265	1234 703-2120	2148 1787-2411	4431 3573-6564	4887 2920-7550
AUC* (ng/mL*h)	17023 13653-20932	19811 11733-33108	33742 25840-42499	68286 57225-97691	77257 48503-132378
t½ (h)	23.8	n.d.	23.9	20.8	n.d.

There were additional clinical pharmacology related issues with Trial 015 (Please refer to Sect. 2.5.2 of this QBR and the Clinical Pharmacology Individual Study review for more details). The clinical pharmacology team recommended that the sponsor exclude Study 015 PK data from the population PK analysis, and instead included PK data from other studies (i.e., MOTION and SETTLE) in the population PK/PD analysis. This was addressed by the sponsor in the NDA re-submission.

2.2.13 Based on the pharmacokinetic parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

Results of a single ascending dose study ((b) (4)-PNU-194-99) suggest that safinamide pharmacokinetics are dose proportional over the range of 2.5, 5.0, 10.0 mg/kg safinamide oral dose.

Non-compartmental analysis

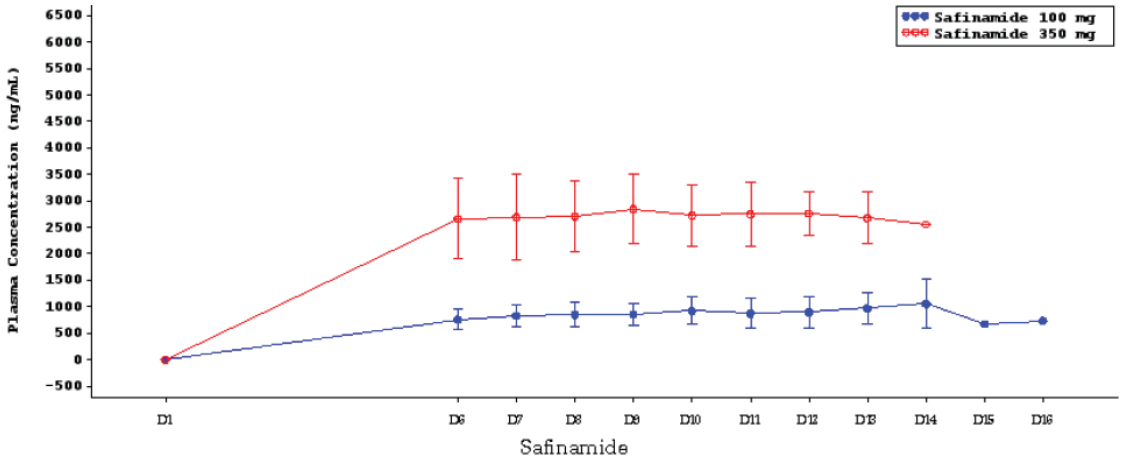
Parameter	2.5 mg/kg			5.0 mg/kg			10.0 mg/kg		
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%
C_{max} (ng·ml^{-1})	1255.76	400.41	31.9	2943.28 (1471.64)	322.49	11.0	6315.74 (1578.94)	1488.82	23.6
AUC_{0-t} (ng·ml^{-1}·h)	26015	5227	20.1	61859 (30930)	7832	12.7	127983 (31996)	24696	19.3
AUC (ng·ml^{-1}·h)	32492	7320	22.5	77803 (38902)	12983	16.7	166134 (41534)	38046	22.9
t_{max} (h)	2.83	2.21	78.1	1.83	1.76	96.2	2.75	2.30	83.6
$t_{1/2\beta}$ (h)	20.63	3.83	18.6	20.22	3.02	14.9	23.39	4.37	18.7
Cl_p (L·h^{-1})	5.53	1.01	18.3	4.84	0.89	18.4	4.72	0.95	20.1

The linear regression analysis performed on Cmax and AUC showed a dose-linearity with the following regression parameters:

Parameter	Slope	Intercept	r	p
C_{max}	674.6392	-430.4712	0.9271	< 0.001
AUC_{0-t}	13542.75	-7047.25	0.9472	< 0.001
AUC	17797.0536	-11673.1875	0.9292	< 0.001

The test for deviations from linearity was non-significant (confirmation of the dose linearity).

In addition, in a multiple-dose study (28558) in healthy subjects, mean (SD) predose safinamide concentrations on Day 6 were 759.9 (184.53) ng/mL in the safinamide 100 mg group and 2661.7 (762.55) ng/mL in the safinamide 350 mg group, i.e., a 3.5-fold increase indicating dose-proportionality of safinamide PK.



In a Phase II, dose titration study (NW-1015/010/II/2002) in patients with epilepsy, safinamide was titrated according to the schedule in the table below and safinamide trough plasma levels were determined at weeks 2, 4, 6, 8 and 12.

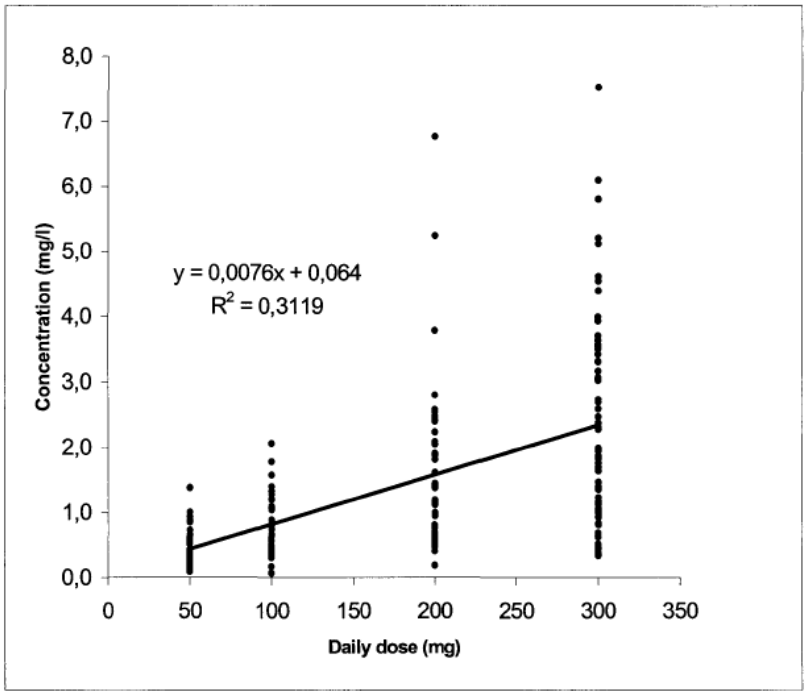
Safinamide Titration Schedule

Days	Weeks	A.M. no. of capsules	Total Daily Dose
1-14		-	0
14-28	0-2	1	50
28-42	2-4	2	100
42-56	4-6	4	200
56-70	6-8	6	300

Descriptive statistics of safinamide plasma concentrations (mg/L)

VISIT	DOSE	N	MEAN	SD	MIN	MEDIAN	MAX
Week 2	50 mg	41	0.472	0.327	0.090	0.360	1.390
Week 4	100 mg	39	0.781	0.450	0.070	0.670	2.060
Week 6	200 mg	38	1.679	1.342	0.310	1.410	6.770
Week 8	300 mg	36	2.185	1.422	0.340	1.910	5.210
Week 12	300 mg	37	2.239	1.718	0.190	1.830	7.520

Scatter plot of dose vs. concentration of safinamide (mg/L)



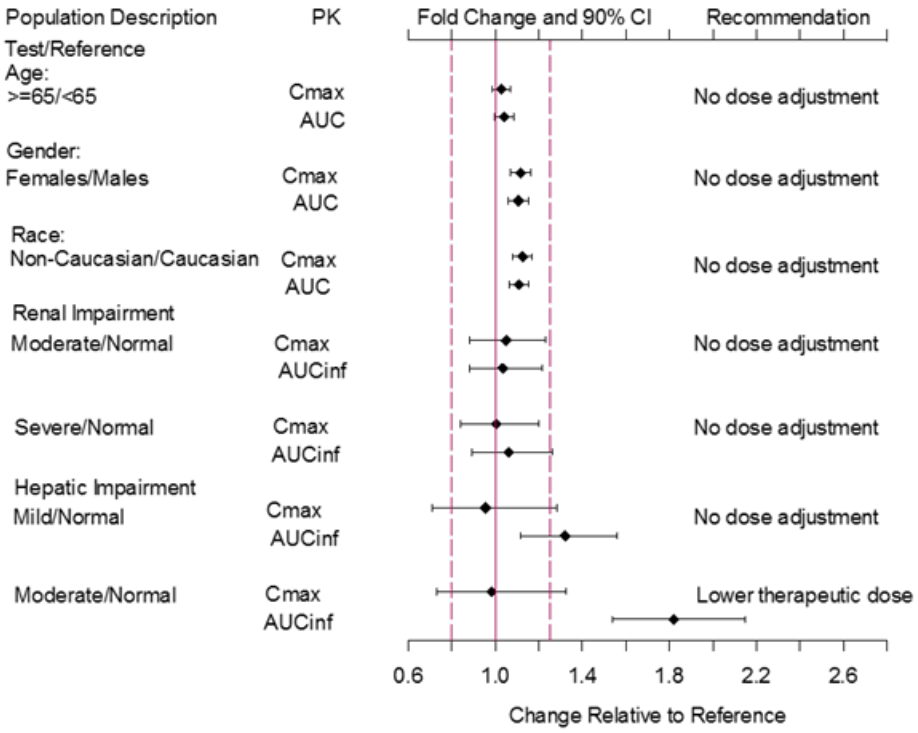
The linear dose-concentration relationship was demonstrated by the results of regression analysis (P<0.000l).

2.3 INTRINSIC FACTORS

2.3.1 What intrinsic factors influence exposure and/or response and what is the impact of any differences in exposure on the pharmacodynamics? Based on what is known about exposure response relationships and their variability, is dosage adjustment needed for any of the subgroups?

The impact of intrinsic factors was studied in a population PK analysis using the concentration data collected from studies 016, 27918 and 27919 (POP PK PD Study Report (016_27918_27919).
Population PK analysis showed that age, gender and race did not influence the pharmacokinetics of safinamide.
In addition, the effect of hepatic and renal impairment on safinamide PK was assessed in dedicated phase 1 PK studies.

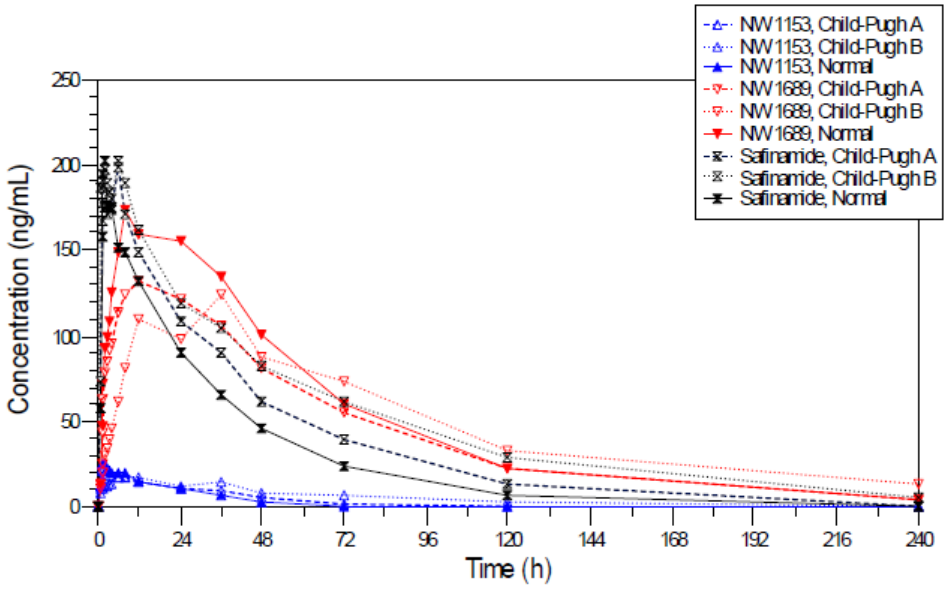
Impact of Intrinsic Factors on Safinamide Pharmacokinetics



2.3.1.6 Hepatic Impairment:

A single dose study (Protocol 28696) was conducted to assess the effect of hepatic impairment on safinamide PK. A single 50 mg safinamide dose was administered to 24 male/female subjects with different grades of hepatic function (subjects with mild, Child-Pugh A, and moderate, Child-Pugh B, hepatic impairment, and age-, gender- and BMI-matching control healthy subjects with normal hepatic function).
The results of this study are presented below.

Mean Plasma Pharmacokinetic Parameters of Safinamide and Metabolites Following a Single Dose of 50-mg Safinamide Administered to Patients with Hepatic Impairment and to Healthy Matched Control Subjects



Summary of Pharmacokinetic Parameters of Safinamide in Subjects with Hepatic Impairment Compared to Healthy Controls

	Normal	Child-Pugh A	Child-Pugh B
C_{max} [ng/mL]	218.7 (33.0) 156 – 408	208.6 (36.0) 142 – 375	214.7 (38.1) 120 – 311
t_{max} [h]	1.75 (0.5 – 4.0)	2.00 (1.0 – 6.0)	6.0 (0.5 – 12.0)
$AUC_{0-\infty}$ [ng/mL*h]	6447.8 (14.9) 5322 – 7856	8520.0 (16.8) 6634 – 12176	11735.5 (25.6) 8828 – 19199
AUC_{0-t} [ng/mL*h]	6086.6 (13.4) 5019 – 7294	7986.2 (17.5) 6152 – 11546	10587.9 (27.6) 8135 – 18289
$t_{1/2}$ [h]	26.80 (21.4) 18.9 – 35.6	31.47 (22.4) 24.7 – 48.2	46.53 (22.1) 34.6 – 67.6
V_z//f [L]	299.8 (26.1) 174 – 413	266.5 (30.5) 161 – 417	286.0 (37.4) 176 – 450
CL/f [L/h]	7.75 (14.9) 6.36 – 9.40	5.87 (16.8) 4.11 – 7.54	4.26 (25.6) 2.60 – 5.66

The total exposure (measured as AUC) of safinamide increased with the degree of hepatic impairment, whereas the C_{max} values were comparable between the hepatic impairment subjects and healthy subjects. Longer half-lives and lower clearance were observed in subjects with hepatic impairment (Child-Pugh A and Child-Pugh B).

Hepatic impairment did not affect safinamide unbound fraction as indicated by the comparable mean values for safinamide protein binding in all three groups.

Mean (SD) and Range (n=8) of Safinamide Fraction Unbound (%)

Scheduled time	Normal	Child-Pugh A	Child-Pugh B
2.5 h	11.0 (1.2) 9.0 – 13.0	9.9 (1.8) 7.5 – 13.4	12.8 (4.8) 7.8 – 19.9
4 h	10.7 (0.9) 9.6 – 12.3	9.6 (1.9) 6.8 – 12.8	12.5 (3.7) 7.7 – 18.0
24 h	10.5 (1.0) 9.0 – 12.2	9.6 (1.9) 7.0 – 12.9	12.4 (3.1) 7.9 – 17.1

The formation of safinamide metabolites NW-1689 and NW-1153 was reduced in subjects with hepatic impairment as indicated by a decrease in C_{max}.
Terminal $t_{1/2}$ of NW-1689 was prolonged by approximately 20 h in Child-Pugh B subjects (from 35.4 to 56.1 h) compared to controls demonstrating that the elimination of this metabolite is also affected; no difference in $t_{1/2}$ of NW-1689 was observed in Child-Pugh A subjects. Overall, the net effect on $AUC_{0-\infty}$ and AUC_{0-t} was marginal.

ANOVA Results for Ratio Mild Impairment (Child-Pugh A) / Healthy Subjects and Moderate Impairment (Child-Pugh B) / Healthy Subjects of Main PK Parameters for Safinamide and its Metabolites NW1153 and NW1689

Analyte	Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
Safinamide	$AUC_{0-\infty}$	Mild imp./Healthy	132.14	111.81 - 156.16
		Moderate imp./Healthy	182.01	154.01 - 215.10
	C_{max}	Mild imp./Healthy	95.40	70.80 - 128.54
		Moderate imp./Healthy	98.16	72.85 - 132.27
	$t_{1/2}$	Mild imp./Healthy	117.44	97.44 - 141.56
		Moderate imp./Healthy	173.62	144.04 - 209.27
	V_z/f	Mild imp./Healthy	88.88	68.14 - 115.93
		Moderate imp./Healthy	95.39	73.13 - 124.42
	CL/f	Mild imp./Healthy	75.68	64.03 - 89.44
		Moderate imp./Healthy	54.94	46.49 - 64.93
NW1689	$AUC_{0-\infty}$	Mild imp./Healthy	74.00	44.26 - 123.73
		Moderate imp./Healthy	89.62	53.60 - 149.84
	C_{max}	Mild imp./Healthy	68.38	43.53 - 107.43
		Moderate imp./Healthy	61.88	39.39 - 97.21
NW1153	AUC_{0-t}	Mild imp./Healthy	108.53	72.60 - 162.26
		Moderate imp./Healthy	168.58	112.76 - 252.03
	C_{max}	Mild imp./Healthy	85.40	65.11 - 112.01
		Moderate imp./Healthy	69.10	52.69 - 90.63

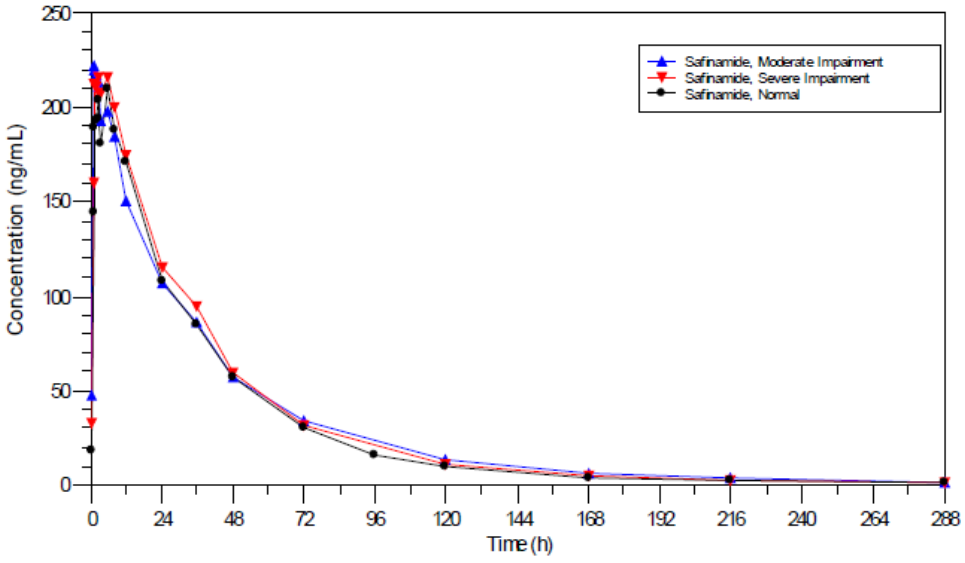
The marginal increase in exposure of safinamide (approx. 30% increase in AUC) observed in subjects with mild hepatic impairment (Child-Pugh A) is considered not to be clinically meaningful based on safinamide safety profile. Therefore, no dose adjustment is required for patients with mild hepatic impairment.

In subjects with moderate hepatic impairment (Child-Pugh B), exposure of safinamide increased about 80% (CI: 154-215%); therefore the lower dose of the two proposed therapeutic safinamide doses (50 mg/day) is recommended in subjects with moderate hepatic impairment.
Safinamide pharmacokinetics were not evaluated in subjects with severe hepatic impairment (Child-Pugh 10-15 points) and safinamide is not recommended for this patient population.
Recommendations:
Safinamide is not recommended in subjects with severe hepatic impairment.
No dose adjustment for safinamide is needed in subjects with mild hepatic impairment.
In subjects with moderate hepatic, the lower dose of the two proposed therapeutic safinamide doses (50 mg/day) is recommended.

2.3.1.7 Renal Impairment:

The effect of renal impairment on safinamide pharmacokinetics was investigated in an open-label, parallel-group, single oral dose (50 mg) study in 24 male and female subjects with moderately impaired, severely impaired, and normal renal function (Protocol EMR701165-025).
Mean plasma concentration-time profiles for safinamide in healthy subjects and subjects with moderate and severe renal impairment are shown in the figure below.

Mean Plasma Pharmacokinetic Parameters of Safinamide Following a Single Dose of 50-mg Safinamide Administered to Patients with Renal Impairment and to Healthy Matched Control Subjects



Mean plasma concentration-time profiles of safinamide were similar between all three groups: moderate and severe renal impairment and healthy subjects with normal renal function. Safinamide PK was not affected by renal function.

Summary of Pharmacokinetic Parameters of Safinamide in Plasma

	Normal	Moderate renal impairment	Severe renal impairment
C_{max} [ng/mL]	237.8 (17.0) 173 - 308	247.6 (24.7) 164 - 353	238.6 (16.9) 187 - 284
t_{max} [h]	4.25 (1.0 - 8.0)	1.50 (1.0 - 3.0)	4.00 (1.5 - 8.0)
$AUC_{0-\infty}$ [ng/mL*h]	8235.8 (14.9) 7093 - 10056	8516.1 (23.8) 5689 - 12053	8734.1 (18.1) 6579 - 10605
AUC_{0-t} [ng/mL*h]	8140.3 (15.0) 7024 - 9940	8396.2 (23.5) 5639 - 11939	8635.2 (18.0) 6518 - 10462
$t_{1/2}$ [h]	62.91 (12.5) 47.8 - 70.2	55.05 (29.1) 29.6 - 72.0	56.46 (23.6) 37.8 - 76.6
V_z/f [L]	551.0 (16.4) 476 - 701	466.3 (29.1) 264 - 620	466.3 (17.4) 351 - 594
CL/f [L/h]	6.07 (14.9) 4.97 - 7.05	5.87 (23.8) 4.15 - 8.79	5.72 (18.1) 4.71 - 7.60

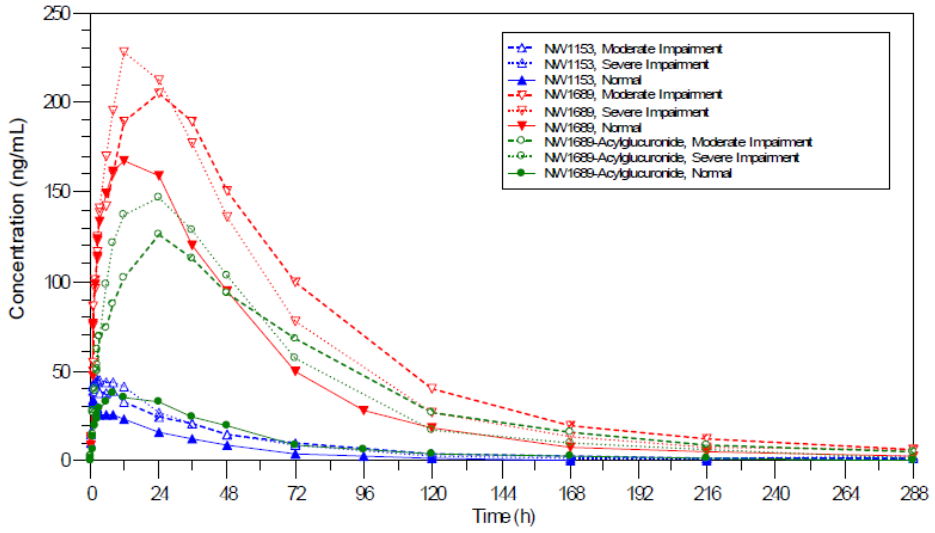
ANOVA Results for Ratio Moderate Versus Normal and Severe Versus Normal of Main Plasma Pharmacokinetic Parameters for Safinamide

Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
$AUC_{0-\infty}$	Moderate imp./normal	103.40	87.95 - 121.57
	Severe imp./normal	106.05	89.26 - 126.01
C_{max}	Moderate imp./normal	104.09	88.04 - 123.07
	Severe imp./normal	100.32	83.93 - 119.91

imp=impairment

Increased exposure of safinamide, metabolites NW-1153, NW-1689 and NW-1689 AG was observed in subjects with moderate and severe renal impairment in comparison with subjects with normal renal function, see figure and table below. This effect was most pronounced for NW-1689 acylglucuronide with 4 to 5 times higher $AUC_{0-\infty}$ and 3 to 4 times higher C_{max} values in subjects with renal impairment than in subjects with normal renal function. For NW-1689 and NW-1153, the effect ranged from 1.2-fold to 1.8-fold increase for C_{max} and $AUC_{0-\infty}$.

Mean Plasma Concentration-Time Profiles for Metabolites NW-1153, NW-1689 and NW-1689 acylglucuronide and for subjects with moderate and severe renal impairment and subjects with normal renal function



ANOVA Results for Ratio Moderate Versus Normal and Severe Versus Normal of Main Pharmacokinetic Parameters for Safinamide Metabolites

NW-1689-AG

Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
$AUC_{0-\infty}$	Moderate imp./normal	428.86	301.14 - 610.75
	Severe imp./normal	463.84	318.29 - 675.95
C_{max}	Moderate imp./normal	289.39	222.05 - 377.15
	Severe imp./normal	366.73	276.59 - 486.26

NW-1689

Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
$AUC_{0-\infty}$	Moderate imp./normal	143.31	98.23 - 209.06
	Severe imp./normal	130.50	87.28 - 195.11
C_{max}	Moderate imp./normal	119.21	84.20 - 168.77
	Severe imp./normal	136.21	94.06 - 197.25

NW-1153

Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
$AUC_{0-\infty}$	Moderate imp./normal	173.52	140.08 - 214.94
	Severe imp./normal	178.94	142.46 - 224.77
C_{max}	Moderate imp./normal	131.80	109.08 - 159.26
	Severe imp./normal	139.56	114.09 - 170.72

These three metabolites have been shown to be pharmacologically inactive; however, there was a concern that the increased exposure of safinamide metabolites NW-1153, NW-1689 and NW-1689 AG in subjects with moderate and severe renal impairment could affect the safety profile of safinamide in this patient population.
A Request for Information was sent to the sponsor to provide an analysis of the most frequently-reported TEAEs, by renal function status (based on the Cockcroft-Gault estimated creatinine clearance).
The analysis of TEAEs by baseline renal status in ESPD and LSPD patients did not show any consistent evidence of an association between the incidence of adverse events and renal status. Small changes were observed for nausea in ESPD patients associated with the moderately impaired group; it is likely that these changes are due to the small sample size of the moderately impaired renal group.

The summary of this analysis is shown in the table below.

Summary of most frequently-reported TEAEs, by Baseline renal status subgroups

	Pooled Group 13 ESPD patients (Studies 015/MOTION)				Pooled Group 14 LSPD patients (Studies 016/SETTLE)		
n (%)	Safinamide 50 mg (N=226)	Safinamide 100 mg (N=317)	Safinamide 200 mg (N=89)	Placebo (N=315)	Safinamide 50 mg (N=223)	Safinamide 100 mg (N=498)	Placebo (N=497)
Subjects with at least one event	152 (67.3)	223 (70.3)	52 (58.4)	221 (70.2)	160 (71.7)	363 (72.9)	359 (72.2)
Overall Incidence							
Dyskinesia	0	1 (0.3)	0	0	47 (21.1)	87 (17.5)	44 (8.9)
Fall	5 (2.2)	4 (1.3)	1 (1.1)	13 (4.1)	8 (3.6)	31 (6.2)	19 (3.8)
Parkinson's Disease	0	3 (0.9)	0	4 (1.3)	14 (6.3)	21 (4.2)	23 (4.6)
Headache	13 (5.8)	17 (5.4)	4 (4.5)	22 (7.0)	13 (5.8)	26 (5.2)	27 (5.4)
Nausea	13 (5.8)	30 (9.5)	8 (9.0)	23 (7.3)	7 (3.1)	28 (5.6)	21 (4.2)
Dizziness	18 (8.0)	20 (6.3)	4 (4.5)	18 (5.7)	7 (3.1)	14 (2.8)	14 (2.8)
Somnolence	17 (7.5)	14 (4.4)	4 (4.5)	23 (7.3)	7 (3.1)	16 (3.2)	14 (2.8)
Back Pain	13 (5.8)	16 (5.0)	3 (3.4)	21 (6.7)	10 (4.5)	22 (4.4)	27 (5.4)
Renal Impairment Status							
Normal (eGFR >90 mL/min)	(N=75)	(N=128)	(N=27)	(N=116)	(N=47)	(N=145)	(N=140)
Dyskinesia	0	0	0	0	12 (25.5)	18 (12.4)	18 (12.9)
Fall	1 (1.3)	1 (0.8)	1 (3.7)	3 (2.6)	3 (6.4)	6 (4.1)	4 (2.9)
Parkinson's Disease	0	1 (0.8)	0	1 (0.9)	3 (6.4)	9 (6.2)	7 (5.0)
Headache	5 (6.7)	8 (6.3)	2 (7.4)	10 (8.6)	3 (6.4)	12 (8.3)	9 (6.4)
Nausea	3 (4.0)	15 (11.7)	0	6 (5.2)	1 (2.1)	8 (5.5)	6 (4.3)
Dizziness	5 (6.7)	6 (4.7)	1 (3.7)	4 (3.4)	1 (2.1)	5 (3.4)	1 (0.7)
Somnolence	6 (8.0)	9 (7.0)	1 (3.7)	8 (6.9)	3 (6.4)	3 (2.1)	4 (2.9)
Back Pain	4 (5.3)	3 (2.3)	2 (7.4)	12 (10.3)	3 (6.4)	6 (4.1)	10 (7.1)
Mild (eGFR >59-90 mL/min)	(N=121)	(N=150)	(N=44)	(N=160)	(N=130)	(N=270)	(N=284)
Dyskinesia	0	1 (0.7)	0	0	27 (20.8)	53 (19.6)	19 (6.7)
Fall	3 (2.5)	2 (1.3)	0	6 (3.8)	3 (2.3)	19 (7.0)	13 (4.6)
Parkinson's Disease	0	2 (1.3)	0	3 (1.9)	8 (6.2)	8 (3.0)	14 (4.9)
Headache	6 (5.0)	7 (4.7)	1 (2.3)	10 (6.3)	6 (4.6)	12 (4.4)	16 (5.6)
Nausea	7 (5.8)	9 (6.0)	5 (11.4)	15 (9.4)	3 (2.3)	11 (4.1)	8 (2.8)
Dizziness	11 (9.1)	11 (7.3)	2 (4.5)	14 (8.8)	5 (3.8)	7 (2.6)	10 (3.5)
Somnolence	9 (7.4)	5 (3.3)	2 (4.5)	14 (8.8)	3 (2.3)	12 (4.4)	8 (2.8)
Back Pain	6 (5.0)	12 (8.0)	1 (2.3)	6 (3.8)	5 (3.8)	12 (4.4)	16 (5.6)
Moderate (eGFR 30-59 mL/min)	(N=29)	(N=34)	(N=18)	(N=37)	(N=46)	(N=80)	(N=72)
Dyskinesia	0	0	0	0	8 (17.4)	15 (18.8)	7 (9.7)
Fall	1 (3.4)	1 (2.9)	0	4 (10.8)	2 (4.3)	6 (7.5)	2 (2.8)
Parkinson's Disease	0	0	0	0	3 (6.5)	4 (5.0)	2 (2.8)
Headache	2 (6.9)	1 (2.9)	1 (5.6)	2 (5.4)	4 (8.7)	2 (2.5)	2 (2.8)
Nausea	3 (10.3)	6 (17.6)	3 (16.7)	2 (5.4)	3 (6.5)	8 (10.0)	7 (9.7)
Dizziness	2 (6.9)	2 (5.9)	1 (5.6)	0	1 (2.2)	1 (1.3)	3 (4.2)
Somnolence	2 (6.9)	0	1 (5.6)	0	1 (2.2)	1 (1.3)	2 (2.8)
Back Pain	3 (10.3)	0	0	2 (5.4)	2 (4.3)	4 (5.0)	1 (1.4)
Severe (eGFR <30 mL/min)	(N=0)	(N=2)	(N=0)	(N=0)	(N=0)	(N=1)	(N=0)
Dyskinesia	0	0	0	0	0	1 (100.0)	0
Fall	0	0	0	0	0	0	0
Parkinson's Disease	0	0	0	0	0	0	0
Headache	0	0	0	0	0	0	0
Nausea	0	0	0	0	0	0	0
Dizziness	0	1 (50.0)	0	0	0	1 (100.0)	0
Somnolence	0	0	0	0	0	0	0
Back Pain	0	0	0	0	0	0	0

Recommendations:
No dose adjustment for safinamide is recommended in subjects with renal impairment.

2.4 EXTRINSIC FACTORS

2.4.1 Is safinamide a substrate, inhibitor or inducer of CYP enzymes?

CYP Inhibition:
The direct inhibitory potential of safinamide towards the metabolism of CYP-specific substrates was determined in human liver microsomes pooled from adult males and females in study (b) (4) MET/99/04. Safinamide did not inhibit activities of CYP 2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and, CYP3A4/5 at concentrations of 0.5 to 100 μM. CYP1A1/2 was inhibited by safinamide with an IC_{50} value of 47.7 μM. Major metabolites of safinamide, NW-1153 and NW-1689 did not inhibit activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and, CYP3A4/5 at concentrations of 0.5 to 100 μM.

Time dependent inhibition (TDI) potential of safinamide, NW-1153, and NW-1689 was investigated in HLM in study XT075070. Ten CYPs were evaluated by incubating HLM with a range of concentrations of test compounds. TDI was determined by pre-incubating the test compounds with HLM for 30 minutes prior to the addition of the selective CYP substrate. There was some evidence of direct inhibition and TDI of CYP1A2, CYP2B6, CYP2C19, and CYP2D6 by safinamide. When safinamide was incubated at 100 μM the direct inhibition of CYP1A2, CYP2B6, CYP2C19, and CYP2D6 was 50%, 20%, 33%, and 33%, respectively. The Ki of the competitive inhibition of CYP1A2 by safinamide was determined to be 54 μM.

There was no evidence of direct or TDI of CYP2A6, CYP2C8, CYP2C9, CYP2E1, and CYP3A4/5 by safinamide at concentrations up to 100 μM. There was no direct inhibition by NW-1153 or NW-1689 of any of the CYP enzymes tested. There was some evidence of TDI of CYP1A2 (36% at 100 μM) and CYP2B6 (78% at 100 μM) by NW-1153, but not NW-1689.

Study (b) (4) 085009 further characterized the mechanism underlying the TDI of CYPs by safinamide. The TDI of CYP1A2, CYP2B6, CYP2C19, and CYP2D6 was NADPH-dependent, indicating that safinamide is a metabolism-dependent inhibitor of these enzymes. In addition, the metabolism-dependent inhibition of CYP1A2 and CYP2B6 was unaffected by dilution of the incubate indicating that safinamide is an irreversible or quasi-irreversible inhibitor. The characteristics of the TDI of CYP2C19 and CYP2D6 by safinamide were investigated further in study (b) (4) 095019. This suggested that a metabolite of safinamide is a more potent direct inhibitor of these enzymes than parent safinamide. Overall, the inhibition of CYP1A2, CYP2B6, CYP2C19, and CYP2D6 by safinamide was less potent than positive controls.

The TDI of CYP1A2 and CYP2B6 by the safinamide metabolite, NW-1153, was investigated further characterized in study (b) (4) 095009. This study showed that NW-1153 caused metabolism-dependent (time-dependent and NADPH-dependent) inhibition of CYP1A2 that was partially reversible by re-isolation of microsomal protein from the pre-incubation and by treatment with potassium ferricyanide. CYP2B6 activity was completely recovered. The characteristics of NW-1689 AG inhibition was determined in study (b) (4) 105058. CYP2C8 activity in HLM was directly inhibited by NW-1689 AG; whereby 22% of CYP2C8 activity was inhibited by 30 μM NW-1689 AG. There was little or no evidence of time-dependent or metabolism-dependent inhibition of CYP2C8 activity at concentrations of NW-1689 AG up to 30 μM. Overall, TDI of the CYP enzymes was weak and at very high concentrations when compared to clinically relevant concentration.

Inhibition of Other Enzymes

In study XT085097, the ability of the safinamide metabolites, NW-1153, NW-1689, and NW-1199, to inhibit MAO-A was evaluated in human liver mitochondria. The concentrations of NW-1153, NW-1689, and NW-1199 tested were 0.1 to 100 μM. There was no evidence of direct inhibition of MAO-A activity by NW-1153, NW-1689 or NW-1199 and the IC_{50} values for this enzyme were >100 μM. In addition, there was also no evidence of time-dependent inhibition of MAO-A activity by NW-1153, NW-1689, or NW-1199 at concentrations up to 100 μM.

The effect of safinamide, NW-1153 and NW-1689 on the decarboxylation of levodopa to dopamine by levodopa decarboxylase was determined in human liver homogenates in study NWR/16. Levodopa metabolism was inhibited by 50 μM safinamide, 50 μM NW-1153, and 50 μM NW-1689 by 2.8%, 3.1%, and 6.0%, respectively, compared to solvent control activities. The positive control, 50 μM benserazide, inhibited levodopa metabolism by 97.9% compared to solvent control activities. Therefore, safinamide, NW-1153 and NW-1689 have no inhibition potential on levodopa decarboxylase.

NW-1689 AG was identified as an inhibitor for human ALDH1A1, having an IC_{50} of 35 μM when propionaldehyde (100 μM) was used as the aldehyde substrate (study DMPK 125-07). Both metabolites NW-1689 and NW-1689 AG were inhibitors of ALDH1B1, with the IC_{50} values were greater than 200 μM.

The average maximal plasma concentration of Safinamide was about 4μM in Parkinson's disease patients treated with the highest dose of 100 mg/day. Plasma exposures of NW-1689, NW-1689 AG, NW-1153 are 160%, 18% and 11% of parent respectively. Based on the Ki and IC_{50} values for inhibition of CYP enzymes or the other enzymes, safinamide or its major metabolites are not inhibitors of CYP, ALDH, levodopa decarboxylase or MAO-A enzymes at clinically relevant concentrations. Therefore, the inhibition potential of safinamide is low.

Following table provides summary of *in vitro* inhibitory effects of safinamide and its main metabolites.

Enzyme	Safinamide		NW-1153		NW-1689		NW-1689 AG	
	Direct	**TDI**	**Direct**	**TDI**	**Direct**	**TDI**	**Direct**	**TDI**
CYP1A2	K_i = 54 µM	K_i = 33.5 µM K_{inact} = 0.075 s^{-1} NADPH-dependent Quasi- or irreversible	No	IC_{50} > 100 µM NADPH-dependent Partially reversible	No	No	-	-
CYP2A6	No	No	No	No	No	No	-	-
CYP2B6	No	IC_{50}= 57 µM NADPH-dependent Quasi- or irreversible not kinetically characterizable	No	IC_{50} = 47 µM NADPH-dependent Reversible	No	No	-	-
CYP2C8	No	No	No	No	No	No	No	No
CYP2C9	No	No	No	No	No	No	-	-
CYP2C19	No	IC_{50} = 63 µM NADPH-dependent	No	No	No	No	-	-
CYP2D6	No	IC_{50} > 100 µM NADPH-dependent Reversible	No	No	No	No	-	-
CYP2E1	No	No	No	No	No	No	-	-
CYP3A4/5 [a]	No	No	No	No	No	No	-	-
MAO-A	-	-	No	No	No	No	-	-
L-dopa decarbox.	No	-	No	-	No	-	-	-
ALDH1A1							K_i = 4.1 µM	
ALDH1B1					IC_{50} > 200 µM		IC_{50} > 200 µM	

No: Inhibition was ≤50% up to the highest concentration investigated -: not investigated.
a: investigated using testosterone, midazolam and nifedipine

CYP Induction:

Safinamide's induction potential for CYP1A1/2, CYP3A4/5, and CYP2C9 activities were evaluated in the hepatocyte monolayers or in microsomes prepared from cell homogenates treated daily with 3, 30, and 100 µM safinamide for 72 hours. The increase in CYP1A2 mRNA expression never exceeded 4.9-fold higher than control treated cells and was negligible compared to the increase in CYP1A2 mRNA expression due to omeprazole, which was induced 132- to 266-fold. The increase in CYP3A4 activity above at 30 and 100 µM, was increased from 1.7- to 2.5-fold and 2.8- to 5.9-fold, respectively, which was 6.5% to 53% and 22% to 84% of positive control values, respectively. Induction of CYP2C9 activity was increased above 20% of the positive

control, 10 μM rifampicin, at mid and high concentrations in hepatocytes from two out of three donors.

The effects of safinamide on CYP2B6 activities and mRNA expression were investigated in human hepatocytes using 3, 30, and 100 μM safinamide. The maximum induction of CYP2B6 mRNA expression in Donors 1, 2, and 4 were 27% (at 3 μM), 60% (at 30 and 100 μM) and 35% (at 30 μM) of the positive control, respectively.

The effect of safinamide on CYP1A2, CYP2B6, UDP-glucuronosyl transferase 1A1 (UGT1A1), and sulfotransferase 2A1 (SULT2A1) in primary human hepatocyte cultures was investigated further in study MS-032010-02 (safinamide 1 to 80 μM). Enzymatic activities and mRNA were measured at the end of the treatment. CYP1A1/2 activities were calculated to be 18% and 28% of the positive control. CYP1A2 mRNA expression was increased by safinamide and the maximum induction in the two donors was 30 μM and 45 μM and corresponded to 10% and 9% of the positive control, respectively. Safinamide increased CYP2B6 activity and the responses were above the threshold of 20% of the positive control (1000 μM phenobarbital) at 30 μM and at 60 μM. CYP2B6 mRNA expression was also increased by safinamide and maximum induction in the two donors was at 60 μM salt and 80 μM salt and corresponded to 25% and 31% of the positive control. There was no concentration dependence or increase in UGT1A1 activity or mRNA expression by safinamide. Similarly, safinamide not affect SULT2A1 activity or mRNA expression.

In summary, based on *in vitro* studies, safinamide is a weak inducer of CYP1A2, CYP2B6, CYP2C9 and CYP3A4 at approximately 10 times clinically relevant concentrations. Safinamide did not induce UGT1A1 or SULT2A1.

2.4.2 Is the drug or its major metabolite, a substrate, an inhibitor and/or an inducer of transporter processes?

The sponsor characterized safinamide transport *in vitro*. Safinamide is not a substrate of P-gp. No polarization of transport was observed over the concentration range (10 -2500 μM) investigated and safinamide transport was unaffected by inhibition of active transport (sodium azide in combination with 2-deoxyglucose). Safinamide, NW-1689, NW-1153 and NW-1689 AG were shown not to be P-gp inhibitors at clinically relevant concentrations. Safinamide and its metabolites did not inhibit the renal drug transporter OCT2, organic anion transporter proteins OATP1B1 and OATP1B3 or the canalicular bile salt export pump BSEP. No inhibition >20% of the renal drug transporter OAT1/3/4 was observed, except for safinamide and NW-1689 AG on OAT3 (<40% inhibition).

Safinamide decreased BCRP mediated 2-Amino-1-methyl-6-phenylimidazol [4, 5-b] pyridine (PhIP) transport in a concentration-dependent manner with an IC_{50} of 43 ± 23 μM. NW-1689 also inhibited BCRP with an IC_{50} of 3.7 ± 0.5 μM. The Agency's guidance recommends a clinical drug-drug interaction study to be conducted in cases where the estimated intestinal concentration C_{int} (calculated as dose/250 mL) divided by the $IC_{50, app}$ is greater than 10. For safinamide, the estimated intestinal concentration

greater 10×IC50, app is about 430±230 µM. Safinamide mesylate is freely soluble in water, if the given dose was fully dissolved in intestinal fluid, this would be equivalent to a dose of about 108 µmol or 43 mg safinamide salt. However, safinamide is absorbed throughout the GI tract which is evident from the Tmax range from 1.8 to 2.8 hours after the drug administration. Therefore the concentration in the small intestine and colon would be below the threshold concentration achieved at 50 mg QD dose. The intestinal concentration of safinamide may be higher than the threshold concentration at 100 mg QD dose which requires in vivo evaluation of DDI potential.

NW-1689 is a major metabolite of safinamide found in plasma at the concentration of 160% of parent compound, safinamide. The average maximal plasma concentration of Safinamide was approximately 4 µM in Parkinson's disease patients treated with the highest dose of 100 mg/day. For NW1689 the R value ($R=1+ [I]/IC_{50}$) calculated per DDI guidance is approximately 2.25 (>1.1) indicating that an *in vivo* clinical study is needed. Substrates of BCRP include methotrexate, mitoxantrone, imatinib, irrinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan.
In summary, safinamide has a potential to inhibit intestinal BCRP at 100 mg dose. Safinamide's metabolite NW1689 also has a potential to inhibit BCRP. Therefore, a clinical DDI study should be conducted to evaluate BCRP inhibition.
Safinamide and its metabolites did not inhibit other transporters OCT2, OATP1B1, OATP1B3, BSEP or OAT1/3/4 at clinically relevant concentrations.

Safinamide and its Main Metabolites: *In Vitro* Inhibitory Effects on Drug Transporters

Transporter	Location[a]	Safinamide	NW-1153	NW-1689	NW-1689 AG
P-gp	Kidney Liver Intestine	No	No	No	No
BCRP	Liver Intestine	IC_{50} = 43 ± 23 µM	No	IC_{50} = 3.7 ± 0.5 µM	No
BSEP	Liver	No	No	No	No
OATP1B1	Liver	No	No	No	No
OATP1B3		No	No	No	No
OCT2	Kidney	Weak (IC_{50} = 130 ± 20 µM)	-	-	-
OAT1	Kidney	No	No	No	No
OAT4		No	No	No	No
OAT3		No	No	No	No

No: Inhibition was ≤50% up to the highest concentration investigated and was not relevant compared to the positive control --: Not investigated.

Late-Cycle Meeting Discussion:
The Clinical Pharmacology reviewer stated that an in vivo drug interaction study would be requested as a post-approval commitment to assess the potential for DDI in light of BCRP inhibition by safinamide's major metabolite, NW-1689. The Sponsor mentioned that Newron has answered a similar request from the EMA; per the discussion, the Clinical Pharmacology reviewer sent an information request following LCM regarding this topic.

The sponsor submitted a Response to the Clinical Pharmacology IR on October 14, 2015 containing three reports. The submission was reviewed by OCP and OCP determined that it does not address the concern about the BCRP inhibition by safinamide's major metabolite, NW-1689.

The reports in the submission, and the reasons why these reports are not adequate to address the the BCRP inhibition potential of NW-1689, are listed below:

HLS-16-02Apr2014: In vitro Interaction Studies of Safinamide and NW-1153 with Selected human BCRP Efflux (ABC) Transporter, and with human OATP1B1, OATP1B3, OATP1A2, OAPT2B1, OCT1, OCT2, OAT1, OAT3, MATE1 and MATE2-K Uptake Transporters
This report does not address the DDI potential of NW-1689.

Study Z7219J01: Drug interaction study of safinamide and a BCRP substrate, diclofenac, concomitantly administered to healthy volunteers. Single dose, open-label, 2-period, cross-over, randomised, interaction, bioavailability study.
This study is not suitable to assess the inhibition potential of NW-1689 for BCRP substrate for the following reasons:

- Diclofenac can not be used as a BCRP substrate for understanding BCRP-mediated DDI in vivo as it is a "weak" BCRP substrate with an efflux ratio (ER) of 1.25 (our guidance recommend an ER ≥2). The following sensitive BCRP substrates are recommended to be used for in vivo studies: methotrexate, mitoxantrone, imatinib, irrinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan.
- The study design (single dose) is not suitable for assessing the DDI potential of the metabolite which is formed much later than safinamide (Tmax of NW-1689 is about 10-12h).

Summary Report RA27820002_01: Evaluation of Time course of Safinamide in Gastrointestinal (GI) Lumen using a Mechanism-Based Approach for Absorption Modeling: The Gastro-Intestinal Transit Time (GITT) Model
The GI transit model in this report is not relevant as the sponsor is using the model to predict the amount of safinamide in the proximal small intestine. There is no estimation for NW-1689. As this safinamide major metabolite is a concern, NW-1689 needs to be part of the model in order to justify why a model approach can be adequate to replace an in vivo DDI study.

In summary, these reports submitted following LCM did not address the inhibition potential of NW-1689 on BCRP substrate. A PMR regarding this will be requested from a clinical pharmacology perspective.

2.4.4 What extrinsic factors (such as herbal products, diet, smoking and alcohol) influence exposure and or response and what is the impact of any differences in exposure on pharmacodynamics?

I. Tyramine-containing food or beverages

A major problem of unspecific MAO inhibitors is hypertensive crisis ("cheese effect") after ingestion of tyramine rich foods, due to inhibtion of the main tyramine catalizing enzyme MAO-A in the gastrointestinal tract. As many MAO-B inhibitors lose their specificity at higher doses and may then cause "cheese effect", the assessment of the potentiation of the tyramine pressor effect is an important part of the evelopment of MAO-B inhibitors.

Three Phase I trials in healthy volunteers were conducted to assess the pressor effect of tyramine during safinamide administration.

In study (b) (4) NW1015/TYR-268-00, tyramine was administered intravenously, and in CRO-PK-03-101, oral tyramine was given under fasting conditions. These 2 trials did not show any effect of safinamide on tyramine induced increase in BP compared to placebo. However, due to some design limitations of these trials (tyramine administrated intravenously in trial (b) (4) NW1015/TYR-268-00, endpoint not reached in any subject of trial CRO-PK-03-101), a dedicated third study was designed to confirm these results.

The third study in healthy subjects (28558) followed a double-blind, parallel-group design with five randomized groups, including a placebo group, a positive control (phenelzine 30 mg) group, a comparator (selegiline 10 mg) group and two treatment groups: safinamide of 100 mg (therapeutic dose) and 350 mg (supratherapeutic dose).

The primary endpoint, Tyramine Sensitivity factor (TSF), was defined as the dose of oral tyramine that increased systolic blood pressure by 30 mm Hg (Tyr30) for a subject as subject-specific Tyr30 at screening, divided by the subject-specific Tyr30 under treatment. The following results for TSF (geometric mean) were reported:

- ☐ Placebo: 1.515
- ☐ Safinamide 100 mg: 2.152
- ☐ Safinamide 350 mg: 2.74
- ☐ Selegiline 10 mg: 3.115
- ☐ Phenelzine 30 mg: 9.977

Assay sensitivity of the trial was confirmed. The geometric least square mean ratios of TSF for the treatment groups versus placebo followed the same ranking as above of treatment effect. Safinamide, at the dose of 100 mg and 350 mg, respectively, potentiated the pressor effect of tyramine by 1.6-fold and 1.8-fold versus placebo, compared to 2.2-fold for selegiline 10 mg and 6-fold for phenelzine 30 mg.

Safinamide at 100 mg (therapeutic dose) and at 350 mg (supratherapeutic dose) induced a mild increase in the tyramine sensitivity factor. This effect appeared to be slightly dose-dependent. However, the potentiation of the pressor effect by safinamide at both doses remained lower than that of the comparator (selegiline), which has no label warning for tyramine at the dose level (10 mg) tested in the study.

Recommendation: Safinamide (at the recommended dose) can be administered without any dietary restrictions related to tyramine-containing food or beverages.

2.4.5 Are there any in-vivo drug-drug interaction studies that indicate the exposure alone and/or exposure response relationships are different when drugs are coadministered? If yes, is there a need for dosage adjustment?

2.4.5.1 Influence of other drugs on safinamide:

II. Proton pump inhibitors (PPI)

The solubility of safinamide is pH-depedent; however a drug interaction study with a proton pump inhibitor has not been conducted.
A Request for Information was sent to the sponsor to provide justification why a drug interaction study with a PPI is not needed. The sponsor's response is discussed below.

The use of PPIs, including Pantoprazole was allowed in patients in safinamide clinical trials. The sponsor evaluated the plasma levels of safinamide in patients treated with these drugs and compared it with safinamide levels in patients who did not take PPIs to evaluate the effect of these drugs on the absorption of safinamide. The potential effect of concomitant administration of PPIs and/or the other stomach acid regulators (collectively referred to as PPI) as a covariate on the rate and extent of absorption of safinamide was evaluated using an existing population pharmacokinetic (popPK) model. A summary of individual steady-state C_{max} and AUC of safinamide derived following administration of 100 mg dose of safinamide in a typical 60 year old weighing 70 kg Asian and a White subject is presented below (Report RB27820002, 26 June 2015).

Summary of Individual Steady-State Cmax (ng/mL) and AUC (ng*hr/mL) of Safinamide following 100 mg dose (in a typical Asian subject)

Dose	100 mg			
	PPI Absent	PPI Present	PPI Absent	PPI Present
Statistics	C_{max} (ng/mL)	C_{max} (ng/mL)	AUC_τ (ng*hr/mL)	AUC_τ (ng*hr/mL)
N	1000	1000	1000	1000
Mean	1645	1638	22228	22497
SD	385	370	4995	5066
Minimum	714	839	9204	10506
Median	1604	1595	21930	21721
Maximum	3341	3024	43234	39507

F value from a Typical Asian subject was used to calculate AUC_τ

Summary of Individual Steady-State Cmax (ng/mL) and AUC (ng*hr/mL) of Safinamide following 100 mg dose (in a typical White subject)

Dose	100 mg			
	PPI Absent	PPI Present	PPI Absent	PPI Present
Statistics	C_{max} (ng/mL)	C_{max} (ng/mL)	AUC_τ (ng*hr/mL)	AUC_τ (ng*hr/mL)
N	1000	1000	1000	1000
Mean	1708	1703	23075	23395
SD	400	385	5185	5268
Minimum	741	872	9555	10925
Median	1665	1659	22766	22587
Maximum	3468	3145	44881	41083

F value from a Typical White subject was used to calculate AUC_τ

Reviewer's Comments: Although the population pharmacokinetic analysis does not include information on dose and timing of PPI intake relative to safinamide, the various evidences suggest that the interaction potential is low. Please refer to the Pharmacometric Review for details.

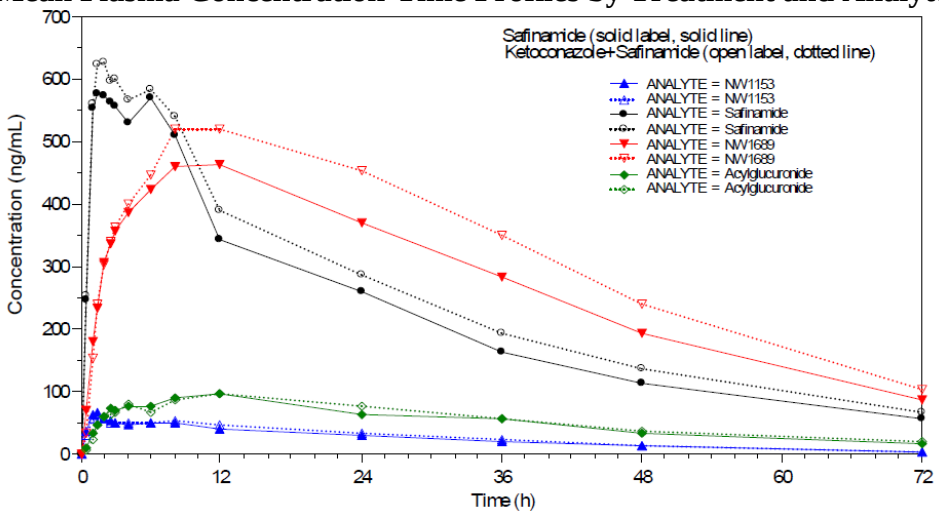
III. Ketoconazole DDI Study (Study 28778)

In vitro data indicated that the initial step of a major metabolic pathway of safinamide was catalyzed by CYP3A4, therefore an in vivo DDI study was conducted to assess the effect of CYP3A4 inhibition on safinamide pharmacokinetics (PK).

The trial was designed to investigate the effect of repeated oral doses of ketoconazole, a potent inhibitor of CYP3A4 isoenzyme, on the PK profile of safinamide and its main circulating metabolites. In order to ensure maximal inhibition of CYP3A4 at the time of safinamide administration, ketoconazole (200 mg twice daily) dosing was started two days before safinamide dosing to be at steady state and was maintained over 4 days (i.e., 4 half-lives of safinamide) following the safinamide dose.

Overall, mean plasma concentration-time profile for safinamide and its metabolites NW1153, NW1689 and NW1689-AG were similar in both treatments A and B (without and with ketoconazole co-administration).

Mean Plasma Concentration-Time Profiles by Treatment and Analyte



The exposure to safinamide when safinamide was administered in combination with ketoconazole was increased by 13% (see ANOVA table below).

ANOVA Results for Ratio Treatment B / Treatment A of $AUC_{0-\infty}$ and C_{max} for Safinamide and Metabolites NW-1153 and NW-1689

Analyte	Safinamide	NW1153	NW1689
	Ratio 90 % CI	Ratio 90 % CI	Ratio 90 % CI
C_{max} (ng/mL)	106.6 (101.0-112.4)	89.2 (83.6-95.2)	112.5 (108.1-117.1)
$AUC_{0-\infty}$ (ng.h/mL)	112.9 (109.8-116)	107.6 (104.1-111.2)	118.5 (111.5-126.0)

CI: Confidence Interval
Treatment B: Safinamide plus ketoconazole
Treatment A: safinamide alone; Source Report 28778 Table 11-5

However, the 90% CI for $AUC_{0-\infty}$ and C_{max} fell within bioequivalence range for all analytes (the 90% CI upper boundry for the AUC of NW1689 was slightly above the upper limits of the BE range).
Conclusion: no relevant drug-drug interaction with CYP3A4 inhibitors is to be expected with safinamide and safinamide can be co-administered with drugs known to inhibit the CYP3A4 iso-enzyme without dose adjustment.

IV. Enzyme Inducers

A Phase II, open-label dose titration study (NW-1015/010/II/2002) was conducted to investigate the tolerability and potential interactions of safinamide with antiepilepsy drugs (AEDs) in patients with epilepsy. Patients had to be under stable doses of at most three AEDs for at least 2 weeks prior to screening.
Information concerning possible interactions between safinamide and the AEDs was obtained by evaluating dosage modifications of AEDs and the trend of blood concentrations of safinamide and AEDs.
Patients took 1 to 3 AEDs (mean 1.9; median 2). Most patients continued their AED treatment without changes in dosage until the study end.
Safinamide levels were linearly related to dose, and there was a trend for safinamide levels to be about 30% lower in patients co-medicated with enzyme inducers compared with those not on enzyme inducers.
The results suggested no evidence of large effect of strong CYP inducers on safinamide exposure (however, this analysis should be considered exploratory). Since safinamide is metabolized mostly by MOA-A and cytosolic amidases, and only minimally by CYPs (see ketoconazole DDI study), no *in vivo* drug-drug interaction study with strong CYP inducers is required to confirm these results.

2.4.5.2 Influence of safinamide on other drugs

V. Interaction with Levodopa (Study 28780 and EMR701165-027)

Safinamide was developed as adjunctive therapy for the treatment of patients with PD in both early stage patients treated with a single dopamine agonist and mid- to late stage patients receiving levodopa (LD).
The effect of safinamide on the PK profile of LD was investigated in patients with idiopathic PD (28780) and in elderly healthy subjects (EMR701165-027) after single safinamide doses and at steady-state.
Study 28780 was a placebo-controlled, two-period, two-sequence crossover trial in patients with idiopathic PD to assess the effect of safinamide, administered once daily at the dose of 100 mg, on the PK of LD and its metabolites. Subjects received safinamide

100 mg and placebo for 6 days concomitantly with an immediate release formulation of LD (Nacom®, 100 mg LD +25 mg carbidopa) with a washout period of at least 14 days between the two periods.
The ANOVA point estimates for the ratio Treatment A/Treatment B on Day 1 and Day 6 were close to 100% for C_{max} and AUC_{0-6} of LD. The 90% CI for both parameters on Day 6 at safinamide steady-state were within the bounds of clinically acceptability (0.75 to 1.33) that were pre-defined based on known safety profile and PK variability of LD.

Estimates for Ratios of Geometric Means with 90% Confidence Intervals for C_{max} and AUC_{0-6} of Levodopa on Day 1 and Day 6 (n=23)

	Least Square Geometric Mean		Ratio Estimate [%] (90% CI)
	Treatment A (Levodopa + Safinamide)	Treatment B (Levodopa + Placebo)	Treatment A/ Treatment B
Day 1			
C_{max} (ng/mL)	974.895	950.327	102.6 (88.6 -118.8)
AUC_{0-6} (ng.h/mL)	1999.9	1734.8	115.3 (95.3-139.5)
Day 6			
C_{max} (ng/mL)	944.322	950.325	99.4 (77.9 -126.8)
AUC_{0-6} (ng.h/mL)	1792.2	1931.6	92.8 (82.1 -104.8)

The PK profile of the main circulating LD metabolite, homovanillic acid (HVA) was also not affected by safinamide treatment.

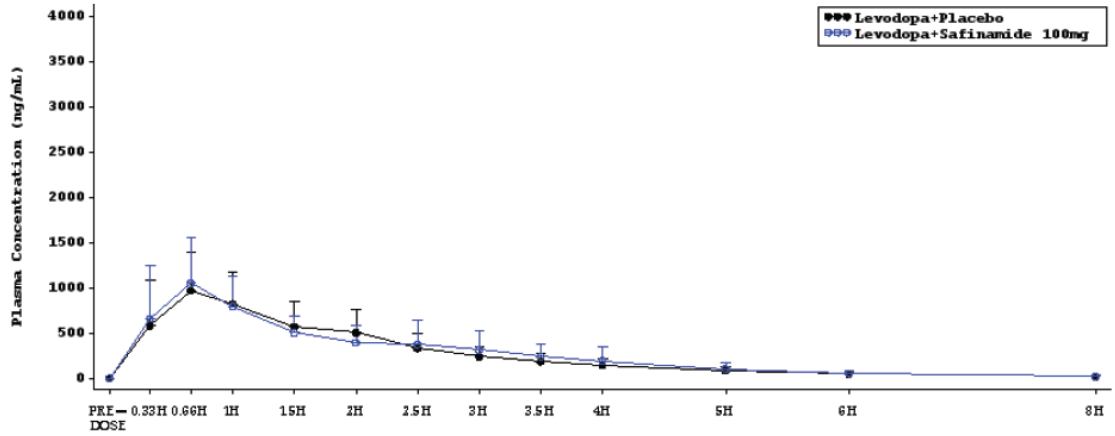
Estimates for Ratios of Geometric Means with 90% Confidence Intervals for C_{max} and AUC_{0-6} of HVA on Day 1 and Day 6

	Least Square Geometric Mean		Ratio Estimate [%] (90% CI)
	Treatment A (Levodopa + Safinamide)	Treatment B (Levodopa + Placebo)	Treatment A/ Treatment B
Day 1			
C_{max} (ng/mL)	243.734	255.541	95.4 (85.6 – 106.2)
AUC_{0-6} (ng.h/mL)	732.8	692.9	105.7 (89.7 -124.7)
Day 6			
C_{max} (ng/mL)	219.848	250.532	87.8 (73.9 -104.2)
AUC_{0-6} (ng.h/mL)	635.3	658.4	96.5 (83.9 -110.9)

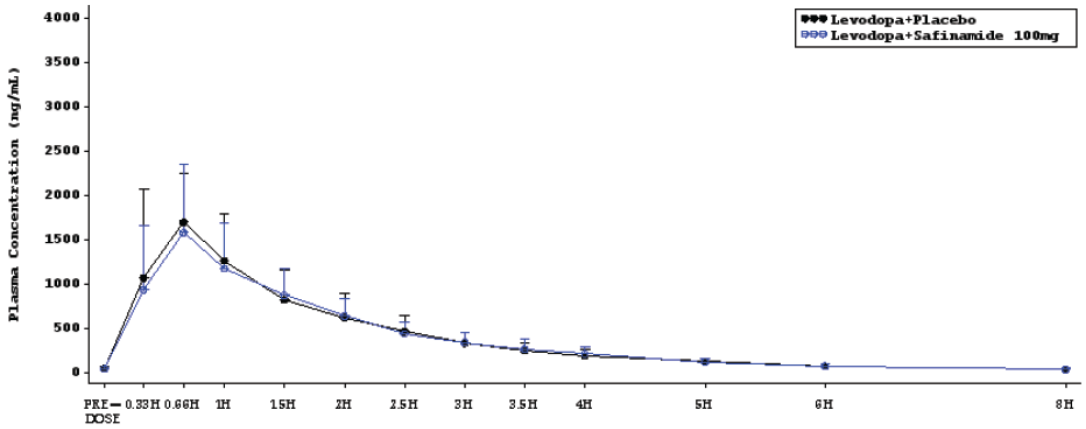
In addition, the plasma concentrations of safinamide and its major metabolites NW-1153 and NW-1689 on Day 5 and Day 6 were in line with previous PK data on safinamide after 100 mg dose (in the range of mean 800-1000 ng/mL for safinamide). Concentrations of NW-1689 and NW-1153 appeared consistent with previous data, with NW-1689 showing concentrations in a range 1.4x safinamide and NW-1153 in a range of 10% of parent.

Study EMR701165-027 was a randomized, double blind, placebo-controlled, two-period, two-sequence-crossover trial in elderly healthy subjects to evaluate the effect of safinamide (100 mg), compared to placebo, on LD pharmacokinetics.
Overall, mean LD concentration profiles obtained after co-administration with safinamide and after co-administration with placebo were super-imposable, both after single dose (Day 1) and at steady state (Day 6).

LD: Day 1



LD: Day 6



The ANOVA analysis for primary PK parameters C_{max} and AUC_{0-8} of LD on Day 1 and Day 6 resulted in 90% CIs for the treatment ratios (LD + safinamide 100mg/ LD + placebo) lying within the clinically acceptabile boundaries (0.75-1.33), that were predefined based on known safety profile and PK variability of levodopa. For AUC_{0-8} Day 1, AUC_{0-8} Day 6 and C_{max} Day1 the BE criteria (90% CI within the range of 0.8 to 1.25) were met.

Estimates for Ratios of Geometric Means with 90% Confidence Intervals for LD C_{max} and AUC_{0-8} on Day 1 and Day 6, Study EMR 701165-027

	Least Squares Geometric Mean		Ratio Estimate [%] (90% CI)
	Levodopa + safinamide	Levodopa + placebo	
$C_{max\ DAY\ 1}$ (ng/mL)	1197	1150	104.1 (94.1;115.1)
$AUC_{0-8\ DAY\ 1}$ (ng.h/mL)	2054.3	1968.1	104.4 (99.6;109.4)
$C_{max\ DAY\ 6}$ (ng/mL)	1652	1883	87.7 (77.6;99.1)
$AUC_{0-8\ DAY\ 6}$ (ng.h/mL)	2891.1	2958.1	97.7 (93.3;102.4)

In addition, the observed predose concentrations of safinamide after repeated doses on Day 5 and Day 6 were consistent with previous data for safinamide.

Recommendation: The following labeling language, proposed by the sponsor, is acceptable:
In vivo drug-drug interaction studies performed with levadopa did not demonstrate any clinically significant effects on the pharmacokinetic profile of XADAGO or on the pharmacokinetic profile of co-administered levadopa.

VI. Interaction with CYP1A2 Substrate (Caffeine) and CYP3A4 Substrate (Midazolam), Study EMR701165_026

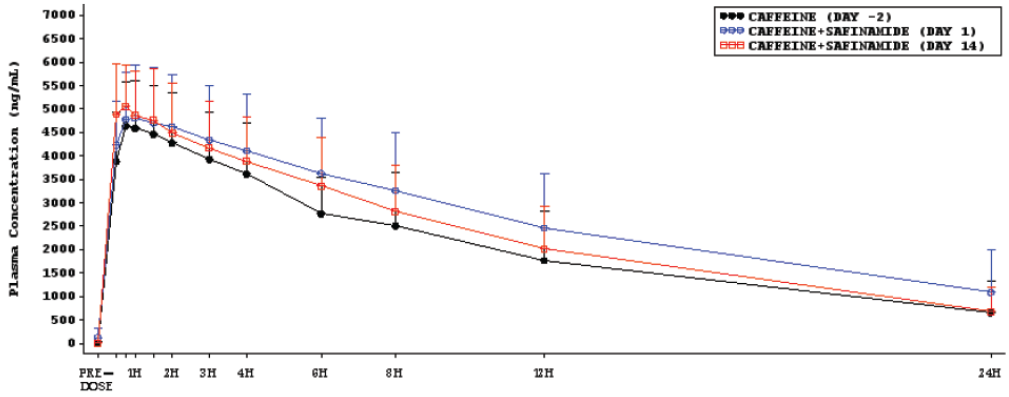
The results of the *in vitro* inhibition studies using human liver microsomes indicated that safinamide was a weak mechanism-based and competitive inhibitor of CYP1A2. In addition, safinamide showed low potency to induce CYP1A2 activity in human hepatocytes. The *in vitro* data investigating the induction potential of safinamide showed also that CYP3A4/5 activity was dose-dependently increased in human hepatocytes.
The clinical DDI-study was designed to investigate the effect of safinamide on the PK of CYP1A2 and CYP3A4 substrates caffeine and midazolam, which are FDA recommended probe substrates for CYP1A2 and CYP3A4, respectively. These 2 probe substrates are validated as cocktail administration, (i.e., the substrates are specific for the individual CYP enzyme and there are no interactions among these substrates).
The treatment duration of 14 days of safinamide was considered adequate to obtain potential maximum induction and mechanism-based inhibition of CYP3A4 and CYP1A2, respectively, since:

- the turnover rate for CYP3A4 $t_{1/2}$ is estimated to be 23-70 hours
- the turnover rate CYP1A2 is estimated to be 105 hours
- time to reach steady-state of safinamide : after 4 doses

Due to the combination of induction and inhibition on CYP1A2 (*in vitro* study results indicated that safinamide was a weak mechanism-based and competitive inhibitor and a weak inducer of CYP1A2), the trial investigated a first dose effect caused by inhibition [i.e., after single dose on Study Day 1] and the effect at later time point when the

potential induction was assumed to be complete (i.e., after multiple doses (m.d.) on SD14) during safinamide treatment.
For safinamide, the highest recommended dose for the treatment of PD was used (100 mg/day). For the probe substrates, the commercial available maximum dose was administered as single dose (7.5 mg midazolam [Dormicum®] and 200 mg caffeine [Coffeinum N® 0.2 g]).

Caffeine Mean Plasma Concentration-Time Curves



The results of this study demonstrated that safinamide is a weak competitive inhibitor of CYP 1A2.

Effect of Safinamide on the AUC_{0-t} (ng.h/mL) of Caffeine - Summary of ANOVA Results

Day	Treatment	Least Squares Geometric Mean	Ratio Estimate [%] (90% CI)	
SD-2	Caffeine	44934		
SD1	Caffeine + safinamide single dose	58396	SD1 / SD-2	130 (124.7-135.5)
SD14	Caffeine + safinamide multiple-dose	50789	SD14 / SD-2	113 (100.3-127.4)

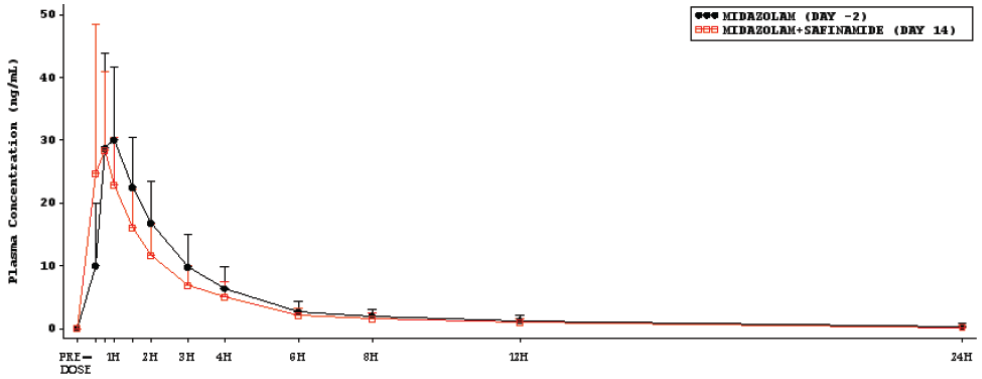
The single dose effect was more pronounced, with an increase in AUC_{0-t} of 30% (ANOVA point estimate), compared to a 13% increase after 14 days of safinamide 100 mg administration.
Single dose effect suggests that safinamide at the dose of 100 mg is a weak CYP1A2 inhibitor, whereas lower increased caffeine exposure after multiple dose of safinamide indicate an additional weak CYP1A2 induction effect.

Effect of safinamide on midazolam PK:
A 20% decrease in exposure of midazolam (ANOVA point estimate) was observed when administered together with safinamide 100 mg after 14 days of safinamide administration.

Effect of Safinamide SD14 Administration on the AUC_{0-t} (ng.h/mL) of Midazolam – Summary of ANOVA Results

Day	Treatment	Least Squares Geometric Mean	Ratio Estimate [%] (90% CI)	
SD-2	Midazolam	80.4	SD14 / SD-2	80.0 (69.2-92.6)
SD14	Midazolam + safinamide multiple dose	64.4		

Midazolam Mean Concentration-time Profile



Recommendation: The following labeling language, proposed by the sponsor, is acceptable:

(b) (4)

VII. Potential pharmacodynamics interactions

The risk of safinamide in combination with specific drugs of interest was explored in the pooled safety dataset of the main placebo-controlled trials. An increased risk of fractures and falls was observed in patients treated with safinamide and concurrent use of anxiolytics and antihypertensive drugs and an increased risk of psychoses with amantadine use. However, the analyses on pharmacodynamic interactions in the pooled dataset should be interpreted with caution, as there was no stratification for co-medication at baseline, and co-medication was not kept constant over time. Therefore, a negative signal from this analysis could not definetly exclude interactions.

Serious adverse reactions have been reported with the concomitant use of meperidine and MAO inhibitors. The sponsor proposes the concomitant use of safinamide with meperidine, or with other MAOIs to be contraindicated, which is acceptable.
The development of a potentially life-threatening serotonin syndrome has been reported with MAOIs, including selective MAO-B inhibitors, when used concomitantly with serotonergic drugs like serotonin selective re-uptake inhibitors (SSRIs). The combination with SSRI's did not induce serotonergic symptoms like dizziness, hypertension or neuropsychiatric events in the safinamide trials (117 subjects used both drugs), however, since serious adverse events have been reported for other MAO-B inhibitors used in combination with SSRI and other antidepressants, the sponsor proposes the following to be included in the label for the use of SSRI's; this is acceptable.

(b) (4)

2.5 GENERAL BIOPHARMACEUTICS

2.5.1 Based on the BCS principles, in what class is this drug and formulation? What solubility, permeability and dissolution data support this classification?

Safinamide can be classified as a Class 2 agent according to the Biopharmaceutical Classification System (BCS), based on solubility and permeability characteristics.

Solubility

The solubility of safinamide is pH-dependent, as determined across the pH range of 1.2 – 6.8 by the shake flask method at 37°C ± 1°C utilizing USP standard buffers. In addition, an aqueous solution of pH 6.8 adjusted with acetic acid and sodium hydroxide solution was used to investigate potential ionic induced influences on the solubility. Equilibrium solubility in bio-relevant media FaSSIF (Fasted State Simulated Intestinal Fluid, pH 6.5) and FeSSIF (Fed State Simulated Intestinal Fluid, pH 5) was determined to estimate solubility in human gastrointestinal fluids (see Table below).

The drug substance has high solubility at lower pH but low solubility at higher pH.

At the highest anticipated dosage strength of 100 mg at pH 6.8, the solubility is less than 0.4 mg per mL, poorly soluble according to BCS criteria.

pH-dependent Solubility of Safinamide

Solvent (Buffer)	pH-Value	Concentration* [mg/mL]
SGFsp pH 1.2	1.2	285
USP acetate buffer pH 4.5	4.5	288
USP phosphate buffer pH 6.8	6.8	0.28
pH 6.8 aqueous, adjusted with acetic acid and sodium hydroxide	6.8	0.27
USP-phosphate buffer pH 7.5	7.5	0.11
FaSSIF (Fasted State Simulated Intestinal Fluid, pH 6.5)	6.5	365
FeSSIF (Fed State Simulated Intestinal Fluid, pH 5.0)	5.0	281

Permeability:

Safinamide's high permeability was demonstrated in standard in vitro permeability studies using Caco-2 cells. In these studies, the apparent permeability (Papp) was compared with 3 reference compounds exhibiting low and high permeability and high efflux transport. Mean values (n = 3) of Papp for safinamide were 230 ± 10 and 260± 17 nm/s in the apical to basolateral and basolateral to apical directions, respectively, at a nominal concentration of 10 µM. At the higher concentration, 2500 µM, the corresponding values were 210 ± 57 and 200± 8 nm/s. These values, when compared to the value for the high permeability reference substrate, propranolol (PappA—B = 240 ± 28 nm/s), indicate that safinamide is highly permeable.

In addition, the oral systemic bioavailability is almost complete (about 95% compared to intravenous administration [Report EMR701165_022]), therefore safinamide can be classified as highly permeable.

2.5.2 Is the proposed to-be-marketed formulation bioequivalent to the formulation used in the clinical trials and pharmacokinetic studies?

Yes. A powder in bottle formulation was used in the two initial Phase I studies [(b) (4)-PNU-194-99 and (b) (4)NW/SS-215-99] and in the human mass balance study [CRO-02-33], to allow for individualized, body-weight adjusted dosing at the low dose range (25-150 µg/kg).
The early Phase I - III clinical trials were performed with capsules (10 mg, 50 mg & 150 mg, calculated as free base). In most of these trials the 10 and 50 mg capsule strengths were employed; the 150 mg capsule strength was used only in the initial safety/tolerability and PK studies in healthy adult subjects [(b) (4)-PNU-194-99 and (b) (4)-NW/SS-215-99].
A (b) (4) tablet (both 50 mg and 100 mg, (b) (4) (b) (4)) with a (b) (4) coating was developed initially. A (b) (4) step was introduced to optimize the manufacturing process performance and make it suitable (b) (4) (b) (4) white tablets are identical in terms of quantitative and qualitative composition as well as tablet shape. Overviews of the tablet compositions of the (b) (4) tablet formulations are provided in the table below.

Ingredient	100 mg (b) (4) White Tablet [mg/Tablet]	100 mg (b) (4) White Tablet [mg/Tablet]	100 mg (b) (4) Tablet [mg/Tablet]	100 mg (b) (4) (b) (4) Tablet [mg/Tablet]
Safinamide methanesulfonate	131.76[a]	131.76[a]	131.76[a]	131.76[a]
Microcrystalline cellulose	(b) (4)			
Crospovidone				
Magnesium stearate				
Colloidal silicon dioxide				
				(b) (4)
Hypromellose*				(b) (4)
Polyethylene glycol 6000*				
				(b) (4)

Comparative *in vitro* dissolution data for (b) (4) tablets displayed differences associated with the change in the manufacturing process. As the pivotal clinical Phase III trials were performed using (b) (4) tablets, a bioequivalence (BE) study comparing 100 mg (b) (4) and 100 mg (b) (4) tablets was conducted and demonstrated bioequivalence between 100 mg (b) (4) tablets and 100 mg (b) (4) tablets [Report EMR701165-021].
During market formulation development, the composition of the film-coating (b) (4) (b) (4) was further modified (b) (4) (b) (4). The (b) (4)

(b) (4) film coated immediate release tablets are available in two strengths (50 mg and 100 mg) and represent the proposed market formulation.
According to the sponsor, comparative dissolution profiles showed that (b) (4) (b) (4) tablets are equivalent in terms of dissolution profiles as well as (b) (4) white tablets which show a slightly faster in vitro dissolution performance compared to (b) (4) tablets.
Note: This needs to be confirmed by the ONDQA reviewer.

Overview of (b) (4) Tablet Formulations used in Safinamide Clinical Trials

EudraCT #	Study #	Phase	Dosage Strength(s)	Process / Formulation	Study Name
2009-011987-13	EMR-701165-021	I	100mg	(b) (4)	Bioequivalence*
2009-015062-60	EMR 701165-022	I	50mg		Bioavailability food effect
2011-000927-34	EMR701165_025	I	50mg		Renal Impairment study
2011-000932-27	EMR701165_026	I	100mg		DDI (CYP1A2, CYP3A4)
2011-001934-41	EMR701165_027	I	100mg		DDI L-dopa
Not Applicable	28849	II	50mg		DA Transporter
2010-020109-34	EMR 701165-024	II	50mg		Cognition
2008-004146-88	27938 MOTION Ext	III	50mg, 100mg		MOTION extension
2008-005492-94	28850 Open Label	III	50mg, 100mg		Open Label

* Bioequivalence between (b) (4) White tablets

Reviewer's Comments:
In addition to the studies listed in the table above, the following studies supporting labeling claims have been conducted with safinamide tablets:
(b) (4) 28696 (hepatic impairment study)
28778 (DDI study with ketoconazole)
28558 (DDI study with oral Tyramine)
(b) (4) 28559 (QTc study)
Therefore, all major clinical pharmacology studies supporting labeling claims have been conducted with safinamide tablets.

The process developed (b) (4) was transferred to another qualified (FDA approved, according to the sponsor) manufacturing site for the commercial production (b) (4) (b) (4) without any changes to the product composition and with only minor adaptations to the manufacturing process.

Overview of Safinamide Formulations used During Development

Reviewer's Comments:

The ONDQA team has determined that the biowaiver criteria are not met and an *in vivo* BE bridging study needs to be conducted comparing clinical trial capsules to the tablets; this has been conveyed to the sponsor at the preNDA CMC meeting: If the capsule was used in any pivotal studies, a BE study should be conducted to bridge the capsule and the to-be-marketed tablet. However, no *in vivo* BE bridging study has been conducted comparing capsules to the tablets.

The capsules were used in the Phase 3 Trial 015 and the extension trial 017, which support the following indication: Early PD, non-fluctuator add-on to dopamine agonist. However, another placebo-controlled phase 3 trial (MOTION), in which tablets were used, supports the same indication; therefore this was not considered a filing issue.

Table 3-39: Overview of Efficacy and Safety Phase II/III Clinical Trials that Used Safinamide

EudraCT #	Study #	Dose strength	Process / Formulation	Study name	Indication
n.a.	009	10mg, 50mg	Capsules	009	Add-on DA -agonist
2004-000833-12	015	50 mg, 100 mg	Capsules	015	Add-on DA-agonist
2006-005860-14	016	50 mg, 100 mg	(b) (4) Tablets	016	Add-on L-dopa
2004-000835-27	017	50 mg	Capsules	017	Add-on DA-agonist
2006-005861-21	018	50 mg	(b) (4) ablets	018	Add-on L-dopa
2007-002964-90	27919	50 mg, 100 mg	ablets	SETTLE	Add-on L-dopa
2007-002963-28	27918	50 mg, 100 mg	ablets	MOTION	Add-on DA

In addition, two more major issues with Trial 015 were found:

1. A 30% difference in Safinamide concentrations between the phase 3 studies.

Safinamide concentrations in the active dose groups of the follow-up (to Study 015) Study NW1015_017-III, were consistently approximately 30% lower over all visits in comparison to Study 015.
2. Safinamide plasma (and metabolites) levels observed in subjects randomized to the placebo group. Overall, 588 samples from the placebo group (90 patients) were analyzed for safinamide and its two main circulating metabolites P2 (NW1153) and P3 (NW1689). Quantifiable safinamide concentrations were present in 155 samples (26%). Measurable safinamide concentrations in samples from placebo subjects were not specific to a certain center.
Considering these major issues, the Clinical pharmacology team recommended that the sponsor exclude Study 015 PK data from the population PK analysis; this has been addressed by the sponsor (in Sect. 5.3.4.2 popPK/PD) in the re-submission of the NDA. Trial 015 will not be reviewed by the clinical pharmacology team.

2.5.3 What is the effect of food on the bioavailability of the drug from the dosage form? What dosing recommendations need to be made regarding the administration of safinamide in relation to meals or meal types?

The effect of co-administration of high fat and high caloric meal on the bioavailability of safinamide has been investigated in two trials for the 10 mg capsule (N=6) [Study (b) (4) Food-257-00] and the 50 mg (b) (4) tablet formulation (N=7M/7F) [Study EMR701165-22]. The food interaction trial using the 10 mg capsule formulation was an early exploratory study that was performed to rationalize and support dosing recommendations in early clinical Phase II/III trials; considering the issues with bridging described above in 2.5.2, the results of the study will not be discussed.
The comparison of orally administered safinamide 50 mg (b) (4) tablets in fed (T) and fasted (R) demonstrated a lack of a relevant food effect: geometric mean T/R ratios of $AUC_{0-\infty}$ and C_{max} values were 100% and 95%, respectively, and the corresponding 90% CIs met standard bioequivalence acceptance criteria. A slight delay in time to achieve maximum safinamide plasma concentrations (T_{max}) was observed in the fed state relative to the fasted condition (mean difference fed-fasted of 0.75 hours, and 90% CI of 0.50 to 1.25 hours).

Summary of Pharmacokinetic Parameters for Safinamide (n=14)

	Treatment A (Fasted)	Treatment B (Fed)	Treatment C* (IV Infusion)
C_{max} (ng/mL)	322.3 (17.9) 227.0 – 421.0	306.3 (21.9) 204.0 – 413.0	411.6 (18.8) 305.0 – 591.1
t_{max} (h)	2.5 2.0 – 5.0	3.25 1.5 – 8.0	0.5 0.25 – 5.0
AUC_{0-t} (ng/mL*h)	9811 (25.4) 6584 – 16737	9846 (25.5) 5862 – 14845	10340 (20.8) 7173 – 13815
$AUC_{0-\infty}$ (ng/mL*h)	10205 (26.0) 6779 – 17012	10230 (25.9) 6058 – 15107	10785 (22.0) 7314 – 14801
$t_{1/2}$ (h)	26.2 (19.2) 20.0 – 40.8	26.0 (17.9) 19.4 – 40.8	26.0 (11.3) 20.7 – 30.7
CL/f (L/h)	4.9 (26.0) 2.9 – 7.4	4.9 (25.9) 3.3 – 8.3	4.6 (22.0) 3.4 – 6.8
V_z/f (L)	185.0 (17.7) 142.9 – 260.5	183.7 (22.0) 132.8 – 292.2	174.1 (15.6) 146.6 – 222.7
F (%)	94.98 (9.25) 83.93 – 119.33	95.30 (10.0) 78.61 – 107.89	-

* In Treatment C, dose adjusted C_{max} and AUC are displayed.
For C_{max}, $AUC_{0-\infty}$, AUC_{0-t}, $t_{1/2}$, CL/f and V_z/f: Geomean (GeoCV %) and range (min-max)
For t_{max}: median and range (min – max)
For F: mean (CV%) and range (min – max)

Ratios of Geometric Means with 90% Confidence Intervals for Primary PK Parameters of Safinamide in Plasma -fed/fasted

Parameter	Treatment	Least Squares Means	Ratio T/R and 90% confidence interval [%]
$AUC_{0-\infty}$ (h*(ng/mL))	Tablet fasted (T)	10205.28	94.62 (90.09 - 99.39)
	Solution i.v. (R)	10785.15	

Recommendation: Safinamide can be administered with or withoud food.

2.6 ANALYTICAL

2.6.1 What bioanalytical method is used to assess concentrations of active moieties and is the validation complete and acceptable?

Plasma concentrations of safinamide and the major human circulating metabolites NW-1153 and NW-1689, were determined using validated liquid chromatography-tandem mass spectrometric detection (LC-MS/MS) methods. A method report was also generated for each clinical study.
Safinamide, NW-1153 and NW-1689 and their corresponding internal standards (b) (4) - (b) (4) were extracted from plasma after protein precipitation with acetonitrile, the supernatant was injected onto a CN column, and detected using MS/MS. An assay method was validated with an LLOQ of 20 ng/mL for safinamide, NW-1153 and NW-1689 [(b) (4) Report R-173/04], that was subsequently improved to LLOQ of 15 ng/mL and 5 ng/mL, respectively [(b) (4) Report RE7810, (b) (4) Report 8203838, (b) (4) Reports (b) (4) 27175 and (b) (4) 29081]. A lower LLOQ of 0.5 ng/mL for

safinamide and NW-1153 was validated to increase the period of time after drug administration over which levels remain quantifiable [(b) (4) Report 8244033].
A separate LC-MS-MS method for quantification of the NW-1689 acyl glucuronide (AG) was validated for analysis of NW-1689 in acidified human plasma (LLOQ of 5 ng/mL, then further decreased to 0.5 ng/mL) [(b) (4) Report 8224857, (b) (4) Report DMPK 8908, (b) (4) Report 8214594, and 8244036]. For details see below Notes: (b) (4) Report number 8214594 (NW-1689 before and after).

Two method validations in human urine were completed, initially for quantification of safinamide, NW-1153 and NW-1689 [Report RE8670] and subsequently for quantification of safinamide and NW-1153 only [(b) (4) Report (b) (4) 28103].
The LLOQs for these assays were 5 ng/mL (all three analytes) and 3.8 ng/mL (both analytes), respectively.
The LC-MS-MS method for quantification of the NW-1689 acyl glucuronide (AG) as described above was also validated in acidified human urine (LLOQ of 10 ng/mL) [DMPK 110-08].

A list of validation data and assay performance characteristics for the quantification of safinamide and its main metabolites in human plasma and urine is provided in the table below.

Overview of Validation Studies for Safinamide and Its Metabolites in Human Matrices

Study No. (Alternative Report No.) [Test Facility]	Human Matrix	Analyte(s)	LLOQ	Method of Detection	Internal Standard	Clinical Study No.
70/99 (b) (4)	plasma	safinamide	20 ng/mL	LC-MS/MS	(b) (4)	(b) (4) NW/SS-215-99 (b) (4) NW/LD-231-00 (b) (4) PNU-194-99 (b) (4) NW/FOOD-257-00 (b) (4) NW/PAR-254-00 NW-1015-005-II-2000
78/99 (b) (4)	plasma	safinamide	0.5 ng/mL	LC-MS/MS		(b) (4) NW/PAR-254-00 (b) (4) NW/SS-215-99 (b) (4) NW/LD-231-00
844464 (b) (4)	plasma	safinamide (b) (4)	10 ng/mL 10 ng/mL	LC-MS/MS		(b) (4) 844437
R-173/04 amended version (b) (4)	plasma	safinamide NW-1153 NW-1689	20 ng/mL 20 ng/mL 20 ng/mL	LC-MS/MS		NW1015/015/III/2003
(b) (4) 27175 (b) (4)	plasma	safinamide NW-1153 NW-1689	15.2 ng/mL 20 ng/mL 20 ng/mL	LC-MS/MS		NW/1015/016/III/2006 NW-1015/017/III/2003

Study No. (Alternative Report No.) [Test Facility]	Human Matrix	Analyte(s)	LLOQ	Method of Detection	Internal Standard	Clinical Study No.
8203838 (b) (4)	plasma	safinamide NW-1153	5 ng/mL 5 ng/mL	LC-MS/MS	(b) (4)	EMR701165-021 EMR701165-022 (b) (4) 27918 27919
(b) (4) 29081 (b) (4)	plasma	safinamide NW-1153 NW-1689	5 ng/mL 5 ng/mL 5 ng/mL	LC-MS/MS		28696 28559 28780 28558 27918 27919
8244033 (b) (4)	plasma	safinamide NW-1153	0.5 ng/mL 0.5 ng/mL	LC-MS/MS		EMR701165-26 EMR701165-25
(b) (4) 28102B (b) (4)	plasma	safinamide free-fraction	5 ng/mL	LC-MS/MS		(b) (4) 28696
(b) (4) 4370 (b) (4)	plasma	safinamide	5 ng/mL	LC-MS/MS		EMR701165-023
DMPK 36-08 (b) (4)	acidified plasma	NW-1689 (+ NW-1689-1-O-Glucuronide)	5 ng/mL	LC-MS/MS	(b) (4)	(b) (4) 28778
8214594 (b) (4)	acidified plasma	NW-1689 (NW-1689 + NW-1689 AG) [a]	5 ng/mL	LC-MS/MS		EMR701165-022 (b) (4) 28559
8244036 (b) (4)	acidified plasma	NW-1689 (NW-1689 + NW-1689 AG) [a]	0.5 ng/mL	LC-MS/MS		EMR701165-26 EMR701165-25
(b) (4) 8670 (b) (4)	urine	safinamide NW-1153 NW-1689	5ng/mL 5ng/mL 5ng/mL	LC-MS/MS	(b) (4)	(b) (4) 28778
(b) (4) 28103 (b) (4)	urine	safinamide NW-1153	3.8 ng/mL 5 ng/mL	LC-MS/MS		(b) (4) 28696
8253226 (b) (4)	urine	safinamide NW-1153	5 ng/mL 5 ng/mL	LC-MS/MS		EMR701165-25
DMPK 110-08 (b) (4)	acidified urine	NW-1689 (NW-1689 + NW-1689 AG)	10 ng/mL	LC-MS/MS		EMR701165-25
8244034 (b) (4)	buffer	safinamide free fraction	5 ng/mL	LC-MS/MS	(b) (4)	EMR701165-25

a: NW-1689 before and after alkaline hydrolysis

Notes: (b) (4) Report number 8214594 (NW-1689 before and after)
As the sponsor has estabilised that NW-1689-1-O-glucuronide (NW-1689AG) is unstable in human plasma, study samples were acidified shortly after collection. NW1689 AG concentrations were derived in an indirect method through LC/MS/MS analysis of the samples before and after conversion of NW1689 AG to NW1689 (alkaline hydrolysis of the acylglucuronide) and appropriate correction was applied according to the molecular weight of NW1689 AG.

Aliquot A was evaporated to dryness under a stream of nitrogen. The residues were reconstituted in acetonitrile: 10 mM ammonium acetate pH 4.0 (50: 50 v/v, 100 µL). The extracts were vortexed; centrifuged (ca. 10 minutes, 3000 g, nominal 22°C) and submitted for LC-MS/MS analysis of NW-1689 (before).
Aliquot B was evaporated to dryness under a stream of nitrogen. The residues were reconstituted in 0.2 mM sodium hydroxide solution (100 µL) and incubated (45 minutes, 50°C). Acetonitrile: formic acid (98: 2 v/v, 50 µL) was added and the extracts were vortexed, centrifuged (ca. 10 minutes, 3000 g, nominal 22°C) and submitted for LC-MS/MS analysis of NW-1689 (after).

Validation of an enantioselective LC-MS-MS method using a chiral column for the determination of safinamide enantiomers in plasma was carried out with an LLOQ of 10 ng/mL for each of both enantiomers, to support metabolite identification during the mass balance study in man [Report 844464].

A method for determination of safinamide free fraction in human plasma was validated using equilibrium dialysis followed by LC-MS-MS [(b) (4) Repor (b) (4)-28102B, (b) (4) Report 8244034]. This bioanalytical method was validated in the range 5.00-1000 ng/mL for safinamide with a 200 µL plasma sample volume.

Following therapeutic doses of safinamide (100 mg/day) maximum plasma steady-state concentrations are in the range of 700-2200 ng/mL for safinamide, 60-200 ng/mL for NW-1153, 1800-13000 ng/mL for NW-1689 and 300-5000 ng/mL for NW-1689 AG. The calibration ranges of standard curves of the validated assays covered the concentrations of safinamide and its metabolites observed in clinical studies.
The validation results from safinamide and its major metabolites bioanalytical assays are acceptable and the results are presented in the table below.

Assay Validation Report (Alternative Report No.) [Test Facility]	Assay Method	Human Matrix	Analyte(s)	Inter-day and Intra-day Precision and Inter-day and Intraday Accuracy			
70/99 (b) (4)	LC-MS/MS	plasma	safinamide	Robustness	Intra-assay accuracy - %Diff (n=6)	6000 ng/mL	3.5%
						600 ng/mL	1.2%
						60 ng/mL	-6.9%
						LLOQ: 20 ng/mL	-5.1%
					Intra-assay precision - CV% (n=6)	6000 ng/mL	4.1%
						600 ng/mL	2.1%
						60 ng/mL	4.4%
						LLOQ: 20 ng/mL	2.6%
					Inter-assay accuracy - %Diff (n=18)	6000 ng/mL	2.9%
						600 ng/mL	-1.4%
						60 ng/mL	-2.3%
					Inter-assay precision - CV% (n=18)	6000 ng/mL	4.0%
						600 ng/mL	3.9%
						60 ng/mL	5.8%
				Recovery	Intra-assay accuracy - %Diff (n=3)	6000 ng/mL	1.4%
						600 ng/mL	4.8%
						60 ng/mL	8.4%
					Intra-assay precision - CV% (n=3)	6000 ng/mL	2.9%
						600 ng/mL	4.0%
						60 ng/mL	6.5%
				Stability	In autosampler (24 h at 4°C in plasma) (n=4)	6000 ng/mL	stable
						600 ng/mL	stable
						60 ng/mL	stable
					Freeze / thaw (3 cycles) (n=4)	6000 ng/mL	stable
						600 ng/mL	stable
						60 ng/mL	stable
					In plasma (24 h at RT) (n=4)	6000 ng/mL	stable
						600 ng/mL	stable
						60 ng/mL	stable
78/99 (b) (4)	LC-MS/MS	plasma	safinamide	Robustness	Intra-assay accuracy - %Diff (n=6)	15 ng/mL	4.0%
						7.5 ng/mL	3.2%
						1.5 ng/mL	-0.7%
						LLOQ: 0.5 ng/mL	0%
					Intra-assay precision - CV% (n=6)	15 ng/mL	3.7%
						7.5 ng/mL	3.2%
						1.5 ng/mL	5.5%
						LLOQ: 0.5 ng/mL	7.6%
					Inter-assay accuracy - %Diff (n=18)	15 ng/mL	2.9%
						7.5 ng/mL	1.5%
						1.5 ng/mL	-1.3%
					Inter-assay precision - CV% (n=18)	15 ng/mL	2.6%
						75 ng/mL	4.7%
						1.5 ng/mL	4.9%
844464 (b) (4)	LC-MS/MS	plasma	safinamide (b) (4)	Sensitivity	Intra-assay accuracy - Mean% (n=3 batches)		96.0 – 109%	98.3 – 108% (b) (4)
					Intra-assay precision - CV% (3 batches)		1.9 – 5.5%	1.9 – 4.0%
					Overall accuracy - Mean% (n=18)		NC	NC
					Overall precision - CV% (n=18)		NC	NC
				Repeatability	Intra-assay accuracy - RE% (n=3 batches)	1200 ng/mL	101 – 103%	104 – 105%
						200 ng/mL	99.0 – 105%	105 – 110%
						50 ng/mL	98.2 – 103%	102 – 105%
					Intra-assay precision - CV% (n=3 batches)	1200 ng/mL	3.5 – 7.2%	3.2 – 6.7%
						200 ng/mL	0.8 – 3.7%	1.5 – 4.6%
						50 ng/mL	2.2 – 6.4%	1.6 – 5.8%

Assay Validation Report (Alternative Report No.) [Test Facility]	Assay Method	Human Matrix	Analyte(s)	Inter-day and Intra-day Precision and Inter-day and Intraday Accuracy					
				Extraction efficacy	n=3		1200 ng/mL	98%	98%
							200 ng/mL	98%	98%
							50 ng/mL	97%	97%
				Effect of dilution	Intra-assay accuracy - Bias% (n=4)		50000 ng/mL (DF=50)	0.2%	4.1%
					Intra-assay precision - CV% (n=4)		50000 ng/mL (DF=50)	0.6%	0.4%
					Intra-assay accuracy - Bias% (n=4)		70000 ng/mL (DF=25)	3.7%	-1.6%[a]
					Intra-batch precision - CV% (n=4)		70000 ng/mL (DF=25)	8.2%	2.0%[a]
				Stock solution stability	4 weeks at 4°C		1200 ng/mL	stable	stable
							50 ng/mL	stable	stable
				Plasma stability	4 weeks at -80°C		1200 ng/mL	stable	stable
							50 ng/mL	stable	stable
					Freeze / thaw (3 cycles)		1200 ng/mL	stable	stable
							50 ng/mL	stable	stable
R-173/04 amended version (b) (4)	LC-MS/MS	plasma	safinamide NW-1153 NW-1689				safinamide	NW-1153	NW-1689
				Robustness	Intra-assay accuracy - %Diff (n=6)	6000 ng/mL	-0.3%	-1.2%	0.1%
						1200 ng/mL	3.6%	-2.5%	-2.6%
						60 ng/mL	-1.0%	-7.2%	-7.5%
						LLOQ: 20 ng/mL	-5.5%	-2.0%	-3.5%
					Intra-assay precision -CV% (n=6)	6000 ng/mL	1.4%	1.5%	1.9%
						1200 ng/mL	1.6%	1.1%	2.6%
						60 ng/mL	1.6%	1.7%	4.2%
						LLOQ: 20 ng/mL	3.5%	5.1%	10.2%
					Inter-assay accuracy - %Diff (n=18)	6000 ng/mL	-1.3%	-2.3%	-0.2%
						1200 ng/mL	2.1%	-3.5%	-1.8%
						60 ng/mL	1.5%	-3.7%	-6.0%
						LLOQ: 20 ng/mL	0%	0%[a]	1.0%[a]
					Inter-assay precision - CV % (n=18)	6000 ng/mL	2.1%	1.6%	2.9%
						1200 ng/mL	2.7%	1.3%	3.0%
						60 ng/mL	3.9%	3.4	5.7%
						LLOQ: 20 ng/mL	7.7%	6.4%[a]	9.8%[a]
				Recovery	Intra-assay accuracy - Mean% (n=3)	6000 ng/mL	88.9%	86.5%	86.5%
						1200 ng/mL	90.1%	89.3%	88.6%
						60 ng/mL	94.7%	94.0%	89.2%
					Intra-assay precision - CV% (n=3)	6000 ng/mL	1.1%	1.7%	3.1%
						1200 ng/mL	1.2%	0.6%	2.0%
						60 ng/mL	1.0%	1.2%	1.8%
				Plasma stability	In autosampler (24 h at 4°C)	6000 ng/mL	stable	stable	stable
						1200 ng/mL	stable	stable	stable
						60.0 ng/mL	stable	stable	stable
					In autosampler (8 h at 4°C)	6000 ng/mL	stable	stable	stable
						1200 ng/mL	stable	stable	stable
						60.0 ng/mL	stable	stable	stable
					24 h at RT	6000 ng/mL	stable	stable	stable
						1200 ng/mL	stable	stable	stable
						60.0 ng/mL	stable	stable	stable
					Freeze / thaw (3 cycles)	6000 ng/mL	stable	stable	stable
						1200 ng/mL	stable	stable	stable
						60.0 ng/mL	stable	stable	stable

Assay Validation Report (Alternative Report No.) [Test Facility]	Assay Method	Human Matrix	Analyte(s)	Inter-day and Intra-day Precision and Inter-day and Intraday Accuracy					
(b) (4)27175 (b) (4)	LC-MS/MS	plasma	safinamide NW-1153 NW-1689				Safinamide	NW-1153	NW-1689
				Sensitivity	Intra-assay precision - CV% (n=3 batches)		2.5%	2.8%	5.7%
					Inter-assay precision - CV% (n=3 batches)		2.1%	5.1%	1.6%
					Overall accuracy - Diff% (n=18)		-4.3%	-8.0%	-9.7%
					Overall precision - CV% (n=18)		3.0%	5.1%	5.8%
				Intermediate precision and accuracy	Intra-assay precision - CV% (n=3 batches)	7600[a] / 10000 ng/mL	1.9%	2.6%	2.2%
						5700[a] / 7500 ng/mL	2.3%	1.5%	2.1%
						912[a] / 1200 ng/mL	1.3%	2.1%	2.5%
						45.6[a] / 60 ng/mL	1.7%	2.0%	2.7%
					Intra-assay precision - CV% (n=3 batches)	7600[a] / 10000 ng/mL	N/A	1.2%	3.6%
						5700[a] / 7500 ng/mL	1.0%	0.8%	3.9%
						912[a] / 1200 ng/mL	1.4%	6.1%	4.9%
						45.6[a] / 60 ng/mL	1.6%	1.4%	2.5%
					Overall accuracy - Diff% (n=18)	7600[a] / 10000 ng/mL	-1.1%	-3.4%	-3.4%
						5700[a] / 7500 ng/mL	-5.1%	-5.9%	-4.4%
						912[a] / 1200 ng/mL	-3.2%	-2.3%	2.6%
						45.6[a] / 60 ng/mL	-4.8%	-3.3%	-0.5%
					Overall precision - CV% (n=18)	7600[a] / 10000 ng/mL	1.8%	2.8%	3.8%
						5700[a] / 7500 ng/mL	2.4%	1.6%	3.9%
						912[a] / 1200 ng/mL	1.7%	5.6%	4.8%
						45.6[a] / 60 ng/mL	2.1%	2.3%	3.4%
				Recovery	Mean% (n=6)	5700[a] / 7500 ng/mL	95.8%	97.4%	90.1%
						912[a] / 1200 ng/mL	100%	101%	95.3%
						45.6[a] / 60 ng/mL	96.2%	94.1%	96.8%
				Selectivity			no interfering peaks	no interfering peaks	no interfering peaks
				Carryover	% of LLOQ		2.1%	10.1%	6.1%
				Effect of dilution (10-fold)	Overall accuracy - Diff% (n=6)	15369[a] / 20000 ng/mL	5.7%	1.0%	5.8%
					Overall precision - CV% (n=6)	15369[a] / 20000 ng/mL	4.2%	5.2%	3.2%
				Stability	Plasma in autosampler (24 h at 8°C)	5700[a] / 7500 ng/mL	stable	stable	stable
						45.6[a] / 60 ng/mL	stable	stable	stable
					Plasma in autosampler (48 h at 8°C)	5700[a] / 7500 ng/mL	stable	stable	stable
						45.6[a] / 60 ng/mL	stable	stable	stable
					Stock solution (5 weeks at +2-8°C)	760[a] /1000 µg/mL	stable	stable	stable

								Safinamide	NW-1153
8203838 (b) (4)	LC-MS/MS	plasma	safinamide NW-1153	Sensitivity	Intra-batch accuracy - Mean% (n=3 batches)			90.4 – 104%	90.2 – 94.0%
					Intra-batch precision - RSD% (n=3 batches)			2.4 – 5.6%	2.3 – 6.9%
					Overall accuracy - Mean% (n=18)			94.8%	91.8%
					Overall precision - RSD% (n=18)			8.0%	5.2%
				Repeatability	Intra-batch accuracy - Mean% (n=3 batches)		800 ng/mL	98.3 – 100%	97.0 – 102%
							400 ng/mL	101 – 102%	97.5 – 102%
							15.0 ng/mL	98.0 – 102%	97.3 – 99.3%
					Intra-batch precision - RSD% (n=3 batches)		800 ng/mL	1.5 – 3.1%	1.8 – 2.8%
							400 ng/mL	2.8 – 3.4%	2.0 – 9.4%
							15.0 ng/mL	2.1 – 3.2%	2.5 – 4.4%

Assay Validation Report (Alternative Report No.) [Test Facility]	Assay Method	Human Matrix	Analyte(s)	Inter-day and Intra-day Precision and Inter-day and Intraday Accuracy				
				precision and accuracy	(n=18)	400 ng/mL	101%	99.8%
						15.0 ng/mL	100%	98.0%
					Overall precision - RSD% (n=18)	800 ng/mL	2.8%	2.9%
						400 ng/mL	3.0%	5.7%
						15.0 ng/mL	3.2%	3.6%
				Recovery	Mean % (n=6)	800 ng/mL	99.2%	97.7%
						400 ng/mL	103%	102%
						15.0 ng/mL	105%	101%
				Matrix effect	% modification (n=6)	400 ng/mL	-8.3 – 8.4%	-9.7 – 5.3%
				Effect of dilution	Intra-batch accuracy - Mean% (n=6)	5000 ng/mL (DF = 10)	98.6%	99.4%
					Intra-batch precision - RSD% (n=6)	5000 ng/mL (DF = 10)	2.1%	2.2%
				Bench top stability	6 and 24 h at RT	800 ng/mL	stable	stable
						15.0 ng/mL	stable	stable
				Freeze / thaw stability	3 cycles	800 ng/mL	stable	stable
						15.0 ng/mL	stable	stable
				Re-injection feasibility	after 4 days at 4°C	All QC levels	acceptable	acceptable

							Safinamide	**NW-1153**	**NW-1689**
(b) (4)	LC-MS/MS	plasma	safinamide NW-1153 NW-1689	**Sensitivity**	Intra-assay precision - CV% (n=3 batches)		11.4%	8.1%	12.2%
					Inter-assay precision - CV% (n=3 batches)		9.8%	1.8%	NA
					Overall accuracy - Diff% (n=18)		-6.1%	-16.7%	-2.7%
					Overall precision - CV% (n=18)		14.1%	8.3%	12.2%
					Inter-assay precision - CV% (n=3 batches)	7500 ng/mL	1.0%	2.2%	1.4
						1000 ng/mL	1.1%	1.1%	NA
						15 ng/mL	1.9%	2.0%	1.4
					Overall accuracy - Diff% (n=18)	7500 ng/mL	-5.8%	-6.1%	-4.0%
						1000 ng/mL	-5.3%	-9.7%	-12.6%
						15 ng/mL	-4.3%	-4.3%	-3.0%
					Overall precision - CV% (n=18)	7500 ng/mL	2.0%	3.1%	2.4%
						1000 ng/mL	2.8%	2.3%	2.6%
						15 ng/mL	4.4%	3.9%	3.4%
				Cross-validation frozen (-20°C) versus freshly prepared samples	Intra-assay accuracy - Diff% (n=6)	7500 ng/mL frozen	-7.8%	-2.0%	-1.2%
						7500 ng/mL fresh	-2.2%	-13.6%	13.0%
						15 ng/mL frozen	-8.6%	-1.4%	-1.7%
						15 ng/mL fresh	-8.6%	-13.8%	13.0%
					Intra-assay precision - CV% (n=6)	7500 ng/mL frozen	2.0%	2.8%	2.6%
						7500 ng/mL fresh	3.0%	3.2%	2.5%
						15 ng/mL frozen	4.3%	2.6%	3.1%
						15 ng/mL fresh	11.7%	4.7%	2.3%

							Safinamide	**NW-1153**
8244033 (b) (4)	LC-MS/MS	plasma	safinamide NW-1153	**Sensitivity (LLOQ)**	Intra-run accuracy - Mean% (n=5/3 runs)		85.8 – 106.8%	98.6 – 112.4%
					Intra-run precision - RSD% (n=5/3 runs)		5.0 – 11.5%	6.1 – 15.4%
					Overall accuracy - Mean% (n=18)		97.0%	106.4%
					Overall precision - RSD% (n=18)		12.1%	13.4%
				Repeatability	Intra-batch accuracy - Mean% (n=5/3 batches)	350 ng/mL	94.9 – 101.1%	94.3 – 107.1%
						20 ng/mL	92.0 – 102.5%	95.5 – 99.5%
						1.5 ng/mL	92.0 – 102.7%	95.3 – 98.7%
					Intra-batch precision - RSD% (n=5/3 batches)	350 ng/mL	3.4 – 5.3%	4.9 – 5.3%
						20 ng/mL	4.3 – 9.7%	7.0 – 8.6%
						1.5 ng/mL	6.0 – 12.7%	10.1 – 13.9%
				Recovery	Mean % (n=6)	350 ng/mL	98.1%	90.8%
						20 ng/mL	95.1%	84.7%
						1.5 ng/mL	96.3%	95.1%
				Matrix effect	Mean normalized (n=6)	1.5 ng/mL	0.98	0.96
					RSD% (n=6)	1.5 ng/mL	7.1%	8.3%

Assay Validation Report (Alternative Report No.) [Test Facility]	Assay Method	Human Matrix	Analyte(s)	Inter-day and Intra-day Precision and Inter-day and Intraday Accuracy				
				Effect of dilution	Intra-batch accuracy - Mean% (n=6)	2500 ng/mL (DF=10)	90.4%	101.6%
					Intra-batch precision - RSD% (n=6)	2500 ng/mL (DF=10)	2.7%	4.2%
				Effect of presence of co-administered drugs	Accuracy – Mean%	1.5 ng/mL	106.0%	96.7%
					Precision – RSD%	1.5 ng/mL	8.7%	7.7%
				QC samples spiked with safinamide/NW-1153 only	Accuracy – Mean%	1.5 ng/mL	112.7%	106.7%
					Precision – RSD%	1.5 ng/mL	7.5%	6.6%
				Bench top stability	25 h at RT	1.5 ng/mL	stable	stable
				Freeze / thaw stability	4 cycles	1.5 ng/mL	stable	stable
				Re-injection feasibility	After 4 days at 4°C	All QC levels	acceptable	acceptable
				Whole blood	1 h at RT	350 ng/mL	stable	stable
				Long term stability	1, 4 and 6 month at nominal -20°C	1.5 ng/mL	stable	stable
(b) (4) 28102B (b) (4)	LC-MS/MS	plasma	safinamide free-fraction				validation samples in treated phosphate buffer saline pH 7.4	plasma validation samples
				Sensitivity	Overall accuracy - Diff% (n=18)		-5.5	
					Overall precision - CV% (n=18)		11.9	
				Repeatability	Intra-batch precision - CV% (n=3 batches)	1000 ng/mL	5.4	
						750 ng/mL	6.9	8.1
						200 ng/mL	10.1	
						15 ng/mL	4.8	7.2
				Intermediate precision and accuracy	Overall accuracy - Mean% (n=18)	1000 ng/mL	-0.9	
						750 ng/mL	-5.2	
						200 ng/mL	-2.5	
						15 ng/mL	-2.7	
					Overall precision - CV% (n=18)	1000 ng/mL	7.1	
						750 ng/mL	9.0	13.8
						200 ng/mL	13.0	
						15 ng/mL	8.8	14.3
				Selectivity	Mean % of LLOQ (n=10)			1.4%
				Matrix effect	Overall precision - CV% (n=12)	1000 ng/mL		8.9
				Carryover (% of peak area for LLOQ)	Chromatographic system	1000 ng/mL		2.2%
					Tecan pipettingrobot	1000 ng/mL		1.7%
				Recovery	Mean% (n=6)	1500 ng/mL	92.6%	
					Mean% (n=6)	400 ng/mL	93.8%	

Assay Validation Report (Alternative Report No.) [Test Facility]	Assay Method	Human Matrix	Analyte(s)	Inter-day and Intra-day Precision and Inter-day and Intraday Accuracy				
DMPK 36-08 (b) (4)	LC-MS/MS	acidified plasma	NW-1689 (+ NW-1689-1-O-Glucuronide)				**Aliquot A** (before conversion)	**Aliquot B** (after conversion)
				Sensitivity	Intra-batch accuracy - Mean% (n=5 batches)		90.5 – 103%	87.5 – 99.1%
					Intra- batch precision - CV% (n=5 batches)		2.43 – 9.56%	2.98 – 9.65%
					Overall accuracy - Mean% (n=25)		96.9%	94.5%
					Overall precision - CV% (n=25)		8.22%	7.45%
				Repeatability	Intra-batch accuracy - Mean% (5 batches)	4000 ng/mL	93.0 – 101%	NA
						2000 / 4000 ng/mL	93.5 – 101%	91.0 – 99.8%
						200 / 400 ng/mL	92.5 – 99.5%	88.0 – 97.3%
						20 / 40 ng/mL	94.5 – 100%	89.0 – 100%
					Intra-batch precision - CV% (5 batches)	4000 ng/mL	2.67 – 4.44%	NA
						2000 / 4000 ng/mL	1.89 – 2.68%	2.54 – 3.95%
						200 / 400 ng/mL	1.62 – 6.52%	1.51 – 3.35%
						20 / 40 ng/mL	3.44 – 7.29%	3.28 – 4.36%
				Bench top stability	at RT	4000 ng/mL	stable	stable
					24 h	20 ng/mL	stable	stable
				Short term stability	at -20°C	4000 ng/mL	stable	stable
					4 weeks	20 ng/mL	stable	stable
					at -80°C	4000 ng/mL	stable	stable
					4 weeks	20 ng/mL	stable	stable
				Freeze / thaw stability	3 cycles	4000 ng/mL	stable	stable
						20 ng/mL	stable	stable
				Re-injection feasibility	72 h at RT	All QC levels	acceptable	acceptable
8214594 (b) (4)	LC-MS/MS	acidified plasma	NW-1689 (NW-1689 + NW-1689 AG) [a]				**Aliquots A** (befor conversion)	**Aliquots B** (after conversion)
				Sensitivity (LLOQ)	Intra-batch accuracy – Mean% (n=3 batches)		92.0 – 100.8%%	88.0 – 102.0%
					Intra-batch precision – RSD% (n=3 batches)		8.3 – 9.5%	7.0 – 7.5%
					Overall accuracy – Mean% (n=18)		96.0%	92.8%
					Overall precision – RSD% (n=18)		9.1%	9.8%
				Repeatability	Intra-batch accuracy – Mean% (n=3 batches)	4000 ng/mL	98.5 – 111.3%	90.5 – 106.3%
						2000 ng/mL	99.0 – 114.5%	-
						200/400 ng/mL[a]	98.5 – 113.5%	91.0 – 107.3%
						15/30 ng/mL[a]	92.0 – 108.7%	92.3 – 107.7%
					Intra-batch precision - RSD% (n=3 batches)	4000 ng/mL	3.8 – 4.9%	4.2 – 4.7%
						2000 mg/mL	2.0 – 5.6%	-
						200/400 ng/mL[a]	3.2 – 9.1%	4.5 – 6.2%
						15/30 ng/mL[a]	3.4 – 10.6%	2.3 – 10.4%
				QC samples spiked with NW-1689 only	Accuracy – Mean% (n=6)	2000 ng/mL	91.0%	91.5%
						4000 ng/mL	88.5%	90.8%
					Precision – RSD% (n=6)	2000 ng/mL	4.4%	5.3%
						4000 ng/mL	3.8%	6.1%
				QC samples spiked with NW-1689 AG only	Accuracy – Mean% (n=6)	3430 ng/mL	-	85.0%
						6850 ng/mL	-	85.0%
					Precision – RSD% (n=6)	3430 ng/mL	-	2.0%
						6850 ng/mL	-	2.3%
				QC samples spiked with 6850 ng/mL NW-1689	% of mean Aliquot B response (n=6)	2000 ng/mL	2.1%	-
						4000 ng/mL	2.0%	-
				Freeze / thaw stability	6 cycles	15/2000	stable	-
						30/4000 ng/mL	-	stable
				Re-injection feasibility	6 days after storage at 4°C	15/200/2000 ng/mL	acceptable	-
						30/400/4000 ng/mL	-	acceptable

Several drug-drug interaction studies were conducted with medications that were coadministered with safinamide. Information regarding the analytical methods used to support the analysis of co-administered compounds can be found in the individual study reviews (EMR701165_027, 28780, EMR701165-026, and 28558).

3.0 DETAILED LABELING RECOMMENDATION

The reviewer's labeling recommendations are shown by track changes to the sponsor proposed label. These labeling changes should be incorporated in the revised label.

18 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

Clinical Pharmacology Individual Studies Review

PRODUCT (Generic Name):	Safinamide (NW-1015)
PRODUCT (Brand Name):	XADAGO
NDA:	207145
DOSAGE FORM:	Tablets
DOSAGE STRENGTH:	50 mg and 100 mg
INDICATION:	Add-on therapy for the treatment of patients with Parkinson's disease
NDA TYPE:	Standard
RESUBMISSION DATE:	Dec 29, 2014
SPONSOR:	Newron Pharmaceuticals
PRIMARY REVIEWER:	Hristina Dimova, Ph.D.
REVIEWER (in vitro studies)	Jagan Parepally, Ph.D.
TEAM LEADER:	Angela Men, M.D, Ph.D.
OCPB DIVISION:	DCP-I
OND DIVISION:	HFD-120

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1.1 PK and Initial Tolerability Studies

I. (b) (4)-PNU-194-99: A first-in-human study of PNU-151774E (NW-1015), an anticonvulsant agent, following ascending single oral doses, Safety, pharmacokinetic (PK) and pharmacodynamics (PD) evaluations.

Objectives:
To evaluate the tolerability, the pharmacokinetic profile, the MAO-B and MAO-A inhibitory activity of NW-1015 following oral administration of single ascending doses in healthy volunteers.

Study Design	Double-blind, 5-period, placebo-controlled, single ascending dose
Study Population	8 healthy male subjects, 18-45 years old , Caucasian
Treatment Group	Five single-treatments, separated by a 7 day washout: NW-1015: 2.5, 5.0, 10.0 mg/kg Placebo: 50 and 150 mg capsules
Dosage and Administration	NW-1015: 2.5, 5.0, 10.0 mg/kg oral, single-dose (50 and 150 mg capsules) Placebo: 50 and 150 mg capsules All treatments were in the fasting state.
PK Sampling: plasma	Plasma samples were collected prior to NW-1015 dosing at 0 (pre-dose), 20, 40 min, 1, 2, 4, 6, 10, 16, 24, 36 and 48 hours post-dosing.
PD Sampling	• Platelet rich plasma (PRP) at pre-dose, 20, 40 min, 1, 2, 4, 6, 10, 24 and 48 h post-dosing (for MAO-B inhibitory activity) • Phenylethylamine (PEA) in urine at -24-0, 0-24 and 24-48 h • 3-methoxy-4-hydroxy-phenilglycol (MHPG) in plasma for MAO-A (sampling times 0, 0.33, 0.66, 1, 4, 10, 24 and 48h)
PK Analysis	Plasma: LC-MS/MS method for NW-1015 Range: 20 to 20000 ng/mL
PK Assessment	C_{max}, t_{max}, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and CL of NW-1015
PD Assessment	Evaluation of MAO-B inhibitory activity in PRP PEA excretion in urine Evaluation of plasma levels of MHPG for MAO-A activity.
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

NW-1015 in Plasma Bioanalytical Assay:
Plasma samples were analyzed for NW-1015 using a validated assay (Bioanalytical Validation Protocol NW-1015 70/99).
Plasma samples were analyzed for NW-1015 using reversed phase HPLC and tandem mass spectrometry in the Multiple Reaction Monitoring (MRM) mode after a liquid-liquid extraction of the drug from plasma. The lower limit of quantitation (LLOQ) was 20 ng/mL with a linear calibration range from 20 to 20000 ng/mL.

The assay performance during the validation was acceptable; details of the validation are presented below.

LC-MS-MS
The ions corresponding to the protonates analytes ($[M-H]^{-1}$) for NW-1015 and the internal standard (I.S.) were chosen as precursor ions. The collision energy was optimized in order to achieve the highest sensitivity for one prominent fragment ion. For NW-1015 one fragment ion was produced (109.1 m/z).
Calibration curve
The calibration range was from 20 ng/mL to 20000 ng/mL of plasma with seven calibrators. The mean percentage of error (ACC%) of back-calculated concentrations ranged from -2.3 to+ 1.6 and the CV% was ≤4.8. The mean coefficient of correlation (r) was 0.9993.
Intra-assay precision and accuracy
Precision (CV%) for all samples analysed was ≤4.4 and accuracy (ACC%) ranged from -6.9 to + 3.5 of the nominal concentration.
Inter-assay precision and accuracy
The CV% was ≤ 5.8 and ACC% ranged from -2.3 to +2.9.
Limit of quantitation (LOQ)
The limit of quantitation was 20 ng/mL. At this concentration the CV% and the ACC% were 2.6 and -5.1.
Specificity
No significant interfering peaks were found at the retention times of NW-1015 and its internal standard.
Stability
No significant change was detected in the measurement of NW-1015 concentrations in plasma samples stored at room temperature for 24 hours and after three freeze/thaw cycles. The analytes (NW-1015 and I.S.) were stable for at least 24 hours in final solution when stored in the autosampler vials at 4°C.
Recovery (extraction efficiency)
The recovery from plasma of NW-1015 was, on average, 104.9% and 106.6% for the I.S.

Evaluation of MAO-B activity
MAO-B activity was measured in PRP by liquid scintillation according to the method of Tipton et al. (1984).

PEA urinary excretion
PEA in urine samples (intervals -24-0 h, 0-24 h, 24-48 h) was determined by HPLC, according to the method of La Croix et al (1996).

Evaluation of MAO-A activity
MAO-A activity was measured by determining plasma levels of MHPG, which a sensitive index of MAO-A activity. Plasma levels of MHPG were determined after NW-1015 administration at 10 mg/kg, according to the method of Rizzo et al. (1987) using HPLC with coulometric electrochemical detection.

Pharmacokinetic Results:

The NW-1015 peak concentrations was reached on average from 1.83 to 2.83 h. Concentrations then decreased being evaluable in all the subjects, and with all the three doses, until the last blood sampling at 48 h.

Subjects 1 and 8 showed measurable concentrations at time 0 (40 and 30 $ng \cdot ml^{-1}$, respectively), just before the treatment with the dose of 10 mg/kg; this was a residue of the previous NW-1015 dose of 5.0 mg/kg. Note: This suggests longer $t_{1/2}$ than the estimated $t_{1/2}$ of 24.33 and 23.60 h, respectively, for these subjects.

Pharmacokinetic parameters were obtained with both non-compartmental model and a two-compartmental model; they overlapped. Mean values are summarized in table below (values normalized to the dose of 2.5 mg/kg are reported in parenthesis).

Non-compartmental analysis

Parameter	2.5 mg/kg			5.0 mg/kg			10.0 mg/kg		
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%
C_{max} ($ng \cdot ml^{-1}$)	1255.76	400.41	31.9	2943.28 (1471.64)	322.49	11.0	6315.74 (1578.94)	1488.82	23.6
AUC_{0-t} ($ng \cdot ml^{-1} \cdot h$)	26015	5227	20.1	61859 (30930)	7832	12.7	127983 (31996)	24696	19.3
AUC ($ng \cdot ml^{-1} \cdot h$)	32492	7320	22.5	77803 (38902)	12983	16.7	166134 (41534)	38046	22.9
t_{max} (h)	2.83	2.21	78.1	1.83	1.76	96.2	2.75	2.30	83.6
$t_{1/2\beta}$ (h)	20.63	3.83	18.6	20.22	3.02	14.9	23.39	4.37	18.7
Cl_p ($L \cdot h^{-1}$)	5.53	1.01	18.3	4.84	0.89	18.4	4.72	0.95	20.1

C_{max} and AUCs showed proportionally increasing values with the dose, or only marginally higher values at the dose of 5.0 and 10.0 mg/kg than the normalized values. The linear regression analysis performed on C_{max} and AUC showed a dose-linearity with the following regression parameters:

Parameter	Slope	Intercept	r	p
C_{max}	674.6392	-430.4712	0.9271	< 0.001
AUC_{0-t}	13542.75	-7047.25	0.9472	< 0.001
AUC	17797.0536	-11673.1875	0.9292	< 0.001

The test for deviations from linearity was non-significant (confirmation of the dose linearity of kinetic profile).

Pharmacodynamic (PD) Results:

MAO-B inhibition

Administration of NW-1015 (2.5, 5.0 and 10.0 mg/kg) caused complete inhibition of platelet MAO-B activity. A marked effect was observed already 20 minutes after the administration (27.59, 79.91, 57.51 % of baseline activity at 2.5, 5 and 10 mg/kg, respectively). Almost complete inhibition was reached within 60 minutes (1.84, 8.39, 6.39 % of baseline at 2.5, 5 and 10 mg/kg, respectively). The inhibitory effect was still high 48 hours post-treatment (6.35, 19.03, 12.52 % of baseline at 2.5, 5 and 10 mg/kg, respectively).

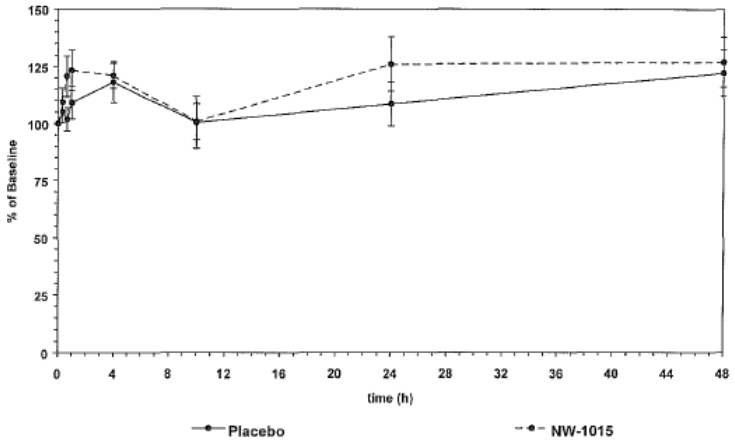
The inhibition of MAO-B, indirectly measured from PEA urinary excretion, was evident at all the tested doses and differences between doses and placebo were statistically significant ($p < 0.01$).

MAO-A inhibition

The inhibitory potential of NW-1 015 on MAO-A activity was evaluated only at the highest dose (10mg/kg) by measuring the plasma levels of MHPG.

The administration of NW-1015 10 mg/kg did not modify MHPG plasma levels when compared to placebo, indicating no inhibitory activity on MAO-A. The statistical analysis showed that any variation in MHPG plasma levels was statistically not significant.

Effect of oral administration of 10 mg/kg NW-1015 or placebo on MHPG plasma levels (Mean values± SEM)



Safety Results:

No serious adverse effects (AEs) were reported and no subject discontinued because of an adverse experience. All objective parameters (vital signs, body temperature, blood/urine biochemical tests and the neurological examination) remained unchanged and normal throughout the study.

The AEs experienced were mild: sensation of decreased attention, somnolence, light-headedness, headache.

II. [redacted]NW/SS-215-99: A parallel design study for evaluation of tolerability and pharmacokinetics of single and multiple dosing of the anticonvulsant and anti-parkinson agent NW-1015 in healthy male volunteers

Objectives:

To evaluate the tolerability and the PK of NW-1015, following oral multiple-dosing of 2.5 mg/kg and 5.0 mg/kg daily for seven consecutive days and evaluate the MAO-B inhibition activity and the plasma concentrations of NW-1015 following four single oral doses (25, 50, 75 and 150 μg/kg).

Study Design	Open label, single- and multiple-dosing, sequential treatment study
Study Population	16 healthy male subjects, 18-45 years, with BMI within 10% of ideal (according to Height and Weight tables from the Metropolitan Life Insurance)
Treatment Group	6 sequential treatments
Dosage and Administration	NW-1015, powder: 25, 50, 75 or 150 μg/kg administered as extemporaneous water solution on day -1*; NW-1015, 50 and 150 mg capsules: 2.5 or 5.0 mg/kg body weight once a day for 7 days*
PK Sampling	Day -1: 0 (pre-dose), 10, 20, 40 min, 1, 2, 4, 6, 10 and 24 hours post-dosing. Day 1 to 6: 0 (pre-dose). Day 7: 0 (pre-dose), 10, 20, 40 min, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours post-dosing.
Analysis	Plasma: LC-MS/MS method for NW-1015 Range: 20 to 20000 ng/mL
PK Assessment	NW-1015 C_{max}, t_{max}, AUC_{0-24} for single dosing NW-1015 C_{min}, C_{max}, t_{max}, AUC_{ss} for multiple dosing
PD Assessment	Evaluation of MAO-B inhibitory activity in platelet rich plasma (PRP) at time 0 (pre-dose), 10, 20, 40 min, 1, 2, 4, 6, 10 and 24 hours post-dosing.
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

* NW-1015 powder was administered, as extemporaneous water solution, at day -1 to evaluate the MAO-B inhibition activity and plasma levels of NW-1015. NW-1015 capsules were administered starting from the following day, for seven consecutive days. Subjects were in fasting condition starting from 12 hours before until 4 hours post-dosing on day -1. From day 1 to day 6, administration occurred before a standardized light breakfast. On day 7, the same restrictions of day -1 were observed.

Assays:

Plasma samples were analyzed for NW-1015 using a validated assay (Bioanalytical Validation Protocol NW-1015 70/99). For details, see under [redacted]-PNU-194-99, p 3. MAO-B activity was measured in PRP by liquid scintillation according to the method of Tipton et al. (1984).

Pharmacokinetic Results:

The following treatments were administered:

Table B – Scheme of treatment

VOL. NR.	INITIALS	TREATMENT	
		SINGLE-DOSING	MULTIPLE-DOSING
1	SL	25 µg/kg	2.5 mg/kg
2	SM	25 µg/kg	2.5 mg/kg
3	BI	25 µg/kg	2.5 mg/kg
4	DM	25 µg/kg	2.5 mg/kg
5	LR	50 µg/kg	2.5 mg/kg
6	RM	50 µg/kg	2.5 mg/kg
7	GG	50 µg/kg	2.5 mg/kg
8	PJ	50 µg/kg	2.5 mg/kg
9	VA	75 µg/kg	5.0 mg/kg
10	LB	75 µg/kg	5.0 mg/kg
11	MM	75 µg/kg	5.0 mg/kg
12	TE	75 µg/kg	5.0 mg/kg
13	HR	150 µg/kg	5.0 mg/kg
14	PG	150 µg/kg	5.0 mg/kg
15	VM	150 µg/kg	5.0 mg/kg
16	PA	150 µg/kg	5.0 mg/kg

A summary of the NW-1015 PK parameters after single- and multiple dosing is presented below.

Single-dosing

PARAMETER	25 µg/kg			50 µg/kg			75 µg/kg			150 µg/kg		
	MEAN	SD	CV%	MEAN	SD	CV%	MEAN	SD	CV%	MEAN	SD	CV%
C_{max} (ng•ml^{-1})	2.92	0.51	17.5	5.54	1.44	26.0	8.81	1.62	18.4	22.54	2.39	10.6
t_{max} (h)	2.00	0.00	/	3.50	2.89	82.6	4.00	1.63	40.8	3.50	1.00	28.6
AUC_{0-24} (ng•ml^{-1}•h)	43.91	6.87	15.6	89.23	9.78	11.0	154.92	34.30	22.1	397.91	55.03	13.8

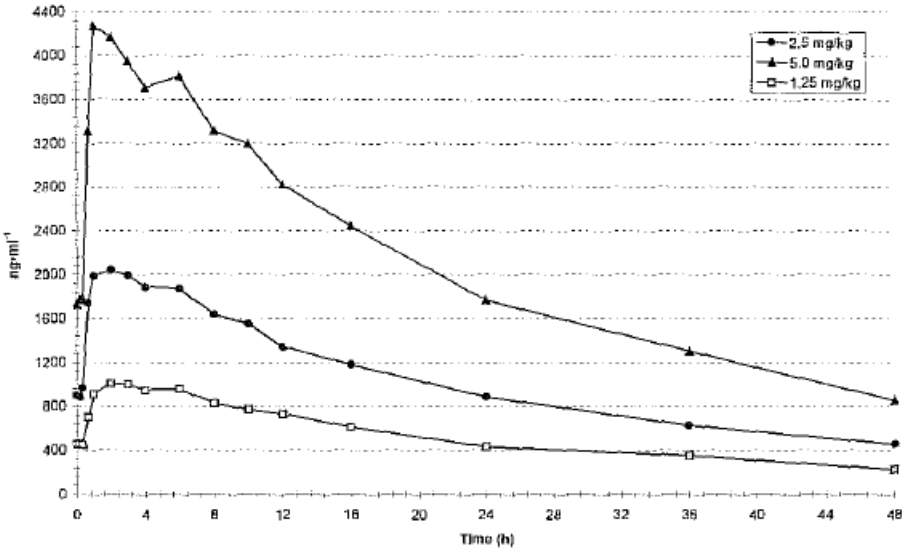
Multiple-dosing

PARAMETER	2.5 mg/kg			5.0 mg/kg		
	MEAN	SD	CV%	MEAN	SD	CV%
C_{max} (ng•ml^{-1})	2158.36	225.93	10.5	4522.40	1030.01	22.8
C_{min} (ng•ml^{-1})	855.31	147.12	17.2	1657.78	356.38	21.5
t_{max} (h)	2.17	1.75	80.6	2.25	1.83	81.3
$AUC_{SS,\tau}$ (ng•ml^{-1}•h)	34040	4773	14.0	69394	14005	20.2
$AUC_{SS,\tau}$ normalized*	36907	4226	11.5	72954	12348	16.9
PTF	0.93	0.12	12.9	0.99	0.13	13.1
AUC_{0-48} (ng•ml^{-1}•h)	49632	7368	14.8	100799	22011	21.8
$t_{1/2}$ (h)	24.08	3.35	13.9	21.07	3.57	16.9

PTF peak to trough fluctuation

The variability in the NW-1015 pharmacokinetic parameters, (except for t_{max}) was low. The $t_{1/2}$ values evaluated with multiple-dosing study after 2.5 and 5.0 mg/kg, were very similar to those previously obtained with 2.5, 5.0 and 10.0 mg/kg in the previous single ascending dosing study in healthy volunteers ((b) (4) -PNU-194-99). The overlapping $t_{1/2}$ values after single- and multiple-dosing trials suggest that neither auto-induction nor auto-inhibition of NW-1015 metabolism occur.

Mean plasma concentration-time profiles of NW-1015 (ng/mL) on study day 7 after repeated oral administration of 1.25, 2.5 or 5.0 mg/kg daily



The dose-related PK parameters C_{max} and AUC_{0-24} after single-dosing (25, 50, 75 or 150 μg/kg dose) were subjected to a linear regression analysis to test dose-linearity. One-way ANOVA was also applied to the same parameters after dose-normalization, in order to test dose-proportionality.
After multiple-dosing, a Student "t" test for paired data was applied to C_{max}, C_{min} and AUC_{ss} after data normalization to the 2.5 mg/kg dose.
The tests for C_{max} led to dose-linearity and dose-proportionality and AUC led to dose linearity and borderline statistics on dose-proportionality (a borderline statistically significant degree ($p < 0.05$) was obtained). According to the sponsor, the lack of statistical demonstration of dose-proportionality on AUC_{0-24} is attributable to the short period during which plasma concentrations were measured (24 h). In this case a relevant part of area was not considered, and this could have caused the borderline statistics on AUC dose-proportionality. In addition, the four doses were given to four different groups of volunteers, which is an additional cause of possible bias.

For the multiple-dosing phase of the study, it has not been possible to evaluate the dose linearity of NW-1015 (only two dose levels were investigated), however no statistically significant difference on C_{max}, C_{min} and AUC_{ss}, was evidenced by Student "t" test.

Pharmacodynamic Results:

The sponsor claims that the results on MAO-B are reported and discussed in another parallel report, however does not indicate which one. There are other studies to demonstrate the PD effects, so this one is not critical.

Safety Results:

No serious adverse experiences AEs were reported in this study. Eight volunteers reported adverse events.

The most frequent adverse event was headache (6 subjects); other discomforts were subjective decrease of attention (4 volunteers), somnolence (3 subjects), nausea (1 subject), gastric pyrosis (1 subject), and muscular pain (1 subject).

In 3 volunteers out of 8 treated with the 2.5 mg/kg dose, there was a slight increase of the ALAT value (maximum value 78 U/I, normal value < 51), however the values returned to normal after 2 weeks.

III. [redacted]NW/FOOD-257-00: Open, randomized, food interaction study of NW-1015 following oral administration of 900 µg/kg dose to healthy male volunteers.

Objective: To evaluate the plasma concentration-time profile of NW-1015 in order to assess the influence of food on its rate and extent of absorption.

Study Design	Open-label, single-dose, two-treatment, two-sequence, two-period, randomized, cross-over study
Study Population	6 healthy subjects (males only), 18-45 years, non-smokers, Caucasians, within 10% of the ideal BW
Treatment Group	900 µg/kg body weight in fasting and fed conditions separated by a 14 days wash-out period
Dosage and Administration	10 mg capsules of NW-1015: 900 µg/kg body weight in fasting and fed conditions separated by a 14 days wash-out period For the administration in fasting conditions, subjects were fasted starting from 12 hours before until 4 hours post-dosing. Fed conditions: subjects assumed a standardized high-fat breakfast during 20 minutes, drug was administered ten minutes after its end.
PK Sampling: plasma	0 (pre-dose), 10, 20, 40 min, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 h post-dosing.
Analysis	Plasma: LC-MS/MS method for NW-1015 Range: 20-20000 ng/mL
PK Assessment	C_{max}, t_{max}, AUC_{0-inf}, AUC_{0-t}, t_{lag} , $t_{1/2}$ of NW-1015 Descriptive statistics, cross-over ANOVA
PD Assessment	none
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry, neurological examinations

Bioanalytical Assay:
Plasma samples were analyzed for NW-1015 using a validated assay (Bioanalytical Validation Protocol NW-1015 70/99). For details, see under [redacted]-PNU-194-99, p 3.

Reviewer's Comments: The assay performance during the validation was acceptable. The assay performance during the analysis of the plasma samples was acceptable.

Pharmacokinetic Results:
After the high fat meal NW-1015 appeared in plasma at 1 h in two volunteers (V3 and V6), at 2 h in V1, V2 and V4 and at 3 h in V5. The peak was achieved on average at 4.83 h (range 2-8 h).
In fasting conditions, NW-1015 appeared in plasma at 0.667 h (40 min) in all the volunteers; the peak was reached on average at 1.50 h (range 1-2 h).
In both the cases, concentrations were evaluable 48 h after dosing in all the subjects.

NW-1015 PK Parameters

PARAMETER	TEST (NON-FASTING)			TEST (FASTING)		
	MEAN	SD	CV%	MEAN	SD	CV%
C_{max} ($ng \cdot ml^{-1}$)	319.18	90.33	28.3	380.83	108.60	28.5
AUC_{0-t} ($ng \cdot ml^{-1} \cdot h$)	7141.64	1727.52	24.2	7527.91	1972.5	26.2
AUC ($ng \cdot ml^{-1} \cdot h$)	9032.78	2108.85	23.3	9264.11	2464.18	26.6
t_{max} (h)	4.83	2.23	46.1	1.50	0.55	36.5
$t_{1/2}$ (h)	21.22	4.53	21.4	20.46	2.62	12.8
t_{lag} (h)	1.83	0.75	41.1	0.67	0.00	//
C_{max} (norm.) ($ng \cdot ml^{-1}$)	343.51	104.84	30.5	409.30	123.44	30.2
AUC_{0-t} (norm.) ($ng \cdot ml^{-1} \cdot h$)	7659.33	1937.53	25.3	8079.34	2222.53	27.5
AUC (norm.) ($ng \cdot ml^{-1} \cdot h$)	9690.62	2389.94	24.7	9944.51	2792.23	28.1

The rate of absorption of NW-1015, when given in fasting conditions, was faster than that after a meal. The extent of absorption (expressed by AUC) in non-fasting and fasting conditions seems to overlap.

ANOVA for cross-over design was performed on the logarithmically transformed and dose-normalized C_{max}, and AUCs. Results are reported below.

PARAMETER	FORMULATION		SEQUENCE		PERIOD	
	F	P	F	P	F	P
C_{max} (norm.)	15.964	< 0.05	12.010	< 0.05	0.107	NS
AUC_{0-t} (norm.)	2.450	NS	5.906	NS	0.978	NS
AUC (norm.)	0.955	NS	5.689	NS	3.929	NS

C_{max}, showed a borderline statistically significant degree ($p<0.05$) for formulation and sequence effect. The formulation effect was expected considering the difference in mean values between fasting and non-fasting. There was no sequence or period effect on the three parameters.

Safety:
The vital signs were measured at screening and at the study end. No abnormalities occurred in blood pressure, heart rate, respiratory rate, body temperature and ECGs. No changes were evidenced in neurological examination.

Reviewer's Comment: The formulation tested in this study is not the to-be-marketed formulation (TBM). The food effect after administration of the tablet formulation (clinical and to-be-marketed formulation) has been evaluated in Study EMR701165_022.

IV. CRO-02-33: Pharmacokinetics and metabolism of ^{14}C-safinamide in healthy male volunteers administered a single oral dose

Objectives:

Primary objective: To describe the absorption, blood and plasma time course and the urinary, and fecal excretion of total radioactivity (total balance of ^{14}C) after a single 400 mg oral dose of ^{14}Csafinamide

Secondary objectives: To describe the PK profile of unchanged safinamide in plasma. Identification of safinamide metabolism pathways

Study Design	Single-dose, open-label study to investigate the absorption, distribution, metabolism, and excretion (ADME) of ^{14}C-safinamide
Study Population *	6 healthy male subjects, 18-45 years, BMI 19-30 kg/m^2
Treatment Group	None, all subjects received 400 mg oral dose of ^{14}C-safinamide
Dosage and Administration	A single oral dose of 400 mg of ^{14}C-safinamide (as salt) lyophilized powder to be reconstituted in water, corresponding to a radioactive dose of about 63 μCi and to a safinamide free base dose of 303.53 mg.
PK Sampling: plasma, whole blood, urine, fecal, saliva	Plasma and whole blood: pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 168 and 192 h post administration**; Urine collection: 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168 and 168-192 h***; Feces collection: 0-24, 24-48,48-72, 72-96, 96-120, 120-144, 144-168 and 168-192 h Saliva samples: 2, 4, 8, 12 and 24 h after dosing
Analysis	Radioactivity determinations by liquid scintillation counting Validated LC-MS-MS method for (b) (4) enantiomers in plasma Metabolic profiling: the number and relative amounts of the unchanged drug and of its metabolites were assessed by TLC
PK Assessments	PK variables: Total radioactivity in plasma, whole blood, urine, feces and saliva for blood and plasma PK profiles and elimination (total balance of ^{14}C); Levels of safinamide enantiomers (unchanged drug) in plasma and their PK profiles; Analysis of safinamide metabolites in plasma and urine samples.
PD Assessment	none
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

* No medications, including OTC drugs, were allowed from 2 weeks before the start of the study. No drug treatment with any P450 3A inhibitors or inducers was allowed for 1 month before the start of the study. No diagnostic examinations with radiation during 3 months before the start of the study, no participation in any radioactivity study for the last 10 years.

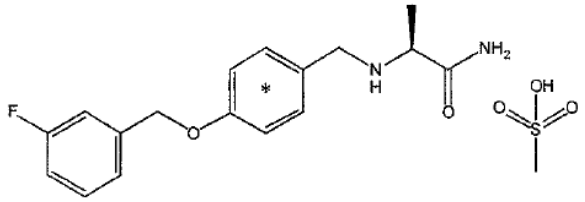
** One plasma sample (5 ml) was also obtained at 1, 2, 6, 12 and 24 h for metabolic profiling.

*** For subjects No 5 and 6, urine was also collected at the time interval 192-200 h post due to detectable radioactivity still present at the last scheduled time (168h). Further collections were done until radioactivity was less than 0.5 % of the administered dose.

Urine samples for metabolic profiling were collected at 0-12, 12-24 and 24-48 h

Bioanalytical Procedures:

^{14}C-safinamide, (S)-(+)- was synthesized by [redacted] by introducing the 14 carbon in a metabolically stable position: (*) indicates ^{4}C-uniformely labelled ring.



Safinamide and its metabolites were identified in four related studies ([redacted] 844437, [redacted] 844439, CRO/02 and CRO-03-51).

Study [redacted] 844439 describes the determination of [redacted] enantiomers in plasma from the ADME study CRO-02-33 using a validated LC-MS-MS method.

Report CRO/02 describes in detail the chromatographic analyses and metabolite identification using LC-MS/MS in urine and plasma of the ADME study CRO-02-33.

Report [redacted] 844437 describes by liquid scintillation counting method and the preliminary analytical work for the identification of human safinamide metabolites using thin layer chromatography (TLC).

In Report CRO-03-51, the metabolism determination described in CRO/02 is summarized and discussed with the results of Report [redacted] 844437.

The radioactivity determinations were performed by liquid scintillation counting.

Sample preparation for radioactivity counting

Whole blood:

Hydrogen peroxide (30%, 100 μL) was added into a glass scintillation vial containing 0.25 ml of whole blood; 1 ml of a mixture of Soluene-350 and isopropyl alcohol at 1:1 was then added. The samples were incubated at 60^{o} C for 2 h and then cooled to room temperature, 10-15 ml of Ultima Gold™ were added and samples were counted for 1 h. The analysis was performed in triplicate.

Urine and Saliva:

An aliquot (0.5-2 ml) of each urine or saliva sample was mixed with 10 ml Ultima Gold™. Samples were vortexed and counted. Analyses were performed in duplicate.

Plasma samples were analyzed for the content of [redacted] enantiomers separately, using a validated LC-MS-MS method.

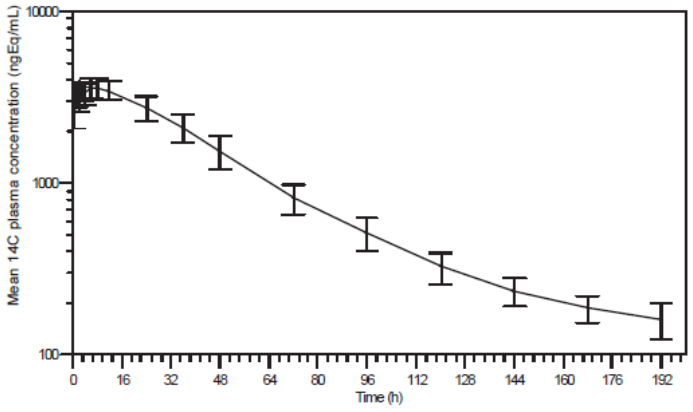
Samples for metabolic profiling were immediately frozen after collection and kept at - 20°C until analysis. Determination of metabolic profiles in plasma, urine and feces was performed by TLC. In addition, the relative concentrations of the metabolites were determined.

The number and relative amounts of the unchanged drug and of its metabolites were assessed by TLC by using three solvent systems, i.e. in neutral, basic and acidic conditions.

In addition, urine samples at 0-48 h for each volunteer were analyzed by TLC after enzymatic incubation with β-glucuronidase/arylsulfatase or arylsulfatase activity only followed by partitioning into ethyl acetate.
For enzymatic incubations, 1% of the total volume of the 3-time intervals (0-12, 12-24 and 24-48 h) were pooled together in order to obtain one sample representative of the 0-48 h time interval for each subject.
An aliquot of each urine sample was incubated at 37°C for 17 h with β-glucuronidase /arysulfatase enzymes which catalyses the de-esterification of glucuronide and sulfate conjugates. For volunteers 1-2, a second urine aliquot was incubated with the same enzymatic mixture + D-saccharo-1.4-lactone, a specific inhibitor of glucuronidase enzyme. An aliquot of urine samples from all subjects was incubated under the same conditions but only contained enzyme buffer as control.
After incubations, radioactivity in the urine samples was extracted with ethyl acetate. The organic phase (50-63% of total radioactivity) was concentrated and analyzed by TLC using the neutral and basic solvent systems. For volunteers 1 and 2, also the remaining aqueous phase was concentrated and analyzed by TLC.

Pharmacokinetic Results:

^{14}C concentration in plasma
Radioactivity was detected in plasma of all volunteers at the first sampling time, i.e. 0.5 h after administration, at an average level of 2446.7 ngEq/mL. ^{14}C was still present 192 h post-dose in all plasma samples at a mean level of 161.1 ngEq/mL.
Mean levels of total ^{14}C in plasma samples of the 6 subjects are presented below.



Main PK parameters of total ^{14}C, calculated from the plasma concentration vs. time profiles, are summarized in the Table below.

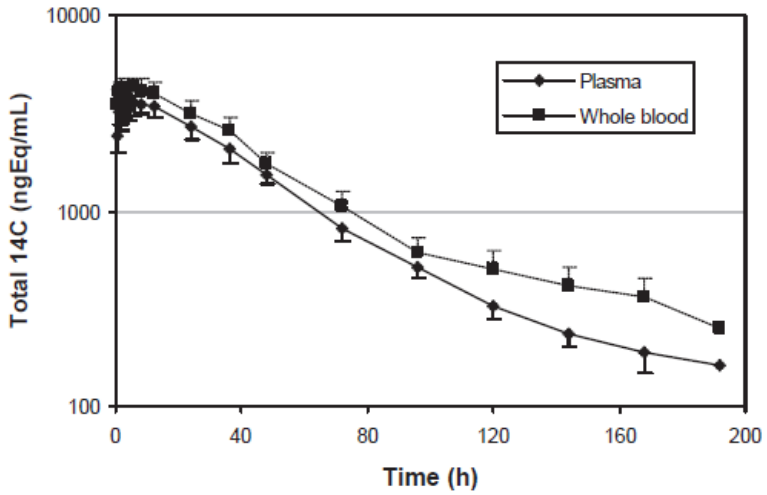
	C_{max} (ngEq/mL)	t_{max} (h)	AUC_t (ngEq×h/mL)	$AUC_{0-\infty}$ (ngEq×h/mL)	AUC_{0-96} (ngEq×h/mL)	initial $t_{½}$ (h)	terminal $t_{½}$ (h)	MRT (h)
MEAN	3722.0	7.67	197808.3	215911.3	171740.5	39.6	77.4	67.5
SD	436.3	3.88	28286.3	33410.4	24248.7	2.1	24.6	11.8
CV%	11.7	50.6	14.3	15.5	14.1	5.4	31.8	17.5
MIN	3000.1	2	159722	168085	138913	37.6	44.1	55.1
MAX	4217.3	12	246853	272669	213708	42.9	114.1	86.7
N	6	6	6	6	6	6	6	6

T_{max} ranged from 2 to 12 h with a mean value of 7.67 h post dose.
The wide T_{max} values range was characterized by two consecutive peaks, the first between 1-2.5 h (average 1.67), and the second between 6-12 h (average 8.33).
Two C_{max} and T_{max} values were therefore calculated for all subjects:

	C_{max1} (ngEq/mL)	t_{max1} (h)	C_{max2} (ngEq/mL)	t_{max2} (h)
MEAN	3445.6	1.67	3711.6	8.33
SD	437.9	0.7	422.6	2.9
CV%	12.71	41.92	11.39	34.81
MIN	2925.8	1	3000.1	6
MAX	4217.3	2.5	4155.1	12
N	6	6	6	6

AUC up to 96 h post-dose (AUC_{0-96}) was calculated in order to compare it with the AUC_t of the unchanged [redacted] (b) (4) (determined in Study [redacted] (b) (4) 844439).

<u>^{14}C concentration in whole blood</u>
Mean ^{14}C profiles in plasma and whole blood are shown below.



T_{max} ranged from 1 to 8 h with a mean value of 3.25 h. Similar to plasma ^{14}C results, two T_{max} value ranges were also observed in whole blood:

	C_{max1} (ngEq/mL)	t_{max1} (h)	C_{max2} (ngEq/mL)	t_{max2} (h)
MEAN	4414.5	1.33	4360.3	7.33
SD	423.9	0.5	425.9	2.4
CV%	9.60	37.59	9.77	32.74
MIN	3962.5	0.5	3957.7	6
MAX	5122.2	2	4981.4	12
N	6	6	6	6

Main PK parameters calculated for plasma and whole blood (WB) are summarized in the table below.

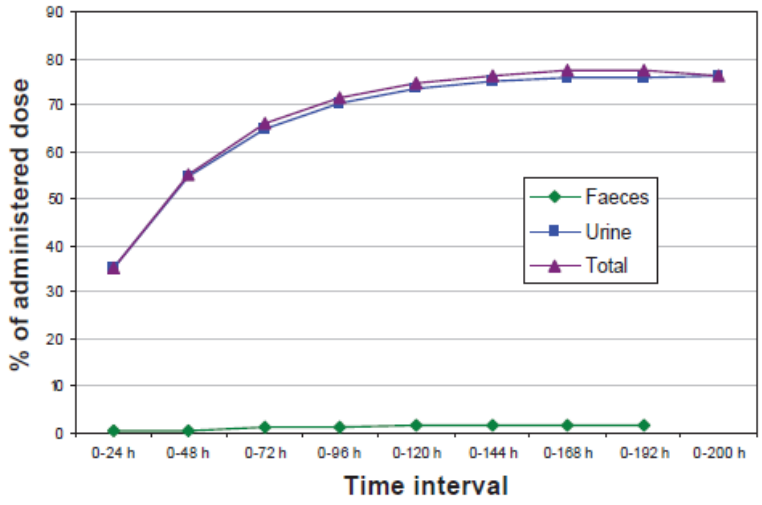
	C_{max} (ngEq/mL)	t_{max} (h)	t_{max1} (h)	t_{max2} (h)	AUC_t (ngEq×h/mL)	$AUC_{0-\infty}$ (ngEq×h/mL)	Initial $t_{½}$ (h)	Terminal $t_{½}$ (h)
Plasma	3722.0±436.3	7.67±3.88	1.67±0.75	8.33±2.94	197808±28286	215911±33410	39.6±2.1	77.4±24.6
WB	4459.4±423.7	3.25±2.98	1.33±0.52	7.33±2.42	244850±39976	277901±55342	46.6±3.2	81.1±33.0

Initial and terminal half-lives did not differ significantly between plasma and whole blood.

<u>^{14}C concentration in urine samples</u>

For all volunteers, radioactivity at the last scheduled time interval (144-168 h) was slightly above 0.5% of the administered dose. Therefore, urine samples (as well as blood and feces) were collected up to 192 h post-dose. For two volunteers, i.e. subjects No. 5 and 6, a further sample was collected at the time interval 192-200h post-dose, i.e. until radioactivity values detected were less than 0.5 % of the dose administered.

Results of the ^{14}C determination in urine samples were presented in individual time intervals and in cumulative values (expressed as µgEq).



As an average, <u>76% of the initial dose of ^{14}C-(S) safinamide (mean dose 303.06 mg, as free base) was recovered in urine up to 192 h</u> after oral single dose administration(urine samples for two volunteers were measured up to 200h post-dose). The individual recovery ranged from 58.07% to 86.30%.

The main PK parameters calculated for total ^{14}C radioactivity in urine are presented in the table below.

Subject	ΣXu (µgEq)	Recovery (% of dose)	dXu/dt Max (µgEq/h)
1	175892.6	58.07	4313.6
2	225430.5	74.43	5663.0
3	242765.7	80.11	6739.3
4	261708.9	86.30	6366.8
5	234399.6	77.32	5020.0
6	243757.2	80.42	5221.6
MEAN	230659.08	76.11	5554.04
SD	29403.81	9.68	895.73
CV%	12.75	12.71	16.13
MIN	175892.60	58.07	4313.57
MAX	261708.90	86.30	6739.25
N	6	6	6

^{14}C concentration in feces

Total recovery in feces at the end of the study was 1.51 (range 0.80-1.81) of the administered dose, as summarized in table below (n=6).

Total amount of ^{14}C eliminated in feces by the 6 volunteers, calculated by cumulative fecal excretion, was in average 4579.1 µgEq ^{14}C-(S)safinamide free base.

Subject	ΣXf (µgEq)	Recovery (% of dose)	dXf/dt Max (µgEq/h)
1	5163.94	1.70	107.99
2	4746.12	1.57	86.90
3	2423.54	0.80	50.32
4	4854.70	1.60	94.48
5	5473.16	1.81	137.83
6	4813.16	1.59	100.06
MEAN	4579.10	1.51	96.26
SD	1090.54	0.36	28.57
CV%	23.82	23.78	29.68
MIN	2423.54	0.80	50.32
MAX	5473.16	1.81	137.83
N	6	6	6

^{14}C levels in saliva samples were very high at 2h post-dose, i.e. 2239.3 ngEq/mL, due to the fact that ^{14}C-safinamide was orally administered as a water solution. However, saliva levels quickly decreased to 701.5 ngEq/mL at 4 h after drug administration. Levels decreased slowly to an average level of 234.9±50.8 ngEq/mL at the last sampling time (24 h).

Total ^{14}C balance

Total ^{14}C balance, i.e. sum of total ^{14}C elimination in urine and feces up to the last sampling time, after single oral administration of about 400 mg ^{14}C-(S) safinamide methanesulfonate, corresponding to about 303.53 mg safinamide free base (63 µCi/dose), ranged from 59.78 to 87.90% of actual administered dose.

Mean total recovery was 77.62±9.58% (n=6.)

Reviewer's Comment: Considering the long $t_{1/2}$ of total ^{14}C (77.4 h), complete recovery is not expected with collection only up to 192 h.

Subject	Urine (%)	Faeces (%)	Total (%)
1	58.07	1.70	59.77
2	74.43	1.57	76.01
3	80.11	0.80	80.92
4	86.30	1.60	87.9
5	77.32	1.81	79.13
6	80.42	1.59	82.01
MEAN	76.11	1.51	77.62
SD	9.68	0.36	9.58
CV%	12.71	23.78	12.34
MIN	58.07	0.80	59.78
MAX	86.30	1.81	87.9
N	6	6	6

Plasma concentrations of safinamide enantiomers

Plasma concentrations of safinamide enantiomers in human plasma after administration of about 400 mg ^{14}C-(S)-safinamide methanesulfonate were determined using a validated LC/MS/MS method with an enantioselective chromatographic procedure.

Levels of the [redacted] (b) (4) enantiomer were below the limit of detection of the analytical assay (10 ng/ml) at all the pre-defined times, suggesting no detectable in vivo transformation of the active isomer [redacted] (b) (4) of safinamide.
[redacted] (b) (4) was detected in plasma of all volunteers, 0.5 h after ^{14}C-(S) safinamide administration (range 1444 - 2045.4 ng/ml) and was still present 96 h post-dose in all plasma samples. AUC_t mean value was 62701 ngxh/mL.

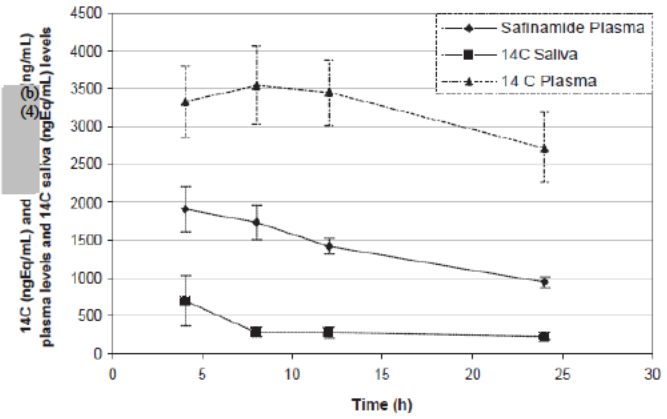
Subject	C_{max} (ng/mL)	t_{max} (h)	AUC_t (ng×h/mL)	$AUC_{0-\infty}$ (ng×h/mL)	AUC_{extra} (%)	$t_{½}$ (h)	MRT (h)	Clearance (mL/hxkg)	Vd (mL/kg)
MEAN	2367.30	1.08	62701	65917	4.86	22.20	30.74	57.0	1813.3
SD	382.16	0.38	4347.6	4818.80	1.01	1.38	2.55	8.5	204.3
CV%	16.14	34.74	6.93	7.31	20.82	6.19	8.29	14.84	11.27
MIN	1861.87	0.5	58590.1	61802.00	3.60	20.34	27.71	43.2	1502.5
MAX	2934.97	1.5	69648.2	74449.50	6.45	24.09	34.79	68.1	2104.1
N	6	6	6	6	6	6	6	6	6

Comparison of ^{14}C levels and levels of [redacted] (b) (4) in plasma
Main PK parameters derived from plasma levels of total ^{14}C-radioactivity and unchanged [redacted] (b) (4) are summarized below.

Main PK parameters of total ^{14}C and unchanged [redacted] (b) (4) in plasma

	C_{max} (ngEq/mL)	t_{max} (h)	AUC_{0-96h} (ngEq×h/mL)	$AUC_{0-\infty}$ (ngEq×h/mL)	$t_{1/2}$	Vd
^{14}C	3722.0±436.3	7.67±3.88	171740±24249	215911±33410	77.4±24.6	1944.6±673.1
Unchanged	2367.30±382.2	1.08±0.38	62701±4347.6	65917±4818.8	22.20±1.38	1813.3±204.3

Comparison of ^{14}C concentrations in plasma and saliva samples
Rationale: Only free molecules can diffuse into the saliva. Therefore, saliva drug levels tend to approximate free drug and/or metabolites fraction rather than total plasma drug concentration.
In the present study, the saliva/plasma ratio of ^{14}C-radioactivity levels at 4, 8, 12 and 24 h was 0.21, 0.08, 0.08 and 0.09 respectively. It is likely that at 4 h post-dose saliva ^{14}C levels were still quite high due to ^{14}C contamination in the mouth following oral drug administration. This result suggests that free safinamide and/or its metabolites accounted for about 8-9% of the total drug/metabolites present in the central compartment, therefore in plasma more than 90% of radioactivity was protein bound.



Metabolic pattern in plasma

Plasma samples of the 6 volunteers were pooled per each time point (1, 2, 6, 12 and 24 h). Samples were extracted twice with acetone. The radioactivity in the extracted acetone phase accounted for 93.26±1.5% of total radioactivity in the plasma samples.

The organic (acetone) extracts of the 5 plasma samples (i.e. pools at 1, 2, 6, 12 and 24 h) were analyzed by TLC using three solvent systems, i.e. in neutral (SS2), basic (SS4) and acidic (SS5) conditions.

Chromatograms showed that at least 5 radioactive fractions (P1 to P5) were detected. Fraction P3 was identified as the parent compound ^{14}C-safinamide by co-migration with the test item (safinamide standard). P3 was present at a relative radioactivity of 48.3% at 1 h post-dose, which decreased to 33.4% at 24 h.

Plasma metabolite fractions and parent compound

TLC fraction	% radioactivity at different times post-dose				
	1 h	2 h	6 h	12 h	24 h
P1	20.6	8.1	5.0	4.1	ND
P2	13.5	25.9	31.2	46.0	55.3
P3 (parent)	**48.3**	**51.5**	**48.2**	**37.5**	**33.4**
P4	6	ND	4.6	ND	ND
P5	4.4	6.4	6.4	4.4	5.5

Metabolic pattern in urine samples

Urine samples from each volunteer from time intervals 0-12, 12-24 and 24-48 h were directly analyzed by TLC using the 3 solvent systems described above.

In urine samples, at least 5 metabolite fractions, i.e. U1-U5, were detected.

Metabolite patterns of the 6 volunteers were similar.

Fraction U1 was identified as the parent drug by co-migration with the test item and by LC-MS.

TLC fraction	% radioactivity at different times post-dose		
	0-12 h	12 –24 h	24-48 h
U1 (parent)	**16.33±4.30**	**10.68±4.66**	**8.75±4.50**
U2	38.30±5.64	34.47±2.17	31.43±3.50
U3	3.63±2.32	0.45±1.10	0.40±0.98
U4	20.90±1.74	30.23±2.63	32.72±2.69
U5	20.83±3.38	24.17±4.26	26.70±1.66

Enzymatic hydrolysis of urine samples

For enzymatic incubations, 1% of the total volume of the 3-time intervals were pooled together in order to obtain one sample representative of the 0-48 h time interval for each subject. After incubation with glucuronidase/arysulfatase, two hydrolytic products, H1 and H2, were detected in the TLC separations. No difference between incubation with glucuronidase/arysulfate or with only arylsulfatase activity was observed, thus suggesting that U4 and U5 probably represented ester-derivatives of phase I metabolites. Hydrolytic fraction H1 may correspond to plasma metabolite fraction P2.

Metabolic pattern in feces

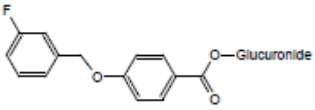
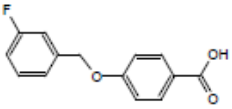
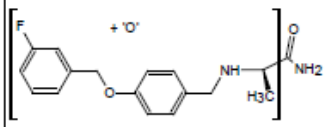
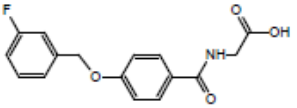
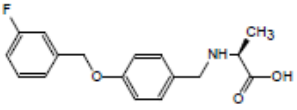
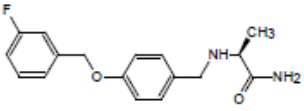
In the organic phase of feces extracts of subjects 1-6, at least 9 radioactive fractions (F1-F9), were detected. No fraction corresponded to the unchanged drug.

LC-MS analysis

HPLC separations, using two HPLC system conditions, as well as LC-MS analysis were performed on selected urine samples for the characterization of radioactive fractions. Radioactive fraction U1: Based on LC-MS results, radioactive fraction U1 proved to be identical to the reference standard (safinamide) with a specific signal of $[M+H]^+$ 303. LC-MS results showed MW of 303 and 318 for U2 and H2 respectively. MS/MS fragmentation patterns suggested replacement of safinamide terminal amino-group with an OH- group for U2 and hydroxylation at one of the phenyl rings for H2.

Further analysis by LC-MS/MS of isolated metabolic fractions was conducted at (b) (4) (study CR0-03-51).

A summary of components identified in urine and plasma using LC-MS/MS

Component	Molecular Weight [a]	Nominal Retention Time (min) [b]	Human Urine	Deconjugated Human Urine	Human Plasma
Glucuronide of N-dealkylated acid metabolite 	422	23.9 (U2)	++	+ where reaction incomplete	+ ? minor
N-dealkylated acid metabolite (NiK-14134) 	246	24.3 (U2a)	ND	++	+
Mono-hydroxy Safinamide 	318	18.4 19.3 24.8 25.9	+ ? + ? + ? + ?	+ ?	ND
Glycine conjugate of N-dealkylated acid metabolite (NiK-15009) 	303	23.7	+ ?	+ ?	-
Acid metabolite 	303	24.9 (U3)	++	++	+
Safinamide 	302	33.5	++	+	++

a Molecular weights quoted are for non-radiolabelled components

b Retention times are taken from [14C] radiochromatogram traces where possible; otherwise retention times are taken from product ion analyses
+ Component present (++ = significant quantities present by LC-MS/MS; ? = tentative assignment)
- Component not present or below limit of detection
ND = not determined or not possible to confirm presence of metabolite

Analysis of human plasma samples

Based on the product ion spectra already obtained, a minimum of three characteristic fragment ions were selected for each component of interest. The selected transitions were used to create specific Multiple Reaction Monitoring (MRM) methods in positive and negative ion modes for the components of interest. MRM was used to confirm the presence of the supplied reference standards, and any additional identified components. Based on the results obtained, safinamide, N-dealkylated acid and the acid metabolite were confirmed as being present in human plasma. The glucuronide of NiK-14134 may also be present as a minor component.

PK Conclusions:

Safinamide undergoes fast and complete absorption after oral administration in healthy male volunteers. The elimination half-life was about 22 h.
^{14}C levels were eliminated in a bi-phasic manner, both from plasma and whole blood, with a terminal half-life of about 80 h.
The volume of distribution calculated in the present study both for the unchanged safinamide and for total ^{14}C-radioactivity (> 100 L) indicate an extensive tissue distribution of the parent drug and metabolite(s). Total balance of radioactivity was not complete at the end of the study (192 h), accounting for 77.6% of the administered dose. About 76% of the dose was recovered in urine and only 1.5% in feces.
Safinamide was extensively metabolized, most of the administered dose was recovered as metabolites in urine.
Safinamide, N-dealkylated acid and the acid metabolite were confirmed as being present in human plasma. The glucuronide may also be present as a minor component.
Levels of the [redacted] (b) (4) enantiomer were below the limit of detection of the analytical assay (10 ng/ml) at all times, suggesting no detectable *in vivo* transformation of the active isomer [redacted] (b) (4) of safinamide.

Safety:

No clinically relevant effects of treatment on laboratory parameters, vital signs and ECGs, were observed. Four AEs were reported, of which only 1, i.e. headache, was judged by the Investigator as possibly related to study drug. All AEs were of mild intensity.

1.2 Biopharmaceutics Studies

V. EMR701165_022: Open-label, randomized, three-way crossover trial to investigate absolute bioavailability of safinamide and to assess food effects on a single dose administration of 50 mg safinamide in healthy volunteers

Primary objective: to determine the absolute bioavailability of safinamide at a dose of 50 mg and to investigate food effects on safinamide pharmacokinetics at a dose of 50 mg.
Secondary objectives:

- To investigate the pharmacokinetics (PK) of safinamide and its metabolites after intravenous (i.v.) and oral (p.o.) administration of safinamide in the fed and fasted conditions
- To assess the safety and tolerability of the formulations (solution for i.v. and tablet) in the fed and fasted conditions

Study Design	Randomized, open-label, crossover design trial with 3 single-dose safinamide treatments
Study Population	14 healthy male and female subjects aged between 18 years and 55 years with a BMI between 18 – 30 kg/m^2
Treatment Groups	The subjects were randomized to one of the following two treatment sequences: • A⟶C⟶B • B⟶C⟶A Treatment A: 50 mg single dose safinamide after an overnight fast Treatment B: 50 mg single dose safinamide after a high-fat, high-calorie breakfast Treatment C: 25 mL solution containing 50 mg safinamide given as an i.v. infusion over 30 minutes after an overnight fast
Dosage and Administration	Treatment A and B: 50 mg safinamide film-coated tablet Treatment C: 25 mL solution for I.V. containing 50 mg safinamide There was a washout period of at least 17 days between treatments.
PK Sampling: plasma	Plasma samples were collected safinamide and of its metabolites at: Treatment A and B: at pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120 and 240 hours post-dose Treatment C: at pre-dose and 0.25, 0.5 (equal to end of infusion period), 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 24, 36, 48, 72, 96, 120 and 240 hours post start of infusion
PK Analysis	Plasma: LC-MS/MS assays for safinamide, NW-1153 and NW-1689 (before and after)
PK Assessment	C_{max}, t_{max}, AUC_{0-t}, AUC_{0-inf}, $t_{1/2}$ and CL CL/f (CL for i.v.), Vz /f (Vz for i.v.), Vss / f (Vss for i.v.), and F of safinamide
PD Assessment	none
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

Bioanalytical Assays

The method for the determination of Safinamide and NW-1153 was previously validated ([(b) (4)] report number 8203838, Sponsor reference number DMPK 63-09) and has a lower limit of quantification (LLOQ) of 5 ng/mL for Safinamide and NW-1153, using 25 µL of plasma.

The analytical procedure used for the determination of Safinamide and NW-1153 in human plasma was the current version of [(b) (4)] HB-09-058-V2. Long term stability has previously been confirmed for Safinamide and NW-1153 in plasma samples (lithium heparin) for up to 22 months at nominal -20°C.

NW-1689 (before and after):

As the sponsor has confirmed that NW-1689-1-O-glucuronide (NW-1689AG) is unstable in human plasma, the clinical study samples were acidified shortly after collection. Therefore the analytical procedure utilizes acidified human plasma (plasma: o-phosphoric acid (20% in water), 10:1, v/v) as control matrix.

The method was previously validated at [(b) (4)] ([(b) (4)] Report number 8214594) and has a lower limit of quantification (LLOQ) of 5 ng/mL for NW-1689 (before) and NW-1689 (after) using 50 µL of acidified plasma.

The analytical procedure used for acidified human plasma samples was the current version of [(b) (4)] HB-09-064-V8. Long term stability has previously been confirmed for NW-1689 (before) and NW-1689 (after) in in acidified human plasma (lithium heparin) for up to 8 months at nominal -20°C.

Aliquot A was evaporated to dryness under a stream of nitrogen at nominal 50°C. The residues were reconstituted in acetonitrile: 10 mM ammonium acetate pH 4.0 (50: 50 v/v, 100 µL). The extracts were vortex mixed; centrifuged (ca. 10 minutes, 3000 g, nominal 22°C) and submitted for LC-MS/MS analysis of NW-1689 (before).

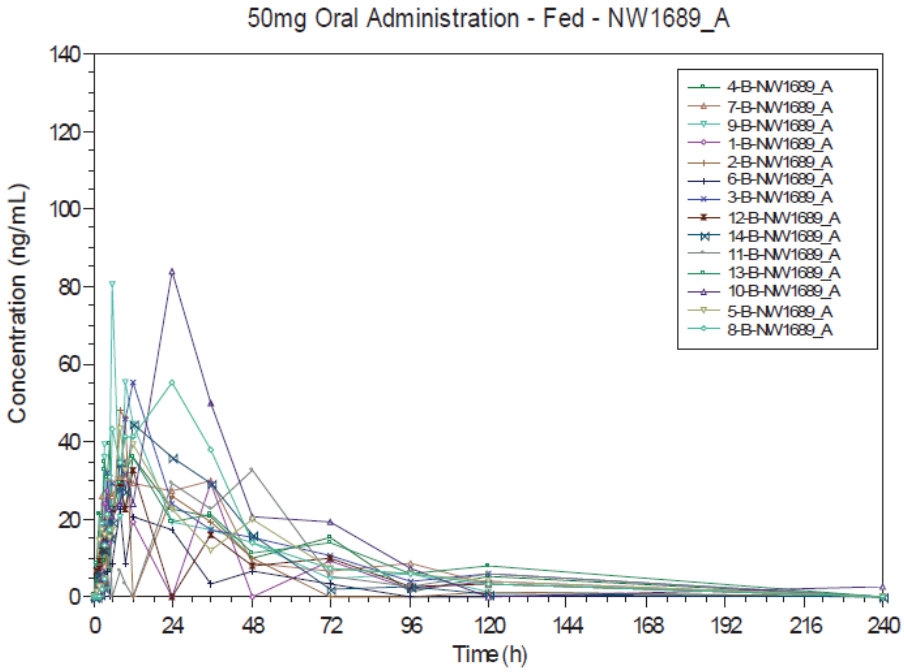
Aliquot B was evaporated to dryness under a stream of nitrogen at nominal 50°C. The residues were reconstituted in 0.2 mM sodium hydroxide solution (100 µL) and incubated (45 minutes, 50°C). Acetonitrile: formic acid (98: 2 v/v, 50 µL) was added and the extracts were vortex mixed, centrifuged (ca. 10 minutes, 3000 g, nominal 22°C) and submitted for LC-MS/MS analysis of NW-1689 (after).

A total of 910 samples were analyzed for safinamide and NW-1153 in eight batches per analyte. The mean accuracy of the back-calculated concentrations for the calibration standards varied between 92.6% and 107.8% for safinamide and between 95.4% and 105.0% for NW-1153.

A total of 910 samples were analyzed for NW-1689 (before) in 16 batches and NW-1689 (after) in 14 reported batches. The correlation coefficients (r) of the calibration curves used in this study phase were between 0.9963 and 0.9996. The mean accuracy of the back-calculated concentrations for the calibration standards varied between 97.2% and 104.2% for NW-1689 (before) and between 97.0% and 103.0% for NW-1689 (after).

Note 1: All calibration standards were prepared in o-phosphoric acid (20% in water), 100:1, v/v rather than plasma: o-phosphoric acid (20% in water), 10:1, v/v, as stated in the protocol. However, a QC samples were prepared using plasma: o-phosphoric acid (20% in water, 10:1 v/v) (as required by the protocol) and all reported batches met the batch acceptance criteria.

Note 2: NW1689 AG concentrations were derived in an indirect method through LC/MS/MS analysis of the samples before and after conversion of NW1689 AG to NW1689 (alkaline hydrolysis of the acylglucuronide) and appropriate correction was applied according to the molecular weight of NW1689 AG. In case the calculation resulted in negative values for NW1689 AG concentration, the values were set to zero. Isolated negative NW1689 AG concentrations (set to zero) were observed in individual PK profiles amongst all treatments, see fig below.



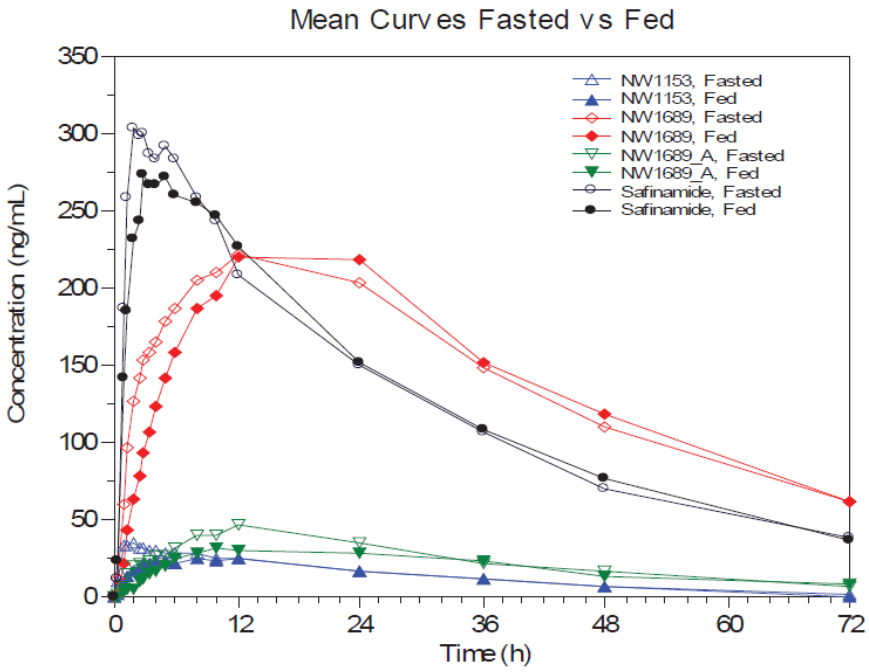
Reviewer's Comment: the assay performance during the study sample analysis for safinamide and NW-1153 is acceptable, however the results for NW1689 and NW1689 AG should be interpreted with caution (see notes above).

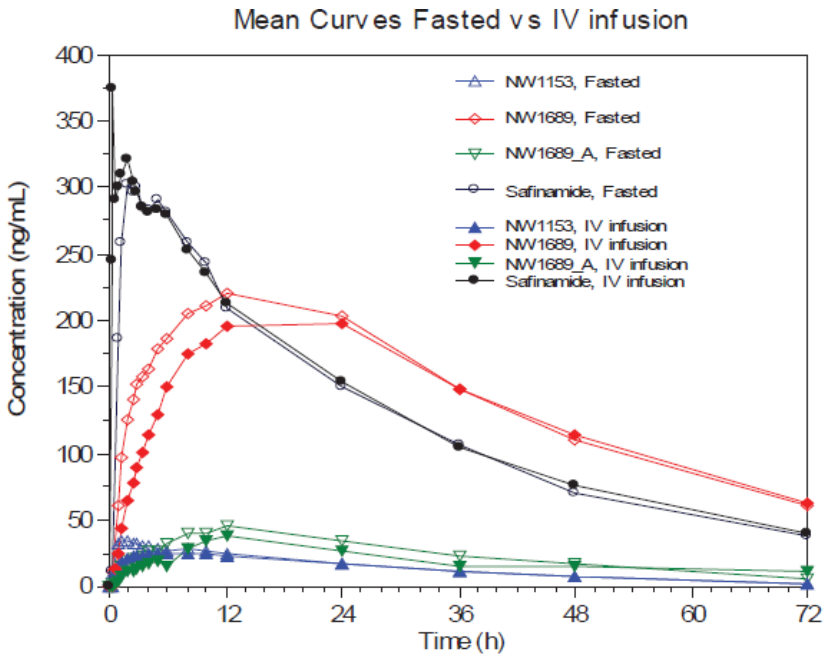
Pharmacokinetic Results

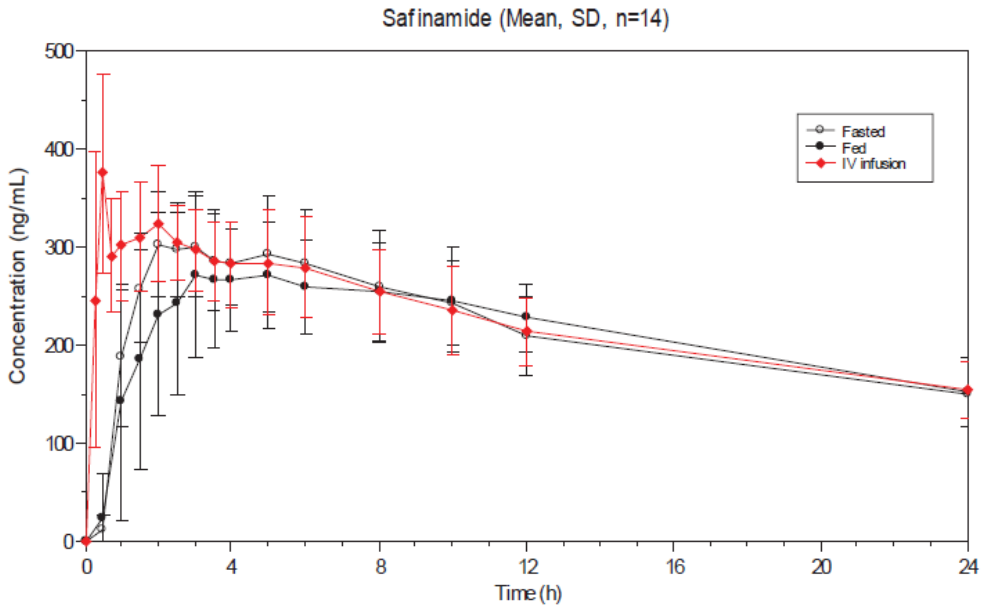
Mean plasma concentration-time curves for safinamide and its metabolites after Treatment A and B and after Treatment A and C in Figure are shown in the figures below.

Peak plasma concentrations of safinamide were observed 2.5 h (range 2.0-5.0 h) after administration of 50 mg oral safinamide to fasted subjects. In some of the PK profiles, a second peak in plasma concentrations was observed at around 5-6 hours. For 3 subjects, the second peak at 5 h defined C_{max} and t_{max}. Safinamide concentrations declined with quantifiable concentrations up to 120 h post-dose for most subjects (Subject number 13 had quantifiable concentration values up to 240 h post-dose).

Peak safinamide plasma concentrations were observed approximately 45 min later after a high-fat, high-calorie breakfast (median t_{max} 3.25 h, range 1.5-8.0 h) compared to the fasted administration and were at a slightly lower than after fasted administration (geometric C_{max} 306 ng/mL in fed state vs. 322 ng/mL in fasted state).


Mean Curves Fasted vs Fed
Concentration (ng/mL)
Time (h)
NW1153, Fasted
NW1153, Fed
NW1689, Fasted
NW1689, Fed
NW1689_A, Fasted
NW1689_A, Fed
Safinamide, Fasted
Safinamide, Fed


Mean Curves Fasted vs IV infusion
Concentration (ng/mL)
Time (h)
NW1153, Fasted
NW1689, Fasted
NW1689_A, Fasted
Safinamide, Fasted
NW1153, IV infusion
NW1689, IV infusion
NW1689_A, IV infusion
Safinamide, IV infusion



After start of infusion, safinamide plasma concentrations increased rapidly with measurable concentrations at 15 minutes post-start of infusion. Peak plasma concentrations were observed at the end of i.v infusion (median t_{max}= 30 min) for most subjects. They were approximately 30% higher than those observed after oral administration in fasted state. After the initial drop in concentration, the majority of subjects experienced increase in concentration. For four subjects (Subjects number 2, 10, 11, 13), this resulted in higher concentrations than observed at end of infusion.

Summary of Pharmacokinetic Parameters for Safinamide (n=14)

	Treatment A (Fasted)	Treatment B (Fed)	Treatment C* (IV Infusion)
C_{max} (ng/mL)	322.3 (17.9) 227.0 – 421.0	306.3 (21.9) 204.0 – 413.0	411.6 (18.8) 305.0 – 591.1
t_{max} (h)	2.5 2.0 – 5.0	3.25 1.5 – 8.0	0.5 0.25 – 5.0
AUC_{0-t} (ng/mL*h)	9811 (25.4) 6584 – 16737	9846 (25.5) 5862 – 14845	10340 (20.8) 7173 – 13815
$AUC_{0-\infty}$ (ng/mL*h)	10205 (26.0) 6779 – 17012	10230 (25.9) 6058 – 15107	10785 (22.0) 7314 – 14801
$t_{1/2}$ (h)	26.2 (19.2) 20.0 – 40.8	26.0 (17.9) 19.4 – 40.8	26.0 (11.3) 20.7 – 30.7
CL/f (L/h)	4.9 (26.0) 2.9 – 7.4	4.9 (25.9) 3.3 – 8.3	4.6 (22.0) 3.4 – 6.8
V_z/f (L)	185.0 (17.7) 142.9 – 260.5	183.7 (22.0) 132.8 – 292.2	174.1 (15.6) 146.6 – 222.7
F (%)	94.98 (9.25) 83.93 – 119.33	95.30 (10.0) 78.61 – 107.89	-

* In Treatment C, dose adjusted C_{max} and AUC are displayed.
For C_{max}, $AUC_{0-\infty}$, AUC_{0-t} , $t_{1/2}$, CL/f and V_z/f: Geomean (GeoCV %) and range (min-max)
For t_{max}: median and range (min – max)
For F: mean (CV%) and range (min – max)

Based on $AUC_{0\text{-}inf}$ after p.o. (fasted) and i.v. administration, absolute bioavailability (F) was determined to be 95% (range 84-119%). The high bioavailability of safinamide indicates that safinamide absorption is almost complete, therefore safinamide can be classified as highly permeable. In addition, high bioavailability shows that first pass metabolism of safinamide is negligible.
The geometric mean Vss of 165 L (Vz of 174 L) was found to be 2.3-fold of body volume (as approximated by mean body weight of 71 kg) indicating that safinamide is distributed well within the body. The geometric mean total clearance is found to be 4.6 L/h, classifying safinamide as a low clearance drug.

Summary of Pharmacokinetic Parameters for NW1153 (n=14)

	Treatment A (Fasted)	Treatment B (Fed)	Treatment C* (IV Infusion)
C_{max} (ng/mL)	37.2 (19.9) 25.1 – 49.2	26.9 (18.9) 21.4 – 41.1	27.0 (15.3) 21.2 – 35.6
t_{max} (h)	1.5 1.0 – 8.0	6.5 2.5 – 12.0	8.0 2.5 – 12.0
$AUC_{0\text{-}t}$ (ng/mL*h)	853 (14.4) 688 – 1174	747 (10.7) 622 – 902	794 (17.7) 506 – 1019
$t_{1/2}$ (h)#	20.5 (23.7) 12.9 – 31.9	21.3 (23.4) 14.7 – 31.2	22.5 (23.8) 14.8 – 31.0

* In Treatment C, dose adjusted C_{max} and $AUC_{0\text{-}t}$ are displayed.
For C_{max}, $AUC_{0\text{-}t}$, $t_{1/2}$: Geomean (GeoCV %) and range (min-max)
For t_{max}: median and range (min – max)
exploratory analysis; the terminal phase of NW1153 could not reliably be determined based on the detection limit of this analyte (for details refer to Section 11.5.2).

The terminal $t_{1/2}$ of NW1153 could not be reliably estimated in the current trial based on the bioanalytical detection limit (5 ng/ml). Nevertheless, the value obtained for the terminal $t_{1/2}$ is in line with the data observed in Study 28778 (geo mean $t_{1/2}$ of 19.5 h). The mean metabolic ratio of NW1153/safinamide based on $AUC_{0\text{-}t}$ was 0.088, therefore NW1153 is a minor metabolite. A small difference in the metabolic ratios of NW1153 was observed after i.v. and p.o administration; the mean metabolic ratios of NW1153 were 0.067 and 0.078 based on C_{max} and $AUC_{0\text{-}t}$, respectively and the corresponding ratios after p.o administration (fasted) were 0.120 and 0.088.

Summary of Pharmacokinetic Parameters for NW1689 (n=14)

	Treatment A (Fasted)	Treatment B (Fed)	Treatment C* (IV Infusion)
C_{max} (ng/mL)	223.9 (27.3) 168.0 – 343.0	227.5 (30.3) 152.0 – 378.0	214.5 (31.3) 144.0 – 392.0
t_{max} (h)	12.0 8.0 – 24.0	18.0 10.0 – 24.0	18.0 8.0 – 24.0
AUC_{0-t} (ng/mL*h)	11744 (40.5) 7849 – 24246	11697 (44.1) 7162 – 25042	11150 (52.0) 6352 – 27631
$AUC_{0-\infty}$ (ng/mL*h)	12296 (39.5) 8263 – 24924	12308 (42.4) 7867 – 25537	11721 (49.6) 6721 – 28447
$t_{1/2}$ (h)	31.1 (26.9) 22.9 – 48.1	31.6 (25.3) 21.2 – 46.2	32.9 (20.5) 24.9 – 45.0

* In Treatment C, dose adjusted C_{max} and AUC are displayed.

The formation of NW1689 occurred slowly: peak plasma concentrations were reached between 12-24 h. NW1689 concentrations declined mono-exponentially with measurable plasma concentrations up to 120 h (10 subjects) or 240 h (4 subjects) post-dose. Metabolic ratio based on AUC_{0-t} (mean 1.55) indicates that NW1689 is a major metabolite of safinamide.
Individual and mean PK profiles of NW1689 under Treatment B and C were comparable to those obtained under Treatment A. In addition, metabolic ratios based on AUC_{0-t} were similar regardless of the treatment.

Summary of Pharmacokinetic Parameters for NW1689 Acylglucuronide (n=14)

	Treatment A (Fasted)	Treatment B (Fed)	Treatment C* (IV Infusion)
C_{max} (ng/mL)	54.5 (32.3) 37.7 – 132.0	42.5 (38.3) 22.3 – 84.0	45.4 (41.1) 18.9 – 90.9
t_{max} (h)	12.0 6.0 - 24.0	11.0 5.0 - 48.0	10.0 4.0 - 48.0
AUC_{0-t} (ng/mL*h)	1790 (35.5) 1121 – 3894	1456 (41.0) 606 – 3083	1386 (79.7) 177 – 2881
$AUC_{0-\infty}$ (ng/mL*h)	1890 (35.1) 1289 – 3904	1557 (40.6) 689 – 3289	1440 (79.8) 181 – 2902
$t_{1/2}$ (h)	27.3 (24.4) 18.2 – 41.5	26.9 (40.7) 15.1 – 58.1	23.7 (37.8) 12.9 – 39.9

* In Treatment C, dose adjusted C_{max} and AUC are displayed.

Due to the indirect analysis method of NW1689-AG (see Bioanalytical assay notes), the NW1689-AG concentrations as well as the derived PK parameters should be interpreted with caution. This is reflected by the high variability in PK parameters observed (geometric CV% up to 80% for the main PK parameters).
The plasma concentration-time profile of NW1689-AG follows the profile of NW1689, the metabolite from which it is formed by glucuronidation. NW1689-AG showed similar t_{max} values as observed for NW1689 (between 12-24 h post-dose (median tmax 12 h), suggesting a rapid formation. The half-life of NW1689 acylglucuronide was around 27 h. Mean metabolic ratio for NW1689-AG was 0.13 and 0.14 based on C_{max} and AUC_{0-t}, respectively.

Ratios of Geometric Means with 90% Confidence Intervals for Primary PK Parameters of Safinamide in Plasma -fed/fasted

Parameter	Treatment	Least Squares Means	Ratio T/R and 90% confidence interval [%]
$AUC_{0-\infty}$ (h*(ng/mL))	Tablet fasted (R)	10205.28	100.24 (95.44 - 105.28)
	Tablet fed (T)	10229.68	
C_{max} (ng/mL)	Tablet fasted (R)	322.26	95.06 (89.21 - 101.30)
	Tablet fed (T)	306.35	

Ratios of Geometric Means with 90% Confidence Intervals for Primary PK Parameters of Safinamide in Plasma -oral/i.v.

Parameter	Treatment	Least Squares Means	Ratio T/R and 90% confidence interval [%]
$AUC_{0-\infty}$ (h*(ng/mL))	Tablet fasted (T)	10205.28	94.62 (90.09 - 99.39)
	Solution i.v. (R)	10785.15	

When analyzing the data by gender, a trend for higher exposure (C_{max} and AUC) and lower clearance and volume of distribution for safinamide and its metabolites was observed in females compared to males in all treatments, suggesting a slight gender effect (see Table below).

Descriptive Statistics of PK Parameters of Safinamide by Gender

PK Parameters following Oral Administration of 50 mg Safinamide under Fasted Conditions - by gender

MED	Analyte	SEX		Cmax (ng/mL)	Tmax (h)	AUClast (ng/mL*h)	AUCtot (ng/mL*h)	AUCextra (%)	Lz (1/h)	t1/2 (h)	MRT (h)	CL/f (L/h)	Vss/f (L)	Vz/f (L)
A	Safinamide	Female	N	7	7	7	7	7	7	7	7	7	7	7
			Mean	364.3	3.50	11273	11632	3.06	0.0273	26.6	37.0	4.5	161.3	166.3
			SD	35.7	1.44	2932	3009	1.42	0.0056	6.8	8.3	1.1	19.1	20.3
			%CV	9.8	41.2	26.0	25.9	46.4	20.5	25.7	22.4	24.2	11.8	12.2
			SEM	13.5	0.55	1108	1137	0.54	0.0021	2.6	3.1	0.4	7.2	7.7
			Min	313.0	2.00	7769	7960	1.59	0.0170	20.0	28.8	2.9	140.1	142.9
			Median	353.0	3.00	10127	10490	3.21	0.0281	24.7	34.4	4.8	156.7	172.8
			Max	421.0	5.00	16737	17012	5.73	0.0346	40.8	53.3	6.3	198.1	201.4
			GeoMean	362.8	3.24	10972	11319	2.80	0.0267	25.9	36.3	4.4	160.4	165.3
			Geo CV	9.8	44.9	25.2	25.4	48.3	23.2	23.2	20.8	25.4	11.4	12.1
			LCL 90% GM	337.6	2.36	9145	9421	2.00	0.0226	21.9	31.2	3.7	147.6	151.3
			UCL 90% GM	389.8	4.44	13163	13599	3.92	0.0316	30.7	42.2	5.3	174.3	180.5
		Male	N	7	7	7	7	7	7	7	7	7	7	7
			Mean	289.4	2.43	8946	9424	4.63	0.0265	26.7	37.7	5.6	203.2	209.1
			SD	46.6	0.35	1979	2330	2.38	0.0041	4.4	6.9	1.2	25.2	32.3
			%CV	16.1	14.2	22.1	24.7	51.5	15.4	16.6	18.3	21.8	12.4	15.4
			SEM	17.6	0.13	748	881	0.90	0.0015	1.7	2.6	0.5	9.5	12.2
			Min	227.0	2.00	6584	6779	2.17	0.0199	21.1	30.0	3.6	177.3	177.1
			Median	296.0	2.50	8611	8943	3.72	0.0273	25.4	36.1	5.6	201.9	200.0
			Max	365.0	3.00	12620	13892	9.15	0.0328	34.9	49.2	7.4	234.6	260.5
			GeoMean	286.3	2.41	8773	9201	4.18	0.0262	26.4	37.2	5.4	201.9	207.0
			Geo CV	16.1	14.4	21.3	23.5	51.1	16.1	16.1	18.0	23.5	12.4	15.2
			LCL 90% GM	254.5	2.17	7515	7761	2.93	0.0233	23.5	32.6	4.6	184.4	185.3
			UCL 90% GM	322.0	2.67	10241	10908	5.95	0.0295	29.7	42.4	6.4	221.1	231.4

In the population PK model, body weight was found to be a covariate factor of clearance and volume of distribution. Thus, lower clearance and volume of distribution observed in female subjects can be mainly explained with the lower mean body weight of female

subjects compared to male subjects (mean body weight of female subjects in this trial was 65 kg and of male subjects 77 kg).

Safety Results:

No deaths or SAEs were reported during the trial. No subject withdrew from the trial prematurely due to a TEAE.

No clinically relevant changes from baseline conditions were recorded in ECGs, vital signs, and physical examinations.

Twenty-five TEAEs were reported; of these, 7 were mild and 18 were moderate in intensity.

The most frequently reported TEAE was headache (n=14), and infusion site pain (n=2) (Treatment C only).

VI. EMR701165_021: A randomized, open-label, two-period crossover bioequivalence trial of two different oral tablets of 100 mg safinamide, utilizing different manufacturing processes, in healthy volunteers

This study will be reviewed by ONDQA.

APPEARS THIS WAY ON ORIGINAL

1.3 Intrinsic Factor PK Studies

VII. (b) (4) 28696: An open-label, parallel-group, single center, single oral dose study to investigate the pharmacokinetics of 50 mg safinamide in subjects with mild and moderate hepatic impairment as compared to matched subjects with normal hepatic function

Primary objective: To investigate the pharmacokinetics (PK) of safinamide in subjects with mild and moderate hepatic impairment as compared to a control group

Secondary objectives:

- To investigate the PK of the main safinamide metabolites (NW1689, NW1153) in subjects with hepatic impairment and in subjects with normal hepatic function.
- To evaluate the safety and tolerability of a single dose of safinamide in both populations
- To determine the unbound fraction of safinamide

Study Design	Open-label, parallel group, single center, single oral dose study
Study Population	24 male/female subjects with different degrees of hepatic function, 18 years to 75 years old with a BMI from 18 to 34 kg/m^2 All 24 subjects completed the study.
Treatment Groups	Trt Groups: ☐ 8 subjects with mild (Child-Pugh grade A) hepatic impairment. ☐ 8 subjects with moderate (Child-Pugh grade B) hepatic impairment. ☐ 8 subjects with normal hepatic function within the same range of age, weight and with about the same ratio of males/females as in the hepatic impairment subject groups.
Dosage and Administration	Each subject received a single dose of 50 mg safinamide (tablet) on Day 1 after an overnight fast, followed by a 10 day PK sampling.
PK Sampling	Plasma samples for safinamide and of its metabolites concentrations at: 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 120, 240 h after dosing. For determination of unbound fraction: 2.5, 4 and 24 h post dose. Urine samples were collected in the following time intervals for Safinamide, NW1153, NW1689 and NW1689-AG: pre-dose (0), 0-4, 4-8, 8-12, 12-24, and 24-48 h post dose
PK Analysis	LC-MS/MS assay for safinamide, NW-1153 and NW-1689 in plasma LC-MS/MS assay for safinamide and NW-1153 in urine LC-MS/MS assay for NW1689 and NW1689-AG in urine
PK Assessment	Primary: C_{max} and $AUC_{0-\infty}$ of safinamide Secondary: C_{max} and $AUC_{0-\infty}$ of safinamide metabolites (NW1689, NW1153); AUC_{0-t}, t_{max}, $t_{1/2}$, λz, Vz/f, CL/f, CL_R, CL_{NonR} /f, Ae, MRT for safinamide and its metabolites as appropriate Fraction unbound of safinamide
PD Assessment	none
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

Bioanalytical Assays

The concentrations of safinamide and its metabolites, NW-1153 and NW-1689 in human plasma samples were determined by LC-MS/MS. Safinamide, NW-1153 and NW-1689 and their corresponding internal standards (b) (4)) were extracted from plasma after protein precipitation with acetonitrile, the supernatant was injected onto a CN column, and detected with MS/MS. The analytical method (AS M-207, version 2) was validated prior to this project with a LLOQ of 5.00 ng/mL per analyte and an upper limit of quantification (ULOQ) of 10000 ng/mL per analyte with a 100 µL sample volume.

The performance of the assay during the analysis of study samples was satisfactory. Seven analytical runs out of eight were within the acceptance criteria for the analytical run when first analyzed. For one run safinamide and NW-1689 results were accepted, but NW-1153 was rejected, and reanalyzed. The mean accuracy of the assay as determined from the analysis of quality control (QC) samples was within ±8.7% for safinamide, ±9.5% for NW-1153 and within ±4.7% for NW-1689.

Determination of Free Concentration of Safinamide

The free (unbound) fraction safinamide in human plasma samples from study (b) (4) 28696 was determined by an HPLC method with MS/MS detection (method AS M-199, version 1). The free fraction of safinamide was obtained by equilibrium between plasma and a buffer solution via a dialysis membrane of 10KDa in cartridges of a 96-well format and injection onto a reversed phase LC column. This bioanalytical method was validated in the range 5.00-1000 ng/mL for safinamide with a 200 µL plasma sample volume.

The total concentration of safinamide in the plasma samples was determined as described above and the free concentration of safinamide was calculated from the free fraction and the total concentration.

The study samples were analyzed in one run. The calibration and QC samples were accepted according to the run acceptance criteria. A mandatory run for incurred sample reanalysis was also performed for 20 of the study samples. The run acceptance criteria were fulfilled for the analytical run and the mandatory run.

The concentrations of Safinamide and its metabolite NW-1153 in human urine were determined by liquid chromatography (LC) with tandem mass spectrometric (MS/MS) detection (method AS M-194, version 1). Safinamide, the metabolite and the two internal standards (b) (4)) in urine ware extracted by solid phase extraction (SPE), injected onto a reversed phase LC-column and detected with MS/MS. The method has been validated in the range 5.00-5000 ng/mL for safinamide and NW-1153 using a 200 µL sample volume.

The concentration of safinamide and NW-1153 were determined in a total of 143 human urine samples obtained from study (b) (4) 28696.

The performance of the assay during the analysis of study samples was satisfactory. All analytical runs fell within the run acceptance criteria. The mean accuracy of the assay as determined from the analysis of quality control (QC) samples was within ±5.1% for safinamide and within ±3.9% for NW-1153.

NW-1689 and NW-1689-1-O-glucuronide (NW-1689-AG) in human urine (Method DMPK 70-09): Urine was collected in polypropylene tubes and the total volume measured. 10 mL urine were transferred into 13 mL tubes and acidified with 210 mg citric acid monohydrate. Samples were stored frozen at -80°C until shipment and analysis. Stability of NW-1689 and NW-1689-AG in human urine at -20±5°C and -80±10°C was demonstrated for at least 6 months.
Sample analysis was performed using a validated HPLC-MS/MS method. Calibration and QC samples were prepared in blank acidified urine (0.1 M citric acid, i.e. 10.5 g citric acid will be added to 500 mL urine). Conversion from NW-1689-AG to NW-1689 was performed by the addition of 100 µL 0.5 M NaOH and incubation for at least 1 h at 50°C (Aliquots B).
A single analysis was performed for all study samples. Repeat analyses were performed for "Apparently incomplete conversion of glucuronides despite adherence to assay protocol". Samples were reanalyzed, for repeat analysis, volumes of NaOH were increased to achieve complete hydrolysis.

Reviewer's Comment: the assays' performance during the study sample analysis is acceptable.

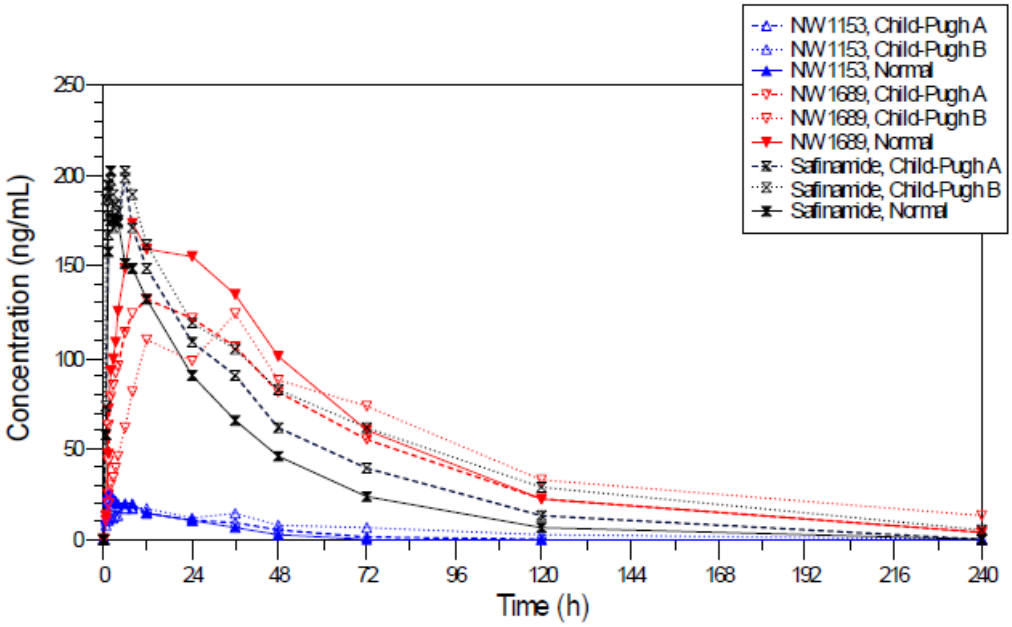
Pharmacokinetic Results

For healthy subjects, no concomitant medication, including multi-vitamins was allowed within 21 days prior to the drug administration, or within six elimination half-lives, whichever was longest, except combined oral contraceptives and occasional use of acetaminophen or ibuprofen within 14 days before study drug administration.
For subjects with mild and moderate hepatic impairment, no change in used medications was allowed within 7 days prior to safinamide administration and no treatment was allowed with any agent known to significantly inhibit or induce drug-metabolising enzymes (e.g., ketoconazole, barbiturates, phenothiazines, etc.) within 4 weeks preceding the screening visit.
All 24 subjects (16 male and 8 female) enrolled were treated according to protocol, and fulfilled the criteria for inclusion into the PK dataset. None of the subjects was excluded from PK analysis.
The bioanalytical method for the determination of NW1689 in plasma was not designed to distinguish between NW1689 and its acylglucuronide. Thus, the determination of NW1689 also includes NW1689-AG concentrations.

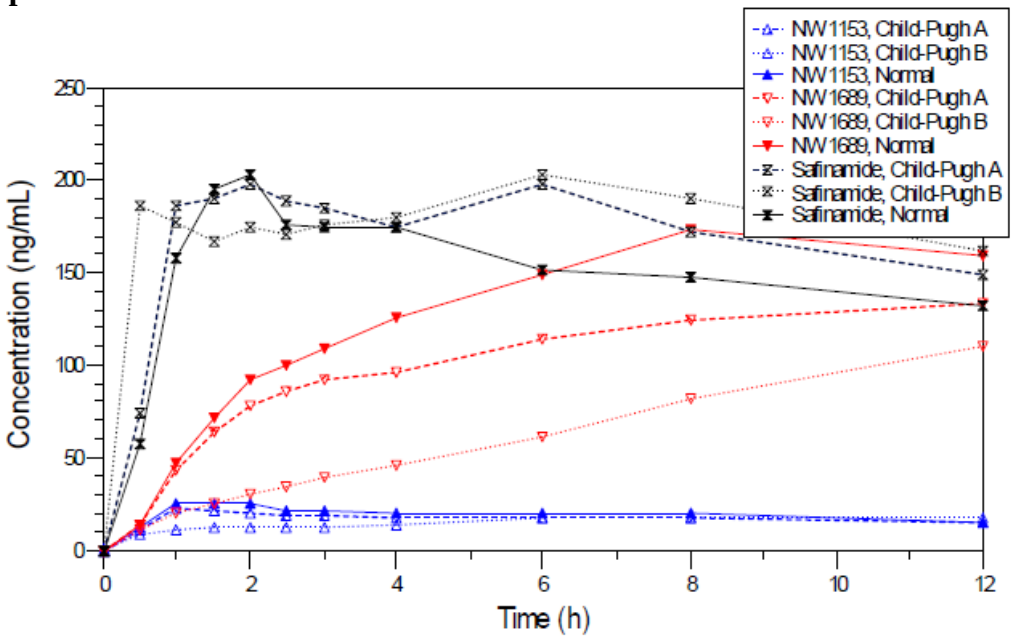
The non-compartmental PK evaluation of all individual concentration-time profiles resulted in reliably estimated PK parameters for safinamide and NW1689. For the metabolite NW1153 in 21 of the 24 PK profiles, the AUC_{extra} exceeded 20% of $AUC_{0-\infty}$ (AUC_{extra} values up to 47%), resulting in unreliable estimation of the terminal phase of NW1153.
Safinamide was rapidly absorbed in both hepatic impairment groups and in healthy subjects. However, higher plasma concentrations of safinamide were observed at the first sampling time point (30 minutes) in both hepatic impairment groups in comparison to healthy subjects [normal- mild- moderate (mean): 57.5 ng/mL - 73.3 ng/mL - 186

ng/mL]. A second concentration peak was observed around 6 h post-dose in both hepatic impairment groups (see figure below). For Child-Pugh B subjects, the second peak was slightly higher then the first peak and therefore the resulting median t_{max} was delayed (median t_{max} = 6 h) in comparison to the other groups. A second plasma concentration peak of safinamide around 6 h has also been described for healthy subjects in previous studies at dosages above 50 mg (e.g. Study 28778, EMR701165_021) and therefore might not be related to the hepatic status of the subjects.

Mean plasma concentration-time profiles for all analytes and for all groups



And up to 12 hours after safinamide administration



The total exposure (AUC) of safinamide increased with the degree of hepatic impairment, whereas the C_{max} values were comparable between the hepatic impairment subjects and healthy subjects. Longer half-lives and lower total body clearance were observed in subjects with hepatic impairment.

Summary of Pharmacokinetic Parameters of Safinamide

	Normal	Child-Pugh A	Child-Pugh B
C_{max} [ng/mL]	218.7 (33.0) 156 – 408	208.6 (36.0) 142 – 375	214.7 (38.1) 120 – 311
t_{max} [h]	1.75 (0.5 – 4.0)	2.00 (1.0 – 6.0)	6.0 (0.5 – 12.0)
$AUC_{0-\infty}$ [ng/mL*h]	6447.8 (14.9) 5322 – 7856	8520.0 (16.8) 6634 – 12176	11735.5 (25.6) 8828 – 19199
AUC_{0-t} [ng/mL*h]	6086.6 (13.4) 5019 – 7294	7986.2 (17.5) 6152 – 11546	10587.9 (27.6) 8135 – 18289
$t_{1/2}$ [h]	26.80 (21.4) 18.9 – 35.6	31.47 (22.4) 24.7 – 48.2	46.53 (22.1) 34.6 – 67.6
V_z/f [L]	299.8 (26.1) 174 – 413	266.5 (30.5) 161 – 417	286.0 (37.4) 176 – 450
CL/f [L/h]	7.75 (14.9) 6.36 – 9.40	5.87 (16.8) 4.11 – 7.54	4.26 (25.6) 2.60 – 5.66

For C_{max}, $AUC_{0-\infty}$, AUC_{0-t}, $t_{1/2}$, V_z/f and CL/f : Geometric mean (Geometric CV %) and range (min-max)

For t_{max} : median and range (min – max)

For NW-1153 overall, the plasma concentration curves are comparable between healthy and Child-Pugh A subjects. However, a slight decrease in C_{max} and delay in t_{max} was observed in Child-Pugh B subjects in comparison to healthy subjects indicating that the formation of this metabolite is slowed in this population.

Summary of Pharmacokinetic Parameters of NW1153

	Normal	Child-Pugh A	Child-Pugh B
C_{max} [ng/mL]	27.38 (35.2) 18.7 – 54.1	23.38 (33.3) 14.7 – 35.1	18.92 (28.2) 13.2 – 27.5
t_{max} [h]	1.25 (0.5 – 2.0)	1.00 (1.0 – 8.0)	10.00 (2.5 – 36.0)
AUC_{0-t} [ng/mL*h]	481.0 (35.4) 266 – 865	522.0 (48.8) 271 – 1053	810.8 (61.9) 342 – 1732
$t_{1/2}$ # [h]	22.37 (30.0) 14.0 – 31.8	34.10 (43.7) 15.1 – 52.0	48.56 (50.1) 24.5 – 102.8

The formation of NW1689 appeared to be reduced in subjects with hepatic impairment as indicated by a decrease in NW1689 C_{max}. Individual and mean plasma concentration curves indicated a slower formation rate of NW1689 in comparison to healthy subjects. Terminal $t_{1/2}$ was prolonged by approximately 20 h in Child-Pugh B subjects compared to controls indicating that the elimination of this metabolite is also affected.

Summary of Pharmacokinetic Parameters of NW1689

	Normal	Child-Pugh A	Child-Pugh B
C_{max} [ng/mL]	175.6 (26.8) 119 – 255	120.06 (60.5) 63.8 – 240.0	108.64 (75.0) 36.6 – 291.0
t_{max} [h]	16.00 (8.0 – 24.0)	12.0 (2.0 – 24.0)	12.00 (12.0 – 36.0)
$AUC_{0-\infty}$ [ng/mL*h]	10981.5 (41.3) 5036 – 17272	8126.3 (76.4) 3870 – 21073	9841.7 (75.8) 4317 – 33509
AUC_{0-t} [ng/mL*h]	10311.1 (42.6) 4731 – 16758	7493.2 (82.7) 3324 – 20730	8953.4 (74.4) 3863 – 27691
$t_{1/2}$ [h]	35.42 (26.8) 24.6 – 57.1	37.32 (31.0) 25.8 – 65.5	56.08 (27.2) 36.1 – 80.7

ANOVA Results for Ratio Mild Impairment (Child-Pugh A) / Healthy Subjects and Moderate Impairment (Child-Pugh B) / Healthy Subjects of Main PK Parameters for Safinamide and its Metabolites NW1153 and NW1689

Analyte	Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
Safinamide	$AUC_{0-\infty}$	Mild imp./Healthy	132.14	111.81 - 156.16
		Moderate imp./Healthy	182.01	154.01 - 215.10
	C_{max}	Mild imp./Healthy	95.40	70.80 - 128.54
		Moderate imp./Healthy	98.16	72.85 - 132.27
	$t_{1/2}$	Mild imp./Healthy	117.44	97.44 - 141.56
		Moderate imp./Healthy	173.62	144.04 - 209.27
	V_z/f	Mild imp./Healthy	88.88	68.14 - 115.93
		Moderate imp./Healthy	95.39	73.13 - 124.42
	CL/f	Mild imp./Healthy	75.68	64.03 - 89.44
		Moderate imp./Healthy	54.94	46.49 - 64.93
NW1689	$AUC_{0-\infty}$	Mild imp./Healthy	74.00	44.26 - 123.73
		Moderate imp./Healthy	89.62	53.60 - 149.84
	C_{max}	Mild imp./Healthy	68.38	43.53 - 107.43
		Moderate imp./Healthy	61.88	39.39 - 97.21
NW1153	AUC_{0-t}	Mild imp./Healthy	108.53	72.60 - 162.26
		Moderate imp./Healthy	168.58	112.76 - 252.03
	C_{max}	Mild imp./Healthy	85.40	65.11 - 112.01
		Moderate imp./Healthy	69.10	52.69 - 90.63

A summary of safinamide unbound fraction per scheduled time and group is presented in the Table below.

Mean (SD) and Range (n=8) of Safinamide Fraction Unbound (%)

Scheduled time	Normal	Child-Pugh A	Child-Pugh B
2.5 h	11.0 (1.2) 9.0 – 13.0	9.9 (1.8) 7.5 – 13.4	12.8 (4.8) 7.8 – 19.9
4 h	10.7 (0.9) 9.6 – 12.3	9.6 (1.9) 6.8 – 12.8	12.5 (3.7) 7.7 – 18.0
24 h	10.5 (1.0) 9.0 – 12.2	9.6 (1.9) 7.0 – 12.9	12.4 (3.1) 7.9 – 17.1

Hepatic impairment did not affect safinamide unbound fraction as indicated by the comparable mean values for safinamide protein binding in all three groups. A marginal increase (2%) in safinamide fraction unbound was observed in Child-Pugh B subjects which is not considered clinically relevant.

Pharmacokinetics of Safinamide and its Metabolites in Urine

Summary of Urine Pharmacokinetic Parameters of Safinamide, NW1153, NW1689 and NW1689-AG per Group

	Normal	Child-Pugh A	Child-Pugh B
Safinamide			
Ae_{0-48} (% dose)	6.6 (39.4) 4.6 – 12.9	4.0 (38.0) 2.2 – 5.8	2.9 (76.8) 1.0 – 7.4
CL_R # (L/h)	0.715 (52.8) 0.360 – 1.707	0.364 (50.6) 0.147 – 0.659	0.244 (74.1) 0.095 – 0.608
CL_{NonR} /f (L/h)	7.0 (12.5) 6.0 – 8.4	5.5 (15.4) 4.0 – 6.9	4.0 (26.7) 2.3 – 5.2
NW1153			
Ae_{0-48} (% dose)	22.5 (17.8) 17.3 – 29.2	16.2 (58.8) 7.1 – 26.1	13.8 (49.9) 7.8 – 27.4
CL_R # (L/h)	23.4 (37.0) 12.5 – 42.9	16.0 (72.9) 4.2 – 31.7	11.7 (91.1) 4.8 – 40.1
NW1689			
Ae_{0-48} (% dose)	0.032 (43.7) 0.02 – 0.07	0.098 (40.1) 0.06 – 0.16	NC (NC) 0.00 – 0.26
CL_R (L/h)	0.0025 (57.0) 0.001 – 0.006	0.011 (70.2) 0.005 – 0.036	NC (NC) 0.000 – 0.076
NW1689 acylglucuronide			
Ae_{0-48} (% dose)	11.6 (27.0) 7.1 –18.5	13.9 (38.1) 7.1 – 22.0	7.7 (29.1) 5.3 – 12.2

For Ae_{0-48}, CL_R and CL_{NonR} /f: Geometric mean (Geometric CV %) and range (min-max)

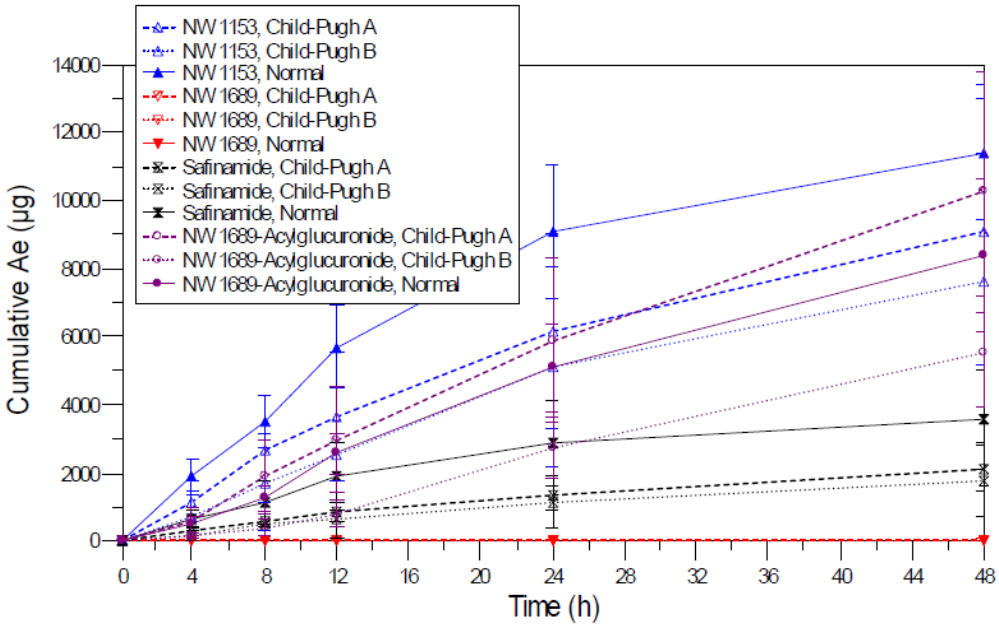
NC, not calculated

#, based on Ae_{0-48} / AUC_{0-48}

CL_{NonR} = Non-renal appearance

Safinamide was excreted unchanged in urine to low extent (6.6%) within a period of 48 h. Overall, a decrease in total dose recovered within the 48-h collection interval was observed in hepatic impairment subjects in comparison to healthy subjects (Child-Pugh A: 34.2%, Child-Pugh B: 24.4%).
However, the relative levels were unchanged in the hepatic impairment groups: NW1153 was the major metabolite recovered in urine, followed by NW1689-AG and safinamide while NW1689 was recovered in urine in negligible amounts (<0.1%).
In line with the amount excreted in urine, renal clearance of safinamide decreased in Child-Pugh A and Child-Pugh B subjects. In addition, non-renal clearance decreased in hepatic impairments subjects. The decrease in non-renal and renal clearance (and total clearance) is in line with the observed increase in AUC in the hepatic impairment groups.

Mean Cumulative Amounts Excreted in Urine for all Analytes and for all Groups within the Collection Period of 0-48 h



PK Conclusions:
The results of this study demonstrated that PK of safinamide and its metabolites is affected by the degree of hepatic impairment. However, the marginal increase in exposure of safinamide in mild hepatic subjects (about 30% increase in AUC) should not be clinically relevant based on safinamide safety profile. No dose adjustment is needed for subjects with mild hepatic impairment.
In Child-Pugh B subjects, exposure of safinamide increased about 80% (90% CI: 154-215%), therefore patients with moderate hepatic impairment need to be treated with the lower of the two therapeutic safinamide doses (50 mg).
Safinamide pharmacokinetics were not evaluated in subjects with severe hepatic impairment (Child-Pugh 10-15 points) and safinamide is not recommended for this patient population.

Safety Results:

Eight adverse events occurred during the trial in 6 subjects, 7 of them (in 5 subjects) were treatment-emergent. Only one of the seven treatment-emergent adverse events, myalgia in a 64-year-old female subject with moderate hepatic impairment, was assessed as possibly related to safinamide. One serious adverse event (SAE), dilative cardiomyopathy in a 52 year-old male subject, occurred during the study, which was accompanied by electrocardiogram (ECG) changes such as QT prolongation and ST-T change. This SAE was considered to be due to the underlying disease of arterial hypertension, diabetes mellitus type II and ethyl toxic liver cirrhosis.
No impact of safinamide on safety laboratory parameters, vital signs and ECG parameters was observed. Individual abnormal values were related to the underlying disease in the subjects with mild or moderate hepatic impairment.

APPEARS THIS WAY ON ORIGINAL

VIII. EMR701165_025: An open-label, parallel-group, single-center, single oral dose trial to investigate the pharmacokinetics (PK) of 50 mg safinamide in subjects with moderate and severe renal impairment compared to matched subjects with normal renal function

Primary objective: To investigate the pharmacokinetics (PK) of safinamide in subjects with moderate and severe renal impairment as compared to a control group

Secondary objectives:

- To investigate the PK of the main safinamide metabolites (NW1689, NW1689-AG, NW1153) in subjects with renal impairment and in subjects with normal renal function.
- To evaluate the safety and tolerability of a single dose of safinamide in both populations
- To determine the unbound fraction of safinamide

Study Design	Open-label, parallel group, single center, single oral dose study
Study Population	24 male/female subjects with different degrees of renal function: 9 subjects with moderate renal impairment, 7 subjects with severe renal impairment, 8 matched healthy subjects; 38 years to 75 years old with a BMI from 20 to 35 kg/m^2 All 24 subjects completed.
Treatment Groups	☐9 subjects with moderate renal impairment* ☐7 subjects with severe renal impairment* ☐8 matched healthy subjects (same range of age, weight and with about the same ratio of males/females as in the hepatic impairment subject groups)
Dosage and Administration	Each subject received a single dose of Film-coated 50 mg safinamide tablet on Day 1 after an overnight fast.
PK Sampling	Plasma samples for safinamide and of its metabolites concentrations at: 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 120, 168, 216, 264** h after dosing. For determination of unbound fraction: 2.5, 4 and 24 h post dose. Urine samples were collected in the following time intervals: pre-dose (0), 0-4, 4-8, 8-12, 12-24, 24-48 and 48-72 h post dose
PK Analysis	LC-MS/MS assay for safinamide and NW-1153 in plasma LC-MS/MS assay NW-1689 and NW1689-AG in plasma LC-MS/MS assay for safinamide and NW-1153 in urine LC-MS/MS assay for NW1689 and NW1689-AG in urine
PK Assessment	Primary: C_{max} and $AUC_{0-\infty}$ of safinamide in plasma Secondary: C_{max} and $AUC_{0-\infty}$ of safinamide metabolites (NW1689, NW1153); AUC_{0-t} , t_{max} , $t_{1/2}$, λz , Vz/f , CL/f , CL_R , CL_{NonR} /f, Ae , MRT for safinamide and its metabolites as appropriate Fraction unbound of safinamide
PD Assessment	none
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

* Male and female subjects with moderate (eGFR 30-59 mL/min [inclusive]) and severe renal impairment (eGFR < 30 mL/min, not requiring dialysis).

**The last blood sample was taken at the End-of-Trial Visit, between 264 and 312 hours after dosing

Bioanalytical Assays

Safinamide, NW-1153 and NW-1689 (before and after) in Human Plasma

The concentrations of safinamide and its metabolite NW-1153 in human plasma (lithium heparin) and NW-1689 and it's potentially formed glucuronides (in acidified human plasma /lithium heparin) were determined using LC-MS/MS. Acidified plasma samples from the clinical trial EMR701165_025 were analyzed for NW-1689 before and after conversion of glucuronides into NW-1689.

The methods using lowered calibration ranges 0.5-500 ng/mL, have been validated at (b) (4) [current versions of (b) (4) HB-11-030-V2 (Safinamide and NW-1153) and HB-11-028-V3 (NW-1689 before and after)].

The methods use 25 µL of plasma for Safinamide and NW-1153 and 100 µL of plasma for NW-1689 (before and after).

Long term stability has previously been assessed in human plasma samples for up to 22 months at nominal -20°C for Safinamide and NW-1153 and for up to 205 days at nominal -20°C for NW-1689 (before and after) in acidified human plasma samples.

A total of 464 plasma samples were analyzed in seven reported batches for Safinamide, eight batches for NW-1153 and nine batches for NW-1689 (before) and eight batches for NW-1689 (after).

Calibration standard data, QC sample data, incurred sample reproducibility data (ISR) and chromatograms indicate that the methods performed acceptably during the study sample analysis.

Safinamide free fraction in plasma samples

The non-protein bound (unbound) concentration of safinamide in dialyzed buffer samples obtained from human plasma samples of study EMR701165_025 was determined using equilibrium dialysis with LC-MS/MS detection.

The method has been validated at (b) (4) ((b) (4) HB-11-059).

The method had a lower limit of quantification (LLOQ) of 5 ng/mL and used 200 µL of plasma for equilibrium dialysis and 100 µL of the dialyzed buffer for analysis.

Long term stability of safinamide in human plasma samples stored for 22 months at nominal -20°C has previously been assessed under (b) (4) Study Number P-172/04.

A total of 72 plasma samples were successfully analyzed in two reported batches. All samples were analyzed within the established stability period of 22 months at -20°C.

The concentration of unbound Safinamide ranged between 5.54 ng/mL to 21.1 ng/mL.

There was one sample where the observed concentration was below the LLOQ.

The calibration data, QC data and ISR data indicate that the method performed satisfactorily during the analyses of all reported batches of study samples. All reported batches complied with the calibration and QC sample acceptance criteria.

The analytical procedure used for the Safinamide and NW-1153 in urine has been validated at (b) (4) ((b) (4) HB-11-074). The assay had a LLOQ of 5.00 ng/mL for Safinamide and NW-1153 using 50 µL of urine.

A total of 168 urine samples were analyzed for safinamide and NW-1153 in three batches and one incurred sample reproducibility data (ISR) batch. Calibration standard data, QC

sample data, ISR and chromatograms indicate that the method performed acceptably during the study sample analysis.

The metabolite NW-1689 and its acylglucuronides (NW-1689-AG) in urine samples were quantified using a validated bioanalytical assay with LC-MS/MS detection (MOP/AP) DEV/NCE-MSC2226765A-03-03D. Calibration standards and quality control samples were prepared in pooled acidified human urine. Conversion from NW-1689-AG to NW-1689 was performed by the addition of 100 µL 0.5 M NaOH and incubation for at least 1 h at 50°C (Aliquots B). The LOQ of NW-1689/NW-1689 converted in urine was 10 ng/mL.
Study samples were analyzed in 16 batches. Results of the QC samples:
For Aliquot A the overall accuracy range was from 103% to 112% for all QC levels. Overall precision for all QC levels was ≤7.87%.
For Aliquot B the overall accuracy range was from 93.1% to 96.8% for all QC levels. Overall precision for all QC levels was ≤8.36%.

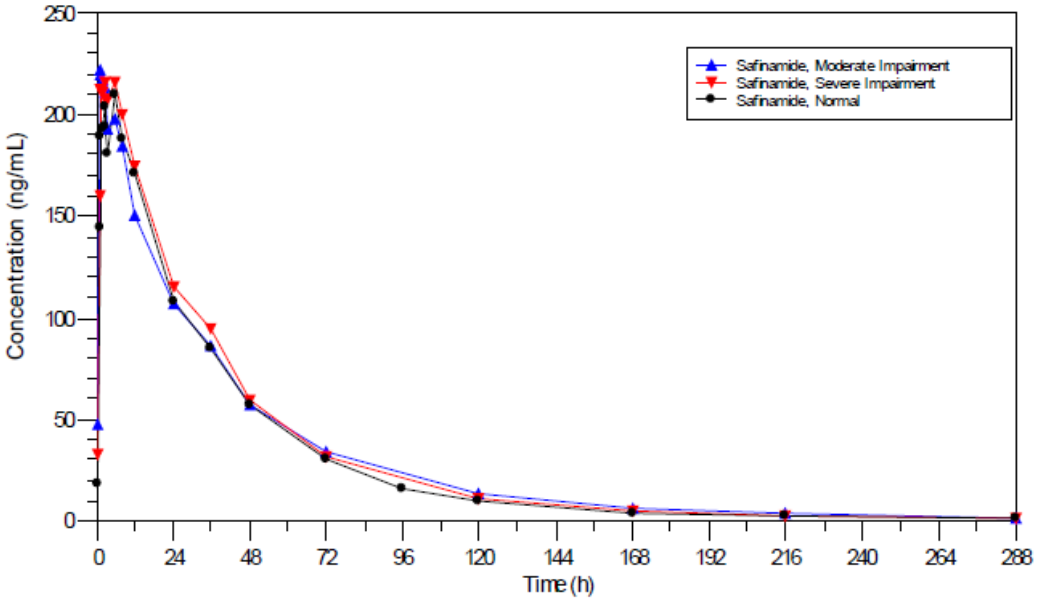
Reviewer's Comment: the assays' performance during the study sample analysis is acceptable.

Pharmacokinetic Results

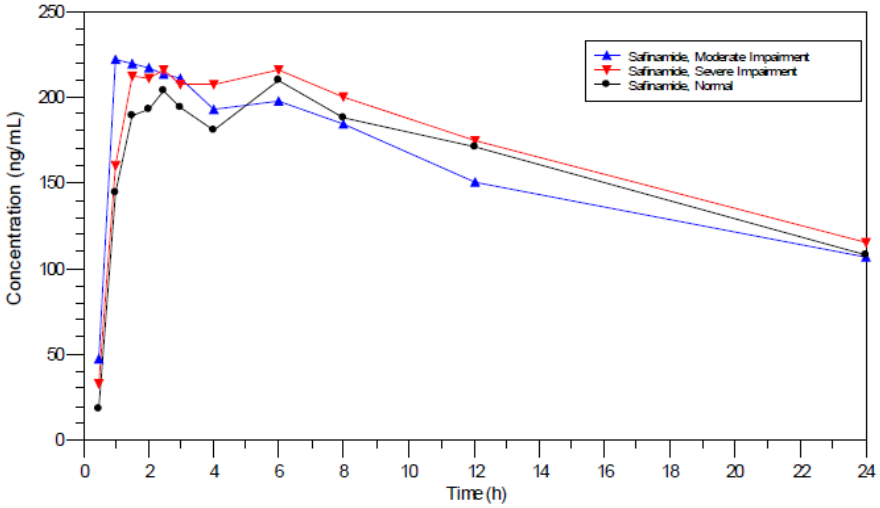
For healthy subjects, no concomitant medication, including multi-vitamins was allowed within 21 days prior to the drug administration, or within six elimination half-lives, whichever was longest, except combined oral contraceptives and occasional use of paracetamol or ibuprofen within 14 days before study drug administration.
For subjects with renal impairment, no change in used medications was allowed within 14 days prior to safinamide administration. No treatment was allowed with drugs affecting gastric acid secretion, such as proton pump inhibitors and antacids, within 48 hours prior to drug administration and for 24 hours after administration.
All 24 subjects (19 male and 5 female) enrolled were treated; none of the subjects was excluded from the PK analysis.

Pharmacokinetics of Safinamide and its Metabolites in Plasma
Mean plasma concentration-time profiles for safinamide in healthy subjects and subjects with moderate and severe renal impairment are shown in the figures below (PK profiles up to 288 hours and up to 24 hours, linear scale).

Plasma Pharmacokinetic Parameters of Safinamide Following a Single Dose of 50-mg Safinamide Administered to Patients with Renal Impairment and to Healthy Matched Control Subjects



Mean Plasma Concentration-Time Profiles for Safinamide for all Groups (up to 24 h post dose)



Overall, mean plasma concentration-time profiles of safinamide were similar between all three groups: moderate and severe renal impairment and healthy subjects with normal renal function.

Safinamide was rapidly absorbed; as observed in previous trials, plasma concentration profile showed two peaks: the first at around 1 to 1.5 hours and the second at around 6 hours. Depending which peak was highest, t_{max} across groups ranged from 1 hour to 4.25 hours; accordingly the apparent shorter median t_{max} in subjects with moderate renal impairment compared to the other two groups unlikely reflects a true effect.

In line main safinamide PK parameters C_{max} and $AUC_{0-\infty}$ were comparable across the three groups, i.e. moderate renal impairment, severe renal impairment and normal renal function.

Summary of Pharmacokinetic Parameters of Safinamide in Plasma

	Normal	Moderate renal impairment	Severe renal impairment
C_{max} [ng/mL]	237.8 (17.0) 173 - 308	247.6 (24.7) 164 - 353	238.6 (16.9) 187 - 284
t_{max} [h]	4.25 (1.0 - 8.0)	1.50 (1.0 - 3.0)	4.00 (1.5 - 8.0)
$AUC_{0-\infty}$ [ng/mL*h]	8235.8 (14.9) 7093 - 10056	8516.1 (23.8) 5689 - 12053	8734.1 (18.1) 6579 - 10605
AUC_{0-t} [ng/mL*h]	8140.3 (15.0) 7024 - 9940	8396.2 (23.5) 5639 - 11939	8635.2 (18.0) 6518 - 10462
$t_{1/2}$ [h]	62.91 (12.5) 47.8 - 70.2	55.05 (29.1) 29.6 - 72.0	56.46 (23.6) 37.8 - 76.6
V_z/f [L]	551.0 (16.4) 476 - 701	466.3 (29.1) 264 - 620	466.3 (17.4) 351 - 594
CL/f [L/h]	6.07 (14.9) 4.97 - 7.05	5.87 (23.8) 4.15 - 8.79	5.72 (18.1) 4.71 - 7.60

ANOVA Results for Ratio Moderate Versus Normal and Severe Versus Normal of Main Pharmacokinetic Parameters for Safinamide Derived From Plasma

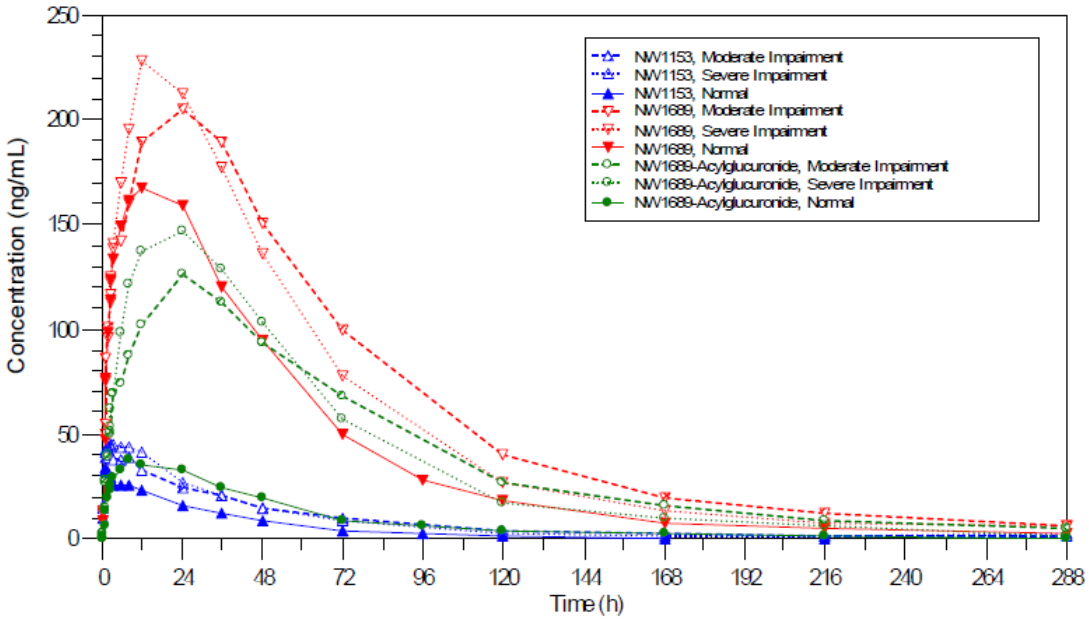
Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
$AUC_{0-\infty}$	Moderate imp./normal	103.40	87.95 - 121.57
	Severe imp./normal	106.05	89.26 - 126.01
C_{max}	Moderate imp./normal	104.09	88.04 - 123.07
	Severe imp./normal	100.32	83.93 - 119.91

imp=impairment

The analysis in plasma demonstrated that safinamide PK is not affected by renal function.

Pharmacokinetics of Safinamide Metabolites in Plasma

Mean Plasma Concentration-Time Profiles for Metabolites NW-1153, NW-1689 and NW-1689 acylglucuronide and for all Groups



Summary of Pharmacokinetic Parameters of NW-1153 in Plasma

	Normal	Moderate renal impairment	Severe renal impairment
C_{max} [ng/mL]	34.85 (27.5) 24.0 - 55.7	45.94 (16.6) 38.3 - 63.0	48.64 (24.4) 34.0 - 75.8
t_{max} [h]	1.25 (1.0 - 2.0)	1.50 (1.0 - 8.0)	4.00 (2.0 - 12.0)
$AUC_{0-\infty}$ [ng/mL*h]	1176.6 (20.6) 1001 - 1756	2041.6 (32.7) 1159 - 3400	2105.4 (21.5) 1557 - 2851
AUC_{0-t} [ng/mL*h]	1142.9 (20.7) 963 - 1698	1984.9 (33.4) 1095 - 3265	2064.8 (21.7) 1528 - 2807
$t_{1/2}$ [h]	38.33 (14.6) 31.8 - 50.6	43.71 (31.7) 28.6 - 74.4	41.06 (20.6) 30.6 - 53.5

ANOVA Results for Ratio Moderate Versus Normal and Severe Versus Normal of Main Pharmacokinetic Parameters for NW-1153 Derived From Plasma

Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
$AUC_{0-\infty}$	Moderate imp./normal	173.52	140.08 - 214.94
	Severe imp./normal	178.94	142.46 - 224.77
C_{max}	Moderate imp./normal	131.80	109.08 - 159.26
	Severe imp./normal	139.56	114.09 - 170.72

NW-1153 $AUC_{0-\infty}$ and C_{max} were increased in subjects with moderate or severe renal impairment compared to healthy subjects. There were no major differences in NW-1153 exposure between subjects with moderate renal impairment and subjects with severe renal impairment.

Summary of Pharmacokinetic Parameters of NW-1689 in Plasma

	Normal	Moderate renal impairment	Severe renal impairment
C_{max} [ng/mL]	166.3 (29.2) 99 - 274	198.3 (55.5) 92.6 - 458	226.5 (40.4) 138 - 405
t_{max} [h]	10.00 (8.0 - 24.0)	24.00 (4.0 - 36.0)	12.00 (8.0 - 24.0)
$AUC_{0-\infty}$ [ng/mL*h]	10729.3 (26.7) 6607 - 16058	15375.8 (57.7) 8931 - 45672	14001.6 (53.3) 7523 - 32580
AUC_{0-t} [ng/mL*h]	10534.5 (26.7) 6466 - 15848	14877.4 (57.9) 8700 - 44027	13781.1 (53.3) 7448 - 32165
$t_{1/2}$ [h]	68.41 (18.5) 44.9 - 80.4	68.99 (19.9) 47.6 - 81.4	53.29 (15.8) 40.9 - 66.0

ANOVA Results for Ratio Moderate Versus Normal and Severe Versus Normal of Main Pharmacokinetic Parameters for NW-1689 Derived From Plasma

Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
$AUC_{0-\infty}$	Moderate imp./normal	143.31	98.23 - 209.06
	Severe imp./normal	130.50	87.28 - 195.11
C_{max}	Moderate imp./normal	119.21	84.20 - 168.77
	Severe imp./normal	136.21	94.06 - 197.25

Increase in NW-1689 $AUC_{0-\infty}$ and C_{max} was observed in subjects with moderate or severe renal impairment compared to healthy subjects. There were no major differences in NW-1689 exposure between subjects with moderate renal impairment and subjects with severe renal impairment.

A pronounced increase in NW-1689 acylglucuronide $AUC_{0-\infty}$ and C_{max} was observed in subjects with moderate or severe renal impairment compared to healthy subjects. There was no major difference in NW-1689 AG exposure between subjects with moderate renal impairment and subjects with severe renal impairment.
The ANOVA analysis of the treatment ratios (moderate vs. normal, severe vs. normal) for NW-1689-AG $AUC_{0-\infty}$ and C_{max} confirmed the pronounced effect of renal impairment on NW-1689 AG exposure. There were 3-fold to 4-fold increase for C_{max} and 4-fold to 5-fold increase for $AUC_{0-\infty}$.

Summary of Pharmacokinetic Parameters of NW-1689-AG in Plasma

	Normal	Moderate renal impairment	Severe renal impairment
C_{max} [ng/mL]	42.56 (24.9) 30.9 - 65.2	123.2 (45.8) 70.3 - 249	156.1 (16.5) 118 - 197
t_{max} [h]	10.00 (6.0 - 12.0)	24.00 (4.0 - 48.0)	24.00 (12.0 - 36.0)
$AUC_{0-\infty}$ [ng/mL*h]	2224.3 (22.5) 1596 - 3118	9539.0 (66.6) 3658 - 32823	10317.2 (28.5) 7032 - 14714
AUC_{0-t} [ng/mL*h]	2198.0 (22.2) 1580 - 3052	9236.3 (65.3) 3513 - 30480	10115.0 (27.2) 6980 - 13912
$t_{1/2}$ [h]	53.25 (23.3) 40.3 - 75.5	59.28 (23.9) 35.5 - 74.7	46.16 (25.7) 36.8 - 66.6

ANOVA Results for Ratio Moderate Versus Normal and Severe Versus Normal of Main Pharmacokinetic Parameters for NW-1689-AG Derived From Plasma

Pharmacokinetic parameter	Ratio	Point estimate [%]	90% Confidence interval [%]
$AUC_{0-\infty}$	Moderate imp./normal	428.86	301.14 - 610.75
	Severe imp./normal	463.84	318.29 - 675.95
C_{max}	Moderate imp./normal	289.39	222.05 - 377.15
	Severe imp./normal	366.73	276.59 - 486.26

Unbound Fraction of Safinamide

A summary of the unbound fraction of safinamide per scheduled time and group is provided in the table below.

Mean (SD) and Range (min – max) of the Unbound Fraction of Safinamide (%)

Scheduled Time	Normal (n=8)	Moderate renal impairment (n=9)	Severe renal impairment (n=7)
2.5 h	7.9 (0.8) 7.2 - 9.7	7.9 (1.3) 6.5 - 10.6	7.9 (1.2) 6.0 - 9.7
4 h	8.5 (1.7) 7.1 - 12.4	8.0 (1.5) 4.9 - 9.5	7.8 (0.7) 6.3 - 8.4
24 h	7.5 (0.5) 6.7 - 8.1	6.8 (2.8) 0.0 - 9.7	7.2 (0.7) 6.1 - 8.2

In healthy subjects, the mean fraction unbound of safinamide was approximately 8%, independent of the sampling times. This value is in line with the reported unbound

fraction (11%) from in vitro trials. The mean values for the unbound fraction in subjects with moderate or severe renal impairment were similar to those observed in healthy subjects with normal renal function. Renal impairment does not appear to have any effect on safinamide unbound fraction. Therefore, the PK parameters were not estimated based on unbound concentrations.

Pharmacokinetics of Safinamide and its Metabolites in Urine
Subjects No 0010 and 0018 (severe renal impairment), 0011 (moderate renal impairment), and 0023 (normal renal function) had NW-1689 concentrations in urine below LOQ at all sampling time points. In accordance to the planned analysis, these subjects were excluded from geometric mean calculation of PK parameters in urine.
In addition, Subject No. 0005 (moderate renal impairment) had remarkably higher NW-1689 concentrations at all quantifiable sampling time points compared to the other subjects in the same group.
Descriptive statistical analyses of NW-1689 in urine are presented with and without these subjects.

Summary of Urine Pharmacokinetic Parameters of Safinamide, NW-1153, NW-1689, and NW-1689-AG per Group

	Normal	Moderate renal impairment	Severe renal impairment
Safinamide			
Ae_{0-72} (% dose)	4.8 (55.6) 1.9 - 8.5	4.3 (54.6) 2.5 - 10.4	4.9 (22.6) 3.5 - 7.4
CL_R (L/h)	0.357 (44.5) 0.176 - 0.597	0.325 (65.5) 0.167 - 0.805	0.348 (15.8) 0.281 - 0.465
CL_{NonR}/f (L/h)	5.7 (16.6) 4.6 - 6.7	5.5 (23.5) 4.0 - 8.3	5.4 (19.3) 4.4 - 7.3
NW-1153			
Ae_{0-72} (% dose)	17.6 (19.6) 12.0 - 22.2	10.2 (59.0) 2.9 - 16.8	6.9 (40.2) 4.3 - 12.2
CL_R (L/h)	9.3 (33.8) 4.6 - 13.1	3.4 (78.7) 0.7 - 6.3	2.1 (42.4) 1.2 - 3.9
NW-1689			
Ae_{0-72} (% dose)	0.10 (104.3) # 0.03 - 0.21 #	0.24 (73.0) ## 0.10 - 0.51 ##	0.31 (25.8) ### 0.23 - 0.43 ###
CL_R (L/h)	0.007 (125.1) # 0.002 - 0.020 #	0.013 (78.5) ## 0.005 - 0.037 ##	0.016 (58.2) ### 0.009 - 0.037 ###
NW-1689 acylglucuronide			
Ae_{0-72} (% dose)	19.2 (12.8) 15.2 - 23.4	16.3 (40.3) 8.9 - 25.3	15.7 (20.8) 12.1 - 19.7
CL_R (L/h)	5.9 (26.3) 3.5 - 8.5	1.3 (91.5) 0.3 - 3.2	1.1 (46.8) 0.6 - 1.8

Source: Table 15.4.6
For Ae_{0-72}, CL_R, CL_{NonR}/f: Geometric mean (Geometric CV %) and range (min – max)
CL_{NonR} = Non-renal clearance
#, Subject No. 0023 excluded from analysis
##, Subjects Nos. 0005 and 0011 excluded from analysis
###, Subjects Nos. 0010 and 0018 excluded from analysis

In subjects with normal renal function, approximately 41.7% of a dose was recovered within 72 hours post-dose, mainly as safinamide metabolites (4.8% parent compound, 37.0% as metabolites).
In subjects with renal impairment, the recovery of parent compound was similar to that of subjects with normal renal function: 4.3% in subjects with moderate renal impairment, and 4.9% in subjects with severe renal impairment.
Cumulative recovery of the metabolites was lower in subjects with renal impairment: 26.7% and 22.9% of the dose were recovered as metabolites within 72 hours post-dose in subjects with moderate and severe renal impairment, respectively.

Overall, urinary excretion of the metabolites NW-1153, NW-1689, and NW-1689-AG in subjects with renal impairment compared to subjects with normal renal function was in line with the observed effect of renal impairment on plasma exposure of these metabolites. Only for NW-1689, an apparent increase in urinary excretion was noticed in the mean cumulative amounts excreted within 72 hours. However, given the very low levels of NW-1689 in urine and bioanalytical assays limited sensitivity (a few subjects had to be excluded from the descriptive statistics of NW-1689 PK in urine due to levels below LOQ), it is unlikely that this observation reflects a true effect.

Safety Results

Six subjects reported 6 TEAEs of mild or moderate intensity (2 subjects with normal renal function, 1 subject with moderate renal impairment, 3 subjects with severe renal impairment). Of the 6 reported TEAEs, 4 were gastrointestinal disorders (nausea, diarrhea, vomiting, and stomatitis). No TEAEs related to central nervous system disorders were reported.

All TEAEs were assessed as unrelated to treatment.

No SAEs, and no TEAEs leading to discontinuation occurred.

No clinically relevant changes in safety laboratory values, ECG parameters or vital signs were observed. Individual abnormal values were mostly related to the underlying diseases of subjects with moderate or severe renal impairment.

No dose adjustment is needed for renal impaired pts. Dialysis patients have not been studied (not recommended).

Since exposure of safinamide metabolites was increased in subjects with moderate or severe renal impairment compared to healthy subjects, additional analysis was requested by the sponsor. The discussion is provided in the QBR.

1.4 Extrinsic Factor PK Studies

IX. EMR701165_027: A randomized, double-blind, placebo-controlled, two-period, two-sequence-crossover interaction trial to assess the effect of safinamide on levodopa pharmacokinetics in elderly healthy volunteers

Objectives:

Primary Objective: To investigate a possible effect of safinamide on levodopa (LD) PK at steady state.

Secondary Objectives:

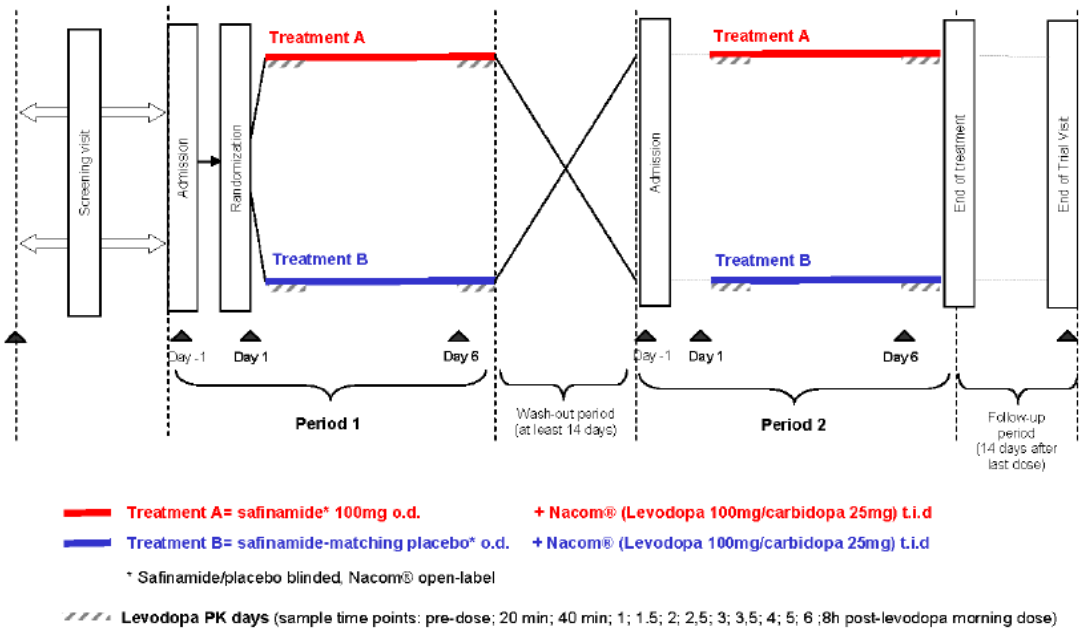
- To investigate a possible effect of safinamide on LD PK after single dose.
- To assess the safety and tolerability of safinamide when given together with LD in the applied regimen.

Study Design	randomized, double blind, placebo-controlled, two-period, two-sequence-crossover study
Study Population*	24 healthy male (12) and female (12) subjects*, 55-80 years of age, BMI 18-32 kg/m^2; all 24 completed the study
Treatment Groups (for details see Trial Design below)**	Treatment A: Safinamide, Film-coated tablets, 100 mg + Levodopa/carbidopa (LD/CD 100 mg/25 mg) (Nacom®) Immediate release tablets Treatment B: Safinamide-matching placebo + Levodopa/carbidopa (LD/CD 100 mg/25 mg) (Nacom®) Immediate release tablets
Dosage and Administration**	Safinamide / placebo: once daily in the morning from Day 1 to Day 6, in each period, according to randomization. Levodopa/carbidopa: t.i.d. (q8h) from Day 1 to Day 5, one administration on Day 6 morning in each period. The morning trial medications were administered under fasting conditions on Day 1 and Day 6 with 240 mL water
PK Sampling: plasma	Plasma samples were collected for the PK assessments of safinamide (Pre-dose / 2 h after safinamide dose) and LD**
Analysis (Plasma)	LC-MS/MS method for LD, Range: 10 to 2000 ng/mL LC-MS/MS method for safinamide, Range: 5 to 1000 ng/mL
PK Assessment	Primary: C_{max} and AUC_{0-8} for LD on Day 6 of the two periods Secondary: t_{max}, $t_{1/2}$, AUC_{0-tmax} of LD on Day 1 and Day 6 of the two periods; $C_{predose}$ of safinamide.
PD Assessment	none
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

* Subjects were equally randomized to both sequences. There was a balanced ratio of male and female subjects in both sequences.

** Trial Design

No food, drink or beverage containing alcohol, grapefruit or grapefruit juice from 72 h prior to drug administrations until last PK sampling was allowed. Caffeine and xanthine from 48 h prior to drug administration until discharge were not allowed. No co-medications, except for acetaminophen, contraceptives, hormone replacement therapy, L-Thyroxin replacement therapy, antihypertensive treatment (single agent), were allowed within 2 weeks of the study and during the study.



Bioanalytical Assays

Levadopa (Bioanalytical Study Number 0155-2011):

Human plasma concentrations of LD were determined using a validated LC-MS-MS assay in the calibration range of 10 - 2000 ng/mL.

Twenty-eight batches were run for this study. All analytical batches, from which data are reported, met acceptance criteria.

Results of the incurred samples reproducibility test for LD: Seventy-four values out of eighty (92.5%) presented an RE% within the acceptance limit of ±20%. Six values presented a %RE higher than 20% but lower than 30%. As the percentage of the reassay results with %RE < ±20% was higher than 67%, the method has been judged reproducible for the analysis of LD.

%RE = ((Reanalysis – Original)/((Original + Reanalysis)/2)) * 100

Safinamide (Bioanalytical Study No RF3981):

Safinamide was measured in plasma by a validated LC-MS-MS assay.

Eight calibration samples at different levels (5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0, 1000.0 ng/mL) were analyzed in each run to establish the calibration curve. All mean values of the calibration curve points related to CV% and BIAS% were within ± 15.0%.

The intra-assay BIAS% was from -7.3% to 8.0% for the QC-Low, from -5.6% to 8.0% for QC-Medium and from 2.2% to 10.5% for the QC-High.

The inter-assay BIAS% was -0.7%, -0.3% and 6.2% for the Low, Mid and High QC respectively.

Incurred Sample Reanalysis (ISR) test successfully passed (90.0% of samples fulfilled the acceptance criteria).

Reviewer's Comment: The bioanalytical assays for LD and Safinamide and the performance of these assays during the analysis of the PK samples are acceptable.

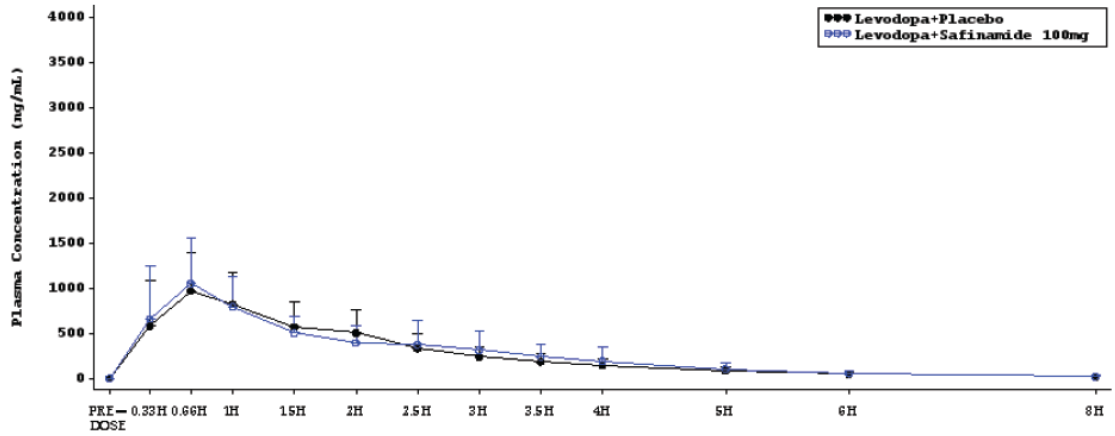
Pharmacokinetic Results:

All subjects completed the study. Only minor protocol deviations (minor delays in PK blood sampling, in the timing of the study treatment administration, isolated missing PK samples, etc.) were observed, which had no impact on the conduct of the study or on the analysis of data.

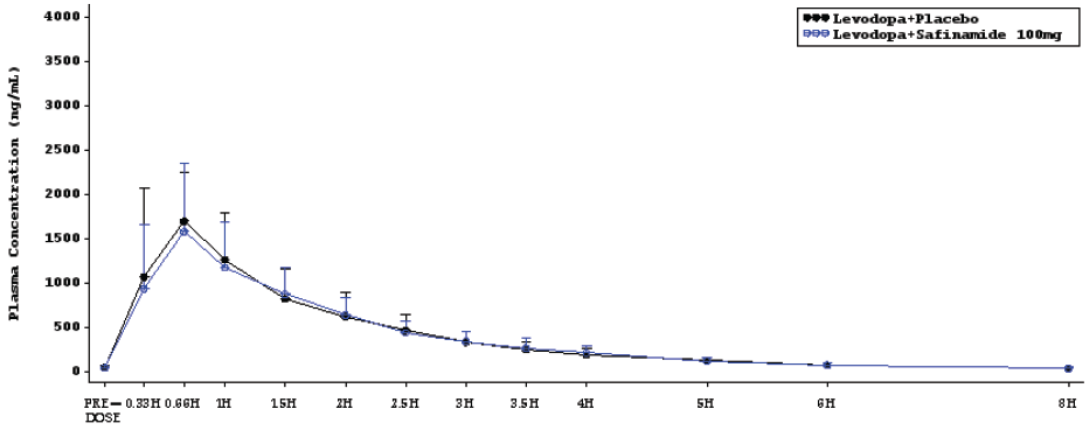
Levadopa

Mean concentration-time curves for levodopa on Day 1 and 6 are shown below.

LD: Day 1



LD: Day 6



Overall, mean LD concentration profiles obtained after co-administration with safinamide and after co-administration with placebo were super-imposable, both after single dose and steady state.

The PK parameters of levodopa on Day 1 and Day 6 were comparable between the two treatments. In addition, LD pharmacokinetics in this trial is consistent with the literature data in healthy volunteers [Khor SP, Hsu A. The pharmacokinetics and pharmacodynamics of levodopa in the treatment of Parkinson's disease. Curr Clin Pharmacol 2007 Sep;2(3):234-43].

ANOVA results comparing C_{max} and AUC_{0-8} of LD between safinamide 100 mg and placebo, on both Day 1 and Day 6, are presented below.

Descriptive Statistics of Pharmacokinetic Parameters in Plasma of LD by Day and Treatment (PK Population)

	Treatment A (levodopa + safinamide 100 mg) (n=24)	Treatment B (levodopa + placebo) (n=24)
Day 1		
C_{max} (ng/mL)	1196.7 (34.1) 510 - 1940	1149.9 (27.8) 788 - 1820
AUC_{0-8} (ng/mL*h)	2054.28 (29.1) 1124.0 - 3297.9	1968.09 (27.6) 1220.2 - 3082.7
AUC_{0-tmax} (ng/mL*h)	340.29 (58.1) 100.7 - 884.0	395.83 (61.4) 174.9 - 1331.9
t_{max} (h)	0.660 0.33 - 2.50	0.660 0.33 - 2.00
$t_{1/2}$ within a 8-h interval (h)	1.334 (12.6) 1.04 - 1.83	1.359 (9.7) 1.06 - 1.58
Day 6		
C_{max} (ng/mL)	1652.2 (40.3) 687 - 2880	1883.3 (34.5) 805 - 3750
AUC_{0-8} (ng/mL*h)	2891.14 (28.1) 1798.9 - 4172.3	2958.15 (26.7) 1994.0 - 4328.4
AUC_{0-tmax} (ng/mL*h)	631.00 (48.8) 266.4 - 1208.2	561.28 (46.2) 255.6 -1243.9
t_{max} (h)	0.660 0.33 - 2.00	0.660 0.33 - 2.00
$t_{1/2}$ within a 8-h interval (h)	1.502 (11.6) 1.20 - 1.85	1.497 (6.1) 1.34 - 1.70

For C_{max}, AUC_{0-8}, AUC_{0-tmax}, $t_{1/2}$: Geomean (GeoCV%) and range (min-max)

For t_{max}: median and range (min – max)

Comparison of C_{max} and AUC_{0-8} of Levodopa on Day 1 and Day 6 between Safinamide 100 mg and Placebo (PK Population)

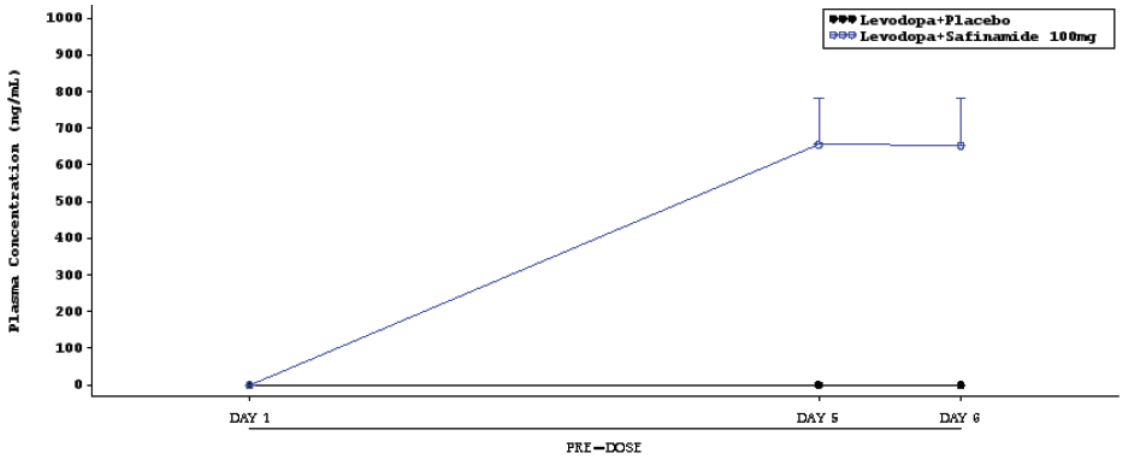
	Least Squares Geometric Mean		Ratio Estimate [%] (90% CI)
	Levodopa + safinamide	Levodopa + placebo	
$C_{max\ DAY\ 1}$ (ng/mL)	1197	1150	104.1 (94.1;115.1)
$AUC_{0-8\ DAY\ 1}$ (ng.h/mL)	2054.3	1968.1	104.4 (99.6;109.4)
$C_{max\ DAY\ 6}$ (ng/mL)	1652	1883	87.7 (77.6;99.1)
$AUC_{0-8\ DAY\ 6}$ (ng.h/mL)	2891.1	2958.1	97.7 (93.3;102.4)

The primary parameters C_{max} and AUC_{0-8} of LD on Day 1 and Day 6 were in 90% CIs for the treatment ratios (levodopa + safinamide 100mg/ levodopa + placebo) lying within the boundaries of clinically acceptability (0.75-1.33), that were pre-defined based on known safety profile and PK variability of levodopa. For $AUC_{0-8\ DAY\ 1}$, $AUC_{0-8\ DAY\ 6}$ and

$C_{max\ DAY\ 1}$ the common BE criteria (90% CI within the range of 0.8 to 1.25) were met. The comparison of safinamide 100 mg and placebo showed no difference in LD t_{max} (Hodges-Lehman shift estimates for the difference safinamide 100mg and placebo and the corresponding 90% CIs according to Tukey for Day 1 and Day 6).
Conclusion: The PK results of this study show that safinamide does not affect levodopa pharmacokinetics.

Safinamide
The mean predose concentration-time curves for safinamide are shown below.

Figure 11-3 **Mean (+SD) Predose Plasma Concentration of Safinamide (PK Population)**



Conclusion: Observed predose concentrations of safinamide after repeated doses on Day 5 and Day 6 is consistent with previous data for safinamide. Additionally, upon visual inspection, attainment of steady state was reached by Day 6.

Safety Results
The TEAEs were more frequent in the treatment LD co-administration with safinamide, compared to the LD co-administration with placebo treatment: 24 TEAEs in 12 (50.0%) subjects versus 10 TEAEs in 7 (29.2%) subjects, respectively.
A majority of TEAEs were from the nervous system disorders (dizziness, headache, presyncope): 8.3% subjects receiving levodopa with placebo treatment, 10 TEAEs in 8 (33.3%) subjects receiving LD with safinamide treatment and gastrointestinal disorders: 1 (4.2%) subject receiving LD with placebo treatment, 11 TEAEs in 7 (29.2%) subjects receiving LD with safinamide treatment. All AEs were of mild to moderate intensity.
According to the sponsor, this TEAE profile is consistent with the known profile of LD and safinamide and, given the nature of AEs and mild to moderate intensity, the imbalance in frequency between the groups is considered not clinically relevant.
Reviewer's Comment: This needs to be confirmed by the MO.

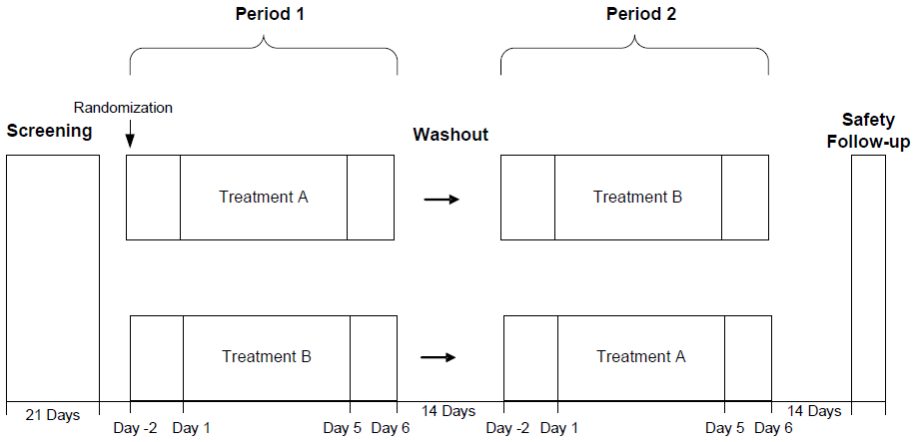
X. Study 28780: A randomised, double-blind, placebo-controlled, two-period, two-sequence-crossover interaction study to assess the effect of safinamide on levodopa pharmacokinetics in subjects with Parkinson's disease (PD)

Objectives:
Primary: to investigate the effect of safinamide on the PK of LD, both after single and steady state dosing of safinamide.
Secondary: to assess the safety and tolerability of safinamide when given together with LD in the applied regimen.

Study Design	randomized, double-blind, placebo-controlled, two-period, two-sequence, cross-over trial in patients with idiopathic PD
Study Population*	24 patients with PD (10 females and 14 males), treated with a stable dose of LD/CD*; the mean (SD) age of all subjects was 64.2 (9.11) years. 23 subjects completed treatment with safinamide and 22 subjects completed treatment with placebo, in the respective periods
Treatment Group	Two 6-day treatment periods separated by a washout period of at least 14 days. Each subject received both treatments: Treatment A: safinamide 100 mg once daily administration for 6 days + levodopa Treatment B: placebo matching safinamide once daily administration for 6 days + levodopa
Dosage and Administration	Safinamide 100 mg p.o. once daily administration 6 days Nacom® immediate release formulation (100 mg levodopa +25 mg carbidopa) **
PK Sampling	For LD, CD and LD metabolites: Day-1 and Day 5: pre-dose sample before each LD dose Day 1 and Day 6 (PK profile): pre- dose; 20 min; 40 min; 1; 1.5; 2; 2.5; 3; 3.5; 4; 5; 6 hours (= prior to second levodopa dose) For safinamide and its metabolites: prior (within 30 min) to safinamide dose on Day 5 as well as on the PK days, i.e. Day 1 and Day 6 pre-safinamide dose
Analysis	LD, CD and LD metabolites (including dopamine [DA] homovanillic acid [HVA], dihydroxyphenylacetic acid [DOPAC], 3-O-methyl-dopa [3OMD])
PK Assessment	Primary: C_{max} and AUC_{0-6} of LD on Day 1 and Day 6 Secondary: t_{max}, $t_{1/2}$ within a 6-h interval, AUC_{0-tmax} of LD on Day 1 and Day 6 C_{max}, AUC_{0-6}, $t_{1/2}$ within a 6-h interval, t_{max} of CD and LD/dopamine metabolites, including HVA, DOPAC and 3OMD on Day 1 and Day 6
PD Assessment	none
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

* treated with a stable dose of levodopa/decarboxylase-inhibitor and are not taking any other MAO-B inhibitor nor any drug causing dopamine release or affecting levodopa metabolism.

** On the main PK days 1 and 6, Nacom® dose was administered in the morning after an overnight fast with the last LD dose on the previous evening at approximately 18:00 (Day -1 and 5, respectively). Thereafter, PK samples were taken for 6 hours before the second dose of LD.



Bioanalytical Assays:

Plasma concentrations of levadopa were determined using a validated LC-MS/-MS method; methods for determination of plasma concentrations of carbidopa, DA, 3MT, DOPAC and HVA were partially validated.

Calibration ranges were 10-2000 ng/mL for LD, 5-500 ng/mL for DA and 3MT and 50-2000 ng/mL for CD, DOPAC and HVA.

The incurred samples reanalysis (ISR) for LD did not pass acceptance criteria. Levodopa samples were not stable under the storage conditions provided in the lab manual. Accordingly, there was no possibility for reanalysis the samples using another method. Due to analytical problems, no concentration data are reported for carbidopa. Due to extensive matrix effects observed from endogenous plasma, calibration curves and QCs of almost all batches of CD failed indicating a poor reliability of this method for this analyte. Therefore, no concentration data for CD are reported.

For dopamine, DOPAC and 3-MT, the number of samples with measurable concentrations was low and not sufficient to perform PK evaluation.

Reviewer's Comment: The performance of the assay for LD and CD during the analysis of the study samples is not acceptable. These bioanalytical limitations (failed incurred sample analysis/unknown LD stability in actual clinical samples) must be taken into account when interpreting the PK results.

Plasma concentrations of safinamide and its metabolites were determined using a validated LC-MS/MS method. The lower limit of quantification (LLOQ) was 5.00 ng/mL for safinamide, NW-1153 and NW-1689. Concentrations of NW-1689 may be slightly overestimated as the samples were not acidified in this trial, such that likely a proportion of NW-1689 acylglucuronide had been degraded to NW-1689.

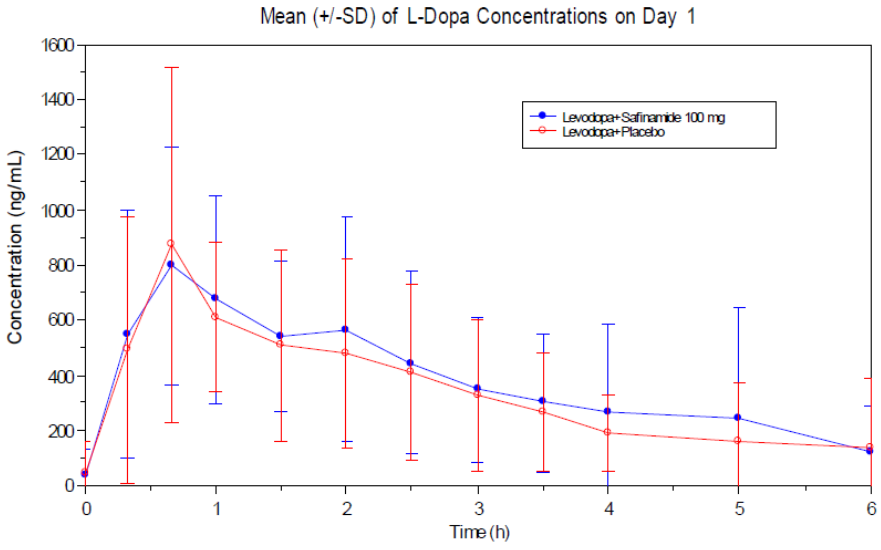
In all but one subject, pre-dose concentrations of safinamide, NW-1153 and NW-1689 on Day 1 in Period 1 were below LLOQ.

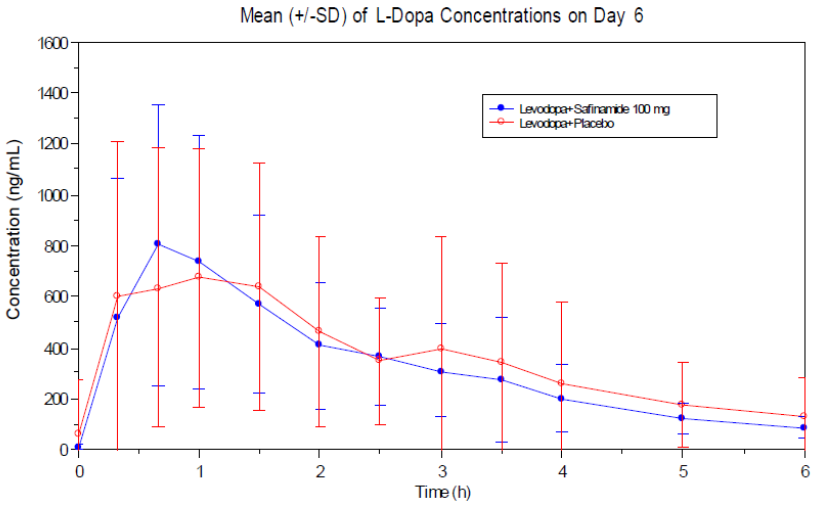
For Subject 001-0007, pre-dose concentrations of safinamide (401 ng/mL), NW-1153

(40.5 ng/mL) and NW-1689 (433 ng/mL) were measured on Day 1 Period 1 and confirmed by repeated analysis. No reasonable explanation for these pre-dose concentrations could be found.
In Period 2, pre-dose concentrations of safinamide, NW-1153 and NW-1689 on Day 1 were below LLOQ for all subjects having taken placebo in Period 1. Among subjects who had taken safinamide in Period 1 (i.e., 10 subjects remaining in Period 2; two subjects discontinued in between periods in this treatment sequence), measurable concentrations of safinamide were found in 3 subjects, while measurable concentrations of NW-1689 were found in 8 subjects. For both analytes, these concentrations were in the low range of 9 ng/mL for safinamide and 6-40 ng/mL for NW-1689. Given the wash-out of at least 14 days in between periods in this trial, this observation suggests a longer terminal $t_{1/2}$ in some subjects (than the reported mean $t_{1/2}$ of 23h for safinamide and 32 h for NW-1689 in other trials).

Pharmacokinetic Results:
For 6 subjects across the two periods, full PK profile was done on Day 5 (3 cases) or on Day 7 (3 cases) instead of Day 6. Given that all assessments scheduled as per protocol on Day 6, including PK sampling time points, were respected on these days and safinamide is already at steady state on Day 5, this was classified as minor protocol deviation.

Mean concentration-time profiles for LD up to 6 hours after Treatment A (levodopa + safinamide) and B (levodopa + placebo) on Day 1 and on Day 6 are shown below.



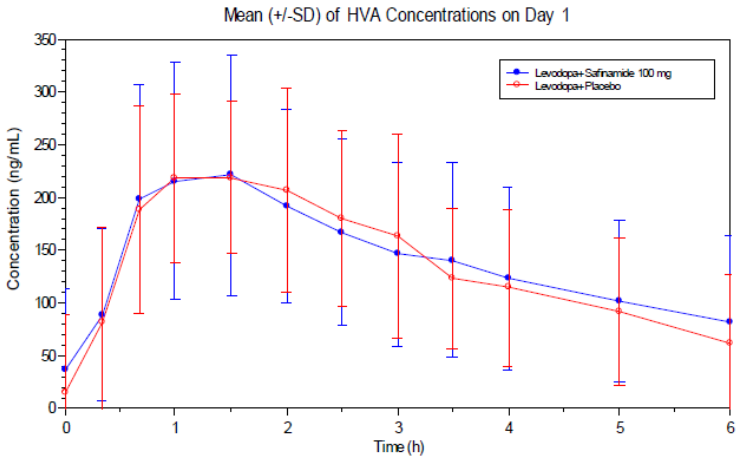


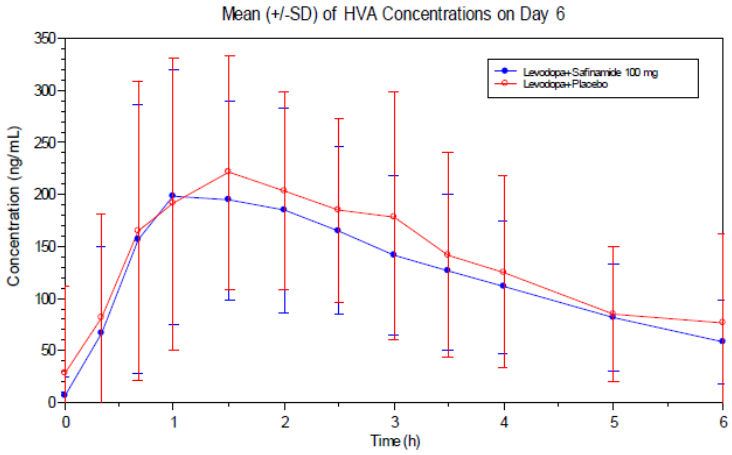
Levodopa PK parameters by Treatment on Day 1 and Day 6

	Treatment A (levodopa + safinamide 100 mg) (n=23*)	Treatment B (levodopa + placebo) (n=22*)
Day 1		
C_{max} (ng/mL)	984.4 (58.2) 149.0 – 2000.0	964.0 (61.2) 206.0 – 3100.0
AUC_{0-6} (ng/mL*h)	2008.5 (65.8) 300.6 – 5047.3	1773.5 (69.5) 262.9 – 4231.2
AUC_{0-tmax} (ng/mL*h)	404.0 (141.0) 24.6 – 4097.3	324.2 (133.0) 41.1 – 3861.1
t_{max} (h)	0.67 (0.33 – 6.00)	0.67 (0.33 – 6.00)
$t_{1/2}$ within a 6-h interval (h)	1.59 (33.88) 0.83 – 4.36	1.53 (22.13) 0.91 – 2.36
Day 6		
C_{max} (ng/mL)	940.7 (70.4) 212.0 – 1990.0	943.0 (66.8) 172.0 – 2330.0
AUC_{0-6} (ng/mL*h)	1794.5 (54.3) 453.1 – 3841.9	1915.5 (59.5) 459.8 – 5417.2
AUC_{0-tmax} (ng/mL*h)	466.4 (66.7) 155.2 – 1567.7	383.3 (86.4) 129.8 – 1472.8
t_{max} (h)	1.00 (0.33 – 3.5)	0.67 (0.33 – 5.00)
$t_{1/2}$ within a 6-h interval (h)	1.55 (26.26) 1.00 – 2.49	1.56 (38.56) 0.85 – 2.90

For C_{max}, AUC_{0-6}, AUC_{0-tmax}, $t_{1/2}$: Geomean (GeoCV %) and range (min-max)

The mean concentration-time profiles for HVA up to 6 hours after Treatment A (levodopa + safinamide) and B (levodopa + placebo) on Day 1 and on Day 6 are shown below.





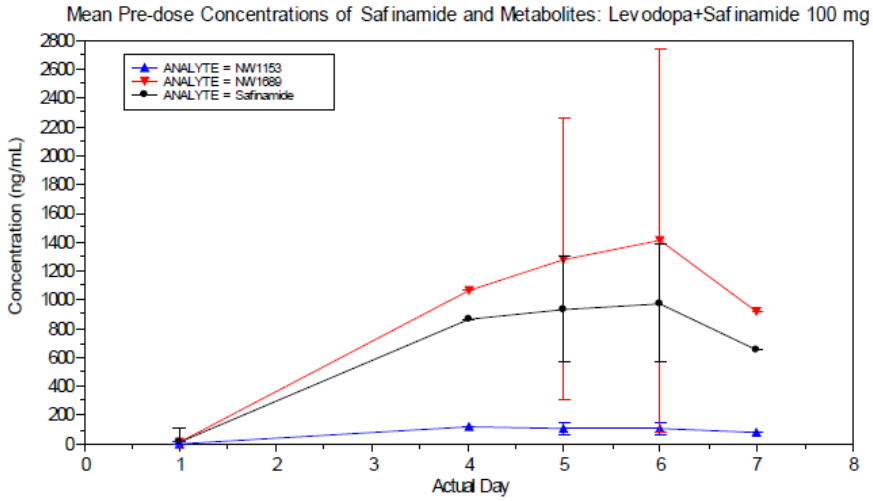
Overall, mean plasma concentration-time profiles for LD main metabolite HVA on Day 1 and Day 6 up to 6h after dosing were similar in both treatments A and B.
Both on Day 1 and on Day 6, peak plasma concentrations of HVA were observed between 1h and 1.5h after LD administration.
C_{max} and AUC_{0-6} of HVA up to 6h after LD administration were comparable in Treatment A and Treatment B, and no difference was noticed between Day 1 and Day 6, see table below).

HVA PK Parameters by Treatment on Day 1 and Day 6

	Treatment A (levodopa + safinamide 100 mg) (n=23*)	Treatment B (levodopa + placebo) (n=22*)
Day 1		
C_{max} (ng/mL)	241.7 (44.2) 87.0 – 505.0	262.9 (32.5) 135.0 – 477.0
$AUC_{0\text{-}6}$ (ng/mL*h)	727.6 (69.5) 123.0 – 1819.3	740.2 (58.1) 207.7 – 1559.0
$AUC_{0\text{-}tmax}$ (ng/mL*h)	140.2 (106.0) 32.3 – 555.1	172.5 (64.7) 64.6 – 600.9
t_{max} (h)	1.00 (0.67 – 3.00)	1.50 (0.00 – 3.00)
$t_{1/2}$ within a 6-h interval (h)	3.00 (60.83) 1.31 – 19.07	2.33 (76.02) 0.96 – 13.36
Day 6		
C_{max} (ng/mL)	218.5 (55.2) 51.0 – 485.0	257.0 (41.6) 127.0 – 528.0
$AUC_{0\text{-}6}$ (ng/mL*h)	626.1 (99.8) 21.2 – 1516.4	750.9 (53.3) 309.4 – 1772.9
$AUC_{0\text{-}tmax}$ (ng/mL*h)	124.2 (140.1) 8.4 – 513.1	253.6 (90.1) 41.3 – 900.8
t_{max} (h)	1.00 (0.33 – 3.5)	1.50 (0.67 – 6.00)
$t_{1/2}$ within a 6-h interval (h)	2.30 (45.48) 0.85 – 5.82	2.36 (45.80) 1.18 – 6.72

Reviewer's Comments: The LD concentration /time profiles on Day 1 and on Day 6 appeared to be similar between treatments, however the performance of the assay for LD during the analysis (see Bioanalytical Assays) makes these results difficult to interpret. However, the PK profile of LD main circulating metabolite HVA (which was stable under the storage conditions), was also not affected by safinamide treatment. This result and the results from the levodopa DDI study EMR701165_027 in healthy subjects support the conclusion that safinamide does not affect LD pharmacokinetics.

Mean concentration-time profiles for safinamide and its metabolites NW-1153 and NW-1689 after safinamide treatment are shown below. Visual examination of individual pre-dose safinamide concentrations on Day 5 and Day 6 was in line with previous PK data on safinamide after 100 mg dose, in the range of 800-1000 ng/mL (mean). Concentrations of NW-1689 and NW-1153 appear consistent with previous data, with NW-1689 showing concentrations in a range 1.4x safinamide and NW-1153 in a range of 10% of parent.



Safety Results:

Safinamide was well tolerated when co-administered with levodopa. No SAEs were reported during the trial. No subjects discontinued from the trial due to an AE.

A total of 4 AEs were reported in 3 subjects (vomiting, dizziness, dyskinesia and anemia), the relationship to the treatment was assessed as unrelated or unlikely related for all 4 AEs.

The clinical laboratory parameters, vital signs, ECG, and results from physical examinations showed no clinically relevant changes, with the exception of one subject who experienced a clinically significant decrease in hematology parameters, which was recorded as an AE (anemia).

Reviewer's Comment

The following labeling language, proposed by the sponsor, is acceptable:

In vivo drug-drug interaction studies performed with L-dopa did not demonstrate any clinically significant effects on the pharmacokinetic profile of XADAGO or on the pharmacokinetic profile of co-administered L-dopa.

XI. Study 28778: A randomized, open label, two-period, two-sequence, cross-over study to investigate the pharmacokinetic interaction of ketoconazole at steady-state with a single dose of safinamide in healthy volunteers

Objectives:

Primary To investigate the effect of repeated oral doses of ketoconazole, a strong CYP3A4 inhibitor, on the PK profile of safinamide after single administration

Secondary:

- To investigate the effect of repeated oral doses of ketoconazole on the PK profile of the main circulating safinamide metabolites (NW1689, NW1689-AG and NW1153) after single administration of safinamide
- To investigate the exposure and urinary excretion to the metabolite NW1689-AG
- To assess the clinical and laboratory safety and tolerability of a single dose of safinamide given alone and co-administered with ketoconazole

Study Design	randomized, open-label, 2-period cross-over trial in healthy subjects
Study Population	14 healthy subjects (7 male, 7 female) aged 18 to 45 years, with a body mass index (BMI) in the range of 18-30 kg/m^2. All 14 subjects completed the study.
Treatment Group*	2 periods and 2 treatments with wash-out period of at least 21 days* Treatment A (safinamide alone): a single 100 mg safinamide tablet Treatment B (ketoconazole co-administered with safinamide): 200 mg of ketoconazole b.i.d. for 6 days and concomitant administration of a single 100 mg tablet of safinamide on the third day of ketoconazole administration (on that day, the single dose of safinamide was administered 30 min after ketoconazole).
Dosage and Administration	Safinamide: 100 mg tablet, single dose oral administration in the morning under fasting conditions Ketoconazole: 200 mg tablet (Nizoral), bid for 6 days, fasting (only in the morning of the day of concomitant administration with safinamide)
PK Sampling**	Plasma for safinamide and its main metabolites: pre-dose and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 120 and 240 hours post-dose** Urine samples for safinamide and its main metabolites (NW1689, NW1153 and NW1689-AG) collected at the following intervals: 0-2, 2-4, 4-6, 6-8, 8-10, 10-12, 12-24, 24-26, 26-28, 28-30, 30-32, 32-34, 34-36 and 36-48 post-dose, in each period
Analysis	Validated LC/MS/MS methods for analysis of safinamide and metabolites NW1689, NW1689-AG and NW1153 in plasma and urine
PK Assessment	Primary: C_{max} and $AUC_{0-\infty}$ of safinamide Secondary: C_{max} and $AUC_{0-\infty}$ of safinamide metabolites NW1689, NW1153 C_{max}, $AUC_{0-\infty}$ and Ae_{0-48} of NW1689-acylglucuronide
PD Assessment	none
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

* Treatments were administered according to a cross-over design, the subjects being randomized to one of the following treatment sequences: treatment A/wash-out/treatment B or treatment B/wash-out/treatment A.

**Blood samples (at least 5 ml collected at each timepoint) were collected into Li heparin tubes and centrifuged. The plasma was then split in 2 aliquots: one for the analysis of safinamide and NW1153, and one for the analysis of NW1689 and NW1689-AG. The latter was acidified using o-phosphoric acid (20% solution in water).

Bioanalytical Assays:

Concentrations of safinamide and its metabolite NW-1153 in plasma and urine were determined using two validated LC-MS/MS methods: No. RE7810 ((b) (4) 28875) and RE8670 ((b) (4) 29066) for plasma and urine, respectively.

For each run a freshly prepared calibration curve in plasma or urine was prepared. Quality Control samples, previously prepared at three different concentration levels and stored at approximately –20°±5°C, were thawed and analyzed with each run.

Eight calibration samples at different levels (5.0, 10.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 ng/mL) were analyzed in each run to establish the calibration curve. All mean values of the calibration curve points were within ± 15.0%.

Safinamide: The intra-assay accuracy were from 0.0% to 14.0% for QCL; from 5.5% to 11.9% for QC-M and from -12.7% to 5.7% for QC-H.

The inter-assay accuracy was 8.7%, 9.5% and -2.6% for QC-L, QC-M and QC-H, respectively. The inter-assay precision was 3.7%, 1.8% and 4.3% for QC-L, QC-M and QC-H, respectively.

NW-1153: The intra-assay accuracy were from -2.0% to 14.7% for QCL; from 3.3% to 13.1% for QC-M and from –4.9% to 9.8% for QC-H.

The inter-assay accuracy were 8.0%, 7.2% and 1.3% for QC-L, QC-M and QC-H, respectively. The inter-assay precision was 4.3%, 2.5% and 3.6% for QC-L, QC-M and QC-H, respectively.

The plasma concentrations of NW1689 and NW1689-1-O-glucuronide (NW1689-AG) were determined using a validated LC-MS/MS method (Report DMPK 59-09). As no direct method was available for measurement of NW1689-AG, an indirect method involving bioanalysis of the samples before and after conversion of NW1689-AG to NW1689 (alkaline hydrolysis of the acylglucuronide), has been previously developed and validated (Study No DMPK 36-08). NW1689-AG concentrations were derived in a further calculation step and appropriate correction was applied according to the molecular weight of NW1689-AG. The range for both analytes was from 5.00 to 5000 ng/mL.

Stability of NW1689 and NW1689-AG in acidified human plasma at -20°C and -80°C was demonstrated for at least 8 months. Calibration samples were prepared in pooled, acidified human plasma (1000 µL plasma spiked with 100 µL o-phosphoric acid 20% in water), whereas QC samples were prepared in native human plasma and acidified with phosphoric acid immediately after preparation.

13 batches were measured including in-study partial validation batches. One batch was rejected due to an instrument failure. All predefined acceptance criteria were met.

• Coefficient of correlation (r) for the calibration curve had to be ≥0.990.

• At least 2/3 of the quality control samples had to be within ±15% of the nominal concentrations including at least one sample at each concentration.

• Additionally, for demonstrating appropriate selectivity, co-eluting peaks had to have a response of ≤20% of the mean analyte response at the LLOQ. For the internal standard, the maximum response in blank samples and samples spiked with ketoconazole had to be

less than or equal to 5% of the peak response at the actual concentration used in the assay.
In addition, incurred sample reanalysis (ISR) was performed using 40 samples from subjects 0001 to 0010. ISR was investigated for sample aliquots analyzed prior to and after conversion and demonstrated good reproducibility of the assay.

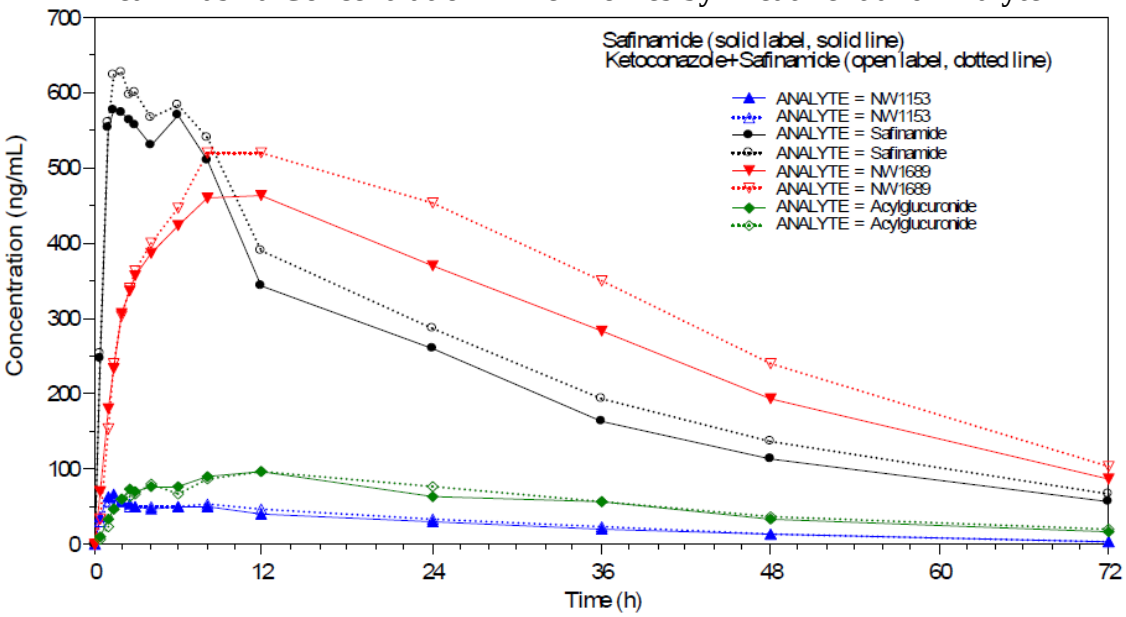
Reviewer's Comment: The performance of the assays during the analysis of plasma/urine samples from Study 28778 is acceptable.

All pre-dose plasma concentrations on Day 1 in each period were below the LLOQ for safinamide and its metabolites NW1153 and NW1689.
Safinamide and its metabolites NW1153 and NW1689-AG were measured in all post dose urine samples up to 48 h, with the exception of 2 unquantifiable concentrations at the 36- 48 h collection interval for safinamide and NW1153 following treatment A.
All safinamide and NW1689 pre-dose urine samples resulted in values below LLOQ.
NW1153 and NW1689-AG were detected in the period 2 pre-dose urine collection fraction in some subjects (9 samples out of 28 for each analyte and both treatments). These concentrations were however very low (in the range of 1.5- to 2-fold higher than LLOQ levels).

Pharmacokinetic Results:
For ketoconazole a daily dose of 400 mg for multiple days is recommended in the DDI guidance. Due to the short half-life of ketoconazole, its daily dose was split into two administrations to maintain constant levels. Dosing was started two days before safinamide dosing to be at steady state and was maintained over 4 days (i.e., 4 half-lives of safinamide) following the safinamide dose. Also, ketoconazole was given 30 min before safinamide dosing in order to have maximum inhibition at the time of safinamide administration. All medications were prohibited except oral combined contraceptive (OCC).
Reviewer's Comment: the study design is adequate for assessing a potential DDI with ketoconazole.
Concentration-time profiles and PK parameters for safinamide and its metabolites NW1153, NW1689 and NW1689-AG for treatments A and B are presented below.
Additionally, analysis of key PK parameters by gender was performed by the sponsor on an ad-hoc basis. PK profile of safinamide and its metabolites NW1153 and NW1689 appeared similar in males and females, showing that there is no gender effect.
The PK profile of safinamide and its metabolites NW1153 and NW1689 appeared consistent with data from previous studies and confirmed that these two metabolites are major metabolites of safinamide in plasma. NW1689 had mean metabolic ratio of 1.61 in terms of $AUC_{0-\infty}$ after administration of safinamide alone. Exposure to NW1153 was much lower, with a metabolic ratio of around 0.1 both for $AUC_{0-\infty}$ and C_{max}.

Overall, mean plasma concentration-time profile for safinamide and its metabolites NW1153, NW1689 and NW1689-AG were similar in both treatments A and B (without and with ketoconazole co-administration).

Mean Plasma Concentration-Time Profiles by Treatment and Analyte



Although a marginal increase in the exposure to safinamide was observed when safinamide was administered in combination with ketoconazole, this effect should not be clinically significant, as the 90% CI for $AUC_{0-\infty}$ and C_{max} fell within bioequivalence range for all analytes (CI for NW1689 $AUC_{0-\infty}$ was slightly outside, see below

ANOVA results for ratio treatment B / treatment A of $AUC_{0-\infty}$ and C_{max} for safinamide and its metabolites NW1153 and NW1689

Analyte	Safinamide	NW1153	NW1689
	Ratio 90% CI	Ratio 90% CI	Ratio 90% CI
C_{max} (ng/mL)	106.6 (101.0-112.4)	89.2 (83.6-95.2)	112.5 (108.1-117.1)
$AUC_{0-\infty}$ (ng.h/mL)	112.9 (109.8-116)	107.6 (104.1-111.2)	118.5 (111.5-126.0)

CI: Confidence Interval

In this trial, the PK profile of NW1689-AG was investigated for the first time. This metabolite, with an exposure in plasma (in terms of $AUC_{0-\infty}$) of more than 10% relative to parent, should be considered a major metabolite of safinamide. Moreover, its excretion in urine represents more than 10% of the fraction of dose recovered for the whole collection period 0-48 h after safinamide administration.

Summary of urine pharmacokinetic parameters of safinamide, NW1153, NW1689 and NW1689 acylglucuronide (treatments A and B)

	Treatment A (safinamide only)	Treatment B (safinamide + ketoconazole)
Safinamide		
Ae_{0-48} (% dose)	4.3 (18.8) 3.0 – 6.2	4.4 (23.8) 2.9 – 6.8
CL_R (L/h)	0.32 (18.4) 0.25 – 0.47	0.30 (28.4) 0.19 – 0.53
NW1153		
Ae_{0-48} (% dose)	20.3 (16.8) 16.2 – 27.3	16.3 (18.3) 11.2 – 23.4
CL_R (L/h)	13.9 (24.7) 9.4 – 18.8	10.4 (26.8) 6.5 – 14.8
NW1689		
Ae_{0-48} (% dose)	NC (NC) 0.000 – 0.173	0.044 (153.1) 0.002 – 0.148
CL_R (L/h)	NC (NC) 0.000 – 0.016	0.002 (156.7) 0 – 0.011
NW1689 acylglucuronide		
Ae_{0-48} (% dose)	18.2 (17.6) 13.5 – 23.4	20.4 (15.4) 17.3 – 26.5
CL_R (L/h)	6.2 (21.6) 4.1 – 9.2	6.9 (34.4) 4.7 – 15.9

For Ae_{0-48} and CL_R: Geomean (GeoCV %) and range (min-max)

Conclusion: The results of this trial showed that CYP3A4 is not the major enzyme involved in the pathways to NW1689, NW1689-AG and NW1153.
This was demonstrated with the marginal increase in exposure observed when safinamide was administered with ketoconazole showing similar extent for safinamide and its metabolites, and was further supported by stable metabolic ratios for all analytes in both treatments.

Reviewer's Comments: This study adequately assessed the drug-drug interaction (DDI) potential of a CYP3A4 strong inhibitor on safinamide PK. No relevant DDI was observed. Safinamide can be co-administered with drugs known to inhibit the CYP3A4 isoenzyme without dose adjustment.

Safety Results:
Safinamide was well tolerated, alone or when co-administered with ketoconazole. A total of six TEAEs were reported in five subjects: four TEAEs in four subjects following treatment A (safinamide alone), and two TEAEs in two subjects following B (safinamide + ketoconazole). All TEAEs were of mild intensity and all subjects recovered without sequelae. The TEAEs were nervous system disorders (four headaches), one urinary tract infection and one oropharyngeal pain.
There was no clinically significant out-of-range value in hematology, blood chemistry, urinalysis or coagulation parameters, and no clinically significant out-of-range value in vital signs or electrocardiogram parameters were observed.

XII. EMR701165-026: A sequential interaction trial to assess the effect of Safinamide on the pharmacokinetics of CYP1A2 and CYP3A4 probe substrates in healthy volunteers

Objectives:

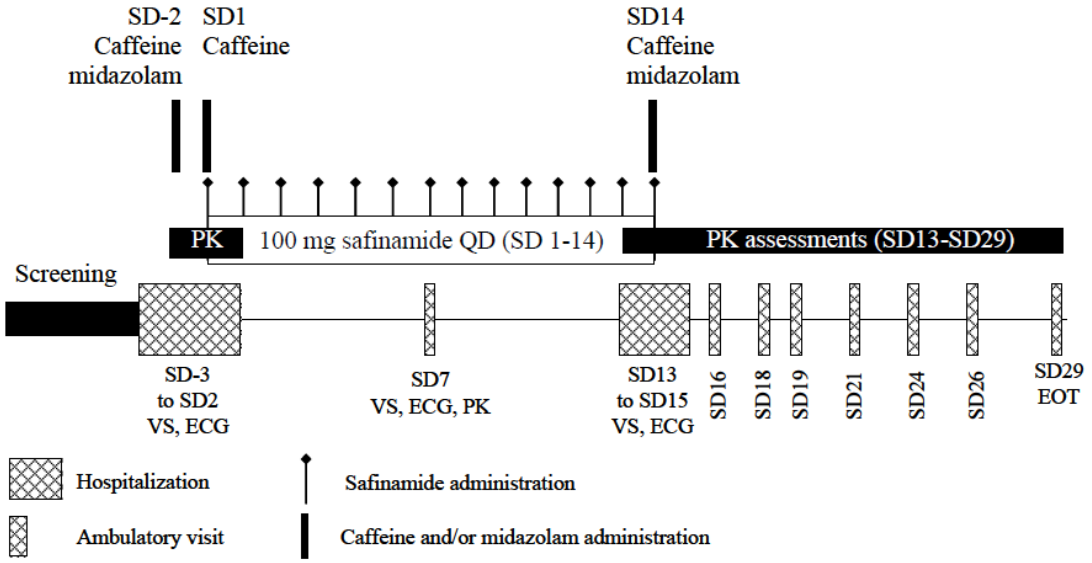
Primary: To investigate a possible effect of safinamide on caffeine (CYP1A2 probe substrate) and midazolam (CYP3A4 probe substrate) pharmacokinetics.

Secondary:

- To investigate the pharmacokinetics (PK) of caffeine/midazolam and its metabolites when co-administered with safinamide after first dose (caffeine and its metabolite) and at steadystate (caffeine, midazolam and their metabolites)
- To investigate the pharmacokinetics of safinamide and its metabolite at steady-state
- To assess the safety and tolerability of safinamide in multiple-dose regimen when coadministered with single doses of caffeine and midazolam.

Study Design*	Open-label, sequential design study in healthy subjects
Study Population	16 healthy subjects (8 male, 8 female) aged 18 to 55 years, with a body mass index (BMI) in the range of 18-30 kg/m^2. 15 subjects completed the study, 1 withdrew due to AE
Treatment Group*	
Dosage and Administration	Safinamide: 14 days (once daily morning administration from Study Day (SD) 1 to SD14). Caffeine: 200 mg single dose on Day-2, Day1 and Day14; Midazolam: 7.5 mg single dose on Day-2 and Day14
PK Sampling**	Plasma samples were collected for the PK assessments of caffeine and 1,7-dimethylxanthine, of midazolam and 1-hydroxymidazolam, and of safinamide and NW-1689.
Analysis	Validated LC/MS/MS assays for analysis of safinamide and metabolite NW1689 Validated LC/MS/MS assays for analysis of Caffeine and its metabolite Paraxanthine (1, 7-dimethylxanthine) and Midazolam and its metabolite 1-Hydroxymidazolam
PK Assessment	Primary: AUC_{0-t} for caffeine and midazolam Secondary: C_{max}, t_{max}, $t_{1/2}$ and $AUC_{0-\infty}$ of midazolam and caffeine and their metabolites (1-hydroxymidazolam, 1,7-dimethylcaffeine) C_{max}, $t_{1/2,}$ $AUC_{0-\infty}$ and AUC_{0-24} of safinamide and NW1689 at steady state
PD Assessment	none
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

*** Trial Schematic Diagram**



** PK assessments of caffeine, 1,7-dimethylxanthine on SD-2, SD1 and SD14: pre-dose, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 h (SD-1/SD2/SD15) post-dose
PK assessments of midazolam, 1-hydroxymidazolam on SD-2 and SD14: pre-dose, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 h (SD-1/SD15) post-dose
PK assessments of safinamide and NW-1689: pre-dose on SD1, SD2, SD7 and SD13.
On SD14: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 h, 24 h (SD15), 48 h (SD16), 96 h (SD18), 120 h (SD19), 168 h (SD21), 240 h (SD24), 288 h (SD26), 360 h (SD29) post-dose.

Discussion of the study design:

The relevant endpoints were based on bioanalytical analysis (i.e., PK parameters), therefore the open label design is justified.
Caffeine and midazolam are FDA recommended probe substrates for CYP1A2 and CYP3A4, respectively. These 2 probe substrates are validated as cocktail administration, (i.e., the substrates are specific for the individual CYP enzyme and there are no interactions among these substrates).
The treatment duration of 14 days of safinamide was considered adequate to obtain potential maximum induction and mechanism-based inhibition of CYP3A4 and CYP1A2, respectively, since:

- the turnover rate for CYP3A4 $t_{1/2}$ is estimated to be 23-70 hours
- the turnover rate CYP1A2 is estimated to be 105 hours
- time to reach steady-state of safinamide : after 4 doses

Due to the combination of induction and inhibition on CYP1A2 (in vitro study results indicated that safinamide was a weak mechanism-based and competitive inhibitor and a weak inducer of CYP1A2), the trial investigated a first dose effect caused by inhibition [i.e., after single dose on Study Day 1] and the effect at later time point when the potential induction was assumed to be complete (i.e., after multiple doses (m.d.) on SD14) during safinamide treatment.
For safinamide, the highest planned dose for the treatment of Parkinson's disease was used (100 mg/day). For the probe substrates, the commercial available maximum dose

was administered as single dose (7.5 mg midazolam [Dormicum®] and 200 mg caffeine [Coffeinum N® 0.2 g]).
No co- medications (including multivitamins and herbal products) were allowed 2 weeks before and during the study with the exception of hormonal contraceptive treatment for females. Subjects were to abstain from the ingestion of any food, drink or beverage containing alcohol, grapefruit or grapefruit juice from 72 h prior to drug administrations until last PK sampling.
On days with intense PK sampling (i.e., SD-2, SD1 and SD14) the subjects fasted overnight for at least 10-hours before drug administration.
Reviewer's Comment: the study design is adequate for assessing the induction potential of safinamide on CYP3A4 and CYP1A2 substrates.

Bioanalytical Assays:

Safinamide and NW1689

Two LC-MS/MS methods (range 0.5-500 ng/mL) for quantification of safinamide and NW1689 (before methods) in acidified human plasma (lithium heparin) have been validated ((b) (4) study numbers 8244033 and 8244036). The methods have a lower limit of quantification (LLOQ) of 0.5 ng/mL for Safinamide and NW1689 (before) using 25 µL of plasma for safinamide and 100 µL of plasma for NW1689.
NW1689 is a metabolite of safinamide, known to form glucuronides in vivo. The method was validated to quantify levels of NW1689 in acidified human plasma before and after the conversion of glucuronides to NW1689. In this study, only the concentrations of NW1689 before the conversion of glucuronides to NW1689 (defined as NW1689 (before)), were determined.
The metabolite NW-1153 concentrations were not determined in this study.

Determination of NW1689 (before)

NW1689 (before) calibration standard and QC sample data were reported as ng/mL acidified plasma. NW1689 (before) study sample data are reported as ng/mL acidified plasma and ng/mL plasma. The plasma concentration was calculated by multiplying the measured concentrations in acidified plasma by 1.10 (to account for the additional volume of o-phosphoric acid for the acidification of the samples).
A total of 333 samples were analyzed for safinamide in human plasma in six batches. A total of 334 samples were analyzed for NW-1689 (before) in acidified human plasma in a total of eight batches.
During the analysis of study samples, it was noted that in several samples from each subject profile the concentration of the analytes were above the calibration range and the samples were analyzed following dilution with control matrix. The data from diluted samples are considered acceptable as the QC samples analyzed in each batch, from which the diluted sample data are reported, were acceptable and incurred samples reanalysis (ISR) data from diluted samples were also acceptable.
The calibration, QC sample data and ISR data indicate that the methods performed adequately during the analyses of all reported batches of study samples. All reported batches complied with the calibration and QC sample acceptance criteria.
A total of 35 samples were re-analyzed. All of the repeat results (100%) and original results were within 20% of each other; this is acceptable.

Caffeine and its metabolite Paraxanthine (1, 7-dimethylxanthine), Midazolam and its metabolite 1-Hydroxymidazolam

Two LC-MS/MS methods have been validated in human plasma samples containing EDTA as an anticoagulant ([(b) (4)] study numbers 8244044 and 8224322).

The methods have lower limits of quantification (LLOQ) of 25 ng/mL for caffeine and paraxanthine (using 25 µL of plasma) and 0.1 ng/mL for midazolam and 1-hydroxymidazolam (using 100 µL of plasma).

A total of 564 plasma samples were analyzed for Caffeine and for Paraxanthine in eight batches, including the assessment of incurred sample reproducibility (ISR) and specificity of the method in the presence of co-administered drugs and metabolites.

A total of 372 plasma samples were analyzed for Midazolam and 1-Hydroxymidazolam in four batches, including the assessment of ISR and specificity of the method in the presence of co-administered drugs and metabolites.

Calibration standard data, QC sample data, ISR data and chromatograms indicate that the performance of the methods during the study sample analysis was acceptable.

Pharmacokinetic Results:

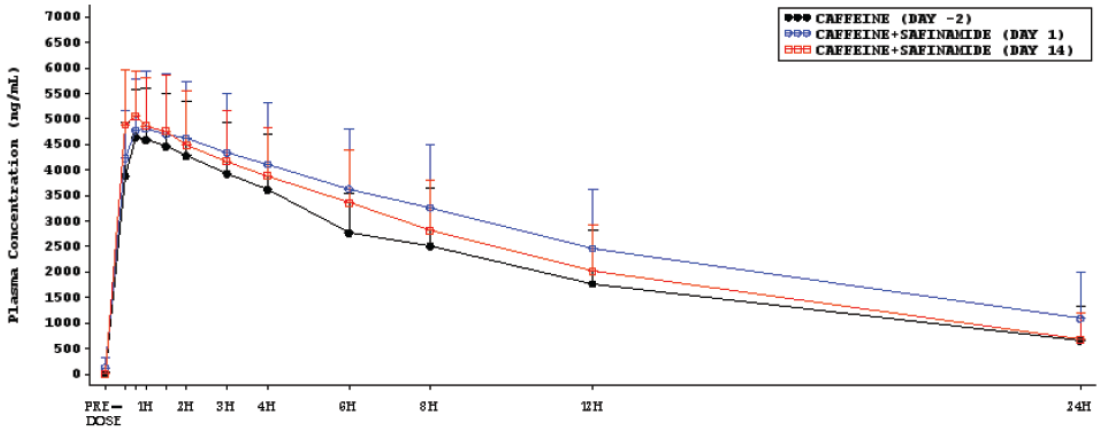
Subject 10016 was withdrawn after SD1 administration of safinamide, following an AE (atrial fibrillation). The other 15 subjects completed the trial.

Four minor protocols deviations were reported during the course of the trial (one PK concentration missing on SD14, two minor delays in PK blood sampling on SD14, one minor delay in safinamide self-administration on SD4). None of these were considered to have an impact on the study endpoints.

Subject 10024 showed unexpected low concentrations for safinamide and its metabolite NW1689 on SD13 and SD14, suggesting that the subject was not compliant with ambulant drug intake. However, neither drug accountability nor the subject confirmed this. Therefore, the subject was kept in the PK analysis set, but a sensitivity analysis for the ANOVA was additionally performed withdrawing this subject from the population.

Effect of safinamide on caffeine PK

Caffeine Mean Plasma Concentration-Time Curves



Increased plasma exposure of caffeine was observed when administered together with

safinamide 100 mg single dose and multiple dose. Single dose effect was more pronounced, with an increase in AUC_{0-t} of 30% (ANOVA point estimate), compared to a 13% increase after 14 days of safinamide 100 mg o.d. administration.
Single dose effect suggests that safinamide at the dose of 100 mg is a weak CYP1A2 inhibitor as per guideline whereas lower increased caffeine exposure after multiple dose of safinamide indicate an additional weak CYP1A2 induction effect.

Caffeine Plasma PK Parameters

	Caffeine alone (SD-2)	Caffeine + safinamide single dose (SD1)	Caffeine + safinamide multiple dose (SD14)
AUC_{0-t} (ng.h/mL)	44933.9 (43.6) 24525 – 101365	58395.9 (41.4) 29270 - 125690	51237.2 (37.0) 27441 - 91965
$AUC_{0-\infty}$ (ng.h/mL)	41021.3 (40.2) 25039 - 90041	51583.6 (31.8) 30637 - 83591	53326.7 (41.0) 28335 - 104909
C_{max} (ng/mL)	4949.3 (17.3) 3690 - 7180	4976.9 (20.2) 3650 - 7280	5346.3 (18.2) 3860 - 7010
t_{max} (h)	0.750 0.5 - 2.0	0.875 0.5 - 2.0	0.500 0.5 - 1.50
$t_{1/2}$ (h)	5.587 (29.6) 3.56 - 9.90	7.150 (19.5) 5.34 - 9.39	6.708 (26.1) 4.52 - 10.01
MR	0.354 (47.1) 0.16 - 0.59	0.279 (52.6) 0.11 - 0.51	0.336 (35.5) 0.16 - 0.53
Individual ratio AUC_{0-t} (SD1 or SD14)/ SD-2 (.)	NA	1.300 (9.5) 1.14 - 1.70	1.127 (26.9) 0.75 - 2.11

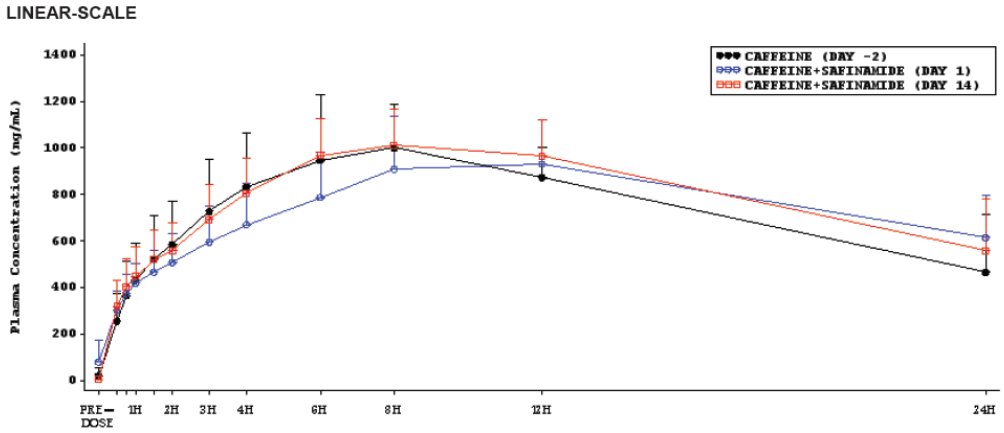
For C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, MR and individual AUC_{0-t} ratios : Geomean (GeoCV %) and range (min-max)
For t_{max}: median and range (min – max)
NA: Not Applicable

Effect of Safinamide on the AUC_{0-t} (ng.h/mL) of Caffeine - Summary of ANOVA Results (PK Population)

Day	Treatment	Least Squares Geometric Mean	Ratio Estimate [%] (90% CI)	
SD-2	Caffeine	44934		
SD1	Caffeine + safinamide single dose	58396	SD1 / SD-2	130 (124.7-135.5)
SD14	Caffeine + safinamide multiple-dose	50789	SD14 / SD-2	113 (100.3-127.4)

Similar results were obtained when the ANOVA was conducted without Subject 10024, as per sensitivity analysis (SD14/SD-2 Ratio 111%).
Mean concentration-time curves for 1,7-dimethylxanthine on SD-2, SD1 and SD14 are shown below. The results for 1,7-dimethylxanthine were difficult to interpret likely due to the overall small effects and due to combined inhibition and induction.

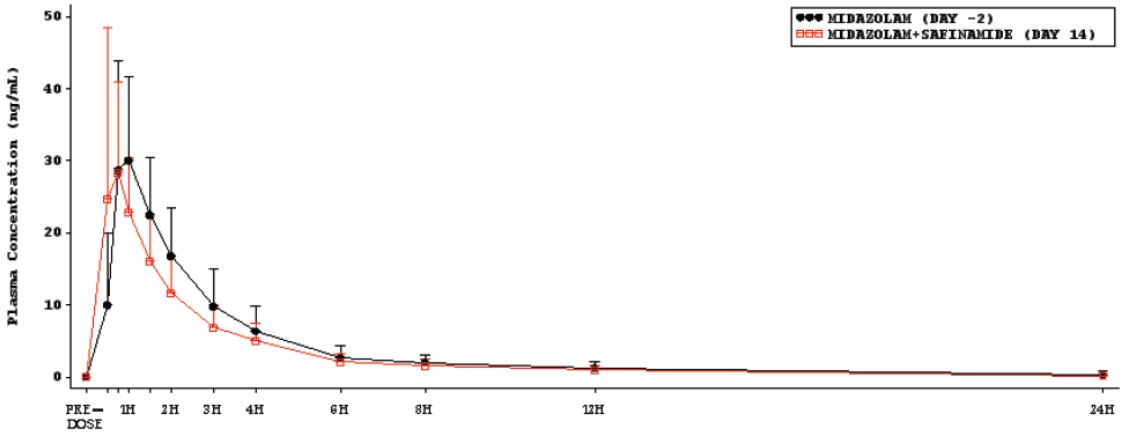
Mean Plasma Concentrations for Caffeine Metabolite (1,7-DIMETHYLXANTHINE)



Effect of safinamide on midazolam PK

Mean concentration-time curves for midazolam on SD-2 and SD14 are shown below.

Midazolam Mean Concentration-time Profile



Plasma concentration-times profiles and PK parameters for midazolam on SD-2 and SD14 indicated a shorter t_{max} and $t_{1/2}$ when midazolam was administered together with safinamide 100 mg after multiple administration compared to when administered alone. C_{max} was comparable.

A 20% decrease in exposure of midazolam (ANOVA point estimate) was observed when administered together with safinamide 100 mg after 14 days of o.d. administration.

Midazolam Plasma PK Parameters

	Midazolam alone (SD-2)	Midazolam + safinamide multiple dose (SD14)
AUC_{0-t} (ng.h/mL)	80.41 (37.1) 43.3 - 167.0	64.81 (43.2) 33.3 - 159.2
$AUC_{0-\infty}$ (ng.h/mL)	82.80 (37.9) 44.2 - 176.1	66.91 (43.3) 34.9 - 165.7
C_{max} (ng/mL)	33.94 (35.2) 19.2 - 66.5	33.13 (42.9) 14.9 - 93.5
t_{max} (h)	0.875 0.50 - 1.50	0.750 0.50 - 1.50
$t_{1/2}$ (h)	4.716 (33.3)	4.064 (41.5) 2.31 - 7.53
MR	0.408 (37.6) 0.25 - 0.84	0.581 (45.5) 0.31 - 1.40
Individual ratio AUC_{0-t} (SD14/ SD-2) (.)	NA	0.798 (33.2) 0.39 - 1.23

For C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$, MR and individual AUC_{0-t} ratios : Geomean (GeoCV %) and range (min-max)

For t_{max}: median and range (min – max)
NA: Not Applicable

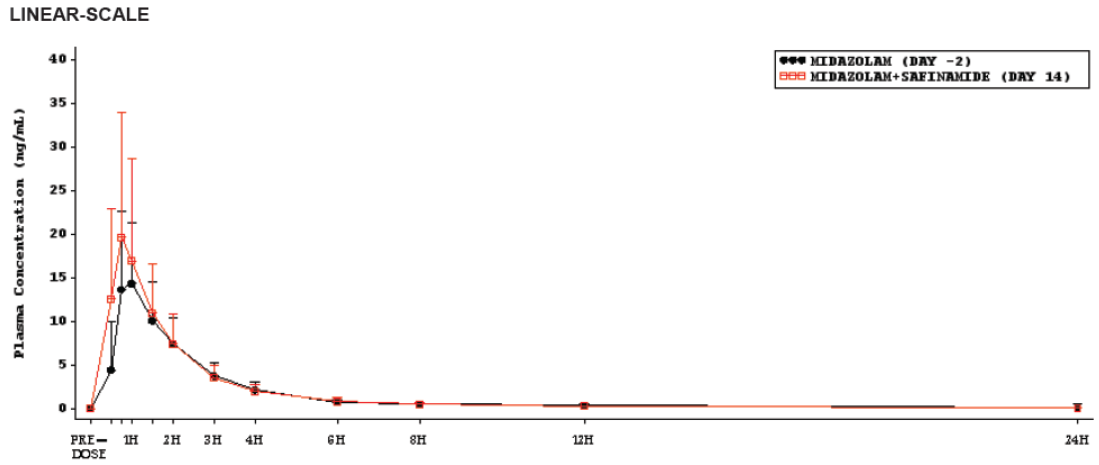
Effect of Safinamide SD14 Administration on the AUC_{0-t} (ng.h/mL) of Midazolam - Summary of ANOVA Results (PK Population)

Day	Treatment	Least Squares Geometric Mean	Ratio Estimate [%] (90% CI)	
SD-2	Midazolam	80.4	SD14 / SD-2	80.0 (69.2-92.6)
SD14	Midazolam + safinamide multiple-dose	64.4		

Similar results were obtained when the ANOVA was conducted without Subject 10024, as per sensitivity analysis (Ratio 77.6%).
These results suggest that safinamide at the dose of 100 mg o.d. is a weak CYP3A4 inducer per the FDA DDI guideline.

Mean concentration-time curves and descriptive statistics of PK parameters for 1-hydroxymidazolam on SD-2 and SD14 are shown below.
Consistent with the weak CYP3A4 inducing effect, exposure of 1- hydroxymidazolam, was slightly elevated.

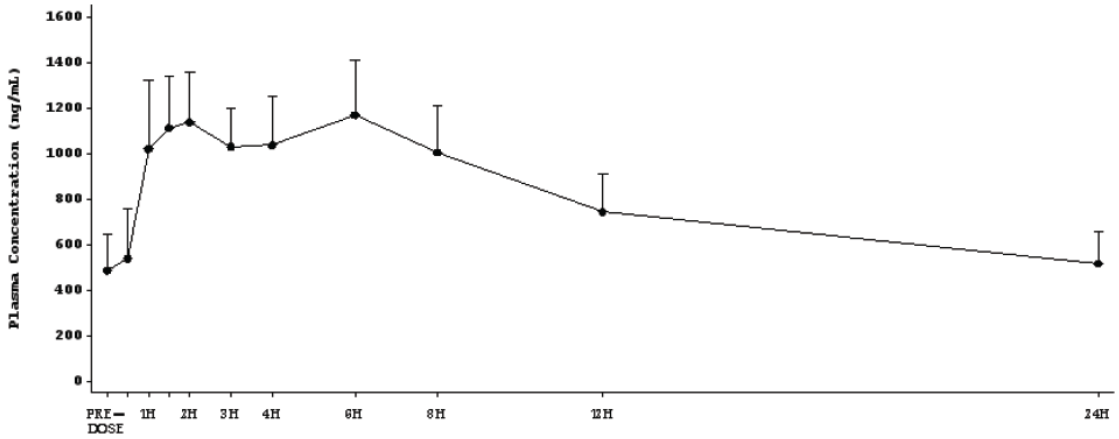
Mean Plasma Concentration-Time Profile of 1-HYDROXYMIDAZOLAM)



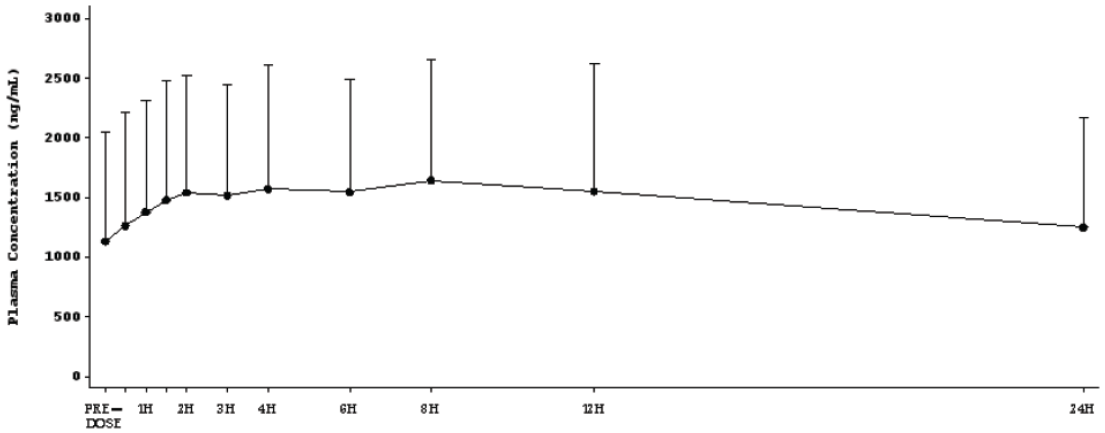
Safinamide PK profile

Overall, observed exposure to safinamide and its metabolite NW1689 appeared consistent with previous data at steady-state. A longer half-life could be determined which is consistent with the terminal $t_{1/2}$ reported from the mass-balance study and is likely due to longer sampling time period and lower limit of quantification.

Safinamide Mean Plasma Concentration-Time Profile on SD14



NW-1689 Mean Plasma Concentration-Time Profile on SD14



PK Parameters for Safinamide and NW1689 (PK Population)

	Analyte	
	Safinamide	NW1689
AUC_{0-t} (ng.h/mL)	35503.8 (25.9) 25313 – 56153	66584.5 (72.1) 31231-248673
$AUC_{0-\infty}$ (ng.h/mL)	35582.4 (25.9) 25383-56272	67006.3 (72.1) 31402-250327
C_{max} (ng/mL)	1207.7 (18.0) 930-1760	1457.6 (59.1) 755-4650
t_{max} (h)	2.000 1.00-6.00	6.000 2.00-12.00
$t_{1/2}$ (h)	61.26 (12.2) 50.6-78.0	62.65 (41.0) 38.7-126.4

Reviewer's Comment
The following labeling language, proposed by the sponsor, is acceptable:

(b) (4)

Safety Results:

Overall, a total of 25 treatment emergent adverse events (TEAEs) were reported in 11 subjects during the study. There was no serious adverse event (SAE) and no TEAE of severe intensity. One subject was withdrawn from the study, due to a moderate atrial fibrillation that started before SD1 dosing; therefore it is not considered related to the safinamide administration. When only safinamide was administered (between SD2 dosing and SD14 pre-dose) 4 TEAEs were reported in 3 subjects; only one of which was considered related to safinamide alone: a moderate orthostatic hypotension.
The most frequent TEAE was somnolence (11 cases in 8 subjects i.e. 50% of the safety population); in all cases it was considered related to midazolam.
Mean and change from baseline of hematology and blood chemistry were similar between treatments at all timepoints. No clinically relevant change was observed.

XIII. Study 28558: A Randomised, Double-Blind, Phase I, Placebo-controlled, Active-controlled (Phenelzine), Comparator-controlled (Selegiline), Multiple-dose Parallel-group Study to Investigate the Pressor Effect of Oral Tyramine During Safinamide Treatment in Healthy Subjects

Objectives:

Primary: To evaluate the potentiation of the pressor effect of oral tyramine by safinamide 100 mg (therapeutic dose) at steady-state versus placebo.

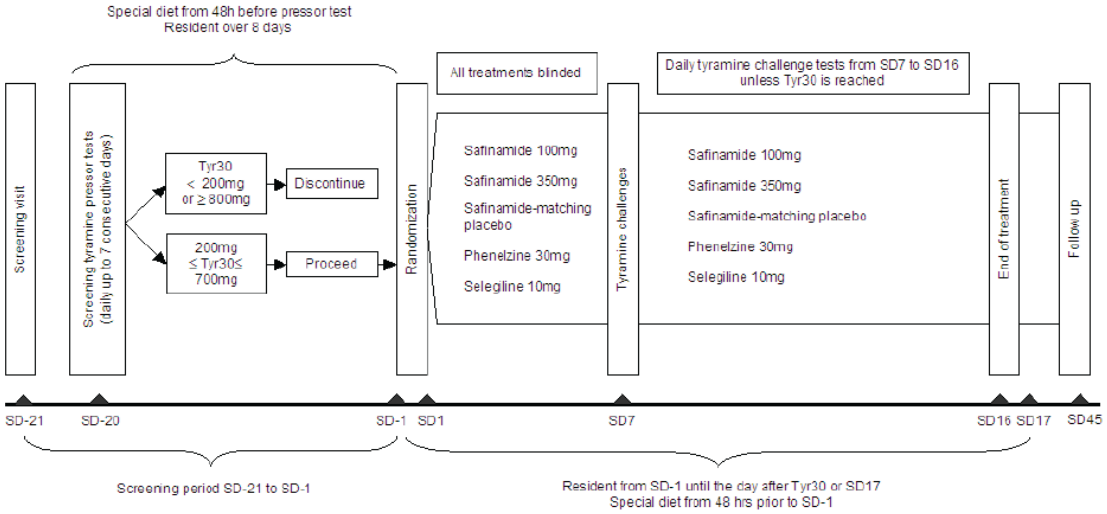
Secondary:

- To evaluate the potentiation of the pressor effect of oral tyramine by safinamide 350 mg (supratherapeutic dose) at steady-state versus placebo,
- To demonstrate the sensitivity of the trial via the potentiation of the pressor effect of oral tyramine by phenelzine 30 mg versus placebo,
- To evaluate the potentiation of the pressor effect of oral tyramine by selegiline (comparator control) at steady-state versus placebo,
- To evaluate the safety of safinamide in a multiple-dose regimen, at the expected therapeutic dose as well as at a supratherapeutic dose.

Study Design*	randomized, double-blind, placebo-, active-, and comparator-controlled parallel-group trial
Study Population**	89 healthy** subjects (18 subjects in each of the safinamide, selegiline, and placebo treatment groups, and 17 subjects in the phenelzine treatment group).
Treatment Groups	5 treatment groups: safinamide 100 mg once per day (o.d.), safinamide 350 mg o.d., phenelzine 30 mg/day (active control), selegiline 10 mg /day (comparator), or placebo.
Dosage and Administration	Subjects received either 100 mg safinamide o.d., 350 mg safinamide o.d., placebo o.d., 15 mg phenelzine twice a day (b.i.d.) (morning and evening) or 5 mg selegiline b.i.d. (morning and lunch) from SD1 until SD16 or until Tyr30 was reached.
PK Sampling	Plasma for safinamide and its main metabolites: predose (safinamide) of SD1 and SD6 as well as at predose and 120 and 150 minutes after safinamide dosing (i.e., together with tyramine) from SD7 until Tyr30 was reached or until SD16. Plasma samples for tyramine levels were collected predose (tyramine), 30 minutes and 60 minutes after tyramine dosing on SD7 and every day until Tyr30 was reached or until SD16.
Analysis	Validated LC/MS/MS method for analysis of safinamide and metabolites NW1689 and NW1153 in plasma Validated LC/MS/MS method for analysis of tyramine in plasma
PK Assessment	Plasma conc of safinamide, NW1689 and NW1153 Plasma conc of tyramine
PD Assessment	Systolic and Diastolic Blood Pressure, Heart Rate
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

*Challenge Agent: Daily escalating doses of tyramine were administered under fasting conditions, during the screening period and during the study period (from SD7 until SD16 or until Tyr30 was reached):
☐ at screening, daily escalating doses of tyramine were from 100 mg up to a maximum of 700 mg.

☐ during the study period, the tyramine dose escalation scheme applied to the phenelzine treatment group was different from that of the safinamide, selegiline and placebo treatment groups. In the phenelzine group, daily tyramine escalating doses were from 6.25 mg to a maximum of 200 mg dose. In the other treatment groups, tyramine doses were from 25 mg to a maximum of 700 mg.
To maintain blinding, according to this different escalation scheme across treatment groups, combinations of tyramine and tyramine-matching placebo capsules were administered at each of the tyramine challenges performed during the treatment period.



** Healthy males and females, aged 18 to 70 years and with a body mass index (BMI) of 18 to 30 kg/m^2, who successfully reached Tyr30 at doses ≥ 200, but ≤ 700 mg of tyramine during the screening oral tyramine pressor tests

Discussion of Trial Design

A parallel-group trial design for this study was justified by the irreversible nature of the MAO inhibition by the positive control, phenelzine, therefore long wash-out periods (between 2 and 7 weeks) between treatment groups would be necessary to allow MAO enzyme activity to recover.
To compensate for the inter-subject variability and to ensure a homogeneous trial population, the subjects included were screened according to their response to an oral tyramine pressor test. In order to fully validate the sensitivity of the trial and characterize the effect of safinamide on tyramine pressor effect, both controls, i.e., the positive control phenelzine and the comparator selegiline, were administered in a blinded manner, as well as safinamide and placebo.
Due to the high potency of phenelzine and the expected significant increase in tyramine pressor effect, doses of tyramine administered to this positive control group were different from those administered to the 4 other groups, starting with a much lower dose and escalating with smaller increments: in the phenelzine group the starting dose of tyramine was 6.25 mg and escalating to a maximum of 200 mg, compared to doses of 25 to 700 mg in the other groups).
The pressor effect of oral tyramine was investigated at safinamide steady-state. According to previous trials, PK and PD (MAO inhibition) steady-state of safinamide is reached within 5 to 6 days with one daily dose and low interindividual variability. In addition, safinamide main metabolites NW-1153 and NW-1689 have not shown any

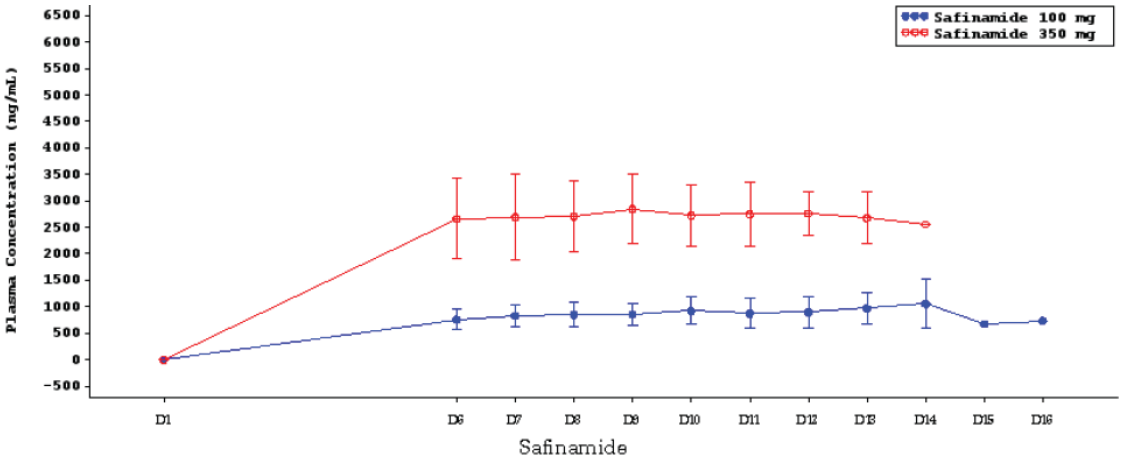
MAO-A inhibition potential and would potentiate the pressor effect of tyramine by safinamide.
Reviewer's Comment: The study design is adequate to evaluate the potentiation of the pressor effect of oral tyramine by safinamide.

Bioanalytical Assays:
Tyramine was isolated from plasma by solid phase extraction (SPE) and determined by reversed phase liquid chromatography (LC) with tandem mass spectrometric (MS/MS) detection. The analytical method was validated prior to this project with a LLOQ of 1.00 ng/mL and an upper limit of quantification (ULOQ) of 2000 ng/mL (Validation report (b) (4) 28095). In this study, the range was reduced after three performed runs to better reflect the concentration levels of the study samples. The ULOQ was changed to 250 ng/mL; a partial within-study validation was performed.
The performance of the assay for tyramine during the analysis of study samples was satisfactory. Out of 26 analytical runs, 23 runs were within the acceptance criteria for the analytical run when first analyzed. Three runs were rejected due to QC samples outside criteria, incorrect sample preparation and interfering peaks in the blank samples. The samples in the rejected runs were re-analyzed. The mean accuracy of the assay as determined from the analysis of quality control (QC) samples was within ±5.6%.

The concentration of **Safinamide and its metabolites NW-1153 and NW-1689** in human plasma were determined by LC-MS/MS. The analytical method was validated with a lower limit of quantification (LLOQ) for safinamide, NW-1153 and NW-1689 of 5.00 ng/mL and a upper limit of quantification (ULOQ) of 10000 ng/mL with a 100 µL sample volume, (Validation report (b) (4) 29081).
An interference test of tyramine was performed in this project. Tyramine did not interfere with the analysis of safinamide or NW-1689. The results were within criteria for the interference test. [Criteria for Interference of Tyramine: Intra- assay precision should not exceed 15% for the six replicates of the QC L samples (without and with tyramine). The mean accuracy should be within ±15%]. For NW-1153, tyramine interfered with the assay. The peak area for the internal standard (b) (4) was increased (+30.0%) when tyramine was included in the IS working solution. However, no such effects were observed in the runs with study samples and the interference is judged to have no impact on the study results for NW-1153.
The study contained five different randomization groups. All study samples were analyzed from the two groups that were administered safinamide, with a total of 36 subjects. For the placebo group with 18 subjects, the 120 min post dose samples on Day 7 to Day 16 were analyzed. No samples were analyzed for the two groups that were administered phenelzine or selegiline.
The performance of the assay for safinamide, NW-1689 and NW-1153 during the analysis of study samples was satisfactory.

Pharmacokinetic Results:
The concentration profiles (mean [s.d.]) for safinamide by treatment group from SD1 to up to SD16 are presented below.

Mean (s.d.) Safinamide Plasma Concentration (ng/mL)



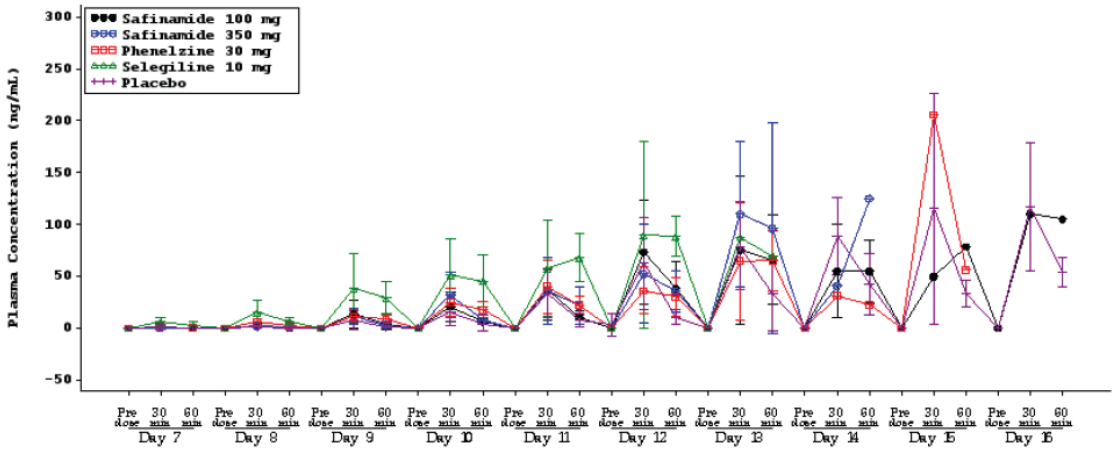
The concentrations of safinamide and the two metabolites were in agreement with the previously reported PK data of safinamide.

Mean (s.d.) predose safinamide concentrations on SD6 were 759.9 (184.53) ng/mL in the safinamide 100 mg group and 2661.7 (762.55) ng/mL in the safinamide 350 mg group, i.e., a 3.5-fold increase indicating dose-proportionality of safinamide PK. Predose concentrations were stable after SD6, indicating that steady-state had been reached at the time when tyramine challenges were started.

Mean (s.d.) predose plasma concentrations of NW-1153 on SD6 were 73.67 (16.978) ng/mL for safinamide 100 mg and 266.06 (46.970) ng/mL for safinamide 350 mg. Mean (s.d.) predose plasma concentration of NW-1689 on SD6 was 1209.944 (777.4947) ng/mL for safinamide 100 mg and 3340.556 (1577.2331) ng/mL for safinamide 350 mg. Predose concentrations of these metabolites were also stable after SD6.

Tyramine

Mean (s.d.) Plasma Concentration of Tyramine by Treatment Group (ng/mL)



When interpreting the tyramine plasma concentration data over time, the following should be taken into consideration:

- The tyramine dose escalation scheme applied to the phenelzine treatment group was different from the escalation scheme assigned to the safinamide, selegiline and placebo treatment groups (see Challenge Agent).
- In each treatment group, the number of subjects decreases with time, since further daily escalation of tyramine dose was stopped once the subjects reached Tyr30.

The visual examination of the plasma concentrations of tyramine and the concentration profiles suggested that the peak of tyramine was reached between the 2 post-dose assessment time points of 30 minutes and 60 minutes.
Overall, subjects received 1 of the 4 treatments for a minimum of 7 days and a maximum of 16 days, until Tyr30 was reached; the duration of exposure is therefore documented by the time to reach Tyr30.

Pharmacodynamic (PD) Results:

A total number of 89 subjects were randomized, 88 subjects completed the treatment. One subject in the safinamide 350 mg treatment group was discontinued due to TEAE (a moderate isolated alanine amino transferase (ALT) increase that resolved without sequelae).
The primary endpoint of the trial was the TSF, i.e. the ratio of Tyr30 at screening to the Tyr30 under treatment. The summary descriptive statistics of the TSF results are summarized in the table below.
High inter-individual variability was observed in most of the PD parameters, e.g., TSF and Tyr30. Nevertheless, results were consistent across analyses and with the literature.

Descriptive Statistics of TSF (Safety Population, N=89)

Parameter	Statistics	Safinamide 100 mg (n=18)	Safinamide 350 mg (n=18)	Phenelzine 30 mg (n=17)	Selegiline 10 mg (n=18)	Placebo (n=18)
TSF	n (missing)	18 (0)	17 (1)	17 (0)	18 (0)	18 (0)
	Mean (s.d.)	2.994 (2.965)	4.775 (4.962)	13.843 (14.400)	3.861 (3.269)	2.459 (3.700)
	Geometric Mean	2.152	2.740	9.977	3.115	1.515
	Median	1.833	2.500	10.667	2.667	1.000
	Min ;Max	0.80 ;12.00	0.75 ;16.00	3.33 ;64.00	1.67 ;12.00	0.71 ;16.00

The sensitivity of the trial was confirmed by the comparator group (selegiline 10 mg) and the positive control group (phenelzine 30 mg), both showing a treatment effect over placebo.
For safinamide at both doses, a mild increase in TSF was observed. This effect appeared to be slightly dose-dependent. However, the potentiation of the pressor effect by safinamide at both doses remained lower than that of the comparator (selegiline 10 mg).
The ANOVA analysis of log-transformed TSF for the different groups versus placebo showed the same ranking of treatment effect as the descriptive statistics analysis; safinamide, at the dose of 100 mg and 350 mg, respectively, potentiated the pressor effect of tyramine by 1.6-fold and 1.8-fold over placebo, compared to 2.2-fold for selegiline 10 mg and 6-fold for phenelzine 30 mg.
As part of the primary endpoint analysis, an ANOVA model was applied to the log-transformed TSF, with fixed effects for treatment, gender and site. A summary of the results is provided below.

Summary of Back-transformed ANOVA Results for TSF (Safety Population)

Contrasts	Ratio (%)	90% CI of Ratio(L)	90% CI of Ratio(U)
Safinamide 100 mg vs. Placebo	155.9	102.5	237.0
Safinamide 350 mg vs. Placebo	175.3	114.9	267.6
Phenelzine 30 mg vs. Placebo	598.2	391.1	914.7
Selegiline 10 mg vs. Placebo	218.7	144.0	332.1

L: Lower limit of 90%CI
U: Upper Limit of 90%CI

The back-transformed results of the ANOVA on the TSF were consistent with the descriptive statistics analyses. Assay sensitivity of the trial was confirmed. The geometric least square mean ratios of TSF for the treatment groups versus placebo followed the same ranking of treatment effect. Safinamide, at the dose of 100 mg and 350 mg, respectively potentiated the pressor effect of tyramine by 1.6-fold and 1.8-fold versus placebo, compared to 2.2-fold for selegiline 10 mg and 6-fold for phenelzine 30 mg. Similar results were also observed when evaluating the ED50 (the dose of oral tyramine necessary for 50% of the subjects to show a sustained increase in SBP of at least 30 mmHg under the conditions of the test i.e., at screening or in response to one of the treatments), and the ED50 ratio (the ratio of ED50 under placebo to the ED50 under treatment). The ED50 analysis was in agreement with the primary analysis results, with safinamide at both doses showing higher ED50 (200 mg for both doses) than selegiline

(150 mg) and phenelzine (37.5 mg). The ED50 for the placebo treatment group was 400 mg, resulting in ED50 ratios of 2 for safinamide 100 mg and 350 mg, 2.67 for selegiline 10 mg, and 10.67 for phenelzine 30 mg.
Other PD results such as maximum SBP, DBP, time to return to baseline, and area under blood pressure curve at Tyr30 were similar across treatment groups and for the screening and treatment period assessments.

Maximum Increase in SBP (mmHg) at Tyr30 (Safety Population)

Time point	Statistics	Safinamide 100 mg (n=18)	Safinamide 350 mg (n=18)	Phenelzine 30 mg (n=17)	Selegiline 10 mg (n=18)	Placebo (n=18)
Tyr30 Screening	Mean (s.d.)	50.3 (11.4)	59.3 (15.6)	59.9 (15.2)	55.3 (14.8)	54.8 (13.8)
	Min ;Max	31 ;68	36 ;82	34 ;94	37 ;94	36 ;82
Tyr30 Under treatment	Mean (s.d.)	57.3 (19.0)	53.2 (11.8)	55.5 (19.4)	50.9 (11.4)	54.4 (11.7)
	Min ;Max	39 ;119	34 ;76	33 ;97	41 ;85	39 ;77

As shown in the table above, maximum increase in SBP at Tyr30 at screening was similar across the treatment groups, which confirmed that there was no clinically relevant difference between the treatment groups at the screening Tyr30 assessment.
Maximum increase in SBP at Tyr30 under treatment was also similar across treatment groups and was not different from the maximum increase in SBP at screening

PD Summary: Results of the different analyses were consistent, showing a mild increase in TSF after administration of safinamide at 100 mg (therapeutic dose) and 350 mg (supratherapeutic dose). The safinamide effects appeared to be slightly dose-dependent, however the potentiation of the pressor effect by safinamide at both doses remained lower than that of the comparator (selegiline 10 mg), for which there is no tyramine warning in the label at the dose level tested in the current study.

Reviewer's Comment
The following labeling language is proposed by the sponsor; this is acceptable:
Dietary tyramine restriction is not required with recommended doses of XADAGO tablets.

Safety Results:
Of all TEAEs reported by 46 subjects during the study, only 2 were of severe intensity: a severe fibula fracture in the phenelzine 30 mg group (not related to treatment) and a severe headache in the placebo group (considered possibly related to treatment). All the other TEAEs were mild to moderate in intensity. Following the administration of safinamide, selegiline or phenelzine only (SD1 to SD6), headaches, nausea, and insomnia were the most frequent TEAEs. Following the administration of IMPs with tyramine (SD7 to the day where Tyr30 was reached), the most frequent TEAE were nervous system disorders (headaches, dizziness), cardiac disorders (palpitations), gastrointestinal disorders (nausea, abdominal pain, diarrhea, and vomiting) and general disorders (chest pain). Cardiac TEAEs (palpitations, specifically) were associated with tyramine challenges and not observed when safinamide (or selegiline or phenelzine) was administered alone. Headaches also tended to be more frequent during the tyramine challenge periods.

XIV. Study NW-1015/010/II/2002: A Phase II, Dose Titration, Open-Label Study to Investigate the Tolerability and Potential Interactions with Anticonvulsant Medications of Safinamide as Add-on Therapy in Patients with Refractory Partial and/or Generalised Epilepsy

Objectives:

- to investigate the tolerability of safinamide in patients with refractory partial and/or generalized epilepsy when administered concomitantly with at most three other AEDs at stable doses.
- to collect further information concerning possible interactions among safinamide and other AEDs.

Study Design	open, non-comparative study, assessing tolerability of increasing doses of safinamide
Study Population*	33 male and 10 female patients with epilepsy aged 18 to 75 years, body weight 50-90 kg. 38 patients completed the 12 weeks treatment period, 5 withdrew before the study end.
Treatment Group**	none
Dosage and Administration	Safinamide: gelatine capsules containing 50 mg safinamide (powder) **
PK Sampling	Blood samples for determination of plasma safinamide and AEDs levels. ***
Analysis	No bioanalytical information provided
PK Assessment	Blood concentrations of safinamide were analyzed by ANOVA followed by Tukey's test. Values at the end of titration were compared with values at the end of stabilization by paired t test. Correlation between safinamide plasma concentrations and AEDs plasma concentrations were performed by Pearson correlation test or Spearman correlation test as appropriate.
PD Assessment	Patients who obtained at least a 50% reduction of seizure frequency, described by means of frequencies and percentages
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

* Patients had to be under stable doses, for at least 2 weeks prior to screening, of at most three AEDs. Exclusion Criteria: Clinically significant gastrointestinal, renal, hepatic, endocrine, pulmonary or cardiovascular disease; Use of variable doses of AEDs in the 2 weeks prior to the screening visit, treatment with SSRis, tetracyclic antidepressants, MAO inhibitors, meperidine derivatives in the 4 weeks prior to the screening visit.

**Safinamide Titration Schedule

Days	Weeks	A.M. no. of capsules	Total Daily Dose
1-14		-	0
14-28	0-2	1	50
28-42	2-4	2	100
42-56	4-6	4	200
56-70	6-8	6	300

***AEDs plasma levels were measured at the end of the baseline period and after 2, 4, 6, 8 and 12 weeks, or in case of premature withdrawal. Safinamide plasma levels were determined at weeks 2, 4, 6, 8 and 12, before the drug administration. At week 4, five PK samples were collected for safinamide concentrations: before drug intake and 3, 6, 12 and 24 hours after drug administration.

Bioanalytical Assays:
The only information provided by the sponsor is the following: Plasma samples and urine samples for assay of AEDs and safinamide concentrations were shipped to the centralized pharmacokinetic laboratory for evaluation.

Pharmacolokinetic Results:
Descriptive statistics of plasma concentrations of safinamide at each visit (before drug administration) are presented below.

Descriptive statistics of safinamide plasma concentrations (mg/L)

VISIT	DOSE	N	MEAN	SD	MIN	MEDIAN	MAX
Week 2	50 mg	41	0.472	0.327	0.090	0.360	1.390
Week 4	100 mg	39	0.781	0.450	0.070	0.670	2.060
Week 6	200 mg	38	1.679	1.342	0.310	1.410	6.770
Week 8	300 mg	36	2.185	1.422	0.340	1.910	5.210
Week 12	300 mg	37	2.239	1.718	0.190	1.830	7.520

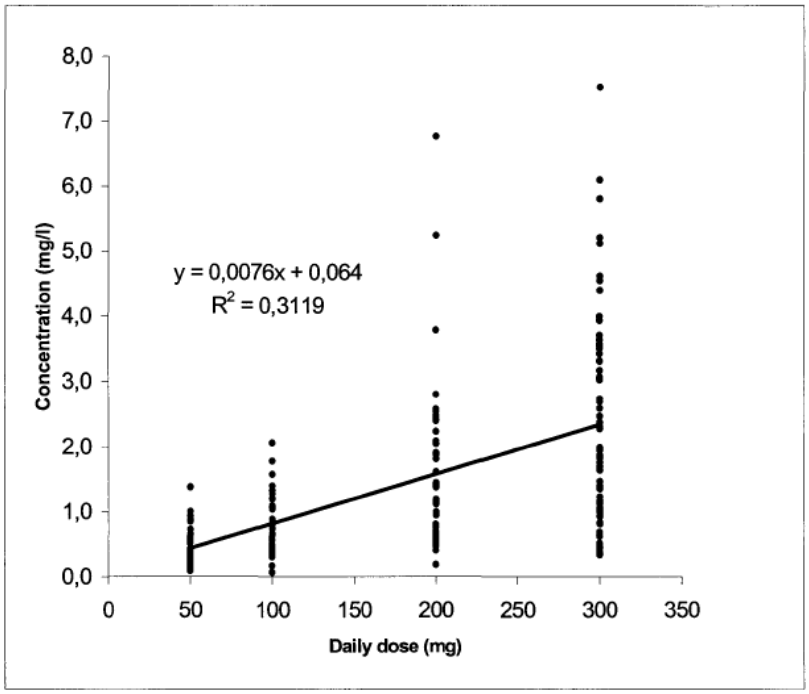
Note: In table above, at Week 12 the dose should reflect the 300 mg, however some patients were taking 200 mg at Week 12, see table below.

VISIT (Period)	50 mg	100 mg	200 mg	300 mg	Total
V3 (Weeks 1-2)	43				43
V4 (Weeks 3-4)	1*	40			41
V5 (Weeks 5-6)		1*	38		39
V6 (Weeks 7-8)			2 (*,&)	37	39
V7 (Weeks 9-12)			5 (&,$,#,@,£)	34§	39

* Subject 33 of centre no. 8. Slow titration probably due to concomitant unrelated AEs, he reached the 300 mg at V7
& Subject 21 of centre 5 did not reach the 300 mg dose due to AE (Headache).
$ Subject 19 of centre 2 reduced the dose due to AE (Vertigo)
# Subject 22 of centre 5 reduced the dose due to AE (Vertigo, dizziness, blurred vision)
@ Subject 39 of centre 9 reduced the dose due to AE (partial evolving to secondarily generalized seizure)
£ Subject 14 of centre 7 reduced the dose probably due to AE (Vertigo)
§ Subject 18 of centre n. 2 reduced the 300 mg dose to 200 mg on day 1 of week 11 because of AE (increase in seizure frequency).

A scatter plot of dose vs. safinamide plasma concentrations at each visit (before drug administration) is presented below.

Scatter plot of dose vs. concentration of safinamide (mg/L)



The linear dose-concentration relationship was demonstrated by the results of regression analysis (P<0.000l).

Safinamide plasma concentrations at Week 4, measured from blood samples taken before drug administration and at 3, 6, 12 and 24 hours after oral administration of 200 mg safinamide, are presented in the figure below.

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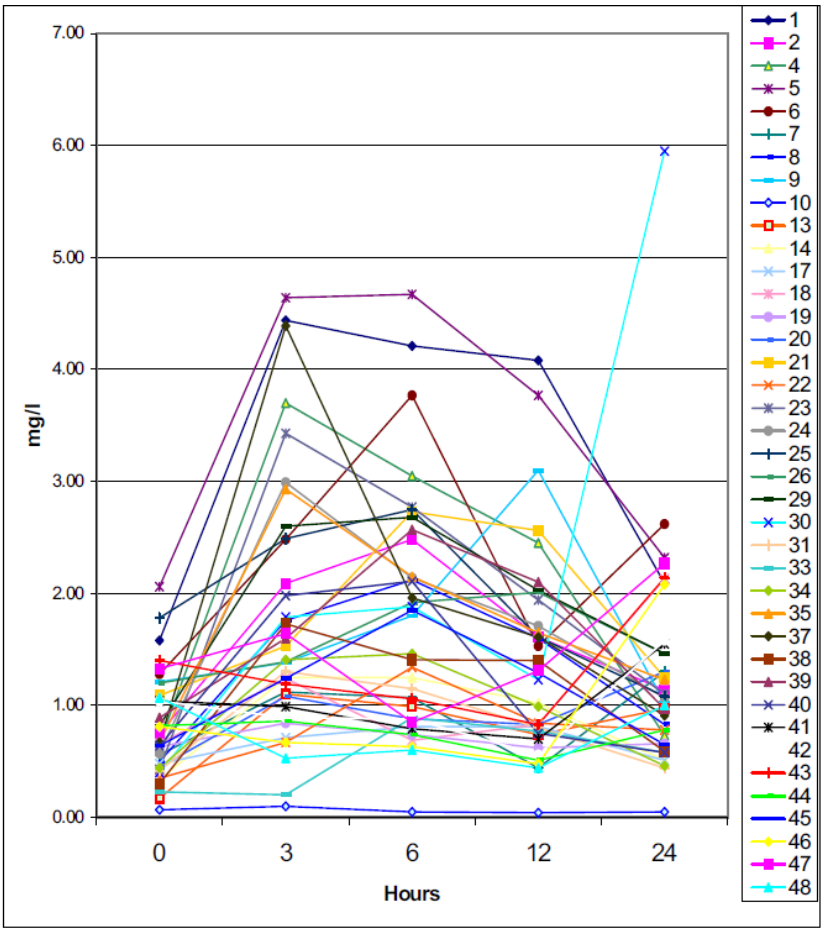
Safinamide PK: Individual time-concentration curves at Week 4



Safinamide PK Parameters After Oral Administration of 200 mg Safinamide at Week 4

PARAMETER	MEAN	SD	MIN	MEDIAN	MAX
AUC (mg-h/l)	34.345	17.978	1.290	28.515	85.875
C_{max} (mg/l)	2.251	1.248	0.100	2.110	5.950
T_{max} (h)	9.308	8.452	0.000	6.000	24.000

N. of patients = 39

In ten patients, the peak plasma levels were observed on the second day, 24 hours after drug administration. According to the sponsor, it is likely that these patients took the safinamide dose on the second day before blood sampling.
This lead to inaccurate estimates of some PK parameters, such as T_{max} and $t_{1/2}$.
An additional analysis was performed by the sponsor after excluding the values of these subjects from the descriptive statistics; this analysis is reported below.

PK parameters calculated from the concentration-time curves after oral administration of 200 mg of safinamide at week 4, after exclusion of patients having taken the medication before blood sampling at 24 hours

PARAMETER	MEAN	SD	MIN	MEDIAN	MAX
AUC (mg-h/l)	36.354	19.231	1.290	39.885	85.875
C_{max} (mg/l)	2.290	1.213	0.100	2.120	4.670
T_{max} (h)	5.069	2.419	3.000	6.000	12.000

N. of patients = 29

Mean safinamide plasma half life was around 16 hours, with a wide variability (from 14 to 29 hours), likely due to blood sampling only up to 24 hours post-dose.

Information concerning possible **interactions between safinamide and the AEDs** was obtained by evaluating dosage modifications of AEDs and the trend of blood concentrations of safinamide and AEDs.
Patients took 1 to 3 AEDs (mean 1.9; median 2), see tables below. Most patients continued their AED treatment without changes in dosage until the study end.
In two patients the AED dose was reduced because of the onset of adverse events: in patient 25 topiramate daily dose was reduced from 500 mg to 400 mg from week 8 to week 12; in subject 33 carbamazepine dose was reduced from 1600 to 1400 mg/day from week 2.

Number of AEDs per patient	n	%
One AED	8	18.6
Two AEDs	17	39.5
Three AEDs	18	41.9

TYPE OF AED	n	(%)
Barbiturates	13	30.2
Phenytoin	1	2.3
Clonazepam	3	7.0
Carbamazepine	19	44.2
Oxacarbazepine	11	25.6
Valproic Acid	9	20.9
Vigabatrin	1	2.3
Lamotrigine	7	16.3
Felbamate	1	2.3
Topiramate	16	37.2
Levetiracetam	8	18.6
Diazepam	1	2.3
Clobazam	6	14.0

N = number of patients in the ITT population
n = number of patients taking the AED
Calculation of percentages based on N

As shown in the table, carbamazepine, topiramate, barbiturates and oxacarbazepine were the most frequently used anti-epileptics.

Safinamide plasma concentrations were assessed by stratifying by AED class:
• Enzyme Inducers (barbiturates, carbamazepine, oxcarbazepine, phenytoin);
• Non-inducers of enzymes (all other AEDs).
Within each of the above 2 classes, an additional stratification was made, according to the presence of concomitant treatment with valproic acid.
Note: No justification for why the additional stratification (according to the presence of concomitant treatment with valproic acid) was needed was provided by the sponsor.

Descriptive statistics of safinamide AUC by AED type. P value of factors included into the complete ANOVA model

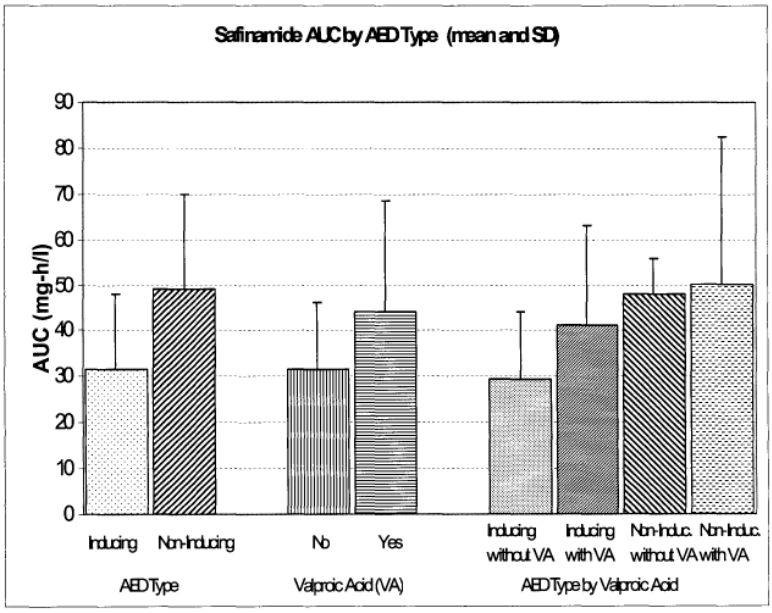
FACTOR	Class	N	MEAN	SD	P value
AED Type	Inducing	33	31.6727272	16.3308956	0.0931
	Non-Inducing	6	49.0424994	21.0498002	
VA	No	30	31.3934999	14.9475097	0.3868
	Yes	9	44.1833329	24.1600797	
AED type by VA					
	Inducing without VA	27	29.5566667	14.4668439	
	Inducing with VA	6	41.1949995	22.0357516	0.5563
	Non-Inducing without VA	3	47.9249992	7.9660588	
	Non-Inducing with VA	3	50.1599996	32.2572582	

VA = Valproic Acid

Descriptive statistics of safinamide AUC by AED type.
P value of comparison between AED class (Reduced ANOVA)

FACTOR	Class	N	MEAN	SD	P value
AED Type	Inducing	33	31.6727272	16.3308956	0.0274
	Non-Inducing	6	49.0424994	21.0498002	

Mean values and SD of Safinamide AUC of in each sub-group determined by the type of AED



Safinamide levels were linearly related to dose, and there was a trend for safinamide levels to be about 30% lower in patients co-medicated with enzyme inducers compared with those not on enzyme inducers.

Reviewer's Comments:
This analysis should be considered exploratory only. The results suggest that there is no evidence of large effect of strong CYP inducers on safinamide exposure.

Efficacy Results:
According to the sponsor, the percentage of "responders", defined as patients who showed at least a 50% reduction of seizures during the 12 weeks of treatment with safinamide, in comparison with the previous 3 months, was 28.2 %. Compared with the 2-week prospective baseline, 16 of 39 evaluable patients (41%) had a greater than 50% reduction in seizure frequency during the treatment period with safinamide.

Safety:
The majority of AEs were rated of mild or moderate intensity.
One serious AE was reported: Patient Number 35 developed severe thrombocytopenia at week 8, probably not related to the study drug. According to the sponsor, the low platelet count (11,000/ml) is likely attributable to a laboratory error, because the previous (two weeks before) and subsequent (one week later) hematological examinations showed normal platelet counts.
One patient (Number 27) withdrew because of visual disturbances (diplopia) after 17 days of treatment with safinamide.

1.4 Healthy Subjects PD and PK/PD Studies

XV. (b) (4)-NW/PAR-254-00: Pharmacodynamic and pharmacokinetic study of the anticonvulsant and antiparkinsonian agent NW-1015 after oral administration of 300, 600 µg/kg and placebo in healthy male volunteers aged 55-65.

Objective:
To evaluate the pharmacokinetics (systemic exposure) and pharmacodynamics (MAO-B inhibition and cholinesterase activity) of NW-1015 following a single oral doses

Study Design	Three-treatment, six-sequence, three-period, randomized, balanced, cross-over study
Study Population*	6 healthy male subjects (55-65 years), Caucasians, non-smokers, within 10% of ideal BW
Treatment Groups	Placebo NW-1015, 300 µg/kg body weight NW-1015, 600 µg/kg body weight
Dosage and Administration	Three single-treatment sessions in the fasting state, each separated by a 14 days wash-out period. NW-1015 (300 or 600 µg/kg body weight) was administered as 10 mg capsules.
PK Sampling: plasma	NW-1015 plasma concentrations at pre-dose, 10, 20, 40 min, 1, 2, 3, 4, 6, 8,10,12,16, 24, 36, 48, 72, 96, 120 h post-dosing. For V1R (first period), V6 (second period) and V3, V4, V5 (third period) plasma concentrations were also evaluated at 168 and 312 h post-dosing
Analysis	Plasma: validated LC-MS/MS method for NW-1015 Range: 20 to 20000 ng/mL
PK Assessment	C_{max}, , t_{max}, $AUC_{0\text{-}inf}$, $t_{1/2}$ of NW-1015
PD Assessment	MAO-B inhibitory activity in platelet rich plasma (PRP) at day -1: 0 h and at day 1: (predose), 10, 20, 40 min, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96,120 h post-dosing. For V1R (first period), V6 (second period) and V3, V4, V5 (third period) plasma concentrations were also evaluated at 168 and 312 h post-dosing.
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry, neurological exam

* No medications were permitted starting from 7 days prior to enrolment and for the entire duration of the study period, except ACE-inhibitors. Treatment with any known enzyme inhibiting or inducing agents (barbiturates, phenothiazines, etc.) in the 4 weeks prior to study initiation was not allowed.
Avoidance of fresh grapefruit or grapefruit juice from 24 hours prior to and until the end of the study (Comment: should have been 72 h before).

Bioanalytical Assay:
Plasma samples were analyzed for NW-1015 using a validated assay (Bioanalytical Validation Protocol NW-1015 70/99). For details, see under (b) (4)-PNU-194-99, p 3.

Assay performance during analysis of Study samples:

Study specific validation of NW-1015 assay in plasma (high concentrations)

		Theoretical concentrations ng/ml							Calibration curve parameters weight : $1/c^2$			QCs ng/ml			
		20000	5000	1000	250	100	50	20	Slope $X10^{-3}$	Interc. $X10^{-2}$	r	6000	600	60	Volunteers Assayed *
Date	Curve no.	Back-calculated concentrations ng/ml													
15/12/2000	1	20750.23	5173.08	1020.32	237.84	97.38	47.82	20.52	8.3000	2.3869	0.9991	6252.25	674.42	59.76	V02(P1,P2),V03(P2,P3),V04(P2,P3)
												6873.15	575.47	64.24	
												6767.50	630.95	57.32	V05(P1,P3),V06(P1,P2)
													582.44		
19/12/2000	2	21702.91	5345.00	994.17	221.56	96.24	48.89	20.51	8.1888	-1.8968	0.9970	6628.27	591.91	52.75	V01R(P1,P3)
												6575.67	559.06	58.23	
	N	2	2	2	2	2	2	2	2	2	2	5	6	5	
	MEAN	21226.57	5259.04	1007.25	229.70	96.81	48.36	20.52	8.2444	0.2461	0.9981	6623.37	602.38	58.46	
	SD	673.646	121.566	18.491	11.512	0.806	0.757	0.007	0.0786			239.457	42.694	4.155	
	CV%	3.2	2.3	1.8	5.0	0.8	1.6	0.0	1.0			3.6	7.1	7.1	
	ACC%	+6.1	+5.2	+0.7	-8.1	-3.2	-3.3	+2.5				+10.4	+0.4	-2.6	

*: For each volunteers samples with plasma concentration ≤20ng/ml were assayed with the more sensitive method (range of calibration curve 20-0.5 ng/ml);other samples were analysed with the method working in the 2000-20 ng/ml range

Reviewer's Comments: The assay performance during the validation was acceptable. The assay performance during the analysis of the plasma samples was acceptable.

Pharmacokinetic Results:

Three subjects had pre-dose NW-1015 concentrations (from the previous period):

	Dose	Concentration at time "0"	Sequence
Vol. 3	300 µg/kg	1.02 ng / ml	P, D_2, **D_1**
Vol. 4	600 µg/kg	1.23 ng / ml	P, D_1, **D_2**
Vol. 6	300 µg/kg	0.91 ng / ml	D_2, **D_1** P

Reviewer's Comments:

- This suggests a longer $t_{1/2}$ (than the 23h reported in the label) for NW-1015. The $t_{1/2}$ estimated by the 3-compartment method (61 h, see below) seems a better estimate.
- The following appears in the report: All these concentrations are however very low, close to the LOQ, which is 0.5 ng/ml. However, everywhere in the bioanalytical reports (validation and in-study) the LOQ is reported to be 20 ng/ml.

Table E – Half-life values estimated with the three compartment procedure in five cases

	$t_{1/2}$ β (h)	$t_{1/2}$ deep compartment (h)
Vol. 3 – 300 mg	23.48	68.44
Vol. 6 – 300 mg	19.33	69.51
Vol. 1 – 600 mg	16.63	60.34
Vol. 4 – 600 mg	36.70	56.47
Vol. 5 – 600 mg	20.36	49.33
Mean	**23.33**	**60.82**

Mean Pharmacokinetic parameters of NW-1015

PARAMETER	300 µg/kg			600 µg/kg		
	MEAN	SD	CV%	MEAN	SD	CV%
C_{max} (ng·ml^{-1})	96.78	14.33	14.8	208.11	57.27	27.5
AUC_{0-t} (ng·ml^{-1}·h)	3187.77	351.92	11.0	7066.72	1525.73	21.6
AUC (ng·ml^{-1}·h)	3534.37	478.26	13.5	7214.26	1573.79	21.8
t_{max} (h)	3.17	2.32	73.2	3.83	2.40	62.6
$t_{1/2}$ (h)	38.4	6.00	15.6	32.1	2.94	9.2
C_{max} (norm.) (ng·ml^{-1})*	103.64	17.32	16.7	222.03	56.88	25.6
AUC_{0-t} (norm.) (ng·ml^{-1}·h) *	3422.55	514.75	15.0	7621.37	1962.87	25.8
AUC (norm.) (ng·ml^{-1}·h)*	3798.59	668.79	17.6	7767.22	1968.79	25.3

Pharmacodynamic Methods:

MAO-B activity was radiochemically measured as follows: platelet rich plasma (PRP) samples were rapidly thawed by placing in a 37^{0}C water bath for approximately 1.5-2 min. In a final volume of 600 µl of Na-phosphate buffer 0.1 M, pH 7.4, 110 µl of PRP and 50 µl of ^{14}C-PEA were added to obtain a final concentration of 0.5 µM ^{14}C-PEA and a 1:5 dilution of original PRP.

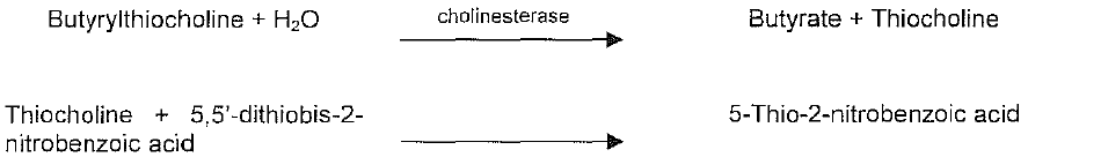
Samples were transferred in a water bath at 37^{0}C. The PEA deamination was carried out in air for 15 minutes (linear kinetics against time) at 37^{0}C under agitation. The reaction was stopped by addition of 200 µl of 65% perchloric acid.

Samples were extracted with 3.5 ml of toluene for 20 min under gentle agitation. The aqueous and the organic phases were separated by centrifugation (1500 g for 10 min).

3 ml of the organic phase were transferred into scintillation vials and 10 ml of Insta-Fluor Plus added as liquid scintillation cocktail.

The radioactivity in each sample was determined by the liquid scintillation counting technique.

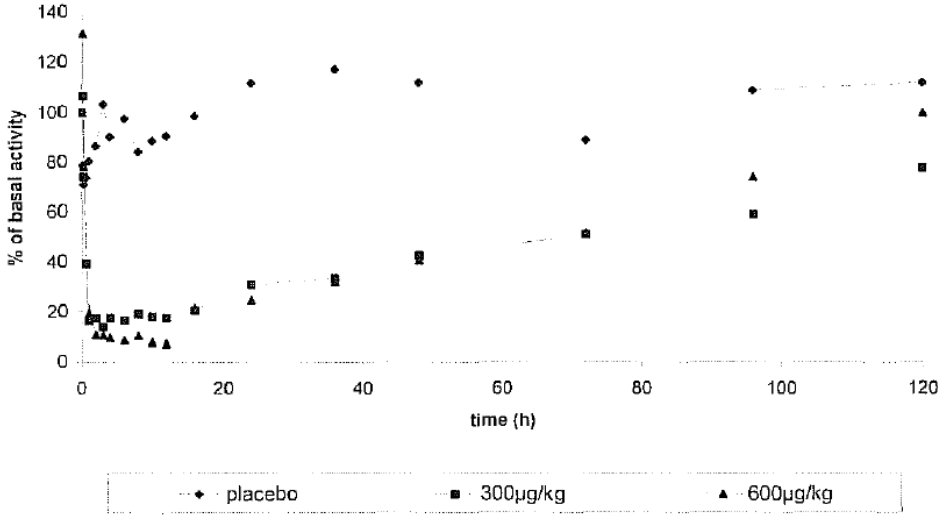
Cholinesterase assay:



Cholinesterase hydrolyzes butyrylthiocholine (BTC) to yield thiocholine which reacts with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) to form the yellow 5-thio-2-nitrobenzoate with an absorbance maximum at 405 nm. The rate of change in absorbance at 405 nm is directly proportional to cholinesterase activity.

Pharmacodynamic Results:

The time course of the platelet MAO-B activity inhibition (expressed as % of basal activity) following each NW-1015 administration is shown in below.



Maximum variations from basal activity were 13.9 ± 2.72 and 7.13 ± 0.86 (Mean ± SD) of basal activity at 300 and 600 µg/kg, respectively, and occurred within 3-12 hours from the administration.
The MAO-B platelet activity after administration of placebo to male healthy volunteers, did not show significant difference (Dunnett's test) from baseline activity (activity at time zero) at all sampling times.
The MAO-B platelet activity after administration of 300 and 600 µg/kg of NW-1015 to male healthy volunteers, resulted significantly different from baseline activity ($p<0.05$) at all sampling times starting from 10 and 20 minutes, respectively.
MAO-B inhibition was still statistically significantly different at 120 h both at 300 and 600 µg/kg. The activity appeared restored after 168 h at 600 µg/kg.
The one-way ANOVA showed that the 300 and 600 µg/kg doses caused a statistically different effect on MAO-B activity with respect to placebo ($p<0.05$).
The effects induced by the 600 µg/kg dose were not statistically different from those of 300 µg/kg.
No serum cholinesterase activity variations have been observed after the administration of NW-1015 at 300 and 600 µg/kg and placebo.

PD Conclusions: Platelet MAO-B activity was inhibited by approximately 84% and 92% after single administration of 300 and 600 µg/kg safinamide, respectively. It can be assumed that after chronic administration of such doses the inhibition will be almost complete.

Safety:
Systolic and diastolic BP, HR and body temperature did not show significant changes. In some cases systolic and diastolic BP decrease at 2 h after dosing. This decrease, however was not clinically relevant. Only Vol. 3 (treated with 300 µg/kg) showed a BP decrease, from systolic 160 to 110 and diastolic from 100 to 80. When treated with 600 µg/kg, this volunteer did not show any BP changes.

XVI. (b) (4) 28559: A randomized, double-blind, phase I, placebo-controlled, multiple-dose parallel groups study with an open-label, single-dose positive control (moxifloxacin 400 mg) to investigate the effect of safinamide (100 mg and 350 mg) on the QT/QTc interval in healthy subjects

Objectives:

Primary Objective: To rule out a QTcF interval prolongation of 10 ms or greater after multiple doses of 100 mg safinamide (therapeutic dose) compared to placebo at all defined time points on Study Day (SD) 6 of drug administration.

Secondary Objectives:

☐ To determine the effect of multiple doses of supratherapeutic dose (350 mg) of safinamide on QTcF interval compared to placebo on SD 6 of drug administration.

☐ To determine the effect of a positive control, single 400 mg dose moxifloxacin on QTcF interval compared to placebo at all defined time points on SD6 of drug administration.

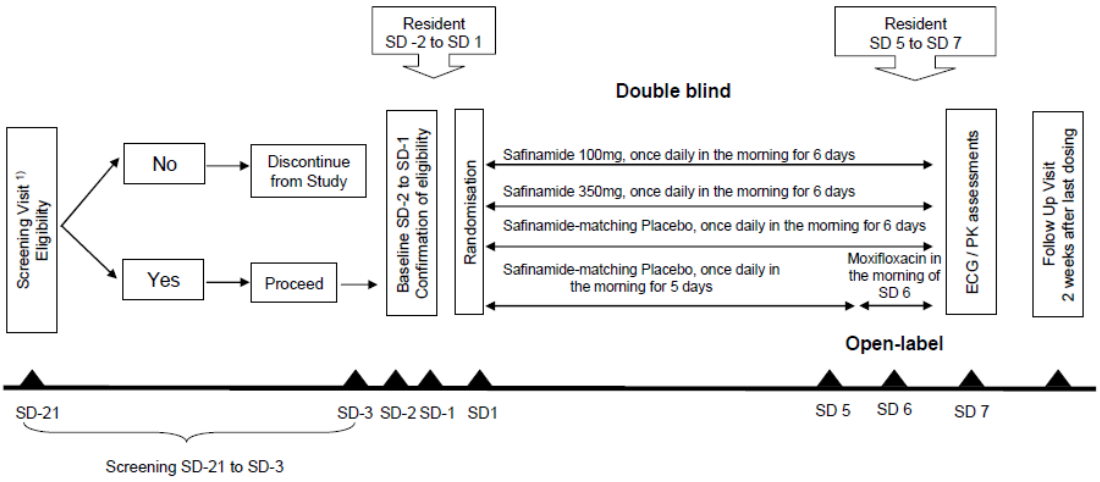
☐ To determine the effect of safinamide (100 mg and 350 mg) on other QT variables

☐ To investigate the relationship between plasma concentration of safinamide (after 100 mg and 350 mg) and QTcF.

☐ To evaluate the pharmacokinetics (PK) of safinamide, safinamide metabolites and moxifloxacin.

Study Design*	Randomized, active- and placebo-controlled parallel groups study. The study was double-blind for the safinamide and matching placebo groups and was open-label for the moxifloxacin group.
Study Population	240 healthy male and female subjects (18-45 years), Pharmacokinetic population: 177 subjects
Treatment Groups	4 groups: Placebo, Safinamide (100 mg and 350 mg) and Moxifloxacin 400 mg
Dosage and Administration**	Safinamide was administered as film-coated tablets 50-mg and 100-mg Moxifloxacin was provided as Avalox® (Bayer)
PK Sampling: plasma	Plasma samples were collected pre-dose on the morning of SD1 to SD5 and at the following time points on SD6: predose, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h postdose (after the administration on SD6).
PK Sampling: urine	24-h urine collection for 6β-hydroxycortisol/cortisol ratio started in the morning of SD –1 and SD6
Analysis	Plasma: 2 validated LC-MS/MS assays (safinamide - NW-1153 and NW1689 - NW1689AG). Range: 5-10000 ng/ml for safinamide and NW-1153 Moxifloxacin: validated LC-MS/MS assay; Range: 1-1000 ng/ml
PK Assessment	C_{max}, , t_{max}, AUC_{0-24} of safinamide, its major metabolites and moxifloxacin Fold-change to baseline in 6β-OHF/free-cortisol ratio***
PD Assessment	QTcF interval at 11 time points on SD 6 (-1, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h) Absolute QT, QTcB and QTcI. PR, QRS, QT, RR, T-wave morphology, U wave, heart rate (HR).
Safety Assessment	Adverse events, vital signs, electrocardiograms, clinical chemistry

* **Study Schematic Design**



** No co-medication allowed with the exception of oral contraceptives.
***To show the absence of clinically relevant CYP3A4 induction, the log-transformed fold-change to baseline in 6β-OHF/free-cortisol ratio was compared between placebo and the two different safinamide dose groups using an ANOVA model with fixed effects for treatment and gender; 95% confidence intervals for the ratio of geometric means (safinamide / placebo) were calculated.

Bioanalytical Assays:

Each PK blood sample was collected into a lithium heparin tube. The samples were immediately placed on wet ice and centrifuged within 15 minutes at about 4°C and 2500 g for 10 minutes. Plasma samples were then separated into 2 aliquots series.
One series (neutral series) was split into 2 further individual aliquots A&B for analysis of safinamide and NW1153 or moxifloxacin. The second series (acidified series) consisted in 1 aliquot C, which was acidified using o-phosphoric acid (20% solution in water) for analysis of NW1689 and NW1689-glucuronide. All plasma aliquots were frozen immediately at –20 °C.

Two bioanalytical methods were validated for the analyses of safinamide and its major metabolites: safinamide - NW-1153 and NW1689 - NW1689AG.
Safinamide and NW-1153 were quantified in plasma (without being acidified first; Analytical Report (b) (4) 28100). Safinamide and NW-1153 and their internal standards were extracted from plasma into acetonitrile by protein precipitation. The supernatant was injected onto a CN column. The compounds were eluted with a mobile phase containing acetonitrile and water with 10 mM ammonium acetate and detected with MS/MS (method AS M-207, version 2). The analytical method was validated prior to this project with a LLOQ of 5.00 ng/mL for both safinamide (free base) and NW-1153 and a ULOQ of 10000 ng/mL with a 100 µL sample volume.
The study PK samples were analyzed in 36 runs, including two runs with incurred samples (runs 20 and 33). 32 runs were within the run acceptance critera when first analyzed. Runs 2, 4, 5 and 8 was rejected due to QC samples outside criteria.
The samples from the rejected runs were reanalyzed in other runs. In Run 7, calibration point No 1 was excluded for both safinamide and NW-1153 and all samples with results

BLOQ in this run was reanalyzed as technical problems in Run 10.
Repeat analysis of 20 samples from 10 subjects (first analyzed in runs 3, 12, 13, 15, 17, 18, 19 and 21) were performed in runs 20 and 33. For safinamide, 17 samples (85%) were within 20% of the first value; for NW-1153, 19 samples (95%) were within 20% of the first value. Therefore, the incurred sample repeatability was adequate, confirming the acceptability of the method performance during the analysis of study samples.

NW1689 and NW1689AG were quantified in acidified plasma samples (Bioanalytical Study Report (b) (4) 28559 (b) (4) 1231981 (before) and (b) (4) 1231981 (after) in Acidified Human Plasma) according to the validated analytical procedure (b) (4) HB-09-064. Note: (b) (4) 1231981 is equivalent to NW-1689.
For measurement of NW-1689AG, an indirect method through bioanalysis of the samples before and after conversion of NW-1689AG to NW-1689 (alkaline hydrolysis of the acylglucuronide) was used. NW-1689AG concentrations were derived as the difference between the concentrations of NW-1689 (after) and NW-1689 (before) and appropriate correction factor according to the molecular weight of NW-1689AG.
The correction factor to transform concentrations of NW-1689 into corresponding concentrations of NW- 1689AG is equal to the molecular weight of NW-1689AG / molecular weight of NW-1689 = 422.36 / 246.23 = 1.715
Notes:
1) The LOQ for NW1689 in plasma was 5 ng/mL; there was no defined LOQ of NW1689AG; NW1689 acylglucuronide concentrations below 0 were set to 0.
2) In several batches, peaks were observed in the carryover blank samples, which were > 20.4% of the LOQ, and up to 55.7% of the LOQ. Therefore, the LOQ was raised to 10 ng/ml or to 30 ng/ml.
3) During the validation of the analytical method, NW-1689AG was found to be stable in acidified human plasma when stored at ambient temperature for up to 6 h or for up to 24 h when stored in a refrigerator. When the stability of NW-1689AG was assessed for up to 24 hours (analysis of (b) (4) 1231981 (after)), the data were acceptable at the LoQC level but were outside the acceptance criteria at the HiQC level (83.2% accuracy).
However, during the analysis of study samples the period at which each sample was stored at room temperature was not recorded. The sponsor claims that if samples were analyzed on up to three different occasions they were not subjected to more than 24 h storage at room temperature. Samples which were analyzed on four or more occasions may have been stored for 24 h at room temperature. Therefore, (b) (4) 1231981 (before) and (after) results which were generated on the fourth analysis occasion or greater cannot be confirmed and have been flagged in the study sample tables.

Reviewer's Comment: Considering all of the above problems, the results for NW-1689 and NW-1689AG should be interpreted with caution.

In total, 7610 plasma concentration data were obtained for safinamide and metabolites, corresponding to 476 complete plasma concentration-time profiles in the 2 safinamide groups. In addition, 116 samples of 58 subjects from the placebo group were analyzed at predose and 2 hours post dose on SD 6, they were all below the LOQ.

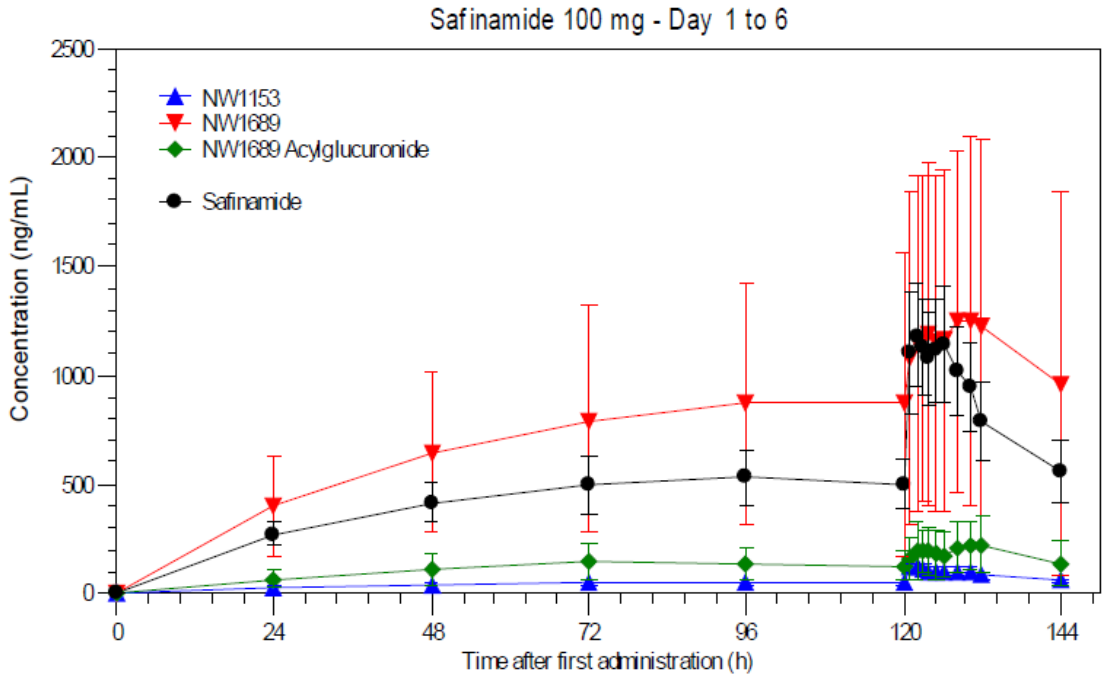
Cortisol and 6-Beta Hydroxycortisol in human urine were determined using a validated analytical method ((b) (4) HB-08-027-V3). This is a LC-MS/MS method with a LOQ of 3 ng/mL for Cortisol and 50 ng/mL for 6-Beta Hydroxycortisol using 25 µL of urine. Long term stability of Cortisol and 6-Beta Hydroxycortisol in urine was found to be acceptable for up to 112 days at nominal -20°C and nominal -70°C.
All study samples were successfully analyzed in four reported batches per analyte. Batch UB001 did not meet acceptance criteria for 6-Beta Hydroxycortisol, the samples were re-analyzed in batch UB001R and acceptance criteria was met upon batch re-injection. The calibration and QC sample data indicate that the method performed satisfactorily during the analyses of all reported batches of study samples.

Moxifloxacin was isolated from plasma by solid phase extraction (SPE) and determined by LC-Ms/MS (Report (b) (4) 28101). The analytical method was validated prior to this project with a LLOQ of 1.00 ng/mL and an upper limit of quantification (ULOQ) of 1000 ng/mL with a 250 µL sample volume (method AS M-196, version 1).
The quality of the determination of moxifloxacin was satisfactory. Out of 20 analytical runs, 15 were within the acceptance criteria for the analytical run when first analyzed. Five runs were rejected and re-analyzed (1, 2, 3, 11 and 12) due to interfering peaks in the blank samples, carry-over and problems in the sample preparation. The mean accuracy of the assay as determined from the analysis of quality control (QC) samples was within ±4.0%. Repeat Analysis of Incurred Samples (20 samples from 10 different subjects analyzed in two separate runs) was acceptable as at least 67% of the samples were within 20% of the first value or within 10% of the mean value.

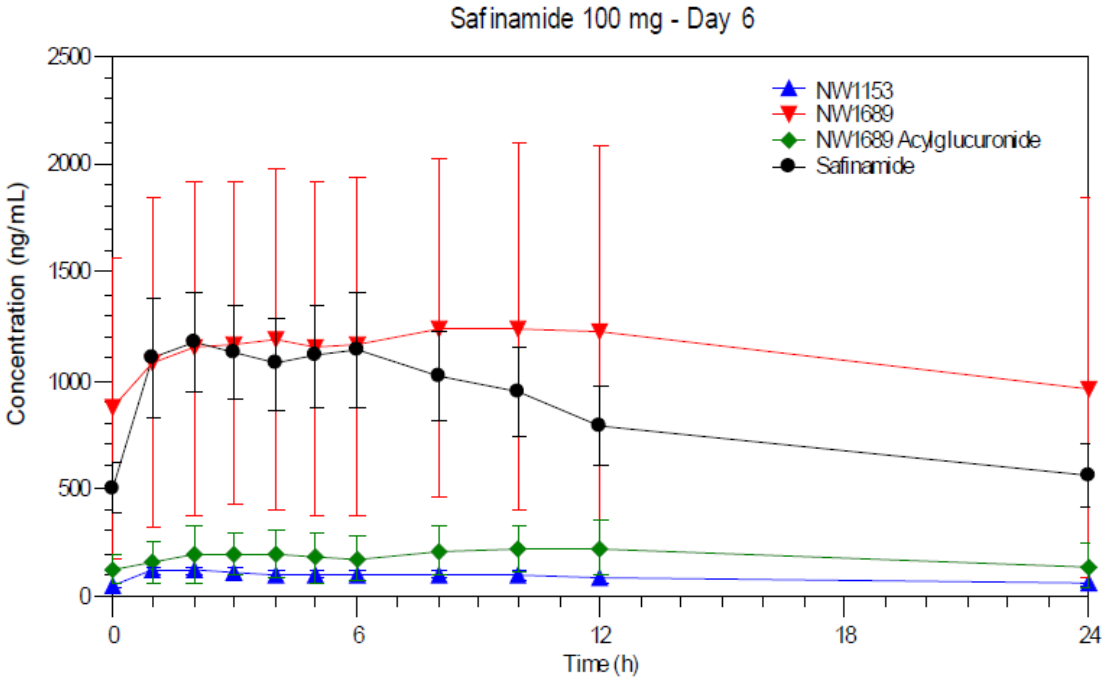
Pharmacokinetic Results:

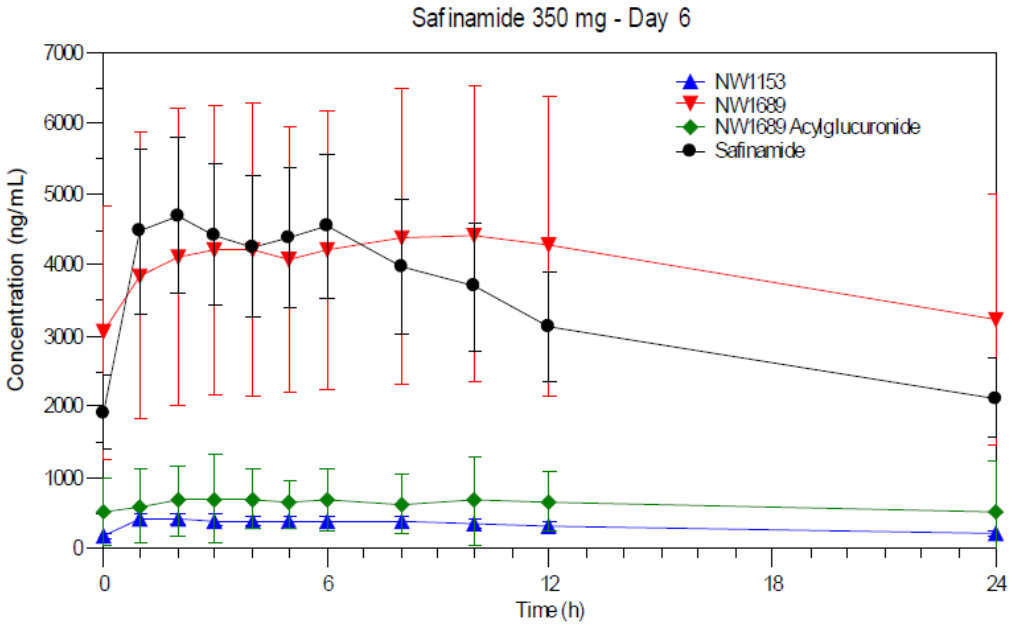
Visual examination of the pre-dose concentrations from SD 1 to SD 6 indicated that steady-state was reached by Day 5 (see figure below) as expected given the safinamide half-life of 22 to 24 hours and a dosing interval of 24 hours.

Mean (SD) Plasma Concentration-Time Profiles of Safinamide and its metabolites in Linear Scale from Day 1 to Day 6 – Safinamide 100 mg



Mean (SD) plasma concentration-time profiles of safinamide and its metabolites in linear scale on Day 6 (SD6) after safinamide 100 mg and 350 mg administration are shown below.





Summary of key PK parameters for safinamide and its metabolites NW1153, NW1689, and NW1689 acylglucuronide is presented in the Table below.

Summary of Pharmacokinetic Parameters of Safinamide and its Metabolites after Multiple Dosing of Safinamide 100 mg and 350 mg on SD 6

Parameter	Safinamide	NW1153	NW1689	NW1689 Acylglucuronide
Safinamide 100 mg (N=61)				
C_{max} [ng/mL]	1234 (20.8) 703-2120	121.2 (18.0) 64.1-166	1149 (49.5) 481-6170	297.7 (41.6) 89.2-738
t_{max} [h]	2.1 (1.1-8.0)	1.3 (1.1-8.0)	8.0 (2.1-24.0)	8.0 (0.0-12.0)
AUC_{0-24} [ng/mL*h]	19811 (21) 11733-33108	2000 (18) 1167-2660	23274 (54) 9727-138730	3787 (63) 473-10711
C_{pre} [ng/mL]	489.6 (21.6) 321-916	49.4 (19.8) 31.0-68.2	730.8 (57.8) 294-4700	120* 0-334
Safinamide 350 mg (N=58)				
C_{max} [ng/mL]	4887 (22.3) 2920-7550	416.9 (19.3) 248-616	4309 (44.0) 1780-12700	1021 (58.5) 292-4854
t_{max} [h]	2.1 (1.1-6.0)	2.1 (1.1-12.0)	8.0 (1.2-12.0)	4.6 (0.0-24.0)
AUC_{0-24} [ng/mL*h]	77257 (23) 48503-132378	7136 (20) 4288-10762	85932 (45) 34601-272963	11968 (72) 2116-51673
C_{pre} [ng/mL]	1849 (29.6) 595-3670	176.6 (24.9) 61.1-279	2671 (53.7) 1060-9390	437.5* 0-2762

For C_{max}, AUC_{0-24}, C_{pre} : GeoMean (GeoCV %) and range (min-max); For t_{max}: median and range (min – max)
* For c_{pre} only median with range is presented as GeoCV and GeoMean could not be calculated.

A 3.5-fold increase in safinamide dose (from 100 mg to 350 mg) resulted in a similar increase in C_{max} and AUC_{0-24} for safinamide and its metabolites NW1153, NW1689, and

NW1689 acylglucuronide (range of fold-change increase for C_{max} and AUC_{0-24} was 3.1-3.9 across all analytes).
Comparison of the steady-state AUC within one dosing interval (AUC_{0-24}, SD6) of safinamide and its metabolites NW1153, NW1689, and NW1689 acylglucuronide after multiple dosing of 100 mg, with the respective single dose $AUC_{0-\infty}$ data after 100 mg safinamide (results from Trial 28778) showed comparable values (Geo Mean):
Safinamide (AUC_{0-24}: 16786 ng/mL*h; $AUC_{0-\infty}$: 17305 ng/mL*h)
NW1153 (AUC_{0-24}: 1555 ng/mL*h; $AUC_{0-\infty}$: 1813 ng/mL*h)
NW1689 (AUC_{0-24}: 21006 ng/mL*h; $AUC_{0-\infty}$: 21660 ng/mL*h)
NW1689 acylglucuronide (AUC_{0-24}: 3787ng/mL*h; $AUC_{0-\infty}$: 4089ng/mL*h)
These results suggest no time dependency of safinamide PK.

Summary of Pharmacokinetic Parameters of Moxifloxacin

Parameter	Moxifloxacin 400 g (N=58)
C_{max} [ng/mL]	2726 (20.7); 1700-4160
t_{max} [h]	1.2 (1.0-12.0)
AUC_{0-24} [ng/mL*h]	29475 (18); 21327-48631
C_{pre} [ng/mL]	NC

For C_{max}, AUC_{0-24}, C_{pre} : GeoMean (GeoCV %) – range (min-max); For t_{max}: median and range (min – max); NC=not calculated.

Moxifloxacin reached t_{max} at around 1 hour (median t_{max} 1.2 hours), with a mean C_{max} of 2726 ng/mL, consistent with published data (t_{max} ranging from 1 hour to 3 hours postdose under fasting conditions, Bayer. Avelox 400 mg film-coated tablets: SmPC. 1-14. 2006. Berkshire). These PK data support the selection of time points (1, 2 and 4 hours postdose) for statistical analysis of QTc values to show assay sensitivity.

PK Conclusions: Overall all analytes showed t_{max} within the period of intensive ECG collection. All analytes were at steady-state on SD 6 demonstrating that ECG timing in this study was well designed for the QTc assessment at the time of maximum exposures to safinamide and its metabolites.

Analysis of CYP3A4 Induction
The fold-change to baseline of 6β-hydroxycortisol/cortisol (6β-OHF/free-cortisol) ratio was compared between placebo and both 100 and 350mg safinamide doses in order to investigate if there might be any clinically relevant CYP3A4 induction.
As these investigations were included as an amendment in the ongoing clinical trial, not all subjects could take part in the analyses. The analysis set included a total of 82 subjects, 28 in the 100 mg safinamide group, and 27 each in the 350 mg safinamide group and in the placebo group.
The analysis of the fold changes to baseline of 6β-OHF/free-cortisol ratios between placebo and both safinamide doses resulted in ratios of 1.11 for the 100 mg safinamide group and 1.08 for the 350 mg safinamide group, showing absence of CYP3A4 induction under safinamide treatment. In addition, the results of the ANOVA model of the

comparison of the log transformed fold change to baseline did not reveal any differences between any of the doses and placebo, as shown by point estimates close to 1 and the p-values (see Table below). Literature data for known inducers show consistently ratios ≥2 [Galteau, 2003].

Analysis of CYP3A4 Induction - Summary of Back-transformed ANOVA Results of Log-transformed Fold-change from Baseline

				95% Confidence Interval		
Comparison	Statistic	n	Estimate	Lower	Upper	p-value
Treatment group						
Safinamide (100 mg)	Geometric Mean	28	0.93	0.82	1.06	
Safinamide (350 mg)	Geometric Mean	27	0.91	0.80	1.04	
Placebo	Geometric Mean	27	0.84	0.74	0.97	
Comparison						
Safinamide (100 mg) versus Placebo	Ratio		1.11	0.92	1.34	0.267
Safinamide (350 mg) versus Placebo	Ratio		1.08	0.89	1.31	0.414

Pharmacodynamic (PD) and PK/PD Results (provided by the QT Team):
No significant QTc prolongation effect of safinamide (100 mg and 350 mg) was detected in this TQT study. The largest upper bounds of the 2-sided 90% CI for the mean difference between safinamide (100 mg and 350 mg) and placebo 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, and the moxifloxacin profile over time was adequately described, indicating that assay sensitivity was established.
The supratherapeutic dose (350 mg) produces mean Cmax 3.9-fold the mean Cmax for the therapeutic dose (100 mg). These concentrations are above those for the sponsor's predicted worst case scenario (low body weight patient with hepatic impairment) and show that at these concentrations there are no detectable prolongations of the QT interval. The data suggests a concentration dependent shortening of QT interval.
PROPOSED LABEL
12 (b) (4) Cardiac Electrophysiology
The effect of XADAGO on the QTc interval was evaluated in a (b) (4) randomized placebo and positive controlled double-blind, multiple-dose parallel thorough QTc study in 240 healthy subjects. (b) (4) XADAGO did not prolong QTc (b) (4).
Safety Results:
More subjects reported TEAEs in the placebo (41.7% of the subjects) and moxifloxacin (45.0% of the subjects) treatment groups than in either the safinamide 100 mg (26.2% of the subjects) or 350 mg (27.1% of the subjects) treatment groups.
The most commonly reported AEs (occurring in ≥4% of subjects overall) were headache, dizziness, nausea and nasopharyngitis.
Two serious adverse events (SAEs) were reported during the trial; both under placebo and assessed as not related to the treatment.

1.5 Efficacy and Safety Studies

XVII. NW1015-009-II-2001: A phase II dose finding, double-blind, placebo controlled study to investigate the efficacy and safety of safinamide, a MA0-B Inhibitor, in patients affected by idiopathic early Parkinson's disease

Objective:
To evaluate the efficacy and safety of orally administered safinamide at two different doses (0.5 mg/kg and 1.0 mg/kg) in parkinsonian patients de-novo or treated with one single dopamine agonist at stable dose.

Study Design	This was a dose finding, double-blind, placebo-controlled, randomised, multicenter, 12-week trial
Study Population	172 de-novo or treated with one single dopamine agonist at stable dose PD patients
Treatment Groups*	Safinamide (0.50 and 1.00 mg/kg) versus placebo as monotherapy or as adjunct therapy to one single dopamine agonist* Placebo: 58 patients Safinamide 0.5 mg/kg: 57 patients Safinamide 1.0 mg/kg: 57 patients
Dosage and Administration	Group 1: placebo (capsules) Group 2: daily final dose of safinamide 0.5 mg/kg (capsules) Group 3: daily final dose of safinamide 1.0 mg/kg (capsules)
PK Sampling: plasma	none
Analysis	NA
PK Assessment	none
PD Assessment	Primary efficacy variable: Percentage of patients with an improvement of at least 30% in the unified Parkinson's disease ratings scale (UPDRS) section III score between baseline (Visit 2) and the end of the study (Visit 9 or early study termination). Secondary Criteria: Percentage of patients with an improvement of at least 30% in the UPDRS section III score between baseline (Visit 2) and Visit 5 and Visit 7; changes in the UPDRS sections II and III scores between baseline (Visit 2) and Visit 5, Visit 7 and the end of the study (Visit 9 or early study termination); clinical global impression (CGI)
Safety Assessment	Adverse events, vital signs, ECG, clinical chemistry, urinanalysis

* There was a 10 to 24 days run-in period, between the screening visit (Visit 1) and the baseline visit (Visit 2), followed by a 12-week treatment period. In the run-in period, no adjustment to the dopamine agonist dose was to be made for patients already treated with a dopamine agonist. Patients did not receive any study medication in the run-in period.
During the treatment phase, the dopamine agonist dose was not to be increased, but could be decreased, according to the patient's condition.
Patients who were eligible for inclusion in the treatment period, were randomly assigned to one of the three treatment groups at baseline (Visit 2).

Dose Rationale:
The two tested doses of safinamide (0.5 mg/kg or 1.0 mg/kg) were chosen on the basis of preclinical and phase I findings suggesting multiple mechanisms of action for safinamide

and a clear dose-response curve obtained for platelet MAO B inhibition with an ED_{50} of 90 mg/kg single oral dose and full inhibition with doses greater than 300 mg/kg at steady state (Cattaneo C, Caccia C, Marzo A, Maj R, Fariello RG.: Pressor response to intravenous tyramine in healthy subjects after safinamide, a novel neuroprotectant with selective, reversible monoamine-oxidase 8 inhibition. Neuropharmacology).
Thus, the expectations were that the 0.5 mg dose should provide maximal MAO B inhibition, whereas the 1.0 mg/kg dose should approach cerebral concentrations at which some degree of dopamine uptake and glutamate release inhibitions were possible.
The safety margin (calculated according to IGH guidelines as Human Equivalent Dose (HED) the maximum allowed dose) was 6.45 mg/kg. In addition, according to the sponsor, single doses of 10 mg/kg or seven days repeated doses of 5 mg/kg have not presented any clinically relevant side effects in previous phase 1 studies.

De-novo patients took the study medication as monotherapy, while patients already treated with one single dopamine agonist took the study medication as adjunct therapy.

Note: This study will not be reviewed in detail by the clinical pharmacologist as there was no PK assessment and an early safinamide formulation (capsules) was used in the study. The capsule formulation has not been tested for BE to the to-be-marketed tablet formulation. Only highlights of the efficacy results are provided below.

	Safinamide 0.5 mg/kg/day		Safinamide 1.0 mg/kg/day		Placebo			
UPDRS III: Change from Baseline							**P-value vs. Placebo group (ANCOVA analysis)**	
Population	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Saf 0.5 mg/kg**	**Saf 1.0 mg/kg**
Total population	-2.6	5.5	-3.3	5.5	-0.6	5.4	**0.030**	**0.005**
Single DA-agonist (n=101)	-4.0	5.9	-4.7	5.7	-1.4	4.5	**0.045**	**0.006**
De novo (n=66)	-0.5	4.2	-1.0	4.5	0.6	6.6	0.485	0.303

Efficacy Conclusions:

- No benefit in de-novo PD patients.
- Benefit shown at 1 mg/kg dose group (~80 mg) in patients taking single DA-agonist.

XVIII. NW- 1015/015/III/2003: A Phase III, Double-Blind, Placebo-Controlled Study to Determine the Efficacy and Safety of a Low (50-100 mg/day) and High (150-200 mg/day) Dose Range of Safinamide, as Add-On Therapy, in Patients with Early Idiopathic Parkinson's Disease Treated with a Stable Dose of a Single Dopamine Agonist

Objectives:

Primary: to evaluate the safety and efficacy of two dose ranges of safinamide (High Dose: 150 to 200 mg/day and Low Dose: 50 to 100 mg/day) orally (p.o.), as compared to Placebo, as add-on therapy in patients with early idiopathic Parkinson's disease who are currently receiving a stable dose of a single dopamine agonist.
The efficacy objective was regarded as met, if the primary efficacy measure, the Unified Parkinson's Disease Rating Scale (UPDRS) Section III (ME) change from Baseline to Endpoint was superior for the treatment group of interest, as compared to Placebo.

Pharmacokinetic Objectives: to determine the relationship between the dose of safinamide and the pharmacokinetics (PK) variables for safinamide and its metabolites using a sparse-sampling, population kinetics procedure.
A secondary PK objective was to determine the relationship between the PK of safinamide and the key efficacy and safety outcomes.

Notes: This study was reviewed by the clinical pharmacologist and the following major issues were found:

1. Formulation (capsules) was used in the study. The capsule formulation has not been evaluated for BE to the to-be-marketed tablet formulation.

2. A 30% difference in Safinamide concentrations between the phase 3 studies.
Safinamide concentrations in the active dose groups of the follow-up (to Study 015) Study NW1015_017-III, were consistently approximately 30% lower over all visits in comparison to Study 015.
In NW1015_017-III-Other reports (page 674), the sponsor claims the following:
A pharmacokinetic explanation (such as time dependent PK) appears unlikely for the following reasons:
• Safinamide plasma concentration are at a constant level across visits in each study
• There is no apparent difference in the sample time points between the two studies.
• The study population in both studies is the same with the population in Study 017 representing a subset of Study 015.
• Plasma concentrations in the follow up Study 017 are comparable to another 6 month study (Study 016 :NW-1015/016/III/2006).
Interestingly this difference is consistent with the difference in molecular weight between safinamide free base and safinamide methanesulfonate, suggestive for systematic error either in medication or bioanalytics. The same capsule formulation was used in the two studies but different batches of different manufactures were involved.
However, the analysis certificates do not point to any deviation in safinamide content.

Furthermore, samples from the two studies were analyzed at different laboratories, [redacted] in Study 015 and [redacted] in Study 017. Both labs used the very comparable analytical method and print outs of weighted references were checked for mistakes. Both labs took into account the salt factor [redacted] during sample preparation whereas [redacted] applied a correction factor in the end. The [redacted] method has been cross evaluated with a [redacted] method. Thirty samples, which have had been analyzed before at [redacted], were sent to [redacted]. All 30 samples were in the range of 97.9-108.9% of [redacted] method. Overall, neither on the CMC part nor at the bioanalytical part a systematic mistake has been detected.

3. Safinamide plasma (and metabolites) levels observed in subjects randomized to the placebo group.

Overall, 588 samples from the placebo group (90 patients) were analyzed for safinamide and its two main circulating metabolites P2 (NW1153) and P3 (NW1689). Quantifiable safinamide concentrations were present in 155 samples (26%). Measurable safinamide concentrations in samples from placebo subjects were not specific to a certain center. Most frequently, safinamide containing samples were observed at visits 5 (Week 8) and 6 (Week 12), 66 [78%] and 48 [57%] patients, respectively.

In Sect. 11.4 (page 99 of CSR 015) the sponsor claims:
To determine the root cause of active drug exposure in the placebo group, an audit was performed on the laboratory in charge of the pharmacokinetic analysis. The conduct of this audit demonstrated that 2 out of 51 bulk placebo bottles used to produce IMP kits actually contained a mixture of active and placebo capsules; the audit did not detect any errors in randomization, labeling, or dispensing of kits. These data indicate that a portion of patients randomized to placebo were inadvertently exposed to safinamide at various time points throughout the study.

Considering these 3 major issues, the Clinical pharmacology team recommended that the sponsor exclude Study 015 PK data from the population PK analysis.
The following was sent to the sponsor in the Refuse to File Letter (28 July RTF Letter), Additional Comments & Recommendations:
We recommend that you exclude Study 015 PK data from your population PK analysis. If you plan to use a population analysis to support labeling language, we suggest that you include PK data from other studies (i.e., MOTION and SETTLE) in your population pharmacokinetic pharmacodynamic analysis".

This has been addressed by the sponsor (in Sect. 5.3.4.2 popPK/PD) in the re-submission of the NDA.

XIX. MOTION (Study 27918): A Phase III, Double-Blind, Placebo-controlled, Randomized Trial to Determine the Efficacy and Safety of a Low (50 mg/day) and High (100 mg/day) Dose of Safinamide, as Add-on Therapy, in Subjects with Early Idiopathic Parkinson's Disease Treated with a Stable Dose of a Single Dopamine Agonist

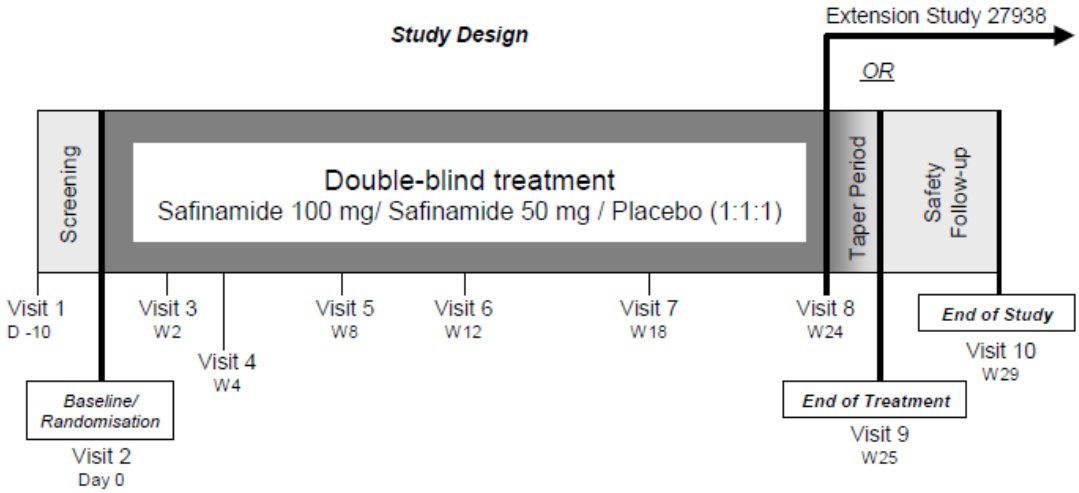
Objectives:

Primary: Evaluate the changes from Baseline to Week 24 in motor symptoms (Unified Parkinson's Disease Rating Scale [UPDRS] Section III).

Secondary: Evaluate the changes from Baseline to Week 24 in activities of daily living, cognition, change in global clinical status, responder rates with regard to motor symptoms, and health related quality of life.

Study Design*	Double-blind, placebo-controlled, parallel-group, randomized, multicenter, Phase III trial, comparing two doses of safinamide (50 and 100 mg p.o. QD) versus placebo as add-on therapy to a stable dose of a single DA in subjects with early idiopathic PD
Study Population	679 patients were randomized to treatment (227 to safinamide 50 mg/day; 227 to safinamide 100 mg/day; and 225 to placebo), and 610 completed the study.
Treatment Groups	Safinamide 50 mg/day Safinamide 100 mg/day Placebo
Dosage and Administration*	Safinamide 50 mg/day (small – 7 mm): two tablets (one small safinamide tablet and one large placebo tablet) QD in the morning with breakfast/Taper Phase: two placebo tablets (one small tablet and one large tablet) once per day* Safinamide 100 mg/day (large – 9 mm): two tablets (one small placebo tablet and one large safinamide tablet) QD in the morning with breakfast/Taper Phase: 50 mg/day (one small safinamide tablet and one large placebo tablet) once per day
PK Sampling: plasma	Sparse blood samples for population PK, see * on next page
Analysis	Validate LC-MS/MS assays for safinamide and ropinirole
PK Assessment	popPK (separate report)
PD Assessment	Primary efficacy variable: Percentage of patients with an improvement of at least 30% in the unified Parkinson's disease ratings scale (UPDRS) section III score between baseline (Visit 2) and the end of the study (Visit 9 or early study termination). Secondary Criteria: Percentage of patients with an improvement of at least 30% in the UPDRS section III score between baseline (Visit 2) and Visit 5 and Visit 7; changes in the UPDRS sections II and III scores between baseline (Visit 2) and Visit 5, Visit 7 and the end of the study (Visit 9 or early study termination); clinical global impression (CGI)
Safety Assessment	Adverse events, vital signs, ECG, clinical chemistry, urinanalysis

* Summary of Study Design and Extension Study



Period	Screening Period	Baseline/ Randomization	Treatment Period	Taper Phase	Extension Study
Duration	10 days	1 day	24 weeks	1 week	18 months
Study Days	–10 to –1	0	1 to 168	169 to 175	169 to 760
Treatment			Study drug (safinamide 50 mg or 100 mg/day) or placebo; and other PD medication (single dopamine agonist)	Subjects who discontinued at Week 24 had their dose of study drug tapered and then discontinued	Continued on same dose of study drug

Evaluation	Screening (From day –10±7d)	Baseline/ Randomisation Day 0		Day 7	Wk 2 (±3d)	Wk 4 (±3d)	Wk 8 (±7d)	Wk 12 (±7d)	Wk 18 (±7d)	Wk 24 (±7d) or Premature Treatment Discontinuation	Wk 25 (Taper phase)	Wk 29 (Follow-up visit)
Visit number	1	2		NA	3	4	5	6	7	8	9	10
		Pre-rand	*Post-rand*									
Informed consent [a]	X											
Inclusion/exclusion criteria	X	X										
Demography/Medical history	X											
Physical examination	X									X		
Neurological exam	X									X		
Ophthalmological exam	X							X[n]		X[o]		
Dermatological exam	X									X[o]		
Blood and urine sampling	X	X			X	X	X	X	X	X		X
Virology tests	X											
Pregnancy test [b]	X(serum)	X(urine)										
PK sampling		X[c]	X[c]			X[d]	X[e]	X[e]	X[e]	X[e]		
Vital signs	X	X[f]	X[f]		X	X	X	X	X	X	X	X
12-lead ECG [g]	X	X[f]	X[f]					X		X		X
Ambulatory Blood Pressure Monitoring [j]	X[k]					X[l]			X[m]			

c The first dose of trial medication will be administered in the clinic, and PK samples will be taken prior to dosing and 5 hr post-dose.
d Trial medication will be taken in the clinic. PK sampling will be done pre-dose and 2-4 hrs post-dose.

e An attempt should be made to obtain an even distribution of sampling times throughout the first 8-hour post-dose during the course of the patient's visits. At least one sampling time 8 hours post-dose should be collected.
f Vital signs and ECG assessments must be performed prior to dosing and 5 hr after the first dose, just prior to PK sampling.

Bioanalytical Assays:

Safinamide

The concentrations of Safinamide in human plasma containing lithium heparin as an anticoagulant were determined by a validated method ((b) (4) study number 8203838) using protein precipitation for sample extraction, followed by liquid chromatography with tandem mass spectrometric detection (LC-MS/MS). The analytical procedure for the determination of Safinamide was validated to include the determination of the metabolite NW-1153. During this analytical study data for both analytes were acquired, however, NW-1153 concentrations data were not processed, reviewed or reported.
The method had a lower limit of quantification (LLOQ) of 5 ng/mL using 25 µL of plasma. The analytical procedure was (b) (4) HB-09-058-V2.
Prior to the initiation of this analytical study at (b) (4) samples from the clinical study were analyzed by (b) (4) ((b) (4) study number Q27178). However, due to the closure of the (b) (4) laboratory the remaining analysis was performed at (b) (4). A cross validation of the analytical methods used at both laboratories was successfully conducted in (b) (4) study 8254461.
The concentration of Safinamide was determined in a total of 4926 human plasma samples obtained from the study No. 27918.
The performance of the assay during the study sample analysis was acceptable, demonstrated by the calibration standard data, QC sample data, ISR and chromatograms. The mean accuracy of the assay as determined from the analysis of QC samples was within ±1.7%.
In addition, it was demonstrated that haemolysis does not affect the quantification of Safinamide in human plasma. The selectivity data confirmed the acceptable quantification of Safinamide in human plasma in the presence of Ropinirole.
Long term stability of Safinamide in human plasma has previously been assessed and the stability of Safinamide in plasma samples (lithium heparin) for up to 22 months at -20°C has been demonstrated. All study samples were analyzed within this period.

Ropinirole (b) (4) Analytical Study Number (b) (4) 29056)
Ropinirole was extracted from plasma using liquid/liquid extraction and determined by LC-MS/MS detection. The LOQ for ropinirole was 0.200 ng/mL and the upper limit of quantification (ULOQ) was 20.0 ng/mLwith a 200 µL sample volume.
The concentration of Ropinirole was determined in a total of 1167 human plasma samples obtained from the study No. 27918. However, due to stability issues (see below), the concentration of ropinirole was reported for only 488 samples.
Stability of ropinirole in human plasma samples stored at -70°C and -20°C was established for up to 6 months. Therefore, only samples analyzed within this time frame had concentration reported and were used for PK evaluation. The concentrations in the samples analyzed outside proven stability were presented in the report for information only.

Runs 24-27 were analyzed with calibration and quality control (QC) samples prepared from stock solutions being prepared with an expired test material. Therefore these data are reported separately for information only.
The quality of the determination of ropinirole was satisfactory in the reported runs. The mean accuracy of the assay as determined from the analysis of QC samples was within ±2.7%.

Pharmacokinetic Results:

Concomitant Medications

Most subjects (83.8%) were taking at least one concomitant medication in addition to PD medications. Concomitant medications taken by greater than 15% of subjects in any treatment group were agents acting on the renin-angiotensin system, lipid-modifying agents, antithrombotic agents, anti-inflammatory and antirheumatic products, beta-blocking agents, analgesics, antibacterials for systemic use, drugs for acid-related disorders, and vitamins. The treatment groups were generally similar with regard to the use of concomitant medications.

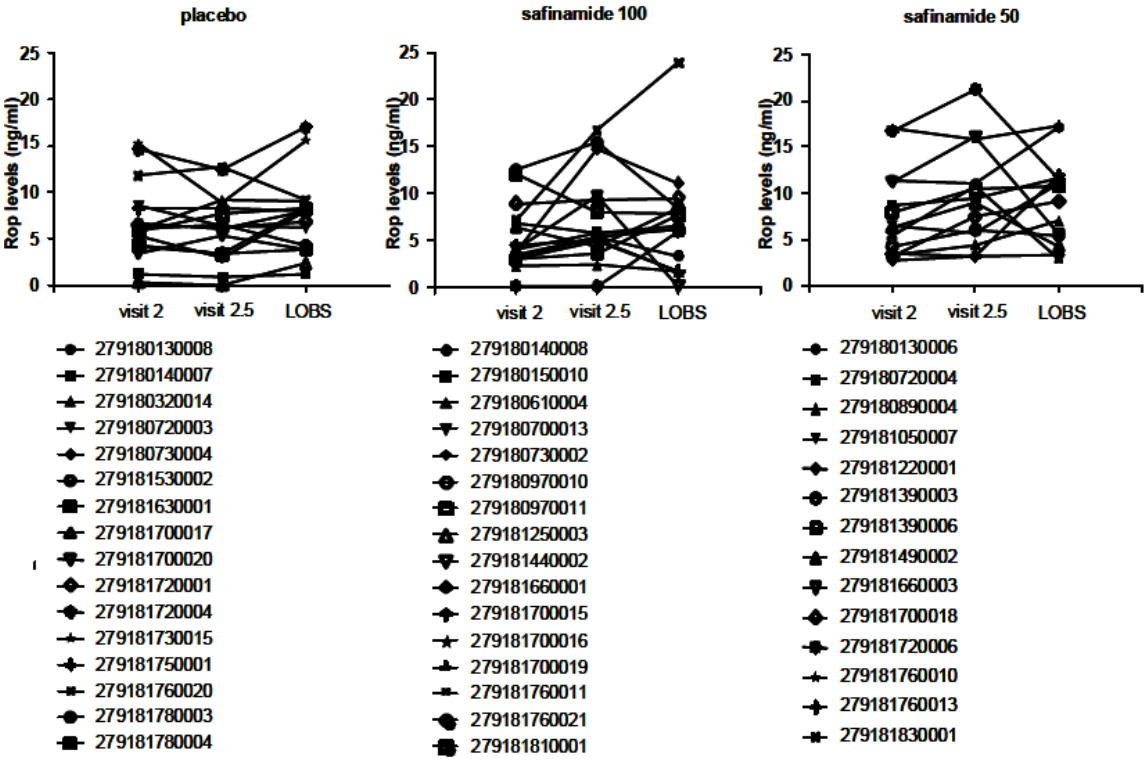
Drug-drug interaction analysis: Effect of safinamide treatment on ropinirole concentrations measured in human plasma samples from Clinical Study 27918

Ropinirole, a concomitantly administered dopamine agonist, is a CYP1A2 substrate and safinamide is a weak inhibitor of this enzyme.
A drug-drug interaction analysis was performed to investigate the impact of safinamide treatment on the plasma concentrations of a concomitant dopamine agonist, ropinirole.
In Study 27918 (MOTION) a total of 210 subjects were treated with ropinirole as a concomitant PD medication (n=65 placebo, n=73 safinamide 100 mg/day, and n= 72 safinamide 50 mg/day. Of the 210 subjects, 100 had samples with problems of stability, therefore the concentration of ropinirole was determined in 110 subjects with a total of 488 samples (n=33 placebo, n=38 safinamide 100 mg/day, and n= 39 safinamide 50 mg/day).
For the evaluation of a possible drug-drug interaction, only data from subjects who fulfilled the following criteria were used:

- Subjects (any treatment group) whose ropinirole concentration values were available at baseline, prior to the first administration of study drug (visit 2), 5 hours post-dose(visit 2.5), and at one LOBS visit, defined as at least one post-dose visit from week 4 to week 24
- Subjects on a stable dose of ropinirole before the baseline visit
- Subjects whose daily dose of ropinirole remained unchanged until the LOBS visit
- Subjects whose PD medications remained unchanged at least until the LOBS visit.

Only 46 subjects fulfilled the above criteria (n=16 placebo, n=16 safinamide 100 mg/day, and n= 14 safinamide 50 mg/day).
The analyses showed that the ropinirole levels measured at visits 2.00, 2.50 and LOBS, showed no significant differences across visits in the three treatment groups (placebo, safinamide 100 mg/day, and safinamide 50 mg/day) indicating that safinamide does not have an effect on ropinirole metabolism.

Ropinirole plasma levels by subject at visit 2.00, 2.50, LOBS in the three groups (n=16 in the placebo group; n=16 in the safinamide 100 mg/day group; n= 14 in the safinamide 50 mg/day group)



The ropinirole plasma level of one subject (279181760011, safinamide 100 mg/day group), was recognized as a significant outlier at LOBS (23.9 ng/ml, $p<0.005$, Grubbs' test). In a further analysis, all data of subject 279181760011 were removed from the data set of the safinamide 100 mg/day group and data were re-analyzed.
A two-way ANOVA was performed followed by Bonferroni's post hoc test for multiple comparison: no significant differences were found among visits in the three groups.
The data set of the safinamide 100 mg/day group was also re-analyzed with a Repeated Measures ANOVA followed by Dunnett's post hoc analysis for comparing post dose visits (visit 2.50, LOBS) vs control baseline pre-dose visit (visit 2.00). No significant differences were found.

Reviewer's Comments:
This DDI was evaluated in only 46 out of the 210 subjects receiving safinamide and ropinirole. However, a dedicated DDI study (EMR701165-026) had been conducted with a sensitive CYP1A substrate. The results from the current analysis support the conclusion from study EMR701165-026 that safinamide has no clinically significant effect on the PK of CYP1A substrates.
The results of Drug Dose, Drug Concentration, and Relationships to Response analyses are presented in a separate report (popPK). This report will be reviewed by the pharmacometrics reviewer.

XX. NW-1015/016/III/2006: A Phase 3, Double-Blind, Placebo-Controlled Study to Determine the Efficacy and Safety of a Low (50 mg/day) and High (100 mg/day) Dose of Safinamide, as Add-on Therapy, in Patients with Idiopathic Parkinson's Disease with Motor Fluctuations, Treated with a Stable Dose of Levodopa and Who May be Receiving Concomitant Treatment with Stable Doses of a Dopamine Agonist, and/or an Anticholinergic

Objective: to evaluate the efficacy and safety of 2 oral (PO) doses of safinamide (50 and 100 mg/day) compared with placebo, as add-on therapy in patients with idiopathic PD with motor fluctuations, who are currently receiving a stable dose of levodopa (LD)

Study Design*	Double-blind, placebo-controlled, parallel-group study in patients with idiopathic PD with motor fluctuations, who were receiving a stable dose of LD (mid-to-late phase PD patients)
Study Population	A total of 669 patients with idiopathic PD of more than 5 years duration (222 placebo, 223 safinamide 50 mg/day, and 224 safinamide 100 mg/day). 594 completed the study.
Treatment Groups	Safinamide 50 mg/day Safinamide 100 mg/day Placebo
Dosage and Administration*	Safinamide 50 mg/day: 2 tablets once daily (one 50-mg tablet and 1 placebo tablet) for 24 weeks during the treatment phase; 2 tablets once daily (2 placebo tablets) during the optional 1-week taper phase Safinamide 100 mg/day: 2 tablets once daily (two 50-mg tablets) for 24 weeks during the treatment phase; 2 tablets once daily (one 50 mg tablet and 1 placebo tablet) during the optional 1-week taper phase
PK Sampling: plasma**	Sparse blood samples for population PK**
Analysis	Validated LC-MS/MS assay for safinamide, NW-1153 and NW-1689
PK Assessment	popPK (separate report)
PD Assessment	Primary efficacy variable: the mean total daily on time without troublesome dyskinesia over 18 hours. Secondary efficacy variables: decrease in total daily off time, UPDRS Section 3 (motor), Clinical Global Impression–change from Baseline (CGI-C), Cognitive Test Battery (Cogtest), decrease in mean off time after first morning dose of LD, Dyskinesias Rating Scale (DRS) during on phase, UPDRS Section 2 (activities of daily living [ADL]) during on phase, CGI–severity of illness (CGI-S), and mean percentage reduction in LD dose.
Safety Assessment	Adverse events, vital signs, ECG, clinical chemistry, neurological, dermatological, and ophthalmological examination findings; and Epworth Sleepiness Scale (ESS) scores.

* The total duration of the study was approximately 30 weeks, including the Screening period (10 days), a LD stabilization phase (4 weeks), the treatment period (24 weeks), and an optional 1-week taper phase. Patients who met the entry criteria at Baseline were randomized (1:1:1) to receive 1 of the 2 doses of safinamide or placebo for a total of 24 weeks.
Patients returned for regularly scheduled visits at Weeks 4, 8, 12, 18, and 24 (or at early discontinuation).
All randomized patients completing their participation in the double-blind treatment period in this study could enter a 78-week, double-blind extension study (Study NW-1015/018/III/2006 [Study 018]).

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Table 5. Schedule of Evaluations											
Evaluation	**Screening**	**Run-in Period**	**Levodopa Stabilization Phase**	**Baseline[e]**		**Wk 4**	**Wk 8**	**Wk 12**	**Wk 18**	**Wk 24 (or Early Discontinuation)**	**Wk 25 (Optional Taper Phase)[h]**
Visit number	1		2	3		4	5	6	7	8	9[i]
Study day	–38	–38 to –29	–28	0	1	28	56	84	126	168	175
Informed consent[a]	X										
Inclusion/exclusion criteria	X			X							
Demography/medical history	X										
Physical examination	X			X						X	
Neurological examination	X			X						X	
Ophthalmological examination	X							X		X	
Dermatological examination	X									X	
Laboratory tests	X			X		X	X	X	X	X	
Virology tests	X										
Pregnancy test[b]	X										
Safinamide levels				X[f]	X[f]	X		X		X	
Vital signs	X			X[g]	X[g]	X	X	X	X	X	
ECG	X			X[g]	X[g]			X		X	
Levodopa dose record	X		X	X	X	X	X	X	X	X	

f The first dose of study drug was administered in the office, and a blood sample was taken prior to dosing and 5 hours post-dose for measurement of levels of safinamide and its metabolites.

Bioanalytical Assay:

Safinamide, NW-1153 and NW-1689

The concentrations of safinamide and its metabolites NW-1153 and NW-1689 were determined in a total of 3179 human plasma samples from the study using a validated method (validation report (b) (4) 27175).

The analytes were isolated from plasma by protein precipitation and determined by reversed phase HPLC with MS/MS detection (method AS M-187, version 1). The lower limit of quantification (LOQ) was 20.0 ng/mL in human plasma for all three compounds.

All study samples were kept frozen below at or -20ºC prior to analysis. There were not complete sample set (5 samples/subject) for all subject and a number of unscheduled samples were not analyzed according to agreement with the Sponsor.

The study samples were analyzed in runs with all study samples from one subject being prepared on the same occasion when it was possible. With each run, separate calibration samples, blank plasma samples and QC samples of three different concentrations were analyzed. QC samples were analyzed in duplicate in each run. Injection order of the runs: Mobile phase, blank plasma samples without and with internal standard, calibration samples, blank for carry-over, QC samples (QC H, QC M and QC L), study samples and QC samples (QC L, QC M and QC H).

Four runs out of 58 runs were rejected (4, 14, 39 and 40). Runs 4, 39 and 40 did not fulfilled QC acceptance criteria and run 14 was rejected due to possibility of misplaced samples. The samples from these runs were re-analyzed.

All remaining analytical runs fell within the run acceptance criteria. The mean accuracy of the assay as determined from the analysis of quality control (QC) samples was within ±3.6%. 20 samples from 10 different subjects were re-analyzed in two separate runs. At least 75% of the samples were within 20% of the initial value for all compounds. The repeat re-analysis of incurred samples was accepted.

Pharmacokinetic Results:

Concomitant Medications:

PD co-medications: Per the protocol, 100% of patients were receiving LD, primarily with carbidopa (as Sinemet).

Overall, 60.8% of patients were receiving DA agonists, 37.1% of patients were receiving tertiary amines, 24.4% of patients were receiving other DA agents (ie, entacapone, a COMT inhibitor), 15.8% were receiving entacapone in Stalevo (carbidopa, levodopa, and entacapone), and 13.9% were receiving adamantine derivatives.

Most patients (approximately 77%) were taking 1 or more concomitant medication other than PD medications. Concomitant medications taken by greater than 15% of patients in any treatment group were agents acting on the renin-angiotensin system, analgesics, beta-blocking agents, drugs for acid-related disorders, drugs used in diabetes, lipid-modifying agents, and vitamins.

The treatment groups were generally similar with regard to concomitant medications.

Reviewer's Comments:

The results of Drug Dose, Drug Concentration, and Relationships to Response analyses are presented in a separate report (popPK). This report will be reviewed by the pharmacometrics reviewer.

Efficacy Results:

Primary Efficacy Measure: Mean Total Daily On Time

The primary efficacy endpoint was the increase in mean total daily on time (on time without dyskinesia plus on time with minor dyskinesia) during the 18-hour diary recording period, analyzed using Mixed- Effect Model Repeated Measures (MMRM): SAS PROC MIXED procedure with the model using treatment, center, visit, and the treatment by visit interaction as fixed effects and baseline as covariate. The multiplicity issue for treatment groups was handled by using a "sequence of comparisons" approach, testing the 100 mg/day group vs placebo first; if a significant difference was detected, then the 50 mg/day group vs placebo was tested.

At Visit 8 (Week 24), the mean increase from Baseline in total daily on time was statistically significantly greater for the safinamide 50 mg/day (1.37 hours; P = 0.0223) and safinamide 100 mg/day (1.36 hours; P = 0.0130) groups compared with the placebo group (0.97 hour).

An ad-hoc responder analysis revealed that a statistically significantly higher percentage of patients in the safinamide 50 mg/day group (60.99%; P = 0.0108) and the safinamide 100 mg/day group (63.39%; P = 0.0019) had ≥ 30-minute improvement in on time than the placebo group (50.45%).

Additionally, the proportion of patients with improvement in on time with no increase in troublesome dyskinesia was statistically significantly higher in the safinamide 100 mg/day group (55.80%; P = 0.0122) than in the placebo group (45.95%).

Safety Results:

The percentage of patients with one or more TEAE was generally similar among the 3 groups (68.5% of patients in the placebo group, 65.9% of patients in the safinamide 50 mg/day group, and 65.6% of patients in the safinamide 100 mg/day group).
The most commonly reported TEAEs that occurred in > 5.0% of patients in any treatment group were dyskinesia, worsening of PD, cataract, back pain, headache, hypertension and depression. Except for dyskinesia, these AEs occurred in a similar or higher percentage of patients in the placebo group than in either of the safinamide treatment groups.
Dyskinesia, the most frequently reported TEAE, occurred more often in patients in the safinamide 50 mg/day group (21.1%), compared with the safinamide 100 mg/day group (18.3%) and the placebo group (12.6%).
Dyskinesia was generally mild to moderate in intensity, with very few patients reporting severe dyskinesia (placebo: 5 [2.3%]; safinamide 50 mg: 2 [0.9%]; safinamide 100 mg: 4 [1.8%]).

XXI. SETTLE (27919): A phase III, double-blind, placebo-controlled, randomized trial to determine the efficacy and safety of a dose range of 50 to 100 mg/day of safinamide, as add-on therapy, in subjects with idiopathic Parkinson's disease with motor fluctuations, treated with a stable dose of levodopa and who may be receiving concomitant treatment with stable doses of a dopamine agonist, an anticholinergic and/or amantadine

Objective: to evaluate the efficacy and safety of 2 oral (PO) doses of safinamide (50 and 100 mg/day) compared with placebo, as add-on therapy in patients with idiopathic PD with motor fluctuations, who are currently receiving a stable dose of levodopa (LD)

Study Design*	Double-blind, placebo-controlled, parallel-group, randomized, Phase III trial, comparing a dose range of 50 -100 mg of safinamide, p.o. QD versus placebo as add-on therapy to a stable LD dose in idiopathic PD subjects with motor fluctuations (mid-to-late phase PD patients)
Study Population	A total of 549 patients were randomized (1:1): 274 to safinamide (50-100 mg/day) and 275 to placebo, and 478 completed the study.
Treatment Groups	Safinamide 50-100 mg/day Placebo
Dosage and Administration	Subjects took one tablet per day in the morning with breakfast for the duration of the treatment period. They started at Dose Level 0 (50 mg/day or matching placebo), and the dose was increased on Day 14 to Dose Level 1 (100 mg/day or matching placebo) if well tolerated. Dose Level 0 – Safinamide 50 mg/day (small – 7 mm): 1 tablet once per day in the morning with breakfast/Taper Phase: 1 placebo tablet (small – 7 mm) once per day Dose Level 1 – Safinamide 100 mg/day (large – 9 mm): 1 tablet once per day in the morning with breakfast/Taper Phase: 1 Safinamide 50 mg/day tablet (small – 7 mm) once per day
PK Sampling: plasma**	Sparse blood samples for population PK**
Analysis	Validated LC-MS/MS assay for safinamide
PK Assessment	popPK (separate report)
PD Assessment	Primary efficacy variable: the change in daily on time (on time without dyskinesia plus on time with minor dyskinesia) from baseline to Week 24. Secondary efficacy variables: change in total daily off time from baseline to Week 24, UPDRS Section 3 (motor), Clinical Global Impression–change from Baseline (CGI-C), Cognitive Test Battery (Cogtest), decrease in mean off time after first morning dose of LD, Dyskinesias Rating Scale (DRS) during on phase, UPDRS Section 2 (activities of daily living [ADL]) during on phase, proportion of subjects with scores of 1, 2, or 3 (showing improvement) on the CGI - Change scale at Week 24.
Safety Assessment	Adverse events, vital signs, ECG, clinical chemistry, neurological, dermatological, and ophthalmological examinations, impulse control disorders, and level of daytime sleepiness.

* The trial included up to 24 weeks of treatment in the double-blind phase, followed by a 1-week taper phase before treatment was discontinued, or subjects may have entered a 3-year, open-label extension trial (28850) for continuing treatment.

Period	Screening Period	Stabilization Phase	Baseline/ Randomization	Treatment Period	Taper Phase	Extension Study
Duration	10 days	4 weeks	1 day	24 weeks	1 week	3 years
Study Days	-38 to -29	-28 to -1	0	1 to 168	169 to 175	
Treatment				Study drug (safinamide 50-100 mg/day) or placebo; and other PD medication plus levodopa	Subjects who discontinued at Week 24 had their dose of study drug tapered and then discontinued	Safinamide 50-100 mg/day, open-label

At the end of the stabilization phase, subjects must have 1) achieved their optimum dose and regimen of levodopa, 2) continued to experience end-of-dose wearing off, and 3) demonstrated the ability to accurately maintain a diary

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Evaluation	Screening (Day -38 ± 7d)	Levodopa Stabili-zation (Day -28)	Baseline/ Randomization (R) Day 0		Wk 1	Wk 2 (± 3d)	Wk 3	Wk 4 (± 3d)	Wk 8 (± 7d)	Wk 12 (± 7d)	Wk 18 (± 7d)	Wk 24 (± 7d) or Premature Treatment D/C	Wk 25 (Taper Phase)	Wk 29 (Follow -up Visit)
Visit number	1	2	3		NA	4	NA	5	6	7	8	9	10	11
			Pre-R	Post-R										
Informed consent[a]	X													
Inclusion/ exclusion criteria	X		X											
Demography/ medical history	X													
Physical examination	X		X									X		
Neurological examination	X		X									X		
Ophthalmo-logical examination	X[p]									X[q]		X[r]		
Dermatological examination	X[p]											X[r]		
Blood & urine sampling	X		X			X		X	X	X	X	X		
Virology tests	X													
Pregnancy test[b]	X (serum)		X (urine)											
PK sampling			X[c]	X[c]				X[d]	X[e]	X[e]	X[e]	X[e]		
Vital signs	X		X[f]	X[f]		X		X	X	X	X	X	X	X

c The first dose of trial medication was administered in the clinic, and PK samples were taken prior to dosing and 5 hours post-dose
d Trial medication was taken in the clinic. PK sampling was done pre-dose and 2-4 hours post-dose
e An attempt should have been made to obtain an even distribution of sampling times throughout the first 8-hour post-dose during the course of the subject's visits. At least one sampling time 8 hours post-dose should have been collected.
f Vital signs and ECG assessments must have been performed prior to dosing and 5 hours after the first dose, just prior to PK sampling

Bioanalytical Assay:

The concentrations of safinamide in human plasma were determined using a validated method (validation report (b) (4)-27176).
Prior to the initiation of this analytical study at (b) (4) the clinical study samples were analyzed by (b) (4) ((b) (4) study number (b) (4)27176). However, due to the closure of

the [redacted] laboratory, the remaining analysis was conducted at [redacted] A cross validation of the analytical methods used at both laboratories was successfully conducted in [redacted] study 8254461.
The method ([redacted]HB-09-058-V3) has a LOQ of 5 ng/mL using 25 µL of plasma.
The analytical procedure for the determination of safinamide was validated to include the determination of the metabolite NW-1153. During this analytical study data for both analytes were acquired, however, NW-1153 concentrations were not required, therefore, NW-1153 data were not processed, reviewed or reported.
A total of 451 plasma samples were analyzed for safinamide in five sample runs and the specificity experiment in one run. The selectivity data confirm the acceptable quantification of safinamide in human plasma in the presence of levadopa.
The performance of the assay during the study sample analysis was acceptable, demonstrated by the calibration standard data, QC sample data, and chromatograms.
The mean accuracy in the determination of the QC samples was 105.3% at 15 ng/mL, 103.5% at 400 ng/mL, 96.6% at 800 ng/mL, 102.0% at 5000 ng/mL (ten-fold dilution).

Pharmacokinetic Results:

Concomitant Medications:

PD co-medications: nearly 100% of subjects were taking LD or dopa derivatives; about 74% were also taking a DA agonist.
Concomitant medications other than PD medications: Concomitant medications taken by greater than 15% of subjects in any treatment group were psycholeptics, antithrombotic agents, analgesics, drugs for acid-related disorders, psychoanaleptics, anti-inflammatory and antirheumatic products, lipid-modifying agents, beta blocking agents, agents acting on the renin-angiotensin system, and antibacterials. The treatment groups were generally similar with regard to use of concomitant medications.

Reviewer's Comments:

The results of Drug Dose, Drug Concentration, and Relationships to Response analyses are presented in a separate report (popPK). This report will be reviewed by the pharmacometrics reviewer.

Efficacy Results:

The primary efficacy parameter was the change from baseline to Week 24 in daily on time (on time without dyskinesia plus on time with minor dyskinesia), as measured by subject diary cards.
The analysis of the primary endpoint (daily on time) was performed using an analysis of covariance (ANCOVA) model, with treatment and region effects and the baseline value of the daily on time as the covariate. A full ANCOVA model including treatment by region was performed to check possible treatment and region interaction. Regions were defined in the statistical analysis plan.
The same ANCOVA model used for the primary efficacy parameter was used for the continuous secondary clinical parameters using the baseline value of the parameter to be analyzed as a single covariate.
The results for the primary efficacy measure showed that at Week 24, the mean (SD) change from baseline in daily on time for the safinamide group was 1.42 hours (2.80)

compared with a change of 0.57 hours (2.47) in the placebo group. The LS mean (SE) difference between the safinamide and placebo groups was 0.96 (0.21), indicating increased daily on time with safinamide. Using a parametric ANCOVA (LOCF) model, the ITT Population, and On-Treatment data, there was a significant difference ($p<0.001$) between the safinamide group and the placebo group, in the change in daily on time from baseline to Week 24.

Analysis of the first key secondary efficacy measure, change in daily off time from baseline to Week 24, yielded statistically significant ($p<0.001$) results. The safinamide group's mean daily off time decreased by about an hour and a half (-1.56 [2.35]), while the placebo group decreased by about half an hour (-0.54 (2.21)) (LS mean (SE) difference vs. placebo = -1.03 (0.19) in On-Treatment ANCOVA [LOCF]).

Safety Results:

Four deaths were reported during the study, one of which occurred during screening. Three subjects died during the randomized treatment period; two of the deaths occurred in subjects randomized to the placebo group (Subject 312-0003 and 501-0002), and one occurred in a subject randomized to the safinamide group (Subject 230-0002).

In the safinamide treatment group, 15 (5.5%) subjects withdrew from the study due to TEAEs, compared with 11 (4.0%) subjects in the placebo group. There was no pattern of association of specific types of AEs with discontinuation of treatment, except that a statistical trend ($p=0.068$) showed that a greater proportion of safinamide subjects withdrew due to events in the Nervous System Disorders compared to the placebo group.

Changes from baseline and abnormal shifts in laboratory results, vital signs, and ECG parameters, as well as physical, neurological, and dermatological findings, were similar between treatment groups.

Clinical Pharmacology Individual In Vitro Studies Review

TABLE OF CONTENTS

Study Title	Interspecies comparison of the in vitro plasma protein binding of Safinamide, NW-1689 and NW-1153 in mouse, rat and human
Study number	NWR-12
Study Period	May 2005
Study Director	Richard J. Cole
Objective	To investigate in vitro plasma protein binding of Safinamide, NW-1689 and NW-1153 was investigated in mouse, rat and human plasma in this study.

METHODS

The extent of plasma protein binding of Safinamide, NW-1689 and NW-1153 in mouse, rat and human plasma at 3 concentrations (nominal 0.5, 2 and 20 µg/mL plasma) was determined by an equilibrium dialysis technique. At each concentration, duplicate determinations of the extent of plasma protein binding were performed.

RESULTS

The extent of plasma protein binding was generally independent of concentration with similar results found across the 40-fold concentration range for each species. The extent of plasma protein binding of Safinamide, NW-1689 and NW-1153 in the three species is given below.

	Mouse	Rat	Human
Safinamide	88.1 ± 1.8	89.4 ± 1.6	92.0 ± 1.3
NW-1689	97.5 ± 0.3	99.5 ± 0.1	99.8 ± 0.0
NW-1153	72.1 ± 3.2	78.1 ± 3.5	70.7 ± 1.9

Data expressed as mean % bound ± standard deviation

CONCLUSIONS

Safinamide was bound to plasma proteins by approximately 88% to 92%. In all species, the most highly protein bound component in this study was the metabolite NW-1689, followed by parent Safinamide. The metabolite NW-1153 was least protein bound.

Study Title	In Vitro Identification of Human Plasma Proteins Binding [14C]-Safinamide and its Major Metabolite NW-1689
Study number	DMPK 127-07
Study Period	October 2010
Study Director	Friedrich Kratzer

Objective	To investigate the non-covalent protein binding of [14C]-safinamide and its major metabolite NW-1689 in human plasma, human serum albumin (HSA), α1- acid glycoprotein (AAG), Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL).

METHODS

The protein binding of both test items was investigated by equilibrium dialysis in pooled plasma of human and solutions containing purified individual protein components of human plasma. Before start of the experiment, human plasma was dialyzed against phosphate buffer (pH 7.4) in order to maintain the physiological pH throughout the experiment.

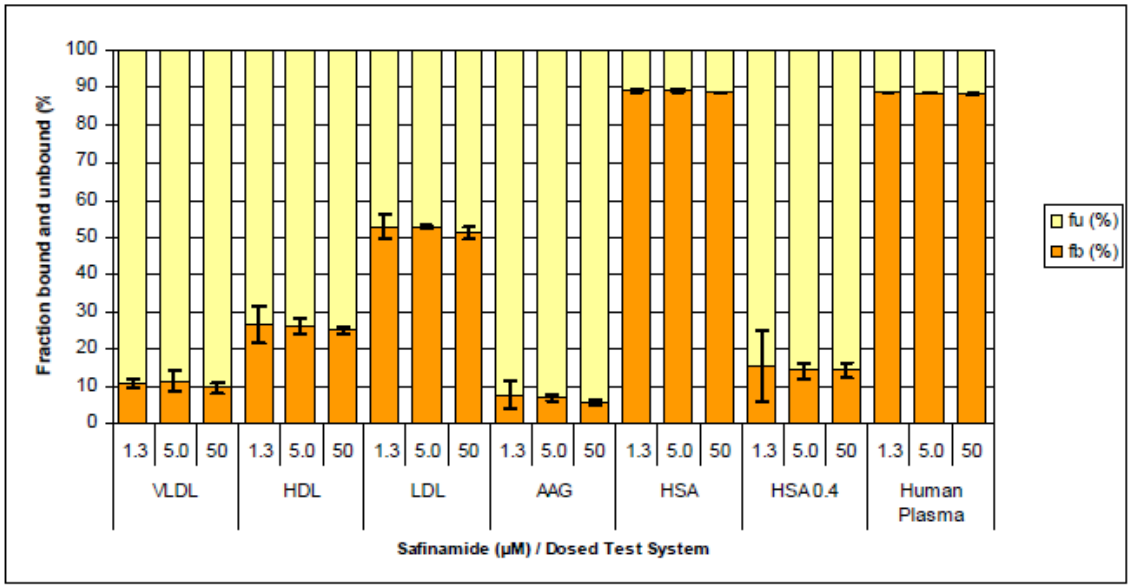
Human plasma or protein solutions containing [^{14}C]-safinamide or NW-1689 were dialyzed against phosphate buffer (70 mM, pH 7.4) at 37°C for 1 hour Afterwards, the radioactive concentrations were measured in both compartments by LSC for [^{14}C]-safinamide and by HPLC-MS/MS for NW-1689. Metabolic stability of [^{14}C]-safinamide over the incubation period was investigated by radio-HPLC analysis.

The concentrations for safinamide investigated were 1.25, 4.99, and 49.9 µM and for NW-1689 were 2.03, 8.12 and 81.2 µM.

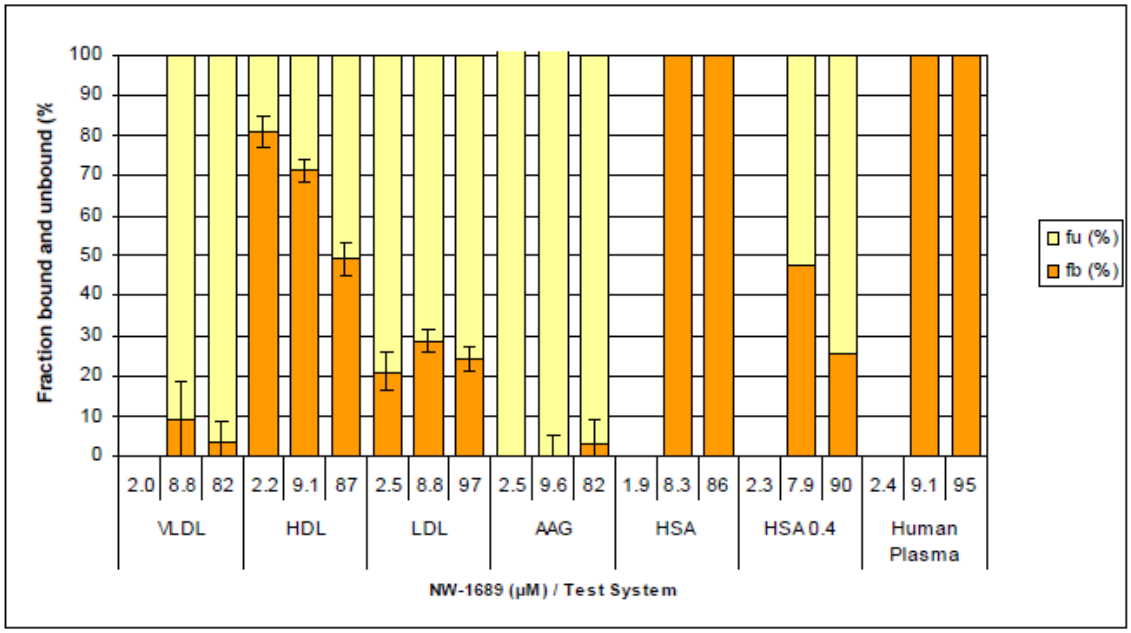
RESULTS

These results indicate that safinamide and NW-1689 are bound predominantly to the albumin protein fraction in human plasma. Since HSA is by far the most abundant protein in human plasma, clinically relevant DDI due to saturation of or displacement from binding sites appear to be remote.

<u>Binding of Safinamide to Human Plasma Proteins</u>



Binding of NW-1689 to Human Plasma Proteins



CONCLUSIONS

- Safinamide and NW-1689 are bound predominantly to the albumin protein fraction in human plasma.
- The affinity of safinamide to plasma protein components was HSA>LDL>HDL >VLDL>AAG.
- For NW-1689 the affinity to plasma protein components was HSA>HDL>LDL >VLDL>AAG.

Study Title	Evaluation of ^{14}C-NW1015 In Vitro Human Plasma Protein Binding
Study number	(b) (4) MET09-01
Study Period	January 2002
Study Director	Paola Tocchetti
Objective	To investigate in vitro the human plasma protein binding of NW-1015 using the radiolabelled compound at different doses covering the plasma concentrations found in clinical studies.

METHODS

^{14}C-NW1015 has been incubated with human plasma at the concentrations of 5, 50, 500, 1000 and 2000 ng/ml. After 30 minutes at 37°C in water bath, 1 ml plasma in quintuplicate from each concentration, has been transferred into Amicon Centrifree and centrifuged at approximately 1200 xg for 20 min to have approximately 200 µl of

ultrafiltrate. Aliquots of 100 µ1 of the ultrafiltrate have been submitted to liquid scintillation counting.

RESULTS
The results showed that the binding of NW1015 to plasma protein is approximately 88% at all the concentrations tested. The unspecific binding to the Centrifree membrane resulted 9.75% ±1.92 (mean ± SD, n=5).

CONCLUSIONS
NW1015 plasma protein binding is approximately 88%.

Study Title	In Vitro Non-Covalent Protein Binding of NW-1689 18-0-Acyl Glucuronide in Human and Monkey Plasma
Study number	DMPK20-11
Study Period	November 2011
Study Director	Friedrich Kratzer
Objective	To determine the in vitro protein binding of NW-1689 1β-0-acyl glucuronide (NW-1689 AG, MSC2357341A) in monkey and human plasma at concentrations of 0.5, 2.0 and 20 µM.

METHODS
The in vitro plasma protein binding of the test item was investigated by ultrafiltration in pooled plasma of human and Cynomolgus monkey. Before start of the experiment, the plasma was dialyzed against phosphate buffer (pH 7.4) in order to maintain the physiological pH throughout the experiment. Plasma of both species was incubated with NW-1689 AG at 37°C for 10 or 20 minutes and afterwards ultra-filtrated at 20°C. Concentration of NW-1689 AG was measured by LC-MS/MS in unfiltrated plasma (dosing solution) and in the filtrate fraction. To stabilize the test item, the solutions were acidified immediately after filtration. A kinetic experiment was performed to demonstrate, that the distribution of the test item in plasma was at equilibrium at the chosen incubation conditions. The in vitro plasma protein binding of [^{14}C]-testosterone was investigated as positive control to demonstrate the validity of the applied test system and experimental procedure.

RESULTS
The results for [^{14}C]-testosterone were in the range of the historical data of the test facility and confirmed the validity of the experimental system. In plasma of monkey and human the unbound fraction of NW -1689 AG was not concentration dependent within the investigated concentration range. Therefore, saturation of plasma protein binding of NW -1689 AG is not to be expected in vivo up to 20 µM. On average, the unbound fraction ofNW-1689 AG was 2.6 ± 0.3% in human and 6.3 ± 0.3% in monkey plasma. The species difference in plasma protein binding should be considered during safety ratio evaluations.

Non-covalent Binding of NW-1689 AG to Plasma Proteins of Monkey and Human Plasma

Species	Human						Monkey		
Incubation time (min)	10			20			10		
Concentration (µM)	0.5	2	20	0.5	2	20	0.5	2	20
f_u (%)	3.2	2.2	2.5	2.7	2.4	2.6	5.9	6.6	6.3
Mean f_u (%)	2.6 ± 0.3						6.3 ± 0.3		

CONCLUSIONS

On average, the unbound fraction of NW-1689 AG was 2.6 ± 0.3% in human and 6.3 ± 0.3% in monkey plasma.

Study Title	Determination of the in vitro plasma protein binding of Safinamide to human alpha-1 acid glycoprotein
Study number	NWR-15
Study Period	May 2006
Study Director	Richard J. Cole
Objective	To investigate in vitro binding of Safinamide to alpha-1 acid glycoprotein.

METHODS

The extent of binding of Safinamide to alpha-1 acid glycoprotein at 3 concentrations (nominal 0.5, 2 and 20 µg/mL alpha-1 acid glycoprotein solution) was determined by an equilibrium dialysis technique. At each concentration, duplicate determinations of the extent of binding were performed.

RESULTS

Determination of the extent of protein binding of Safinamide in alpha-1 acid glycoprotein solutions

Nominal Safinamide conc., µg/mL*	Rep	Degree of protein binding, % bound
0.5	1	15.5
	2	-6.1
2	1	7.4
	2	1.5
20	1	0.1
	2	1.6
Mean ± SD		3.3 ± 7.4

* for actual concentrations (ng/mL) see Table 1
SD – standard deviation

CONCLUSIONS

The results of these investigations showed that there was negligible binding of Safinamide to alpha-1 acid glycoprotein in vitro.

Study Title	In Vitro Distribution of [^{14}C]-Safinamide and its Major Metabolites [^{14}C]-NW-1153 and [^{14}C]-NW-1689 in Whole Blood of Mouse , Rat, Rabbit, Dog, Monkey and Human
Study number	DMPK 126-07
Study Period	January 2010
Study Director	Friedrich Kratzer
Objective	To compare the in vitro distribution of [^{14}C]-Safinamide and its major metabolites [^{14}C]-NW-1153 and [^{14}C]-NW-1689 in whole blood of mouse, rat, rabbit, dog, monkey and human.

METHODS

The distribution of the test items was investigated in fresh whole blood of mouse, rat, rabbit, dog, monkey, and human. To obtain the equilibration time, the distribution kinetics of [^{14}C]-Safinamide and its major metabolites [^{14}C]-NW-1153 and [^{14}C]-NW-1689 were determined in human whole blood in advance. In addition, the item stability in whole blood of the six species was investigated. To maintain physiological conditions the pH was stabilized by addition of 7% (v/v) phosphate buffer (70 mM, pH 7.4 containing 0.11 M glucose). Whole blood aliquots were spiked with radioactive test item and incubated for 5 min at 37°C to achieve distribution equilibrium. Following separation of blood cells (BC) from plasma, radioactivity in whole blood and packed blood cells was measured as $^{14}CO_2$ after combustion; radioactivity in plasma and application solutions was measured by liquid scintillation counting. Metabolic stability of the test items over the incubation period was investigated by radio-HPLC analysis.

RESULTS AND DISCUSSION

Based on the distribution kinetics of Safinamide, NW-1153 and NW-1689 in human whole blood an incubation time of 5 min was chosen for the distribution experiments. All three test items were found to be stable in whole blood during the incubation period of 5 minutes. The whole blood distribution of Safinamide was investigated at 2.5 µM and 100 µM and found to be concentration-independent. In whole blood of mouse, Safinamide was predominantly found in blood cells. In blood of rat, rabbit and dog Safinamide was distributed almost equally between both compartments whereas in blood of monkey and human Safinamide was mainly found in the plasma. The average distribution coefficients K (BC/plasma) were 1.8 (mouse), 1.2 (rat), 1.3 (rabbit), 1.0 (dog) 0.6 (monkey) and 0.4 for human.

The whole blood distribution of NW-1153 was investigated at 0.5 µM and 20 µM and found to be concentration-independent. In whole blood of mouse, NW-1153 was predominantly found in the blood cell compartment whereas in rat, rabbit, dog, monkey and human the larger portion was found in plasma. The average distribution coefficients K(BC/plasma) were 2.2 (mouse), 1.1 (rat), 1.1 (rabbit), 0.9 (dog) 0.7 (monkey) and for human 0.6. The whole blood distribution of NW-1689 was investigated at 2.5 µM and 100 µM and found to be concentration-independent. In whole blood of all investigated species NW-1689 was found in the plasma compartment to a large extent. The average distribution coefficients K (BC/plasma) were 0.06 (mouse), 0.06 (rat), 0.05 (rabbit), 0.06 (dog) 0.06 (monkey) and 0.05 for human.

Species	Mouse		Rat		Rabbit		Dog		Monkey		Human	
Safinamide (µM)	**2.5**	**100**	**2.5**	**100**	**2.5**	**100**	**2.5**	**100**	**2.5**	**100**	**2.5**	**100**
Fraction in plasma (%)	39	37	54	53	52	51	53	52	67	66	73	71
Fraction in BC (%)	61	63	46	47	48	49	47	48	33	34	27	29
$K_{(BC/plasma)}$	1.8	1.9	1.2	1.2	1.3	1.3	0.9	1.0	0.5	0.6	0.4	0.5
max. uncertainty of K	0.05	0.05	0.07	0.06	0.05	0.06	0.04	0.09	0.06	0.07	0.07	0.07
NW-1153 (µM)	**0.5**	**20**	**0.5**	**20**	**0.5**	**20**	**0.5**	**20**	**0.5**	**20**	**0.5**	**20**
Fraction in plasma (%)	35	34	61	60	54	54	53	53	57	57	63	63
Fraction in BC (%)	65	66	39	40	46	46	47	47	43	43	37	37
$K_{(BC/plasma)}$	2.2	2.3	1.0	1.1	1.1	1.1	0.9	0.9	0.7	0.7	0.6	0.6
max. uncertainty of K	0.05	0.06	0.13	0.05	0.09	0.08	0.05	0.04	0.06	0.04	0.07	0.07
NW-1689 (µM)	**2.5**	**100**	**2.5**	**100**	**2.5**	**100**	**2.5**	**100**	**2.5**	**100**	**2.5**	**100**
Fraction in plasma (%)	95	94	97	96	98	96	96	95	95	95	96	96
Fraction in BC (%)	5	6	3	4	2	4	4	5	5	5	4	4
$K_{(BC/plasma)}$	0.05	0.07	0.05	0.07	0.03	0.06	0.05	0.06	0.06	0.06	0.05	0.05
max. uncertainty of K	0.16	0.26	0.24	0.14	0.27	0.25	0.14	0.21	0.17	0.16	0.21	0.17

Study Title	To investigate the permeability of Safinamide across Caco-2 monolayers
Study number	DIXG1000

Study Period	May 2003
Study Director	Victoria Eagling, PhD
Objective	To investigate the permeability of Safinamide across Caco-2 monolayers.

METHODS

Apparent permeability coefficients for transport in both the apical to basolateral and basolateral to apical directions were determined and compared to investigate the involvement of active efflux of Safinamide across the monolayer. The involvement of active transport processes was further investigated by determining the effect of performing transport studies at 40°C or in the presence of sodium azide in combination with 2-deoxyglucose.

The transport of [^{14}C]-Safinamide a cross Caco-2 monolayers (grown on polyethylene terephthalate membranes) was assessed in both the apical to basolateral (AP-BL) and basolateral to apical (BL-AP) directions at two concentrations (10.0 and 2500 µM) at various time points (30, 60, 90, 120 and 150 minutes). Appearance of [^{14}C]-Safinamide the receiver chamber was assessed by liquid scintillation counting. The ratio of [^{14}C]-Safinamide transport in both directions was calculated to determine if active efflux had occurred. The effect of sodium azide (15 mM) in combination with 2-deoxyglucose (50 mM) and performing transport experiments at 4°C was also investigated to confirm the lack of involvement of active processes in the transport of [^{14}C]-Safinamide (10 and 2500 µM).

RESULTS

Mean Papp values for the transport of [^{14}C]-Safinamide (10.0 µM) in the AP-BL and BL-AP directions were 22.9 ± 0.971 x 10^{-6}cm.s^{-1} and 25.7 ± 1.65 x 10^{-6}cm.s^{-1} (n = 3), respectively. Corresponding values at a [^{14}C]-Safinamide concentration of 2500 µM were 21.3± 5.70 x 10^{-6}cm.s^{-1} and 19.7 ± 0.839 x 10^{-6}cm.s^{-1}.

The ratios of BEAP/AP-BL transport for 10.0 and 2500 µM [^{14}C]-Safinamide were 1.12 and 0.928, respectively.

[^{14}C]-Safinamide (10.0 and 2500 µM) transport was also unaffected by the presence of sodium azide (15.0 mM) in combination with 2-deoxyglucose (50.0 mM), screened as inhibitors of active transport processes. Mean Papp values for the transport of [^{14}C]-Safinamide (10.0 µM) in the AP-BL and BL-AP directions were 25.4± 3.35 x 10^{-6}cm.s^{-1} and 27.3 ± 0.961 x 10^{-6}cm.s^{-1} (n = 3), respectively.

Corresponding values at a [^{14}C]-Safinamide concentration of 2500 µM were 29.9 ±1.33 x 10^{-6}cm.s^{-1} and 32.2 ± 0.404 x 10^{-6}cm.s^{-1}. The ratios of BL-AP/AP-BL transport for 10.0 and 2500 µM [^{14}C]-Safinamide were, 1.07 and 1.08, respectively.

Permeability coefficients (Papp) for the transport of [14C]-Safimamide across Caco-2 monolayers in both the apical to basolateral and basolateral to apical direction (mean of three determinations, in each direction). Transport studies were performed in the presence

of HBSS (containing HEPES [l .00 mM]; pH 7.4). The effects of sodium azide + 2-deoxyglucose in both the donor and receiver chambers and incubations at 40°C were also determined.

Conditions	[^{14}C]-Safinamide Concentration	replicate	Papp cm.s^{-1} (x 10^{-6}) AP-BL	Papp cm.s^{-1} (x 10^{-6}) BL-AP	Mean Papp cm.s^{-1} (x 10^{-6}) AP-BL	s.d. (x 10^{-6}) AP-BL	Imprecision (%) AP-BL	Mean Papp cm.s^{-1} (x 10^{-6}) BL-AP	s.d. (x 10^{-6}) BL-AP	Imprecision (%) BL-AP	ratio BL-AP/AP-BL
Control 37°C	10.4 µM	1	22.7	26.5							
		2	24.0	26.8							
		3	22.1	23.8	22.9	0.971	4.24	25.7	1.65	6.43	1.12
Control 37°C	2498 µM	1	27.8*	19.2							
		2	18.7	20.7							
		3	17.3*	19.3	21.3	5.70	26.8	19.7	0.839	4.25	0.928
NaN_3 + 2-DG	10.4 µM	1	29.2	26.4*							
		2	22.9	27.1							
		3	24.1	28.3*	25.4	3.35	13.2	27.3	0.961	3.52	1.07
NaN_3 + 2-DG	2500 µM	1	29.1*	32.6							
		2	29.1*	31.8*							
		3	31.4	32.1*	29.9	1.33	4.45	32.2	0.404	1.26	1.08

CONCLUSIONS

[^{14}C]-Safinamide is not a substrate for P-glycoprotein as no polarization of transport was observed over the concentration range investigated and [^{14}C]-Safinamide transport was unaffected by inhibition of active transport (sodium azide in combination with 2-deoxyglucose).

Study Title	Uptake of Safinamide into Human Hepatocytes
Study number	DMPK 15-10
Study Period	December 2010
Study Director	Dieter Gallemann
Objective	To investigate to which extent safinamide (MSC2191632B) is taken up into human hepatocytes (suspension) and whether hepatic uptake transporters may be involved. Furthermore potential trapping of safinamide into hepatocyte lysosomes was investigated.

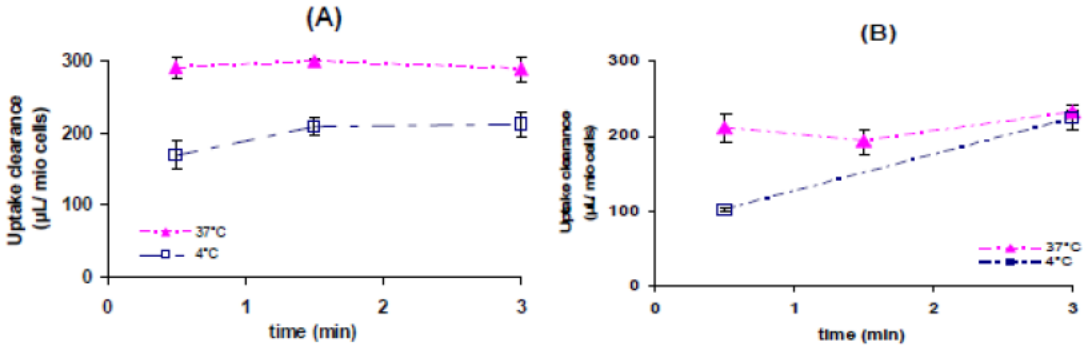
METHODS

The uptake of the radio-labeled test item safinamide into human hepatocytes was determined by incubating the test item with cryopreserved hepatocytes in suspension. The uptake was studied by separating the cells from incubation buffer using the oil-layer method and monitoring the time-dependence of compound concentration in both compartments. The amount of compound was quantified by liquid scintillation counting.

RESULTS

A 29 to 62-fold accumulation of safinamide into human hepatocytes compared to the extracellular dosing solution was observed at 37°C. No time dependency of the uptake was observed (25 seconds as first sampling time), so the equilibrium was reached very quickly.

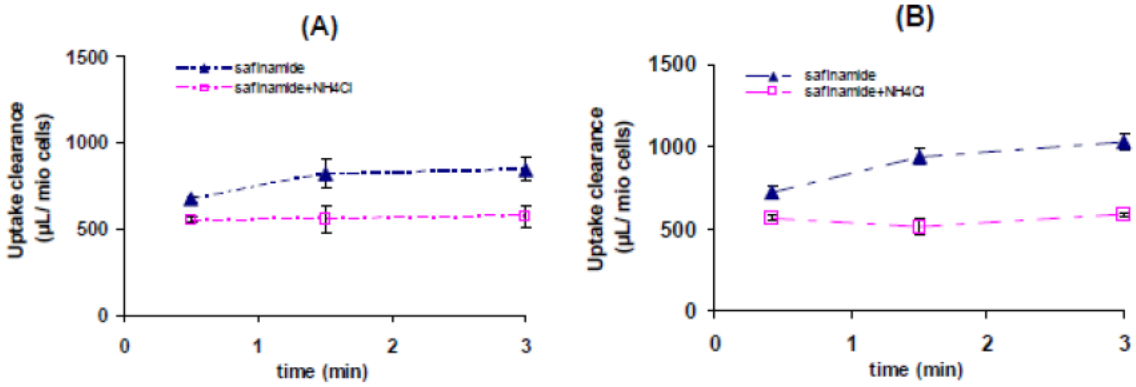
[14C]-Safinamide Uptake in Human Hepatocytes



Uptake in cryopreserved hepatocytes was measured by incubating cells with 7.3 µM [14Cl-safinamide (panel A, hepatocytes [redacted](b) (4) lot no. Hu8075 and panel B, hepatocytes from [redacted](b) (4) lot no. HH206) at 37°C or 4°C. Each point represents the mean±SD or average deviation (n=2 or 3).

The addition of ammonium chloride as an inhibitor for lysosomal uptake showed 32% and 45% maximum inhibition of safinamide accumulation in two independent experiments, which indicated lysosomal trapping of safinamide. Due to the high passive permeability of safinamide, potential involvement of hepatic uptake transporters was regarded not relevant with respect to potential drug-drug interactions in the liver.

[14C]-Safinamide Uptake Clearance in Human Hepatocytes with and w/o Ammonium Chloride



CONCLUSIONS

Safinamide is accumulated in human hepatocytes very quickly by about 40-fold, which has been shown to be partly due to lysosomal trapping.

Study Title	Evaluation of Intrinsic Clearance of ^{14}C-Safinamide in Primary Human Hepatocyte Cultures
Study number	DMPK 30-08
Study Period	October 2011
Study Director	Friedrich Krätzer

Objective	To investigate in vitro the metabolic clearance of ^{14}C-safinamide during a longer incubation period in primary human hepatocyte cultures at a non-saturating concentration. To analyze metabolic profile of prominent metabolites and to perform structural elucidation.

INTRODUCTION

A previous in vitro study already investigated the metabolism of safinamide in hepatocyte suspensions with an incubation time up to 4h. Only a low turn-over of safinamide has been seen and an important metabolite, the acyl glucuronide of NW-1689 has not been observed. Moreover, the previous study was performed at a high concentration of 50 µM safinamide which could have saturated the involved drug metabolizing enzymes. This study was conducted to evaluate the metabolic clearance of safinamide during a longer incubation period in primary human hepatocyte cultures.

METHODS

Metabolism of safinamide was studied in fresh hepatocyte monolayer cultures during 24h incubation. Supernatants were analyzed using a radio-HPLC method for quantification of parent drug and metabolites. Intrinsic clearance was calculated using the half-life approach. In addition the metabolic profile was evaluated for time-dependent formation of metabolites. Allocation of peaks to safinamide and the respective metabolites was performed by LC-MS/MS and by comparison with a reference solution containing safinamide and NW-1689, NW-1153, NW-1689 acyl glucuronide (AG), Met-A and Met-X.

RESULTS AND CONCLUSIONS

Evaluation of metabolic profiles showed that in all donors the metabolites appeared in a nearly identical chronological order. The first metabolite found after 2-4 h was NW-1153, followed by the NW-1689 acyl glucuronide (M422) after 4-6 h. Acylmigrated rearrangements of the NW-1689 glucuronide (M422-1 to 3) were detected after 6 and 24 hours. Met-A or Met-X, possible intermediate products on the pathway to NW-1689, were not observed in the supernatants. Also a N-glucuronide conjugate of safinamide was not detected. The chronological order of metabolite appearance may indicate that NW-1153 is an intermediate product in one pathway leading to NW-1689 and its glucuronide conjugates. NW-1199 was formed by a non-metabolic process, since it was found in samples with and without cells in similar amounts.

Intrinsic hepatic clearance based on free fraction in incubation of human hepatocytes (CLuint) was 1.4 µL/min/106 cells. Scaling of CLu,int to hepatic in vivo clearance (CL_H; considering the free fraction in whole blood) gives a value of 0.023 L/h/kg which corresponds to 1.5% liver blood flow.

Safinamide	1 µM			5 µM			Mean of 1 and 5 µM		
Parameters	mean	±	S.D.	mean	±	S.D.	mean	±	S.D.
k_{el} (min^{-1})	0.00082	±	0.00031	0.00074	±	0.00017	0.00078	±	0.00023
$CLu_{int, hep}$ (µL/min/10^6 cells)	1.5	±	0.6	1.4	±	0.3	1.4	±	0.4
CL_H (L/h/kg)	0.024	±	0.009	0.022	±	0.005	0.023	±	0.007
% of $Q_{H,B}$	1.6%	±	0.6%	1.4%	±	0.3%	1.5%	±	0.4%

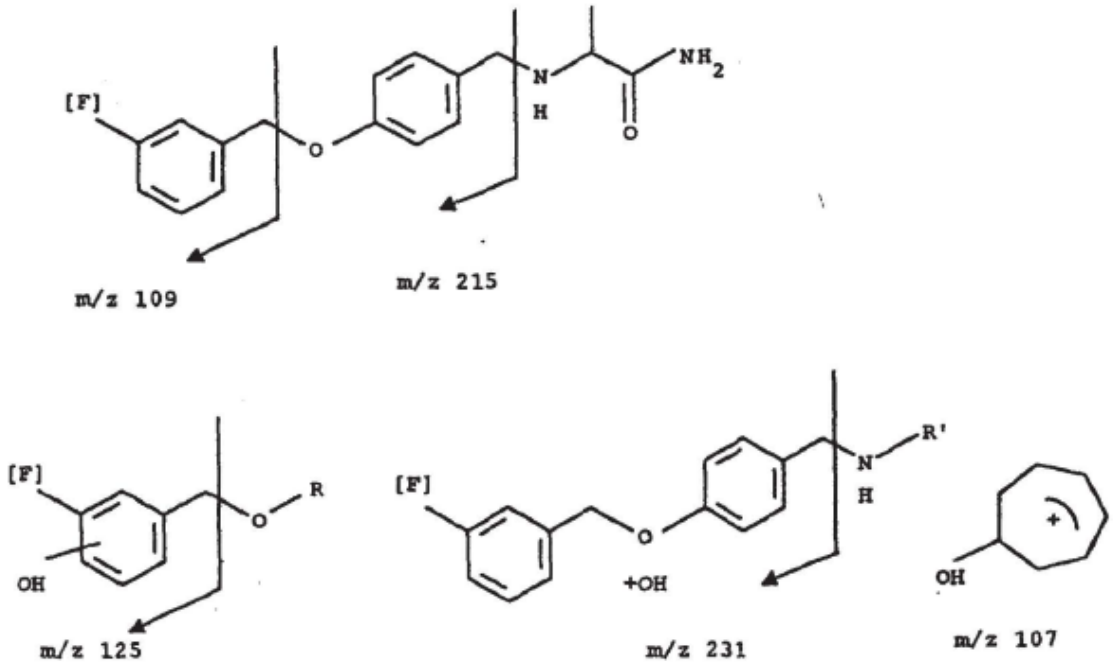
Study Title	Uptake of Safinamide into Human Hepatocytes
Study number	DMPK 15-10
Study Period	December 2010
Study Director	Dieter Gallemann
Objective	To evaluate the metabolism of safinamide by rat, dog, monkey and human microsomes.

METHODS

Control microsomes from dog, monkey and human were incubated with buffer and another set of microsomes from dog, monkey and human were incubated with drug. Supernatants were evaluated using LC-MS/MS.

RESULTS

The drug unchanged had retention time about 8.5 min. No interfering peaks were present in the control samples. The LC/MS profiles represent the total ion current chromatogram (lower trace) and the mass chromatograms of the protonated molecular ions of the drug unchanged (m/z 303; RT = 8.25+ 8.51 min), metabolites A,B,C,D (m/z 319; RT,. 3.5-4-5-5.2min respectively), metabolite E (m/z 317; RT= 15.41 + 16.29 min) and metabolite F (m/z 319; RT= 25.28+25.34min). The CID mass spectra of the drug unchanged (parent ion m/z 303), metabolite A, B, C, D (parent ion m/z 319), metabolite E (parent ion 317) and metabolite F (parent ion m/z 319) respectively.



CONCLUSIONS

A similar metabolic profile was seen in the four species. Monkey was found to be the most similar species compared to human.

Study Title	In Vitro Investigation of Safinamide Metabolism: Contribution of ALDH to the Metabolic Pathway to NW-1689
Study number	DMPK 125-07
Study Period	December 2011
Study Director	Vasilis Vasiliou, PhD
Objective	To investigate the involvement of human ALDH isoenzymes in the in vitro metabolism of safinamide intermediate Met-X to form NW-1689. In addition, the inhibitory potential of safinamide and its metabolites on ALDH isoenzymes was investigated.

METHODS

ALDH catalytic activity was determined by monitoring the formation of NADH at 340 nm during the oxidation of substrates at 25°C. The enzyme kinetics of Met-X oxidation was studied using mouse liver cytosolic fraction and recombinant human ALDH isoenzymes including ALDH1A1, ALDH1B1, ALDH2 and ALDH7A1. Six concentrations of Met-X representing three below the apparent Km and three above the apparent Km were used. Propionaldehyde (10 mM) was used as the positive control of enzyme activity. The inhibition effects of safinamide and its metabolites Met-X, Met-A, NW-1153, NW-1689, NW-1689 AG and NW-1199, towards human ALDH isoenzymes

were investigated. The inhibitory potential of safinamide and its six metabolites (50 μM for each compound) using ALDH-specific substrates at their Km concentrations was assessed using recombinant ALDH enzymes and cell lysate derived from human hepatoma HuH7 cells. The IC50 values of NW-1689 and NW-1689 AG were determined. The inhibition kinetics of NW-1689 AG on ALDH1A1 was evaluated. Four concentrations of inhibitor and three concentrations of substrate were used. Disulfiram, cyanamide and 4-DEAB were used as positive controls inhibitors. The apparent kinetic constants were derived using Sigma Plot Enzyme Kinetic Module by means of the Michaelis-Menten model.

RESULTS

Mouse liver cytosol and four studied human ALDH isoenzymes, namely ALDH1A1, ALDH1B1, ALDH2 and ALDH7A1, were all catalytically active towards Met-X oxidation. Among these enzymes, ALDH2 and ALDH7A1 showed the highest substrate affinity. Since the abundance of individual ALDH isoenzymes in hepatocytes is not known, a prediction of the contribution of individual ALDH isoenzymes to in vivo metabolism was not possible.

CONCLUSIONS

Multiple ALDH isoenzymes are able to oxidize Met-X and therefore a potential drug-drug interaction due to inhibition of a single ALDH enzyme is considered not clinically relevant. Safinamide and six metabolites were screened for an inhibition effect on ALDH activity of several recombinant human ALDHs and of human hepatoma HuH7 cell line. Only NW-1689 AG was identified to have an inhibitory potential for human ALDH1A1. Due to the limited relevance of ALDH1A1 as drug metabolizing enzyme, the risk for drug-drug interactions in patients with Parkinson's Disease is considered remote.

Presumed Pathways from Safinamide to NW-1689 (AG)

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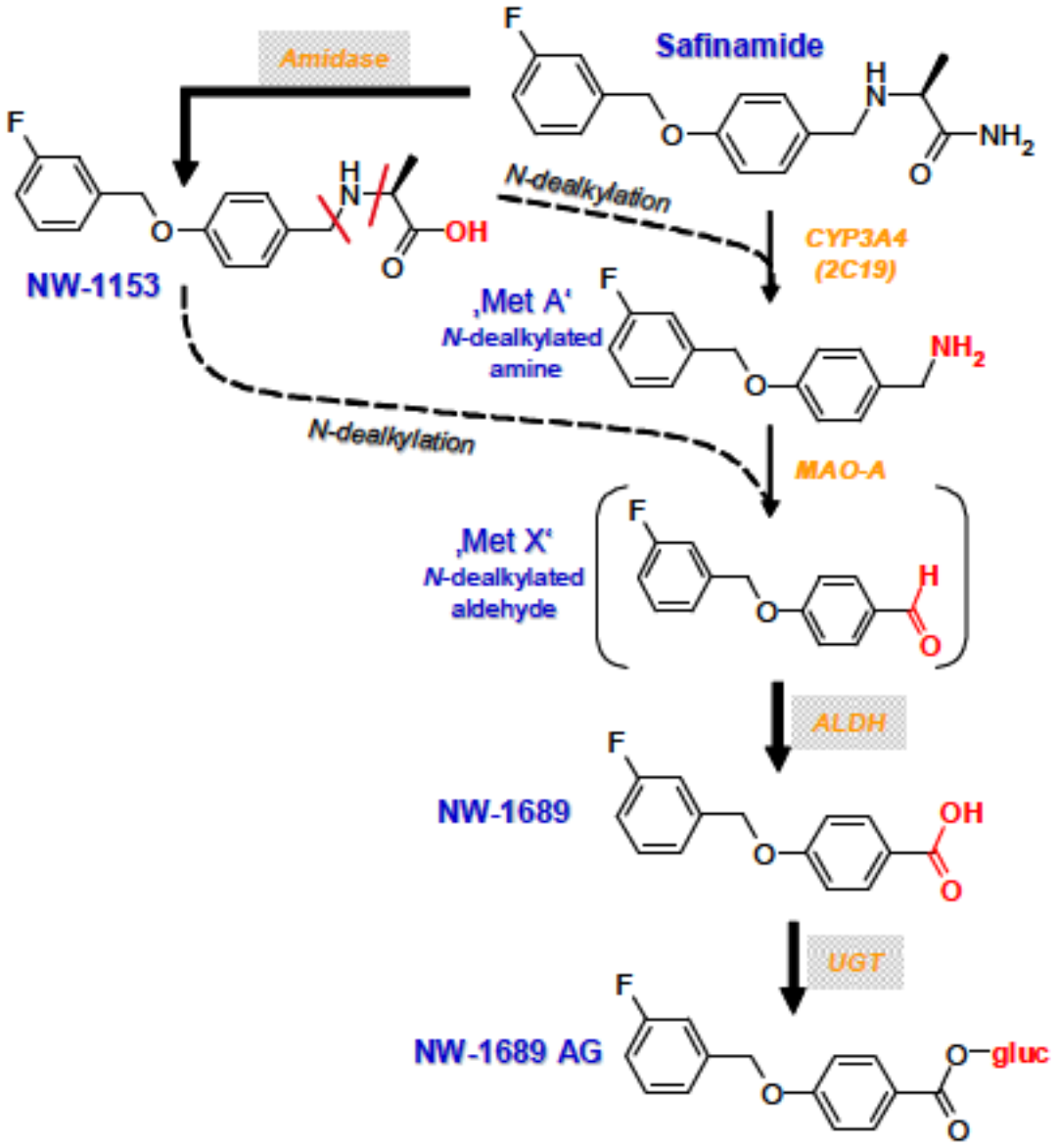


Study Title	In Vitro Investigation of Safinamide Metabolism: Phenotyping of the Metabolic Pathway to NW-1689
Study number	DMPK 123-07
Study Period	November 2011
Study Director	Friedrich Krätzer
Objective	This study aimed at confirming the relevance of some isoenzymes and some intermediate metabolites in the metabolic pathway of safinamide to NW-1689.

INTRODUCTION

Clinical studies have shown that the predominant metabolite of safinamide in human plasma was the N-dealkylated acid NW-1689. By two preceding in vitro studies this metabolite has been indicated to be formed in a multistep pathway with supposed sequential involvement of amidase(s) / cytochrome P450 (CYP) 3A4 and 2C19 enzymes, monoamine oxidase (MAO-A) and aldehyde dehydrogenases (ALDH). This study aimed at confirming the relevance of some isoenzymes and some intermediate metabolites in the metabolic pathway of safinamide to NW-1689. In addition, various experiments were performed to elucidate the putative underprediction of in vivo clearance by previous in vitro studies.

Study Rationale

Rationale	Purpose	Test Item	Test System
Extension of microsomal phenotyping panel	Investigation of capability of HLM to metabolize safinamide; optimization of incubation parameters	[^{14}C]-safinamide	HLM
Low safinamide turnover in HLM under-predicts human *in vivo* metabolic clearance	Investigation of CYP and potential non-CYP mediated extra-hepatic metabolism of safinamide; Test of capability of additional human test systems to metabolize safinamide	[^{14}C]-safinamide	Human liver mitochondria, human liver cytosol, rhCYP3A4, rhCYP3A5, rhCYP2C19, rhCYP4F2, rhCYP 2J2, rhMAO-A, rhMAO-B, human intestinal, renal, and pulmonary microsomes, S9 fraction from human spleen; human whole blood, plasma and simulated organic fluids
	Investigation of microsomal protein binding ($f_{u,mic}$)	[^{14}C]-safinamide	HLM
	Investigation of a potential inhibition of safinamide metabolism by safinamide and its metabolites e.g. NW-1199	[^{14}C]-safinamide	HLM (in absence and presence of plasma and/or NW-1199)
Previous studies indicated contribution of monoamine oxidase enzymes in safinamide metabolism	Investigation of the contribution of MAO-A and MAO-B to formation of Met-X	Met-A	HLM, rhMAO-A, rhMAO-B (in absence and presence of inhibitors)
In vivo experiments with rat showed rapid formation of NW-1689 after administration of NW-1153	Test of several rat test systems to reproduce *in vitro* the *in vivo* results and to gain information which analogous human test system could be promising for further investigation.	[^{14}C]-safinamide [^{14}C]-NW-1153	Rat liver microsomes, rat S9 fraction (brain) and rat whole blood

METHODS

[^{14}C]-safinamide was incubated with HLM and several other in vitro test systems to investigate their possible contribution to NW-1689 formation: human liver mitochondria, human liver cytosol, Supersomes (CYP3A4, CYP3A5, CYP2C19, MAO-A, MAO-B), Bactosomes (CYP4F2, CYP2J2), human intestinal, renal and pulmonary microsomes,

human whole blood, plasma and simulated organic fluids (intestinal fluid and gastric fluid). Potential time-dependent inhibition of safinamide metabolism by parent drug or its metabolites was investigated. Bioanalysis was performed by radio-HPLC.

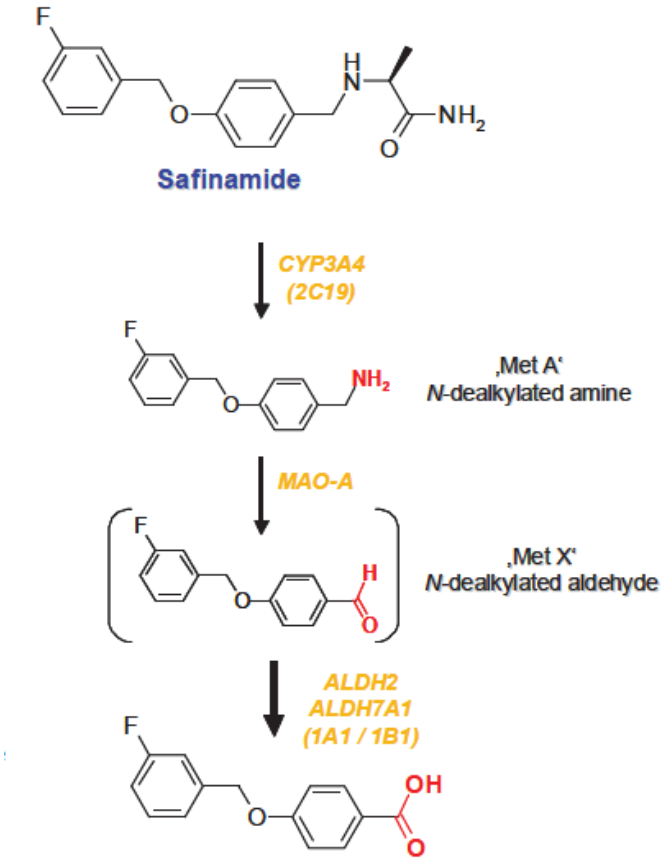
The contribution of MAO-A and MAO-B in absence and presence of inhibitors (ABT and clorgyline) and other test systems to formation of Met-X was investigated. Non-radioactive Met-A was used as substrate and Met-X formation was monitored by LC-MS/MS.

RESULTS
Safinamide was N-dealkylated to form Met-A, mediated mainly by CYP3A4 with minor contribution of CYP2C19, 2J2 and MAO-A, while CYP4F2 as well as liver cytosolic and mitochondrial enzymes appeared to be non-effective. The subsequent reactions of Met-A to form an intermediate aldehyde Met-X and the carboxylic acid NW-1689 were confirmed to be catalyzed mainly by MAO-A and probably ALDH. Potential minor contributions of NADPH dependent CYPs to Met-X oxidation.

The transient formation of an expected Met-X by MAO-A was confirmed for the first time by applying authentic Met-A at high concentration to HLM. Safinamide itself, however, did not yield detectable amounts of Met-X, neither in vitro nor in vivo. Both, the rate limiting formation of Met-A and the high ALDH capacity in hepatocytes suggest that transitory concentrations of the aldehyde Met-X are kept at toxicologically irrelevant levels.

CONCLUSION
This study confirmed results of previous studies that in HLM the formation of the N-dealkylated metabolite Met-A appears to be catalyzed by CYP3A4, with some minor contribution of microsomal CYP2C19 and MAO-A. It could be shown that the step following Met-A, the formation of the intermediate aldehyde Met-X is predominantly mediated by MAO-A. The final step, the oxidation of the aldehyde Met-X to the carboxylic acid NW-1689, was observed in HLM, mediated by ALDH enzymes.

Presumed Pathways from Safinamide to NW-1689



Study Title	In Vitro Investigation of Metabolic Pathways that Lead to Formation and Decomposition of the Safinamide Metabolite NW 1153
Study number	DMPK 124-07
Study Period	April 2012
Study Director	Dieter Gallemann
Objective	To a) characterize the drug metabolizing enzymes (DMEs) that are responsible for safinamide hydrolysis to form NW-1153, b) explore whether NW-1153 is an intermediary product on the pathway to the major safinamide metabolite NW-1689, and c) to identify the DME(s) involved in the shunt from NW-1153 towards NW-1689.

METHODS

^{14}C-Safinamide hydrolysis to form NW-1153 was investigated in human and rat hepatocytes, human liver S9 fraction, RLM, human plasma, and in recombinant hydrolyzing enzymes (hCE-1/2 and FAAH), using in addition commonly used inhibitors of esterases / amidases (BNPP, PMSF). To explore the potential shunt from NW-1153 to NW-1689, 14C-NW-1153 metabolism was studied in human and rat hepatocytes, HLM, recombinantly expressed human enzymes (MAO-A/B, FMO-1/3/5), and some

peroxidases (GS-Px, My-Px). Metabolic patterns in the different test systems were investigated after protein precipitation using radio-HPLC.

RESULTS AND DISCUSSION

In human hepatocyte suspensions, NW-1153 was the major product of safinamide metabolism during 4 h (12 - 43%). When esterases / amidases were deactivated by the generic inhibitors PMSF or BNPP, NW-1153 formation was completely suppressed and safinamide clearance reduced to about 20%, suggesting that the metabolic pathway through NW-1153 is the most important elimination pathway of safinamide. Following amidase inhibition other metabolites such as NW-1199 appeared to be increased to ≤5% indicating a slight takeover of the safinamide elimination by other pathways. Weak inhibition of safinamide metabolism in human hepatocytes was also observed by the non-steroidal anti-inflammatory drugs ibuprofen and indomethacin (by ~1/3), however, without a clear concentration dependence.

According to the sponsor, several attempts to encircle the origin of the safinamide hydrolyzing enzyme(s) were undertaken. Esterases / amidases contained in whole blood, plasma and serum as well as in simulated gastric and intestinal fluid had already been excluded by preceding experiments. While human hepatocytes had been proven to be quite competent in generating NW-1153, in human liver S9 fraction very low amounts were only sporadically detected in presence and in absence of specific cosubstrates. Focusing further investigations to commercially available esterases / amidases, hCE-1 and hCE-2 were excluded as safinamide metabolizing enzymes, while (fatty acid amide hydrolase) FAAH was most likely proven to catalyze the formation of NW-1153 at extremely low rates. Under these conditions measurement of kinetic parameters was not possible and also scaling factors for FAAH are not available. Thus, a well-founded estimation to which extent FAAH may contribute to the elimination of safinamide in humans is not possible at present. Significant contribution of other amidases to NW-1153 formation can therefore not be excluded.

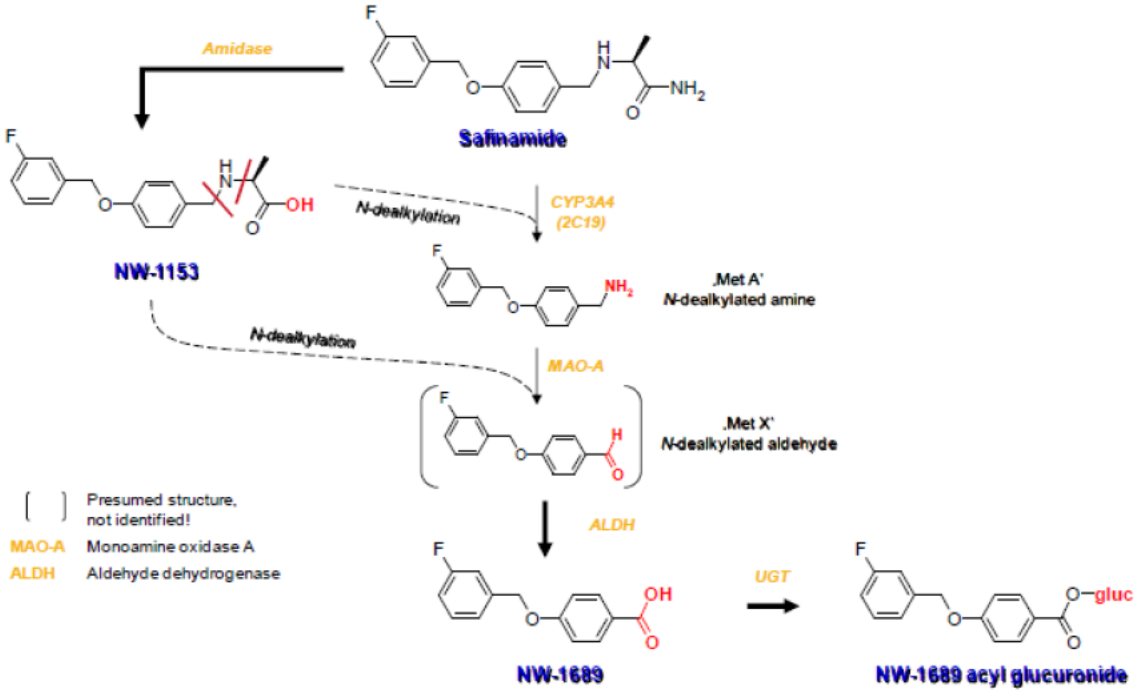
The supposed conversion of NW-1153 into NW-1689 was verified in rat as well as in human hepatocytes, indicating that a major metabolic shunt from safinamide through NW-1153 leading to NW-1689 would exist in both species. Rat as well as human liver microsomes, cytosol and human liver mitochondria did apparently do not catalyze NW-1153 degradation. Moreover, several recombinantly expressed or purified phase I enzymes, i.e. MAO-A, MAO–B, FMO-1, FMO-3, GS-Px and My-Px were not able to catalyze the N-dealkylation of NW-1153. Only FMO-5 apparently showed a very low formation of NW-1689 that could not be further characterized kinetically. Since FMOs are not predominantly involved in N-dealkylation reactions, the in vivo responsible enzyme for NW-1153 to NW-1689 conversion is probably still not identified. Finally, the C-N bond to be broken during that N-dealkylation could not be identified without having ^{14}C-alkyl labelled NW-1153 at hand.

CONCLUSION

Human FAAH was identified as one potential enzyme that may contribute to the main metabolic pathway of safinamide to form NW-1153. Safinamide has been shown to be

cleared by low affinity metabolic reaction(s). Significant contribution of other amidases to NW-1153 formation may be possible.

Main Metabolic Pathways of Safinamide in Human



Study Title	Investigations into the roles of cytochrome P450 CYP3A4 and monoamine oxidases in the metabolism of ^{14}C-Safinamide using freshly isolated human hepatocytes
Study number	NWR14
Study Period	October 2007
Study Director	Richard Cole,
Objective	To investigate the nature of the enzymes involved in these biotransformations.

INTRODUCTION

Previous in vitro studies performed at [redacted] (b) (4) (Study references: NWR/03 and NWR/05) had indicated that Safinamide is metabolized to two carboxylic acid metabolites by human hepatocytes; NW-1689 and NW-1153. This current study was conducted to investigate the nature of the enzymes involved in these biotransformations.

METHODS

^{14}C-Safinamide was incubated at a final concentration of 50 μM with fresh human hepatocytes in suspension for up to 4 hours and the samples analyzed by HPLC with on-line radio-detection. Additional analyses were also performed using LC-MS/MS to determine the chemical structure of any metabolites produced. Two major metabolites were detected in these incubations labelled as MET 1 and MET 2 on the radiochromatograms presented in this report. These metabolites were subsequently identified as comprising of NW-1689 and Safinamide N-glucuronide (these metabolites co-eluted as the single metabolite fraction MET (1) and NW-1153, respectively.

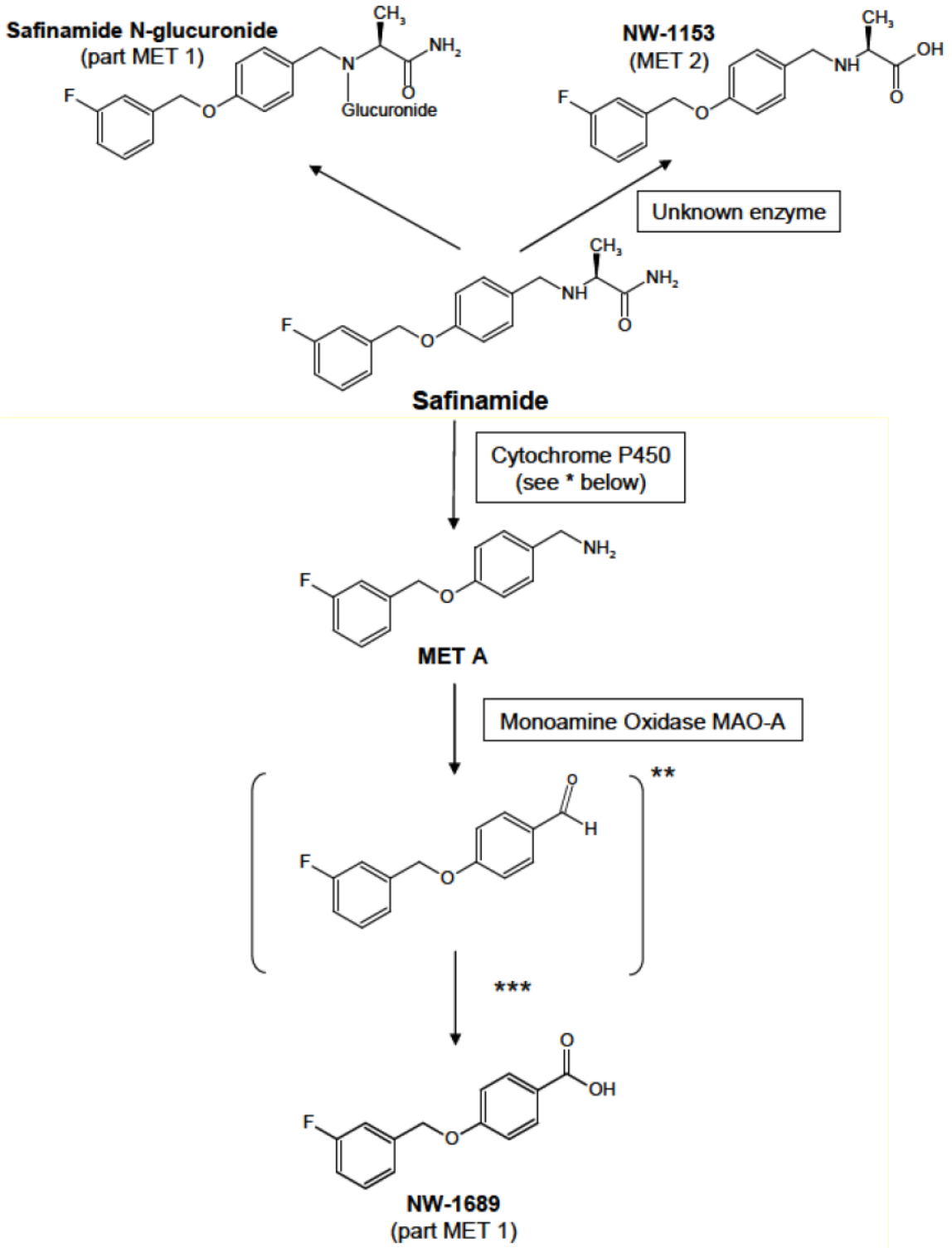
Incubations of ^{14}C-Safinamide with human hepatocytes were also performed in the presence of chemical inhibitors, selective for human monoamine oxidase A and B enzymes and cytochrome P450 enzymes.

RESULTS AND DISCUSSION

In the presence of clorgyline (1 μM) an additional metabolite was detected labelled as MET A. Clorgyline is a chemical inhibitor selective for human monoamine oxidase A (MAO-A) enzymes (Fowler, Mantle and Tipton, 1982). Clorgyline has also been shown to be an inhibitor of CYP1A2 (Polasek et al, 2006) though CYP1A2 has been shown not to be involved in the metabolism of Safinamide. MET-A was not produced to any appreciable extent in the presence of pargyline (a selective monoamine oxidase B inhibitor (Fowler, Mantle and Tipton, 1982)) or in the presence of troleandomycin (triacetyloleandomycin), a chemical inhibitor selective for CYP3A4 enzyme (Clarke, 1998). The chemical structure of MET A was determined using LC-MS/MS and it is likely, based upon its structure, that MET A is an intermediary of NW-1689, that is, formation of NW-1689 proceeds as a result of metabolism of MET A. The observation that MET A is only produced when MAO-A is inhibited (by clorgyline) indicates that MAO-A enzyme is involved in the formation of NW-1689 from MET A.

MET 2 comprised NW-1153 as a single component and its formation was slightly inhibited by the MAO inhibitors, clorgyline (approximately 15% inhibition) and pargyline (4%), but not at all by the CYP3A4 inhibitor troleandomycin. In addition, this metabolite was not observed in previous related (b) (4) studies (NWR/04 and NWR/05) using human liver microsomes and cDNA expressed recombinant human MAO enzymes. Therefore it is concluded (although tentative) that MET 2 formation is catalyzed by unknown enzyme(s) found in hepatocytes but not liver microsomes. A proposed metabolic route for Safinamide metabolism:

Proposed metabolic route for Safinamide in human hepatocytes



* - CYP450 enzymes implicated : CYP2C19 (confirmed in this study – see Sec 3.2), CYP2D6 and CYP3A4 (involvement suggested by results from related [redacted] (b) (4) study NWR/04).

** - Structure not proven in this study but indicated by reaction mechanism of MAO-A. Possibly Met X observed in related [redacted] (b) (4) study NWR/04

*** - Enzyme not proven – possibly aldehyde reductase or aldehyde oxidase

Study Title	Identification of the cytochrome P450 enzymes involved in the human hepatic metabolism of ^{14}C-Safinamide
Study number	NWR04
Study Period	March 2004
Study Director	Richard Cole,
Objective	To investigate in vitro metabolism of 14C-Safinamide

INTRODUCTION

Initially, preliminary studies were conducted using 1 (or 3), 10 and 100 µM ^{14}C-Safinamide and pooled human liver microsomes (0.1 - 5 mg/mL) to optimize the incubation conditions for the subsequent incubation of ^{14}C-Safinamide with liver microsomes from individual donors (correlation analysis). These preliminary results demonstrated that ^{14}C-Safinamide was not extensively metabolized by human liver microsomes. However, a number of radiolabelled metabolites were detected following analysis of the incubation samples by HPLC with on-line radiochemical detection and these have been labelled as MET 1 to MET 13 for the purposes of this report. The major metabolites were MET 1, MET 3, MET 7 and MET 8. There was evidence to suggest that the formation of MET 1 and other minor metabolites, MET 2, 4 and 5, could be catalyzed in the absence of NADPH and therefore may not be cytochrome P450 mediated.

The remainders of the observed metabolites were only formed in the presence of microsomal protein and NADPH, which is indicative of CYP450-mediated metabolism in microsomes.

METHODS

Reaction Phenotyping was undertaken by incubation of 14C-Safinamide with:

i) liver microsomes prepared from 15 individual donors (correlation analysis),
ii) pooled human liver microsomes in the presence of cytochrome P450-selective chemical 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4).

Incubations for experiments
i) and ii) above, were performed under conditions of linear metabolite formation (derived from the preliminary experiments discussed above).

RESULTS

There was a strong and statistically significant correlation between the metabolism of ^{14}C-Safinamide and formation of the three major NADPH-dependant metabolites, MET 3, MET 7 and MET 8 with both;

i) Testosterone 6β-hydroxylation, a marker reaction for CYP3A4/5-mediated metabolism, (correlation coefficients were 0.9773, 0.9676, 0.9363 and 0.8095, respectively); and
ii) Dextromethorphan N-demethylation, a marker for CYP3A4-mediated metabolism, (correlation coefficients were 0.8442, 0.8256, 0.9002 and 0.6565 respectively). Relatively strong correlation was also observed with ^{14}C-

Safinamide metabolism and CYP2B6 activity (S-mephenytoin N-demethylation), where correlation coefficients for the metabolism of ^{14}C-Safinamide and formation of the three major metabolites, MET 3, MET 7 and MET 8 were 0.8520, 0.8355, 0.8719 and 0.6386, respectively.

However, subsequent studies with chemical inhibitors and cDNA expressed CYP450 enzymes suggest that CYP2B6 does not contribute towards the metabolism of Safinamide in human liver microsomes.

Chemical inhibitors
Ketoconazole, a CYP3A4-selective chemical inhibitor, was the only chemical inhibitor to markedly reduce the metabolism of ^{14}C-Safinamide in pooled human liver microsomes (93.7% inhibition compared to control activities). Other chemical inhibitors (quinidine (selective for CYP2D6), diethyldithiocarbamate (selective for CYP2B6), furafylline (selective for CYP1A2) and tranylcypromine (selective for CYP2C19) had little effect on the metabolism of Safinamide).

cDNA expressed P450 enzymes
14C-Safinamide was incubated with cDNA expressed CYP1A2, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4 enzymes at a concentration of 25 µmoles CYP450 enzyme per incubation. Under these conditions, ^{14}C-Safinamide was metabolized to varying extents by CYPs 1A2, 2C19, 2D6 and 3A4. However, the metabolic profile obtained with CYP3A4 most closely resembled the hepatic metabolic profiles obtained using human liver microsomes.

CYPs 2B6, 2C9 and 2E1 displayed no metabolizing activity towards Safinamide. Although exhibiting some metabolizing activity towards Safinamide when using the expressed enzyme, it is unlikely that CYPs 1A2, 2C19 or CYP2D6 contribute to Safinamide metabolism in human liver given the absence of any correlation of ^{14}C-Safinamide metabolism with these enzyme activities (during the correlation analysis) and the absence of metabolic inhibition when chemical inhibitors of these enzymes are used.

Overall, these reaction phenotyping studies indicate that CYP3A4, the major human drug metabolizing cytochrome P450 enzyme, is the only cytochrome P450 enzyme involved in the metabolism of Safinamide to numerous metabolites in human liver.

CONCLUSIONS
14C-Safinamide is metabolized relatively slowly to numerous metabolites by pooled human liver microsomes in vitro. Studies indicate that CYP3A4, is mainly involved in the metabolism.

Study Title	Identification of the cytochrome P450 enzymes involved in the human hepatic metabolism of ^{14}C-Safinamide
Study number	NWR04

Study Period	March 2004
Study Director	Richard Cole,
Objective	To investigate in vitro metabolism of 14C-Safinamide

INTRODUCTION

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Overall, these reaction phenotyping studies indicate that CYP3A4, the major human drug metabolizing cytochrome P450 enzyme, is the only cytochrome P450 enzyme involved in the metabolism of Safinamide to numerous metabolites in human liver.

CONCLUSIONS
14C-Safinamide is metabolized relatively slowly to numerous metabolites by pooled human liver microsomes in vitro. Studies indicate that CYP3A4, is mainly involved in the metabolism.

Study Title	In Vitro Reaction Phenotyping of the UDP-Glucuronosyltransferase (UGT) Isozyme(s) Responsible for O-Glucuronidation of NW-1689
Study number	(b) (4) 104133
Study Period	April 2011
Study Director	Jennifer Skibbe
Objective	To determine the role of human UGT enzymes in the metabolism of NW-1689 to NW-1689 acyl glucuronide in human liver microsomes.

INTRODUCTION

The metabolism of NW-1689 to NW-1689 acyl glucuronide by human liver microsomes (preactivated with alamethicin) required UDPGA and was proportional to incubation time over a wide range of substrate concentrations. Thirteen concentrations of NW-1689, ranging from 15 to 1500 μM, were incubated with human liver microsomes under initial rate conditions to determine the Michaelis-Menten enzyme kinetic constants, which established that human liver microsomes catalyze the glucuronidation of NW-1689 to NW-1689 acyl glucuronide with a Km of 211±10 μM and a Vmax of 4350±78 pmol/mg/min. The in vitro intrinsic clearance (Vmax/Km) was estimated to be 20.6 μL/mg/min.

METHODS

NW-1689 (10 μM) was incubated with a panel of recombinant human UGTs (namely rUGT1A1, rUGT1A3, rUGT1A4, rUGT1A6, rUGT1A7, rUGT1A8, rUGT1A9, rUGT1A10, rUGT2B4, rUGT2B7, rUGT2B15 and rUGT2B17, 0.5 mg protein/mL, preactivated with alamethicin). Formation of NW-1689 acyl glucuronide was detected in incubations with rUGT1A1, rUGT1A3, rUGT1A7, rUGT1A9 and rUGT2B15.
In addition to recombinant human UGT enzymes, the glucuronidation of NW-1689 (10 μM) was evaluated with a bank of human liver microsomes from 16 individual subjects (0.5 mg protein/mL, preactivated with alamethicin) to determine the inter-individual differences in the rate of NW-1689 acyl glucuronide formation.

RESULTS

NW-1689 acyl glucuronide formation was not observed with recombinant UGT2B7 whereas the recombinant UGT2B7 activity was demonstrated by the positive control.

Together with the observation that the correlation between NW-1689 acyl glucuronidation and UGT2B7 activity was modest, the regression line did not pass near the origin and data points were scattered, this correlation with UGT2B7 activity may be an artifact. As described above, formation of NW-1689 acyl glucuronide was detected in incubations with rUGT1A1, rUGT1A3, rUGT1A7, rUGT1A9 and rUGT2B15. However, in a bank of human liver microsomes, the rate of NW-1689 acyl glucuronide formation did not correlate with UGT1A1 and UGT1A9 activities (p value <0.05) and the correlation between NW-1689 acyl glucuronide formation and UGT1A3 and UGT1A7 activities could not be evaluated because characterization data are not available for UGT1A3 and UGT1A7 (not expressed in the liver). Because multiple UGT enzymes may participate in the glucuronidation of NW-1689 (based on the results of recombinant human UGT experiment), correlation analysis may have been deficient of the statistical power to establish the identity of each enzyme.

In addition, UGT2B15 is reported to be one of the highly expressed enzymes in the liver. Although UGT2B15 is abundant in the liver and the results from this study suggest the potential involvement of UGT2B15 (based on the results with recombinant UGT2B15), extrapolation factors (i.e., ISEF values) for the recombinant UGT enzymes and the human liver microsomes used in this study are not available to appropriately determine the relative contribution of the UGT enzymes in the glucuronidation of NW-1689.

CONCLUSIONS
Several UGTs including UGT1A1, UGT1A3, UGT1A7, UGT1A9 and UGT2B15 have the potential to glucuronidate NW-1689.

Study Title	Evaluation of the Inhibitory Potential of NW-1015 (safinamide) on Different CYP450 Isoenzymes
Study number	(b) (4) MET-99-04
Study Period	September 2000
Study Director	Paola Tocchetti
Objective	To investigate the potential inhibitory effect of NW-1015 on several human CYP model substrate reactions.

METHODS
Pooled human liver microsomes were incubated with different concentrations of NW-1015 (0.5, 5, 10, 50, 100 µM). Activity of CYP isozymes were evaluated using probe substrates and positive controls (listed below).

Probe reactions:

Enzyme	Probe reaction	Classical inhibitor
CYP1A1/2	7-ethoxyresorufin O-deethylation	Furafylline
CYP2C9	Tolbutamide 4- hydroxylation	Sulfaphenazole
CYP2C19	S-mephenytoin 4-hydroxylation	Tranylcypromine
CYP 2D6	Dextromethorphan-O-demethylation	Quinidine
CYP2E1	Chlorzoxazone 6-hydroxylation	Diethyldithio-carbamic acid
CYP3A4	Testosterone 6β-hydroxylation	Ketoconazole

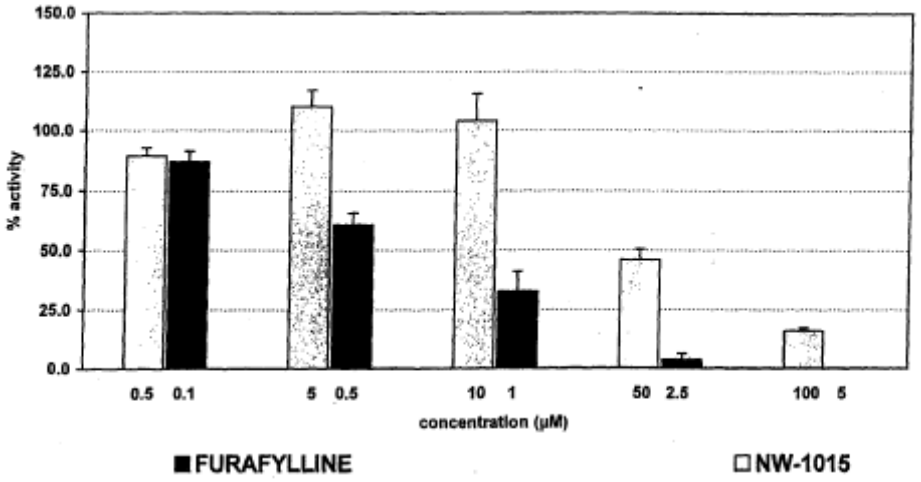
Incubation Mixture and Test Conditions
Microsomes (1mg protein /ml) were incubated at 37°C in a shaking water bath in a final volume of 500 µl of 0.1 M Na-phosphate buffer pH 7.4 containing NW-1015 0, 0.5, 5, 10, 50 and 100 µM. After 3 minutes of equilibration at 37C, a preincubation period of 15 min was initiated by the addition of a NADPH regenerating system . After 15 min, the reaction was started by adding tolbutamide at the final concentration of 400 µM. The solvent concentration was kept constant throughout alt the incubations and never exceeded 1% v/v. After 30 min (linear kinetics against time), the reaction was stopped by adding 100µlperchloric acid 65%. Samples were centrifuged at 2500g for 15 min and an aliquot of the supernatant was analyzed using HPLC-UV analysis. A similar incubation was carried out in the presence of a classical inhibitor of the probe reaction (sulfaphenazole 0-50 µM).

RESULTS

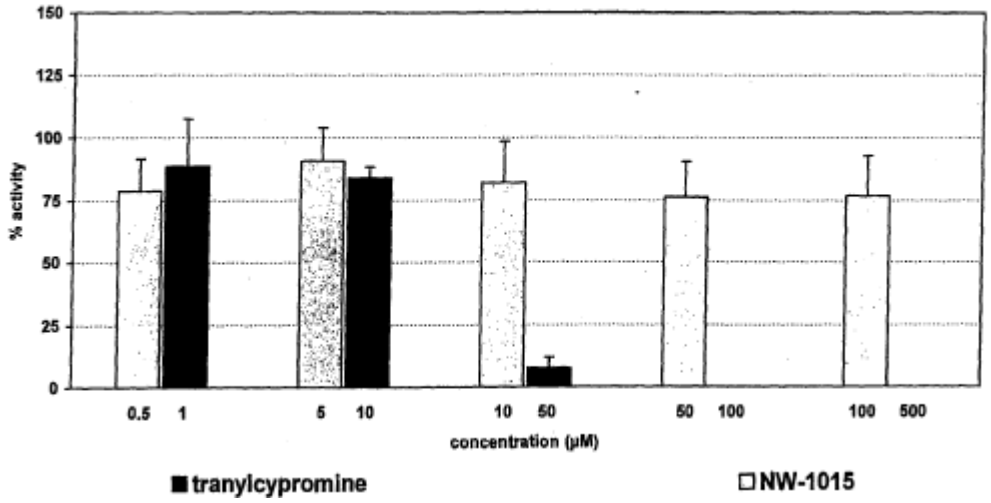
No inhibitory effect of NW-1015, at all the concentrations tested was found for probe reactions for CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4.

NW-1015 inhibited 7-ethoxyresorufin O-deethylation (CYP1A1/2) with an IC50 value of 47.7 μM.

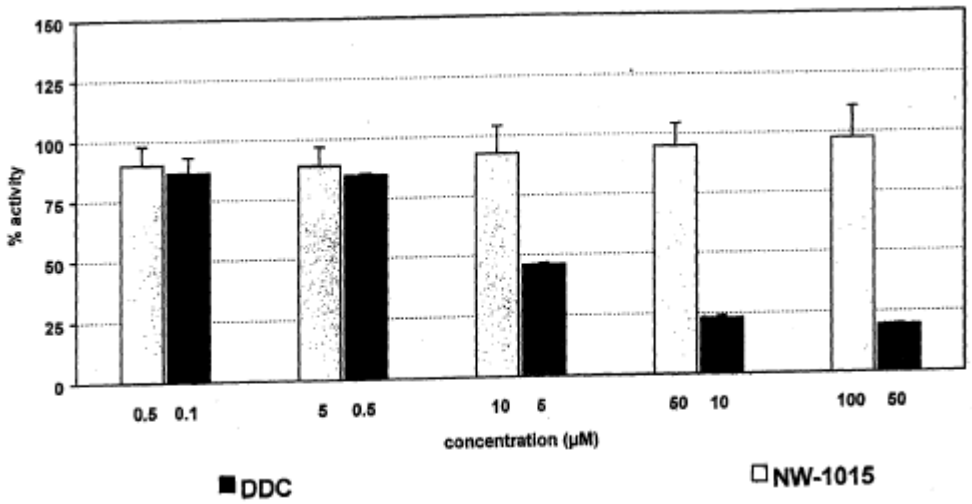
Inhibition of 7-ethoxyresorufin de-ethylation (marker for CYP1A1/2) by NW-1015 and furafylline at different concentrations



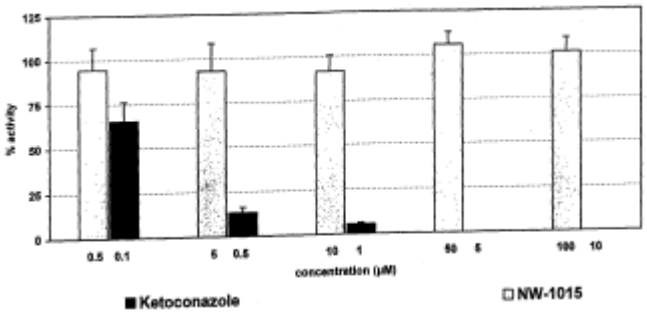
Inhibition of S-mephenytoin hydroxylation (marker for CYP2C19) by NW-1 015 and tranylcypromine at different concentrations



Inhibition of chlorzoxazone hydroxylation (marker for CYP2E1) by NW-1 015 and diethyldithiocarbamlc acid (DOC)



Inhibition of testosterone hydroxylation (marker for CYP3A4) by NW-1015 and ketoconazole at different concentrations



Note: The average maximal plasma concentration of Safinamide was < 4µM in Parkinson's disease patients treated with the highest dose of 100 mg/day, the in vitro data determined in this study did not result in considerable induction at these concentrations.

CONCLUSIONS

Safinamide has no inhibitory effect on CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. NW-1015 inhibited CYP1A1/2 with an IC50 of 47.7 µM. The IC50 value for CYP1A1/2 inhibition is more than 10 times the average maximal plasma concentration of safinamide seen in clinical studies.

Study Title	Investigation of the potential for Safinamide, NW-1689 and NW-1153 to inhibit human CYP450-mediated metabolism in vitro
Study number	NWR17
Study Period	January 2006
Study Director	Richard J. Cole
Objective	This study was designed to obtain information on the effect of Safinamide, NW-1689 and NW-1153 on human CYP450 activity using

	in vitro methods.

METHODS

The effect of NW-1689 and NW-1153 (the major circulating metabolites of Safinamide in man) at concentrations of up to 100 µM on the activities of CYP1A2, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 in pooled human liver microsomes was investigated in this study. NW-1689 and NW-1153 at concentrations of 0, 0.1, 1, 10, 30 and 100 µM were incubated with pooled human liver microsomes, NADPH, phosphate buffer and CYP450-selective chemical substrates as shown below:

P450 Isoform	Selective substrate	Reaction	Selective inhibitor*
CYP1A2	Phenacetin (20 µM)	O-Deethylation to acetaminophen	Furafylline (10 µM)
CYP2C8	Paclitaxel (15 µM)	Hydroxylation to 6α-hydroxy paclitaxel	Quercetin (50 µM)
CYP2C9	Tolbutamide (100 µM)	Hydroxylation to 4-methylhydroxy tolbutamide	Sulphaphenazole (30 µM)
CYP2C19	S-Mephenytoin (100 µM)	Hydroxylation to 4-hydroxy S-mephenytoin	Tranylcypromine (50 µM)
CYP2D6	Bufuralol (5 µM)	Hydroxylation to 1-hydroxy bufuralol	Quinidine (5 µM)
CYP2E1	Chlorzoxazone (5 µM)	Hydroxylation to 6-hydroxy chlorzoxazone	Diethyldithiocarbamate (22.8 µM)
CYP3A4**	Testosterone (50 µM)	Hydroxylation to 6β-hydroxy testosterone	Ketoconazole (2 µM)
	Nifedipine (25 µM)	Nifedipine → oxidative aromatisation to the 2,6-diethyl-4-(2'-nitrophenyl)-3,5-pyridine carboxylic acid diethyl ester derivative	
	Midazolam (60 µM)	Hydroxylation to 1-hydroxy midazolam	

* used as positive controls
** multiple substrates to assess CYP3A4 interactions

In addition, Safinamide, at concentrations of 0, 0.1, 1, 10, 30 and 100 µM, was tested against CYP2C8 activity only.

RESULTS

NW-1689 and NW-1153 did not markedly inhibit any of the cytochrome P450 activities under study. Where inhibition was observed, it was <16% of the relevant control activity at the highest concentration of test compound investigated in the study (100 µM), i.e. at a concentration 25- times higher than the plasma concentrations after therapeutic doses. Safinamide did not inhibit CYP2C8 activity.

Effect of NW-1689 upon 14C-paclitaxel 6α-hydroxylation (CYP2C8 activity)

Concentration of NW-1689 (μM)	Rate of acetaminophen formation (pmol/min/mg)		% of control acetaminophen formation	
	Individual Samples	Mean	Individual Samples	Mean
0	130 130	130	100 100	100
0.1	133 126	130	102 96.9	99.5
1	131 120	126	101 92.3	96.7
10	127 122	125	97.7 93.8	95.8
30	125 120	123	96.2 92.3	94.3
100	121 122	122	93.1 93.8	93.5

Effect of NW-1153 upon 14C-paclitaxel 6α-hydroxylation (CYP2C8 activity)

Concentration of NW-1153 (μM)	Rate of 6α-OH-paclitaxel formation (pmol/min/mg)		% of control 6α-OH-paclitaxel formation	
	Individual Samples	Mean	Individual Samples	Mean
0	66.7 66.3	66.5	100 99.7	100
0.1	68.0 70.6	69.3	102 106	104
1	67.0 68.3	67.7	101 103	102
10	68.2 67.8	68.0	103 102	103
30	66.1 70.9	68.5	99 107	103
100	71.0 65.2	68.1	107 98.0	103

6α-OH-paclitaxel = 6α-hydroxy-paclitaxel

Effect of Safinamide upon 14C-paclitaxel 6α-hydroxylation (CYP2C8 activity)

Concentration of Safinamide (μM)	Rate of 6α-OH-paclitaxel formation (pmol/min/mg)		% of control 6α-OH-paclitaxel formation	
	Individual Samples	Mean	Individual Samples	Mean
0	74.0 69.6	71.8	103 96.9	100
0.1	69.9 72.5	71.2	97.4 101	99.2
1	68.1 71.3	69.7	94.8 99.3	97.1
10	70.9 70.7	70.8	98.7 98.5	98.6
30	73.7 70.8	72.3	103 98.6	101
100	64.7 66.0	65.4	90.1 91.9	91.0

6α-OH-paclitaxel = 6α-hydroxy-paclitaxel

Effect of NW-1689 upon 14C-tolbutamide 4-methyl-hydroxylation (CYP2C9 activity)

Concentration of NW-1689 (μM)	Rate of 4-MeOH-TOL formation (pmol/min/mg)		% of control 4-MeOH-TOL formation	
	Individual Samples	Mean	Individual Samples	Mean
0	17.1 22.8	20.0	85.5 114	100
0.1	16.4 18.9	17.7	82.0 94.5	88.3
1	20.8 22.9	21.9	104 115	110
10	21.2 17.1	19.2	106 85.5	95.8
30	21.6 20.9	21.3	108 105	107
100	19.1 23.2	21.2	95.5 116	106

Effect of NW-1153 upon 14C-tolbutamide 4-methyl-hydroxylation (CYP2C9 activity)

Concentration of NW-1153 (µM)	Rate of 4-MeOH-TOL formation (pmol/min/mg)		% of control 4-MeOH-TOL formation	
	Individual Samples	Mean	Individual Samples	Mean
0	24.7 25.8	25.3	97.6 102	100
0.1	27.9 26.7	27.3	110 106	108
1	23.8 27.9	25.9	94.1 110	102
10	27.6 22.7	25.2	109 89.7	99.4
30	24.2 27.4	25.8	95.7 108	102
100	26.1 27.6	26.9	103 109	106

Effect of NW-1689 upon 14C-S-mephenytoin 4-hydroxylation (CYP2C19 activity)

Concentration of NW-1689 (µM)	Rate of 4-OH-MEPH formation (pmol/min/mg)		% of control 4-OH-MEPH formation	
	Individual Samples	Mean	Individual Samples	Mean
0	20.9 16.8	18.9	111 88.9	100
0.1	15.6 18.0	16.8	82.5 95.2	88.9
1	11.7 17.5	14.6	61.9 92.6	77.3
10	20.6 22.1	21.4	109 117	113
30	19.3 16.0	17.7	102 84.7	93.4
100	17.0 17.9	17.5	89.9 94.7	92.3

Effect of NW-1153 upon 14C-S-mephenytoin 4-hydroxylation (CYP2C19 activity)

Concentration of NW-1153 (µM)	Rate of 4-OH-MEPH formation (pmol/min/mg)		% of control 4-OH-MEPH formation	
	Individual Samples	Mean	Individual Samples	Mean
0	14.9 13.3	14.1	106 94.3	100
0.1	15.9 15.6	15.8	113 111	112
1	13.6 14.4	14.0	96.5 102	99.3
10	16.1 14.8	15.5	114 105	110
30	13.6 18.8	16.2	96.5 133	115
100	14.6 13.4	14.0	104 95.0	99.5

4-OH-MEPH = 4 hydroxy S-mephenytoin

Effect of NW-1689 upon bufuralol 1-hydroxylation (CYP2D6 activity)

Concentration of NW-1689 (μM)	Rate of 1-OHB formation (pmol/min/mg)		% of control 1-OHB formation	
	Individual Samples	Mean	Individual Samples	Mean
0	31.7 29.3	30.5	104 96.1	100
0.1	28.5 30.4	29.5	93.4 99.7	96.6
1	29.2 30.4	29.8	95.7 99.7	97.7
10	26.7 28.2	27.5	87.5 92.5	90.0
30	34.5 30.6	32.5	113 100	107
100	28.9 34.8	31.9	94.8 114	104

Effect of NW-1153 upon bufuralol 1-hydroxylation (CYP2D6 activity)

Concentration of NW-1153 (μM)	Rate of 1-OHB formation (pmol/min/mg)		% of control 1-OHB formation	
	Individual Samples	Mean	Individual Samples	Mean
0	- 32.0	32.0	- 100	100
0.1	34.0 31.8	32.9	106 99.4	103
1	- 31.7	31.7	- 99.1	99.1
10	30.9 30.9	30.9	96.6 96.6	96.6
30	25.7 32.4	29.1	80.3 101	90.7
100	33.2 33.4	33.3	104 104	104

Effect of NW-1689 upon 14C-chlorzoxazone 6'-hydroxylation (CYP2E1 activity)

Concentration of NW-1689 (μM)	Rate of 6'-OH Chlorzox formation (pmol/min/mg)		% of control 6'-OH Chlorzox formation	
	Individual Samples	Mean	Individual Samples	Mean
0	38.4 39.6	39.0	98.5 102	100
0.1	42.5 40.9	41.7	109 105	107
1	39.6 41.3	40.5	102 106	104
10	37.0 42.9	40.0	94.9 110	102
30	36.3 40.8	38.6	93.1 105	99.1
100	40.5 37.9	39.2	104 97.2	101

Effect of NW-1153 upon 14C-chlorzoxazone 6'-hydroxylation (CYP2E1 activity)

Concentration of NW-1153 (µM)	Rate of 6'-OH Chlorzox formation (pmol/min/mg)		% of control 6'-OH Chlorzox formation	
	Individual Samples	Mean	Individual Samples	Mean
0	49.4	49.3	100	100
	49.2		99.8	
0.1	52.5	50.9	106	103
	49.3		100	
1	47.8	47.7	97.0	96.7
	47.5		96.3	
10	47.4	47.5	96.1	96.4
	47.6		96.6	
30	47.9	46.7	97.2	94.8
	45.5		92.3	
100	46.2	45.8	93.7	92.9
	45.4		92.1	

6'-OH Chlorzox = 6'-hydroxy Chlorzoxazone
DEDTC - diethyldithiocarbamate

Effect of NW-1689 upon midazolam 1-hydroxyation (CYP3A4 activity)

Concentration of NW-1689 (µM)	Rate of 1-OH midazolam formation (pmol/min/mg)		% of control 1-OH midazolam formation	
	Individual Samples	Mean	Individual Samples	Mean
0	2147.3	2137.4	100	100
	2127.4		99.5	
0.1	1945.0	1920.9	91.0	89.9
	1896.8		88.7	
1	2015.4	1946.3	94.3	91.1
	1877.1		87.8	
10	1902.4	19.7.1	89.0	89.2
	1911.8		89.4	
30	1920.8	1917.9	89.9	89.8
	1914.9		89.6	
100	1934.4	1952.6	90.5	91.4
	1970.7		92.2	

Effect of NW-1153 upon midazolam oxidation (CYP3A4 activity)

Concentration of NW-1153 (µM)	Rate of 1-OH midazolam formation (pmol/min/mg)		% of control 1-OH midazolam formation	
	Individual Samples	Mean	Individual Samples	Mean
0	- 1812.9	1812.9	- 100	100
0.1	1645.4 1803.9	1724.7	90.8 99.5	95.2
1	1781.7 1832.4	1807.1	98.3 101	99.7
10	1742.3 1768.8	1755.6	96.1 97.6	96.9
30	1789.3 1789.7	1789.5	98.7 98.7	98.7
100	1811.2 1812.9	1812.1	99.9 100	100

CONCLUSIONS

Safinamide did not inhibit CYP2C8 activity. Safinamide's major circulating metabolites, NW-1689 and NW-1153, did not inhibit any of the CYP isoforms.

Study Title	In Vitro Evaluation of Safinamide as a Metabolism-Dependent Inhibitor of Human CYP2C19 and CYP2D6
Study number	(b) (4) 095019
Study Period	January 2010
Study Director	Lois Haupt
Objective	Safinamide has been shown in previous in vitro studies ((b) (4) study codes 121-07 and 10-08, (b) (4) study numbers (b) (4) 075070 and (b) (4) 085009) to inhibit CYP2C19 and CYP2D6 in a metabolism-dependent manner that can be reversed by dilution. This study was designed to further investigate the observed metabolism-dependent inhibition of these enzymes and to confirm the results obtained from the previous studies.

METHODS

General Incubation Conditions

To measure residual CYP activity, marker substrate incubations were conducted in triplicate for each sample group listed above at approximately 37°C in 200-µL incubation mixtures (target pH 7.4) for 5 minutes prior to addition of the appropriate internal standard and stop reagent, acetonitrile.

Positive Controls for Evaluation of Metabolism-Dependent Inhibition of Human CYP2C19 and CYP2D6

For the [redacted] reversibility assay, additional incubations were conducted with known positive controls for CYP3A4/5 since appropriate positive control inhibitors for the enzymes being evaluated have not yet been identified. Inhibition by the positive controls was NADPH-dependent, as expected. The irreversible positive control inhibitor (mibefradil) demonstrated metabolism-dependent inhibition that was irreversible after microsomal re-isolation and treatment with potassium ferricyanide prior to re-isolation. The quasi-irreversible positive control inhibitor (troleandomycin) demonstrated metabolism-dependent inhibition that was irreversible by microsomal re-isolation but reversible after treatment with potassium ferricyanide prior to re-isolation.

Summary of Assay Conditions to Measure Microsomal CYP Enzyme Activity: Metabolism-Dependent Inhibition of CYP2C19 and CYP2D6 by Safinamide

		Preincubation conditions					Marker substrate incubation conditions			
Enzyme	CYP reaction	Safinamide target concentration examined (µM) [a]	Pre-incubation volume (µL)	Solvent volume [b] (µL)	Protein [c] (µg/mL)	Pre-incubation time (min)	Substrate concentration (µM)	Incubation volume (µL)	Protein [c] (µg/mL)	Incubation time (min)
CYP2C19	S-Mephenytoin 4'-hydroxylation	10	8000	80	100	30	400 [d]	200	100	5
CYP2D6	Dextromethorphan O-demethylation	10	8000	80	100	30	75 [d]	200	100	5

Positive Controls for [redacted] Reversibility Assay

Additional incubations containing troleandomycin (a known quasi-irreversible metabolism-dependent inhibitor of CYP3A4/5 that forms a metabolite-inhibitory complex (MIC)) and mibefradil (a known irreversible metabolism-dependent inhibitor of CYP3A4/5) were included as positive controls with the [redacted] reversibility assay.

Table 2 Positive Controls for XenoTech Reversibility Assay

CYP enzyme	Positive control	Solvent	Concentration studied
CYP3A4/5	Troleandomycin	Acetonitrile	0, 25 µM
CYP3A4/5	Mibefradil	High purity water	0, 2 µM

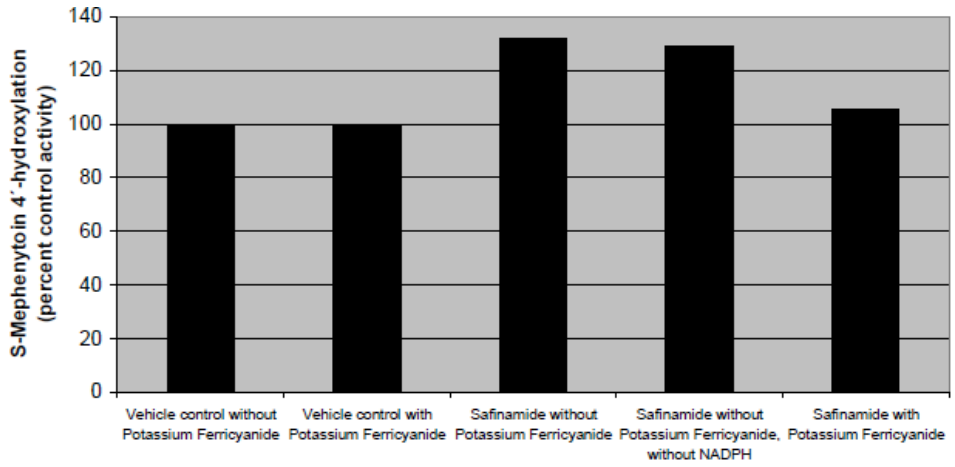
Note: Microsomal re-isolation is useful in determining whether reversible, irreversible or quasi-irreversible metabolism-dependent inhibition is occurring in that it allows removal of any remaining test article and any test article metabolites not bound to the enzyme. Potassium ferricyanide treatment is useful in reversing metabolite-inhibitory complex (MIC) formation, typically referred to as quasi-irreversible inhibition, since it removes any test article metabolites complexed with the heme of CYP enzymes (e.g., troleandomycin-CYP3A4 MIC) thereby restoring enzymatic activity.

RESULTS

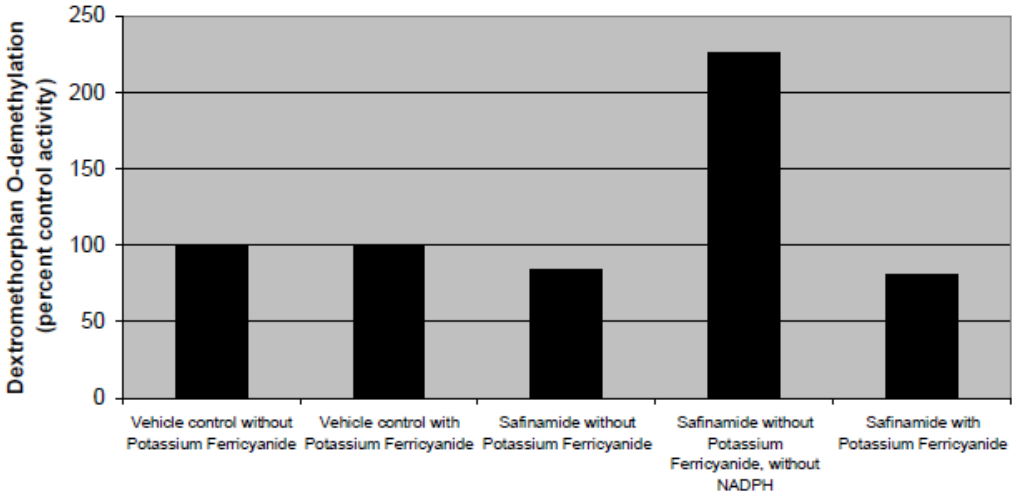
Safinamide caused metabolism-dependent inhibition of CYP2C19 and CYP2D6 that was mostly reversible by re-isolation of microsomal protein after preincubation of Safinamide with NADPH-fortified human liver microsomes. This result is consistent with a metabolite of Safinamide that is a more potent direct inhibitor of these enzymes than Safinamide, itself, since the effects of a direct inhibitor can be minimized either by

dilution or by physical removal of the test article from the incubation mixture (i.e., microsomal protein re-isolation by ultracentrifugation).

Inhibition of CYP2C19 (S-Mephenytoin 4′-Hydroxylation) by Safinamide: Metabolism-Dependent Inhibition



Inhibition of CYP2D6 (Dextromethorphan O-Demethylation) by Safinamide: Metabolism-Dependent Inhibition



According to the sponsor in this case even though reduced NADPH was not added to preincubations, a loss in enzyme activity was not observed, the activity of samples preincubated without reduced NADPH is higher than those preincubated with reduced NADPH. A loss in enzyme activity in the presence of reduced NADPH is attributed to inactivation of CYP enzymes

Note: These studies were conducted at 10 µM target concentration of safinamide which is approximately more than 2.5 fold clinical concentrations.

CONCLUSIONS
Safinamide (10 μM) caused weak reversible metabolism-dependent inhibition of CYP2C19 and CYP2D6.

Study Title	Investigation into the possible inhibition of L-Dopa decarboxylase by Safinamide, NW-1689 and NW-1153
Study number	NWR/16
Study Period	April 2006
Study Director	Richard Cole
Objective	To evaluate the ability of the Safinamide metabolites NW-1689, NW-1153 and NW-1199 to inhibit, L-Dopa decarboxylase activity

METHODS
The effect of Safinamide and its two major circulatory metabolites, NW-1689 and NW-1153, on L-Dopa decarboxylase activity was investigated in this study by incubating ^{14}C-L-Dopa with human liver homogenate (30% (w/v)). Samples were analyzed by radio-HPLC.

Benserazide, 50 μM was used as positive control for the inhibition of L-Dopa decarboxylase.

14C-L-Dopa (10 μM) was further incubated with human liver homogenate in the presence of Safinamide, NW-1689, NW-1153 and Benserazide separately for 5 minutes.

RESULTS
14C-L-Dopa (1, 10 and 100 μM) was rapidly metabolized by human liver homogenate to a number of radiolabelled components. Stability control incubations of 14C-L-Dopa (100 μM) with buffer showed that 14C-L-Dopa was chemically stable over the incubation time period investigated.

Positive control incubations, containing a known L-Dopa decarboxylase chemical inhibitor (Benserazide, 50 μM), demonstrated almost complete inhibition of ^{14}C-L-Dopa (100 μM) metabolism.

Inhibition of L-Dopa decarboxylase activity by Safinamide, NW-1689, NW-1153 and Benserazide (50 μM).

Compound (50 µM)	Rate of L-Dopa metabolism (nmol/min/g liver)		% of control L-Dopa metabolism	
	Individual Samples	Mean	Individual Samples	Mean
Inhibitor solvent (Control)	3.30	3.34	98.8	100
	3.38		101.2	
Safinamide	3.21	3.25	96.1	97.2
	3.28		98.2	
NW-1689	3.15	3.14	94.3	94.0
	3.13		93.7	
NW-1153	3.15	3.24	94.3	96.9
	3.32		99.4	
Benserazide	0.0520	0.0694	1.56	2.08
	0.0867		2.60	

CONCLUSIONS

Safinamide, NW-1689 and NW-1153 have no inhibitory effect on L-Dopa decarboxylase activity in human liver.

Study Title	In Vitro Evaluation of NW-1689, NW-1153 and NW-1199 as Inhibitors of Human MAO-A Enzymes
Study number	DMPK 156-08
Study Period	March 2009
Study Director	Brian Smith
Objective	To evaluate the ability of the Safinamide metabolites NW-1689, NW-1153 and NW-1199 to inhibit, in vitro, MAO-A enzymes in human liver mitochondria

METHODS

To evaluate NW-1689, NW-1153 and NW-1199 as direct and time-dependent inhibitors of MAO-A activity, human liver mitochondria from a pool of five individuals were incubated with a specific marker substrate, at a concentration approximately equal to its apparent Km, in the presence or absence of the test items. To evaluate time-dependent inhibition, NW-1689, NW-1153 and NW-1199 were preincubated with human liver mitochondria for 30 minutes prior to the incubation with the marker substrate to allow for the generation of metabolites that might inhibit MAO-A activity. The target concentrations of NW-1689, NW-1153 and NW-1199 ranged from 0.1 to 100 µM. Known direct and metabolism dependent inhibitors of MAO-A enzymes were included as positive controls.

RESULTS

There was no evidence that NW-1689, NW-1153 or NW-1199 caused direct inhibition of MAO-A activity and the IC50 values for these enzymes were reported to be >100 µM. There was no evidence of time-dependent inhibition of MAO-A activity by NW-1689, NW-1153 or NW-1199 at concentrations up to 100 µM.

In Vitro Evaluation of NW-1689, NW-1153 and NW-1199 as Inhibitors of Human

MAO-A Enzyme

Enzyme	Enzyme reaction	Test article	Direct inhibition: Zero-minute preincubation: IC_{50} (µM) [a]	Direct inhibition: Zero-minute preincubation: Maximum inhibition at 100 µM (%) [b]	Time-dependent inhibition: 30-minute preincubation: IC_{50} (µM) [a]	Time-dependent inhibition: 30-minute preincubation: Maximum inhibition at 100 µM (%) [b]	Potential for time-dependent inhibition [c]
MAO-A	5-Hydroxytryptamine oxidation	NW-1689	>100	0.71	>100	11	Little or no
		NW-1153	>100	13	>100	23	Little or no
		NW-1199	>100	8.5	>100	9.7	Little or no

CONCLUSIONS

NW-1689, NW-1153 or NW-1199 do not cause direct or time-dependent inhibition of MAO-A activity.

Study Title	In Vitro Evaluation of NW-1153 as a Metabolism- Dependent Inhibitor of Human CYP1A2 and CYP2B6
Study number	(b) (4) 095009
Study Period	September 2009
Study Director	Lois Haupt, B.S.
Objective	NW-1153 has been shown in a previous in vitro study ((b) (4) study code 121-07, (b) (4) study no. (b) (4) 075070) to inhibit CYP1A2 and CYP2B6 in a time-dependent manner. This study was designed to further investigate metabolism-dependent inhibition of these enzymes with the aim of ascertaining the potential of NW-1153 to inhibit the metabolism of concomitantly administered drugs.

METHODS

Human Liver Microsomes

Human liver microsomes from donated livers were prepared and characterized by the Testing Facility. A pool of sixteen individual, mixed gender, human liver microsomal samples was used for this study.

Positive Controls for (b) (4) Reversibility Assay

Additional incubations containing troleandomycin (a known quasi-irreversible metabolism-dependent inhibitor of CYP3A4/5 that forms a metabolite-inhibitory complex (MIC) and mibefradil (a known irreversible metabolism-dependent inhibitor of CYP3A4/5) were included as positive controls with the (b) (4) reversibility assay.

CYP enzyme	Positive control	Solvent	Concentration studied
CYP3A4/5	Troleandomycin	Acetonitrile	0, 25 µM
CYP3A4/5	Mibefradil	High purity water	0, 2 µM

Positive Controls for Evaluation of Time-Dependent Inhibition of Human CYP Enzymes

For the (b) (4) reversibility assay experiment, additional incubations were conducted with known positive controls. Inhibition by the positive controls did require NADPH. The irreversible positive control (mibefradil) was irreversible after microsomal re-isolation and treatment with potassium ferricyanide prior to re-isolation. The quasi-irreversible positive control (troleandomycin) was irreversible with microsomal re-isolation; however, the inhibition caused by this control was reversible with treatment with potassium ferricyanide prior to re-isolation.

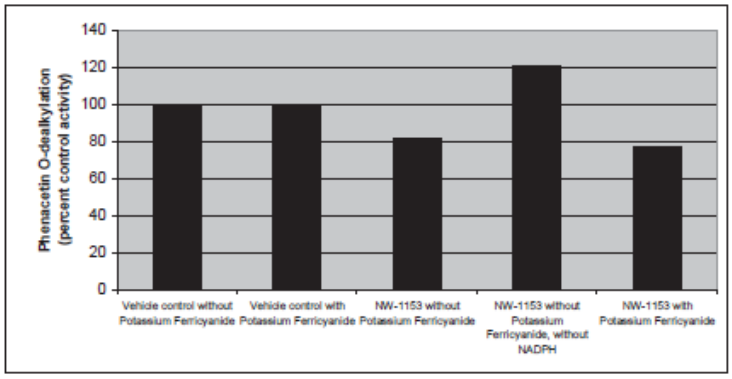
Summary of Assay Conditions to Measure Microsomal CYP Enzyme Activity: Metabolism-Dependent Inhibition of CYP1A2 and CYP2B6 by NW-1153

Enzyme	CYP reaction	Preincubation conditions					Marker substrate incubation conditions			
		NW-1153 target concentration examined (μM) [a]	Pre-incubation volume (μL)	Solvent volume [b] (μL)	Protein [c] (μg/mL)	Pre-incubation time (min)	Substrate concentration (μM)	Incubation volume (μL)	Protein [c] (μg/mL)	Incubation time (min)
CYP1A2	Phenacetin O-dealkylation	100	8000	80	100	30	120 [d]	200	100	5
CYP2B6	Bupropion hydroxylation	100	8000	80	100	30	500 [e]	200	100	5

RESULTS

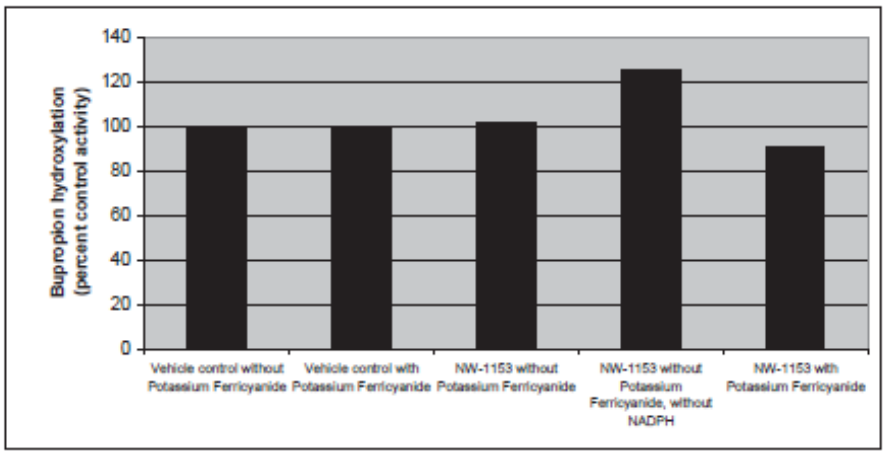
NW-1153 caused metabolism-dependent (i.e., time-dependent and NADPH-dependent) inhibition of CYP1A2 that was partially reversed by re-isolation of microsomal protein from preincubation.

Inhibition of CYP1A2 (Phenacetin O-Dealkylation) by NW-1153: Time-Dependent Inhibition



CYP2B6 activity was recovered after microsomal re-isolation prior to marker substrate incubations. NW-1153 appears to be a reversible metabolism dependent inhibitor of this enzyme. This result is consistent with the formation of a metabolite of NW-1153 that is a more effective direct inhibitor of CYP2B6 than NW-1153.

Inhibition of CYP2B6 (Bupropion Hydroxylation) by NW- 1153: Time-Dependent Inhibition



Note: These studies were conducted at 100 µM target concentration of NW-1153, which is approximately 250 fold higher than the clinically relevant levels.

CONCLUSIONS
NW-1153 (100 µM) caused partially reversible metabolism-dependent inhibition of CYP1A2 and reversible metabolism-dependent inhibition of CYP2B6.

Study Title	In Vitro Evaluation of Safinamide as a Time-Dependent Inhibitor of Human CYP1A2, CYP2B6, CYP2C19, and CYP2D6
Study number	(b) (4) 085009
Study Period	March 2010
Study Director	Lois Haupt, B.S.
Objective	Safinamide has been shown during a preceding study ((b) (4) study code 121-07, (b) (4) study no. (b) (4) 075070) to inhibit CYP1A2, CYP2B6, CYP2C19 and CYP2D6 in a time-dependent manner. This study was designed to characterize the mechanism and kinetic parameters underlying these CYP inhibitions. More specifically, experiments were performed to evaluate whether the time-dependent inhibition of CYP1A2, CYP2B6, CYP2C19 and CYP2D6 was NADPH-dependent and/or resistant to dilution.

METHODS
Safinamide (at target concentrations approximately equal to 10 and 100 µM) was preincubated with pooled human liver microsomes. Preincubations were conducted in either the presence or absence of an NADPH-generating system, with and without dilution, prior to incubation with the marker substrate.

Positive Controls for Time-Dependent Inhibition Experiments (Effects of NADPH and Dilution)

Positive control inhibitors for time-dependent inhibition of the CYP enzymes examined were included at specific concentrations. For these preincubations, each inhibitor was preincubated in duplicate in the presence and absence of an NADPH generating system for zero and 30 minutes, with and without dilution. Marker substrate (at approximately 3 Km for phenacetin and 10 Km for all other substrates examined) was then added, and the incubation was continued for 5 minutes to allow formation of metabolites of the marker substrate. The residual CYP activities were then determined.

Enzyme	Inhibitor	Conditions for 25-fold diluted samples		Conditions for undiluted samples
		Pre-incubation concentration of inhibitor	Final concentration of inhibitor	Final concentration of inhibitor
CYP1A2	Furafylline	0, 1.0 µM	0, 0.04 µM	0, 1.0 µM
CYP2B6	Phencyclidine	0, 30 µM	0, 1.2 µM	0, 30 µM
CYP2C19	Ticlopidine	0, 0.75 µM	0, 0.03 µM	0, 0.75 µM
CYP2D6	Paroxetine	0, 0.3 µM	0, 0.012 µM	0, 0.3 µM

Positive Controls for Time-Dependent Inhibition

Experiments (Determination of K_I and K_{inact} Values) Metabolism dependent inhibitors known to inactivate CYP1A2 and CYP2B6 (i.e., furafylline and phencyclidine, respectively) were included as positive controls. For these preincubations, each inhibitor was preincubated in triplicate with pooled human liver microsomes (2.5 mg/mL) for multiple incubation times (0, 2.5, 5 and 10 minutes). After the preincubation period, marker substrate (at approximately 3 Km for phenacetin and 10 Km for bupropion) and NADPH-generating system were added to the incubation mixtures and treated.

Enzyme	Inhibitor	Preincubation concentration of inhibitor	Final concentration of inhibitor
CYP1A2	Furafylline	0, 0.1, 0.25, 0.5, 1.0, 1.5 µM	0, 0.004, 0.01, 0.02, 0.04, 0.06 µM
CYP2B6	Phencyclidine	0, 1, 3, 10, 30, 60 µM	0, 0.04, 0.12, 0.4, 1.2, 2.4 µM

RESULTS

Under the experimental conditions examined, time-dependent inhibition of CYP1A2, CYP2B6, CYP2C19 and CYP2D6 appeared to be dependent on NADPH indicating that Safinamide is a metabolism-dependent inhibitor of these enzymes. Furthermore, this metabolism-dependent inhibition appeared to be resistant to dilution for CYP1A2 and CYP2B6, but not for CYP2C19 and CYP2D6. These results suggest Safinamide is an irreversible or quasi-irreversible inhibitor of CYP1A2 and CYP2B6, while showing reversibility for CYP2C19 and CYP2D6.

Further characterization of the mechanism-dependent inhibition of CYP1A2 and CYP2B6 was performed to determine to what extent (KI value) and how quickly (kinact value) Safinamide inactivates these enzymes. The data for inactivation of CYP1A2 by Safinamide followed a first-order loss of enzyme activity only through 10 minutes

preincubation; therefore, only these data were included in calculation of inactivation parameters. Under the experimental conditions examined, the K_I value was determined to be 33.5 μM and the k_{inact} was 0.075 min^{-1} for CYP1A2.

In the concentration range studied (10 to 100 μM); Safinamide was found to be a very weak inactivator of CYP2B6. No substantial change in the rate of inactivation was observed with respect to increasing Safinamide concentrations and incubation time. Thus, calculation of K_I and k_{inact} values was not possible for CYP2B6.

DISCUSSION AND CONCLUSIONS

At saturating concentrations of Safinamide, approximately 7.5% of the CYP1A2 activity was inactivated per minute. A Safinamide concentration of 33.5 μM will give one-half the maximum rate of inactivation. For comparison, the KI and kinact values for the positive control, furafylline, were found to be 3.0 μM and 0.18 min^{-1}, respectively. In comparison, Safinamide is a less potent metabolism-dependent inhibitor of CYP1A2 than furafylline.

In the concentration range studied (10 to 100 μM); Safinamide was found to be a very weak inactivator of CYP2B6.

Study Title	In Vitro Evaluation of NW-1689 Acyl Glucuronide as an Inhibitor of CYP2C8 in Human Liver Microsomes
Study number	(b) (4) 105058
Study Period	December 2010
Study Director	Lois Haupt
Objective	To evaluate the ability of the safinamide metabolite NW-1689 1ß-O-acyl glucuronide to inhibit, in vitro, CYP2C8 activity in human liver microsomes with the aim of ascertaining the potential of NW-1689 acyl glucuronide to inhibit the metabolism of concomitantly administered drugs.

METHODS

General Incubation Conditions

To measure residual CYP activity, marker substrate incubations were conducted in triplicate for each sample group listed above at approximately 37°C in 200-μL incubation mixtures (target pH 7.4) for 5 minutes prior to addition of the appropriate internal standard and stop reagent, acetonitrile.

Determination of IC50 Values: Evaluation of NW-1689 Acyl Glucuronide as an Inhibitor of Human CYP2C8

To measure CYP2C8 activity, incubations were conducted at approximately 37°C in 200 μL incubation mixtures (target pH 7.4) containing water, potassium phosphate buffer (50 mM, pH 7.4), $MgCl_2$ (3 mM), EDTA (1 mM, pH 7.4), an NADPH generating system (always the mixture of the following: NADP [1 mM], glucose-6-phosphate [5 mM],

glucose-6-phosphate dehydrogenase [1 Unit/mL]), and marker substrate at the final concentrations indicated. Pooled human liver microsomes (from sixteen individuals) were used as the source of the enzyme. The concentration of marker substrate was based on the Km and Vmax data.

To examine its ability to act as a direct inhibitor of CYP2C8, NW-1689 acyl glucuronide (at concentrations ranging from 0.3 to 30 µM) was co-incubated with marker substrate. To examine its ability to act as a metabolism-dependent inhibitor of CYP2C8, NW-1689 acyl glucuronide (at the same concentrations used to evaluate direct inhibition) was preincubated at 37±1°C, in duplicate, with human liver microsomes and an NADPH-generating system for approximately 30 minutes. Additional duplicate samples at all NW-1689 acyl glucuronide concentrations were preincubated for 30 minutes in the presence of pooled human liver microsomes but in the absence of NADPH to determine if any observed time-dependent inhibition was also NADPH-dependent. After the preincubation period, the marker substrate incubations were continued as described above. Incubations containing no NW-1689 acyl glucuronide (0 µM) and incubations that contained NW-1689 acyl glucuronide but were not preincubated, served as negative controls.

Positive Controls for IC50 Determination

Additional incubations were conducted at the normal incubation time and microsomal protein concentration in the presence of the marker substrate (approximately equal to Km) and the following direct inhibitor at the concentration listed below.

Table 5 Direct Inhibitor Positive Control

Enzyme	Positive control	Vehicle	Concentration studied
CYP2C8	Montelukast	Methanol (0.1%)	0.05 µM

Table 6 Metabolism-Dependent Inhibitor Positive Control

Enzyme	Positive control	Vehicle	Concentration studied
CYP2C8	Gemfibrozil glucuronide	Acetonitrile with 0.1% v/v formic acid (0.5%)	5.0 µM

RESULTS AND DISCUSSION

NW-1689 acyl glucuronide inhibited CYP2C8 activity in human liver microsomes as measured by amodiaquine N-dealkylation causing approximately 22% inhibition in incubations containing 30 µM NW-1689 acyl glucuronide; however, the IC50 value was reported as >30 µM. Furthermore, there was little or no evidence of either time-dependent or metabolism-dependent inhibition of CYP2C8 activity at concentrations of NW-1689 acyl glucuronide up to 30 µM.

In Vitro Evaluation of NW-1689 Acyl Glucuronide as an Inhibitor of Human CYP2C8

Enzyme	Enzyme reaction	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation without NADPH		30-minute preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} (μM) [a]	Inhibition observed at 30 μM (%) [b]	IC_{50} (μM) [a]	Inhibition observed at 30 μM (%) [b]	IC_{50} (μM) [a]	Inhibition observed at 30 μM (%) [b]	
CYP2C8	Amodiaquine N-dealkylation	>30	22	>30	29	>30	29	Little to no

Note: IC50 value for in the inhibition of CYP2C8 (>30 μM) is approximately 24 fold higher than clinically relevant NW-1689 acyl glucuronide concentration.

CONCLUSIONS
NW-1689 acyl glucuronide inhibited CYP2C8 activity in human liver microsomes as measured by amodiaquine N-dealkylation causing approximately 22% inhibition in incubations containing 30 μM NW-1689 acyl glucuronide.

Study Title	In Vitro Evaluation of Safinamide, NW-1153 and NW-1689 as Inhibitors of Human Cytochrome P450 Enzymes
Study number	(b) (4) 075070
Study Period	January 2008
Study Director	Lois Haupt, B.S.
Objective	The objective of this study was to evaluate the ability of Safinamide, NW-1153 and NW-1689 to inhibit, in vitro, the major CYP enzymes in human liver microsomes (namely CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5) in either a direct or a time-dependent manner.

METHODS
To evaluate the three test items as direct inhibitors of CYP activity, pooled human liver microsomes were incubated with marker substrates at concentrations equal to their apparent Km, in the presence or absence of test item. The target concentrations of Safinamide, NW-1153 and NW-1689 ranged from 0.1 to 100 μM.

Positive Controls for IC50 and Ki Determinations
For the following direct inhibition assays, additional incubations were conducted at the normal incubation time and microsomal protein concentration in the presence of the marker substrate (approximately equal to Km) and the following inhibitors at the concentrations listed.

Following table indicates the list of substrates and positive controls for each CYP isoforms tested.

Concentrations of substrates and positive control inhibitors for each CYP isoform

CYP enzyme	Positive control	Vehicle	Concentration studied
CYP1A2	α-Naphthoflavone	Methanol	0.5 μM
CYP2A6	Nicotine	Methanol	300 μM
CYP2B6	Orphenadrine	DMSO	750 μM
CYP2C8	Montelukast	Methanol	0.5 μM
CYP2C9	Sulfaphenazole	Methanol	2.0 μM
CYP2C19	Modafinil	DMSO	250 μM
CYP2D6	Quinidine	High purity	0.5 μM
CYP2E1	4-Methylpyrazole	High purity	15 μM
CYP3A4/5	Ketoconazole	Methanol	0.15 [a] / 0.075 [b] μM

a Testosterone 6β-hydroxylation or Nifedipine oxidation
b Midazolam 1′-hydroxylation

Metabolism-Dependent Inhibitor Positive Controls

CYP enzyme	Positive control	Vehicle	Concentration studied
CYP1A2	Furafylline	DMSO	1.0 μM
CYP2A6	8-Methoxypsoralen	Methanol	0.05 μM
CYP2B6	Phencyclidine	High purity water	30 μM
CYP2C8	Gemfibrozi glucuronide	Acetonitrile with 0.1% (v/v) formic acid (0.5%)	25 μM
CYP2C9	Tienilic acid	Tris base (0.002 mg/mL)	0.25 μM
CYP2C19	Ticlopidine	High purity water	0.75 μM
CYP2D6	Paroxetine	High purity water	0.3 μM
CYP2E1	3-Amino-1,2,4-triazole	High purity water	10,000 μM
CYP3A4/5	Troleandomycin	Acetonitrile	25 [a] / 20 [b] / 7.5 [c] μM

a Testosterone 6β-hydroxylation
b Nifedipine oxidation
c Midazolam 1′-hydroxylation

RESULTS

Safinamide: There was some evidence of direct inhibition of CYP1A2, CYP2B6, CYP2C19, and CYP2D6: at 100 μM 50%, 20%, 33%, and 33% inhibition, respectively, were observed. The IC50 values for these enzymes were reported as >100 μM.

Safinamide was a competitive inhibitor of CYP1A2 with a Ki value of 54 μM (little or no binding to microsomal protein). There was little or no evidence of direct inhibition of CYP2A6, CYP2C8, CYP2C9, CYP2E1, and CYP3A4/5 by Safinamide at concentrations up to 100 μM. Under the experimental conditions examined, there was evidence of time dependent inhibition for CYP1A2, CYP2B6, CYP2C19 and CYP2D6, but little or no evidence for CYP2A6, CYP2C8, CYP2C9, CYP2E1 and CYP3A4/5.

Summary of Results: In Vitro Evaluation of Safinamide as Inhibitors of Human CYP Enzymes

Enzyme	CYP reaction	Direct inhibition					Time-dependent inhibition		
		Zero minute preincubation					30 minute preincubation		Potential for time-dependent inhibition [b]
		IC_{50} (μM)	Maximum inhibition at 100 μM (%) [a]	K_i (μM)	Type of inhibition		IC_{50} (μM)	Maximum inhibition at 100 μM (%) [a]	
CYP1A2	Phenacetin O-deethylation	>100	50	54	competitive		13	79	yes
CYP2A6	Coumarin 7-hydroxylation	>100	NA	ND	ND		>100	NA	little or no
CYP2B6	Bupropion hydroxylation	>100	20	ND	ND		57	70	yes
CYP2C8	Amodiaquine N-dealkylation	>100	6.8	ND	ND		>100	6.3	little or no
CYP2C9	Diclofenac 4´-hydroxylation	>100	0.7	ND	ND		>100	14	little or no
CYP2C19	S-Mephenytoin 4´-hydroxylation	>100	33	ND	ND		63	63	yes
CYP2D6	Dextromethorphan O-demethylation repeat	>100	33	ND	ND		>100	21	yes
CYP2D6	Dextromethorphan O-demethylation repeat 2	>100	33	ND	ND		>100	24	yes
CYP2E1	Chlorzoxazone 6-hydroxylation	>100	NA	ND	ND		>100	5.3	little or no
CYP3A4/5	Testosterone 6β-hydroxylation	>100	13	ND	ND		>100	22	little or no
CYP3A4/5	Midazolam 1´-hydroxylation	>100	NA	ND	ND		>100	NA	little or no
CYP3A4/5	Nifedipine oxidation	>100	NA	ND	ND		>100	12	little or no

Notes: Average data (i.e., percent of control of activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values. IC_{50} values were calculated with XLfit.

NW-1153: Under the experimental conditions examined, there was little or no evidence of direct inhibition for any of the CYP enzymes evaluated. However, there was some evidence of time-dependent inhibition for CYP1A2 and CYP2B6, but little or no evidence for CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5.

Summary of Results: In Vitro Evaluation of NW-1153 as Inhibitors of Human CYP Enzymes

Enzyme	CYP Reaction	Direct inhibition					Time-dependent inhibition		
		Zero minute preincubation					30 minute preincubation		Potential for time-dependent inhibition [b]
		IC_{50} (μM)	Maximum inhibition at 100 μM (%) [a]	K_i (μM)	Type of inhibition		IC_{50} (μM)	Maximum inhibition at 100 μM (%) [a]	
CYP1A2	Phenacetin O-deethylation	>100	8.9	ND	ND		>100	36	yes
CYP2A6	Coumarin 7-hydroxylation	>100	9.3	ND	ND		>100	5.9	little or no
CYP2B6	Bupropion hydroxylation	>100	11	ND	ND		47	78	yes
CYP2C8	Amodiaquine N-dealkylation	>100	NA	ND	ND		>100	NA	little or no
CYP2C9	Diclofenac 4´-hydroxylation	>100	NA	ND	ND		>100	NA	little or no
CYP2C19	S-Mephenytoin 4´-hydroxylation	>100	10	ND	ND		>100	4.4	little or no
CYP2D6	Dextromethorphan O-demethylation	>100	NA	ND	ND		>100	NA	little or no
CYP2E1	Chlorzoxazone 6-hydroxylation	>100	NA	ND	ND		>100	NA	little or no
CYP3A4/5	Testosterone 6β-hydroxylation	>100	NA	ND	ND		>100	NA	little or no
CYP3A4/5	Midazolam 1´-hydroxylation	>100	NA	ND	ND		>100	NA	little or no
CYP3A4/5	Nifedipine oxidation	>100	NA	ND	ND		>100	NA	little or no

NW-1689: Under the experimental conditions examined, there was little or no evidence of direct inhibition for any of the CYP enzymes evaluated. There was also little or no evidence of time-dependent inhibition for any of the CYP enzymes evaluated.

Summary of Results: In Vitro Evaluation of NW-1689 as Inhibitors of Human CYP Enzymes

Enzyme	CYP reaction	Direct inhibition					Time-dependent inhibition		
		Zero minute preincubation					30 minute preincubation		Potential for time-dependent inhibition [b]
		IC_{50} (µM)	Maximum inhibition at 100 µM (%) [a]	K_i (µM)	Type of inhibition		IC_{50} (µM)	Maximum inhibition at 100 µM (%) [a]	
CYP1A2	Phenacetin O-deethylation	>100	NA	ND	ND		>100	NA	little or no
CYP2A6	Coumarin 7-hydroxylation	>100	14	ND	ND		>100	11	little or no
CYP2B6	Bupropion hydroxylation	>100	NA	ND	ND		>100	NA	little or no
CYP2C8	Amodiaquine N-dealkylation	>100	3.9	ND	ND		>100	7.2	little or no
CYP2C9	Diclofenac 4´-hydroxylation	>100	NA	ND	ND		>100	NA	little or no
CYP2C19	S-Mephenytoin 4´-hydroxylation	>100	0.75	ND	ND		>100	4.4	little or no
CYP2D6	Dextromethorphan O-demethylation	>100	NA	ND	ND		>100	NA	little or no
CYP2E1	Chlorzoxazone 6-hydroxylation	>100	5.1	ND	ND		>100	NA	little or no
CYP3A4/5	Testosterone 6β-hydroxylation	>100	4.4	ND	ND		>100	5.4	little or no
CYP3A4/5	Midazolam 1´-hydroxylation	>100	12	ND	ND		>100	5.1	little or no
CYP3A4/5	Nifedipine oxidation	>100	NA	ND	ND		>100	NA	little or no

CONCLUSIONS

Safinamide, NW-1153 and NW-1689 did not inhibit, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 in either a direct or a time-dependent manner below therapeutically relevant concentrations.

Study Title	Determination of CYP3A4 Induction Potential of Safinamide and Ralfinamide, Hereinafter Called New Chemical Entities (NCEs) Using a Transfected Human Liver Cell Line and a Reporter Gene Assay
Study number	030242
Study Period	October 2003
Study Director	Mary Zacour
Objective	To evaluate the potential of Safinamide to induce CYP3A4/5 using a human cell-based reporter gene model.

METHODS

Safinamide and ralfinamide were tested for their potential to induce CYP3A4 in humans, using a reporter gene method. A human cell line was transfected with vectors containing genetic elements involved in xenobiotic induction of CYP3A4 or with control vectors, incubated with test compounds or vehicle for approximately 48 hours, and assayed for reporter gene expression. Data analysis controlled for both vehicle effects and the effects of control vectors.

RESULTS

Safinamide significantly induced CYP3A4 at only the highest concentration, 50 µM, with a maximal induction of only 1.7-fold over the vehicle-only condition (compared to 5-fold induction caused by 10 µM rifampicin). Ralfinamide, on the other hand, significantly induced CYP3A4 at all concentrations tested. Maximal induction with ralfinamide

represented a 2.3-fold increase over the vehicle-only condition, and a 1.6-fold increase in activity was seen at equimolarity to the positive control (10 μM). Therefore, magnitudes of induction were low, relative to rifampicin.

Both compounds showed some potential for induction of CYP3A4, with ralfinamide being the stronger inducer of the two drugs. Statistically significant induction of CYP3A4 by safinamide was only seen at the highest concentration, 50 μM, whereas ralfinamide significantly induced CYP3A4 at all concentrations (1, 10, and 50 μM). The estimated safinamide EC50 was greater than or equal to 30 μM, and ralfinamide EC50 ~gr eater than or equal to 10 μM. Compared to the positive control CYP3A4 inducer, rifampicin (10 μM), magnitudes of CYP3A4 induction by the test compounds were relatively low.

Note: The average maximal plasma concentration of Safinamide was < 4μM in Parkinson's disease patients treated with the highest dose of 100 mg/day, the in vitro data determined in this study did not result in considerable induction at these concentrations.

CONCLUSIONS

- Both compounds are potential inducers of CYP3A4, but have a relatively low induction capacity compared to rifampicin (10 μM).

- Safinamide has low in vivo CYP3A4-inducing potential below 50 μM concentration which is more than 10 times therapeutic levels.

Study Title	In vitro Evaluation of Cytochrome P450 1A2 and 2B6, UDP-Glucuronosyl Transferase 1A1 and Sulfotransferase 2A1 Inducibility by (b) (4) 1195686
Study number	DMPK 31-10
Study Period	August 2010
Study Director	Blanchard N, Richert L.
Objective	To characterize the potential of (b) (4) 1195686 (safinamide, MSC2191632B) to induce CYP1A2, CYP2B6, UGT1A1 and SULT2A1 in human hepatocytes

METHODS

Treatment of Hepatocytes for Measuring Induction of CYP/UGT/SULT Activities
After a 24 or 48 h pre-culture period (T0), hepatocytes cultured in 60 mm dishes and 12-well plates were incubated with incubation medium containing (b) (4) 1195686 at different concentrations, positive control inducers or 0.1% DMSO. The induction incubation period was 72 h. The dosing solutions for each treatment were renewed every day with freshly prepared incubation medium.

Enzyme Activities in Cell Microsomes and Cell Cytosol

CYP and UGT activities were determined by incubating microsomes and SULT activities by incubating cytosol with prototypical substrates, according to SOPs in place at KALY-CELL. Substrate stock solutions were prepared in appropriate solvent and subsequently diluted in incubation buffer to obtain final concentrations.

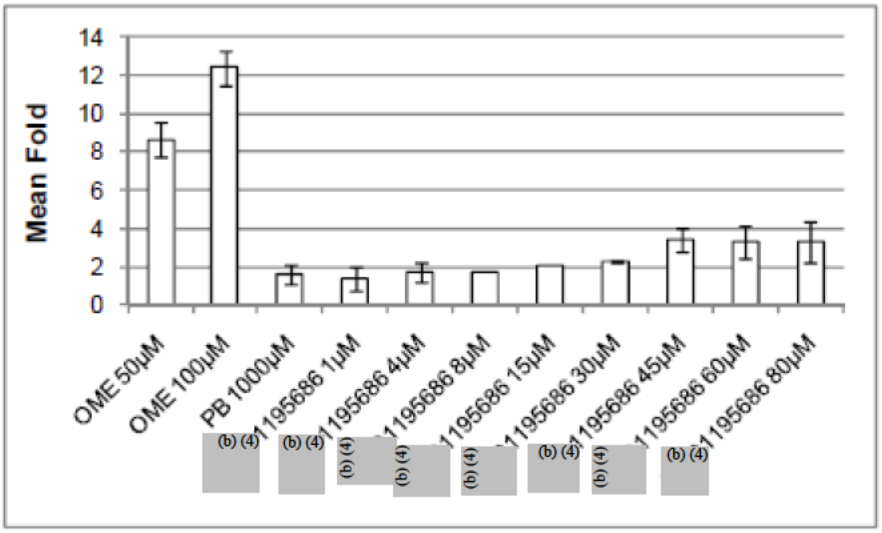
Prototypical Substrates Used for CYP, UGT and SULT Activity Measurements

CYP/UGT/SULT	Substrate	Final substrate concentration (µM)	Reaction
CYP1A2	7-ethoxyresorufin	6.5	Ethoxyresorufin-O-dealkylation
CYP2B6	Bupropion	500	Bupropion hydroxylation
UGT1A1	Estradiol	100	Estradiol 3-*O*-glucuronidation
SULT2A1	Ethinylestradiol	5	Ethinylestradiol 3-*O*-sulfation

Measurement of CYP1A2 and 2B6, UGT1A1 and SULT2A1 mRNA Expression
Total RNA was extracted from hepatocytes by using Trizol reagent according to the manufacturer's instruction. RNA was quantified by spectrophotometry and cDNAs were synthesized from 0.1 to 0.5 µg of total RNA using the iScript kit from Biorad at 42°C for 30 min. A negative control of cDNA synthesis was prepared by omitting reverse transcriptase in one sample for each donor.

RESULTS
(b) (4) 1195686 affected CYP1A2 expression and activity (2/2 donors) as observed in previous studies. More than 1.5-fold activity increase was observed at all concentrations and >20% positive control (PC) activity increase at concentrations ≥45 µM.

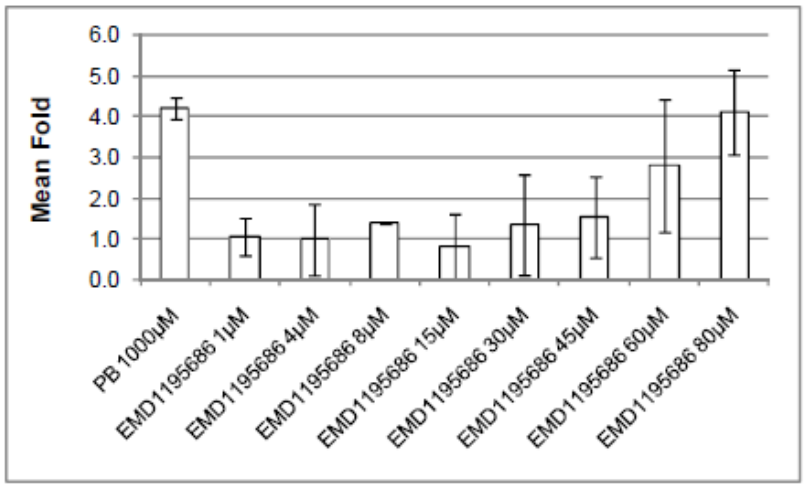
Mean CYP1A2 Fold Activities in Human Hepatocytes from the Two Donors



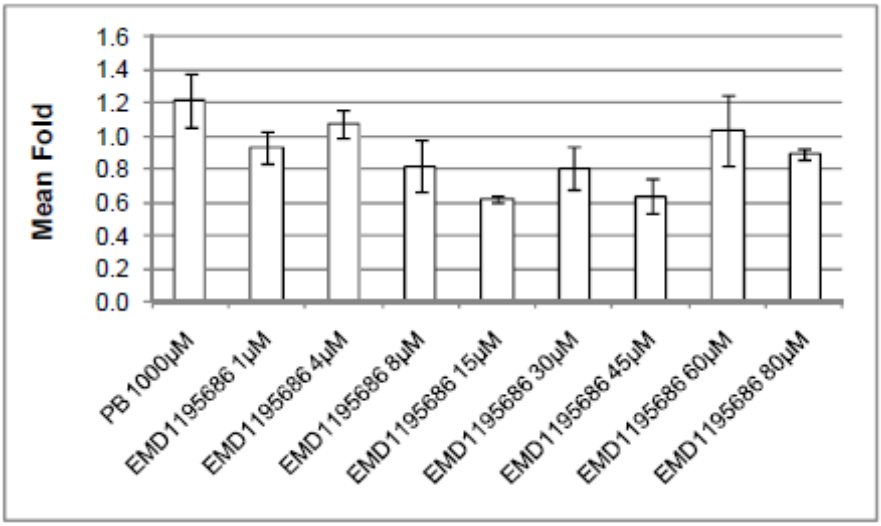
(b) (4) 1195686 increased CYP2B6 activity (2/2 donors), close to or over the FDA threshold of 40% of PC from 30 µM (1/2 donor) and from 60 µM (2/2 donors) and

CYP2B6 mRNA expression. An Emax of 156% and 98% of PC (1000 µM PB) and an EC50 of 58 µM and 66 µM were calculated for donor 1 and donor 2, respectively.

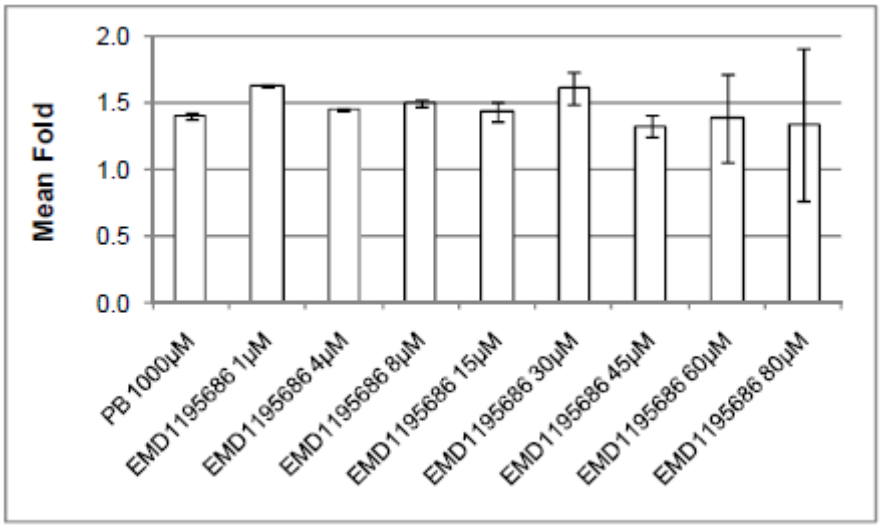
Mean CYP2B6 Fold Activities in Human Hepatocytes from the Two Donors



Mean UGT1A1 Fold Activities in Human Hepatocytes from the Two Donors



Mean SULT2A1 Fold Activities in Human Hepatocytes from the Two Donors



Safinamide treatment overall resulted in slight decrease in UGT1A1 activity and increased SULT2A1 activity by max 1.8-fold at the 30 µM concentration.

Note: The average maximal plasma concentration of Safinamide was < 4µM in Parkinson's disease patients treated with the highest dose of 100 mg/day, the in vitro data determined in this study did not result in considerable induction at these concentrations.

CONCLUSIONS
Safinamide is not a potential inducer of CYP1A2, CYP2B6, UGT1A1 and SULT2A1 in human hepatocytes.

Study Title	Safinamide and its Major Metabolites NW-1153 and NW-1689 Inhibition of P-glycoprotein Investigated in Caco-2/TC7 cells
Study number	DMPK 120-07
Study Period	April 2008
Study Director	F. Krätzer
Objective	To investigate the inhibitory potency of Safinamide and its main metabolites NW-1153 and NW-1689 on P-glycoprotein

METHODS
Caco-2/TC7 cell monolayers were prepared in transwell plates and incubated in a CO_2 containing, water-equilibrated atmosphere at 37°C for 7 to 8 days. Tightness of monolayers was checked by TEER-measurement in each well before and after the experiment. Integrity of the cell model and expression of P-gp was proved by transport experiments with digoxin, which was used as P-gp reference substrate.

To elucidate its inhibitory potential on P-gp in vitro, Safinamide, NW-1153 and NW-1689, respectively, were added to the basal and apical compartment at 0.3 to 1000 µM, and transport rates of [^{3}H]-digoxin were measured. The stability of Safinamide, NW-1153 and NW-1689 in Caco-2 cells during the incubation interval of the experiments was investigated by analyzing samples from the apical and basal receiver chambers after 2 h.

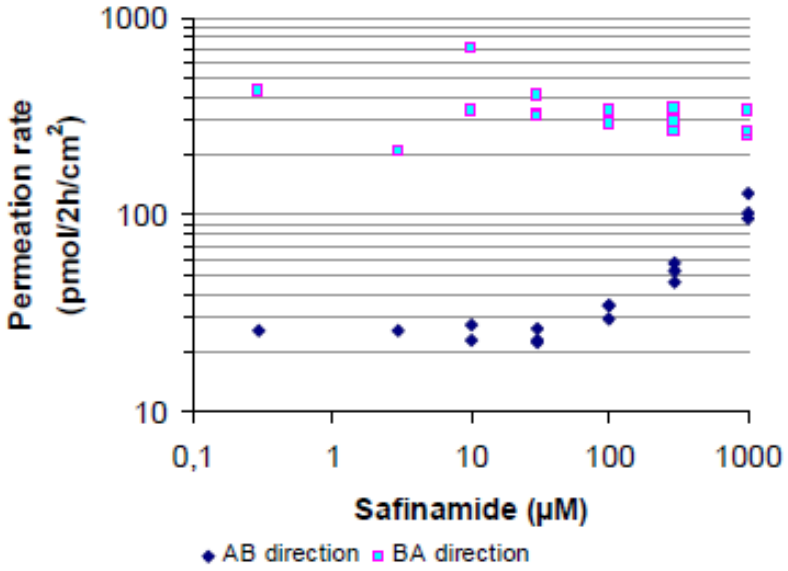
To investigate the influence of Safinamide on tightness of Caco-2 monolayers an additional experiment with mannitol as a marker for stability of the tight-junctions in the presence of Safinamide was carried out.

RESULTS AND DISCUSSION

Safinamide, NW-1689 and NW-1153 were not metabolized to considerable extent during permeation of Caco-2 cells within 2 h.

Safinamide did not change the P-glycoprotein mediated polarized digoxin transport in Caco-2 cell monolayers up to concentrations of 30 µM. At concentrations >100 µM, a gently inclining net transport rate (TRnet) of digoxin was observed. At the highest concentration a reduction in net transport rate of ~45% was measured. This reduction in TRnet was caused by an increase in TR AB rather than by a decrease of TR BA. A significant increase of mannitol permeability in both directions was observed in the presence of 1000 µM Safinamide.
This change in mannitol permeability indicates some influence of Safinamide on the tightness of the Caco-2 monolayers. Consequently, the observed increase in TR AB is due to incremental leakiness of the monolayers and not due to influence on the P-gp mediated digoxin transport.

Permeation Rates of Digoxin in Presence of Safinamide



The metabolites NW-1153 and NW-1689 did not show any influence on [3H]-digoxin transport.

CONCLUSIONS
Safinamide and its main metabolites NW-1153 and NW-1689 are inhibitors of P-glycoprotein transport.

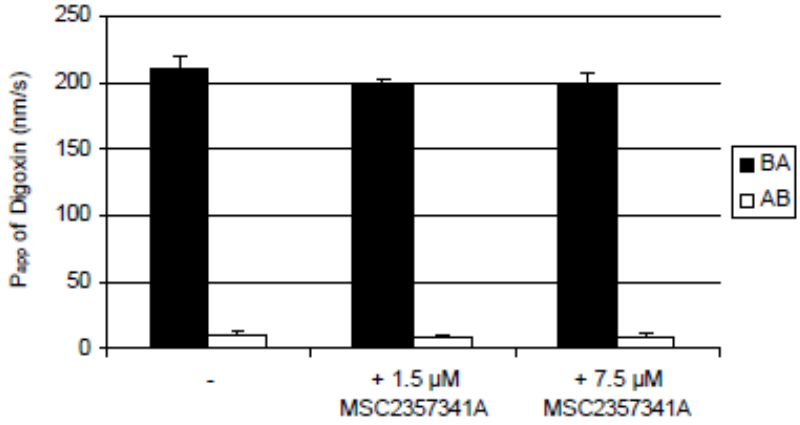
Study Title	P-gp Inhibition in Caco-2 cells
Study number	DMPK 145-11
Study Period	December 2011
Study Director	Tetsuo Yamagata
Objective	To investigate whether MSC2357341A (NW-1689 AG), which is one of the main human metabolites of safinamide, is an inhibitor of P-gp in the in vitro human Caco-2/TC7 cell model.

METHODS
Caco-2 cell monolayers (TC7-clone) were cultured in transwell plates in an 8.5% CO_2 containing, water-equilibrated atmosphere at 37°C for 7 or 8 days. Integrity of the cell model and expression of P-gp were assessed by lucifer yellow (LY) permeation and transport of digoxin, which was used as P-gp reference substrate. To investigate P-gp inhibitory potential, various concentrations of MSC23257341 (NW-1689 AG) or PSC833 (specific P-gp inhibitor) was added to the basal and apical compartment and [^{3}H]-digoxin was added either to the basal or apical side of the monolayer and the appearance of [^{3}H]-digoxin in the other compartment was measured.

RESULTS

NW-1689 AG did not inhibit P-gp-mediated digoxin transport at the test item concentrations of 1.5 and 7.5 µM. Control experiments proved that the Caco-2 cell monolayer was intact and P-gp functioning.

<u>P-gp-mediated Transport of [3H]-Digoxin with and without MSC2357341A</u>



CONCLUSIONS
NW-1689 AG is not a P-gp inhibitor, up to 7.5 µM concentration.

Study Title	BCRP Inhibition in MDCKII-BCRP Cells
Study number	DMPK 103-10
Study Period	January 2011
Study Director	Ulrike Gradhand
Objective	To investigate, in support of the drug-drug interaction package, whether safinamide (MSC2191632B) and/or its human metabolites NW-1689 (MSC2226765A), NW-1689-acylglucuronide (MSC2357341A), NW-1199, NW-1153 (MSC2271526A) exhibit a relevant inhibitory effect on the BCRP- mediated transport of 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the MDCKII-BCRP cell model. BCRP has been proven to be involved in various drug-drug interactions in humans and variable.

METHODS

Parental MDCKII and MDCKII-BCRP monolayers were plated in transwell plates and incubating in an 8.5% CO_2 containing, water-equilibrated atmosphere at 37°C for 7 or 8 days. Integrity of the cell model and expression of BCRP was assessed by lucifer yellow permeation, TEER measurement and transport experiments with PhIP, which was used as BCRP reference substrate. [^{14}C]-PhIP with or without KO134 (specific BCRP inhibitor) was added either to the basal or apical side of the monolayer and its appearance in the other compartment was measured.

To elucidate its inhibitory potential on BCRP in vitro, safinamide or its metabolites were added to the basal and apical compartment at various concentrations and transport rates of [^{14}C]-PhIP in both directions were measured in MDCK-BCRP and/or parental MDCKII cells.

RESULTS

Safinamide reduced BCRP mediated PhIP transport in a concentration-dependent manner with an $IC_{50,app}$ of 43±23 µM. The safinamide metabolite NW-1689 reduced BCRP mediated transport of the reference substrate with an IC50,app of 3.7±0.5 µM.

NW-1689-AG, NW-1153 and NW-1199 showed no inhibitory effect on BCRP mediated transport up to the highest tested concentration of 50 µM. Several control experiments proved that the MDCK-BCRP and control cell monolayers were intact and BCRP was functioning.

CONCLUSIONS

- Safinamide and its human metabolite NW-1689 are BCRP inhibitors with in vitro IC50,app values of approximately 43 and 3.7 µM, respectively.
- Based on the in vitro results NW-1689-AG, NW-1156 and NW-1199 are not expected to influence BCRP mediated transport in vivo.

DISCUSSION
NW-1689 is a major metabolite of safinamide found in plasma at the concentration of 1.4x that of parent compound, safinamide.

The average maximal plasma concentration of Safinamide was < 4μM in Parkinson's disease patients treated with the highest dose of 100 mg/day.

Substrates of BCRP include methotrexate, mitoxantrone, imatinib, irrinotecan, lapatinib, rosuvastatin, sulfasalazine, topotecan.

Sponsor's Position
The International Transporter Consortium, to which the FDA is a contributor, in its recent White Paper recommends that a clinical study to investigate potential drug-drug interactions due to BCRP inhibition should be performed in cases where the quotient $C_{max,ss,u}$ /IC50, app is greater 0.1. Based on this assessment, a clinical drug-drug interaction study would be needed in case $C_{max,u}$ of safinamide was greater than 4.3±2.3 μM or Cmax,ss,u of the metabolite NW-1689 was greater than 0.37±0.05 μM.

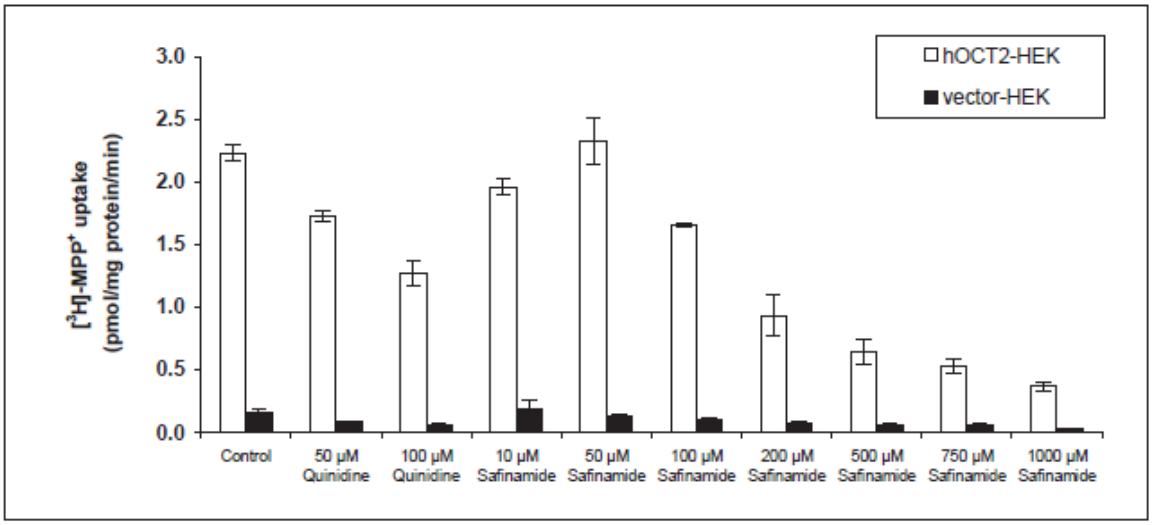
Reviewer's Comment: The sponsor's calculations are based on unbound steady state concentration of safinamide and NW-1689. For NW1689 The R value (**R=1+[I]/IC50) is approximately 2.5. Since R>1.1, evaluation of inhibitory potential of NW-1689 for BCRP in an in vivo study is recommended.**

Study Title	Safinamide-Inhibition of Human Organic Cation Transporter 2(hOCT2) Investigated in hOCT2-Transfected HEK Cells
Study number	DMPK 166-07
Study Period	April 2008
Study Director	Karen Engel
Objective	To determine if Safinamide is an inhibitor of hOCT2 to identify potential drug-drug interactions.

METHODS
Transport assays were performed using human embryonic kidney (HEK) cells stably expressing hOCT2 and vector-transfected control cells. The cells were incubated with the radio-labeled model substrate [^{3}H]-MPP+ and the accumulation in the cells in the absence and presence of Safinamide was quantified by liquid scintillation counting.

RESULTS
Safinamide at 1 μM and 10 μM did not or only marginal inhibit hOCT2-mediated MPP+ transport. At higher concentrations, Safinamide inhibited MPP+ transport concentration dependently. An approximate IC50 value of 130±20 μM was calculated.

Inhibition of MPP+ in hOCT2 transfected HEK Cells



Note: The average maximal plasma concentration of Safinamide was < 4µM in Parkinson's disease patients treated with the highest dose of 100 mg/day.

CONCLUSIONS

The in vitro data determined in this study suggest that hOCT2 is not inhibited by safinamide at clinical relevant plasma concentrations.

Study Title	In vitro Interaction Study of Safinamide and its Metabolites with Human BSEP (ABCB11/sP-gp) ABC (efflux) Transporter
Study number	DMPK 55-09
Study Period	August 2010
Study Director	Mónika Pásztor-Turák, Ph.D.
Objective	To investigate whether safinamide and its metabolites inhibit the human BSEP (ABCB11/sP-gp) ABC (efflux) transporter in vitro at relevant concentrations.

METHODS

Low permeability probe substrates are transported into inside-out membrane vesicles prepared from specific ABC transporter overexpressing cells in an ATP-dependent manner. In the present study possible interactions between human BSEP transporter and safinamide and its metabolites NW-1689, NW-1689-AG, NW-1199 and NW-1153 were investigated in the inhibitory type of vesicular transport assay through the modulation of the probe substrate (taurocholate) transport at two concentrations. The potential of the test articles to modify the uptake of the probe substrate into the human BSEP transporter-containing vesicles was measured. Each experiment was carried out in two independent measurements on two different human BSEP (SB-BSEP-Sf9) membrane batches. Membrane preparations containing beta-galactosidase protein served as a negative control.

RESULTS

No inhibition was observed in case of safinamide, NW-1689-AG, NW-1199 and NW-1153 at the applied concentrations (safinamide and NW-1153: 3 µM and 300 µM; NW-1199 and NW-1689- AG: 1 µM and 100 µM, respectively).

Test compound NW-1689 showed a weak inhibition on the BSEP-mediated taurocholate transport into the vesicles at the investigated concentration of 300 µM, but not at 3 µM. The observed inhibitory effects on the two separate membrane batches at 300 µM were 34% and 43%, respectively.

Compound	Concentration	BSEP mediated TC transport [pmol/mg protein/min] (S.D.)	
		Membrane batch 2 Feb 2009	Membrane batch 9 Apr 2009
Safinamide	3 µM[a]	30.12 (1.32)	28.87 (2.00)
	300 µM	30.60 (2.26)	32.61 (2.45)
NW-1689	3 µM [b]	30.67 (0.50)	31.79 (0.82)
	300 µM	17.77 (1.66)	18.10 (0.93)
NW-1689-AG	1 µM	30.10 (2.44)	32.71 (0.51)
	100 µM	29.48 (1.27)	28.90 (1.03)
NW-1199	1 µM[c]	31.34 (0.76)	32.38 (1.96)
	100 µM	30.38 (1.26)	29.52 (0.63)
DMSO (pH 7.4)	1%	31.18 (0.52)	27.54 (0.31)
Cyclosporine A (pH 7.4)	20 µM	1.87 (1.03)	1.11 (0.37)
NW-1153	3 µM[d]	24.00 (0.91)	28.99 (2.19)
	300 µM	24.47 (0.67)	28.52 (1.47)
MeOH/HCl (pH 7.0)	1%	26.6 (1.08)	23.73 (0.91)
Cyclosporine A (pH 7.0)	20 µM	1.13 (0.79)	1.81 (0.31)
DMSO (pH 7.0)	1%	25.73 (0.70)	25.78 (1.91)
Cyclosporine A (pH 7.0)	20 µM	1.99 (1.11)	1.28 (0.39)

CONCLUSIONS

- Safinamide, NW-1689-AG, NW-1199 and NW-1153 showed no inhibition of BSEP mediated taurocholate transport in vitro at the concentrations investigated.
- NW-1689 showed a weak inhibition of BSEP-mediated taurocholate transport at 300 µM but not at low concentration.

Study Title	In vitro Interaction Study of Safinamide and its Metabolites with Human BSEP (ABCB11/sP-gp) ABC (efflux) Transporter

Study number	DMPK 55-09
Study Period	August 2010
Study Director	Mónika Pásztor-Turák, Ph.D.
Objective	To investigate whether safinamide and its metabolites inhibit the human BSEP (ABCB11/sP-gp) ABC (efflux) transporter in vitro at relevant concentrations.

METHODS

Low permeability probe substrates are transported into inside-out membrane vesicles prepared from specific ABC transporter overexpressing cells in an ATP-dependent manner. In the present study possible interactions between human BSEP transporter and safinamide and its metabolites NW-1689, NW-1689-AG, NW-1199 and NW-1153 were investigated in the inhibitory type of vesicular transport assay through the modulation of the probe substrate (taurocholate) transport at two concentrations. The potential of the test articles to modify the uptake of the probe substrate into the human BSEP transporter-containing vesicles was measured. Each experiment was carried out in two independent measurements on two different human BSEP (SB-BSEP-Sf9) membrane batches. Membrane preparations containing beta-galactosidase protein served as a negative control.

RESULTS

No inhibition was observed in case of safinamide, NW-1689-AG, NW-1199 and NW-1153 at the applied concentrations (safinamide and NW-1153: 3 μM and 300 μM; NW-1199 and NW-1689- AG: 1 μM and 100 μM, respectively).

Test compound NW-1689 showed a weak inhibition on the BSEP-mediated taurocholate transport into the vesicles at the investigated concentration of 300 μM, but not at 3 μM. The observed inhibitory effects on the two separate membrane batches at 300 μM were 34% and 43%, respectively.

Compound	Concentration	BSEP mediated TC transport [pmol/mg protein/min] (S.D.)	
		Membrane batch 2 Feb 2009	Membrane batch 9 Apr 2009
Safinamide	3 µM[a]	30.12 (1.32)	28.87 (2.00)
	300 µM	30.60 (2.26)	32.61 (2.45)
NW-1689	3 µM [b]	30.67 (0.50)	31.79 (0.82)
	300 µM	17.77 (1.66)	18.10 (0.93)
NW-1689-AG	1 µM	30.10 (2.44)	32.71 (0.51)
	100 µM	29.48 (1.27)	28.90 (1.03)
NW-1199	1 µM[c]	31.34 (0.76)	32.38 (1.96)
	100 µM	30.38 (1.26)	29.52 (0.63)
DMSO (pH 7.4)	1%	31.18 (0.52)	27.54 (0.31)
Cyclosporine A (pH 7.4)	20 µM	1.87 (1.03)	1.11 (0.37)
NW-1153	3 µM[d]	24.00 (0.91)	28.99 (2.19)
	300 µM	24.47 (0.67)	28.52 (1.47)
MeOH/HCl (pH 7.0)	1%	26.6 (1.08)	23.73 (0.91)
Cyclosporine A (pH 7.0)	20 µM	1.13 (0.79)	1.81 (0.31)
DMSO (pH 7.0)	1%	25.73 (0.70)	25.78 (1.91)
Cyclosporine A (pH 7.0)	20 µM	1.99 (1.11)	1.28 (0.39)

CONCLUSIONS

- Safinamide, NW-1689-AG, NW-1199 and NW-1153 showed no inhibition of BSEP mediated taurocholate transport in vitro at the concentrations investigated.
- NW-1689 showed a weak inhibition of BSEP-mediated taurocholate transport at 300 µM but not at low concentration.

Study Title	Inhibitory Potential of Safinamide and Four of its Metabolites as Inhibitors on OATP1B1-mediated BSP (sulfobromophthalein) Uptake
Study number	DMPK 84-08
Study Period	April 2009
Study Director	Martin Fromm
Objective	To investigate whether safinamide and / or four metabolites (NW1153, NW1199, NW1689 and NW16891ß-O-acylglucuronide) are inhibitors of OATP1B1-mediated BSP uptake or not.

METHODS

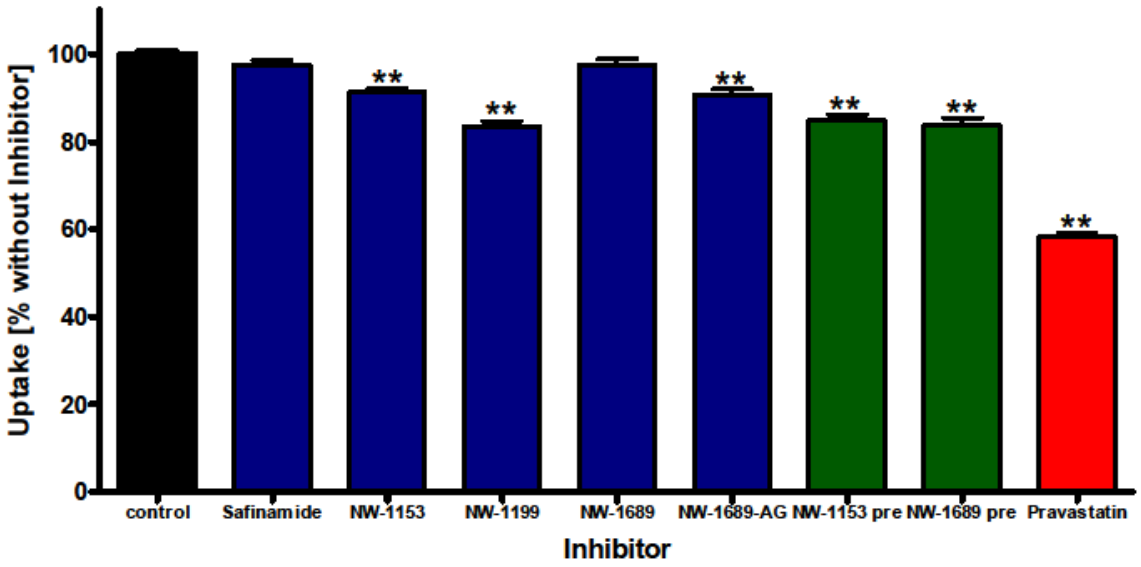
Using stably transfected HEK cells expressing the hepatic uptake transporter OATP1B1, inhibition of OATP1B1-mediated uptake by pravastatin (100 µM) was used in control

experiments. The following concentrations of test items were used which are about 80 or 30 times higher than the expected free or total human plasma concentrations, respectively (safinamide: 50 µM; NW1153: 20 µM; NW1199: 120 µM and NW1689 1ß-Oacylglucuronide: 30 µM). In addition, NW-1153 and NW-1689 were used in preloading experiments.

RESULTS

Safinamide and the metabolite NW-1689 did not inhibit OATP1B1-mediated BSP uptake. Adding NW-1153 into the uptake solution inhibited BSP uptake by approximately 10%. An inhibitory effect has also been observed for NW-1199 (17%) and NW-1689 1ß-Oacylglucuronide (9%). Pravastatin inhibited OATP1B1-mediated BSP uptake by more than 40%. Uptake inhibition was slightly increased when the cells were preincubated for 30 minutes with NW-1153 or NW-1689 before adding labelled BSP (15% and 16%, respectively).

Inhibitory Effect of Safinamide and four of its Metabolites on OATP1B1-Mediated BSP Uptake



CONCLUSIONS

Safinamide and the metabolite NW-1689 did not inhibit OATP1B1-mediated BSP uptake. NW-1153, NW-1199 and NW-1689 1ß-Oacylglucuronide inhibited 10%, 17% and 9% of OATP1B1 respectively; at about 10 to 50 fold higher than clinically relevant plasma concentrations expected in humans.

Study Title	Inhibitory Potential of Safinamide and Four of its Metabolites on OATP1B3- mediated BSP Uptake
Study number	DMPK 142-10

Study Period	February 2011
Study Director	Martin Fromm
Objective	To investigate whether safinamide and / or four metabolites (NW1153, NW1199, NW1689 and NW16891ß-O-acylglucuronide) are substrates or inhibitors of human OATP1B3

METHODS

Using stably transfected human embryonic kidney (HEK) cells expressing the hepatic uptake transporter OATP1B3, it was the goal of this study whether safinamide and / or four metabolites are inhibitors of OATP1B3-mediated sulfobromophthalein (BSP) uptake or not. Inhibition of OATP1B3-mediated uptake by telmisartan was used in control experiments. For safinamide and its metabolites the following concentrations were used:

Safinamide	50 µM
NW-1153	20 µM
NW-1199	120 µM
NW-1689	2 µM
NW-1689 1ß-O-acylglucuronide (NW-1689-AG)	30 µM
Telmisartan	5 µM

RESULTS

Neither safinamide nor its metabolites (NW-1153, NW-NW-1199, NW-1689 and NW-1689-AG) significantly inhibited OATP1B3-mediated BSP uptake. A slight inhibition was detected in the preloading experiments for NW-1153 with a decrease in BSP uptake of 13.2%. Telmisartan (positive control) inhibited OATP1B3-mediated BSP uptake by 82%.

CONCLUSIONS

- Safinamide and its metabolites NW-1689, NW-1153, NW-1199 and NW-1689-AG did not inhibit OATP1B3-mediated BSP-uptake at concentrations being >10 x higher than the plasma concentrations expected in humans.
- Slight inhibition detected for NW-1153.

Study Title	Inhibitory Potential of Safinamide and Four of its Metabolites as Inhibitors on hOAT1, hOAT3 and hOAT4 In Transfected HEK-Cells
Study number	DMPK 53-10
Study Period	March 2011
Study Director	Yohannes Hagos
Objective	To characterize whether safinamide and / or four human metabolites (NW-1153, NW-1199, NW-1689 and acylglucuronide of NW-1689 (NW-1689-AG)) are interacting with hOAT1-mediated paminohippuric acid (PAH) uptake, and with hOAT3- as well as hOAT4-mediated

	estrone sulfate (ES) uptake, respectively, using hOAT-transfected HEK-cell lines.

METHODS
To perform uptake experiments, hOAT-transfected and vector-transfected cells were harvested, plated into 24-well-plates at a density of 2 x 10^5 cells/well and were cultured for 3 days. The uptake of the reference substrate for hOAT1, hOAT3 and hOAT4 was performed at two concentrations (at the Km value and at the 1/10 Km value). To determine the inhibitory effect of safinamide and four metabolites, they were added in one single concentration (Safinamide: 50 µM; NW-1153: 20 µM; NW-1199: 120 µM; NW-1689: 2 µM; NW-1689-AG: 30 µM) to the [^{3}H]-reference substrate. Inhibition of hOAT1-mediated [^{3}H]-PAH uptake by glibenclamide, hOAT3-mediated [3H]-ES uptake by indomethacin and hOAT4-mediated [3H]-ES uptake by sulfobromophthalein (BSP) was performed as control experiments.

RESULTS
Safinamide and four human metabolites (NW-1153, NW-1199, NW-1689 and NW-1689-AG) showed no significant inhibition of hOAT1 and hOAT4 in transfected cells.
In hOAT3-transfected cells, safinamide showed some inhibition on the [^{3}H]-ES uptake of 26±6% (mean±average deviation) at 16 µM ES and 39±2% at 1.6 µM ES. At 1.6 µM ES only the metabolite NW-1689-AG showed inhibition (29±1%) in hOAT3-transfected cells, whereas the experimental set at 16 µM ES showed no effect.

The metabolite NW-1689-AG (30 µM) exhibited ~30% inhibition of hOAT3 only at 1/10 Km of ES, while no inhibition was observed at Km. Free plasma concentration of NW-1689-AG is <3 µM.

The other metabolites NW-1153 (20 µM), NW-1199 (120 µM), and NW-1689 (2 µM) did not show any relevant inhibition in the in vitro test.

CONCLUSIONS
OAT1 and OAT4 mediated PAH uptake was not inhibited by safinamide and its four metabolites NW-1153, NW-1199, NW-1689, NW-1689-AG.

OAT3 mediated ES uptake was inhibited by safinamide at 50 µM by amaximum of ~40%.

Study Title	Inhibitory Potential of Safinamide and Four of its Metabolites as Inhibitors on hOAT1, hOAT3 and hOAT4 In Transfected HEK-Cells
Study number	DMPK 53-10
Study Period	March 2011
Study Director	Yohannes Hagos
Objective	To characterize whether safinamide and / or four human metabolites (NW-1153, NW-1199, NW-1689 and acylglucuronide of NW-1689

	(NW-1689-AG)) are interacting with hOAT1-mediated paminohippuric acid (PAH) uptake, and with hOAT3- as well as hOAT4-mediated estrone sulfate (ES) uptake, respectively, using hOAT-transfected HEK-cell lines.

METHODS
To perform uptake experiments, hOAT-transfected and vector-transfected cells were harvested, plated into 24-well-plates at a density of 2 x 10^5 cells/well and were cultured for 3 days. The uptake of the reference substrate for hOAT1, hOAT3 and hOAT4 was performed at two concentrations (at the Km value and at the 1/10 Km value). To determine the inhibitory effect of safinamide and four metabolites, they were added in one single concentration (Safinamide: 50 µM; NW-1153: 20 µM; NW-1199: 120 µM; NW-1689: 2 µM; NW-1689-AG: 30 µM) to the [^{3}H]-reference substrate. Inhibition of hOAT1-mediated [^{3}H]-PAH uptake by glibenclamide, hOAT3-mediated [3H]-ES uptake by indomethacin and hOAT4-mediated [3H]-ES uptake by sulfobromophthalein (BSP) was performed as control experiments.

RESULTS
Safinamide and four human metabolites (NW-1153, NW-1199, NW-1689 and NW-1689-AG) showed no significant inhibition of hOAT1 and hOAT4 in transfected cells.
In hOAT3-transfected cells, safinamide showed some inhibition on the [^{3}H]-ES uptake of 26±6% (mean ± average deviation) at 16 µM ES and 39±2% at 1.6 µM ES. At 1.6 µM ES only the metabolite NW-1689-AG showed inhibition (29±1%) in hOAT3-transfected cells, whereas the experimental set at 16 µM ES showed no effect.

The metabolite NW-1689-AG (30 µM) exhibited ~30% inhibition of hOAT3 only at 1/10 Km of ES, while no inhibition was observed at Km. Free plasma concentration of NW-1689-AG is <3 µM.

The other metabolites NW-1153 (20 µM), NW-1199 (120 µM), and NW-1689 (2 µM) did not show any relevant inhibition in the in vitro test.

CONCLUSIONS
OAT1 and OAT4 mediated PAH uptake was not inhibited by safinamide and its four metabolites NW-1153, NW-1199, NW-1689, NW-1689-AG.

OAT3 mediated ES uptake was inhibited by safinamide at 50 µM by a maximum of ~40%.

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 Are the proposed labeling statements regarding the influence of age, gender and race on pharmacokinetics of safinamide acceptable?

Yes, with minor modifications.

The influence of age, gender and race on pharmacokinetics of safinamide, from three phase 3 studies: 016, 27918 (MOTION) and 27919 (SETTLE), was quantified using population pharmacokinetic analyses.

Figure 1, Figure 2 and Figure 3 show that the mean steady state concentration-time profile of safinamide (using observed data from three phase 3 studies) are not influenced by age, gender and race to the extent that would warrant dose adjustments. Time after dose was calculated and average concentrations, normalized to 100 mg dose and 70 kg adult, were derived by time bins.

Figure 1. Mean Safinamide Concentration-Time Profile by Age.

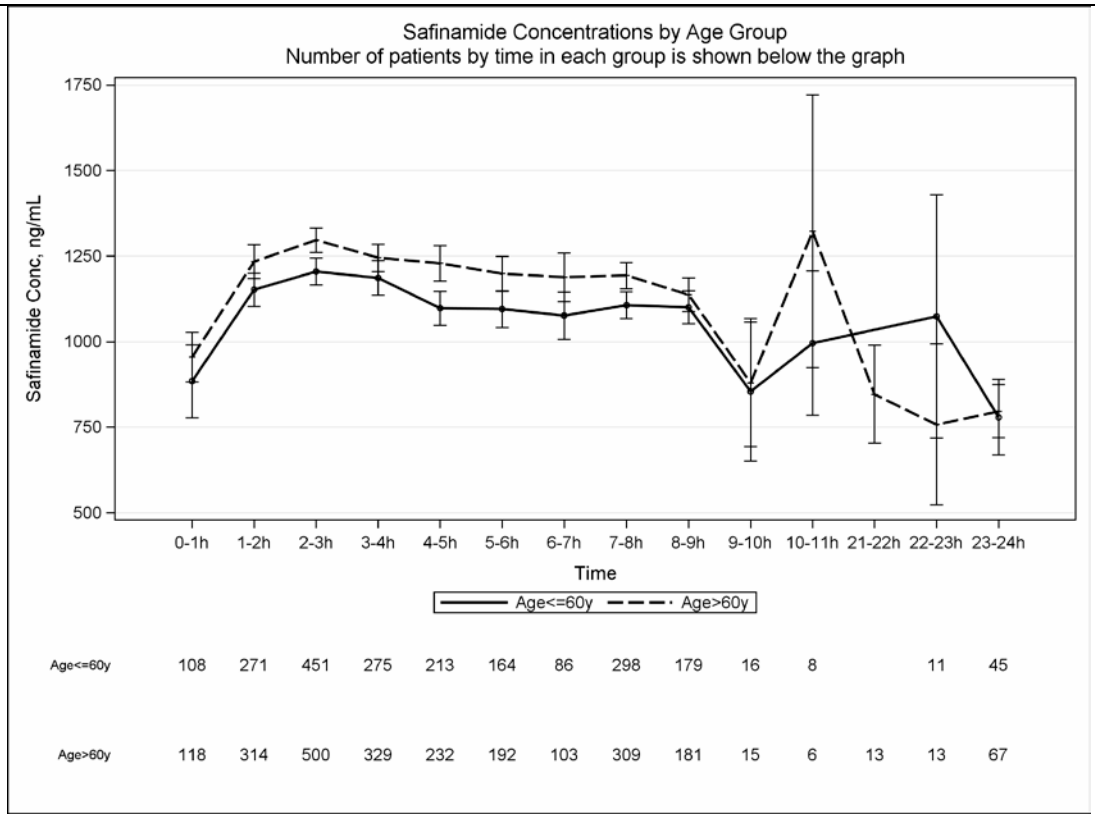


Figure 2. Mean Safinamide Concentration-Time Profile by Gender.

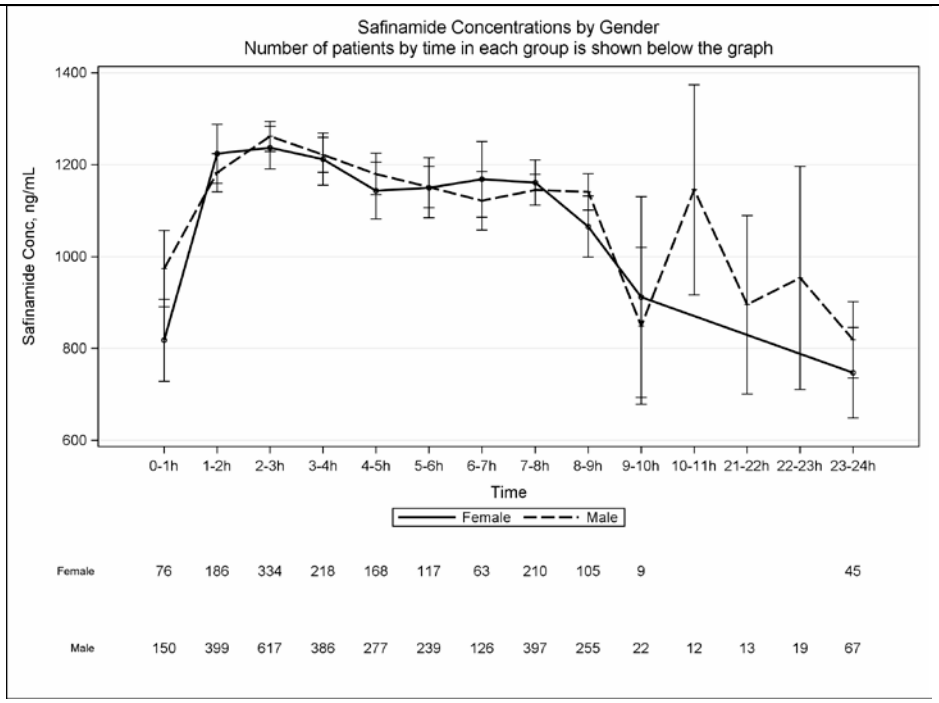
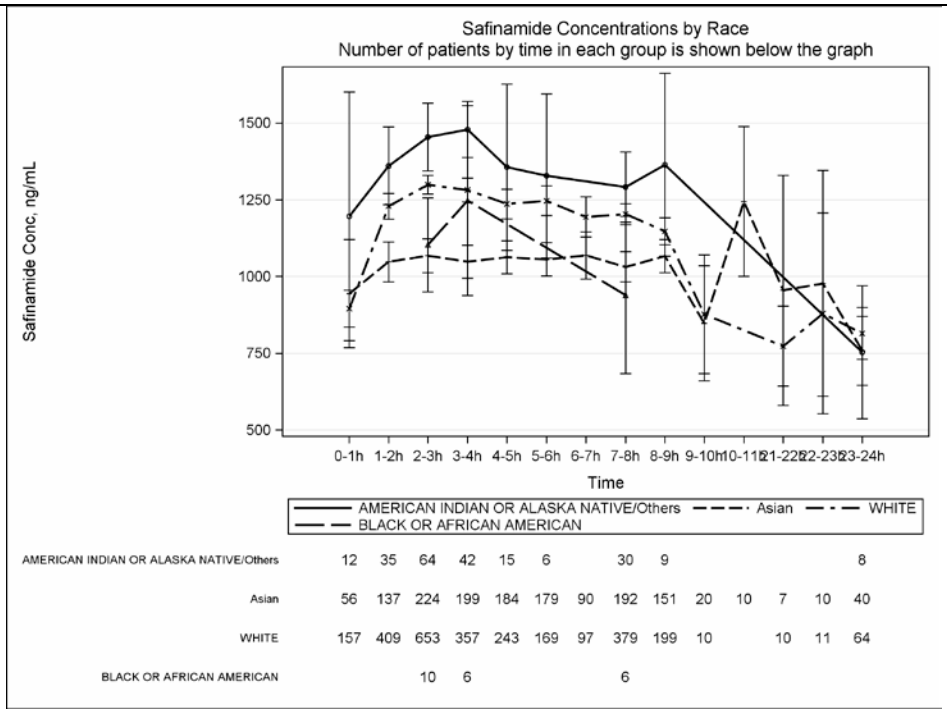


Figure 3. Mean Safinamide Concentration-Time Profile by Race.



1.1.2 Population pharmacokinetic analyses show that the interaction between safinamide and proton pump inhibitors/other acid regulators is unlikely. Are these findings acceptable?

Although the population pharmacokinetic analysis does not include information on dose and timing of PPI intake relative to safinamide, the various evidences (See **Analyses for Identifying Influence of Proton Pump Inhibitors/Other Acid Regulators on Pharmacokinetics of Safinamide**) suggest that the interaction potential is low.

1.2 Recommendations

The Division of Pharmacometrics has reviewed the population pharmacokinetic analyses conducted by the sponsor. Minor changes are suggested for the proposed label.

1.3 Label Statements

Labeling statements to be removed are shown in ~~red strikethrough font~~ and suggested labeling to be included is shown in underline blue font.

12.3 Pharmacokinetics

Pharmacokinetics in Special Populations:

(b) (4)

The pharmacokinetics of XADAGO are not influenced by gender. (b) (4)

(b) (4)

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2 PERTINENT REGULATORY BACKGROUND

Newron Pharmaceuticals is seeking approval for safinamide. The proposed indication is for the treatment of patients with idiopathic Parkinson's disease (PD) as add-on therapy to:

- A single DA-agonist at a stable dose in early-stage, (b) (4) patients, and
- (b) (4) L-dopa alone or in combination with other PD medications in mid-to-late stage (b) (4) patients.

Labeling statements describing the influence of age, gender and race on pharmacokinetics of safinamide are based on population pharmacokinetic analyses.
The population pharmacokinetic (PK) analysis describes the PK of safinamide following administration of safinamide in Parkinson's disease patients in three double-blind, placebo-controlled, parallel-group, randomized, multi-center, multi-national 24-week Phase 3 studies: 016, 27918 (MOTION) and 27919 (SETTLE).

Study 016 was a Phase III, double-blind, placebo-controlled study to determine the efficacy and safety of a low (50 mg/day) and high (100 mg/day) dose of safinamide, add-on therapy, in patients with idiopathic Parkinson's Disease (IPD) with motor fluctuations, treated with a stable dose of levodopa and who may be receiving concomitant treatment with stable doses of a dopamine agonist, and/or an anticholinergic. A total of 446 patients contributed 1620 plasma safinamide concentrations and a total of 668 (including placebo) patients contributed a total of 3603 On Time values.

Study 27918 (MOTION) was a phase III, double-blind, placebo-controlled randomized trial to determine the efficacy and safety of a low (50 mg/day) and high (100 mg/day) dose of safinamide, as add-on therapy, in subjects with early IPD treated with a stable dose of a single dopamine agonist. A total of 452 patients contributed 2913 plasma safinamide concentrations.

Study 27919 (SETTLE) was a phase III, double-blind, placebo-controlled, randomized trial to determine the efficacy and safety of a dose range of 50 to 100 mg/day of safinamide, as add-on therapy, in subjects with IPD with motor fluctuations, treated with a stable dose of levodopa and who may be receiving concomitant treatment with stable doses of a dopamine agonist, an anticholinergic and/or amantadine. A total of 273 patients contributed 1754 plasma safinamide concentrations and a total of 547 (including placebo) patients contributed a total of 3618 On Time values.

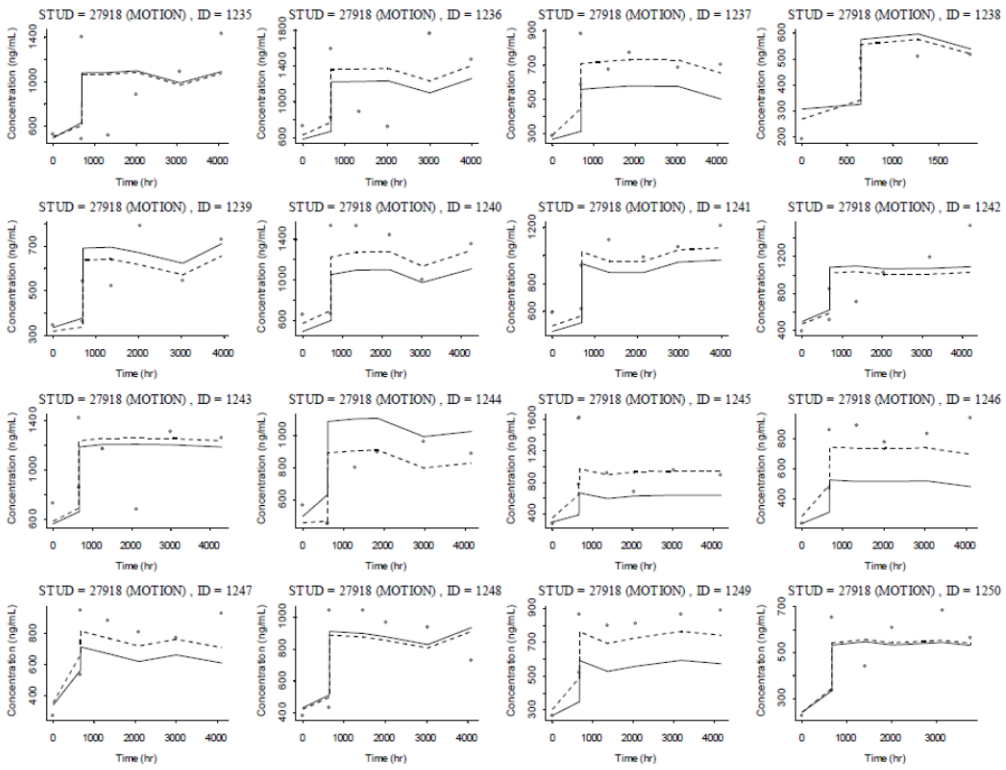
3 RESULTS OF SPONSOR'S ANALYSIS

3.1 Analyses for Identifying Influence of Age, Gender, Race on Pharmacokinetics of Safinamide

Figure 1, Figure 2 and Figure 3 show the representative observed safinamide concentrations along with population and individual level predicted safinamide

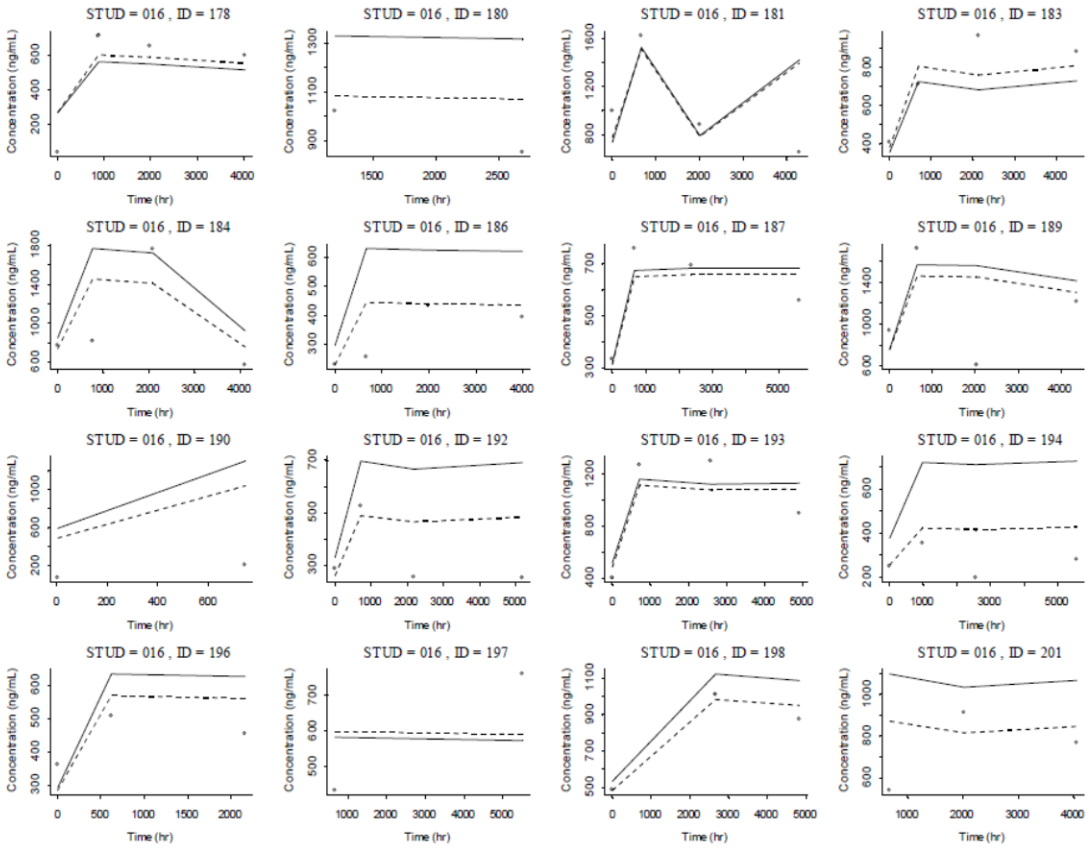
concentrations based on estimated parameters in the three studies. Safinamide concentrations collected in the study show considerable variability.

Figure 4. Representative Observed Population and Individual Predicted Safinamide Concentrations versus Time after Dose Profiles in Study 27918 (MOTION). Open Circles indicate Observed Plasma Concentration data; Solid Lines indicate Population Predictions; Dashed Lines indicate Individual Predictions.



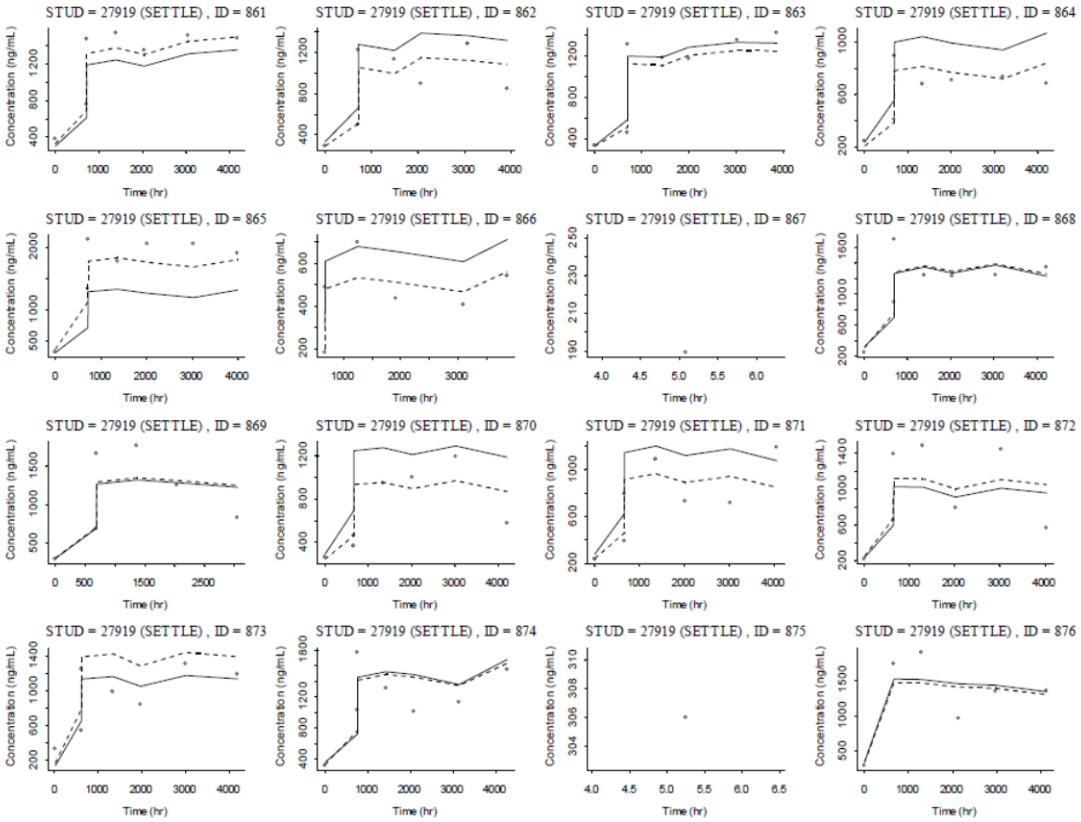
Source : Figure 12-13 on page 98 in safinamide-pop-pk-pd.pdf

Figure 5. Representative Observed Population and Individual Predicted Safinamide Concentrations versus Time after Dose Profiles in Study 016. Open Circles indicate Observed Plasma Concentration data; Solid Lines indicate Population Predictions; Dashed Lines indicate Individual Predictions.



Source : Figure 12-13 on page 96 in safinamide-pop-pk-pd.pdf

Figure 6. Representative Observed Population and Individual Predicted Safinamide Concentrations versus Time after Dose Profiles in Study 27919 (SETTLE). Open Circles indicate Observed Plasma Concentration data; Solid Lines indicate Population Predictions; Dashed Lines indicate Individual Predictions.



Source : Figure 12-13 on page 100 in safinamide-pop-pk-pd.pdf

Table 1 shows the estimates of pharmacokinetic parameters based on the final model. Safinamide CL/F and Vd/F (95% CI) were estimated to be 4.54 (4.43, 4.65) L/hr and 168 (164, 172) L, respectively, and were estimated with good precision (CL/F; RSE=1.22%, Vd/F; RSE=1.17%). KA (95% CI) was estimated to be 1.19 hr-1 (1.00, 1.38) and was also estimated with good precision (RSE=8.03%). The shrinkage of individual random effects was estimated as 13% for CL/F and 31% for Vd/F. The residual error shrinkage was estimated at 8.6%.

Table 1. NONMEM Population Pharmacokinetic Parameter Estimates–for the Final Model (Model 136).

Parameter [Units]	NONMEM Estimates			
	Point Estimate	%RSE	95% CI	
CL/F [L/hr]	4.54	1.22	4.43-4.65	
Vd/F [L]	168	1.17	164-172	
KA [hr^{-1}]	1.19	8.03	1.00-1.38	
CL/F (WGT)	0.75 FIX	NA	NA	
Vd/F (WGT)	1 FIX	NA	NA	
CL/F~AGE	-0.199	24.0	-0.293- -0.105	
F~Whites	1.04	1.40	1.01-1.07	
F~Others	1.16	2.52	1.10-1.22	
Inter-individual variability				**CV%**
ω^2_{CL}	0.0506	7.09	0.0436-0.0576	CV= 22.5%
Covar ω_{CL}, ω_{Vd}	0.0232	17.8	0.0151-0.0313	R=0.569
ω^2_{Vd}	0.0329	24.0	0.0174-0.0484	CV= 18.1
Residual variability				
σ^2_{prop}	0.0648	3.58	0.0603-0.0693	CV= 25.5%

Abbreviations: %RSE: percent relative standard error of the estimate = SE/parameter estimate * 100, CL/F = apparent clearance, Vd/F = apparent volume of distribution, KA = absorption rate constant, F (Whites)=relative bioavailability of Whites, F (Others)=relative bioavailability of Others, σ^2prop = proportional residual error model. The reference population for PK parameters CL/F and Vd/F is a 70 kg patient.

Source : Table 11-15 on page 61 in safinamide-pop-pk-pd.pdf

Plasma concentrations of safinamide were adequately described by a linear one-compartment population PK model with first-order absorption and elimination. The Sponsor concluded the following:

- For a typical 70-kg, 60-year old Asian patient, the estimated mean (95% CI) parameter values were: 4.54 (4.43, 4.65) L/hr for apparent oral clearance (CL/F), 168 (164,172) L for apparent volume of distribution (Vd/F), and 1.19 (1.00, 1.38) hr-1 for absorption rate constant (KA).
- Safinamide CL/F and Vd/F increased allometrically with body weight. For the range of weights in the analysis (35 to 134 kg), CL/F ranged from 41% lower to 63% higher, and Vd/F ranged from 50% lower to 91% higher compared to a 70-kg individual.
- Safinamide CL/F decreased with increasing age. Over the age range of 30 to 80 years, there was an 18% decrease, which is not considered to be clinically relevant.
- Safinamide bioavailability was, on average, 4% and 16% higher in Whites and Others compared to Asian patients, which is not considered to be clinically relevant.
- Gender, renal function and exposure to levodopa did not influence the PK of safinamide, suggesting that dose adjustment in mild to moderate renally impaired patients will not be required.

The findings from population pharmacokinetic analyses were translated into a forest plot (Figure 7).

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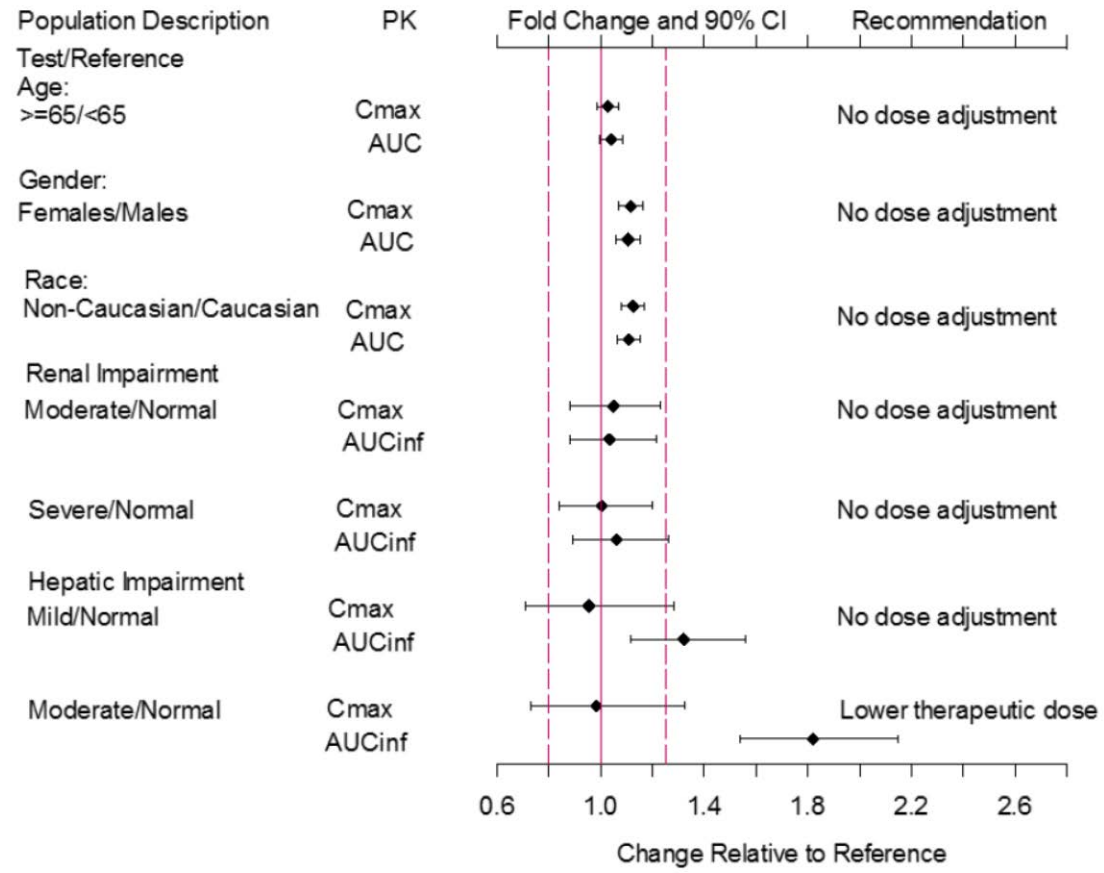
Figure 7. Impact of Other Factors on Safinamide Pharmacokinetics



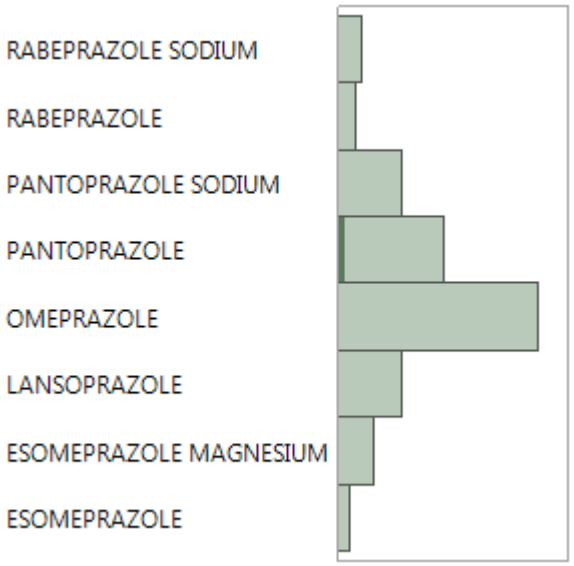
Source : Figure 3 on page 15 in draft-annot-labeling-text.pdf

Reviewer's Comments: The analysis methodology is reasonable. It is not clear why in some patients, the model does not explain the observed concentrations adequately (See Figure 5*).*

3.2 Analyses for Identifying Influence of Proton Pump Inhibitors/Other Acid Regulators on Pharmacokinetics of Safinamide

Out of a total of 1165 patients, only 98 patients had taken a PPI (Pantoprazole, Omeprazole, Rabeprazole, Lansoprazole, Esomeprazole Magnesium) at some point of time during the pharmacokinetic sampling period. The absence and presence of PPI at the corresponding dosing and PK sampling time was set to 0 (absent) and 1 (present), respectively, in the dataset. Figure 8 below shows the PPI's taken by patients and their corresponding dose.

Figure 8. (Left) PPI's Taken by Patients (Right) PPI's and their Corresponding Dose



Frequencies

Level	Count	Prob
ESOMEPRAZOLE	2	0.02247
ESOMEPRAZOLE MAGNESIUM	6	0.06742
LANSOPRAZOLE	11	0.12360
OMEPRAZOLE	34	0.38202
PANTOPRAZOLE	18	0.20225
PANTOPRAZOLE SODIUM	11	0.12360
RABEPRAZOLE	3	0.03371
RABEPRAZOLE SODIUM	4	0.04494
Total	89	1.00000

N Missing 0

8 Levels

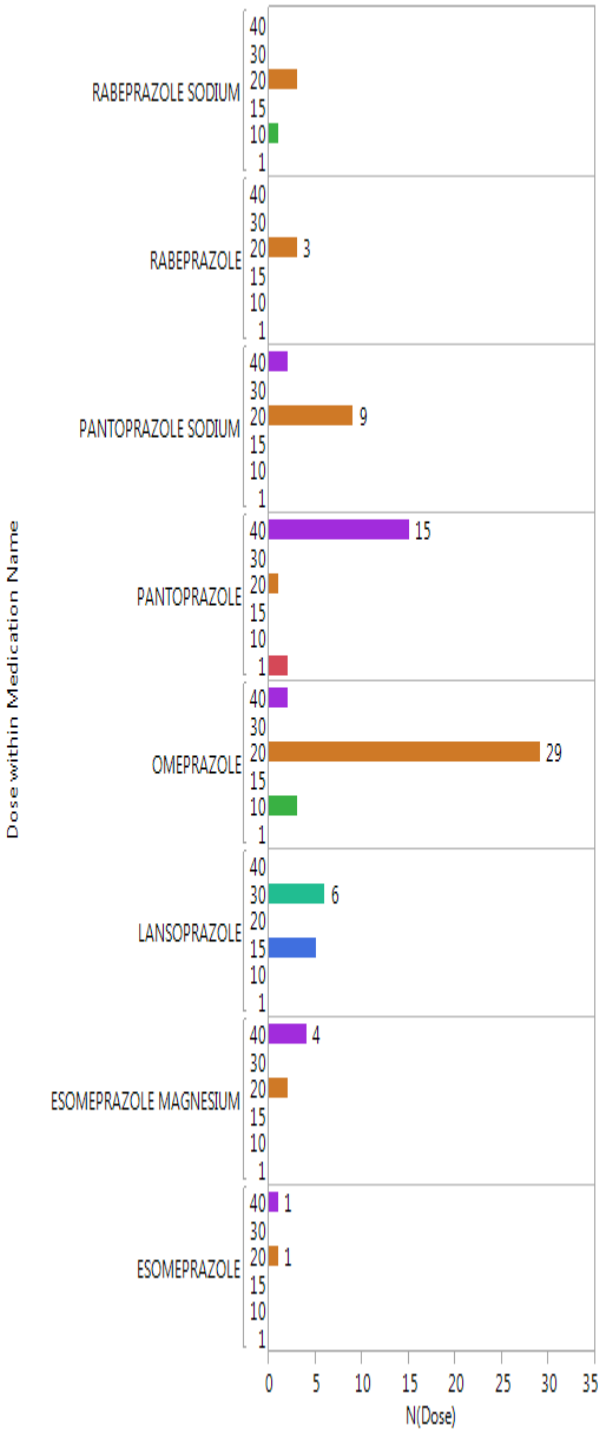
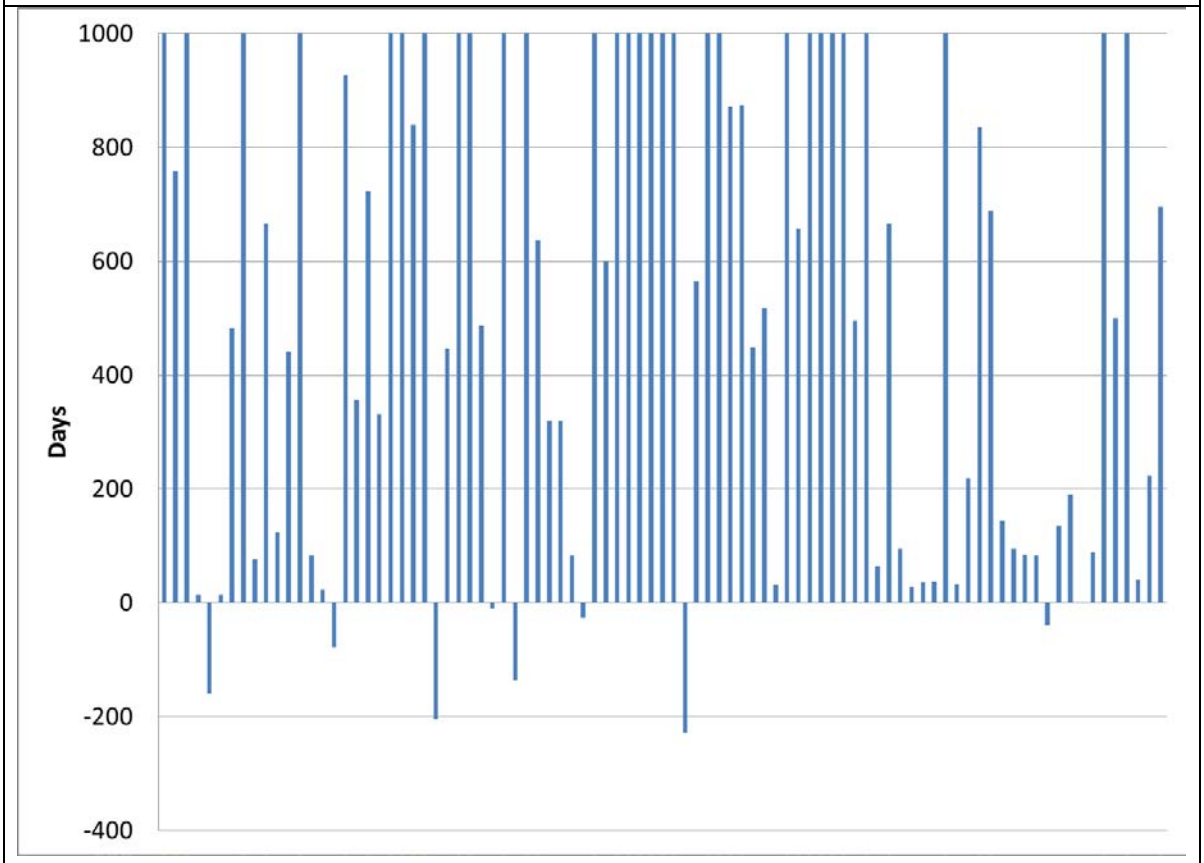


Figure 9 shows the number of days patients were taking PPI prior to or after initiation of safinamide treatment. These values were derived using conmed start date and safinamide start date. Some patients took PPI after the start of safinamide treatment.

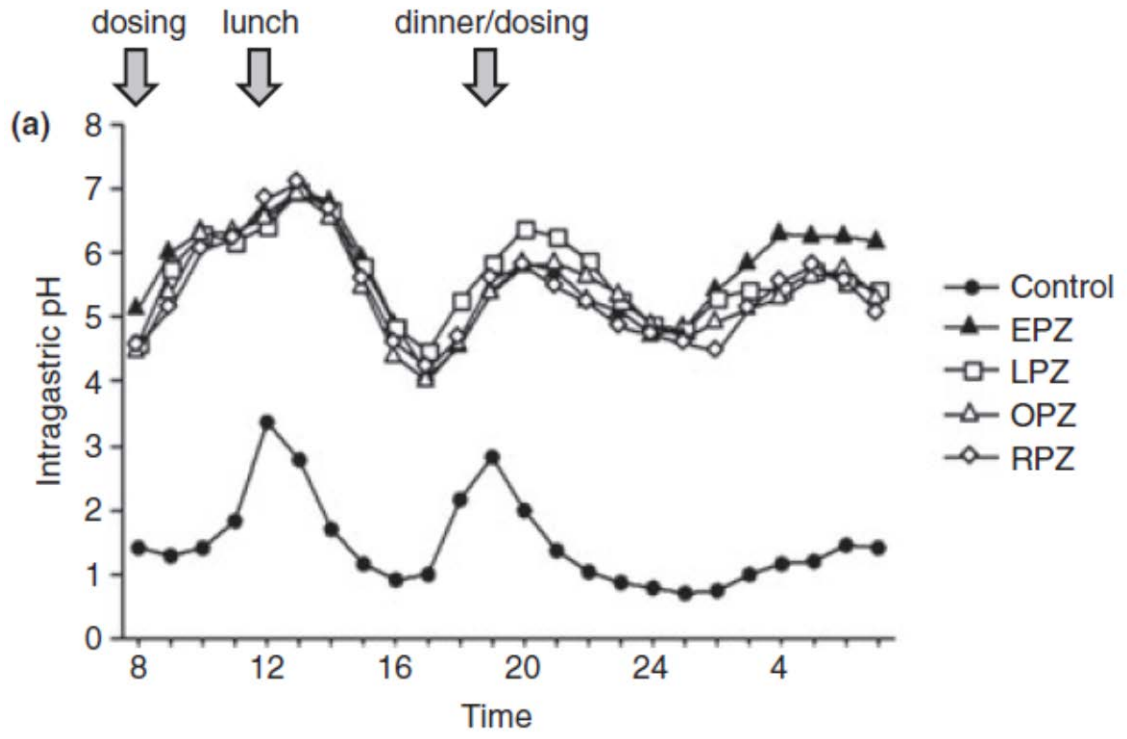
Days = Conmed start date – Safinamide Start Date

Figure 9. Number of Days Patients Were Taking PPI Prior to (Positive Days Values on Y axis) and After Starting (Negative days Values on Y axis) Treatment With Safinamide.



Literature (Sahara et al, Aliment Pharmacol Ther 2013; 38: 1129–1137) suggests that gastric pH changes reach steady state after 7 days of treatment with PPI twice daily (Figure 10). This would suggest that gastric pH in patients enrolled in safinamide clinical studies would be at steady state, if compliance is not an issue. Safinamide is highly soluble at pH 1.2 and 4.5, but low soluble at pH 6.8 and 7.5 for a dosage strength of 100 mg since the solubility is less than 0.4 mg per mL. If an assumption can be made that changes in pH, as shown in Figure 10, are representative in PD patients taking PPI's it can be expected that even if safinamide is taken with PPI it would not affect plasma levels of safinamide due to maximum pH changes occurring 4-5h after PPI administration. These observations suggest that the interaction potential of safinamide with PPI is low.

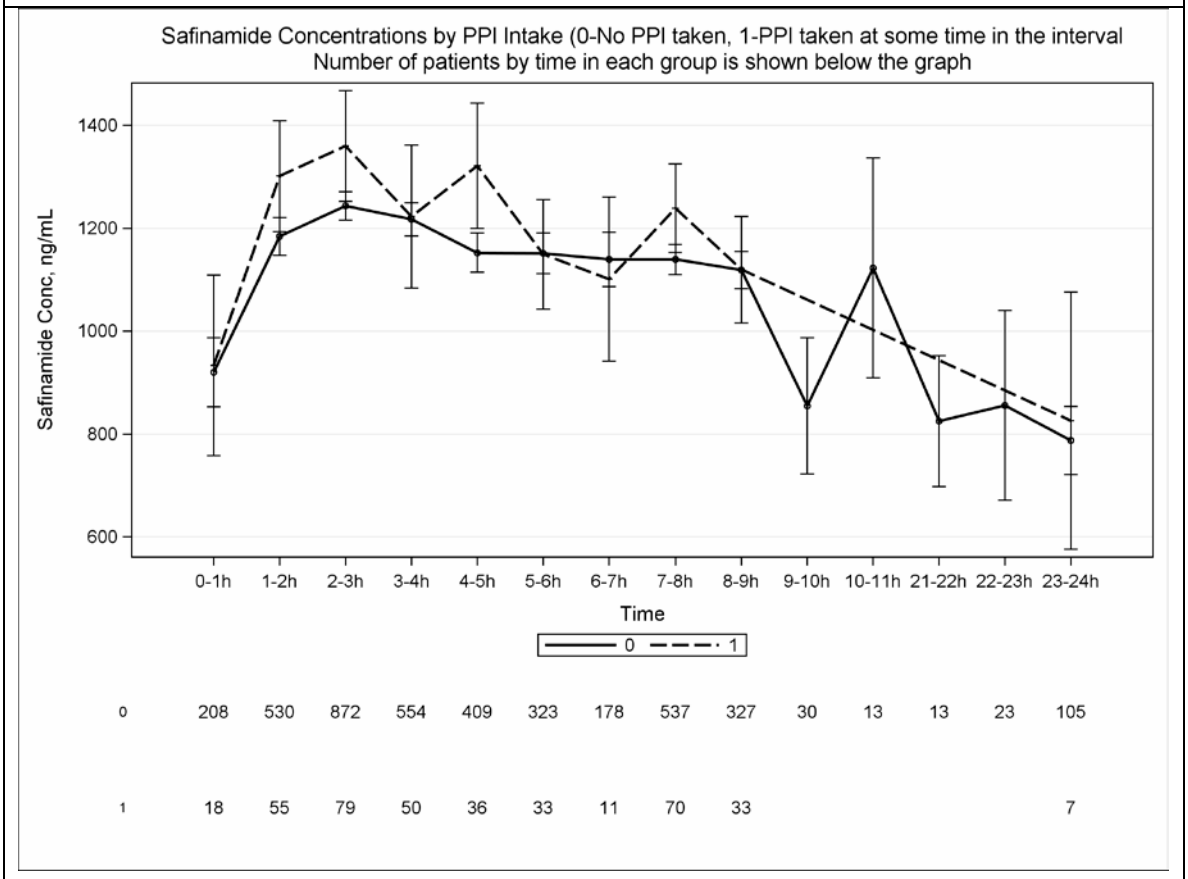
Figure 10. Median 24-h intragastric pH–time curves for omeprazole (OPZ), lansoprazole (LPZ), rabeprazole (RPZ) and esomeprazole (EPZ) (a) their median intragastric pH values



Source: Figure 1 from Twice-daily dosing of esomeprazole effectively inhibits acid secretion in CYP2C19 rapid metabolisers compared with twice-daily omeprazole, rabeprazole or lansoprazole; Sahara et al, Aliment Pharmacol Ther 2013; 38: 1129–1137

Figure 10 shows the mean steady state concentration-time profile in patients (Studies NW-1015/016/III/2006, 27918 (MOTION), 27919 (SETTLE)) taking PPI or not taking PPI. Time after dose was calculated and mean concentrations were derived by time bins. The data suggests no influence of PPI on pharmacokinetics of safinamide.

Figure 11. Mean Safinamide Concentration-Time Profile in Patients Taking PPI (0) and Not Taking PPI (1).



A summary of individual steady-state Cmax and AUC of safinamide derived following administration of 100 mg dose of safinamide in a typical 60 year old weighing 70 kg Asian and a White subject are presented in Table 1. This is based on the population pharmacokinetic model developed by the Sponsor to evaluate interaction potential between safinamide and PPI.

Table 2. (Top) Summary of Individual Steady-State Cmax (ng/mL) and AUC (ng*hr/mL) of Safinamide following 100 mg dose (in a typical Asian subject); (Bottom)Summary of Individual Steady-State Cmax (ng/mL) and AUC (ng*hr/mL) of Safinamide following 100 mg dose (in a typical White subject)

Dose	100 mg			
	PPI Absent	PPI Present	PPI Absent	PPI Present
Statistics	C_{max} (ng/mL)	C_{max} (ng/mL)	AUC_τ (ng*hr/mL)	AUC_τ (ng*hr/mL)
N	1000	1000	1000	1000
Mean	1645	1638	22228	22497
SD	385	370	4995	5066
Minimum	714	839	9204	10506
Median	1604	1595	21930	21721
Maximum	3341	3024	43234	39507

F value from a Typical Asian subject was used to calculate AUC_τ

Dose	100 mg			
	PPI Absent	PPI Present	PPI Absent	PPI Present
Statistics	C_{max} (ng/mL)	C_{max} (ng/mL)	AUC_τ (ng*hr/mL)	AUC_τ (ng*hr/mL)
N	1000	1000	1000	1000
Mean	1708	1703	23075	23395
SD	400	385	5185	5268
Minimum	741	872	9555	10925
Median	1665	1659	22766	22587
Maximum	3468	3145	44881	41083

F value from a Typical White subject was used to calculate AUC_τ

Reviewer's Comments: Although the population pharmacokinetic analysis does not include information on dose and timing of PPI intake relative to safinamide, the various evidences suggest that the interaction potential is low.

4 REVIEWER'S ANALYSIS

4.1 Introduction

To understand the impact of various covariates on pharmacokinetics of safinamide, the reviewer created graphs that show the mean and 95% CI of safinamide plasma concentration data. Time after dose was calculated and average concentrations, normalized to 100 mg dose and 70 kg adult, were derived by time bins. The findings are discussed in Section 1.1.1.

4.1.1 Data Sets

Data sets used are summarized in Table 2.

Table 3. Analysis Data Sets

Study Number	Name	Link to EDR
Safinamide-pop-pk-pd.pdf report	Pk.xpt	\\cdsesub1\evsprod\NDA207145\0000\m5\datasets\pop-pk-pd\tabulations\legacy

5 LISTING OF ANALYSES CODES AND OUTPUT FILES

File Name	Description	Location in \\cdsnas\pharmacometrics\
136.nm7	NONMEM output folder	\\cdsnas\PHARMACOMETRICS\Reviews\Ongoing PM Reviews\Safinamide_NDA207145_VAB\PPK Analyses\Final Model
136.ctl	NONMEM control stream	\\cdsnas\PHARMACOMETRICS\Reviews\Ongoing PM Reviews\Safinamide_NDA207145_VAB\PPK Analyses\Final Model
PKConc.sas	SAS code for creating graphs	\\cdsnas\PHARMACOMETRICS\Reviews\Ongoing PM Reviews\Safinamide_NDA207145_VAB\PPK Analyses\Final Model

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HRISTINA DIMOVA
12/03/2015

JAGAN MOHAN R PAREPALLY
12/03/2015

VENKATESH A BHATTARAM
12/03/2015

KEVIN M KRUDYS
12/03/2015

YUXIN MEN
12/03/2015

MEHUL U MEHTA
12/04/2015