

**CENTER FOR DRUG EVALUATION AND  
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*APPLICATION NUMBER:*

**22-275**

**CLINICAL PHARMACOLOGY AND  
BIOPHARMACEUTICS REVIEW(S)**

## CLINICAL PHARMACOLOGY REVIEW

### Division of Clinical Pharmacology I

NDA 22275

Submission Date: October 22, 2007

Type: NDA, Priority: Standard

Brand Name: Samska

Generic Name: Tolvaptan

Dosage Strength: 15 mg, 30 mg tablets

Sponsor: Otsuka America Pharmaceuticals, Inc.

Indications: Short Term Treatment of Signs and Symptoms of Worsening Heart Failure  
Treatment of Euvolemic and Hypervolemic Hyponatremia and Prevention of  
Hyponatremia

PDUFA Date: August 22, 2008

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## 1. EXECUTIVE SUMMARY

### Characteristics of Submission

The submission contained 50 Clinical Pharmacology study reports. Nine (9) reports related to in vitro studies with human biomaterials and 41 reports reported the results of studies in volunteers and patients. The in vivo studies used different service formulations and were performed in the US, UK and Japan. Included in the submission were also 18 assay reports. In addition, the submission contained a POPPK report that used rich and sparse sampling information from 8 studies in patients with hyponatremia of any origin and CHF with or without hyponatremia. Reviewed were 22 of the 41 in vivo Clinical Pharmacology reports, all 9 reports on human biomaterials and 18 reports on assays. The non-reviewed 19 Clinical Pharmacology reports reported results from discontinued formulations and indications or from small studies conducted in Japan, for which partly only synopses existed and that had used assays that were not cross-validated with those applied in trials conducted in the US and UK.

### Indication for Tolvaptan

Tolvaptan (Samska®) is provided as 15, 30 and 60 mg tablets. Tolvaptan is indicated a) for the short term improvement of signs and symptoms of worsening heart failure beyond that achieved with standard of care b) for the treatment of hypervolemic and euvolemic hyponatremia (including patients with heart failure, cirrhosis, SIADH, etc) and for the prevention of hyponatremia. The proposed dose regimen for the treatment of worsening heart failure is 30 mg qd. The proposed initial dose regimen for the treatment of hyponatremia is 15 mg tolvaptan qd. The dose may be increased to 30 mg at intervals of at least 24 h, and to a maximum of 60 mg/day as tolerated to achieve the desired level of serum sodium. During titration the patients should be monitored for serum sodium and volume status.

Conivaptan, a  $V_{1a}/V_2$  receptor antagonist is the first agent in this pharmacological class and is approved for the treatment of euvolemic hyponatremia in hospitalized patients. In contrast to tolvaptan which is administered by the oral route, conivaptan's mode of administration is by intravenous injection.

## ***Drug Substance and Drug Product***

The drug substance exhibits low and pH independent water solubility. Tolvaptan can be classified preliminarily as a BCS IV drug.

The sponsor used the clinical service formulations of strength 15, 30 and 60 mg in the Phase 2 and 3 clinical trials. The sponsor proposes to market only the 15 and 30 mg tablets. The sponsor has not demonstrated in vivo bioequivalence of the clinical service formulations and the commercial formulations. The clinical service and to be marketed formulations of 30 mg strength are compositionally identical. The respective formulations of 15 mg strength are compositionally different. The sponsor has demonstrated that the 15 mg and 30 mg clinical service formulations are bioequivalent and seeks granting of a biowaiver based on in vitro dissolution data.

## ***Mechanism of Action of Tolvaptan***

Tolvaptan [ $\pm$  -4'-[(7-chloro-2,3,4,5-tetrahydro-5-hydroxy-1H-1-benzazepin-1-yl) carbonyl]-o-tolu-*m*-toluidide] is racemic and has a MW of 448.9. It is a non-peptide, selective vasopressin  $V_2$  receptor antagonist with an affinity for the  $V_2$  receptor that is 1.8 times that of the native arginine vasopressin (AVP). AVP is a neuropeptide synthesized in the para-ventricular and supra-optic nuclei of the hypothalamus, transported to the posterior pituitary gland and released in the general circulation. A decrease in volume or pressure leads to an increase in circulating AVP. Stimulation of the  $V_2$  receptors by AVP leads to synthesis and insertion of aquaporin-2 water channels in the collecting duct cells of the kidney, allowing water re-absorption in the hypertonic medulla and antidiuresis. Tolvaptan's blockage of the  $V_2$ -receptors triggers a chain reaction starting with an increase in urine volume/excretion rate, change in plasma and urine osmolality with an increase in free water clearance resulting in a decrease in extra-cellular volume and body weight and an increase in plasma/serum sodium. However, due to a counter-regulatory increase in AVP levels with thirst and increased fluid ingestion the net reduction in extra-cellular volume is significantly smaller than the increase in urine volume and osmotic clearance. Tolvaptan increases both urine excretion and fluid ingestion resulting in a slightly more negative fluid balance compared to placebo. Serum potassium concentrations and urine excretion of sodium and potassium appear not to be affected significantly.

## ***AVP Dysregulation***

Hyponatremia and Syndrome of Inappropriate Secretion of AVP (SIADH)

Euvolemic hyponatremia is the most common type of hyponatremia in hospitalized patients, and SIADH is the most frequent cause. In SIADH hypo-osmolar conditions fail to suppress secretion of AVP, resulting in elevated levels. The salient features of SIADH include hypotonic hyponatremia, urine less than maximally diluted despite hypo-osmolality, increased natriuresis, absence of edema or volume depletion and normal renal and adrenal function. Cancer, neurological disorders, pulmonary diseases, postoperative state and drugs including barbiturates, morphine and carbamazepine and others may cause SIADH.

Hyponatremia and Heart Failure

Plasma AVP is chronically elevated in patients with congestive heart failure (CHF). Hypo-osmolar hyponatremia occurs frequently in patients with advanced CHF. The pathophysiologic role of AVP in CHF includes water retention and severe fluid overload through effects at the renal  $V_2$ -receptor. Activation of the  $V_{1a}$ -receptor by AVP may increase after-load owing to an increase in peripheral vascular resistance. The impairment of free water excretion and persistence of hyponatremia may exacerbate congestive symptoms and worsen outcomes.

Hyponatremia and Cirrhosis

As many as 75% of patients with cirrhosis have impaired renal water handling, as demonstrated by diminished free water clearance after a water load. Plasma concentrations of AVP are elevated due to baroreceptor-mediated non-osmotic stimuli secondary to reduced effective arterial blood volume characteristic of cirrhosis.

## ***Clinical Pharmacology Studies with Tolvaptan***

The sponsor characterized tolvaptan by in vitro studies with human biomaterials investigating the protein binding of tolvaptan, tolvaptan as substrate of CYPs and transporters as well as its potential as inhibitor of enzymes and transporters. The sponsor performed in vivo single and multiple ascending dose studies to characterize the pharmacokinetics, pharmacodynamics and tolerability/safety of tolvaptan in healthy subjects and in the target population. Additional studies in healthy subjects investigated the mass balance, absolute bioavailability and dose strength equivalence of tolvaptan, the effect of food on the bioavailability of tolvaptan,

the impact of age and gender on the exposure to tolvaptan, the effect of tolvaptan on the QT/QTc interval, and the potential for a drug-drug interaction when tolvaptan is co-administered with ketoconazole, grapefruit juice, rifampin, lovastatin, warfarin, amiodarone, digoxin, furosemide or hydrochlorothiazide. The effect of congestive heart failure, and hepatic impairment on the PK of tolvaptan was also investigated in specific studies as were the hemodynamics and renal effects of tolvaptan in CHF patients. The impact of renal impairment on the exposure to tolvaptan was not investigated in a separate study.

The pharmacokinetic analysis used two-stage and population PK approaches. The population PK analysis was only performed with the patient data. The pharmacodynamic parameters assessed in Phase I and II studies included serum sodium, body weight, urine volume/excretion rate, free water clearance, fluid balance, serum potassium, AVP and components of the renin-angiotensin-aldosterone system.

### *Pivotal Clinical Studies with Tolvaptan*

#### *Hyponatremia*

At present there is no consensus on the degree of hyponatremia requiring treatment and size and optimal rate of the change in serum sodium to be attained. There is evidence that a rapid correction of hyponatremic conditions ( $> 12$  mEq/24 h) can induce the osmotic demyelination syndrome.

The outcome variable in two multi-center, double-blind, placebo-controlled, randomized Phase 3 studies in patients with hyponatremia (mean baseline serum sodium concentration=129.0 mEq) was the mean change from baseline in average daily area under the serum sodium concentration time curve up to Days 4 or 30. A starting dose of 15 mg tolvaptan qd that could be up-titrated to 30 or 60 mg qd as needed was used. The serum sodium levels were monitored and used to guide the titration of tolvaptan.

#### *CHF*

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### *Salient Clinical Pharmacology Findings*

#### *Pharmacokinetics*

##### *Healthy Subjects*

Tolvaptan, after single doses up to 480 mg and multiple doses up to 300 mg, exhibits dose proportional kinetics. Maximum plasma concentrations of tolvaptan are attained 2-4 h post-dose. At doses  $\geq 60$  mg Cmax increases less than dose proportional without affecting bioavailability. The pharmacokinetics of tolvaptan are stereospecific with a steady-state ratio of the S(-) to the R(+) enantiomer of about 3. At least 40 % of an oral dose of tolvaptan is absorbed as tolvaptan or metabolites. Food has no relevant impact on the bioavailability of tolvaptan. In vitro data indicate that tolvaptan is a substrate and inhibitor of MDR1. Tolvaptan is highly plasma protein bound ( $\geq 0.99\%$ ) and distributed in an apparent volume of distribution of 4.8 l/kg. Tolvaptan is eliminated entirely by non-renal routes. In vitro data indicate that tolvaptan is metabolized mainly by CYP3A. The mean oral clearance is 4 mL/min/kg and the mean apparent terminal half-life is 12 h, however, the dominant half-life is shorter. When given every 24 h the accumulation factor of tolvaptan is 1.4. At steady-state the trough concentration is  $< 20$  % of the peak concentration. Body weight increases oral clearance. Age or sex does not significantly change oral clearance. The metabolites of tolvaptan do not exhibit relevant  $V_2$ -receptor antagonism.

##### *Patients*

In the patients with hyponatremia of any origin and in patients with CHF and hyponatremia with severe liver impairment, the oral clearance of tolvaptan is reduced by about 20% and the volume of distribution increased by about 50%. CHF does not impact oral clearance significantly. As in healthy subjects oral clearance increases with body weight in patients, whereas age and sex appear not to be relevant covariates. Mild, moderate or severe renal impairment do not increase exposure to tolvaptan. Dialyzability of tolvaptan has not been determined.

### *Dose-Effect Relationship*

#### *1. Serum Sodium*

### 1.1 Patients with Hyponatremia

In the two pivotal trials in hyponatremic patients (all causes) the titration regimen of tolvaptan gradually increased the serum sodium levels into the lower normal range. Compared to baseline, treatment with tolvaptan increased the average daily area under the serum- $\text{Na}^+$  concentration time curve statistically significantly more than a placebo treatment ( $p < 0.0001$ ). With the pooled data the treatment effect was an increase of 3.73 and 4.57 mEq up to Days 4 and 30, respectively. A complete reversal of the sodium effect was observed 6 days after stopping tolvaptan supporting the notion of a true treatment effect. Modeling showed that the observed maximum change from baseline ( $E_{\text{max}}$ ) increases with decreasing baseline values. The time needed to attain 50%  $E_{\text{max}}$  was estimated to be about 3 days in agreement with the observed gradual upwards shift of serum sodium with the titration regimen used.

In a Phase 2 dose ranging study in patients with hyponatremia secondary to liver disease (SLD) single doses of tolvaptan between 15 and 60 mg elicited peak increases of serum sodium between 3.9 and 6.5 mEq. The peak increases in serum sodium occurred later than the peak plasma concentrations suggesting a lagging of the effect behind the plasma concentrations of tolvaptan. A substantial portion of the peak increase in serum sodium was maintained at trough.

### 1.2 Healthy Subjects

The onset of tolvaptan's effect on serum sodium after administration of single doses between 60 and 480 mg was swift. Peak increases in serum sodium ranging between 4.6-7.8 mEq were seen between 6-12 h post-dose suggesting lagging of the sodium effect behind the plasma concentrations. At trough more than 60 % of the peak effect was sustained. Single doses in excess of 60 mg tolvaptan appeared not to increase peak or trough sodium levels importantly in support of the proposed upper dose limit of 60 mg in hyponatremic patients.

### 1.3. Conclusion

Since a slow increase of serum sodium is a required feature of any dose regimen in hyponatremic patients, an increase of the dose of tolvaptan beyond 60 mg qd appears not to be an option. However, titration schemes for tolvaptan with bid administrations using asymmetrical doses and/or dose intervals could provide more constant levels of serum sodium throughout the dose interval. The sponsor did not explore this option even though the results of a Phase 2 study in CHF patients receiving daily doses of 30 mg either as 30 mg qd or 15 mg bid showed significantly greater morning trough values of serum sodium with the bid regimen than with the qd regimen.

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### 3. Aquaresis

### 3.1 Patients

In hypervolemic CHF patients and patients with hyponatremia SLD the onset of the aquaretic effect of tolvaptan at the proposed therapeutic dose levels occurred swiftly. In both patient groups peak aquaretic effects were seen 6 h post-dose and the effect size increased with dose. The effect size of tolvaptan in the patients with hyponatremia SLD appeared to be greater than in the hypervolemic CHF patients. In the former group polyuria continued into the 12-24 post-dose interval.

### 3.2 Healthy Subjects

The onset of the aquaretic effect after single doses between 60 and 480 mg tolvaptan was fast at all doses tested. Peak urine excretion rates of between 8.5 and 10 mL/min were attained over a range of doses differing by factor of 8 suggesting saturability of the aquaretic effect. Peak effects occurred between 3 and 7 h post-dose suggesting a lagging of the effect behind the plasma concentrations of tolvaptan ( $t_{max}$  2-4 h post-dose). After a single dose of 60 mg no increase in urine output was observed 25 h post-dose even though the urine output in the 12-24 h interval suggested continued aquaresis after 12 h post-dose. The rapid decline in urine output during the night interval can be explained by a stop in the fluid intake by the subjects when they were asleep.

### 3.3 Conclusions

The aquaretic effect in healthy subjects reaches saturability at single doses  $\geq 60$  mg. In patients with hyponatremia or CHF higher doses may be required to reach a ceiling effect. It should be noted that a treatment with tolvaptan has two components: a first one triggered by the initial blockade of the  $V_2$ -receptors and a second one that is triggered to counterbalance the thirst induced increase in water consumption caused by the first component.

At the proposed therapeutic dose levels for the treatment of hyponatremia night time polyuria/pollakisuria may become a QOL concern if a long term treatment of hyponatremia is required. Polyuria would be a less of an issue in a short term effective treatment of worsening heart failure.

#### Concentration-Aquaretic Effect Relationship

The relationship between aquaretic effect and plasma concentration of tolvaptan is saturable with smaller effect increments at higher concentrations in patients with hyponatremia SLD, patients with hypervolemic CHF and healthy subjects. The aquaretic effect lags behind the plasma concentration of tolvaptan.

#### Exposure-Safety Relationship

Tolvaptan within the large dose range tested in healthy volunteers and the more limited dose range tested in patients does not increase serum potassium clinically relevantly. The effects of tolvaptan appear to be limited to its inhibition of water-reabsorption in the collecting tubules of the kidney and subsequent events caused by the perturbation of body water homeostasis. Tolvaptan does not prolong the QT/QTc interval.

### Drug-Drug Interactions

#### PK

Ketoconazole (200 mg qd) increases peak and average exposure to tolvaptan by a factor of 5. It is likely that the increase in exposure to tolvaptan by the highest labeled dose of ketoconazole (400 mg qd) would be even greater. Orange juice increases peak and average exposure to tolvaptan by a factor of 1.8. Rifampin reduces exposure to tolvaptan by 85%. Co-administered lovastatin, digoxin, hydrochlorothiazide or furosemide do not impact the exposure to tolvaptan.

Tolvaptan does not impact the pharmacokinetics of co-administered warfarin. Tolvaptan increases the exposure to digoxin and lovastatin 1.3 fold and 1.4 fold, respectively. Tolvaptan does not affect the exposure to amiodarone, hydrochlorothiazide or furosemide.

#### PD

Co-administration of tolvaptan with warfarin does not impact active partial thromboplastin time, prothrombin time or the international normalization ratio. The aquaretic effect, when furosemide or hydrochlorothiazide are co-administered with tolvaptan, is not importantly greater than when tolvaptan is administered alone. However, the co-administration of furosemide or hydrochlorothiazide with tolvaptan produces a greater effect than the furosemide or hydrochlorothiazide alone treatments.

**The review of the Clinical Pharmacology part of the submission indicated the following deficiencies:**

1. The quantitative aspects of the exposure-response-relationship including time course of the effect of tolvaptan and impact of a shorter than 24 h dose interval with both indications has not been performed adequately.
2. A substantial fraction of the circulating total radioactivity (about 40 %) has not been identified. The unidentified metabolites could be pharmacologically active.
3. The sensitivity of the LC/MS/MS and HPLC/UV assays used is not sufficient for proper determination of  $\lambda_z$  and derived parameters for tolvaptan ( $t_{1/2z}$ ,  $V_z/F$ ,  $AUC_{\infty}$ ) and metabolites ( $t_{1/2z}$  and  $AUC$ ) at the proposed therapeutic dose levels.
4. The drug-drug interaction study with ketoconazole used 200 mg qd instead of the highest labeled dose of 400 mg qd. Therefore, the full inhibitory potential of ketoconazole on the exposure to tolvaptan remains unknown. In the drug interaction study with furosemide or hydrochlorothiazide a 30 mg dose of tolvaptan was used. A 60 mg dose of tolvaptan would have been more appropriate.
5. Evidence for the identity of the postulated metabolites DM-4129-4133 was not provided.

**1.1 RECOMMENDATION**

From a Clinical Pharmacology viewpoint the submission is acceptable.

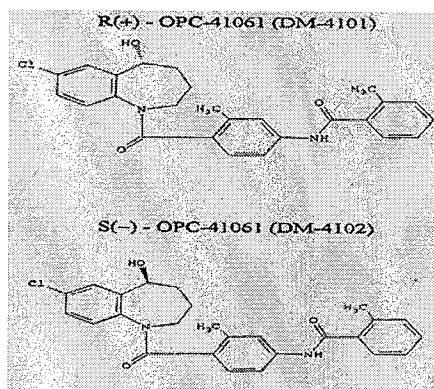
The briefing took place May 6, 2008. In attendance were Drs. N.Stockbridge, D. Unger, S. Targum, A. Thompson, U.A. Meyer, S. Huang, J. Lazor, M. Mehta, A. Raman, C. Sahajwallah, J. Gobburu, A. Dorantes, R. Kumi, D. Brum, G. Iyer, R. Uppoor, N. Mehrorta, K. Busse, S. Mada, I. Jounis, D. Meron-Anderson, J. Parepally, A. Jackson, J. Xu, K. Busse, J. Earp, and P. Hinderling

**2. QUESTION BASED REVIEW**

**2.1 General attributes of the drug**

*2.1.1 What are the highlights of the chemistry and physico-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?*

Tolvaptan { $\pm$  4'-[(7-chloro-2,3,4,5-tetrahydro-5-hydroxy-1H-1-benzazepin-1-yl) carbonyl]-o-tolu-*m*-toluidide} is racemic and has a MW of 448.9. It is practically insoluble in water (0.00005 % w/v) and the solubility is pH independent. The structure of the tolvaptan S(-) and R(+) enantiomers are shown in the below figure:



The composition of the 15, 30 and 60 mg tablets used as clinical service formulations in the Phase 2 and 3 studies and the proposed to be marketed formulation is shown in the below 2 tables:

Table 2.7.1.5.1-3 Composition of Tolvaptan 15-, 30-, and 60-mg Tablets Used in Phase 2 and 3 Clinical Studies			
Component	Composition (mg/Tablet)		
	15-mg Tablets	30-mg Tablets	60-mg Tablets
Tolvaptan	15.0	30.0	60.0
Hydroxypropyl cellulose <sup>a</sup>			
Lactose monohydrate			
Corn starch			
Microcrystalline cellulose			
Hydroxypropyl cellulose			
Low-substituted hydroxypropyl cellulose			
Magnesium stearate			
Tablet shape and color			

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2.7.1 Summary of Biopharmaceutics and Associated Analytical Methods				
Table 2.7.1.5.1-4 Composition of Proposed Marketing Tolvaptan 15-, 30-, and 60-mg Tablets				
Component	Function	15-mg Tablets	30-mg Tablets	60-mg Tablets
Tolvaptan	Active ingredient	15.000	30.000	60.000
Hydroxypropyl cellulose <sup>a</sup>				
Lactose monohydrate				
Corn starch				
Microcrystalline cellulose				
Hydroxypropyl cellulose <sup>c</sup>				
Low-substituted hydroxypropyl cellulose				
FD&C Blue No.2				
Aluminum Lake				
Magnesium stearate <sup>d</sup>				

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The proposed marketing formulations of tolvaptan are blue uncoated tablets containing 15 or 30 mg tolvaptan

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2.1.2 What are the proposed mechanism(s) of action and therapeutic indication(s)?

#### Molecular Level

Native arginine vasopressin (AVP) is a neuropeptide synthesized in the para-ventricular and supra-optic nuclei of the hypothalamus, transported to the posterior pituitary gland and released in the general circulation. Decrease in volume or pressure leads to an increase in circulating AVP and increased re-absorption of water by the collecting tubules in the kidney. Stimulation of the V<sub>2</sub> receptors by AVP increases cyclic adenosine monophosphate (cAMP) production by adenylyl cyclase resulting in the synthesis and insertion of

aquaporin-2 water channels in some cells of thick ascending limb and in all cells of the collecting tubules, allowing water re-absorption in the hypertonic medulla and antidiuresis. The  $V_2$ -receptors are expressed mainly in the basolateral membrane, fewer are found in the luminal membrane. Blockade of the  $V_2$ -receptors is expected to result in a substantial portion of the fluid flowing into the collect duct (12 mL/min) not to be reabsorbed and excreted as urine. AVP can also stimulate  $V_{1a}$  and  $V_{1b}$ -receptors and elicit additional effects as shown in the below table:

Receptors	Location	Effects of AVP
$V_{1a}$	Lymphocytes and monocytes	Coagulation factor release
	Platelets	Platelet aggregation
	Adrenal cortex	Glycogenolysis
	Vascular smooth muscle cells	Vasoconstriction
$V_{1b}$	Anterior pituitary	Adrenocorticotrophic hormone and beta-endorphin release
$V_2$	Principal cells of the renal collecting duct	Free water retention

Abbreviations: AVP = arginine vasopressin.

Both tolvaptan enantiomers are non-peptide, selective vasopressin  $V_2$  receptor antagonists with an affinity for the  $V_2$  receptor that is 1.8 times that native AVP and 29 times greater than that for the  $V_{1a}$ . Systemic Level

#### Systemic Level

Tolvaptan's blockade of the  $V_2$ -receptors triggers a chain reaction starting with an increase in urine volume/excretion rate, change in plasma and urine osmolality with an increase in free water clearance resulting in a decrease in extra-cellular volume and body weight and an increase in serum sodium. However, due to a counter-regulatory increase in AVP levels with thirst and increased fluid ingestion, the net reduction in extra-cellular volume is significantly smaller than the increase in urine volume and free water clearance. Tolvaptan increases both fluid ingestion and excretion resulting in a slightly more negative fluid balance compared to placebo. Tolvaptan's renal effects appear to be largely restricted to blocking the  $V_2$ -receptor. It does not change GFR, sodium or potassium re-absorption/secretion. Tolvaptan increases slightly plasma renal flow and can trigger increased plasma levels of AVP and plasma renin activity (PRA).

#### Pathophysiology

##### Hyponatremia and Syndrome of Inappropriate Secretion of AVP (SIADH)

Euvoletic hyponatremia is the most common type of hyponatremia in hospitalized patients, and SIADH is the most frequent cause. In SIADH hypo-osmolar conditions fail to suppress secretion of AVP, resulting in elevated levels. The salient features of SIADH include hypotonic hyponatremia, urine less than maximally diluted despite hypo-osmolality, increased natriuresis, absence of edema or volume depletion and normal renal and adrenal function. Cancer, neurological disorders, pulmonary diseases, postoperative state and drugs including barbiturates, morphine and carbamazepine and others may cause SIADH.

##### Hyponatremia and Heart Failure

Plasma AVP is chronically elevated in patients with heart failure. Hypo-osmolar hyponatremia occurs frequently in patients with advanced CHF. The pathophysiologic role of AVP in CHF includes water retention and severe fluid overload through effects at the renal  $V_2$ -receptor. Activation of  $V_{1a}$ -receptor by AVP may increase afterload owing to an increase in peripheral vascular resistance. The impairment of free water excretion and persistence of hyponatremia may exacerbate congestive symptoms and worsen outcomes.

##### Hyponatremia and Cirrhosis

As many as 75% of patients with cirrhosis have impaired renal water handling, as demonstrated by diminished free water clearance after a water load. Plasma concentrations of AVP are elevated due to baroreceptor-mediated non-osmotic stimuli secondary to reduced effective arterial blood volume characteristic of cirrhosis.

#### Therapeutic Indications

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Tolvaptan is indicated a) for the short term improvement of signs and symptoms of worsening heart failure beyond that achieved with standard of care b) for the treatment of hypervolemic and euvolemic hyponatremia (including patients with heart failure, cirrhosis, SIADH, etc) and for the prevention of hyponatremia. [REDACTED]

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The proposed initial dose regimen for the treatment of hyponatremia is 15 mg tolvaptan qd. The dose may be increased to 30 mg at intervals of at least 24 h, and to a maximum of 60 mg/day as tolerated to achieve the desired level of serum sodium. During titration the patients should be monitored for serum sodium and volume status

## 2.1 General clinical pharmacology

### 2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

#### Hyponatremia

The sponsor performed 3 randomized, double blind and placebo controlled studies in hyponatremic patients: Two identical pivotal trials and one Phase 2 study. In addition, two open-label studies were performed. Information was also obtained from CHF trials with hyponatremic subpopulations (6 trials, all randomized, double-blind and placebo controlled). All trials were of short duration, the Phase 2 trials lasted 13 and 26 days and the pivotal trials 40 days.

All hyponatremia trials used a dose range of 15 to 60 mg qd. In two trials the dose range tested ranged from 5 to 60 mg qd or 10 to 60 mg tolvaptan qd. A fixed dose regimen was used only in one Phase 2 study. In the other 4 studies including the pivotal studies regimens with dose titration were used. In the pivotal studies the initial dose was 15 mg qd. The dose could then be up titrated to 30 or 60 mg qd as required. The goal of the Phase 3 trials was to increase serum sodium by 5 mEq or normalize serum sodium ( $>135$  mEq). The dose could be reduced at any time if serum sodium exceeded 145 mEq or the increase was too steep ( $>12$  mEq/12 h or  $>8$  mEq/8 h).

#### CHF

Eight studies were conducted in patients with CHF. Seven of the trials were multiple dose safety and efficacy studies and one study was a single dose study investigating the hemodynamics of tolvaptan. All trials were randomized, double-blind and placebo controlled. The 30 mg qd dose was investigated in all studies. Other doses investigated included 60 mg (4 trials), 15 mg (2 trials), 90 mg (2 trials) and ascending doses of between 10 and 120 mg (1 trial). Four trials were relatively short term (7 to 61 days) and 3 trials long term (6 to 32 months). All patients received standard of care treatment.

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### 2.2.3 Are the active moieties in the plasma (or other biological fluids) appropriately identified and measured to assess pharmacokinetic parameters and exposure-response relationships?

The two enantiomers of racemic tolvaptan are the only pharmacologically active moieties among the identified circulating compounds. The sponsor measured the plasma concentrations of the parent drug in all Phase 1 studies and in eight of the Phase 2 and 3 studies. The LC/MS/MS and HPLC/UV assays used for the measurement of racemic tolvaptan are not sensitive enough to determine reliably  $\lambda_z$  and the derived parameters  $t_{1/2z}$  and  $AUC_{\infty}$  after single or multiple dose administration at the proposed therapeutic levels. Because the partial AUC determined by  $\lambda_z$  is relatively small, the bias in  $AUC_{\infty}$  is not major.

The results of the mass balance study indicate that 40% of the circulating radioactivity is not identified. Thus, although the 7 identified metabolites showed no pharmacological activity, the presence of unidentified pharmacologically active metabolites of tolvaptan cannot be excluded.

### 2.2.4 Exposure-response

#### 2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? What is time of onset and offset of the desirable pharmacological response or clinical endpoint?

The sponsor explored in healthy subjects a wide range of doses after single (5-480 mg) and multiple doses (30 to 300 mg qd) that were tolerated. In patients a more restricted dose range was tested. In CHF patients doses ranging from 10 to 120 mg qd (1 trial) and in hyponatremia patients doses ranging between 5 to 60 mg were tested and tolerated.

In the pivotal trial with CHF (NYHA Class III/VI) patients hospitalized for worsening heart failure a single dose level of 30 mg qd was used in the short and long term parts of the trial. 7

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The two pivotal trials in patients with hyponatremia used an initial dose of 15 mg followed by titration to 30 or 60 mg qd as needed for 30 days. Serum sodium was not to exceed 145 mEq. Monitoring of serum sodium levels guided the titration regimen. The outcome variables were the mean change from baseline in average daily area under the serum sodium concentration time curve (AUCNa<sup>+</sup>) up to Days 4 or 30.

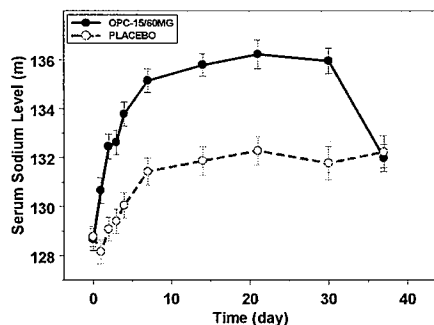
## Dose-Response Relationships for Serum Sodium, Body Weight and Aquaresis

### 1. Serum Sodium

At present there is no consensus on the degree of hyponatremia requiring treatment and on size and optimal rate of the change in serum sodium to be attained. There is evidence that a fast correction of hyponatremic conditions (>12 mEq/24 h) can induce "osmotic demyelination syndrome", a serious encephalopathy.

#### 1.1. Hyponatremia Patients

The sponsor demonstrated efficacy of tolvaptan in hyponatremic patients (all causes) with a mean baseline serum sodium of 129.0 mEq. Compared to baseline, treatment with tolvaptan increased the average daily AUCNa<sup>+</sup> statistically significantly more than placebo (p< 0.0001). With the pooled data the mean treatment effect was an increase of 3.73 and 4.57 mEq up to Days 4 and 30, respectively. The time course of the mean serum sodium concentration in patients treated with tolvaptan or placebo in one of the two pivotal trials (156-02-235) is shown in the below figure:



Tolvaptan increases serum sodium from a hyponatremic level (129 mEq) into the lower normal range ( $\geq 135$  mEq) in both studies. A complete reversal of the effect on sodium is observed 6 days after stopping active treatment supporting the notion of a true treatment effect. The figure shows that the titration regimen with feedback from sodium level monitoring increases the serum sodium gradually over several days. Using an Emax model with time as independent variable it can be shown that the maximum observed change from baseline in serum sodium (Emax) after tolvaptan or placebo is linearly related to the baseline indicating that the lower the baseline serum sodium, the higher Emax will be. The slope of the regression with tolvaptan is significantly greater than with placebo. Using this information in the final Emax model the time to 50 % Emax is estimated to be 3.3 days confirming the gradual increase in serum sodium achieved with the titration scheme used.

The below table summarizes the results of a Phase 2 dose-ranging study in patients with hyponatremia secondary to liver disease (SLD):

#### Serum Sodium Increasing Effect <sup>a</sup> of Tolvaptan in Patients with Hyponatremia SLD<sup>b</sup> on Day 1 of Regimens with Fixed Doses Administered qd

Study	Dose	Epeak <sup>c</sup>	tEpeak <sup>d</sup>	Onset <sup>e</sup>	Etrough <sup>f</sup>	Etrough/Epeak
	mg	mEq	h	h	mEq	

156-96-203						
	5	2.4	4	4	2.0	0.83
	10	4.4	8	4	3.5	0.80
	15	3.9	12	8	2.7	0.69
	30	4.3	8	2	3.3	0.77
	60	6.5	23	4	6.5	1.0

<sup>a</sup> baseline and placebo corrected <sup>b</sup> baseline sodium levels 127-131 mEq <sup>c</sup> observed peak effect <sup>d</sup> time to peak effect <sup>e</sup> First time increase in net serum sodium  $\geq$  1 mEq <sup>f</sup> increase in sodium 23 h post-dose

There is marked variation in onset (2-8 h) and peak time (4-23 h) of the serum sodium increasing effect of tolvaptan in the patients. The peak effects (2.4- 6.5 mEq) appear to increase with dose. A significant portion of the peak effect is maintained at trough.

## 1.2. Healthy Subjects

The below table summarizes the results of the effect of tolvaptan on serum sodium in healthy subjects:

### Serum Sodium Increasing Effect<sup>a</sup> of Tolvaptan after a Single Dose in Healthy Subjects

Study	Dose	Epeak <sup>b</sup>	tEpeak <sup>c</sup>	Onset <sup>d</sup>	Etough <sup>e</sup>	Etough/Epeak
	mg	mEq	h	h	mEq	
156-98-210	60	5.7	6	2	3.6	0.64
&	180	6.7	7	2	5.2	0.76
156-01-229	300	5.5	12	2	4.8	0.87
	480	5.7	12	2	4.3	0.75

<sup>a</sup> baseline and placebo corrected <sup>b</sup> maximum observed effect <sup>c</sup> time to peak effect <sup>d</sup> first time increase in serum sodium  $\geq$  1 mEq <sup>e</sup> net effect 24 h post-dose

The data in healthy subjects are more consistent than those in patients with hyponatremia SLD. The onset of tolvaptan's effect on serum sodium is swift with all doses. Peak increases ranging between 5.7 to 6.7 mEq occur with doses differing by a factor 8 indicating saturability of the sodium increasing effect of tolvaptan. The peak effect occurs between 6-12 h after administration suggesting that the effect of tolvaptan on serum sodium lags behind the plasma concentration (tmax 2-4 h) (counter-clockwise hysteresis). At trough a substantial portion of the peak effect on serum sodium is sustained, as previously seen in the patients with hyponatremia SLD.

1.3 Conclusions: The results of the pivotal trials in the patients with a baseline serum sodium of 129 mEq indicate that the proposed titration regimen with an initial dose of 15 mg qd that can be titrated up to 30 or 60 mg qd normalizes hyponatremia gradually over time as recommended. The findings in patients with hyponatremia SLD indicate that doses between 15 and 60 mg tolvaptan elicit peak effects on serum sodium between 3.9 and 6.5 mEq. The results in healthy subjects suggest that doses  $\geq$  60 qd are unlikely to increase peak or trough serum sodium levels further suggesting saturability of the sodium increasing effect of tolvaptan.

Since a slow increase of serum sodium is a required feature of any dose regimen in hyponatremic patients, an increase of the dose of tolvaptan beyond 60 mg appears not to be an option. However, titration schemes for tolvaptan with bid administration of asymmetrical doses and/or dose intervals could provide more constant levels of serum sodium throughout the dose interval. The sponsor did not explore this option even though the results of a Phase 2 study in CHF patients receiving daily doses of 30 mg either as 30 mg qd or 15 mg bid showed significantly greater morning trough values of serum sodium with the bid regimen than with the qd regimen (study 156-01-231).

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### 3. Aquaretic Effect

#### 3.1 Patients

The below table summarizes the results on the aquaretic effect of tolvaptan in Phase 2 studies in patients with hyponatremia SLD or hypervolemic CHF:

#### Aquaretic Effect<sup>a</sup> of Tolvaptan in Patients with Hypervolemic CHF or Hyponatremia SLD on Day 1 of Regimens with Fixed Doses Administered qd

Study	Population	Dose mg	Epeak <sup>b</sup> mL/min	tEpeak <sup>c</sup> h	Onset <sup>d</sup> h	E <sub>12-24</sub> <sup>e</sup> mL/min
156-97-251	Hypervolemic CHF	10	1.8	6	6	0.25
		15	1.1	6	6	0.89
		30	1.6	6	2	0.55
		60	3.5	6	2	1.0
		90	3.2	6	2	0.68
		120	4.5	6	2	1.5
156-96-203	Hyponatremia due to liver disease	5	2.2	2	2	1.0
		10	3.9	6	2	1.3
		15	3.6	6	2	1.3
		30	4.7	6	2	1.3
		60	4.9	6	2	2.1

<sup>a</sup> baseline and placebo corrected <sup>b</sup> maximum observed effect <sup>c</sup> time to maximum effect

<sup>d</sup> first time increase in excretion rate  $\geq 1$  mL/min <sup>e</sup> effect during the 12-24 h post-dose interval

The onset of the aquaretic effect in the patients at the proposed therapeutic dose levels of 15-60 mg occurs swiftly. In both patient groups peak effects occur at 6 h post-dose, and the effect size increases with dose. The aquaretic effect in hyponatremic patients with liver disease appears to exceed that in the CHF patients. At the proposed dose levels of 15- 60 mg the urine output in the patients with hyponatremia SLD during the 12-24 h interval is increased by between 1 and 2 mL/min indicating maintenance of aquaretic activity with temporary polyuria after 12 h post-dose. No relevant net urine output is observed during the 12-24 h post dose interval in the hypervolemic patients at the proposed dose of 30 mg.

#### 3.2. Healthy Subjects

The below table summarizes the pharmacodynamics of the aquaretic effect of tolvaptan in healthy subjects

#### Aquaretic Effect<sup>a</sup> of Tolvaptan after a Single Dose in Healthy Subjects

Study	Dose	Epeak <sup>b</sup>	tEpeak <sup>c</sup>	Onset <sup>d</sup>	Etrough <sup>e</sup>	Etrough/Epeak	E <sub>12-24</sub> <sup>f</sup>
-------	------	--------------------	---------------------	--------------------	----------------------	---------------	---------------------------------

	mg	mL/min	h	h	mL/min		mL/min
156-98-210 & 156-01-229	60	8.5	5	1	0	0	1.7
	180	10	6	1	0.88	0.088	4.2
	300	8.6	7	1	3.9	0.48	4.6
	480	9.7	3	1	7.7	0.79	5.9

<sup>a</sup> baseline and placebo corrected <sup>b</sup> observed peak effect <sup>c</sup> time to peak effect <sup>d</sup> first time increase in excretion rate  $\geq 1$  mL/min <sup>e</sup> effect at 25 h post-dose <sup>f</sup> effect during the 12-24 h post dose interval

The onset of tolvaptan's aquaretic effect is swift at all doses tested. Peak urine excretion rates ranging between 8.5 and 10 mL/min are observed at dose levels differing by a factor of 8 indicating the aquaretic effect of tolvaptan is saturable. Peak aquaretic activity occurs between 3 and 7 h post-dose, suggesting that the effect lags behind the plasma concentration time curve ( $t_{max}$  2-4 h), as previously noted with the sodium increasing effect of tolvaptan. At doses up to about 180 mg no significant aquaretic activity is observed at trough even though the size of the urine output in the 12-24 h interval suggests continued aquaretic activity after 12 h post-dose. The rapid decline of the aquaresis during the night can be explained by the decreased water intake by the subjects during sleep. A single dose of 60 mg of tolvaptan increases normal urine output (about 1 mL/min) by about 2 mL/min during the 12 h night interval causing temporary polyuria and pollakisuria.

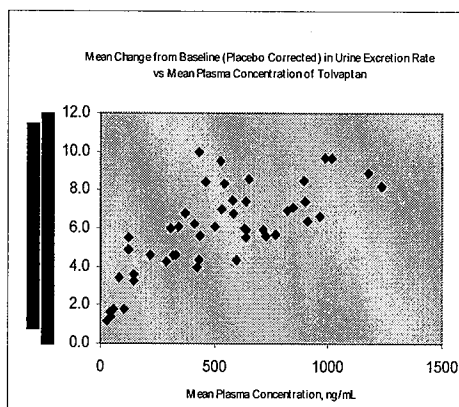
### 3.3 Conclusion:

The aquaretic effect of tolvaptan in healthy subjects reaches saturability at doses  $\geq 60$  mg. In patients with hyponatremia or CHF higher doses may be required to attain a ceiling effect. It should be noted that the aquaretic effect of a treatment with tolvaptan has 2 components: a first one triggered by the initial blockade of the V2 receptors and a second one that is triggered to counterbalance the thirst induced increase in water consumption caused by the first component. A deconvolution of the two aquaretic components, if possible, is certainly beyond the scope of this review.

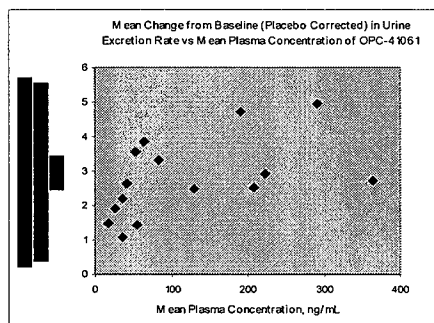
At the proposed therapeutic dose levels for the treatment of hyponatremia night time polyuria/pollakisuria may become a QOL concern if a long term treatment of hyponatremia is required. Polyuria/pollakisuria would be less of a concern for the short treatment of worsening heart failure.

### Plasma Concentration-Aquaretic Effect Relationship

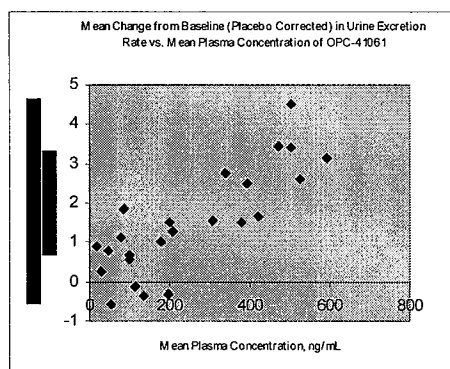
The relationship between aquaretic response and plasma concentration of tolvaptan in healthy volunteers and patients with hyponatremia SLD or hypervolemic CHF investigated is shown below:



Healthy Subjects (Study 156-01-229)



Patients with Hyponatremia SLD (Study 156-96-203)



Patients with Hypervolemic CHF (Study 156-97-251)

The plots indicate saturability of the aquaretic effect with smaller increments with increasing plasma concentrations in healthy subjects, patients with hyponatremia SLD and hypervolemic CHF patients. It should be noted that the lagging of the aquaretic effect behind the plasma concentration (counter-clockwise hysteresis) contributes to the marked variability of the data.

The relationship between the effect on serum sodium and plasma concentration of tolvaptan has not been explored. It is likely that the relationship between plasma concentration and sodium increasing effect displays some of the same characteristics (saturability, hysteresis) seen in the relationship between plasma concentration and aquaretic effect.

#### 2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety?

In patients and volunteers (at supratherapeutic dose levels) the serum potassium concentrations appear not to be clinically relevantly affected by tolvaptan. The risk for a sudden and unexpected hyponatremia during treatment with tolvaptan appears to be remote in patients and healthy subjects if the sodium level is monitored. Treatment with tolvaptan can increase the plasma levels of AVP and PRA.

#### 2.2.4.3 Does the drug prolong the QT/QTc interval?

The thorough QT/QTc study performed in healthy volunteers receiving multiple therapeutic and supra-therapeutic doses of tolvaptan qd is negative. At the supratherapeutic dose level of 300 mg qd the time matched and baseline adjusted mean difference in QTc between drug and placebo at tmax is 3 ms and the one sided 95% upper bound is 7 ms and does not exceed the regulatory threshold of 10 ms. No significant drug effect on QT/QTc is observed at the 30 mg qd dose level (Please refer to 6. PM Review, QTcIRT Report).

#### 2.2.4.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

##### Hyponatremia

The proposed qd regimen with an initial dose of 15 mg that can be increased to 30 or 60 mg normalizes moderate hyponatremia gradually within a few days. A slowly evolving increase of serum sodium is preferable to an abrupt correction. Based on the available evidence it cannot be ruled out that an asymmetrical bid dose regimen using the same daily doses is superior to the sponsor proposed qd regimen.

#### 2.2.5 What are the PK characteristics of the drug and its major metabolite(s)

##### 2.2.5.1 What are the single and multiple dose PK parameters?

##### Parent Drug

The below table summarizes the relevant PK parameters of tolvaptan in healthy subject and hyponatremic and CHF patients:

**Representative Mean PK Parameters of Tolvaptan by a 2-Stage Noncompartmental Model Approach in Healthy Subjects and Patients with Hypervolemic CHF and or Hyponatremia SLD**

Population	Dose	tmax <sup>a</sup>	CL/F	Vz/F	t1/2z	Fluct. <sup>b</sup>	Accum. <sup>c</sup>	% Variability <sup>d</sup>	
								Cmax	AUC
<b>Healthy Subjects</b>	mg	h	mL/min/kg	L/kg	h				
M & F, 18-55 y	240-480	3.2	4.7	4.8	12	na	na	34	31
M, 18-45 y	60 qd	2.0	4.2	na	na	11	1.5	44	55
F, 18-45 y	60 qd	1.8	5.4	na	na	19	1.4	31	38
M ≥ 65 y	60 qd	2.0	3.2	na	na	11	1.3	30	30
F ≥ 65 y	60 qd	3.0	3.8	na	na	6.3	1.3	41	50

Patients									
Hyponatremia <sup>c</sup> , M & F, 38-73 y	10 qd	3.9	1.2	na	na	4.0	2.1	45	40
	30 qd	2.0	1.8	na	na	5.6	1.7	34	20
	60 qd	2.0	2.0	na	na	3.6	2.2	56	49
Hypervolemic CHF <sup>f</sup> , 49-86 y	60 qd	2.0	1.2	na	na	5.1	1.3	28	35
	120 qd	3.1	4.2	na	na	4.2	1.2	62	78

<sup>a</sup> median <sup>b</sup> fluctuation=C<sub>max</sub>/C<sub>trough</sub> <sup>c</sup> Accumulation=AUC<sub>0-τ</sub>(Last Day)/AUC<sub>0-τ</sub>(Day1) <sup>d</sup> percent coefficient of variation about the mean <sup>e</sup> secondary to liver disease <sup>f</sup> NYHA Class I-III na= not available

Due to insufficient sensitivity of the LC/MS/MS and HPLC/UV assays used by the sponsor, only truncated time profiles of tolvaptan and metabolites were available that prevented determination of the full set of the standard parameters after administered of therapeutic doses.

The plasma concentration time profile of tolvaptan is multi-phasic. In healthy subjects the PK of tolvaptan after single doses of 5 to 480 mg and multiple doses of 30 to 480 mg qd are dose proportional. At the proposed therapeutic dose levels of 15 to 60 mg qd, the drug is absorbed with a mean t<sub>max</sub> of about 2 h in healthy subjects and patients. At dose levels of ≥ 60 mg absorption appears to be slowed resulting in C<sub>max</sub> values that are less than dose proportional without affecting absolute bioavailability of tolvaptan. The mean CL/F in young to middle aged and elderly subjects is not significantly different. The value of the pseudo steady-state volume of distribution (V<sub>z</sub>/F) indicates significant extravascular partitioning of the highly protein bound drug (98.9-99.2 %), even though V<sub>z</sub>/F overestimates the true extent of distribution. The accumulation factor of tolvaptan with a qd regimen is small and similar in healthy subjects of different age and sex. The lack of significant accumulation indicates that only small amounts of tolvaptan remain to be eliminated during the terminal elimination phase with an estimated mean half-life of 12 h. The high values for the peak to trough ratio (fluctuation) reiterate that the dominant half life of tolvaptan is shorter than the terminal t<sub>1/2z</sub>. The percent coefficient of variation about mean C<sub>max</sub> and AUC after single and multiple doses indicate marked intersubject variation of the kinetics of tolvaptan.

#### Metabolites

Seven metabolites were followed in plasma. None appears to exhibit significant V<sub>2</sub>-antagonist activity at concentrations reached at therapeutic doses of tolvaptan. DM-4103 is not only the major metabolite, but also the major moiety circulating in plasma. After single doses of tolvaptan up to 480 mg the kinetics of DM-4103 appear to be dose proportional. In contrast to tolvaptan and the other metabolites, DM-4103 is very slowly eliminated from the body with a t<sub>1/2z</sub> in excess of 180 h resulting in substantial accumulation. The time course of the other metabolites appears to be determined by their formation from tolvaptan.

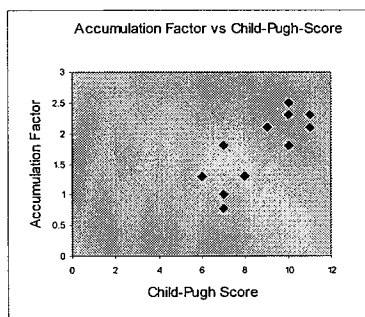
#### 2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

See also Table in 2.2.5.1

#### Two Stage Approach

The kinetics of tolvaptan in patients with hypervolemic CHF or hyponatremia secondary to liver disease receiving qd regimens of the drug for 13 days are compatible with dose proportional kinetics.

In the hyponatremic patients with liver disease CL/F and plasma protein binding (97.7-98.6%) are lower than in healthy subjects. The CL/F in the CHF patients appears to vary significantly. The peak to trough ratio (fluctuation) in both patient groups tends to be lower than in most healthy subjects. The accumulation factor of tolvaptan in the hyponatremic patients with liver disease is greater than in healthy subjects and CHF patients as shown in the below figure:



The accumulation factor increases with increasing Child-Pugh score.

#### Population PK Approach

A population PK analysis was performed using only patient data. A one-compartment model with first order absorption and elimination was used; CHF and liver impairment were identified as covariates. The parameters of tolvaptan in the patients with hyponatremia of any origin and the impact of CHF liver impairment are shown in the below tables:

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#### Impact of Disease State on PK Parameters of Tolvaptan

Disease State	CL/F mL/min/kg	V/F L/kg	t <sub>1/2</sub> h
Hyponatremia of any origin	2.4	2.0	10
CHF	1.7	1.2	8
Moderate/severe liver impairment	1.9	3.0	18

#### Impact of Body Weight on PK Parameters of Tolvaptan

Parameter	Body weight, kg		
	35	70	150
CL/F, mL/min	133	167	217
V/F, L	74	143	276
t <sub>1/2</sub> , h	7	10	14

#### Active Metabolites

None of the identified metabolites of tolvaptan exhibits relevant V<sub>2</sub>-receptor antagonistic activity.

#### 2.2.5.3 What are the characteristics of drug absorption (possible transporters and pH impact)

Tolvaptan is a low solubility drug and the solubility is pH independent. The permeability of tolvaptan is unknown and reliable information on the absolute bioavailability is not available. The results from the mass balance study show that 40% of the radioactivity administered is excreted in urine indicating that at least 40 % of the administered radiolabeled tolvaptan is systemically available as tolvaptan or metabolites. Data on the bioavailability of the clinical service formulations or to be marketed formulations relative to a solution of tolvaptan are not available. At doses ≤ 60 mg tolvaptan is absorbed rapidly in healthy subjects with a mean t<sub>max</sub> of about 2

h. At doses in excess of 60 mg the absorption is slowed and C<sub>max</sub> is less than dose proportional, but the absolute bioavailability of tolavaptan is not affected. In vitro tolavaptan is a substrate and inhibitor of MDR1.

#### 2.2.5.4 What are the characteristics of drug distribution (incl. plasma protein binding)

In healthy subjects the plasma protein unbound tolavaptan is concentration independent and the mean percentage unbound tolavaptan in plasma ranges between 0.83- 1.02. The plasma protein binding of the enantiomers was not reported. The major circulating moiety, the metabolite DM-4103, does not impact the plasma protein binding of tolavaptan. As expected for a drug with extensive plasma protein binding the red cell partitioning in whole blood is minimal with tolavaptan. The pseudo steady-state volume of distribution indicates significant extravascular distribution of the highly plasma protein bound drug.

#### 2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

The below table summarizes the results of the mass balance study performed by the sponsor:

##### Mean Recoveries of Total Radioactivity

Mean Recovery (SD), % Dose		
Total	Fecal	Urine
98.9 (8.1)	58.7 (9.1)	40.2 (8.6)

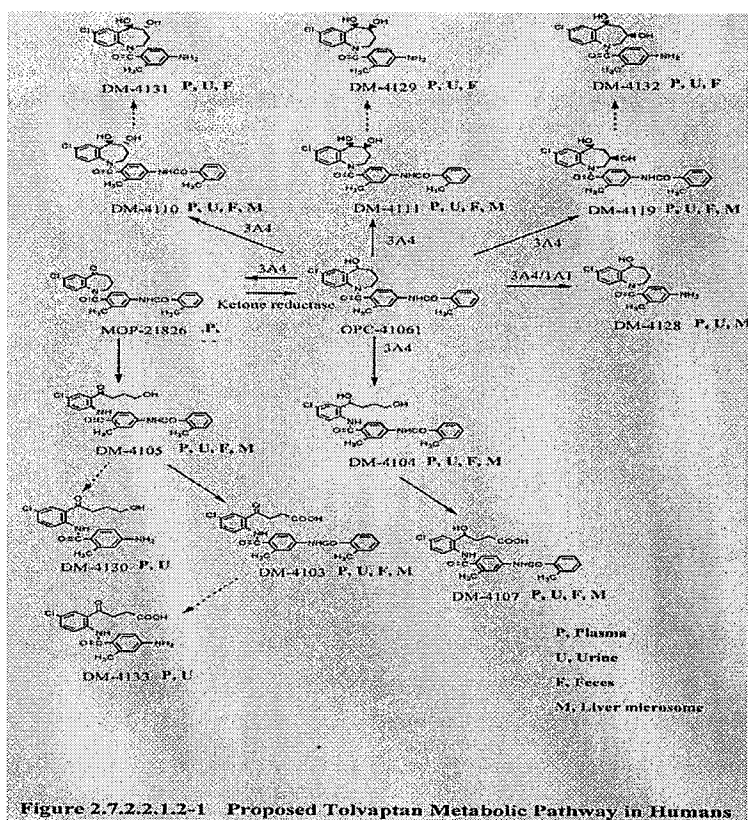
Most of the radioactivity after administration of labeled tolavaptan is excreted in the feces. As little as 0.2 % of the dose is excreted as unchanged tolavaptan in urine up to 36 h after administration. Thus, non-renal elimination by metabolism and/or excretion into bile is the main route of elimination for systemically available tolavaptan.

In plasma tolavaptan and 7 metabolites were identified that together represent on average 60 % of the circulating radioactivity leaving about 40 % unidentified. About 33 % of the total radioactivity administered is excreted in urine up to 36 h after dosing. About 72% of the total radioactivity excreted up to 36 h in urine is identified tentatively or definitively as metabolites and tolavaptan and about 28 % remain unidentified. Five metabolites are identified definitively in urine. About 44% of total radioactivity administered is excreted in the feces up to 36 h after dosing. About 75 % of the total radioactivity eliminated in the feces is identified tentatively or definitively as metabolites and tolavaptan leaving about 25 % of the radioactivity unidentified. Four metabolites are definitively identified in the feces. The most abundant compound in the feces is tolavaptan (about 14 % of the dose). Information on the possible metabolism of tolavaptan by intestinal contents is not available. The relative contributions of metabolism and excretion into the bile to the elimination of tolavaptan are unknown. It should be noted that non-negligible amounts of radioactivity in plasma, urine and feces remain to be identified.

#### 2.2.5.6 What are the characteristics of drug metabolism (extraction ratio, metabolic scheme, enzymes responsible, fractional clearances)?

In vitro studies with human hepatic tissues indicate that CYP3A is the main enzyme involved in the metabolism of tolavaptan. In humans the metabolites DM-4103, DM-4104, DM-4105, DM-4107, DM-4110, DM-4111 and DM-4119 have been identified in one or more matrices.

The below figure shows the sponsor proposed scheme for the metabolism of tolavaptan:



The *in vivo* metabolism of tolvaptan to its first generation metabolites DM-4104, MOP-21826/DM-4105, DM-4110, DM-4111, DM-4119 and DM-4128 is catalyzed largely if not exclusively by CYP3A 4. Glucuronides appear not to be formed during the catabolism of tolvaptan. Data in support of the identity of some of the postulated second and third generation metabolites DM-4129-4133 in the above scheme were not provided.

In healthy subjects the mean CL/F of tolvaptan is about 4 mL/min/kg suggesting low hepatic extraction. From the provided information fractional metabolic clearances for individual metabolites cannot be calculated.

#### 2.2.5.7 What are the characteristics of drug excretion

After oral administration of labeled tolvaptan the fraction of total radioactivity excreted in urine is 40 %. Less than 1% of the dose is excreted as unchanged tolvaptan. The renal clearance of tolvaptan is about 0.006 mL/min/kg in healthy subjects which equals roughly the predicted glomerular clearance if the extensive protein binding and tubular re-absorption of the lipophilic drug is considered. There is no information on the renal clearance of the metabolites.

#### 2.2.5.8 Based on PK parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

In healthy subjects the kinetics (average exposure) of tolvaptan are dose proportionate. C<sub>max</sub> increases less than dose proportional. The data obtained in patients with hyponatremia of any origin and CHF patients with and without hyponatremia are also compatible with dose proportional kinetics.

#### 2.2.5.9 How do the PK parameters change with time following chronic dosing?

There is no evidence that the PK parameters of tolvaptan following chronic dosing change with time.

2.2.5.10 What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

See 2. 2.5.1

### 2.3. Intrinsic factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

The impact of genetic polymorphism, race, pregnancy and lactation on the kinetics of tolvaptan is unknown in healthy subjects and patients.

The following covariates have been identified for tolvaptan:

#### Healthy Subjects

Oral clearance increases with body weight, whereas age and sex appear not to impact CL/F.

#### Patients

See also 2.2.5.2 ' [REDACTED] moderate and severe liver impairment and CHF are covariates of the kinetics in patients. The exposure to tolvaptan is increased in patients with liver impairment or CHF.

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2.3.2 Based on what is known about exposure-response relationships, what dosage regimen adjustments, if any, are recommended for each subgroup listed below?

No dose adjustment is recommended for the elderly, females versus males, race other than Caucasian, patients with mild, moderate or severe liver impairment or with mild or moderate or severe renal impairment. The expected increase in exposure is not expected to be of a magnitude that could jeopardize safety. No recommendations can be made for pediatric populations, because the PK of tolvaptan have not been investigated in this population.

2.3.2 Based on what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations (examples shown below), what dosage adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

#### 2.3.2.1 Elderly (see Study of Drugs likely to be used in the Elderly)

No dose adjustment is necessary

#### 2.3.2.2 Pediatric patients. Also what is status of pediatric studies and/or any pediatric plan for study?

No recommendation can be made. Safety, efficacy and PK of tolvaptan in pediatric patients have not been investigated.

#### 2.3.2.3 Gender

No dose adjustment is necessary

#### 2.3.2.4 Race, in particular differences in exposure and/or response in Caucasians, African Americans, and/or Asians

No information on the impact of race on exposure is available. The sodium increasing effect of tolvaptan appears to be similar in Caucasian and Non-Caucasian subjects.

#### 2.3.2.5 Renal Impairment

A study in patients with varying degrees of renal impairment and subjects with normal renal function was not done. However, a population PK analysis of data from patients with hyponatremia of any origin or CHF with creatinine clearance values ranging between 9.5 and 150 mL/min (561 patients with normal, 1130 with mild, 1057 with moderate and 378 with severe renal impairment) was performed. The analysis showed no impact of renal impairment on the exposure to tolvaptan. Thus, an adjustment of the dose in patients with renal impairment is not necessary.

#### **2.3.2.6 Hepatic Impairment**

A full experimental study in patients with different degrees of hepatic impairment and healthy controls was not done. However, a population PK analysis of data from patients with different degrees of liver impairment obtained in Phase 2 and 3 studies was performed. The results indicate that moderate and severe liver impairment decrease CL/F by about 20% and increase V/F by about 50% in patients with hyponatremia of any origin. An adjustment of the dose in patients with hepatic impairment is not required.

#### **2.3.2.7 Congestive Heart Failure**

The population PK analysis using patient data from patients with hyponatremia of any origin or CHF indicates that CHF decreases CL/F by 30 % and V/F by 40%. An adjustment of the dose of tolvaptan in patients with CHF is not required.

#### **2.3.2.7 What pharmacogenetic information is there in the application and is it important or not**

The submission does not contain pharmacogenetic information on pharmacokinetics or pharmacodynamics of tolvaptan.

#### **2.3.2.7 What pregnancy and lactation use information is there in the application? What other human factors are important to understanding the drug's efficacy and safety?**

The submission does not contain pregnancy or lactation use information on tolvaptan.

### **2.4. Extrinsic Factors**

#### **2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or- response and what is the impact of any differences in exposure on response?**

The submission does not contain information on the possible impact of herbal products, diet, smoking and alcohol use on exposure or response. No dose recommendations can be made regarding these extrinsic factors.

#### **2.4.2 Drug-drug interactions**

##### **2.4.2.1 Is there an in vitro basis to suspect in vivo drug-drug interactions?**

In vitro data indicate that tolvaptan is a substrate and inhibitor of MDR1. Information on the substrate or inhibitor status of tolvaptan for other transporters is not provided. Experiments with human liver tissue and cells expressing the major human cytochrome P450 enzymes indicate that tolvaptan is a substrate of CYPs 3A and a weak inhibitor of CYP3A4 ( $K_i$ = 5.4-10.4 $\mu$ M) and 2C9 ( $K_i$ = 6.7  $\mu$ M).

##### **2.4.2.2 Is the drug a substrate of CYP enzymes? Is metabolism influenced by genetics?**

Tolvaptan is mainly metabolized by CYP 3A4. It is not anticipated that the metabolism of tolvaptan is importantly influenced by genetic differences.

##### **2.4.2.3 Is the drug an inhibitor and/or an inducer of CYP enzymes?**

In vitro tolvaptan inhibits CYPs 3A4 and 2C9, but only at concentrations that exceed the therapeutic range of tolvaptan. No information is provided on the potential of tolvaptan to act as an inducer of the metabolism of other drugs.

##### **2.4.2.4 Is the drug a substrate and/or an inhibitor of P-glycoprotein transport processes?**

Tolvaptan is a substrate and an inhibitor of MDR1.

##### **2.4.2.5 Are there other metabolic/transporter pathways that may be important?**

Excretion of unchanged tolvaptan into bile cannot be ruled out as a secondary route of elimination. About 14% of the radioactivity administered was identified in the feces up to 36 h after dosing. Of course, the tolvaptan recovered in the feces could represent unabsorbed drug.

Tolvaptan as a possible substrate or inhibitor of other efflux- or influx transporters *in vitro* has not been investigated. Thus, the possibility that tolvaptan interacts with other transporters cannot be excluded.

*2.4.2.6 Does the label specify co-administration of another drug (e.g. combination therapy in oncology) and, if so, has the interaction potential between these drugs been evaluated?*

No

*2.4.2.7 What other co-medications are likely to be administered to the target population?*

Other drugs indicated for the treatment of patients with CHF with and without hyponatremia include loop-, thiazide- and other diuretics including spironolactone, antihypertensives, lipid lowering drugs and anticoagulants. Patients with hyponatremia secondary to liver disease, may receive additionally antacids and H2 blockers.

*2.4.2.8 Are there any in vivo drug-drug interaction studies that indicate the exposure alone and/or exposure-response relationships are different when drugs are co-administered?*

The sponsor tested the impact of tolvaptan on the kinetics of lovastatin and lovastatin  $\beta$ -hydroxy acid (CYP 3A substrate), amiodarone and desethylamiodarone (CYP3A substrates), R(+) warfarin (CYP 3A substrate), S(-) warfarin (CYP2C9 substrate) and the metabolites (7-hydroxy and 10- hydroxy warfarin), digoxin (MDR1 substrate), furosemide and hydrochlorothiazide (HCT) (frequently co-administered). In the interaction studies with warfarin, the dynamic endpoints, aPTT, PT and INR, were assessed and in the interaction studies with furosemide and HCT the additive impact on urine volumes/excretion rates were measured. The impact of ketoconazole (CYP3A and MDR1 inhibitor), grapefruit juice (CYP 3A and MDR1 inhibitor), lovastatin CYP 3A inhibitor), rifampin (CYP3A inducer, transport inhibitor), and digoxin, furosemide and hydrochlorothiazide (frequently co-administered) on the kinetics of tolvaptan were investigated. In interaction studies of furosemide and HCT the potential of an additive effect on urine volume/excretion rate when the diuretics are co-administered with tolvaptan was also assessed.

The results of the interaction studies are summarized in the below table:

Tolvaptan Drug-Drug Interactions					
Drug	Dose mg	Impact on	Dose mg	Factor	
				C <sub>max</sub>	AUC
Ketoconazole	200, SD	Tolvaptan	30, SD	3.5	5.6
Rifampin	600, MD qd	Tolvaptan	240, SD	0.1	0.2
Grapefruit Juice	240 mL, SD	Tolvaptan	60, SD	1.9	1.6
Furosemide	80, SD	Tolvaptan	30, SD	1.1	1.2
HCT <sup>a</sup>	100, SD	Tolvaptan	30, SD	0.89	1.1
Lovastatin	80, SD	Tolvaptan	60, SD	1.2	1.2
Digoxin	0.25, MD	Tolvaptan	60, SD/MD	1.1	1.1
Tolvaptan	60, SD	Lovastatin	80, SD	1.4	1.4
		Lovastatin $\beta$ -hydroxy acid		1.2	1.3
	90, SD	Lovastatin	80, SD	1.3	1.4
		Lovastatin $\beta$ -hydroxy acid		1.0	1.1
Tolvaptan	30, SD	Amiodarone	200, MD qd	0.99	0.98
		DEA <sup>b</sup>		0.99	0.99
	90, SD	Amiodarone		1.0	1.0
		DEA <sup>b</sup>		0.98	1.0
Tolvaptan	60, MD qd	(S)-Warfarin	25, SD	1.1	1.1

		(R)-Warfarin		1.1	1.1
		7-Hydroxywarfarin		0.98	1.1
		10-Hydroxywarfarin		1.1	1.1
		aPTT <sup>c</sup>			1.0
		PT <sup>d</sup>			1.0
		INR <sup>e</sup>			1.1
Tolvaptan	60, SD/MD qd	Digoxin	0.25, MD	1.3	1.2
Tolvaptan	30, SD	Furosemide	80, SD	0.84	0.91
Tolvaptan	30, SD	HCT <sup>a</sup>	100, SD	1.1	1.1

<sup>a</sup> hydrochlorothiazide <sup>b</sup> desethylamiodarone <sup>c</sup> activated partial thromboplastin time <sup>d</sup> prothrombin time <sup>e</sup> international normalization ratio

Except for digoxin whose exposure in the presence of tolvaptan increased by a factor of 1.3, tolvaptan had no impact on exposure or response (when measured) on the co-administered other drugs. However, other drugs including ketoconazole and rifampin have clinically relevant effects on the kinetics of tolvaptan, if co-administered. Ketoconazole increases and rifampin reduces C<sub>max</sub> and AUC of tolvaptan clinically importantly. It should be noted that the dose of ketoconazole used is sub-maximal (200 mg qd). Grapefruit juice increases exposure to tolvaptan significantly less than ketoconazole. Digoxin co-administration has no impact on the kinetics of tolvaptan. The 24 h urine volume/excretion rate with furosemide or hydrochlorothiazide when co-administered with tolvaptan is not importantly larger than that with tolvaptan alone treatment. The 24 h urine volume/excretion rate with the furosemide or hydrochlorothiazide alone treatments is significantly smaller than that with the tolvaptan alone or combination treatments. A 60 mg dose of tolvaptan would have been more adequate in the interaction study with furosemide and hydrochlorothiazide

#### 2.4.2.9 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

Tolvaptan and loop-, thiazide- and other type diuretics increase the urine volume/excretion rate. Thus, tolvaptan and diuretics could exert additive aquaretic effects when co-administered.

#### 2.4.2.10 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions, or protein binding?

Significant percentages of total radioactivity remain unidentified in plasma, urine and feces. Evidence for the identity of the postulated second generation metabolites DM-4129-4133 is not provided.

#### 2.4.3 What issues related to dose, dose regimens, or administration are unresolved and represent significant omissions?

There is evidence at the 30 mg dose level that an asymmetrical bid regimen may result in larger sodium concentrations at trough in CHF NYHA Class II-III patients than a 30 mg qd regimen. Thus, is possible that a bid regimen of tolvaptan with asymmetrical doses and/or dose intervals is superior to the proposed qd regimen.

### 2.5 General biopharmaceutics

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

The water solubility of tolvaptan is low (0.00005 % w/v) and pH independent. Tolvaptan's permeability/absolute bioavailability is undetermined. Tolvaptan is a substrate of MDR1.

#### 2.5.2 What is the relative bioavailability of the proposed to-be-marketed formulation relative to the formulation used in the pivotal clinical trial?

An in vivo bioequivalence study comparing the commercial and clinical service formulations was not performed.

#### 2.5.2.11 What data support or do not support a waiver of in vivo BE data?

- BCS classification system
- Formulation ingredient information
- Dissolution profiles
- Others

The respective compositions and weights of the 15, 30 and 60 mg clinical service- and commercial formulations are shown below:

**Table 2.7.1.5.1-3 Composition of Tolvaptan 15-, 30-, and 60-mg Tablets Used in Phase 2 and 3 Clinical Studies**

Component	Composition (mg/Tablet)		
	15-mg Tablets	30-mg Tablets	60-mg Tablets
Tolvaptan	15.0	30.0	60.0
Hydroxypropyl cellulose <sup>a</sup>			
Lactose monohydrate			
Corn starch			
Microcrystalline cellulose			
Hydroxypropyl cellulose <sup>b</sup>			
Low-substituted hydroxypropyl cellulose			
Magnesium stearate			
Total weight	180.0	174.0	
Tablet shape and color	White, round, 8 mm diameter, flat-face, beveled-edge		

b(4)

**Table 3.2.P.1-1 Quantitative Composition of Tolvaptan Tablets**

Component	Quality Standard	Function	15-mg Tablets		30-mg Tablets		60-mg Tablets	
			mg	% w/w	mg	% w/w	mg	% w/w
Tolvaptan	Non compendial	Active ingredient	15.000	17.24	30.000	17.24	60.000	37.04
Hydroxypropyl cellulose <sup>a</sup>	NF							
Lactose monohydrate <sup>c</sup>	NF							
Corn starch	NF							
Microcrystalline cellulose	NF							
Hydroxypropyl cellulose <sup>d</sup>	NF							
Low-substituted hydroxypropyl cellulose	NF							
FD&C Blue No.2	21 CFR							
Aluminum Lake	82.51, 82.102							
Magnesium stearate <sup>e</sup>	NF							
Total weight			87.000		174.000			
Tablet description			Blue, triangular, shallow-convex, beveled-edge tablet, debossed with "OTSUKA" and "15" on one side		Blue, round, shallow-convex, beveled-edge tablet, debossed with "OTSUKA" and "30" on one side			

b(4)

2.5.2.2 What are the safety or efficacy issues, if any, for BE studies that fail to meet the 90% CI using equivalence limits of 80-125%?

An in vivo BE study comparing the clinical service and commercial formulations was not performed by the sponsor.

2.5.2.3 If the formulations do not meet the standard criteria for bioequivalence, what clinical pharmacology and/or clinical safety and efficacy data support the approval of the to be marketed product?

There are no clinical pharmacology data that support approval of the proposed 15 mg commercial formulation.

*2.5.3 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?*

Food has no clinically relevant impact on C<sub>max</sub> or AUC of tolvaptan. Therefore, tolvaptan can be taken in the fasted or fed state.

*2.5.4 When would a fed BE study be appropriate and was one conducted (Refer to Appendix 3-Table 1, When to Request a Fasted BE Study)*

NA

*2.5.5 If different strength formulations are not bioequivalent based on standard criteria, what clinical safety and efficacy data support the approval of the various strengths of the to be marketed product?*

An in vivo BE study demonstrating dose strength bioequivalence of the commercial 15, 30 and 60 mg tablet has not been performed.

*2.5.6 If the NDA is for a modified release formulation of an approved immediate release product without supportive safety and efficacy studies, what dosing regimen changes are necessary, if any, in the presence or absence of PK-PD relationship?*

NA

*2.5.7 If unapproved products or altered approved products were used as active controls, how is BE to the approved product demonstrated? What is the basis for using either in vitro or in vivo data to evaluate BE?*

NA

*2.5.8 What other significant, unresolved issues related to in vitro dissolution or in vivo BA and BE need to be addressed?*

None

## **2.6 Analytical section**

This section should address issues related to the analytical and bioanalytical methods used to support the clinical pharmacology and biopharmaceutics studies

*2.6.1 How are the active moieties identified and measured in the plasma in the clinical pharmacology and biopharmaceutics studies?*

Tolvaptan appears to be the only pharmacologically active species in the circulation. None of the metabolites appears to be pharmacologically active.

*2.6.2 Which metabolites have been selected for analysis and why?*

In several clinical pharmacology studies from one to seven of the identified inactive metabolites were measured in plasma. Among these the major circulating metabolite DM-4103 was assessed most often. A reason for measuring the inactive metabolites is not provided.

*2.6.3 For all moieties measured, is free, bound, or total measured? What is the basis for that decision, if any, and is it appropriate?*

The total (bound + unbound) concentration of tolvaptan and the metabolites was measured. The plasma protein binding of tolvaptan is constant over the clinically significant concentrations range in healthy subjects and in patients with hyponatremia secondary to liver disease. Thus, since the ratio of unbound (=active moiety) to total tolvaptan is constant, measurement of the total plasma concentration of tolvaptan in plasma is justifiable. The plasma protein binding of DM-4103 and the other metabolites was not reported.

*2.6.4 What bioanalytical methods are used to assess concentrations?*

In all clinical pharmacology studies LC/MS/MS or HPLC/UV assays were used to measure tolvaptan alone or together with one or more of the identified and metabolites in plasma. Both assays used an internal standard. The HPLC/UV method was also used to measure tolvaptan in urine. The HPLC/MS/MS method and the HPLC/UV method were not cross-validated.

Lovastatin, Lovastatin- $\beta$ -hydroxyacid, amiodarone and desethylamiodarone, R- and S- warfarin and the metabolites 7-hydroxy and 10-hydroxywarfarin were measured by validated LC/MS/MS assays. Rifampin was assayed by a proprietary HPLC/UV assay.

Ketoconazole in plasma was evaluated by a HPLC/UV method. Furosemide and HCT in plasma and urine were measured by HPLC/UV methods with internal standards.

2.6.4.1 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques are used?

#### LC/MS/MS and HPLC/UV Assays for Tolvaptan

The calibration curve for tolvaptan with the HPLC/MS/MS and HPLC/UV methods is linear in the range 5.00 -1000 ng/mL in plasma. Linear functions with 1/y weighting and quadratic functions with weighting 1/y<sup>2</sup> function were used to fit the calibration curve standard data. The LLOQ (5.0 ng/mL) of both assays in plasma is not sensitive enough to measure λ<sub>z</sub> of the terminal disposition phase of tolvaptan after doses in the clinical range (15-60 mg).

Table 1. Validation Characteristics for the HPLC/MS/MS Assay for Tolvaptan in Plasma

Study	Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
156-05-254	Plasma	5.0-1000	≥ 0.9980	2.38-4.0	≤ 7.65	Yes	Yes
156-01-233	Plasma	5.0-1000	≥ 0.9978	-1.95-6.41	≤ 6.64	Yes	Yes
156-05-256	Plasma	5.0-1000	0.9962	-7.38-3.38	≤ 11.9	Yes	Yes
156-98-202	Plasma	5.0-1000	0.9988	-2.80-5.57	≤ 5.09	Yes	Yes
156-01-231	Plasma	5.0-1000	0.9976	-8.14-1.74	≤ 7.04	Yes	Yes
156-98-210	Plasma	5.0-1000	≥ 0.9957	-5.2-5.5	≤ 10.5	Yes	Yes
156-04-247	Plasma	5.0-1000	0.9980	-5.88-2.00	≤ 8.82	Yes	Yes
156-01-229	Plasma	5.0-1000	≥ 0.9957	-2.82-0.28	≤ 6.23	Yes	Yes
	Urine	5.0-1000	0.9992	0.38-5.89	≤ 6.07	Yes	Yes
156-98-201	Plasma	5.0-1000	No information	-5.76-9.01	≤ 5.83	Yes	Yes
156-03-239	Plasma	5.0-1000	0.9970	-1.13-6.88	≤ 9.32	Yes	Yes
156-03-240	Plasma	5.0-1000	0.9977	1.63-8.87	≤ 7.72	Yes	Yes
156-01-234	Plasma	5.0-1000	0.9989	-7.88-3.33	≤ 11.3	Yes	No information
156-03-245	Plasma	5.00-1000	0.9980	-2.50-8.87	≤ 8.14	Yes	Yes

Table 2. Validation Characteristics for the HPLC/UV Assay for Tolvaptan in Plasma

Study	Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
156-96-203	Plasma	5.0-1000	0.998	-21.8-3.7 <sup>a</sup>	≤ 16.9	Yes	No information
156-95-305	Plasma	5.0-1000	0.998	-0.1-1.8	≤ 9.5	Yes	Yes
156-00-221	Plasma	5.0-1000	0.998	-4.0-0.9	≤ 8.6	Yes	Yes
156-97-251	Plasma	5.0-1000	0.996	-9.8-2.3	≤ 14.9	Yes	Yes <sup>b</sup>
156-01-223	Plasma	5.0-1000	0.998	-3.7-12.6	≤ 8.3	Yes	Yes <sup>b</sup>
156-01-226	Plasma	5.0-1000	0.998	-5.2- (-)2.4	≤ 8.2	Yes	Yes <sup>b</sup>
156-01-225	Plasma	5.0-1000	0.998	-2.5-2.6	≤ 7.0	Yes	Yes <sup>b</sup>
156-96-205	Plasma	5.0-1000	0.999	0.2-7.9	≤ 16.6	Yes	No information
	Urine	2.5-500	0.997	4.4-7.9	≤ 10.0	Yes	Yes <sup>c</sup>

<sup>a</sup> exceeds the upper limit of 20% <sup>b</sup> no information provided on long term freezer and freeze/thaw cycle stability <sup>c</sup> only stability after 3 freeze/thaw reported

Table 3. Validation Characteristics for the Chiral HPLC/UV Assay for the Tolvaptan Enantiomers in Plasma

Study	Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
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156-96-305	S(-)Tolpaptan	2.5-1000	$\geq 0.996$	-16.6-16.0	$\leq 11.2$	Yes	No information <sup>a</sup>
	R(+)-Tolpaptan	2.5-1000	$\geq 0.996$	-19.0-14.7	$\leq 12.9$	Yes	

<sup>a</sup> No information on possible interconversion of enantiomers

#### ***S- and R-Warfarin and 7-Hydroxywarfarin and 10-hydroxywarfarin***

Study Report 156-01-225: "An Open Label, Randomized, Placebo Controlled and Cross-Over Study of the Potential Drug Interaction between Tolpaptan (OPC-41061) and Warfarin in Healthy Male and Female Subjects"

S- and R-warfarin were measured by HPLC/MS/MS assays

Table 4. Validation Characteristics for the HPLC/MS/MS Assay for S-Warfarin in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	1.00-100.00	0.9983	-4.4- (-)1.7	$\leq 9.4$	Yes	No information

<sup>a</sup> no information provided

Table 5. Validation Characteristics for the HPLC/MS/MS Assay for R-Warfarin in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	1.00-100.00	0.9978	-2.6- (-)2.6	$\leq 9.4$	Yes	No information

<sup>a</sup> no information provided

The assays for S- and R-warfarin cannot be considered fully validated.

7-Hydroxy- and 10-hydroxywarfarin were measured by HPLC/MS/MS assays

Table 6. Validation Characteristics of the HPLC/MS/MS Assay for 7-Hydroxywarfarin in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	5.00-1000.00	0.9974	-10.0-0.0	$\leq 9.4$	Yes	Yes <sup>a</sup>

<sup>a</sup> Stability of analyte in plasma after 3 freeze-thaw cycles and storage at room temperature for 5 h reported, long term stability in freezer not reported

Table 7. Validation Characteristics of the HPLC/MS/MS Assay for 10-Hydroxywarfarin in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	5.00-1000.00	0.9968	-4.8.0-(-)1.3	$\leq 8.3$	Yes	Yes <sup>a</sup>

<sup>a</sup> Stability of analyte in plasma after 3 freeze-thaw cycles and storage at room temperature for 5 h reported, long term stability in freezer not reported

The assay for 7-hydroxywarfarin and 10-hydroxywarfarin cannot be considered fully validated.

#### **Assay for Amiodarone and Desethylamiodarone**

Study Report 156-01-226:” An Open-Label, Single Arm, Sequential Design Study to Evaluate the Effects of Tolvaptan (OPC-41061) on the Pharmacokinetics of Oral Maintenance Amiodarone Therapy in Patients with Cardiac Arrhythmias”

Table 8. Validation Characteristics for the LC/MS/MS Assay of Amiodarone in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	100.18-4007.00	Not reported	-4.76-0.60	≤ 6.09	Yes	Yes

Table 9. Validation Characteristics for the LC/MS/MS Assay of Desethylamiodarone in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	99.88-3995.00	Not reported	-8.89-3.25	≤ 6.23	Yes	Yes

OPC-41061 does not interfere with the assay for amiodarone nor desethylamiodarone

The assays for amiodarone and desethylamiodarone can be considered validated

#### Assay for Rifampin

Study Report 156-03-239:” An Open-Label, Sequential Study of the Effects of Rifampin Administration on Tolvaptan Pharmacokinetics and Pharmacodynamics in Normal, Healthy Volunteers”

Table 10. Validation Characteristics for the HPLC/UV Assay for Rifampin in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	1-100	0.9952	-4.13 – 2.33	≤ 11.5	Yes	Yes

The assay for rifampin can be considered validated.

#### Assay for Lovastatin and Lovastatin β-hydroxyacid

Study Report: 156-01-223” A Study to Determine the Effects of Single Dose Administration of OPC-41061 on the Single Dose Pharmacokinetic Profile of Lovastatin in Healthy Male and Female Subjects”

Table 11. Validation Characteristics for the LC/MS/MS Assay for Lovastatin in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	0.10-10.0	0.9986	-6.0(-)0.31	≤ 28 <sup>a</sup>	Yes	Yes

<sup>a</sup> exceeds upper limit of 20%

Table 12. Validation Characteristics for the LC/MS/MS Assay for Lovastatin-β-hydroxyacid in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	1-1.00	0.9982	--3.65-1.67	≤ 23 <sup>a</sup>	Yes	Yes

<sup>a</sup> exceeds upper limit of 20%

#### Assay for Lovastatin and Lovastatin β-hydroxyacid

Study Report 156-01-233: "Open-Label, Randomized, Crossover Study to Assess Dose Strength Equivalence among 15, 30, and 60 mg Strength Oral Tablets of Tolvaptan"

Table 13. Validation Characteristics for the LC/MS/MS Assay for Lovastatin in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	0.10-10.0	0.9978	--3.69-1.00	≤ 11.55	Yes	Yes

Table 14. Validation Characteristics for the LC/MS/MS Assay for Lovastatin-β-hydroxyacid in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	1-1.00	0.9970	-4.00-3.67	≤ 15.11	Yes	Yes

The assays for lovastatin and lovastatin β-hydroxyacid can be considered validated

#### Assay for Digoxin

Study Report 156-01-241: "An Open Label Study of the Pharmacokinetic Interaction between Tolvaptan and Digoxin Following Multiple Oral Doses to Healthy Men and Women"

Table 15. Validation Characteristics for the Turbidimetric Immunoassay for Digoxin in Plasma and Urine

Matrix	Calibration Range ng/mL	Calibration Curve Fit R <sup>2</sup>	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	0.325- 6.0	≥ 0.9966	No information	No information	Yes	No information
Urine	0.325- 6.0	≥ 0.9966	No information	No information	Yes	No information

Based on the information provided the turbidimetric immunoassay for digoxin cannot be considered validated in plasma or urine.

#### Assays for Furosemide and Hydrochlorothiazide in Plasma and Urine

Study Report 156-96-205: "Single Center, Randomized, Open-Label, Safety Study to Assess Potential Pharmacodynamic and Pharmacokinetic Interactions between OPC-41061 and the Diuretics Furosemide and Hydrochlorothiazide"

Table 16. Validation Characteristics for the HPLC/UV Assay for Furosemide in Plasma and Urine

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	20-2000	0.9997	-6.5-3.0	≤ 7.7	Yes	Yes <sup>a</sup>
Urine	1000-100000	0.9961	-10.0-(-)1.3	≤ 6.7	Yes	Yes <sup>a</sup>

<sup>a</sup> Long term freezer stability not reported

Table 17. Validation Characteristics for the HPLC/UV Assay for Hydrochlorothiazide in Plasma and Urine

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	10-500	0.9999	2.0-7.0	≤ 10.2	Yes	Yes
Urine	1000- 50000	0.9996	2.5-3.0	≤ 5.7	Yes	Yes

<sup>a</sup> Long term freezer stability not reported

The assay for hydrochlorothiazide in plasma and urine can be considered validated.

#### Assay for Ketoconazole in Plasma

Table 18. Validation Characteristics for the HPLC/UV Assay for Ketoconazole in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	50-10000	0.9997	-0.68-(-) 0.28	≤ 7.61	Yes	No information

#### Assay for Moxifloxacin in Plasma

Study Report: 156-03-245.” A Parallel Arm, Double-Blind, Placebo Controlled, Multiple Dose Administration Study of Tolvaptan Oral Tablets and Moxifloxacin Oral Tablets on ECG QTc Interval in Healthy Men and Women”

Table 19. Validation Characteristics for the LC/MS/MS Assay for Moxifloxacin in Plasma

Matrix	Calibration Range ng/mL	Calibration Curve Fit r	Accuracy %	Precision %	QC Samples Used	Stability Tested
Plasma	10-1000	0.9982	0.5- 7.7	≤11.4	Yes	Yes

The assay for moxifloxacin in plasma can be considered validated.

#### 2.6.4.2 What are the lower and upper limits of quantification (LLOQ/ULOQ)?

See above tables

#### 2.6.4.3 What are the accuracy, precision, and selectivity at these limits?

See above tables

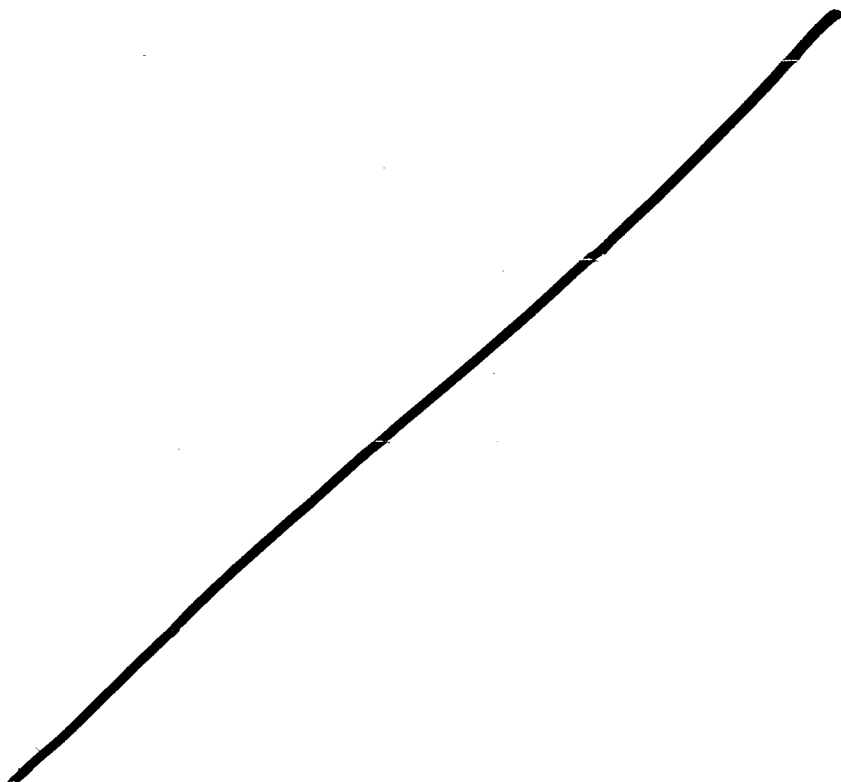
#### 2.6.4.4 What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample handling, sample transport, autosampler)?

Long term freezer, freeze/thaw cycle, bench top-, and auto sampler-stability of tolvaptan in plasma and urine have been demonstrated. The corresponding information on stability is not available or incomplete for R- and S- warfarin and metabolites and ketoconazole in plasma and digoxin in plasma and urine.

#### 2.6.4.5 What is the QC sample plan?

QC samples were co-analyzed along the samples to be assayed for concentrations within the range of the calibration curves for tolvaptan and metabolites and all other drugs co-assayed in the drug-drug interaction studies.

### 3. LABELING RECOMMENDATIONS



b(4)

32 Page(s) Withheld

       Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

       Draft Labeling (b5)

       Deliberative Process (b5)

#### 4. INDIVIDUAL STUDY REPORTS

##### Study Report 009473: "Binding of $^{14}\text{C}$ -OPC-41061 to Human Plasma Proteins"

###### Study Site and Investigator

H. Okumura, M.S.  
Tokushima Research Institute  
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Japan

###### Methods

The purity of  $^{14}\text{C}$ -OPC-41061 was determined by TLC. Blood was collected from 5 healthy adult male volunteers. After addition of heparin plasma was obtained by centrifugation. The plasma from the 5 volunteers was pooled for the binding experiments. The plasma protein binding of radiolabeled tolcapatan was determined by an ultra-filtration procedure. 1 mL of plasma spiked with  $^{14}\text{C}$ -OPC-41061 was added to the ultra-filtration apparatus ( [REDACTED] ) and incubated for 5 min at 37° C. Then the plasma sample was centrifuged at room temperature and an ultrafiltrate of 0.5 mL obtained. The percent of drug bound in plasma, % PB, was obtained from:

b(4)

1- [concentration of radioactivity in ultrafiltrate/concentration of radioactivity in plasma] • 100

The concentrations of tolcapatan in the experiments were 0.1 µg/mL, 1 µg/mL and 10 µg/mL.

The binding of radio-labeled tolcapatan to the individual human plasma proteins serum albumin (HSA, 40 mg/mL), α1-acid glycoprotein (AGP, 10 mg/mL) and IgG (10 mg/mL) was also studied using the same method. The individual proteins were dissolved in phosphate buffer.

The possible impact of lowering the concentration of albumin (down to 1 mg/mL) on the binding of tolcapatan (0.1 µg/mL) was investigated in an additional experiment.

The possible displacement of tolcapatan (0.1 µg/mL (by diazepam, digitoxin and warfarin, 1-100 µM ) was also studied using HSA (0.1 mg/mL) and AGP (1 mg/mL). Diazepam, digitoxin and warfarin are known to be bound to specific bindings sites on HSA.

Triplicate determinations of the binding of OPC-41061 to plasma proteins were made and mean and SD determined.

The concentrations of radio-labeled OPC-41061 were measured by liquid scintillation spectrometry (external standard, channel ratio method)

###### Results

The binding of tolcapatan to plasma, human serum albumin, AGP and IgG are shown in Tables 1 and 2 below:

Table 1 In vitro binding of [ <sup>14</sup> C]-OPC-41061 to human plasma protein	
Concentration( $\mu$ g/ml)	binding rate (%)
0.1	98.5 $\pm$ 0.2
1	98.5 $\pm$ 0.3
10	98.0 $\pm$ 0.3

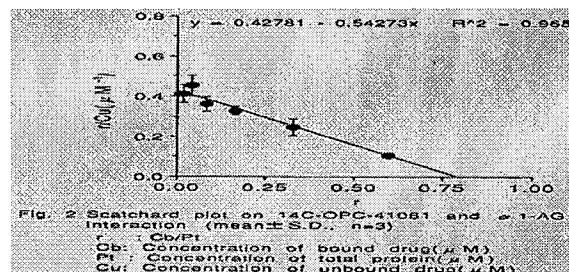
Each value represents the mean  $\pm$  S.D. (n=3)

Table 2 In vitro binding of [ <sup>14</sup> C]-OPC-41061 to isolated human plasma proteins			
Concentration( $\mu$ g/ml)	binding rate (%)		
	HSA (10mg/ml)	$\alpha$ -AG (1mg/ml)	IgG (10mg/ml)
0.1	97.0 $\pm$ 0.3	95.2 $\pm$ 0.3	99.3 $\pm$ 1.8
1	97.7 $\pm$ 0.2	93.9 $\pm$ 0.5	97.5 $\pm$ 2.0
10	97.3 $\pm$ 0.1	92.2 $\pm$ 0.8	93.8 $\pm$ 0.2

Each value represents the mean  $\pm$  S.D. (n=3)

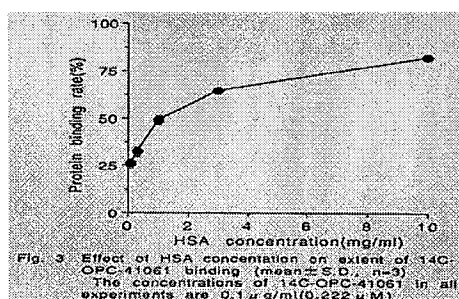
The results indicate that the plasma protein binding of OPC-41061 is about 98 % and the main binding proteins are albumin and AGP. The binding of OPC-41061 to plasma, albumin and IgG at physiological concentrations appears to be concentration independent, whereas the binding of OPC-41061 to AGP tends to decrease with increasing concentrations.

As shown in the figure below the Scatchard plot for OPC-41061 binding to AGP is linear with a negative slope suggesting that a single class of binding sites exists for OPC-41061 on AGP:

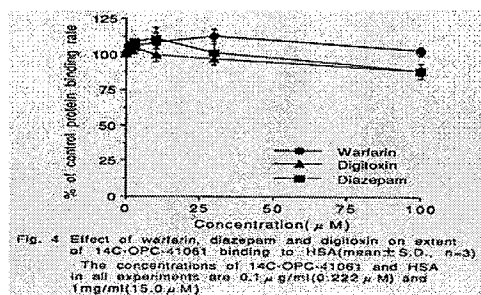


The association constant of OPC-41061 to AGP is  $5.43 \cdot 10^5 \text{ M}^{-1}$ .

The extent of the binding of tolcapton to HSA decreases as the concentrations of the protein are lowered from physiological levels to < 10 mg/ml as shown in the below plot:



The presence of warfarin, digitoxin and diazepam had no effect on the binding of OPC-41061 to plasma as shown below:



## Conclusion

The binding of OPC-41061 at concentrations of  $0.1$ - $10 \mu\text{g/mL}$  appears to be in excess of 90% and concentration independent. The main binding proteins of OPC-41061 are HAS and AGP. The binding of OPC-41061 to HAS in the presence of diazepam, digitoxin or warfarin appears to be similar. However, the results on the plasma protein binding of racemic OPC-41061 using the ultrafiltration method may be biased. The binding of OPC-41061 to the filter, the impact of the ultrafiltrate volume on the ultrafiltrate concentration was not tested, the pH of plasma and ultrafiltrate during filtration was not controlled and the experiments were performed at room temperature.

## Comments

1. The binding of OPC-41061 to the filter of the ultra-filtration apparatus was not tested so that the extent of the plasma protein binding of OPC-41061 may be overestimated.
2. The ultra-filtrate volume of  $0.5 \text{ mL}$  is too large relative to  $1 \text{ mL}$  volume of the plasma sample subjected to filtration. During the filtration process the protein concentration increased by a factor of 2 and could have impacted the equilibrium between bound and unbound tolcapten. Also the increased protein concentration may have impacted the passage of unbound OPC-41061 through the pores of the filter.
3. The report does not state that the pH of the spiked protein solution was controlled.

4. Contrary to the statement on p. 5, third paragraph, of the report a Scatchard plot of the binding of OPC-41061 to HSA is not shown and the explanation given for finding  $r/C_u$  not to depend on  $r$  is erroneous. The binding of OPC-411061 to HSA under the conditions chosen by the sponsor is not saturable.
5. The plasma protein binding of the individual enantiomers should have been investigated.
6. The radiochemical purity of the labeled OPC-41061 is not indicated.

**Study Report 015892:” Effects of OPC-41061 and DM-4103 on Human Plasma Protein Binding of Concomitant Drugs”**

**Investigator and Study Site**

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**Objectives**

To study in vitro the effects of OPC-41061 and the metabolite DM-4103 on the human plasma protein binding of potentially co-administered propranolol, lidocaine and spironolactone

**Methods**

Heparinized blood was obtained from 6 healthy volunteers and plasma was generated by centrifugation. The individual plasma samples were pooled.

The studied drugs and concentrations are shown in the below scheme:

Group	Human Sample	Concomitant drug		Test Substance(Final Concentration)
		Radioactive Chemical	Unlabeled Chemical(Final Concentration)	
A1	Plasma	<sup>3</sup> H-Propranolol	Propranolol (0.3 µg/mL)	Vehicle
A2				OPC-41061 (0.5 µg/mL)
A3				DM-4103 (3 µg/mL)
B1	Plasma	<sup>14</sup> C-Lidocaine	Lidocaine (5 µg/mL)	Vehicle
B2				OPC-41061 (0.5 µg/mL)
B3				DM-4103 (3 µg/mL)
C1	Plasma	<sup>3</sup> H-Spironolactone	Spironolactone (0.5 µg/mL)	Vehicle
C2				OPC-41061 (0.5 µg/mL)
C3				DM-4103 (3 µg/mL)

The selected concentrations of the tested drugs are equivalent to or greater than those reported to occur in vivo.

A possible drug binding of propranolol, lidocaine or spironolactone to the \_\_\_\_\_ apparatus was tested by filtrating samples of 0.5 mL phosphate buffer saline (PBS) containing the radio-labeled drugs. Recovery was calculated from:

b(4)

% Recovery= concentration of radioactivity in filtrate/concentration of radioactivity in PBS

In the definitive experiments 4.5 mL plasma was spiked with drug or vehicle (methanol) at room temperature. Then two portions of each plasma solution were ultra-filtered by centrifugation for 20 min at 24° C.

The percentage of drug bound in plasma was obtained from:

1- [concentration of radioactivity in ultra-filtrate/concentration of radioactivity in plasma] • 100

The effect of the presence of OPC-41061 or DM-4103 on the plasma protein binding of the potentially co-administered drugs was computed from the ratio of the mean percent bound propranolol, lidocaine and spironolactone in the presence of OPC-41061 or DM-4103 to that in the absence of OPC-41061 or DM-4103.

Radioactivity was measured by liquid scintillation spectrometry (channel ratio method using an external standard)

## RESULTS

The recovery of propranolol, lidocaine and spironolactone was 89.0%, 99.5% and 83.0%, respectively, indicating some binding of propranolol and spironolactone to the ultrafiltration apparatus.

The results of the binding of propranolol, lidocaine and spironolactone in the presence of OPC-41061 are shown in the table below:

Table 1 Effects of OPC-41061 and DM-4103 on human plasma protein binding of concomitant drugs (*in vitro*)

Group	Test substance (concentration)	Concomitant drug (concentration)	Protein binding (%)		% of control
				Mean	
A1	Vehicle control	Propranolol (0.3 µg/mL)	92.1	91.8	100
			91.1		
A2	OPC-41061 (0.5 µg/mL)		91.4	90.9	99.2
			90.3		
A3	DM-4103 (3 µg/mL)		91.8	91.8	100.3
			92.0		
B1	Vehicle control	Lidocaine (5 µg/mL)	68.9	68.6	100
			68.3		
B2	OPC-41061 (0.5 µg/mL)		63.7	63.5	95.3
			63.3		
B3	DM-4103 (3 µg/mL)		65.9	66.5	99.8
			67.1		
C1	Vehicle control	Spironolactone (0.5 µg/mL)	88.5	85.7	100
			85.0		
C2	OPC-41061 (0.5 µg/mL)		87.8	87.1	101.6
			86.6		
C3	DM-4103 (3 µg/mL)		84.3	84.7	98.8
			85.1		

Vehicle control: methanol

The results appear to indicate no major impact of OPC-41061 or DM-4103 on the plasma protein binding of propranolol, lidocaine or spironolactone.

## Conclusions

The plasma protein binding of propranolol, lidocaine or spironolactone appears not to be significantly impacted by OPC-41061 or DM-4103 at therapeutic concentrations. However, the results on the plasma protein binding of propranolol, lidocaine or spironolactone using the ultrafiltration method may be biased. The binding of the tested

drugs to the filter, the impact of the ultrafiltrate volume on the ultrafiltrate concentration was not tested, the pH of plasma and ultrafiltrate during filtration was not controlled and the experiments were performed at room temperature.

#### **Comments**

1. The experiments should have been conducted at 37 ° C. Temperature can affect the degree of binding.
2. Control of the pH of the plasma ultra-filtrate during the filtration process was not reported.
3. The volume of the ultra-filtrate was not indicated. The appropriateness of the volume of the ultra-filtrate relative to the volume of the plasma sample filtered cannot be assessed
4. It would have been more meaningful to study the plasma protein binding of propranolol, lidocaine and spironolactone in plasma samples spiked with OPC 41061 and DM-4103.
5. The binding of OPC 41061 and DM4103 to the ultra-filtration apparatus should have been determined.

#### **Study Report 021987: “ Interaction of Protein Binding in Human Plasma between OPC-41061, Metabolites and Concomitant Drugs in Vitro; Method Validation and Protein Binding Measurement”**

#### **Investigator and Study Site**

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Tokushima Research Institute  
Otsuka Pharmaceutical Co., Ltd.  
Tokushima  
Japan

#### **Objectives**

1. To assess the impact of potentially co-administered drugs including propranolol, furosemide, disopyramide, warfarin, lidocaine and spironolactone on the plasma protein binding of OPC-41061, DM-4103 and DM-4107, the major metabolites of OPC-41061.
2. To validate the assay methods to determine OPC-41061, DM-4103 and DM-4107 in plasma and ultrafiltrate.

#### **Methods**

##### ***Protein binding***

Heparinized blood was obtained from five healthy adult men. Plasma was generated by centrifugation. After pooling, the plasma samples were spiked with OPC-41061, DM-4103, DM-4107 or control (DMSO) to attain the following final concentrations:

Group	Sample	Test article (Final concentration)	Concomitant drug (Final concentration)
A1	Plasma	OPC-41061 (0.5 µg/mL)	Control (DMSO)
A2			Furosemide (1 µg/mL)
A3			Spirolactone (0.5 µg/mL)
A4			Propranolol (0.05 µg/mL)
A5			Disopyramide (2 µg/mL)
A6			Lidocaine (5 µg/mL)
A7			Warfarin (10 µg/mL)
B1	Plasma	DM-4103 (3 µg/mL)	Control (DMSO)
B2			Furosemide (1 µg/mL)
B3			Spirolactone (0.5 µg/mL)
B4			Propranolol (0.05 µg/mL)
B5			Disopyramide (2 µg/mL)
B6			Lidocaine (5 µg/mL)
B7			Warfarin (10 µg/mL)
C1			Control (DMSO)
C2			Furosemide (1 µg/mL)
C3			Spirolactone (0.5 µg/mL)

The drug levels used were equivalent or greater than those observed to occur in vivo. The spiked samples were kept at 37° C for 10 minutes. The plasma samples were then centrifuged for 20 minutes at 25° C using \_\_\_\_\_). Replicate determinations of the plasma protein binding were made. Percent protein binding was determined from:

b(4)

1- [concentration of radioactivity in ultra-filtrate/concentration of radioactivity in plasma] • 100

If the concentration in the ultra-filtrate was less than the LLOQ, the value of the LLOQ was used.

The effect of the presence of the potentially co-administered drugs on the binding of OPC-41061, DM-4107 or DM-4103 to plasma proteins was computed from the ratio of the mean percent bound OCP-41061, DM-4107 or DM-4103 in the presence of the co-administered drugs to that in the absence of the co-administered drugs.

#### Assay

A LC/MS/MS method with electrospray ionization and positive ionization mode was employed. The method used an internal standard. The stability of OPC-41061, DM-4103 and DM-4107 in plasma and ultrafiltrate exposed to 4° C for 24 h in the autosampler was investigated.

## RESULTS

### Plasma Protein Binding

The results on the binding of OPC-41061, DM-4107 and DM-4103 to plasma proteins in the absence and presence of potentially co-administered drugs are shown in the below tables:

Table 7 Human plasma protein binding of OPC-41061, DM-4103 and DM-4107					
Compound	Sample	Ultrafiltrate		Plasma Concentration (ng/mL)	Protein binding (%)
		Concentration (ng/mL)	Mean (ng/mL)		
OPC-41061	1	2.525	4.000	525.9	99.2
	2	3.478			
	3	5.998			
DM-4103	1	<5.000	<5.000	2699	> 99.8
	2	<5.000			
	3	<5.000			
DM-4107	1	8.301	7.460	508.2	98.5
	2	7.543			
	3	6.536			

Table 8: Concentration in plasma and stability during the protein binding assay

Compound	Sample	Concentration (ng/mL)	Content (%)	Residual content (%)
OPC-41061	Reference	533.2	106.6	-
	Incubation	525.9	-	98.6
DM-4103	Reference	2630	87.7	-
	Incubation	2699	-	102.6
DM-4107	Reference	570.6	114.1	-
	Incubation	508.2	-	89.1

References: No incubation

Incubation: Incubation at 37°C for 10 minutes

Content: Concentration in plasma / nominal concentration x 100

Residual content: % of control (reference)

Table 9: Effect of the concomitant drug on human plasma protein binding of OPC-41061

Sample	Concomitant drug (Concentration)	Concentration in ultrafiltrate (ng/mL)		Protein binding (%)	% of control (%)
			Mean (ng/mL)		
A1a	Control (DMSO)	5.818	7.230	98.8	100
A1b		8.642			
A2a	Furosemide (1 µg/mL)	6.067	6.773	98.6	100.1
A2b		7.479			
A3a	Spirinolactone (0.5 µg/mL)	6.453	7.943	98.4	99.9
A3b		9.433			
A4a	Propafenol (0.05 µg/mL)	8.249	8.361	98.3	99.8
A4b		8.473			
A5a	Disopyramide (2 µg/mL)	6.791	7.279	98.5	100.0
A5b		7.767			
A6a	Lidocaine (5 µg/mL)	6.957	7.914	98.4	99.9
A6b		8.671			
A7a	Warfarin (10 µg/mL)	6.889	7.535	98.5	99.9
A7b		8.180			

OPC-41061: 0.5 µg/mL

Table 10: Effect of the concomitant drug on human plasma protein binding of DM-4103

Sample	Concomitant drug (Concentration)	Concentration in ultrafiltrate (ng/mL)		Protein binding (%)	% of control (%)
			Mean (ng/mL)		
B1a	Control (DMSO)	< 5.00	< 5.00	> 99.8	100
B1b		< 5.00			
B2a	Furosemide (1 µg/mL)	< 5.00	< 5.00	> 99.8	> 99.8
B2b		< 5.00			
B3a	Spirinolactone (0.5 µg/mL)	< 5.00	< 5.00	> 99.8	> 99.8
B3b		< 5.00			
B4a	Propafenol (0.05 µg/mL)	< 5.00	< 5.00	> 99.8	> 99.8
B4b		< 5.00			
B5a	Disopyramide (2 µg/mL)	< 5.00	< 5.00	> 99.8	> 99.8
B5b		< 5.00			
B6a	Lidocaine (5 µg/mL)	< 5.00	< 5.00	> 99.8	> 99.8
B6b		< 5.00			
B7a	Warfarin (10 µg/mL)	< 5.00	< 5.00	> 99.8	> 99.8
B7b		< 5.00			

DM-4103: 3 µg/mL

Table 11: Effect of the concomitant drug on human plasma protein binding of DM-4107

Sample	Concomitant drug (Concentration)	Concentration in ultrafiltrate (ng/mL)	Mean (ng/mL)	Protein binding (%)	% of control (%)
C1a	Control (DMSO)	4.846	4.632	99.1	100
C1b		4.817			
C2a	Furosemide (1 µg/mL)	8.582	8.447	98.3	99.2
C2b		8.512			
C3a	Spironolactone (0.5 µg/mL)	5.960	6.173	98.8	99.7
C3b		6.386			
C4a	Propranolol (0.05 µg/mL)	5.820	5.776	98.8	99.8
C4b		5.732			
C5a	Disopyramide (2 µg/mL)	6.667	6.253	98.7	99.7
C5b		5.439			
C6a	Lidocaine (5 µg/mL)	4.800	6.329	98.7	99.7
C6b		7.857			
C7a	Warfarin (10 µg/mL)	7.006	7.657	98.6	98.5
C7b		7.107			

DM-4107: 0.5 µg/mL

The results appear to indicate no significant impact of any of the potentially co-administered drugs on the plasma protein binding of OPC-41061 or DM-4107. Because the concentrations of DM-4103 in the ultra-filtrate were below the LLOQ, no conclusion can be drawn regarding the potential impact of the tested potentially co-administered drugs on the plasma protein binding of DM-4103. However, the results on the plasma protein binding of propranolol, lidocaine or spironolactone using the ultrafiltration method may be biased. The binding of the tested drugs to the filter, the impact of the ultrafiltrate volume on the ultrafiltrate concentration was not tested, the pH of plasma and ultrafiltrate during filtration was not controlled and the experiments were performed at room

#### Assay

The calibration curves in plasma were linear over the range of 12 ng/mL to 4000 ng/mL for OPC-41061 and DM-4107. The calibration curve for DM-4103 was linear over the range of 30 ng/mL to 10 000 ng/mL. The coefficients of determination for the analytes exceeded 0.9992.

The precision and accuracy estimated from the QC samples in plasma were for OPC-4106, DM-4103 and DM-4107 as follows:

Compound	Matrix	Precision, %	Accuracy, %
OPC-41061	Plasma	≤ 2.8	-2.6 -13.1
	Ultrafiltrate	≤ 4.2	-8.4 - 3.3
DM-4103	Plasma	≤ 4.7	-6.0 -6.4
	Ultrafiltrate	≤ 9.3	-21.7 -11.8
DM-4107	Plasma	≤ 6.7	-7.6 -14.8
	Ultrafiltrate	≤ 7.2	-18.5 - (-) 0.7

The stability of OPC-41061, DM-4103 and DM-4107 in plasma and ultra-filtrate exposed to 4 °C for 24 h in the autosampler was demonstrated by recoveries that were reasonably close to 100 %.

#### Conclusion

Propranolol, furosemide, spironolactone, disopyramide, lidocaine and warfarin appear not to impact the plasma protein binding of OPC-41061 and its metabolites DM-4103 and DM-4107.

The LC/MS/MS assay measures OPC-41061, DM-4107 and D-4103 in plasma precisely and accurately and with the required sensitivity. The LC/MS/MS assay measures OPC-41061 and DM-4107 also in ultra-filtrate with the required sensitivity, precision and accuracy. However, the LC/MS/MS assay for DM-4103 does not display the required sensitivity. The 3 analytes were found to be stable when exposed to 4° C for 24 h in the auto-sampler.

#### Comments

1. The binding of OPC 41061, DM4103 and DM-4107 to the ultra-filtration filter should have been determined.
2. The experiments should have been conducted at 37° C.
3. The report does not state that the pH during ultra-filtration was kept constant at 7.4.
4. The volume of the ultra-filtrate was not indicated. The appropriateness of the volume of the ultra-filtrate relative to the volume of the plasma to be filtered cannot be assessed and thus a bias in the binding estimates cannot be excluded.
5. The plasma protein binding of the individual enantiomers of OPC-41061 should have been determined.

**Study Report 016298: "In Vitro Metabolism of MOP-21826, DM-4104, DM-4105 and DM-4107 Using Humans Liver Supernatant Fraction (S9)"**

**Investigator and Study Site**

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Tokushima Research Institute  
Otsuka Pharmaceuticals Co. Ltd.  
Tokushima  
Japan

**Objectives**

To elucidate the metabolic pathway of OPC-41061 to DM-4103. DM-4103 is a major circulating metabolite of OPC-41061. The in vitro metabolism of MOP-21826, DM-4104, DM-4105 and DM-4107 to DM-4103 is investigated in this study.

**Methods**

Synthesized MOP-21826, DM-4104, DM-4105, DM-4107 and OPC-41061 were available. Pooled human liver supernatant fractions (protein content 25 mg/mL) were prepared from the livers of 10 donors. The standard mixture contained 0.1 M pH 7.4 phosphate buffer pH 7.4, 2.5 mM NADPH, 2.5 mM NADH, 0.5 mg (total protein) human liver S9 and 5 µg/mL of the test or control article in a final volume of 0.5 mL (n=2). The standard mixture was pre-incubated without the test article at 37° C for 3 min. After addition of the test article, the reactions were carried out at 37° C for 2 h. The reaction was stopped by adding 0.5 mL of acetonitrile. After transfer to polypropylene tubes the mixture was centrifuged the supernatant fraction analyzed by a HPLC assay with UV detector.

The following experiments were conducted in parallel:

Group A (control): No incubation, mixtures contains water instead of S9  
Group B (control): No incubation  
Group C (control): Incubation for 2 h, mixture contains water instead of S9  
Group D: Incubation for 2 h  
Group E: Check of enzyme activity in S9 using OPC-41061 instead of the test substance  
Replicate experiments were performed with Groups A-E.

The results of Groups B and D were compared.

The test substances included MOP-21826, DM-4104, DM-4105 and DM-4107. The standards included DM-4103 and OPC-41061.

The products in the reaction mixture were determined by the retention times of the test-and standard-chromatograms

## Results

Human liver S9 catalyzes the metabolism of OPC-41061 to DM-4104, DM-4107 and other metabolites as shown by the chromatogram:

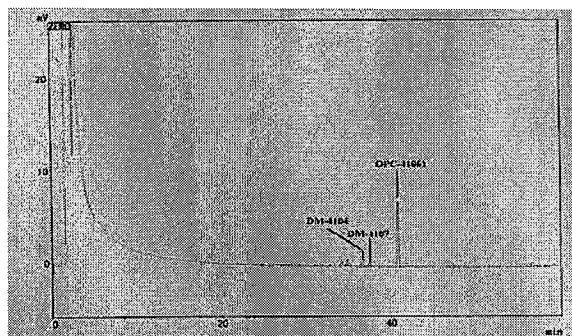


Figure 1 Chromatogram of incubation mixture of OPC-41061 with human liver S-9 fraction using HPLC with ultraviolet detection

DM-4103, DM-4104, DM-4105, DM-4107 and OCP-41061 are produced from MOP-21826 as shown in the next chromatogram:

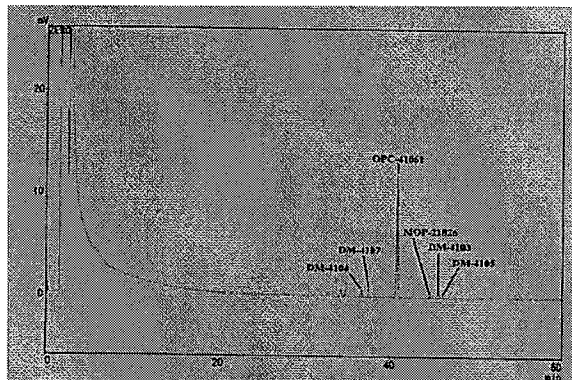


Figure 2 Chromatogram of incubation mixture of MOP-21826 with human liver S-9 fraction using HPLC with ultraviolet detection

DM-4107 is generated from DM-4104 and DM-4103 from DM-4105 as shown by the next 2 chromatograms:

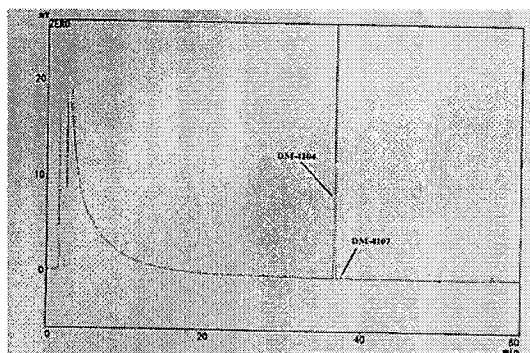


Figure 3 Chromatogram of incubation mixture of DM-4104 with human liver S-9 fraction using HPLC with ultraviolet detection.

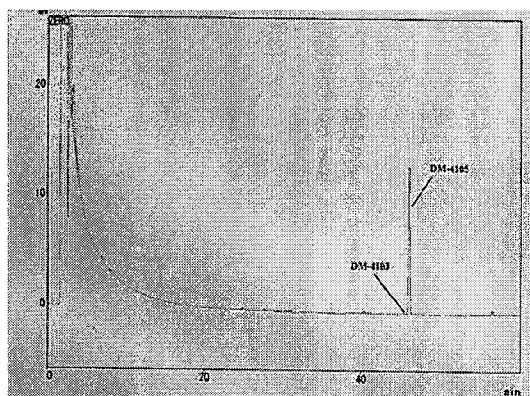


Figure 4 Chromatogram of incubation mixture of DM-4105 with human liver S-9 fraction using HPLC with ultraviolet detection.

However, DM-4103, DM-4104, DM-4105 and OPC-41061 were not detected when DM-4107 was incubated with the reaction mixture:

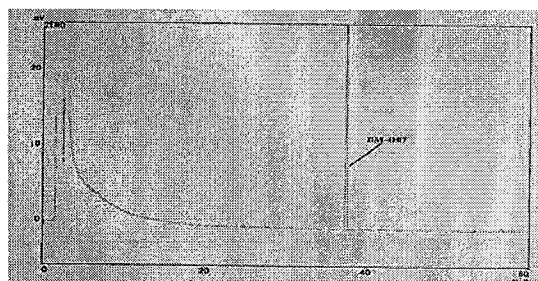
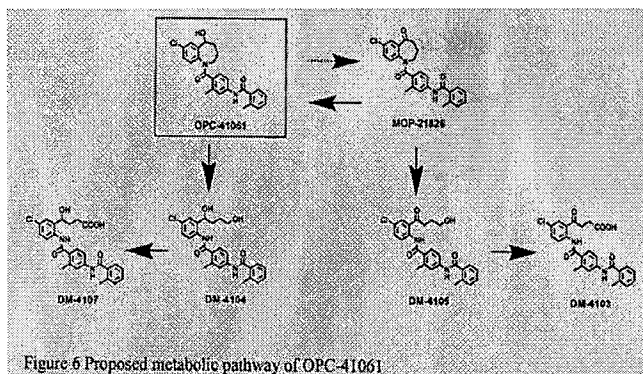


Figure 5 Chromatogram of incubation mixture of DM-4107 with human liver S-9 fraction using HPLC with ultraviolet detection.

Thus, the sponsor claims, that DM-4103 must be produced by MOP-21826 via DM-4105 and DM-4107 via DM-4104. The sponsor proposed metabolic pathway of OPC-41061 is depicted by the scheme below:



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## Conclusion

Before the proposed metabolic scheme for OPC-41061 can be accepted the sponsor should provide evidence that the very small traces of metabolites 4107 and 4103 after incubation of DM-4104 (Figure 3) and DM-4105 (Figure 4), respectively, are significantly greater than background.

## Comments

1. The sponsor should indicate what minimal ratio of LLOQ to background was applied using the HPLC method.
2. The possible stereo-specificity of the metabolism of the enantiomers should have been determined.

## Study Report 017665: "In Vitro Metabolism of DM-4103 Using Human Liver Supernatant Fraction (S9)"

### Investigator and Study Site

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### Objectives

To examine the metabolism of DM-4103 in vitro using the supernatant fraction (S9) prepared from human liver in order to illuminate the metabolic pathway of OPC-41061. DM-4103 is a major circulating metabolite of OPC-41061.

### Methods

Synthesized DM-4103 was available. Pooled human liver supernatant fractions (protein content 25 mg/mL) were prepared from the livers of 10 donors. The standard mixture contained 100 mM potassium phosphate buffer pH 7.4, 2.5 mM NADPH, 2.5 mM NADH, 0.5 mg (total protein) human liver S9 and 5 µg/mL of the test or control article in a final volume of 0.5 mL (n=2). The standard mixture was pre-incubated without the test article at 37° C for 3 minutes. After addition of the test article, the reactions were carried out at 37° C for 2 h. The reaction was stopped

by adding 0.5 mL of acetonitrile. After transfer to polypropylene tubes the mixture was centrifuged at 15 000 rpm for 10 minutes and the supernatant fraction analyzed by a HPLC assay with UV detector.

The following experiments were conducted in parallel:

Group A (control): No incubation, mixtures contains water instead of S9

Group B (control): No incubation

Group C (control): Incubation for 2 h, mixture contains water instead of S9

Group D: Incubation for 2 h

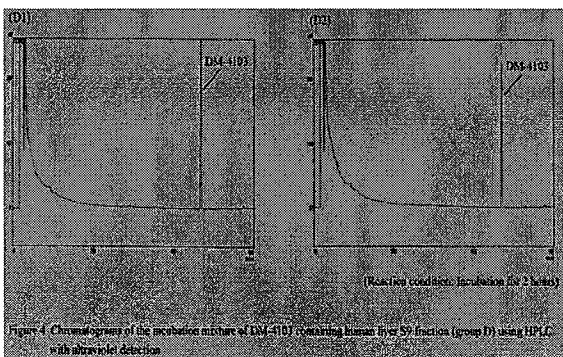
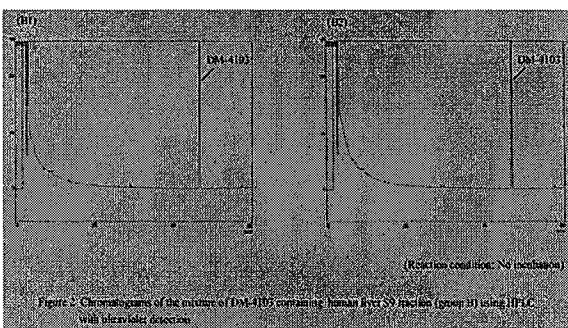
Group E: Check of enzyme activity in S9 using OCP-41061 instead of the test substance

Replicate experiments were performed with Groups A-E.

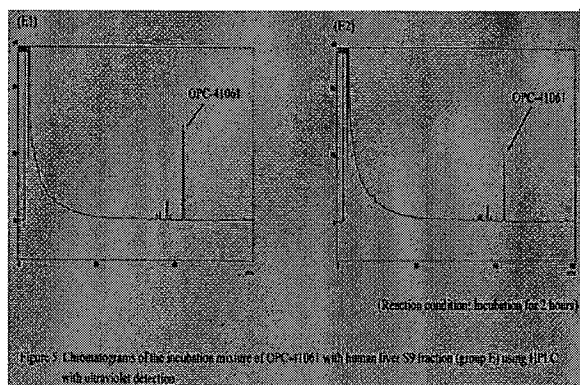
The results of Groups B and D were compared.

## Results

The chromatograms of Groups B, D and E are shown below:



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The chromatograms of Group B (and the other controls) and D are similar and show DM-4103 peaks with retention times ranging between 46.7 to 46.9 min. No traces of metabolites are found in Groups B and D. In Group E traces of metabolites are visible, but none of the retention times of these metabolites corresponds to that of DM-4103.

The sponsor concludes from the results that DM-4103 is a terminal metabolite as shown in the below scheme:

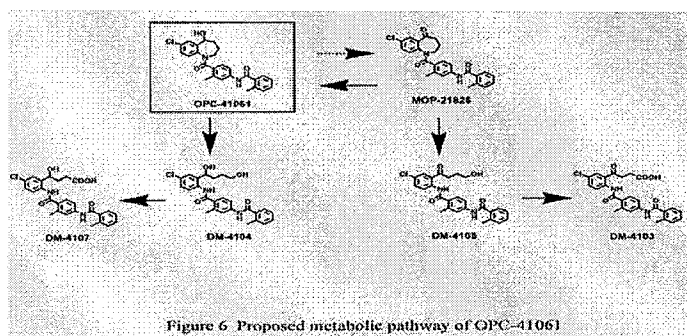


Figure 6 Proposed metabolic pathway of OPC-41061

#### Conclusion

The metabolite DM-4103 appears not to be metabolized by human liver S9 under the conditions used in the experiments.

#### Comments

1. The ratio of the LLOQ to the background of the HPLC method should have been defined for the compounds of interest.

#### Study Report 014276: "Involvement of Cytochrome P450 in the Formation of DM-4128 from OPC-41061"

##### Investigator and Study Site

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## Objectives

To assess the enzyme mediated production of DM-4128.

## Methods

Synthesized OPC-41061, DM-4101, DM-4102, DM-4128 were available.

Pooled human liver microsomes (HLM) (protein content 20 mg/mL, and pooled human liver cytosol (HLC) (protein content 15 mg/mL) were obtained commercially. Cell microsomes (protein content 10 mg/mL) containing human CYP1A1 + P450 reductase, 1A2, 2A6 + P450 reductase, 2B6, 2C8 + P450 reductase, 2C9-Cys + P450 reductase, 2C19, 2D6-Val + reductase, 2E1 + reductase, 3A4 + P450 reductase and 4A11 were obtained commercially as well.

### *Metabolism of OPC-41061 to DM-4128 Using Human Liver Microsomes*

The standard mixture contained 290  $\mu$ L or 340  $\mu$ L 0.1 M pH 7.4 phosphate buffer, 10  $\mu$ L 2.5 mM OPC-41061 dissolved in 50% DMSO, 100  $\mu$ L HLM and 5 mM  $\beta$ -NADPH and 5 mM  $\beta$ -NADH in a final volume of 0.5 mL. The standard mixtures without OPC-41061 were pre-incubated at 37° C for 3 min. After addition of OCP-41061 the reactions were carried out at 37° C for 20 min. After addition of the internal standard the reaction mixtures were centrifuged and the supernatants analyzed by HPLC. The final concentrations of OPC-41061 used were 1, 2.5, 5, 10, 20, 30, 40 and 50  $\mu$ M.

The effect of  $\beta$ -NADPH and  $\beta$ -NADH on the metabolism of OPC-41061 to DM-4128 was evaluated. In determining the  $K_m$  and  $V_{max}$  of the reaction 5 mM of  $\beta$ -NDAPH was used.

### *Metabolism of OPC-41061 to DM-4128 Using Human Liver Microsomes and Human Liver Cytosols*

The standard mixture contained 390  $\mu$ L or 440  $\mu$ L 0.1 M Tris-HCl buffer of pH 7.4, 8.0 or 8.6, 10  $\mu$ L 0.25 mM or 2.5 mM OPC-41061 dissolved in DMSO, 50  $\mu$ L HLM or 100  $\mu$ L HLC. The standard mixtures were incubated at 37° C for 1 h. After addition of the internal standard the reaction mixtures were centrifuged and the supernatants analyzed by HPLC.

### *Metabolism of OPC-41061, DM-4101(=R (+)-OPC-41061) and DM-4102(=S (-) OPC-41061) to DM-4128 Using Cell Microsomes containing Human CYP450s*

The standard mixture contained 240  $\mu$ L 0.1 M pH 7.4 phosphate buffer or Tris-HCl buffer, 200  $\mu$ L microsomes (2 mg protein), 50  $\mu$ L of 50 mM  $\beta$ -NADPH and 10  $\mu$ L 2.5 mM OPC-41061 or DM-4101 or DM-4102, in a final volume of 0.5 mL. The standard mixtures without  $\beta$ -NADPH solution were pre-incubated at 37° C for 3 min. After addition of  $\beta$ -NADPH the reactions were carried out at 37° C for 1 h. After addition of the internal standard the reaction mixtures were centrifuged and the supernatants analyzed by HPLC.

### *Effect of Ketoconazole on the Metabolism of OPC-41061 and DM-4101 (=R-(+) OPC-41061) to DM-4128 Using Human Liver Microsomes*

The standard mixture contained 335  $\mu$ L 0.1 M pH 7.4 phosphate buffer, 100  $\mu$ L HLM (2 mg protein), 50  $\mu$ L of 50 mM  $\beta$ -NADPH and 10  $\mu$ L 2.5 mM OPC-41061 or DM-4101 and ketoconazole in DMSO, in a final volume of 0.5 mL. Ketoconazole was used in final concentrations of 0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 3 and 10  $\mu$ M. The standard mixtures without  $\beta$ -NADPH solution were pre-incubated at 37° C for 3 min. After addition of  $\beta$ -NADPH the reactions were carried out at 37° C for 1 h. After addition of the internal standard the reaction mixtures were centrifuged and the supernatants analyzed by HPLC.

### *Effect of Quinidine and 7,8-Benzoflavone on the Metabolism of OPC-41061 and DM-4101(R-(+)OPC41061) to DM-4128 Using Human Liver Microsomes*

The standard mixture contained 290  $\mu\text{L}$  or 335  $\mu\text{L}$  0.1 M pH 7.4 phosphate buffer, 100  $\mu\text{L}$  HLM (2 mg protein), 50  $\mu\text{L}$  of 100 mM  $\beta$ -NADPH and 10  $\mu\text{L}$  2.5 mM OPC-41061 or DM-4101 and 50  $\mu\text{L}$  quinidine in water or 5  $\mu\text{L}$  7,8-benzoflavone ( $\alpha$ -naphthoflavone) in DMSO, in a final volume of 0.5 mL. Quinidine or 7,8 benzoflavone were used in final concentration of 0, 0.1, 1, and 10  $\mu\text{M}$ . The standard mixtures without  $\beta$ -NADPH solution were pre-incubated at 37° C for 3 min. After addition of  $\beta$ -NADPH the reactions were carried out at 37° C for 20 min. After addition of the internal standard the reaction mixtures were centrifuged and the supernatants analyzed by HPLC.

*Effect of Monoclonal Antibodies against CYP3A4 (MAB-3A4) on the Metabolism of DM-4101(R-+) OPC-41061 to DM-4128 Using Human Liver Microsomes*

The standard mixture contained 190  $\mu\text{L}$  0.2 M pH 7.4 Tris-HCl buffer, 50  $\mu\text{L}$  HLM (1 mg protein), 50  $\mu\text{L}$  of 50 mM  $\beta$ -NADPH and 10  $\mu\text{L}$  2.5 mM DM-4101, MAB-3A4 and 25 mM pH 7.4 Tris-HCl buffer in a final volume of 0.5 mL. MAB-3A4 was used at 0, 10, 25, 50, 100 and 200  $\mu\text{L}$ . The HLM, MAB-3A4 and 25 mM pH 7.4 tris-buffer were mixed and kept room temperature for 20 min. After addition of 0.2 M pH 7.4 Tris-HCl buffer, DM-4101 and  $\beta$ -NADPH the reactions were carried out at 37° C for 20 min. After addition of the internal standard the reaction mixtures were centrifuged and the supernatants analyzed by HPLC.

**Assay**

A HPLC method with UV detection using an internal standard was employed to measure the formation of DM-4128.

**RESULTS**

*Metabolism of OPC-41061 to DM-4128 Using Human Liver Microsomes*

As shown in the below table HLM metabolize OPC-41061 to DM-4128 in the presence of  $\beta$ -NADPH:

Table 4 Kinetics of OPC-41061 to DM-4128 using human liver microsomes in the presence of 5 mM NADPH

OPC-41061 S ( $\mu\text{M}$ )	DM-4128 V (pmol/min/mg)
5	0.985
10	1.754
20	3.235
30	4.504
40	6.008
50	7.159

$Y = 4.8672X + 0.0568, r = 0.9969, X: 1/S, Y: 1/V$   
 $K_m = 85.7 \mu\text{M}, V_{max} = 17.6 \text{ pmol/min/mg}$

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*Metabolism of OPC-41061 to DM-4128 Using Human Liver Microsomes or Cytosols*

The HLM and HLC preparations in reaction mixtures of pH 7.4- 8.in the absence of  $\beta$ -NADPH were not able to produce DM-4128 from OPC-41061.

*Metabolism of DM-4101 (=R-(+) OPC-41061 and DM-4102 (=S-(-)OPC-41061 to DM-4128 Using Human Liver Microsomes*

The results shown in the below table indicate that HLM generate DM-4128 from both DM-4101 and DM-4102:

Table 7 Kinetics of DM-4101 to DM-4128 using human liver microsomes in the presence of 5 mM  $\beta$ -NADPH

OPC-41061 S ( $\mu$ M)	DM-4128 V (pmol/min/mg)
2.5	0.769
5	1.402
10	2.716
20	5.364
30	7.784
40	10.436
50	11.966
$Y = 3.2331 X + 0.0284, r = 0.9988, X: 1/S, Y: 1/V$	
$K_m = 113.7 \mu M, V_{max} = 35.2 \text{ pmol/min/mg}$	

Table 8 Kinetics of DM-4102 to DM-4128 using human liver microsomes in the presence of 5 mM  $\beta$ -NADPH

OPC-41061 S ( $\mu$ M)	DM-4128 V (pmol/min/mg)
10	1.193
20	1.981
30	2.795
40	3.481
50	4.223
$Y = 7.4387 X + 0.1056, r = 0.9952, X: 1/S, Y: 1/V$	
$K_m = 70.4 \mu M, V_{max} = 9.5 \text{ pmol/min/mg}$	

Metabolism of OPC-41061, DM-4101(=R-(+) OPC-41061 and DM-4102 (=S-(-)-OPC41061) to DM-4128 Using Cell Microsomes Containing CYP450s

The below table summarizes the results:

Table 10 Metabolisms of OPC-41061, DM-4101 and DM-4102 to DM-4128 using cell microsome preparations containing human cytochrome P450s

P450	Substrate	DM-4128 (nmol/hr/nmol P450) Mean
CYP1A1	OPC-41061	2.511
	DM-4101	2.715
	DM-4102	1.330
CYP1A2	OPC-41061	ND
	DM-4101	ND
	DM-4102	0.096
CYP2A6	OPC-41061	ND
	DM-4101	ND
	DM-4102	ND
CYP2B6	OPC-41061	ND
	DM-4101	ND
	DM-4102	ND
CYP2C8	OPC-41061	ND
	DM-4101	ND
	DM-4102	ND
CYP2C9	OPC-41061	ND
	DM-4101	ND
	DM-4102	ND
CYP2C19	OPC-41061	ND
	DM-4101	ND
	DM-4102	ND
CYP2D6	OPC-41061	0.182
	DM-4101	ND
	DM-4102	ND
CYP2E1	OPC-41061	ND
	DM-4101	ND
	DM-4102	ND
CYP3A4	OPC-41061	3.067
	DM-4101	4.426
	DM-4102	1.178
CYP4A11	OPC-41061	ND
	DM-4101	ND
	DM-4102	ND

ND=not detected

The results indicate that with cell microsomes expressing human CYPs, OPC-41061 is metabolized to DM-4128 mainly by CYPs 3A4 and 1A1 and to a much smaller extent by CYPs2D6 and CYP1A2. The other tested enzymes were inactive. The metabolism by CYP3A appears to be stereospecific with DM-4101 (R-enantiomer) to produce more DM-4128 than DM-4102 (S-enantiomer).

Effect of Ketoconazole on the Metabolisms of OPC-41061 and DM-4101(=R-(+) OPC-41061) to DM-4128 using Human Liver Microsomes

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As shown in the below tables ketoconazole in a dose dependent manner inhibits the production of DM-4128 from OPC-41061 and 4102:

Table 11: Effect of ketoconazole on the metabolisms of OPC-41061 and DM-4101 to DM-4128 using human liver microsomes

Substrate	Ketoconazole ( $\mu\text{M}$ )	DM-4128 production (% of control)	EC <sub>50</sub> ( $\mu\text{M}$ )
OPC-41061	0	100	0.76
	0.001	105.6	
	0.003	104.3	
	0.01	105.9	
	0.03	106.9	
	0.1	97.0	
	0.3	74.6	
	1	41.2	
	3	27.4	
	10	2.7	
DM-4101	0	100	0.64
	0.001	72.7	
	0.003	89.5	
	0.01	99.6	
	0.03	97.2	
	0.1	68.8	
	0.3	68.2	
	1	33.0	
	3	16.0	
	10	-	

The respective IC<sub>50</sub> value for ketoconazole's inhibition of the metabolism of OPC-41061 and DM-4101 to DM-4128 is 0.76  $\mu\text{M}$  and 0.64  $\mu\text{M}$ .

#### Effect of Quinidine and 7,8 Benzoflavone on the Metabolism of OPC-41061 and DM-4101 to DM-4128

Quinidine, a 2D6 inhibitor, and 7.8 Benzoflavone a CYP1A2 inhibitor do not inhibit the production of DM-4128 from OPC-41061 or DM-4101.

#### Effect of CYP3A4 Monoclonal Antibodies on the Metabolism of DM-4101(R-(+) OPC-41061) to DM-4128 Using Human Liver Microsomes

The below table shows a dose dependent inhibition of the formation of DM-4128 from DM-4101 by the anti-CYP3A4 monoclonal antibodies:

Table 13 Effect of MAB-3A4 on the metabolism of DM-4101 to DM-4128 using human liver microsomes

Substrate	MAB-3A4 ( $\mu\text{L}$ )	DM-4128 production (% of control)
DM-4101	0	100
	10	77.5
	25	59.2
	50	40.8
	100	33.2
	200	30.4

#### Assay

The calibration curve of the HPLC assay for DM-4128 is linear between 1 and 50  $\mu\text{g/mL}$ . The intra-day precision of the HPLC assay is < 4.8% and the accuracy ranges between -10.2%-8.0% in samples containing concentrations between 1 and 50  $\mu\text{g/mL}$ .

#### Conclusions

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The major enzyme involved in the metabolism of OPC-41061, and DM-4101 to DM-4128 is CYP 3A4. The almost complete inhibition of these reactions by ketoconazole, a CYP3A4 inhibitor, and the 70% inhibition by anti-CYP3A4 monoclonal antibodies support this notion. The relevance of CYPs1A1, 1A2 and 2D6 in the production of DM-4128 is uncertain.

#### Comments

1. No positive controls were used in the inhibition experiments with ketoconazole, quinidine and 7,8 benzoflavone.
2. The final protein concentrations in the reaction mixtures are not stated.
3. The report does not provide evidence for the specificity of the anti- CYP3A4 monoclonal antibodies used.
4. An inhibitor of CYP1A1 should have been used to confirm the potential relevance of CYP1A1 for the metabolism of OPC41061, DM-4101 and DM-4102.
5. Linearization of enzyme data (Lineweaver-Burk, Eadie-Hofstee and Dixon-plots) may introduce bias. Software exist that uses untransformed data to determine the enzyme kinetic parameters.
6. The impact on the microsomes by the vehicle DMSO was not tested.
7. Additional experiments with human liver microsomes were performed at pH 7.4, 8.0 and 8.6 with no  $\beta$ -NADPH in the reaction mixture. The report does not state the rationale behind these experiments.

#### Study Report 010760: "In Vitro Metabolism of OPC-41061 by Microsomes Derived from Human AHH-1/TK+/-Cells"

##### Investigator and Study Site

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##### Objectives

1. To analyze in vitro using microsomes from the AHH-1 TK+/-cell line the CYP 450 enzymes involved in the metabolism of OPC-41061
2. The effect of drugs including warfarin, digoxin, quinidine, furosemide, captopril, and amiodarone to interact with the metabolism of OPC-41061.

##### Methods

Synthesized OPC-41061, DM-4101 and DM-4102, OMP-21826, DM-4104, DM-4105 and DM-4111 were available.

##### Metabolism

Cell microsomes from the AHH-1 TK<sup>+</sup>/cell line expressing CYP1A1, 1A2, 2A6 + P450 reductase, 2B6, 2C8 and OR, 2C9 +P450 reductase, 2C9-Arg +P450 reductase, 2C19, 2E1 + reductase, or 3A4 +P450 reductase, cell microsomes over-expressing CYPD6-Val and cell lines containing vector were obtained commercially.

#### *Identification of CYP450 Isozymes*

The reaction system (0.5mL) consisting of microsomal protein (2 mg/mL), 10 mM  $\beta$ -NADPH, 5 mM  $\beta$ -NADH, and 100  $\mu$ M OPC-41061 was incubated at 37° C for 2 h. OPC-41061 was dissolved in DMF (final DMF concentration 2 %). A 0.1 M pH 7.4 Tris-HCl buffer was used in the reaction system for CYP2A6 and 2C9, and a 0.1 M pH 7.4 phosphate buffer was used in the reaction system with the other iso-zymes. Methanol (0.5 mL) was added to terminate the reaction. The solution was then centrifuged and the supernatant analyzed by HPLC and the produced OPC-41061 metabolites quantitated.

#### *1. CYP3A4-Catalyzed Production of OPC-41061 Metabolites*

The reaction system described above was used. OPC-41061 was incubated at 37° C for 5, 10, 20, 30, 45, and 60 min.

The production of DM-4110, MOP-21816, and an unknown product (later identified as DM-4119) with a 10 min incubation time was found to be linear. In subsequent experiments concentrations of 1, 2.5, 5, 10, and 50  $\mu$ M (final concentration in DMF 2%) of OPC-41061 were used and the above described reaction system and incubation time used. After termination of the reaction the internal standard and 5 mL diethyl-ether were added, and the mixture shaken for 10 min and centrifuged. The supernatant was then dried under a stream of air, the residue dissolved in 100  $\mu$ L methanol and DM-4110, DM-4111, MOP-21826, and DM-4119 (in terms of equivalent to unchanged compound) quantitated. V<sub>max</sub> and K<sub>m</sub> for the production of DM-4110, MOP-21816, and DM-4119 were determined.

#### *2. CYP-Catalyzed Metabolism of the OPC-41061 Enantiomers DM-4101 and 4102*

The reaction system (0.5mL) consisting of microsomal protein (2 mg/mL), 10 mM  $\beta$ -NADPH, 5 mM  $\beta$ -NADH, and 100  $\mu$ M OPC-41061 was incubated at 37° C for 5, 10, 20, 30, 45, and 60 min. OPC-41061 was dissolved in DMF to give a final concentration of 2 %. A 0.1 M pH 7.4 phosphate buffer was used in the reaction system. Sodium hydroxide was added to terminate the reaction. After addition of the internal standard and 5 mL diethyl-ether the mixture was shaken for 10 min, centrifuged and the supernatant analyzed by HPLC and the produced OPC-41061 metabolites quantitated.

#### *3. Effects of CYP3A4 Inhibitors on CYP3A4-Catalyzed Metabolism of OPC-41061*

Substrate and inhibitors dissolved in DMF (final concentration 2%) were added to the reaction system (0.5 mL) consisting of microsomal protein (1 mg/mL), 0.1 M pH 7.4 phosphate buffer, 10 mM  $\beta$ -NADPH, 5 mM  $\beta$ -NADH and the mixture was incubated at 37° C for 10 min. The final concentration of the substrates DM-4110, DM-4111, and DM-4119 was 20  $\mu$ M and 50  $\mu$ M for MOP-21826. The inhibitors included the CYP 3A4 inhibitors  $\pm$  miconazole, ketoconazole and troleandomycin and potentially co-administered warfarin, digoxin, quinidine, furosemide, captopril, and amiodarone. Sodium hydroxide was added to terminate the reaction. After addition of the internal standard and 5 mL diethyl-ether, the mixture was shaken for 10 min, centrifuged and the supernatant analyzed by HPLC and the produced DM-4110, DM-4111, MOP-21826 and 4119 quantitated.

#### *4. Effect of OPC-41061 on CYP3A4-catalyzed Metabolism of Warfarin, Quinidine and Amiodarone*

The same reaction system (0.5 mL) consisting of microsomal protein (1 mg/mL), 0.1 M pH 7.4 phosphate buffer, 10 mM  $\beta$ -NADPH, 5 mM  $\beta$ -NADH was used and the mixture was incubated at 37° C for 5, 10, 20, 30, 45 and 60 min in the experiments with warfarin, quinidine and amiodarone in the presence and absence of OPC-41061. The apparent K<sub>m</sub> and V<sub>max</sub> values were determined with an incubation time of 10 min that produced a linear formation of the respective metabolites of warfarin (10-hydroxywarfarin) quinidine (N-oxide, 3-hydroxyquinidine) and amiodarone (N-desethylamiodarone). In the absence of OPC-4106 the final concentrations of warfarin in the

reaction system were 25, 50, 100, 250, and 500  $\mu\text{M}$ , those of quinidine were 5, 10, 25, 50, and 100  $\mu\text{M}$  and those of amiodarone were 2.5, 5, 10, and 25  $\mu\text{M}$  (final concentration of DMF 1%). The reactions were terminated by the addition of 0.2 mL 2N HCl. Ethyl-acetate was added, the mixture shaken for 10 min and then centrifuged and the supernatant dried under a stream of air. The residue was dissolved in methanol and the respectively formed metabolites quantitated by HPLC.

When the potential inhibitor OCPC-41061 was co-administered to warfarin, quinidine and amiodarone the same reaction system and assay methods were used. The final concentrations of warfarin were 100, 250, and 500  $\mu\text{M}$  and those of quinidine and amiodarone were 0, 2, 5, and 10  $\mu\text{M}$  (dissolved in DMF, final concentration 1%). The final concentrations of OPC-41061 were 0, 2, and 5  $\mu\text{M}$ .

#### 5. Inhibitory Effects of OPC-41061 on Enzymes other than CYP3A4

The reaction system (0.5 mL) used consisted of microsomal protein (1 mg/mL), 0.1 M pH 7.4 phosphate buffer, 1mM  $\beta$ -NADPH, 1 mM  $\beta$ -NADH. The mixture was incubated at 37° C. The potential inhibitor OPC-41061 at final concentrations of 0.5, 5 and 50  $\mu\text{M}$  was dissolved in propylene glycol (final concentration 1%). The final concentrations of the substrates were: R-(+) warfarin 200  $\mu\text{M}$ , tolbutamide 200  $\mu\text{M}$ , S-mephenytoin 100  $\mu\text{M}$ , bufuralol 100  $\mu\text{M}$  and chlorzoxazone 150  $\mu\text{M}$ . The incubation time for CYP1A2, 2C19, 2D6 and 2E1 was 20 min and for CYP2C9 was 30 min. Active controls were furafylline for CYP1A2, sulphenazole for CYP2C9, tranlycypromine for 2C19, quinidine for 2D6 and diethylthiocarbamide acid for 2E1. Published Km and Vmax values for CYP1A2 catalyzed R-(+)-warfarin-6-hydroxylation, CYP2C9-catalyzed tolbutamide 4-hydroxylation, 2C19-catalyzed S-mephenytoin 4'-hydroxylation, 2D6-catalyzed bufuralol 1'-hydroxylation and 2E1-catalyzed chlorzoxazone- hydroxylation were considered to be indicators for enzymic activity.

#### Assay

All assay methods used HPLC methods with UV detection. Column switching was used to determine the inhibitory effects of OPC-41061 on the catalytic activities of the iso-zymes other than CYP3A4

#### Data Analysis

Apparent Km and Vmax values were obtained from linear regression in Lineweaver-Burk plots. Ki was obtained from linear regressions in Dixon plots.

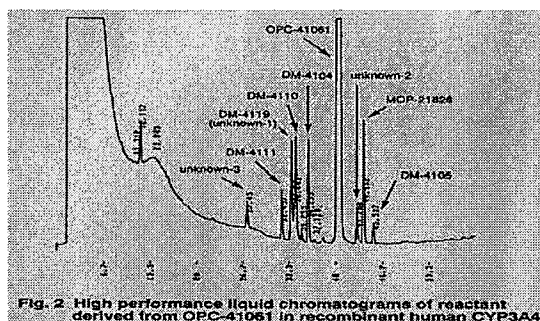
### RESULTS

#### 1. Identification of CYP450 Isozymes Involved in the Metabolism of OPC-41061

As shown in the below table with the human cDNA expressed CYPs only CYP3A4 catalyzed OPC-41061:

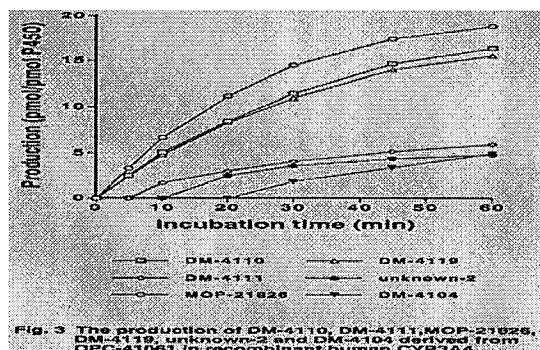
CYP	Activity (pmol/pmol P450/2hr)							
	DM-4104	DM-4105	DM-4110	DM-4111	MOF-21928	DM-4119	unknown-2	unknown-3
CYP1A1	<1.7	<1.7	<1.7	<1.7	<1.8	<1.7	<1.7	<1.7
CYP1A2	<1.7	<1.7	<1.7	<1.7	<1.8	<1.7	<1.7	<1.7
CYP2A6	<1.7	<1.7	<1.7	<1.7	<1.8	<1.7	<1.7	<1.7
CYP2B6	<1.7	<1.7	<1.7	<1.7	<1.8	<1.7	<1.7	<1.7
CYP2C8	<1.7	<1.7	<1.7	<1.7	<1.8	<1.7	<1.7	<1.7
CYP2C9	<1.7	<1.7	<1.7	<1.7	<1.8	<1.7	<1.7	<1.7
CYP2C19	<1.7	<1.7	<1.7	<1.7	<1.8	<1.7	<1.7	<1.7
CYP2D6	<1.7	<1.7	<1.7	<1.7	<1.8	<1.7	<1.7	<1.7
CYP2E1	<1.7	<1.7	<1.7	<1.7	<1.8	<1.7	<1.7	<1.7
CYP3A4	12.9	3.2	23.8	8.4	23.5	19.0	8.7	4.8

CYP 3A4 produces DM-4110, DM-4111, MOP-21826, DM-4104, DM-4105, DM-4119 and unknown metabolites as shown in the below chromatogram:

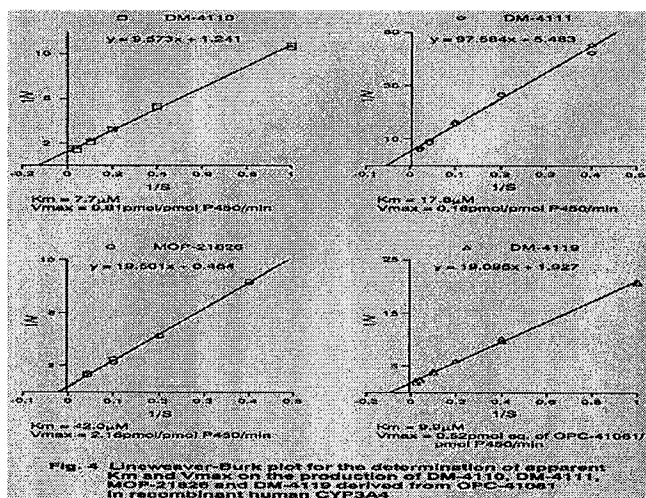


## 2. CYP3A4-Catalyzed Production of OPC-41061 Metabolites

The CYP 3A4 catalyzed production of the metabolites of OPC-41061, DM-4110, MOP-21826, and DM-4119 is linear with an incubation time of 10 min as shown in the figures below:



The respective Lineweaver-Burk plots are linear, and the respective  $K_m$  and  $V_{max}$  values are listed in the figures below:



### 3. CYP3A4 Catalyzed Metabolism of OPC-41061 Enantiomers (DM-4101 and DM-4102)

The apparent mean  $K_m$ ,  $V_{max}$  and intrinsic clearance values for the metabolites DM-4110, DM-4111, and MOP-21826 produced from substrates OPC-41061 and its enantiomers, DM-4101 (=R-(+) enantiomer of OPC-41061), and DM-4102 (=S-(-) enantiomer of OPC-41061), are listed in the below table:

Substrates	Parameters	Metabolites				Sum of $CL_{int}$
		DM-4110	DM-4111	MOP-21826	DM-4119	
OPC-41061	$K_m$ ( $\mu M$ )	7.7	17.8	42.0	9.9	
	$V_{max}$ (pmol/pmol P450/min)	0.81	0.16	2.16	0.82	
	$CL_{int}$ ( $V_{max}/K_m$ )	0.105	0.010	0.051	0.083	
DM-4101	$K_m$ ( $\mu M$ )	---	20.7	18.1	9.0	0.087
	$V_{max}$ (pmol/pmol P450/min)	---	0.17	1.00	0.22	
	$CL_{int}$ ( $V_{max}/K_m$ )	---	0.008	0.055	0.024	
DM-4102	$K_m$ ( $\mu M$ )	7.8	---	14.1	8.2	0.121
	$V_{max}$ (pmol/pmol P450/min)	0.54	---	0.43	0.18	
	$CL_{int}$ ( $V_{max}/K_m$ )	0.069	---	0.030	0.022	

$CL_{int}$ : intrinsic clearance

DM-4111 is selectively produced from DM-4001, and DM-4112 from DM-4002. The intrinsic clearance of the enantiomer DM-4102 tends to be slightly greater than that of the enantiomer DM-4101.

### 4. Effects of Potentially Co-administered Drugs and Known Inhibitors on the CYP3A4-catalyzed Metabolism of OPC-41061

The below table lists the results:

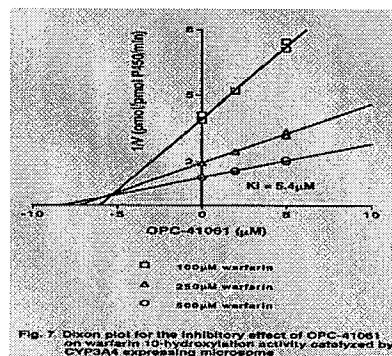
Compounds	Conc. (μM)	% Inhibition DM-4110	% Inhibition DM-4111	% Inhibition DM-4119	Conc. (μM)	% Inhibition MOP-21626
Warfarin	0.2	8.40	7.60	8.88	0.5	8.13
	2	4.48	4.38	5.64	5	8.41
	20	8.91	10.68	8.37	50	8.49
Digoxin	0.2	9.30	9.24	4.17	0.5	8.51
	2	4.28	6.56	1.03	5	19.76
	20	7.87	12.41	3.98	50	6.51
Quinidine	0.2	1.05	3.48	4.59	0.5	0.14
	2	11.08	2.42	2.69	5	2.93
	20	8.08	4.30	1.33	50	18.18
Furazemide	0.2	1.46	2.00	0.52	0.5	7.25
	2	0.50	8.18	7.05	5	0.08
	20	3.88	6.90	5.71	50	0.38
Gabapril	0.2	6.51	6.31	7.70	0.5	0.80
	2	28.63	28.63	27.03	5	1.70
	20	8.18	10.33	7.67	50	0.44
Amiodarone	0.2	14.33	15.34	13.06	0.5	7.83
	2	13.37	15.19	12.68	5	8.47
	20	38.86	36.07	28.85	50	16.18
Miconazole	0.2	30.91	37.86	28.80	0.5	30.86
	2	83.45	77.10	81.18	5	87.68
	20	90.73	100	88.17	50	93.75
Ketoconazole	0.2	34.56	40.81	31.03	0.5	63.15
	2	68.46	60.88	61.80	5	94.47
	20	100	100	100	50	98.44
Troleandomycin	0.2	14.43	15.95	13.23	0.5	14.78
	2	48.91	50.17	51.65	5	43.76
	20	87.58	80.23	87.41	50	84.60

It can be seen that the standard CYP3A4 inhibitors ± miconazole, ketoconazole and troleandomycin inhibit dose dependently the CYP3A4 mediated metabolism of OPC-41061. At the highest concentration the metabolic inhibition of OPC-41061 with these inhibitors was complete. Among the other compounds tested as potential inhibitors only amiodarone (up to 19%) and quinidine (up to 16%) appeared to weakly inhibit the metabolism of OPC-41061.

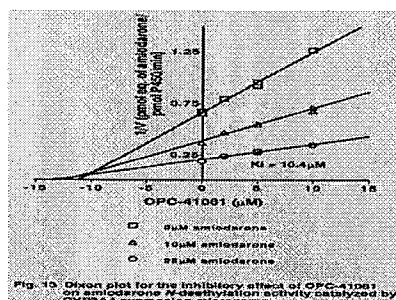
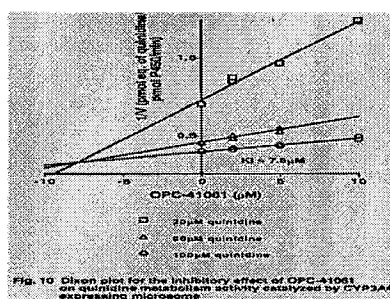
#### 5. Effect of OPC-41061 on CYP3A4-Catalyzed Metabolism of Warfarin, Quinidine and Amiodarone

The metabolism of amiodarone, quinidine and warfarin is known to be mediated by CYP3A4. OPC-41061 as a co-substrate of CYP3A4 could affect the metabolism of these drugs when co-administered.

The respective Dixon plots indicate that OPC-41061 inhibits warfarin 10-hydroxylation with  $K_i = 5.4 \mu\text{M}$ , quinidine 3-hydroxylation or N-oxydation with  $K_i = 7.8 \mu\text{M}$  and amiodarone N-deethylation with  $K_i = 10.4 \mu\text{M}$ :



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#### 6. Inhibitory Effect of OPC-41061 on Isozymes Other than CYP3A4

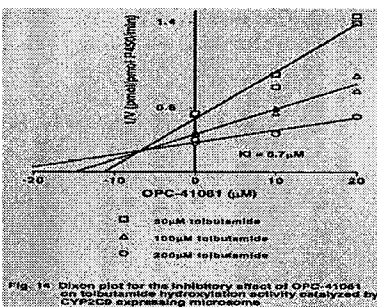
OPC-41061 inhibits importantly only tolbutamide 4-hydroxylation as shown by the table and Dixon plot below:

Reaction			OPC-41061 or Probe	% of inhibition			
CYP450	K <sub>m</sub> (μM)	V <sub>max</sub> (pmol/pmol P450/min)		Concentration (μM)			
				0.5	5	50	500
CYP1A2	<i>R</i> -(+)-Warfarin 6-hydroxylation K <sub>m</sub> =165.4, V <sub>max</sub> =0.014	OPC-41061	8.46	7.08	21.83	not done	
		Furafylline	5.97	27.84	75.93	100	
CYP2C9	Tolbutamide 4-hydroxylation K <sub>m</sub> =189.8, V <sub>max</sub> =18.54	OPC-41061	4.02	19.82	76.30	not done	
		Sulphenazole	82.51	95.77	100	100	
CYP2C19	(S)-Mephenytoin 4'-hydroxylation K <sub>m</sub> =22.1, V <sub>max</sub> =0.19	OPC-41061	2.46	4.20	0.43	not done	
		Tranylcypromine	17.17	53.22	84.57	100	
CYP2D6	Butorolol 1'-hydroxylation K <sub>m</sub> =104.0, V <sub>max</sub> =0.010	OPC-41061	2.21	0.91	12.25	not done	
		Quinidine	55.64	93.70	100	100	
CYP2E1	Chlorzoxazone 6-hydroxylation K <sub>m</sub> =133.2, V <sub>max</sub> =0.23	OPC-41061	7.90	7.08	10.67	not done	
		DDC <sup>a</sup>	24.35	43.81	85.50	100	

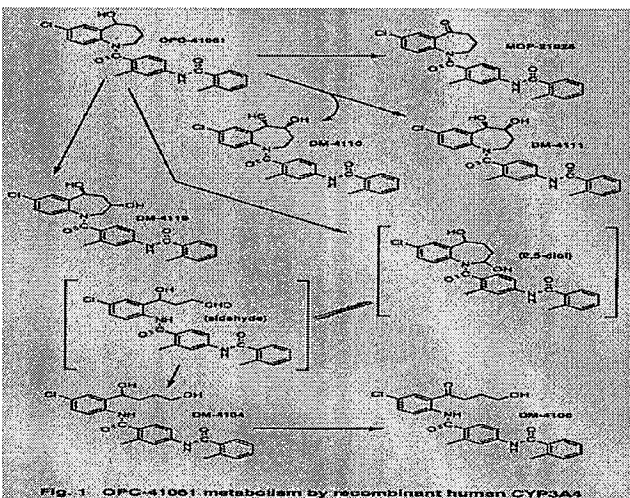
The substrate concentrations used for each assay were 200 μM *R*-(+)-warfarin, 200 μM tolbutamide, 50 μM (S)-mephenytoin, 100 μM butorolol, or 150 μM chlorzoxazone. The control activities were 0.005, 4.430, 0.022, 0.001, 0.075 pmol/pmol cytochrome P450/min for CYP1A2, 2C9, 2C19, 2D6 and 2E1-catalyzed enzyme reaction, respectively. Enzyme incubations and metabolic analysis were carried out in duplicate, and data were expressed as the mean.

<sup>a</sup> 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane

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Based on the findings from this study the sponsor proposes the following metabolic scheme for OCP-41061:



## Conclusions

CYP3A4 appears to be the main enzyme involved in the metabolism of OPC-41061. Classical CYP3A4 inhibitors such as  $\pm$  miconazole, ketoconazole, and troleandomycin inhibit dose dependently the metabolism of OPC-41061. Among the potentially co-administered drugs tested only amiodarone and quinidine, both substrates of CYP3A4, inhibit the metabolism of OPC-41061. OPC-41061 inhibits the metabolism of warfarin, quinidine, amiodarone and tolbutamide.

## Comments

1. The inhibitory potential of the metabolite DM-4103, the major circulating moiety in plasma should be investigated.
2. The protein concentrations in the reaction mixtures in inhibitor experiments should be  $< 1 \text{ mg/mL}$ .
3. Dixon plots cannot differentiate between competitive and un-competitive inhibition