

## ARTICLE

# Pharmacokinetics/pharmacodynamics of L-ornithine phenylacetate in overt hepatic encephalopathy and the effect of plasma ammonia concentration reduction on clinical outcomes

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## Abstract

Hepatic encephalopathy (HE) is a serious neurocognitive complication of liver dysfunction, often associated with elevated plasma ammonia. Ornithine phenylacetate (OP), a potent ammonia scavenger, is being evaluated for the treatment of acute/overt HE. The pharmacokinetics and pharmacodynamics of OP in patients with HE were characterized in this phase IIb study (NCT01966419). Adult patients hospitalized with an overt HE episode, cirrhosis, and plasma ammonia above the upper limit of normal (ULN) who failed to improve after 48 hours' standard care were randomly assigned to continuous intravenous OP (10, 15, or 20 g/day, based on Child–Turcotte–Pugh score) or matching placebo for 5 days. Plasma levels of ornithine and phenylacetic acid (PAA) and plasma/urinary levels of phenylacetylglutamine (PAGN) (primary metabolite of PAA) were regularly assessed; plasma ammonia level was the primary pharmacodynamic variable. PAA demonstrated dose-dependent pharmacokinetics; ornithine and PAGN levels increased with dose. PAGN urinary excretion represented ~50%–60% of administered PAA across all doses. Mean reduction in plasma ammonia with OP at 3 hours postinfusion was significantly greater versus placebo ( $p = 0.014$ ); and time to achieve plasma ammonia less than or equal to the ULN was significantly reduced ( $p = 0.028$ ). Achievement of clinical response based on HE stage was associated with a greater reduction in mean plasma ammonia level ( $p = 0.009$ ). OP effects on plasma ammonia were consistent with its proposed mechanism of action as a primary ammonia scavenger, with a significant association between reduced plasma ammonia and improvement in HE

Rifaat Safadi and Robert S. Rahimi are co-lead authors of this article.

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stage. OP should be further evaluated as a promising treatment for hyperammonemia in patients with overt HE.

### Study Highlights

#### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Overt hepatic encephalopathy (OHE) is characterized by severe liver impairment and signs of neurocognitive dysfunction commonly associated with hyperammonemia. Ornithine phenylacetate (OP), a potent ammonia scavenger, has an acceptable safety profile in patients with decompensated cirrhosis and gastrointestinal bleeding, and lowers plasma ammonia levels.

#### WHAT QUESTION DID THIS STUDY ADDRESS?

What are the pharmacokinetic (PK) and pharmacodynamic (PD) profiles of OP and its metabolites in patients hospitalized due to OHE with cirrhosis and hyperammonemia?

#### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

PK/PD analysis results are consistent with the putative mechanism of action of OP. Mean plasma levels of OP analytes reach steady state in approximately 48–72 hours, regardless of dose, and appear to increase as liver function declines. OP treatment significantly reduces plasma ammonia and improves clinical OHE stage, supporting the proposed central role of ammonia in HE.

#### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

OP treatment may be beneficial as a first-line treatment for patients with OHE; however, additional studies are warranted.

## INTRODUCTION

Hepatic encephalopathy (HE) encompasses a range of neurocognitive signs and symptoms associated with liver injury and/or dysfunction, and, less frequently, with portosystemic shunting. Overt HE (OHE) represents the most severe category in the spectrum and is currently the only form for which guidelines recommend active treatment.<sup>1</sup> Although the pathophysiology of HE is complex and multifactorial, elevated ammonia levels (hyperammonemia) are consistently implicated in its development.<sup>2–4</sup> However, no direct association has been found between ammonia concentration and the severity of HE, which can manifest despite normal ammonia levels.<sup>4</sup> As such, the role of ammonia as a biomarker of HE and the accuracy of ammonia testing have been the subject of ongoing debate.<sup>5</sup>

The primary driver of the development of hyperammonemia secondary to cirrhosis and other liver dysfunction appears to result from gut-derived toxins, primarily ammonia, that bypass or escape detoxification by the failing liver and ultimately cross the blood–brain barrier.<sup>6–8</sup> Hyperammonemia leads to alterations in amino acid homeostasis, particularly elevations in the blood levels of glutamine and glycine.<sup>9–11</sup> The ammoniagenic effects of

increased glutamine and glycine are inadequately compensated by muscle and liver glutamine synthetases, which use ammonia to generate glutamine from glutamate, and are exacerbated by increased activity of gut glutaminase, which breaks down glutamine to generate ammonia.<sup>12</sup>

The fundamental principle of pharmacologic management of OHE is based on reducing gut and systemic levels of ammonia and modulating the intestinal microbial environment.<sup>13</sup> Agents that reduce hyperammonemia vary in their mechanisms of action and include nonabsorbable disaccharides, such as lactulose, nonabsorbable/poorly absorbed antibiotics (rifaximin, neomycin), laxatives such as polyethylene glycol, L-ornithine L-aspartate, glycerol phenylbutyrate, and branched-chain amino acids, some of which are not readily available worldwide.<sup>2,13</sup> Consensus guidelines for treating HE discuss the use of lactulose (despite limited prospective evidence and/or indication), the usual first-line therapy, and rifaximin (in recurrent OHE), with other alternatives including L-ornithine L-aspartate, neomycin, and metronidazole.<sup>1</sup> The use of these and other agents, however, is based primarily on empirical observations.<sup>1</sup>

Ornithine phenylacetate (OP; also known as OCR-002 and MNK-6105) is a potent ammonia scavenger

with potential utility in the treatment of HE. L-OP is a salt that rapidly dissociates in solution to ornithine (ORN) and phenylacetic acid (PAA). It is administered via infusion of a solution containing equimolar amounts of ORN and PAA, which effect ammonia removal via interlinked amino acid metabolic pathways (Figure S1). ORN is converted to glutamate via the  $\omega$ -ORN aminotransferase pathway; glutamine is then generated from glutamate by glutamine synthetase, using an ammonium ( $\text{NH}_4^+$ ) ion in the process. By increasing the provision of glutamate and stimulating the activity of glutamine synthetase, ORN induces body muscle to trap circulating ammonia in the form of glutamine.<sup>10</sup> A primary metabolic pathway for PAA elimination involves its conjugation with glutamine (catalyzed by phenylacetyl coenzyme A: glutamine acyltransferase) to form phenylacetylglutamine (PAGN), which is excreted in the urine.<sup>10,14,15</sup> The conjugation of PAA with glutamine prevents its glutaminase-catalyzed breakdown into glutamate and ammonia and a consequent “rebound” increase in ammonia levels, potentiating OHE.

L-OP has been evaluated in humans in several studies, including phase I and IIa studies of dosing/pharmacokinetics (PK), safety, and tolerability in healthy volunteers and patients with stable cirrhosis.<sup>16,17</sup> In addition, OP has been shown to be safe and well tolerated in patients with decompensated cirrhosis and gastrointestinal bleeding; it was also effective in lowering plasma ammonia levels.<sup>18</sup>

The current study (STOP-HE) was conducted to evaluate the efficacy and safety of OP in the treatment of hospitalized patients with cirrhosis and an acute/overt episode of HE; the primary safety and efficacy results have been published separately.<sup>19</sup> Here, we summarize findings with respect to the PK and pharmacodynamic (PD) characteristics of OP, which was the secondary objective in the STOP-HE study. We also present an analysis of the effect of the reduction in the plasma ammonia concentration on the HE stage.

## METHODS

### Study objectives

The principal objectives of this analysis were to confirm the PK profile of OP when used in the treatment of patients with cirrhosis and hyperammonemia who were hospitalized with an acute episode of OHE; to assess the kinetics of plasma ammonia level reduction associated with administration of OP and urinary PAGN excretion;

and to evaluate the clinical effect of the reduction in plasma ammonia concentration on the HE stage.

### Study design

STOP-HE (ClinicalTrials.gov study number: NCT01966419) was a phase IIb randomized, double-blind, placebo-controlled international study conducted at 68 study centers between January 2014 and December 2016. A complete description of the study methodology has been published separately.<sup>19</sup> Adult patients aged 18–75 years hospitalized with cirrhosis with a venous ammonia level greater than the upper limit of normal (ULN) and an episode of OHE with a score of  $\geq 2$  on the Hepatic Encephalopathy Staging Tool (HEST) despite standard of care for 48 hours were randomly assigned to receive OP or matching placebo via intravenous infusion at a constant rate of 500 ml/24 hours for  $\leq 5$  days, in addition to the usual standard of care. Based on data from preclinical models, OP infusion at a constant rate was chosen over an intermittent dosing schedule.<sup>20</sup> Randomly assigned patients were stratified by the Model for End-Stage Liver Disease (MELD) score ( $\leq 30$  vs.  $>30$ ), categorized by HEST stages 2, 3, or 4, and (for North America only) by liver transplantation centers performing  $\geq 70$  transplants/year versus  $<70$ . Patients randomly assigned to treatment with OP received one of three intravenous dose options according to the baseline total score for four hepatic synthetic and portal elements (ie, ascites, total bilirubin, albumin, and international normalized ratio) from the Child–Turcotte–Pugh (CTP) score. A pharmacist, who was unblinded to the dosage being assigned, prepared the initial OP solution and adjusted the study drug concentration in the infusate (ie, 20 g [0.833 g/h], 15 g [0.625 g/h], or 10 g [0.417 g/h] for 4–6, 7–9, or 10–12 points, respectively) such that 500 ml of solution when infused at a constant rate over 24 hours ( $\sim 20.8$  ml/h) provided the appropriate dose of OP by level of hepatic decompensation based on the CTP score. Encephalopathy was not included in the calculation, because all patients included in the study had HE.

The primary efficacy end point was time to confirmed clinical response—defined as improvement from baseline HEST stage 4 (coma) or 3 (stupor; gross disorientation) to stage 2, or from baseline stage 2 (asterixis and disorientation) to stage 0/1 (no asterixis, no disorientation) from initiation of study drug infusion to 3 hours postinfusion. Safety was evaluated via adverse events, laboratory assessments, vital signs, 12-lead electrocardiography, and

physical and neurological examinations in accordance with standard of care.

The study protocol was approved by the institutional review board or independent ethics committee at each site. The study was conducted in accordance with principles of the Declaration of Helsinki. A legally authorized representative provided surrogate written informed consent for patient participation, with patient consent when possible.

## PK and PD assessments and end points

Characterizations of the PK profile of OP (ORN, PAA, and PAGN PK parameters) and its PD effects (change in plasma ammonia levels) were secondary objectives of the STOP-HE study.

Plasma levels of ORN, PAA, and PAGN were determined at baseline, once daily on treatment days, and at 0.5, 1, and 3 hours after either the end of infusion, early hospital discharge, or early termination. Measurement of total urine output and timed urine collection for PAGN were conducted at baseline, treatment day 1, and 3 hours after either the end of infusion, early hospital discharge, or early termination. Venous blood sampling for ammonia levels was conducted at screening, baseline, twice daily during treatment days, and at 0.5, 1, and 3 hours after either the end of infusion, early hospital discharge, or early termination; all measurements after screening were conducted by a single bioanalytical laboratory. Venous blood samples were collected from the arm opposite to the arm with the intravenous infusion line for dosing and then placed on wet ice until centrifugation. Plasma samples were immediately stored at  $-70^{\circ}\text{C}$  until analysis.

## PK and PD end points

Plasma samples were analyzed to determine mean steady-state concentrations ( $C_{\text{ss}}$ ) of ORN, PAA, and PAGN. Urinary PAGN was also assessed to determine daily quantity of PAGN excretion at steady state and the percentage of PAA dose excreted as PAGN at steady state. Plasma ammonia levels were analyzed to determine PD end points including change from baseline in plasma ammonia; time from OP initiation to achievement of plasma ammonia level  $\leq\text{ULN}$  (ULN defined as  $47.0\ \mu\text{g}/\text{dl}$  per central laboratory [normal reference range,  $16.4\text{--}47.0\ \mu\text{g}/\text{dl}$ ]); and comparison of changes from baseline in plasma ammonia levels between patients achieving and not achieving a confirmed clinical response.

Plasma concentrations of ORN, PAA, PAGN, as well as urinary PAGN concentrations, were evaluated using

validated high-performance liquid chromatography/mass spectrometry techniques, according to the US Food and Drug Administration and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use regulatory guidelines. Ammonia levels were determined at ACM Global Central Laboratories using an enzymatic assay. The calibration ranges of standard curves were  $5.00\text{--}1000\ \mu\text{g}/\text{ml}$  for PAA, PAGN, and ORN in human plasma, and  $0.300\text{--}200\ \text{mg}/\text{ml}$  for PAGN in human urine. The lowest standard in the calibration range was the lower limit of quantification (LLOQ) for each analyte and each matrix. Any experimental samples below the LLOQ were annotated as BLQ (below the limit of quantification).

## Data analysis

The PK analysis was conducted on all patients randomly assigned to OP who demonstrated compliant study drug administration, and for whom sufficient plasma samples for analysis of ORN, PAA, or PAGN concentrations were available to permit reliable and accurate determination of PK parameters. Analysis of PD variables was conducted on all randomized patients who received at least one dose of their assigned study drug and had at least one post-dose PD assessment (plasma ammonia and urinary PAGN). For both populations, samples were excluded from statistical analysis if they were collected in violation of protocol-specified sample collection criteria deemed sufficient to potentially cause spurious results.

The PK parameters were calculated from plasma ORN, PAA, and PAGN concentration–time data, based on exact elapsed times after study drug initiation, using noncompartmental methods. For PD analyses, summary statistics were calculated for change from baseline in ammonia levels to prespecified time windows over time; comparison of between-group differences in change from baseline at 3 hours after the final infusion was conducted using a two-sided van Elteren test ( $\alpha = 0.05$ ), stratified by randomization categories.

Time-to-event analyses employed the Kaplan–Meier method. For time to achieve ammonia levels  $\leq\text{ULN}$ , the elapsed time from start of first drug infusion to achieve ammonia levels  $\leq\text{ULN}$  was determined using actual date/clock of the first blood sample collection with ammonia value within the reference range; treatment groups were compared using a log-rank test (two-sided  $\alpha = 0.05$ ) stratified by randomization categories. Between-group differences in median Kaplan–Meier times to achieve ammonia level  $\leq\text{ULN}$  (and 95% confidence intervals) were used to characterize the estimated reduction in time to normalization of ammonia levels due to the use of OP.

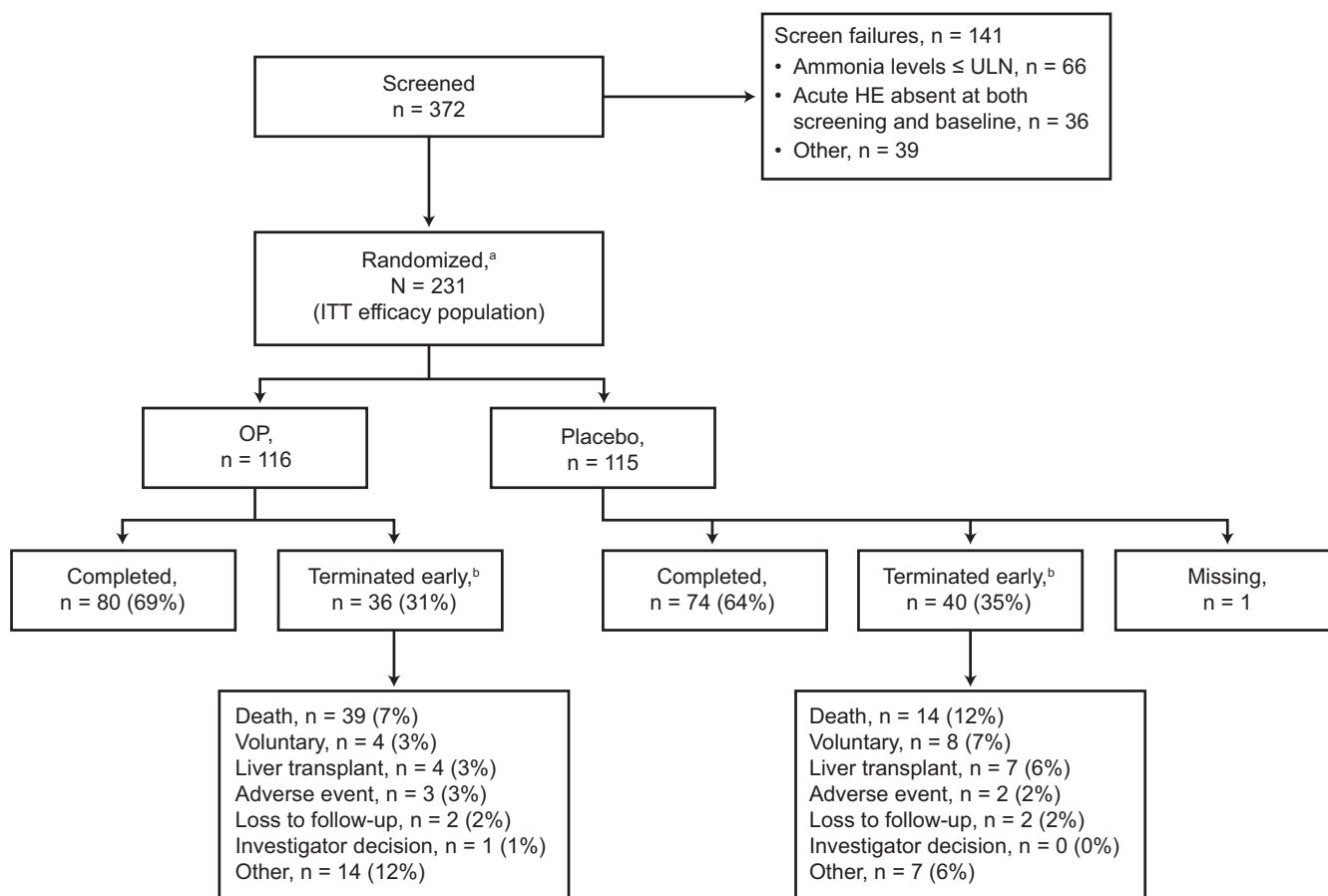
The time-normalized area under the curve (TN-AUC) for plasma concentrations of ammonia, PAA, and ORN over the 5-day treatment period was calculated in individual patients from day 1 to day 6/7 (at 3 hours after the end of the infusion) using the trapezoidal rule and using all available (non-missing) values, including the baseline value (day 1 prior to start of drug infusion). The TN-AUC and TN-AUC-minus-baseline (TN-AUCMB) values were compared between treatment groups using a van Elteren test (two-sided alpha = 0.05), stratified by randomization strata. The TN data from patients in the OP treatment group were also used to generate scatter plots and evaluate correlations (using Pearson's correlation) between TN-AUC plasma ammonia and TN-AUC plasma PAA; between TN-AUCMB plasma ammonia and TN-AUCMB plasma PAA; between TN-AUC plasma ammonia and TN-AUC plasma ORN; and between TN-AUCMB plasma ammonia and TN-AUCMB plasma ORN. Scatter plots were also produced to evaluate correlations between total plasma ammonia and HEST-based HE stage using all paired evaluation times; correlations were assessed using Spearman rank correlation by treatment group.

## RESULTS

A total of 114 patients were included in the PK analysis population, and a total of 231 patients were included in the intent-to-treat (ITT) analysis (placebo,  $n = 115$ ; OP,  $n = 116$ ). Patient disposition is summarized in [Figure 1](#). Demographic variables and baseline characteristics for the ITT population are summarized in [Table 1](#). Treatment groups were well matched for age, gender, race, and body mass index ( $p \geq 0.187$ ). Patients received different doses of OP based on calculations of CTP score at screening. As reported previously, medical history, HEST stage, MELD score, and use of concomitant medications for HE such as lactulose or rifaximin were similar between the OP and placebo groups.<sup>19</sup>

### PK results

Plasma concentrations of ORN and PAA, the principal components of OP, achieved steady-state levels approximately 48 and 72 hours, respectively, after initiation of



**FIGURE 1** Patient disposition flow diagram. <sup>a</sup>The safety population ( $n = 226$ ) included all patients who received any randomly assigned study treatment. <sup>b</sup>Only one reason could be recorded per patient; if death was chosen as the reason, an adverse event could not be chosen as well. HE, hepatic encephalopathy; ITT, intention-to-treat; OP, ornithine phenylacetate; ULN, upper limit of normal

**TABLE 1** Demographics and baseline characteristics (intent-to-treat population)

Attribute	Placebo (n = 115)	OP dose			Overall (n = 116)	Total (N = 231)
		≤10 g/24 h (n = 29)	15 g/24 h (n = 60)	20 g/24 h (n = 27)		
Age, mean (SD), years	60 (9.5)	59 (6.4)	58 (11.0)	62 (9.8)	59 (9.8)	59 (9.6)
Sex, n (%)						
Male	78 (68)	20 (69)	35 (58)	17 (63)	72 (62)	150 (65)
Female	37 (32)	9 (31)	25 (42)	10 (37)	44 (38)	81 (35)
Race, n (%)						
White	54 (47)	15 (52)	30 (50)	13 (48)	58 (50)	112 (48)
Black/African American	8 (7)	1 (3)	3 (5)	1 (4)	5 (4)	13 (6)
Asian	1 (1)	0	1 (2)	0	1 (1)	2 (<1)
Native Hawaiian/Pacific Islander	1 (1)	0	0	0	0	1 (<1)
American Indian/Alaska Native	0	0	1 (2)	0	1 (1)	1 (<1)
Other	1 (1)	0	1 (2)	0	1 (1)	2 (<1)
Missing	50 (43)	13 (45)	24 (40)	13 (48)	50 (43)	100 (43)
Weight, kg, mean (SD)	84.6 (21.6)	77.9 (22.0)	85.5 (22.7)	78.8 (21.7)	82.1 (22.4)	83.3 (22.0)
BMI, kg/m <sup>2</sup> , mean (SD)	29.20 (6.7) <sup>a</sup>	27.63 (7.6) <sup>b</sup>	28.96 (7.1) <sup>c</sup>	27.55 (7.3) <sup>d</sup>	28.31 (7.2) <sup>a</sup>	28.75 (6.9) <sup>e</sup>
Child–Turcotte–Pugh class, n (%)						
A	1 (1)	0	0	2 (7)	2 (2)	3 (1)
B	28 (24)	1 (3)	12 (20)	25 (93)	38 (33)	66 (29)
C	86 (75)	28 (97)	48 (80)	0	76 (66)	162 (70)

Abbreviations: BMI, body mass index; OP, ornithine phenylacetate; SD, standard deviation.

<sup>a</sup>n = 112; <sup>b</sup>n = 27; <sup>c</sup>n = 59; <sup>d</sup>n = 26; <sup>e</sup>n = 224.

constant OP infusion across all dose levels (7, 10, 15, or 20 g per 24 h). Mean plasma PAA levels in the PK population increased with dose, as summarized in [Figure 2a](#); in each dose cohort, mean plasma PAA levels increased with declining liver function (assessed using CTP class). In the 15 g/24 hours dose group, mean PAA C<sub>ss</sub> increased from 93.7 to 165 μg/ml, and in the 20 g/24 hours dose group from 153 to 218 μg/ml, with progressing decline in liver function from CTP class B to C and from A to B, respectively. Mean plasma ORN levels, summarized by dose level in [Figure 2b](#), also increased with dose.

Mean plasma levels of PAGN, the major metabolite of PAA, are illustrated over time and summarized by dose level in [Figure 2c](#). Mean plasma PAGN levels reached steady state approximately 72 hours after the initiation of OP treatment; however, there was substantial intersubject variability at all doses. Mean plasma PAGN levels over time increased with dose, with mean C<sub>ss</sub> in the 20 g/24 hours group about 50% greater than those in the 10 g/24 hours group. With respect to urinary excretion of PAGN, approximately 50% to 60% of the daily PAA dose (both mean and median) was excreted in the form of PAGN

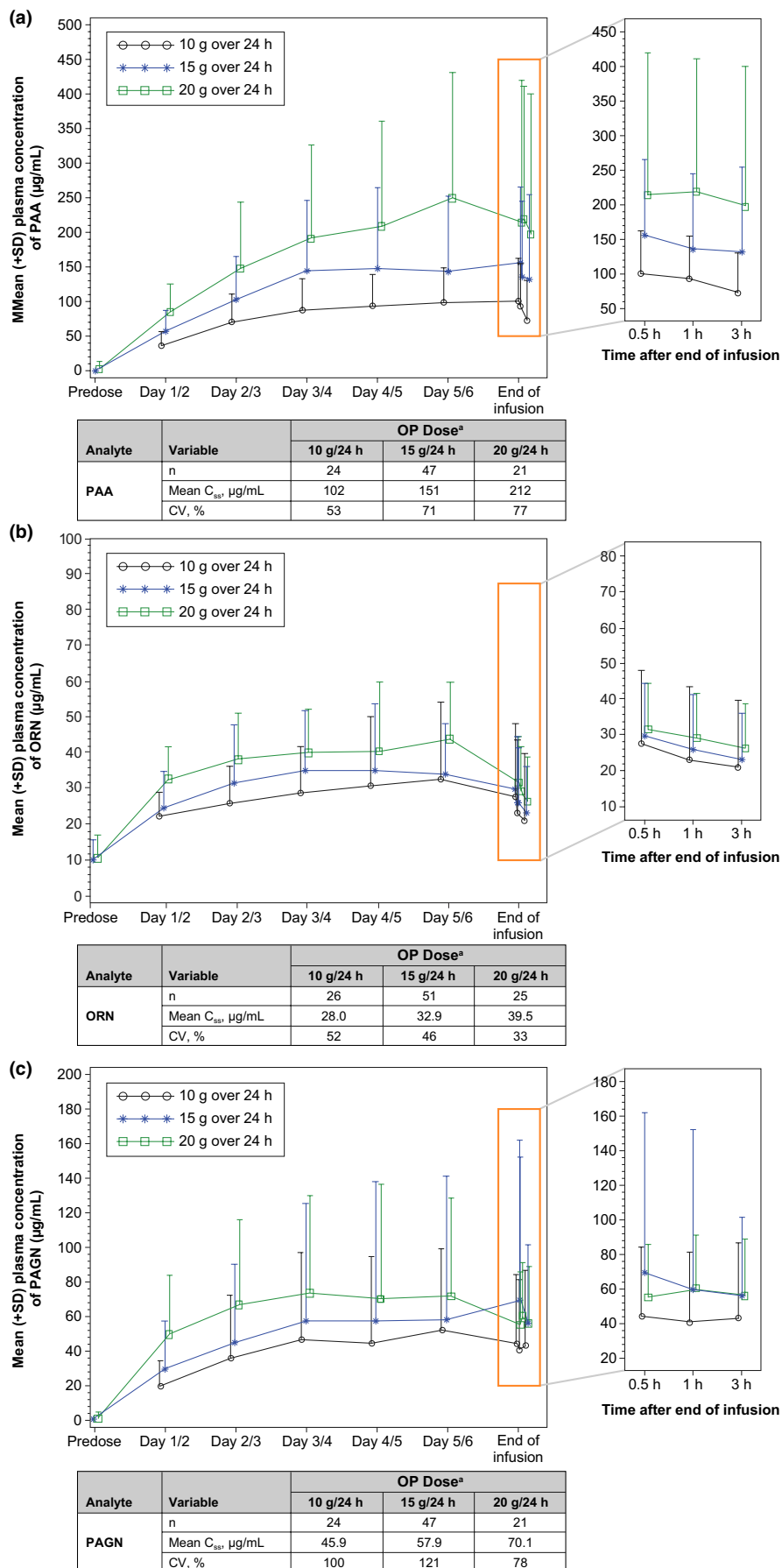
across all days and doses ([Table 2](#)). In addition, there was no observable effect of CTP class on the percentage of the PAA daily dose excreted as urinary PAGN.

## PD results

There was a dose-dependent reduction in mean plasma ammonia levels for all OP doses, compared with placebo, beginning 12 hours after initiation of OP (mean reductions of −4.4, −10.0, −19.3, and −24.8 μmol/L in the placebo and ≤10, 15, and 20 g/24 hours OP groups, respectively). At 3 hours postinfusion, dose-dependent reductions in plasma ammonia concentration in the OP groups were also observed: mean (standard deviation [SD]) reductions were −15.6 (40.9), −28.3 (63.1), and −39.1 (40.3) μmol/L in the 10, 15, and 20 g/24 hours OP groups, respectively, versus −1.2 (106.0) μmol/L in the placebo group. Across all OP doses, the mean reduction (SD) at 3 hours postinfusion was −27.8 (52.6) μmol/L (*p* value vs. placebo = 0.014) ([Figure 3](#)).

Similar patterns were observed with respect to TN, baseline-corrected plasma ammonia AUC levels

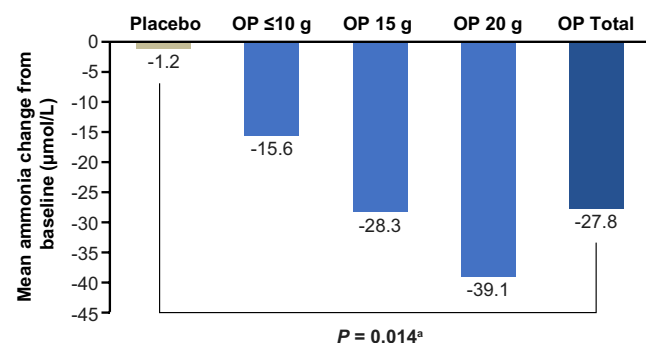
**FIGURE 2** Mean (standard deviation) plasma levels and mean steady-state plasma concentrations of (a) phenylacetic acid (PAA), (b) ornithine (ORN), and (c) phenylacetylglutamine (PAGN) following initiation of ORN infusion, by dose level<sup>a</sup>. <sup>a</sup>Only 1 patient received 7 g/24 hours ornithine phenylacetate (OP) (not shown in the figure). Mean plasma levels of PAA, ORN, and PAGN for this patient were lower than those of the 10, 15, and 20 g/24 hours dose groups across all timepoints evaluated. Mean  $C_{ss}$  values for PAA, ORN, and PAGN after intravenous infusion of OP for this patient were 55.5, 17.9, and 22.2  $\mu\text{g}/\text{mL}$ , respectively.  $C_{ss}$ , steady-state plasma drug concentration; CV, coefficient of variation



OP dose level <sup>a</sup>	Variable	Daily PAA dose excreted in urine as PAGN (%)			
		Days 2–3	Days 3–4	Days 4–5	Days 5–6
10 g/24 h	<i>n</i>	25	20	19	12
	Mean % excreted	42.3	51.9	56.5	47.7
	CV, %	57	67	91	49
15 g/24 h	<i>N</i>	43	35	30	28
	Mean % excreted	51.5	50.4	54.8	62.3
	CV, %	49	46	59	48
20 g/24 h	<i>N</i>	19	20	14	10
	Mean % excreted	48.1	55.8	62.6	64.0
	CV, %	57	60	87	38

Abbreviations: CV, coefficient of variation; OP, ornithine phenylacetate; PAA, phenylacetic acid; PAGN, phenylacetylglutamine.

<sup>a</sup>Only 1 patient received 7 g/24 hours OP. For this patient, 64.4% and 66.6% of the daily PAA dose was excreted in urine as PAGN across days 2–3 and days 3–4, respectively.



**FIGURE 3** Dose-related mean reduction in plasma ammonia level from baseline to 3 hours after the end of infusion. <sup>a</sup>The two-sided *p* value comparing ornithine phenylacetate (OP) and placebo was based on a van Elteren test stratified by randomized strata

(TN-AUCMB) at 3 hours postinfusion, with mean reductions (SD) of  $-6.8$  (33.1),  $-14.4$  (21.2),  $-26.2$  (31.2), and  $-34.2$  (26.2)  $\mu\text{mol/L}$  in the placebo, 10, 15, and 20 g/24 hours OP groups, respectively. Across all OP doses, the mean reduction (SD) at 3 hours postinfusion was  $-25.0$  (28.5)  $\mu\text{mol/L}$  (*p* value vs. placebo,  $<0.001$ ). In addition, there was an overall, statistically significant correlation ( $p = 0.032$ ) between plasma ammonia levels (TN-AUCMB) at 3 hours postinfusion or early hospital discharge and the HEST stage (Figure S2). Correlations between plasma ammonia ( $\mu\text{mol/L}$ ) TN-AUCMB and plasma PAA ( $\mu\text{mol/L}$ ) TN-AUCMB were not statistically significant (Figure S3). Similarly, correlations between plasma ammonia ( $\mu\text{mol/L}$ ) TN-AUCMB and plasma ORN ( $\mu\text{g/ml}$ ) TN-AUCMB were not statistically significant at the 15 g/24 hours and 20 g/24 hours doses (Figure S4). The time to achieve plasma ammonia levels  $\leq\text{ULN}$  was significantly shorter across all OP treatment groups than with placebo treatment ( $p = 0.028$ ) (Figure 4). In addition, the

**TABLE 2** Percentage of daily dose of phenylacetic acid excreted in urine as phenylacetylglutamine, by dose level and study day

probability of achieving a plasma ammonia level  $\leq\text{ULN}$  was significantly greater with OP than with placebo (hazard ratio, 1.691).

### Effect of a reduction in plasma ammonia concentration on clinical outcome

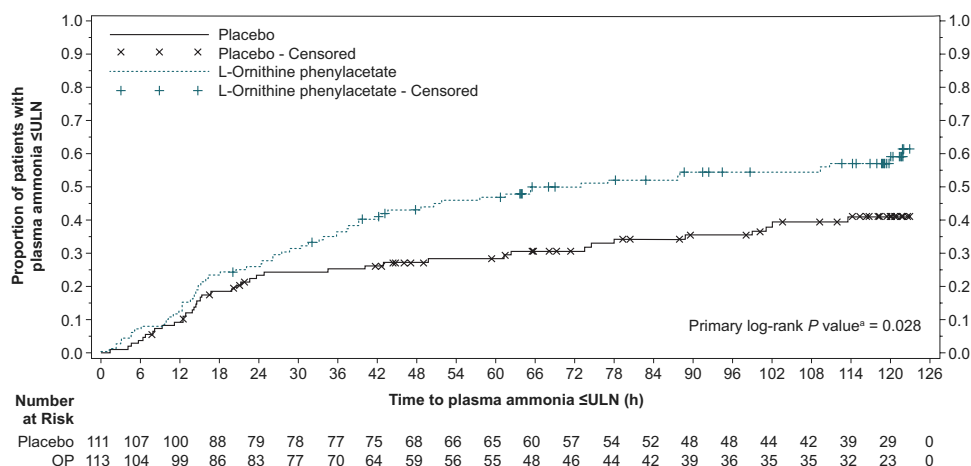
The covariance model for achieving a confirmed clinical response (versus not achieving a clinical response) as a fixed effect, adjusted for randomization strata, demonstrated a statistically significant difference for overall mean plasma ammonia concentration reduction ( $p = 0.009$ ). The confirmed primary clinical response rate at 3 hours postinfusion was 73/115 (63%) in the placebo group, and 21/29 (72%), 42/60 (70%), and 19/27 (70%) in the 10, 15, and 20 g/24 hours OP groups, respectively.

The median time for a confirmed clinical response was 47 hours for patients who received OP; therefore, 48 hours was selected as the landmark timepoint at which to evaluate the clinical effect of plasma ammonia reduction across all patients. At 48 hours, there was a significant correlation observed between plasma-ammonia-level reduction and clinical improvement: mean (SD) plasma-ammonia-level reduction in the 78 placebo- and OP-treated patients who achieved a confirmed clinical response was  $-22.6$  (40.6)  $\mu\text{mol/L}$ , compared with  $-1.7$  (39.7)  $\mu\text{mol/L}$  among the 33 patients not achieving a confirmed clinical response ( $p = 0.009$ ) (Figure 5).

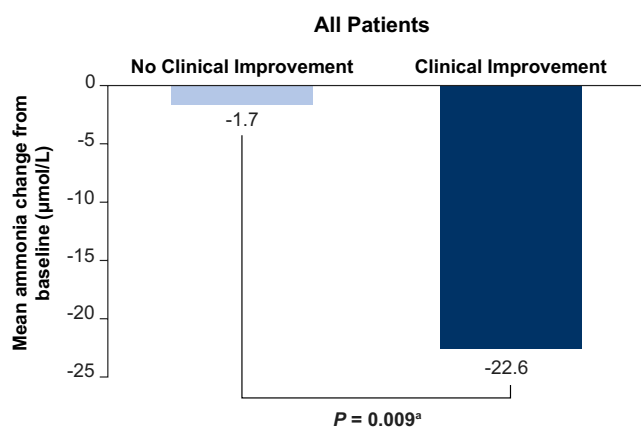
## DISCUSSION

This is the largest-scale, multicenter, prospective study to date assessing the PK and PD effects of OP in patients with





**FIGURE 4** Kaplan–Meier analysis of time to achieve plasma ammonia levels less than or equal to upper limit of normal. Patients who died, had a liver transplant, or discontinued the study were censored. <sup>a</sup>The two-sided *p* value comparing ornithine phenylacetate and placebo is based on a log-rank statistic, stratified by randomization strata. OP, ornithine phenylacetate; ULN, upper limit of normal



**FIGURE 5** Correlation between plasma ammonia concentration reduction and clinical improvement at 48 hours.

<sup>a</sup>The two-sided *p* value was calculated from an analysis of covariance model with a fixed effect for achieving versus not achieving primary clinical response, adjusting for the randomization strata

acute HE/OHE. In this study, mean plasma levels of the two components of OP, ORN, and PAA, reached steady state in approximately 48 and 72 hours, respectively, after study drug initiation regardless of dose. Mean plasma PAA concentrations appeared to increase as patients' liver function declined, as shown by the CTP score progressing from class A to B or from class B to C within the same dose level of OP. Mean plasma levels of PAA and ORN over time increased with dose. One patient who received the 20 g/24 hours dose had plasma PAA concentrations that were greater than 500 µg/ml. None of the patients in the 10 g/24 hours or 15 g/24 hours OP dose group had plasma PAA  $C_{ss}$  greater than 500 µg/ml.

Similar to PAA, PAGN achieved steady-state plasma concentrations about 72 hours after the start of infusion across all OP dose levels; however, there was extensive intersubject variability at all dose levels. Mean plasma levels of PAGN over time also increased with dose: there was an increase of approximately 50% in mean  $C_{ss}$  of PAGN as the dose of OP doubled from 10 g/24 hours to 20 g/24 hours. The percentage of the daily PAA dose excreted in the urine as PAGN was consistent, with the average of PAA excreted in urine as PAGN at approximately 50% to 60% across all three OP dose levels.

OP was significantly superior to placebo with respect to mean reduction from baseline in plasma ammonia levels (*p* = 0.017) and the time required for normalization of plasma ammonia level. Changes from baseline in ammonia levels at 3 hours after the end of the infusion were dose-dependent in the OP treatment group, and overall, there was a statistically significant correlation (*p* = 0.032) between plasma ammonia levels (TN-AUCMB) at 3 hours postinfusion or early hospital discharge and the HEST stage.

The effect of OP on plasma ammonia levels was also shown in a small, open-label study by Ventura-Cots and colleagues.<sup>18</sup> OP treatment for a special group of patients at risk for HE, those with decompensated cirrhosis and upper gastrointestinal bleeding (*n* = 10), were compared to a matched historical cohort (*n* = 10). Beginning at 24 hours after baseline, patients who received treatment with OP at 10 g/day demonstrated significantly greater reductions in plasma ammonia than those in the control group, despite similar baseline ammonia levels.

Reductions in ammonia levels were correlated with reductions in plasma glutamine and with urinary excretion of PAGN. Interestingly, within the OP treatment

group, the plasma ammonia reduction response depended on baseline values: the 7 patients with baseline ammonia  $>50 \mu\text{mol/L}$  achieved a mean 65% reduction at day 5 ( $p < 0.01$ ), while ammonia levels remained relatively unchanged in the 3 patients with baseline ammonia  $<50 \mu\text{mol/L}$ . The inability of OP to significantly reduce plasma ammonia in these patients with lower baseline levels may reflect the additional ammonia load associated with gastrointestinal bleeding.

Importantly, the results demonstrated the dose-dependency of plasma ammonia concentration reduction (Figure 3) and, at the same time, the reduction in plasma ammonia level correlated with clinical response (Figure 5). Of note, the reduction observed in the placebo arm may be because patients in both arms received standard-of-care therapy throughout their hospital stay.

Limitations of this study include the inability to evaluate formally the effect of different OP doses, as the dose level was not randomized but instead calculated based on individual prespecified hepatic and portal parameters. In addition, the inherent subjectivity of HEST scoring, especially across multiple study sites, may have affected initial patient stratification, as well as the ability to achieve the primary efficacy outcome. There may also have been discrepancies in screening/baseline ammonia levels obtained locally (study sites were permitted to use either their own assays or the central laboratory) versus central laboratory-measured values (required for all subsequent assessments).

In conclusion, the results of this PK/PD analysis were consistent with the putative mechanism of action of OP. The significant association between reduced plasma ammonia and improvement in HE stage was consistent with the proposed central role of ammonia in the development and progression of HE. Taken in combination with the primary study results, data from the current analysis support ongoing clinical development of OP as a novel and potent ammonia scavenger for the treatment of patients with OHE. Larger trials are required to further validate these PD findings at various dose levels.

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## CONFLICT OF INTEREST

R.S.: declares no competing interests for this work. R.S.R.: research support and an honorarium from Ocera Therapeutics (now Mallinckrodt Pharmaceuticals). D.T.: speaker for Norgine B.V. J.S.B.: institutional grant from Ocera Therapeutics and payment of personal fees during the conduct of the study; institutional grants from Valeant and Grifols; payment by Ocera and Norgine of personal fees for participation in an advisory board; payment by Valeant of personal fees. K.R.B.: grants from Gilead Sciences, Inc., Vital Therapies, and Mallinckrodt Pharmaceuticals; support as a member of the scientific advisory boards of Merck, Gilead, AbbVie, Inc., and Intercept Pharmaceuticals; and support from Alexion Pharmaceuticals, Inc., for providing disease state education. N.P.: research grants from Beigene, Intercept, Grifols, DURECT, Bayer, BMS, and Mallinckrodt Pharmaceuticals. A.P.: salary as an employee of Ocera Therapeutics at the time of the study. S.B.: salary as an employee of Ocera Therapeutics at the time of the study. L.W. received fees from Ocera Therapeutics for the design of this study and analysis of the pharmacokinetic data. K.J. is an employee of Mallinckrodt Pharmaceuticals. K.R.D. is a former employee of Mallinckrodt Pharmaceuticals who participated in the post hoc pharmacokinetic/pharmacodynamic analysis. The sponsor participated in the review of the manuscript (scientific accuracy and intellectual property only).

## AUTHOR CONTRIBUTIONS

R.S.R., R.S., K.R.B., and S.B. wrote the manuscript. J.S.B., L.W., N.P., and S.B. designed the research. D.T., J.S.B., K.R.B., K.R.D., L.W., S.B., R.S.R., and R.S. performed the research. A.P., K.R.D., K.J., L.W., N.P., R.S.R., and R.S. analyzed the data.

## DATA AVAILABILITY STATEMENT

Discussion of statistical end points and analysis are included in the article. Summary aggregate (basic) results (including adverse events information) and the study protocol will be available on clinicaltrials.gov ([NCT01966419](https://clinicaltrials.gov/ct2/show/study/NCT01966419)) when required by regulation. Individual de-identified patient data will not be disclosed. Requests for additional information should be directed to Mallinckrodt Pharmaceuticals at [medinfo@mnk.com](mailto:medinfo@mnk.com).

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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