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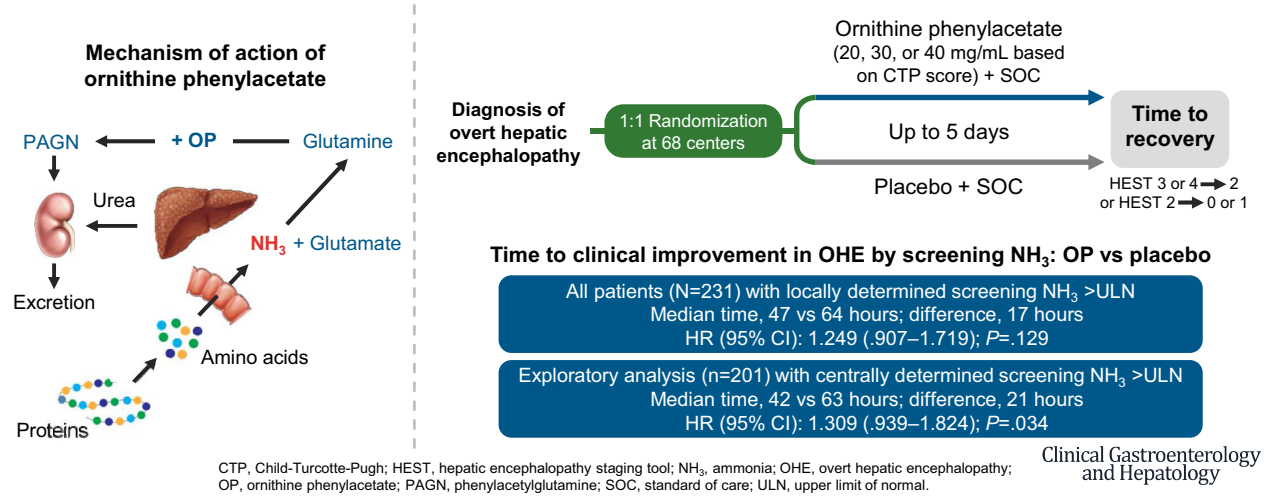

CTP, Child-Turcotte-Pugh; HEST, hepatic encephalopathy staging tool; NH_3, ammonia; OHE, overt hepatic encephalopathy; OP, ornithine phenylacetate; PAGN, phenylacetylglutamine; SOC, standard of care; ULN, upper limit of normal.

BACKGROUND & AIMS: **Hepatic encephalopathy (HE) is associated with increased morbidity, mortality, and health care resource use. In this phase 2b study, we evaluated the efficacy and safety of ornithine phenylacetate (OP), an ammonia scavenger, in hospitalized patients with cirrhosis, increased levels of ammonia at screening, and acute or overt HE.**

METHODS: **We conducted a double-blind study of 231 patients with cirrhosis and HE at multiple sites in North America, Europe, Israel, and Australia from January 7, 2014, through December 29, 2016. Patients were assigned randomly to groups that received placebo or OP (10, 15, or 20 g/d, based on the severity of liver disease), plus each institution's standard of care (eg, lactulose to achieve 2–3 bowel movements with or without rifaximin, in accordance with guidelines). The primary end point was time to confirmed clinical response, defined as reduction to HE staging tool (HEST) stage 2 from baseline HEST stages 3/4 or improvement to HEST stages 0/1 from**

[a]Authors share co-first authorship.

Abbreviations used in this paper: AE, adverse event; BM, bowel movement; CNS, central nervous system; HE, hepatic encephalopathy; HEST, Hepatic Encephalopathy Staging Tool; HR, hazard ratio; ICU, intensive care unit; ITT, intent-to-treat; LOLA, L-ornithine L-aspartate; MELD, Model for End-stage Liver Disease; MO-log, modified-orientation log; OHE, overt hepatic encephalopathy; OP, ornithine phenylacetate; PAA, phenylacetic acid; QTcF, QT interval with Fridericia correction; SOC, standard of care; TEAEs, treatment-emergent adverse events; ULN, upper limit of normal.

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baseline stage 2, in the intent-to-treat population (all patients with increased levels of ammonia at screening, determined by a local laboratory).

RESULTS: **Median times to clinical improvement, based on ammonia measurements at local laboratories, did not differ significantly between the groups given OP vs the placebo group ($P = .129$). Analyses of central laboratory–confirmed increases in levels of ammonia at baseline (n = 201) showed clinical improvement in HE at a median of 21 hours sooner in groups given OP vs placebo. The percentages of patients with any specific adverse event did not differ significantly between groups. Serious adverse events occurred in 25% of patients in the OP group and in 29% in the placebo group ($P = .552$).**

CONCLUSIONS: **In a randomized controlled trial of patients with cirrhosis and HE, we found no significant difference in time to clinical improvement between patients given OP vs placebo. However, OP appears to be safe and should undergo further testing for treatment of hyperammonemia in hospitalized patients receiving treatment for the underlying precipitant of acute or overt HE. ClinicalTrials.gov no: NCT01966419.**

Keywords: Altered Mental Status; Liver Fibrosis; Portal Hypertension; NH3.

See editorial on page 2493.

Overt hepatic encephalopathy (OHE) is a debilitating, generally reversible, neurologic condition that is common in patients with cirrhosis and portal hypertension.[1,2] It is associated with increased hospitalizations, health care costs, and mortality. A history of OHE increases the risk of recurrence within 6 months despite standard lactulose therapy.[2] In a North American cohort of hospitalized patients with cirrhosis, approximately 50% were re-admitted within 3 months, most commonly because of hepatic encephalopathy (HE).[3] Mortality for patients with severe HE in the intensive care unit (ICU) is estimated at 24% to 54%.[4,5] HE lasting 48 hours or longer, regardless of baseline HE grade, may be an independent predictor of mortality.[6] Furthermore, HE episodes among patients with cirrhosis may be associated with persistent, cumulative cognitive deficits despite appropriate therapy.[7] The ongoing morbidity and mortality associated with HE highlight the unmet need for new therapies.

Although the etiology of HE is complex and multifactorial, an increased systemic ammonia concentration is considered key to its pathophysiology.[1,8] It has been proposed that gut-derived toxins, primarily ammonia, bypass a failing liver that normally would detoxify them, and ultimately cross the blood-brain barrier, impairing central nervous system (CNS) function.[8–10] Consequently, a rapid reduction of ammonia is an important approach to treating acute HE and preventing recurrence.[1,8]

The American Association for the Study of Liver Diseases and the European Association for the Study of the Liver recommend a 4-pronged approach to treating acute HE.[2] A key step is controlling precipitating factors with concurrent initiation of pharmacotherapy.[2] The main standard medical therapies are nonabsorbable disaccharides (eg, lactulose, lactitol) and nonabsorbable antibiotics (eg, rifaximin).[2] Guidelines recommend lactulose as the initial therapy for episodic OHE; rifaximin is indicated for preventing recurrence and is recommended in combination with lactulose.[2,11] Lactulose and rifaximin are administered orally and may be unsuitable for patients with gastrointestinal bleeding or severe encephalopathy.

Guidelines also note L-ornithine L-aspartate (LOLA) as an alternative for patients unresponsive to conventional treatment, although evidence from a meta-analysis is tentative.[10] LOLA can lower ammonia levels by trapping ammonia as glutamine, but the effect is not sustained when infusion of LOLA is stopped; rebound may occur, possibly because new ammonia forms.[8,9] Furthermore, LOLA is not readily available worldwide.

Ornithine phenylacetate (OP) is a novel ammonia scavenger that lowers ammonia levels independent of gut action (ie, by a different mechanism) in patients with cirrhosis.[9] L-ornithine stimulates the activity of glutamine synthetase, inducing body muscle to trap circulating ammonia as glutamine. Glutamine then is conjugated with phenylacetic acid (PAA) to form phenylacetylglutamine, which is excreted in the urine, avoiding formation of new ammonia.[12] Three early phase studies in patients with cirrhosis showed that OP could be administered safely and decrease plasma ammonia levels.[12–14]

The primary objectives of this phase 2b study were to evaluate the efficacy and safety of OP in comparison with placebo when used concomitantly with standard of care (SOC) (eg, lactulose to achieve 2–3 bowel movements [BMs] with or without rifaximin, in accordance with guidelines) in patients with cirrhosis hospitalized with an episode of OHE.

Patients and Methods

Study Design

This randomized, double-blind, placebo-controlled, international study (ClinicalTrials.gov NCT01966419)

conducted at 132 sites (68 sites recruited ≥1 patients) evaluated OP in patients hospitalized with cirrhosis, hyperammonemia, and OHE stage 2 or higher on the Hepatic Encephalopathy Staging Tool (HEST), developed under US Food and Drug Administration guidance (Table 1, Supplementary Methods). Investigators were trained to use the HEST. The protocol was approved by the institutional review board or independent ethics committee at each site. The study was conducted in accordance with the principles of the Declaration of Helsinki. A legally authorized representative provided surrogate written informed consent for patient participation, with patient consent when possible.

All authors had access to the study data and reviewed and approved the final manuscript.

What You Need to Know

Background

The efficacy and safety of ornithine phenylacetate, an ammonia scavenger, were evaluated in hospitalized patients with cirrhosis who had increased levels of ammonia at screening and acute or overt hepatic encephalopathy.

Findings

The median time to clinical improvement in ammonia levels, the primary efficacy end point, was similar between groups of patients given ornithine phenylacetate vs placebo. Ornithine phenylacetate was safe and well tolerated overall.

Implications for patient care

The safety profile and trends in efficacy end points support a future trial of ornithine phenylacetate in a similar population but with an enhanced study design.

Patients

Eligible patients were men or nonpregnant women aged 18 to 75 years with cirrhosis (either evidence of or an established diagnosis), hospitalized with acute HE at screening (≥12 hours after HE diagnosis in the hospital, to prevent enrollment of patients who would have responded within 12 hours with SOC only [eg, lactulose]) and at baseline (study day 1 and randomization). Venous ammonia levels had to exceed the upper limit of normal (ULN) at screening, determined locally for rapid turnaround. The original protocol required ammonia levels of ≥1.5 × ULN; this criterion was amended to ≥1.3 × ULN, and, finally, higher than the ULN to achieve full enrollment. Ammonia samples also were analyzed at a central laboratory, but not to confirm eligibility before randomization (see the Supplementary Methods section for more detail).

Study Procedures

Enrollment began January 7, 2014; the last patient completed the study on December 29, 2016. The study included periods for diagnosis, screening, treatment (maximum, 5 days, if clinically indicated), and follow-up evaluation (14 days) (Supplementary Figure 1, Supplementary Methods). Treatment (initiation of infusion) was to begin 1 hour or less (±15 min) after randomization. HE was assessed by the HEST score, Glasgow Coma Scale,[15] and modified-orientation log (MO-log)[16] before starting the infusion, daily during infusion (7 AM and 5 PM, ±1 h), 3 hours after the end of the infusion, and on day 19 (2 weeks after the end of the infusion). Patients could be discharged before 5 days (120 hours) of continuous infusion if medically appropriate. For patients remaining in the hospital, HE parameters were reassessed 24 hours after the end of the infusion and immediately before discharge if discharge occurred during the follow-up period. All patients had a follow-up visit 2 weeks after cessation of study drug.

Treatments

Continuous intravenous infusions of OP or placebo (5% aqueous dextrose) were given for 5 days or fewer (500 mL/24 h [20.8 mL/h] through a separate peripheral venous catheter) in addition to SOC (eg, lactulose to achieve 2–3 BMs with or without rifaximin) based on the investigator's clinical judgment and usual institutional practice. Patients assigned to OP were randomized to 1 of 3 dosages according to baseline Child–Turcotte–Pugh score (40, 30, or 20 mg/mL for 4–6, 7–9, or 10–12 points, respectively, with each element [ascites, total bilirubin, albumin, and international normalized ratio] ranging from 1–3 points, because all patients had HE). An unblinded pharmacist prepared the initial OP solution and adjusted the study drug concentration appropriately. Patients received the randomization dose throughout the study (see the Supplementary Methods section for more detail).

Randomization and Blinding

An interactive voice-response or Web-based response system was used to assign patient numbers and to randomize patients to OP or matching placebo (1:1), labeled to maintain blinding. Randomized patients were stratified by Model for End-stage Liver Disease (MELD) score (≤30 vs >30), and categorized by HEST stages 2, 3, or 4, and, for North America only, by liver transplantation centers performing 70 or more transplants per year vs fewer than 70. An unblinded

Table 1. Hepatic Encephalopathy Staging Tool

Stage	Criteria
0/1	No asterixis[a] and no disorientation based on the following 5 questions (ie, patient provides a correct response to questions 1, 2, 3, 4, and 5): 1. What is your name? 2. What city are we in? 3. What type of place is this? (correct answer: hospital) 4. What is the year? 5. What is the month?
2	Asterixis[a] and disorientation based on the following 5 questions (ie, any single incorrect response qualifies the patient as stage 2 for questions 1, 2, 3, 4, or 5): 1. What is your name? 2. What city are we in? 3. What type of place is this? (correct answer: hospital) 4. What is the year? 5. What is the month?
3	Stupor, arousable but falls asleep, responsive to verbal stimuli Obvious confusion Gross disorientation
4	Coma

NOTE. To qualify for study entry as stage 2 hepatic encephalopathy, both asterixis and ≥1 error in the 5 sentinel questions must have been present at screening and baseline. For recording hepatic encephalopathy response after starting study drug infusion, patients were classified as improved to stages 0/1 only if asterixis resolved *and* all 5 questions were answered correctly.
[a]Three or more flaps/30 s indicates asterixis.

third-party statistician supported the Independent Data Monitoring Committee, identified pharmacokinetic samples, and oversaw data management for special laboratory results.

End Points and Assessments

The primary efficacy end point was time to confirmed clinical response, defined as reduction to HEST stage 2 from baseline HEST stages 3/4, or improvement to HEST stages 0/1 from baseline HEST stage 2. Time to clinical response was from initiation of drug infusion to the first of 2 assessments meeting response criteria, or to the first assessment meeting response criteria if a patient was discharged before any further efficacy assessment. Post hoc exploratory analyses evaluated time to confirmed clinical response for patients with a baseline ammonia level greater than the ULN determined by a central laboratory (results unavailable at randomization) and time to confirmed clinical response, censored at 48 hours after starting drug infusion.

Prespecified secondary efficacy end points, tested sequentially after the primary end point, included time to complete response (confirmed improvement to HEST stages 0/1 after initiating infusion to 3 hours after ending infusion); cumulative proportion of patients fulfilling primary end point response criteria through 3 hours after infusion; change from baseline in MO-log (scores 0–3 for 24 questions; higher scores indicate better orientation to time and place); length of hospitalization (start of infusion until discharge); and length of ICU stay (from start of infusion for ICU patients).

Safety analyses included adverse events (AEs), laboratory assessments, change from baseline MELD score, vital signs, and electrocardiogram changes including QT interval with Fridericia correction (QTcF) and PR interval (interval between the start of the P wave and the start of the QRS complex).

Statistical Analyses

An independent, unblinded third party conducted analyses using the SAS System (SAS Institute Inc, Cary, NC) version 9.2 or higher. The intent-to-treat (ITT) population (all randomized patients) was used to analyze efficacy, patient characteristics, and disposition. The

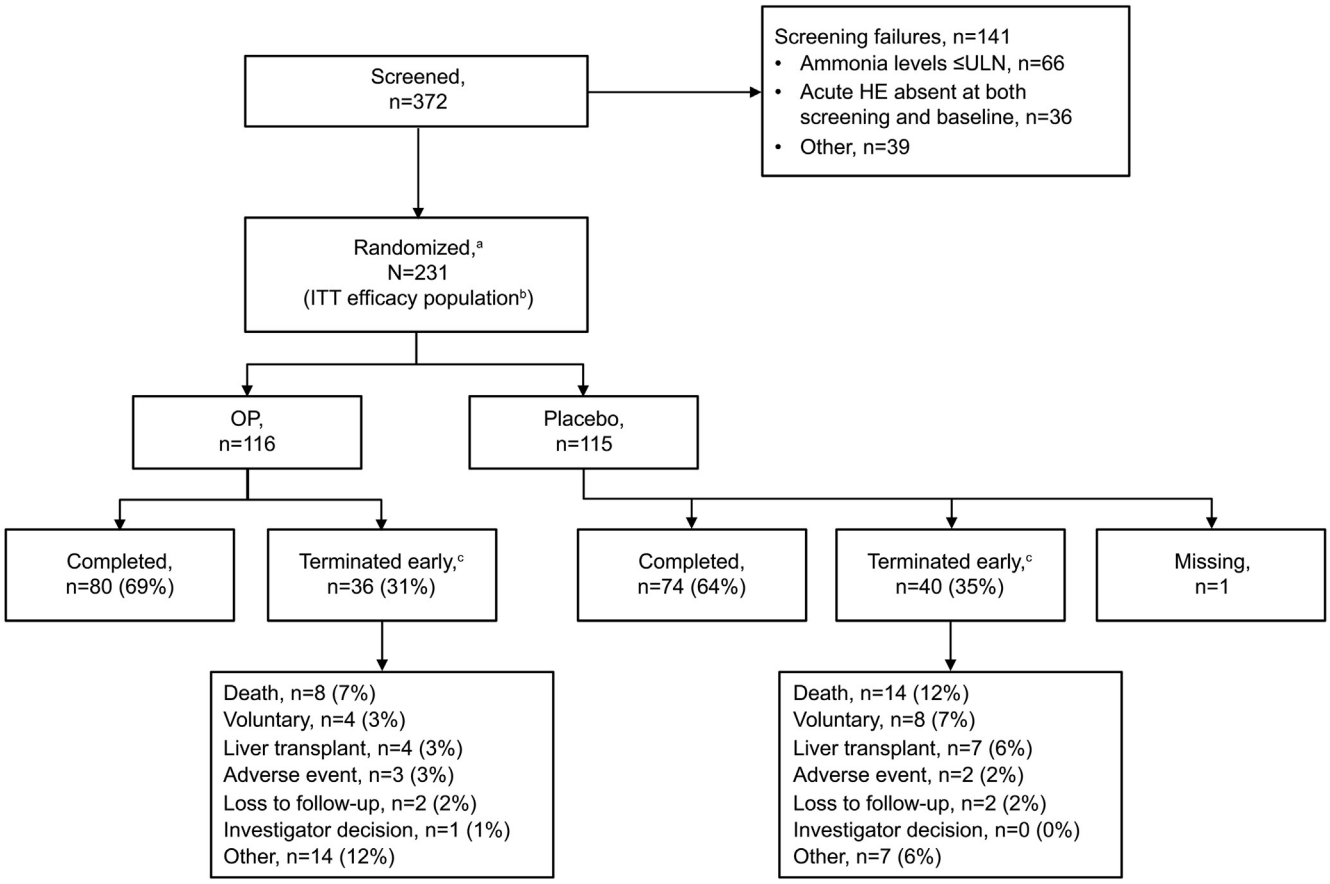

Figure 1. Patient disposition. [a] Safety population (n = 226) included randomized patients who received any study treatment. [b] Five patients were excluded from the primary efficacy analysis because of missing Hepatic Encephalopathy Staging Tool stage at baseline. [c] Only 1 reason was recorded per patient; if death was the reason, an adverse event could not also be chosen. HE, hepatic encephalopathy; ITT, intention-to-treat; OP, ornithine phenylacetate; ULN, upper limit of normal.

safety population included ITT patients who received any study treatment (see the Supplementary Methods section for more detail).

Sample Size

Assuming 70 patients per group and a requirement for 74 primary end point events in total, a 0.05 significance level, 2-sided log-rank test of equality of time-to-event curves was hypothesized to render approximately 80% power to detect a difference between SOC and OP response rates of 45% and 68%, respectively. After a preplanned interim analysis of the primary end point at 25 observed events, the Independent Data Monitoring Committee recommended increasing the sample size to approximately 234 patients, with 146 events required.

Results

Patient Disposition

Of 372 patients screened, 231 were randomized (ITT population) to OP (n = 116) or placebo (n = 115). The safety population included 226 patients (OP, n = 114; placebo, n = 112). The most common reason for screen failure (n = 141) was ammonia level lower than the ULN (n = 66; 47%) (Figure 1). The mean number of days in the study was similar for OP (18 d; SD, 6.7 d) and placebo (17 d; SD, 6.9 d) groups; 121 patients (52%) completed 5 days of infusion (OP, 62 [53%]; placebo, 59 [51%]). The mean volumes infused in the placebo, 10-g (1 patient received 7 g), 15-g, and 20-g groups, respectively, were 1878 (SD, 745), 2024 (SD, 607), 1899 (SD, 701), and 2027 (SD, 704) mL (P = .654 across groups). The most common reasons for discontinuing infusion in fewer than 5 days were similar between groups (P = .812): early hospital discharge (OP, 18%; placebo, 17%), investigator decision (OP, 12%; placebo, 11%), and AEs (OP, 6%; placebo, 7%).

Baseline and screening characteristics. Baseline demographics, disease characteristics, and medical history were similar between groups (Table 2). The median ammonia levels at baseline (determined locally) for patients who received any OP (n = 113) or placebo (n = 111) were as follows: 86.9 μmol/L (range, 23.4–242.7 μmol/L) and 85.9 μmol/L (range, 6.2–398.8 μmol/L), respectively. Ammonia levels at screening differed significantly by disease severity (P = .003); median

Table 2. Baseline Demographic and Disease Characteristics (Intent-to-Treat Population)

Characteristic	OP (n = 116)	Placebo (n = 115)	*P* value
Male, n (%)	72 (62)	78 (68)	.359
Median age, *y* (minimum, maximum)	60 (26, 74)	61 (27, 79)[a]	.704
Child–Turcotte–Pugh score, n (%)			.292
A	2 (2)[b]	1 (<1)[c]	
B	38 (33)	28 (24)	
C	76 (66)	86 (75)	
Hepatic Encephalopathy Staging Tool stage, n (%)			.229
2	68 (59)	71 (62)	
3	40 (34)	30 (26)	
4	6 (5)	11 (10)	
Missing	2 (2)	3 (3)	
Mean (SD) Model for End-stage Liver Disease score at randomization	19 (6.7)	19 (6.3)	.359
Most common inciting etiologic factors, n (%)			.644
Bacterial infection	15 (13)	14 (12)	
Poor compliance (lactulose)	15 (13)	13 (11)	
Dehydration	16 (14)	9 (8)	
Transjugular intrahepatic portosystemic shunt	7 (6)	9 (8)	
Constipation	8 (7)	7 (6)	
Esophageal/gastric variceal bleeding	4 (3)	7 (6)	
Most common concomitant medications for hepatic encephalopathy[d]			
Lactulose			
Use, n (%)	91 (78)	82 (71)	
Mean dose, *mL/d* (SD)	131.4 (145.7)	134.8 (103.3)	
Rifaximin			
Use, n (%)	72 (62)	63 (55)	
Mean dose, *mg/d* (SD)	1072.9 (177.2)	1039.7 (209.9)	

OP, ornithine phenylacetate; SD, standard deviation.
[a]One patient aged 79 years was enrolled; not considered a major protocol violation.
[b]Patients had a value of 4 in the interactive voice-response system for hepatic synthetic and portal elements of the Child–Turcotte–Pugh score.
[c]Patient had a value of 4 in the interactive voice-response system for hepatic synthetic and portal elements of the Child–Turcotte–Pugh score.
[d]Based on safety population (OP, n = 114; placebo, n = 112).

ammonia levels were as follows: 82.3 μmol/L (range, 30.6–209.4 μmol/L), 94.9 μmol/L (range, 33.0–257.3 μmol/L), and 129.0 μmol/L (range, 60.8–287.2 μmol/L) for HEST stages 2, 3, and 4, respectively (Supplementary Table 1). Most patients (89%) had no change in HEST stage from screening to randomization. One placebo and 1 OP patient shifted from stage 2 to stages 0/1. Baseline central ammonia determinations in the ITT population (N = 231) were greater than the ULN (n = 201), normal (n = 24), low (n = 1), and missing (n = 5). Among patients with nonmissing central ammonia levels (n = 226), the median was 86.1 μmol/L (range, 6.2–398.8 μmol/L). Baseline laboratory parameters appeared similar among groups, except for bilirubin and direct bilirubin levels (Supplementary Table 2).

Efficacy Outcomes

Primary efficacy outcome. A statistically significant difference between OP and placebo was not shown in the primary efficacy end point. The median time to confirmed clinical response in the OP and placebo groups was 47 (95% CI, 34–69) hours and 64 (95% CI, 53–98) hours, respectively; the between-group difference was 17 hours ($P = .129$). The hazard ratio (HR) of 1.25 (95% CI, 0.907–1.719) indicated an approximate 25% increase in the probability of confirmed response with OP vs placebo (Figure 2*A*).

Secondary. In the ITT population, the median time to complete response (confirmed improvement to HEST stages 0/1) in the OP and placebo groups was 87 hours and 102 hours, respectively ($P = .361$; HR, 1.16; 95% CI, 0.818–1.651).

The cumulative proportion of patients with a complete response in the OP and placebo groups was 59% and 51%, respectively, 3 hours after the end of infusion or early hospital discharge/early termination ($P = .230$). The cumulative proportion of patients (ITT population) with a confirmed primary clinical response in the OP and placebo groups was 71% and 63%, respectively ($P = .228$), 3 hours after the end of infusion or early hospital discharge/early termination.

The median MO-log baseline scores were 8 (minimum, maximum, 0, 23) and 9 (minimum, maximum, 0, 24) for the OP and placebo groups, respectively. The

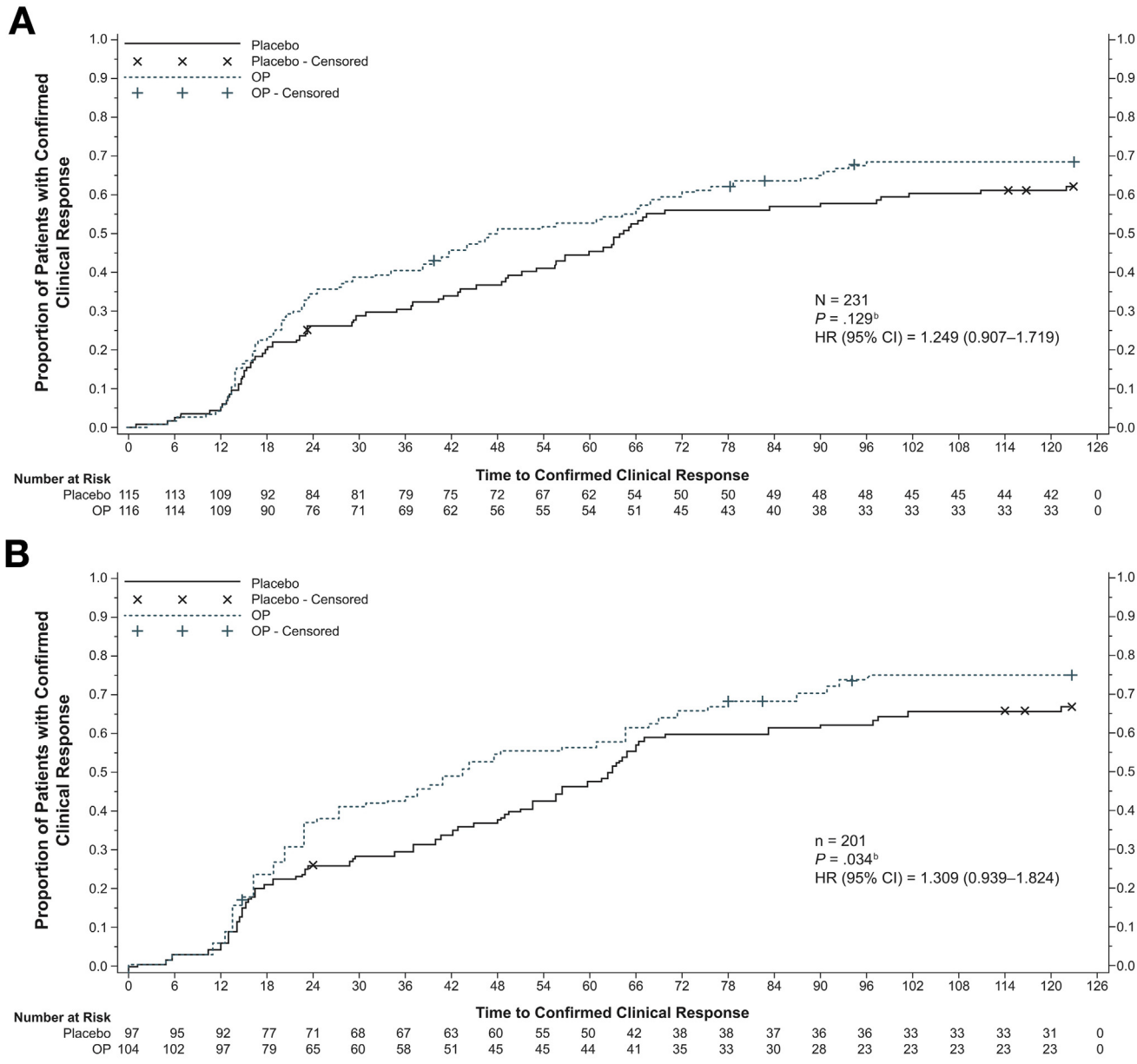

Figure 2. Time to clinical response (Kaplan–Meier plots). (*A*) Time to confirmed clinical response (intent-to-treat population as read by local laboratories). (*B*) Time to clinical response (exploratory, patients with centrally confirmed baseline ammonia greater than the upper limit of normal). [a] Patients who had a liver transplant or died were censored at that time. [b] The 2-sided *P* value comparing ornithine phenylacetate (OP) and placebo was based on a log-rank statistic, stratified by randomization strata. HR, hazard ratio.

median change from baseline in MO-log scores at 3 hours after infusion or early hospital discharge was similar between each OP group and placebo (OP 10 g/24 h: 10 [minimum, maximum, −6, 24], 15 g/24 h: 10 [minimum, maximum, −11, 24], and 20 g/24 h: 10 [minimum, maximum, −2, 24]; placebo: 8 [minimum, maximum, −15, 24]; $P = .750$, $P = .585$, and $P = .226$, respectively).

The median length of hospitalization was similar for OP and placebo groups (170 h, 95% CI, 146–192 h; and 169 h, 95% CI, 142–212 h, respectively; $P = .584$). Among patients starting infusion in the ICU (OP, n = 30; placebo, n = 33), the median time to discharge from the ICU was 155 hours (95% CI, 117–453) and 192 hours (95% CI, 75–377) for OP and placebo groups, respectively ($P = .7$).

Sensitivity analysis. Comparison of subgroups based on the baseline MELD, HEST, and Child–Turcotte–Pugh scores and other factors showed almost no significant differences in efficacy outcomes.

Exploratory analysis. After excluding 30 patients with normal or missing baseline ammonia levels (because OP is an ammonia scavenger) using the central laboratory, the median time to confirmed clinical response was shorter in the OP group (n = 104) than in the placebo group (n = 97) (42 vs 63 h). The HR of 1.31 (95% CI, 0.939–1.824) indicated approximately 30% greater

Table 3. Incidence of Treatment-Emergent AEs (Safety Population)

	OP dose/24 h					
	10 g (n = 29)	15 g (n = 59)	20 g (n = 26)	Total (n = 114)	Placebo (n = 112)	*P* value[a]
Any AE	20 (69)	42 (71)	18 (69)	80 (70)	79 (71)	>.999
Treatment-related AE	5 (17)	10 (17)	3 (12)	18 (16)	14 (13)	.568
Serious AEs	10 (34)	13 (22)	6 (23)	29 (25)	33 (29)	.552
Discontinued because of an AE	3 (10)	4 (7)	0	7 (6)	9 (8)	.614
Most common AEs occurring in ≥5% of patients in the OP or placebo groups						
Anemia	5 (17)	6 (10)	3 (12)	14 (12)	8 (7)	.262
Hepatic encephalopathy[b]	2 (7)	7 (12)	1 (4)	10 (9)	11 (10)	.822
Hypokalemia	3 (10)	4 (7)	2 (8)	9 (8)	8 (7)	>.999
Urinary tract infection	2 (7)	3 (5)	3 (12)	8 (7)	4 (4)	.375
Pyrexia	1 (3)	3 (5)	2 (8)	6 (5)	8 (7)	.593
Hypophosphatemia	2 (7)	2 (3)	1 (4)	5 (4)	9 (8)	.283
Hypotension	1 (3)	3 (5)	1 (4)	5 (4)	4 (4)	>.999
Headache	2 (7)	2 (3)	1 (4)	5 (4)	2 (2)	.446
Nausea	2 (7)	2 (3)	1 (4)	5 (4)	4 (4)	>.999
Pneumonia	2 (7)	3 (5)	0	5 (4)	2 (2)	.446
Edema	0	4 (7)	0	4 (4)	3 (3)	>.999
Peripheral edema	1 (3)	1 (2)	2 (8)	4 (4)	3 (3)	>.999
Hypomagnesemia	1 (3)	3 (5)	0	4 (4)	4 (4)	>.999
Acute renal failure	1 (3)	0	2 (8)	3 (3)	6 (5)	.331
Ascites	1 (3)	0	2 (8)	3 (3)	5 (4)	.497
Pain	2 (7)	0	1 (4)	3 (3)	1 (1)	.622
Abdominal distension	0	3 (5)	0	3 (3)	1 (1)	.622
Hyperkalemia	0	3 (5)	0	3 (3)	2 (2)	>.999

NOTE. The number of patients (%) are shown.
AE, adverse event; OP, ornithine phenylacetate.
[a]2-sided Fisher exact test.
[b]Defined as worsening disease; not statistically significant.

probability of confirmed response with OP vs placebo (Figure 2*B*).

The percentages of patients achieving a confirmed primary clinical response within 48 hours after infusion were 37% with placebo and 51% with OP. Censoring patients at 48 hours after the start of infusion, the Kaplan–Meier estimate of the median time to confirmed primary clinical response was 47 hours for the OP group but too long to be estimated for the placebo group; the difference was significant ($P = .026$). The HR of 1.502 (95% CI, 1.01–2.23) indicated approximately 50% greater probability of confirmed response within 48 hours with OP vs placebo.

Safety

The overall incidence of treatment-emergent AEs (TEAEs), serious TEAEs, and TEAEs leading to discontinuation was similar for the OP and placebo groups (Table 3). Among TEAEs in 5% or more of the patients in either group, only anemia had an incidence of ≥5% greater in the OP than the placebo group; there was no evidence of a dose-response in the OP groups. No clinically important differences were observed between OP and placebo groups for TEAEs related to bleeding (9 [7.9%] and 10 [8.9%], respectively). Among all patients who experienced TEAEs, most events were mild (41 [18%]) or moderate (58 [26%]). There were no statistically significant between-group differences in the incidence of treatment-related AEs (OP, 16%; placebo, 13%; $P = .568$) or any specific TEAE. Two patients in the OP group had CNS-related TEAEs (headache, vascular dementia) and a PAA concentration of 200 μg/mL or greater (208.2 and 399.9 μg/mL). PAA concentrations overlapped between patients who had mild vs moderate CNS-related TEAEs (Supplementary Figure 2). Mean steady-state PAA concentrations ranged from 102 to 212 μg/mL across the OP doses. PAA concentrations at the end of infusion are shown in Supplementary Figure 3.

Twenty-six deaths occurred during the study (OP, 11 [9.5%]; placebo, 15 [13%]; $P = .413$). When assignment to study drug dose by baseline hepatic function (ie, Child–Turcotte–Pugh score) was considered, death rates for OP and placebo were 24% vs 26% for 10 g/24 h, 5% vs 12% for 15 g/24 h, and 4% vs 0% for 20 g/24 h. The most common AEs leading to death were associated with underlying hepatic disease (OP, n = 6; placebo, n = 6). Two TEAEs leading to death were considered possibly related to the study drug by the investigator (OP, 10 g/24 h, ventricular tachycardia; placebo, multi-organ failure).

Most patients experienced grade 3 (severe) or grade 4 (potentially life-threatening) laboratory abnormalities (OP, 75%; placebo, 71%). Treatment-emergent grade 3

(26% vs 17%) and grade 4 (9% vs 4%) hemoglobin level was higher in the OP group than in the placebo group, but no clinically important between-group differences in TEAEs or serious TEAEs related to bleeding were noted. Reticulocyte counts indicated functioning bone marrow. Incidences of other grades 3/4 treatment-emergent laboratory abnormalities, including hepatic (alanine aminotransferase, aspartate aminotransferase, bilirubin) and renal-related (creatinine) values, were similar for OP and placebo groups. No clinically significant differences between OP dose groups were observed.

No clinically significant mean differences from baseline in vital signs, including heart rate, respiratory rate, or temperature, were observed. The only treatment-emergent clinically notable vital sign abnormality was a significant increase in heart rate of 15 or more bpm, with a value greater than 120 bpm in the placebo group vs the OP group (4% vs 0%, respectively; $P = .029$) (see the Supplementary Results section for more details).

Discussion

In totality, this phase 2b study suggests that OP should undergo further evaluation for the treatment of OHE in patients with cirrhosis and hyperammonemia requiring hospitalization. This study used the HEST scale as the primary end point of clinical response; the scale continues to be refined and validated. Although a statistically significant treatment effect was not shown in the primary end point, the median time to clinical improvement in HE symptoms was numerically 17 hours shorter in patients treated with OP vs receiving placebo, which is considered clinically meaningful. The difference was greater in patients with confirmed baseline hyperammonemia. A reduction in time to clinical improvement is important because mortality, and deleterious neurocognitive and neurotoxic consequences, may increase with duration of HE.[6,7]

Ammonia levels were determined locally to expedite results; central laboratory results can require days, limiting their utility in this seriously ill population. Local laboratories, however, introduce variability in collection protocols and timing of analyses. Furthermore, ammonia levels could have normalized between screening and randomization. When the primary end point was reassessed only in patients with centrally confirmed ammonia levels greater than the ULN, the median time to confirmed clinical response was shorter (21-hour difference) in the OP group vs the placebo group. This suggests the potential of OP to improve clinical outcomes in patients with HE and highlights the need for improved point-of-care ammonia measurements. A higher cutoff value for ammonia levels also might have minimized discrepancies between local and central laboratories.

In another exploratory analysis, the response rate at 48 hours was greater with OP than placebo (51% vs 37%), and patients treated with OP had an approximate 50% increase in probability of confirmed clinical response within 48 hours vs placebo. Evidence suggests that a 48-hour duration of HE window exists for improving patient outcomes.[6]

Prespecified secondary clinical end points showed numerically greater benefits with OP vs placebo. The median time to complete response was shorter with OP vs placebo, and cumulative percentages of patients with a confirmed clinical response and confirmed complete response were greater in the OP group than in the placebo group 3 hours after the end of infusion or early hospital discharge/termination. The MO-log, which measures orientation, improved across all treatment groups, but without differences from placebo 3 hours after the end of infusion.

The length of hospitalization and/or ICU stay can be affected by HE status, but also by other medical or social factors; thus, the primary outcome remained time to clinical improvement. Because of the substantial morbidity, mortality, and economic burden of hospitalizations, outcomes such as the median time to discharge from the ICU and length of hospital stay were assessed.[1–3,5–7] Although the overall median time to hospital discharge was similar between the 2 groups, the median time to discharge from the ICU occurred approximately 1.5 days sooner for patients who received OP vs placebo.

OP generally was safe and well tolerated, without significant differences from placebo in the percentage of TEAEs, serious TEAEs, TEAEs leading to discontinuation, deaths, and abnormal laboratory values or vital signs. Although grades 3/4 anemia was more common in OP-treated patients than placebo, this was considered a random finding after extensive review of nonclinical and clinical data identified no reasonable biologic mechanism; no patients discontinued because of anemia. Although not statistically significant vs placebo, a greater percentage of OP-treated patients had a QTcF increase greater than 60 msec and absolute QTcF greater than 500 msec. The relationship of these events to the drug is unclear because patients with cirrhosis are at increased risk of cardiac arrhythmias and frequently have a prolonged QTc interval.[17] Electrolyte imbalances and reduced renal function might influence treatment differences in QT prolongation in patients hospitalized with HE.

Study limitations included issues already mentioned about local vs central laboratory determinations of ammonia levels. A more stringent entry criterion for HEST stage (ie, ≥ 3) might have resulted in more dramatic responses to treatment, but would have impeded patient enrollment. BM frequency was not assessed; however, the use and dose of lactulose (left to the discretion of the patient's primary medical team in consultation with the study investigator) were comparable between the OP and placebo groups. Future OP studies should consider a modified ITT primary analysis to exclude patients whose ammonia levels normalize by baseline or are less than $1.3 \times$ ULN. A planned phase 3 study will take experiences from the present study into account. Finally, patients with a Child–Turcotte–Pugh classification of C might have been underdosed. Dosing in these seriously ill patients was conservative to minimize safety risks.

Conclusions

Overall, OP was safe and well tolerated in hospitalized patients with cirrhosis and OHE. In patients with HE grades of 2 or higher and ammonia levels greater than the ULN confirmed by a central laboratory, OP showed proof of concept of its ammonia-scavenging mechanism with improvement in OHE.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2020.10.019.

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Conflicts of interest

These authors disclose the following: Robert S. Rahimi has served on the advisory board for Mallinckrodt Pharmaceuticals (formerly Ocera Therapeutics), and has received research support and an honorarium from Mallinckrodt; Kalyan Ram Bhamidimarri has received research grants from Gilead Sciences, Inc, Ocera/Mallinckrodt, and Vital Therapies, and has received support as a member of the scientific advisory boards of AbbVie, Inc, Esai Co, Gilead, Intercept Pharmaceuticals, and Merck; Nikolaos Pyrsopoulos has received grants/research support from Allergan, Bayer, BeiGene, Bristol-Myers Squibb, Confirm, Conatus, Exelixis, Genfit, Gilead, Hologic, Intercept, Mallinckrodt, Novartis, Prometheus, Resusix, Saro, Shire, and Valeant, and has consulted for Bayer, Exelixis, Gilead, and Novartis; Amy Potthoff received a salary and owns stock/equities as an employee of Ocera Therapeutics at the time of the study; Stan Bukofzer received a salary as an employee and Section 16 officer of Ocera Therapeutics at the time of the study; and Jasmohan S. Bajaj has served on the advisory board for Mallinckrodt. The remaining authors disclose no conflicts.

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Supplementary Methods

Study Design

In this study, the primary end point was assessed using HEST, developed in accordance with US Food and Drug Administration guidance, which is based on the West Haven Criteria but modified to be easier to administer and more objective. The scale continues to be refined and undergo further validation.

Patients

Patients with a transjugular intrahepatic portosystemic shunt, those intubated only for airway protection not requiring sedation, or those undergoing transitory intubation with sedation anticipated to be less than 24 hours were allowed to enroll.

Patients with serum creatinine concentration greater than 3 mg/dL (265.2 μmol/L) or with the need for hemodialysis, peritoneal dialysis, or continuous venovenous hemofiltration at screening, New York Heart Association class 3 or 4 congestive heart failure, prolonged QTcF greater than 500 msec at screening, or with an immediate poor hospital prognosis were excluded. Patients receiving or planning to receive sodium benzoate, LOLA, sodium benzoate/sodium phenylacetate, sodium phenylbutyrate, or glycerol phenylbutyrate, or those receiving concomitant administration of drugs known to interfere with renal excretion of phenylacetylglutamine, such as probenecid and penicillin antimicrobials, also were excluded. Patients were excluded if they presented with acute alcohol or drug intoxication. Other key exclusion criteria included use of the molecular adsorbent recirculation system, alcoholic hepatitis, and previous solid organ transplantation.

Study Procedures

All plasma for ammonia testing was to be prepared and frozen for storage with the greatest speed feasible. The collection tubes were chilled on ice for at least 10 minutes before drawing blood. Immediately after drawing blood, each tube was inverted gently approximately 8 to 10 times and placed back on wet ice. Within 15 minutes of drawing blood, plasma was separated by refrigerated centrifugation. The plasma specimens were frozen at -70°C or colder immediately after centrifugation and stored in a freezer at -70°C or colder. The frozen specimens were transported to a central laboratory testing site and processed by the laboratory or by trained personnel per facility standard operating procedures.

Safety, laboratory values, physical findings, electrocardiograms, and vital signs were monitored throughout the study period (screening through 24 hours after infusion, if still in the hospital). Neurologic examinations were conducted at screening, baseline, during treatment, and at study end according to standard of care. Institutional and physician standard of care was continued throughout the duration of the study.

Treatments

Concomitant drug administration. Drugs that could cause hyperammonemia, such as sedatives, sleep aids, and other psychoactive medications (barbiturates, opioids, and benzodiazepines), were prohibited during treatment. In addition, sedatives and sleep aids were not permitted through the 3- and 24-hour assessments after the end of the infusion, and other psychoactive medications were to be avoided during that time period.

Statistical Analyses

Demographic and baseline characteristics were summarized descriptively. For the primary efficacy end point and its exploratory analyses, the survival function for the time from the start of the infusion until a response was estimated using the Kaplan–Meier method. A stratified log-rank test was used for between-group comparisons. Hazard ratios were calculated using Cox models. For the primary end point, patients were censored upon liver transplant or death.

Efficacy statistical testing was 2-sided and controlled to a study-wise, type I error value of 0.05 using hierarchical testing: if an end point was not significant, efficacy was not inferred for ensuing end points, and subsequent P values were considered descriptive. Safety statistical testing was 2-sided ($\alpha = .05$) and unadjusted for multiple comparisons; analyses and P values were considered descriptive, not inferential.

Kaplan–Meier estimates were provided for time-based secondary analyses. Descriptive statistics were calculated for the change from baseline in MO-log by the time of collection. Between-group comparisons of change from baseline in MO-log through the 3-hour assessment after the end of the final infusion used a stratified van Elteren test.

The cumulative proportion of patients fulfilling primary end point response criteria through 3 hours after the end of the final infusion was compared between treatment groups using a stratified Cochran–Mantel–Haenszel test.

Safety end points were evaluated by treatment group and/or drug dosage. The Fisher exact test was used for between-group comparisons of TEAE preferred terms, patients with 1 or more TEAEs, treatment-emergent QTcF greater than 30 or greater than 60 msec, patients with QTcF greater than 450 or greater than 500 msec, change from baseline in PR interval (>25% increase if PR interval >200 msec), QRS complex (>25% increase if QRS complex >100 msec), and electrocardiogram heart

rate (>25% decrease from baseline to <50 bpm, or >25% increase from baseline to >100 bpm).

Supplementary Results

Low diastolic blood pressure was observed in both groups throughout the study. TEAEs related to abnormal electrocardiogram findings were QT prolonged in 3 patients (3%) in the placebo treatment group and in 2 patients (2%) in the OP treatment group, and electrocardiogram findings were abnormal (elongation of QT) in 1 patient (1%) in the OP treatment group. A QTcF increase of more than 60 msec was observed in 11% and 7% of patients who received OP and placebo, respectively ($P = .360$); an absolute QTcF of more than 500 msec was observed in 15% and 8% of patients who received OP and placebo, respectively ($P = .144$). No correlation between PAA concentration and QTcF was observed (data not shown).

The mean improvement (decrease from baseline) in the Model for End-stage Liver Disease score, indicating lessening severity of disease, was −4, −1, and −1 in the OP 10-, 15-, and 20-g/24 h groups, respectively, compared with 0 in the placebo group at 3 hours after the end of the infusion or early hospital discharge/early termination; the differences were not statistically significant.

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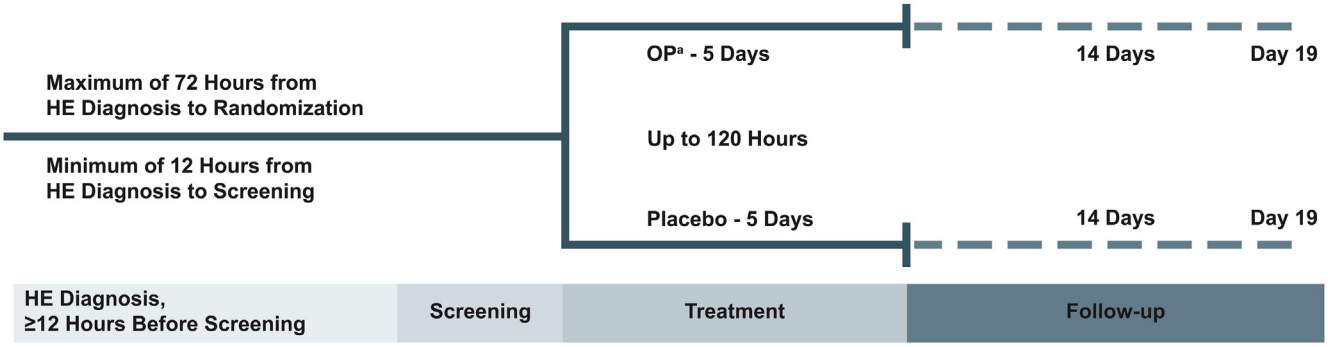
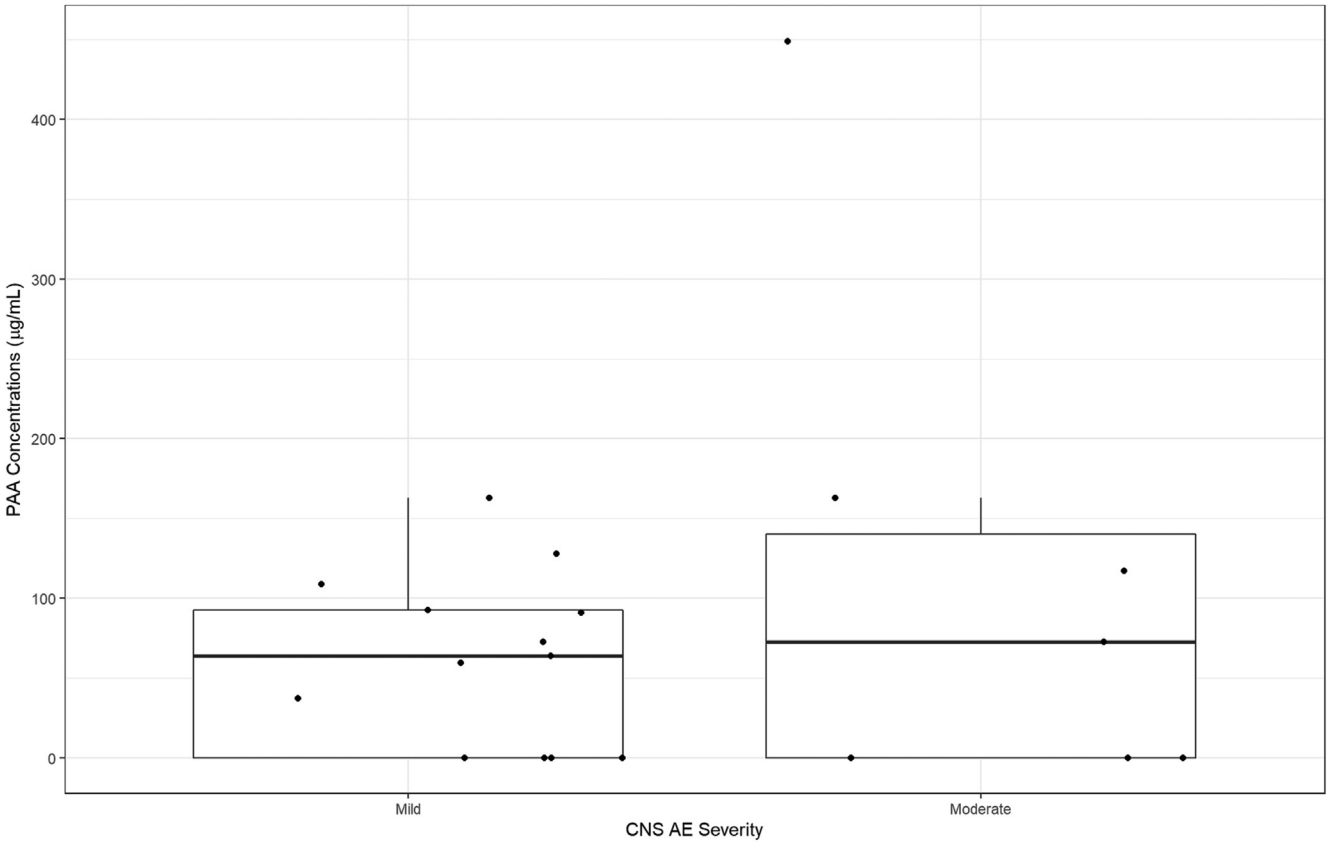
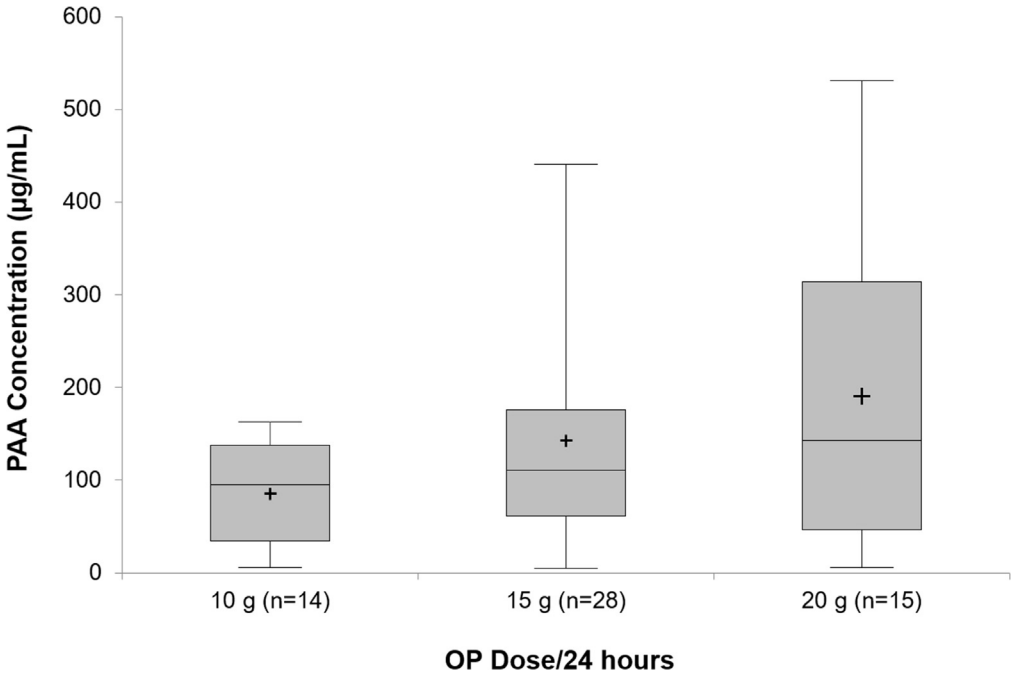


Supplementary Figure 1. STOP-HE study schematic. [a] OP was given as a continuous intravenous infusion for 5 days (500 mL/24 h; 20.8 mL/h), administered in addition to standard of care in accordance with investigator clinical judgment and usual institutional practice. HE, hepatic encephalopathy; OP, ornithine phenylacetate; STOP-HE, Safety and Tolerability of OCR-002 (ornithine phenylacetate) in Hospitalized Patients With Cirrhosis and Associated Hyperammonemia With an Episode of Hepatic Encephalopathy.



Supplementary Figure 2. Relationship between phenylacetic acid (PAA) concentration and the reported severity of all potentially central nervous system (CNS)-related treatment-emergent adverse events (TEAEs). The severity of the TEAEs was plotted against the observed PAA concentrations nearest the TEAE occurrence. This approach is a valid approximation because the TEAEs occurred either when the PAA concentration was at steady state or near the observed concentration. Some TEAEs also occurred when PAA had already been reduced to below the quantifiable level (treated as 0 in the plot). Individual concentrations are shown as **dots.** The lower and upper edges of the **boxes** represent the first and third quartiles, respectively. The **bold horizontal lines** represent the medians.

Supplementary Figure 3. The phenylacetic acid (PAA) concentration by ornithine phenylacetate (OP) dose 3 hours after the end of the infusion. The lower and upper edges of the **boxes** represent the first and third quartiles, respectively. The **horizontal lines** within each **box** represent the medians. The **plus signs** represent the means. The bottom and top **error bars** represent the minimum and maximum values, respectively. Values below the limit of quantitation were excluded.



Supplementary Table 1. Baseline Levels of Ammonia: Pharmacodynamic Population

	OP dose/24 h				
Ammonia, *μmol/L*	10 g (n = 29)	15 g (n = 58)	20 g (n = 26)	Total (n = 113)	Placebo (n = 111)
Median (range)	70.2 (39.2–235.8)	88.9 (23.4–242.7)	104.7 (46.4–196.8)	86.9 (23.4–242.7)	85.9 (6.2–398.8)
Mean (SD)	78.9 (36.08)	94.3 (43.76)	104.7 (37.86)	92.7 (41.28)	95.3 (57.36)

The pharmacodynamic population was defined as all randomized patients who were administered 1 or more doses of the study drug and had 1 or more postdose pharmacodynamic assessments (ie, plasma ammonia or urinary phenylacetylglutamine).
OP, ornithine phenylacetate.

Supplementary Table 2. Baseline Levels of Laboratory Parameters: ITT Population

	OP dose/24 h				
	10 g (n = 29)	15 g (n = 60)	20 g (n = 27)	Total (n = 116)	Placebo (n = 115)
Chemistry parameters, n					
Albumin, *g/dL*	29	59	26	114	112
Median (range)	2.3 (1.5–3.3)	2.6 (1.7–4.5)	3.3 (1.7–4.4)	2.6 (1.5–4.5)	2.7 (1.4–8.3)
Mean (SD)	2.3 (0.43)	2.6 (0.56)	3.2 (0.67)	2.7 (0.63)	2.8 (0.94)
Alkaline phosphatase, *U/L*	27	59	25	111	111
Median (range)	103.0 (39.0–319.0)	104.0 (49.0–254.0)	109.0 (64.0–246.0)	104.0 (39.0–319.0)	122.0 (32.0–530.0)
Mean (SD)	122.1 (67.41)	118.2 (50.09)	127.4 (53.57)	121.2 (55.11)	135.0 (76.29)
Alanine aminotransferase, *U/L*	29	59	26	114	112
Median (range)	28.0 (7.0–120.0)	27.0 (11.0–143.0)	29.5 (11.0–52.0)	28.0 (7.0–143.0)	30.0 (9.0–1392.0)
Mean (SD)	34.7 (22.21)	34.6 (22.98)	29.6 (12.50)	33.5 (20.80)	64.1 (150.74)
Aspartate aminotransferase, *U/L*	26	54	25	105	104
Median (range)	54.0 (16.0–312.0)	52.5 (20.0–192.0)	38.0 (15.0–179.0)	50.0 (15.0–312.0)	52.5 (10.0–950.0)
Mean (SD)	74.8 (62.80)	64.5 (37.05)	44.4 (31.15)	62.3 (44.61)	82.8 (106.55)
Bicarbonate, *mmol/L*	27	56	25	108	106
Median (range)	18.7 (10.7–25.4)	19.3 (11.7–32.4)	18.0 (9.7–26.6)	18.8 (9.7–32.4)	19.1 (10.4–31.0)
Mean (SD)	18.0 (3.83)	19.0 (3.92)	18.0 (4.31)	18.5 (3.98)	18.8 (4.15)
Bilirubin, *μmol/L*	29	59	26	114	112
Median (range)	127.0 (14.0–646.0)	58.0 (7.0–386.0)	26.0 (7.0–149.0)	53.0 (7.0–646.0)	57.5 (9.0–677.0)
Mean (SD)	168.3 (155.17)	77.4 (64.96)	32.3 (27.14)	90.2 (103.52)	102.3 (123.20)
Direct bilirubin, *μmol/L*	22	50	23	95	93
Median (range)	33.5 (7.0–206.0)	21.0 (3.0–141.0)	8.0 (2.0–53.0)	19.0 (2.0–206.0)	21.0 (3.0–206.0)
Mean (SD)	71.3 (64.04)	29.0 (26.89)	10.5 (10.33)	34.3 (42.33)	33.2 (35.90)
Blood urea nitrogen, *mmol/L*	27	59	26	112	111
Median (range)	8.2 (2.6–20.5)	6.8 (1.4–27.8)	7.5 (2.5–21.0)	7.5 (1.4–27.8)	7.1 (2.1–29.3)
Mean (SD)	9.2 (4.78)	8.1 (5.03)	9.0 (4.60)	8.6 (4.85)	8.8 (5.48)
Calcium (EDTA), *mmol/L*	27	59	26	112	111
Median (range)	2.1 (1.7–2.8)	2.1 (1.5–2.5)	2.2 (1.8–2.5)	2.1 (1.5–2.8)	2.2 (1.2–2.6)
Mean (SD)	2.1 (0.23)	2.2 (0.20)	2.2 (0.15)	2.2 (0.20)	2.2 (0.21)
Chloride, *mmol/L*	29	59	26	114	112
Median (range)	103.0 (93.0–123.0)	103.0 (91.0–125.0)	103.0 (85.0–114.0)	103.0 (85.0–125.0)	104.0 (82.0–128.0)
Mean (SD)	103.1 (6.44)	103.3 (6.85)	103.9 (6.91)	103.4 (6.71)	103.3 (7.56)
Creatinine, *mg/dL*	29	59	26	114	112
Median (range)	1.2 (0.4–3.1)	1.1 (0.3–3.1)	1.0 (0.5–1.9)	1.1 (0.3–3.1)	1.0 (0.5–2.7)
Mean (SD)	1.2 (0.64)	1.2 (0.64)	1.1 (0.45)	1.2 (0.60)	1.2 (0.51)
Glucose, *mmol/L*	29	59	26	114	112
Median (range)	6.4 (3.1–19.9)	8.2 (4.3–23.4)	8.5 (4.5–19.4)	7.5 (3.1–23.4)	6.9 (4.1–19.9)
Mean (SD)	7.5 (3.53)	10.4 (5.52)	9.4 (3.94)	9.5 (4.86)	8.3 (3.68)
Potassium, *mmol/L*	29	59	26	114	112
Median (range)	3.9 (2.4–5.1)	4.0 (2.4–6.2)	3.9 (2.5–5.6)	3.9 (2.4–6.2)	3.9 (2.5–7.4)
Mean (SD)	3.9 (0.63)	3.9 (0.74)	3.8 (0.62)	3.9 (0.69)	3.9 (0.68)
Magnesium, *mmol/L*	27	59	26	112	111
Median (range)	0.9 (0.6–1.1)	0.8 (0.3–1.3)	0.8 (0.6–1.2)	0.8 (0.3–1.3)	0.8 (0.2–1.3)
Mean (SD)	0.8 (0.12)	0.8 (0.17)	0.9 (0.15)	0.8 (0.15)	0.8 (0.18)
Phosphate, *mmol/L*	25	58	25	108	108
Median (range)	1.1 (0.5–1.5)	1.0 (0.5–1.8)	1.0 (0.7–1.3)	1.0 (0.5–1.8)	1.0 (0.4–1.6)
Mean (SD)	1.0 (0.29)	1.0 (0.28)	1.0 (0.19)	1.0 (0.26)	1.0 (0.25)
Sodium, *mmol/L*	29	59	26	114	112
Median (range)	136.0 (125.0–150.0)	136.0 (125.0–151.0)	138.0 (121.0–150.0)	136.0 (121.0–151.0)	136.0 (118.0–159.0)
Mean (SD)	135.0 (5.30)	136.2 (5.65)	137.8 (5.37)	136.2 (5.54)	136.6 (6.67)
Hematology parameters					
Hematocrit (%)	29	58	26	113	111
Median (range)	28.0 (21.0–38.0)	29.0 (18.8–41.0)	33.0 (23.8–48.0)	29.0 (18.8–48.0)	30.0 (19.7–50.0)
Mean (SD)	29.1 (4.87)	30.0 (5.54)	32.4 (6.38)	30.3 (5.66)	31.4 (6.62)

Supplementary Table 2. Continued

	OP dose/24 h				
	10 g (n = 29)	15 g (n = 60)	20 g (n = 27)	Total (n = 116)	Placebo (n = 115)
Hemoglobin, *g/dL*	29	58	26	113	112
Median (range)	9.3 (7.4–12.1)	10.0 (3.5–13.9)	10.3 (6.7–17.0)	9.8 (3.5–17.0)	10.3 (6.4–16.4)
Mean (SD)	9.5 (1.35)	10.0 (2.03)	10.6 (2.34)	10.0 (1.98)	10.5 (2.23)
Coagulation parameters					
Prothrombin international normalized ratio	29	58	26	113	111
Median (range)	1.8 (1.2–3.7)	1.5 (1.0–3.4)	1.3 (1.0–2.1)	1.5 (1.0–3.7)	1.6 (1.0–4.9)
Mean (SD)	1.9 (0.60)	1.6 (0.40)	1.3 (0.22)	1.6 (0.48)	1.7 (0.62)
Prothrombin time, *s*	29	57	26	112	110
Median (range)	18.2 (12.6–41.5)	15.4 (11.2–51.3)	13.2 (10.3–66.0)	15.3 (10.3–66.0)	16.0 (10.7–48.0)
Mean (SD)	19.9 (6.42)	17.6 (8.07)	15.7 (10.58)	17.7 (8.41)	18.3 (6.87)

NOTE. The ITT population with laboratory parameters containing data for fewer than 20 patients in the total OP group is not shown.
ITT, intent-to-treat; OP, ornithine phenylacetate.