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Randomized, Double-Blind, Controlled Study of Glycerol Phenylbutyrate in Hepatic Encephalopathy

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Glycerol phenylbutyrate (GPB) lowers ammonia by providing an alternate pathway to urea for waste nitrogen excretion in the form of phenylacetyl glutamine, which is excreted in urine. This randomized, double-blind, placebo-controlled phase II trial enrolled 178 patients with cirrhosis, including 59 already taking rifaximin, who had experienced two or more hepatic encephalopathy (HE) events in the previous 6 months. The primary endpoint was the proportion of patients with HE events. Other endpoints included the time to first event, total number of events, HE hospitalizations, symptomatic days, and safety. GPB, at 6 mL orally twice-daily, significantly reduced the proportion of patients who experienced an HE event (21% versus 36%; $P = 0.02$), time to first event (hazard ratio [HR] = 0.56; $P < 0.05$), as well as total events (35 versus 57; $P = 0.04$), and was associated with fewer HE hospitalizations (13 versus 25; $P = 0.06$). Among patients not on rifaximin at enrollment, GPB reduced the proportion of patients with an HE event (10% versus 32%; $P < 0.01$), time to first event (HR = 0.29; $P < 0.01$), and total events (7 versus 31; $P < 0.01$). Plasma ammonia was significantly lower in patients on GPB and correlated with HE events when measured either at baseline or during the study. A similar proportion of patients in the GPB (79%) and placebo groups (76%) experienced adverse events. *Conclusion*: GPB reduced HE events as well as ammonia in patients with cirrhosis and HE and its safety profile was similar to placebo. The findings implicate ammonia in the pathogenesis of HE and suggest that GPB has therapeutic potential in this population. (Clinicaltrials.gov, NCT00999167). (HEPATOLOGY 2014;59:1073-1083)

See Editorial on Page 764

Hepatic encephalopathy (HE) constitutes a spectrum of neuropsychiatric abnormalities ranging from confusion to coma.[1-7] It is typically

Abbreviations: AEs, adverse events; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHESS, Clinical Hepatic Encephalopathy Staging Scale; CI, confidence interval; DSMB, Data Safety Monitoring Board; ECG, electrocardiogram; GPB, glycerol phenylbutyrate; FDA, U.S. Food and Drug Administration; GI, gastrointestinal; HE, hepatic encephalopathy; HR, hazard ratio; INR, international normalized ratio; IRB, institutional review board; ITT, intention to treat; LT, liver transplantation; MELD, Model for End-Stage Liver Disease; PAA, phenylacetic acid; PAGN, phenylacetyl glutamine; PBA, phenylbutyric acid; QTc, corrected QT interval; SAEs, serious AEs; SD, standard deviation; TNAUC, time-normalized area under the curve; WH, West Haven.

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reversible, although patients do not always recover to the original level of function, and it represents a major burden to patients, families, and the health care system.[1-7]

The pathophysiology of HE is incompletely understood. Though elevated blood ammonia has long been suspected as important, the evidence is largely correlative, other factors have been postulated, and evidence against ammonia has been reported.[1-8] Current treatments, including poorly absorbed disaccharides (e.g., lactulose) and antibiotics (e.g., rifaximin), may act by reducing ammonia production and/or absorption in the intestine,[1,9] but are not ammonia selective.

Glycerol phenylbutyrate (GPB; HPN-100; Hyperion Therapeutics, Inc., South San Francisco, CA) is approved for treatment of urea cycle disorders, inherited disorders manifested by hyperammonemia,[10-14] and is under development for HE.[15] GPB consists of three molecules of phenylbutyric acid (PBA) joined to glycerol by ester linkage and is an odorless, nearly tasteless sodium-free liquid that acts by providing an alternate pathway for ammonia removal and waste nitrogen excretion in the form of urinary phenylacetyl glutamine (PAGN).[10-15]

A 4-week open-label study indicated that 6 mL of GPB given orally twice-daily with food was well tolerated and lowered ammonia in patients with cirrhosis and HE.[15] Therefore, we performed a multicenter, randomized, double-blind, placebo-controlled phase II study to test the hypothesis that lowering ammonia using this agent in patients with HE would decrease the likelihood of patients experiencing HE events.

Patients and Methods

Study Design. Patients were randomly assigned in a blinded fashion in a 1:1 ratio using a computerized central randomization schedule to receive 6 mL of GPB or matching placebo, orally, twice-daily, for 16 weeks. All study personnel were blind to treatment group assignment, as were patients and caregivers. Enrollment was stratified for rifaximin use at baseline. Patients continued to receive their standard of care treatment, including lactulose, rifaximin, or both, until an on-study HE event, after which the patient was allowed to continue on study and have their background standard of care modified. For example, patients on lactulose at enrollment could begin rifaximin treatment only after an on-study HE event. Compliance with study drug and lactulose was monitored using a daily caregiver log; study drug compliance was additionally assessed by monitoring the amount of drug returned.

The protocol was designed by Hyperion Therapeutics, Inc. in consultation with the authors and the U.S. Food and Drug Administration (FDA), was conducted under a U.S. investigational new drug application at study sites in the United States, Russia, and the Ukraine, and was reviewed and approved by the institutional review board (IRB) or ethics committee at each investigative site and/or a central IRB. The protocol conformed to the ethical guideline of the 1975 Declaration of Helsinki. All patients and/or their authorized representatives provided written informed consent. A Data and Safety Monitoring Board (DSMB) met periodically throughout the study to review safety data. Data were collected by the principal investigators and were monitored by Synteract and SPRI Clinical Trials. After commencement of enrollment, in consultation with the FDA and the DSMB, and based on satisfactory safety and the results of a thorough corrected QT interval (QTc) study as well as an interim analysis to which all sponsor and site personnel remained blinded, the QTc exclusion and age cut-off criteria were removed, the Model for End-Stage Liver Disease (MELD) score cutoff was increased to >25, and the enrollment limit was increased from 140 to 200.

Study Population. Eligible patients included adults with cirrhosis who had experienced at least two episodes of HE or West Haven (WH) grade 2 or greater, in the previous 6 months, one of which was within 3

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Additional Supporting Information may be found in the online version of this article.

months of randomization. Exclusion criteria included use of putative ammonia-lowering agents (e.g., L-ornithine-L-aspartate or sodium benzoate), active complications of cirrhosis (e.g., sepsis or bleeding), gastrointestinal (GI) bleeding requiring blood transfusion within 3 months, transjugular intrahepatic portosystemic shunt placement or revision within 90 days, recreational drug use or alcohol consumption for patients with a history of alcohol or drug abuse within 6 months, regular use of benzodiazepines, narcotics, or barbiturates, MELD score >25, serum creatinine >2 mg/dL, serum sodium <125 mEq/L, platelet count <35,000/μL, hemoglobin <8 g/dL, hematocrit <25%, expected liver transplantation (LT) within 6 months, and hypersensitivity to GPB or its metabolites. Patients taking rifaximin were eligible if they had experienced at least one of their two qualifying HE events after taking rifaximin for at least 1 month. All patients were to be followed through study termination.

Safety. Safety assessments included vital signs, electrocardiograms (ECGs), and hematologic and chemistry evaluations. The study included 10 trial-site visits, during which laboratory studies were obtained and adverse events (AEs) assessed, as well as two phone calls. Patients underwent a daily assessment by their designated caregiver based on the Clinical Hepatic Encephalopathy Staging Scale (CHESS).[16] The scale consists of nine "yes" or "no" questions related to the patient's overall awareness and physical and cognitive function, and a score of 3 generally corresponds to WH 2.[16] For patients with a score ≥3, the caregiver was instructed to contact the study site. HE events were in all cases adjudicated by the investigator.

Pharmacokinetics and Ammonia Sampling. Patients underwent blood sampling for venous ammonia, and plasma and urine levels of PBA, phenylacetic acid (PAA), and PAGN. Analyses of metabolites were performed by QPS Holdings LLC (Newark, DE). Ammonia was measured at accredited local laboratories in the United States and a central laboratory in Eastern Europe.

Outcomes. The prespecified primary endpoint for the intention-to-treat (ITT) population was the proportion (expressed as %) of patients experiencing an HE event, defined as either WH grade ≥2 or an increase ≥1 in both the WH and asterixis grades, if baseline WH was 0. The ITT population was predefined as all randomized subjects who received any amount of study drug. Prespecified secondary endpoints included time to first HE events and total HE events. The primary efficacy measure was adjudicated by the blinded investigators during the study and analyzed after unblinding. The DSMB had access to unblinded data upon request. Exploratory and post-hoc analyses included analyses of the treatment effect for HE events WH grade ≥2, the relationship of HE events to blood ammonia, results based on rifaximin use, and HE-related hospitalizations. Decisions regarding hospitalizations were based on standard of care and safety considerations and not stipulated by the protocol.

Sample Size and Statistical Analyses. A sample size of 186 was determined to be sufficient for 80% power to detect an expected 50% treatment effect based on a two-sided significance level of 0.05 (i.e., 18% versus 36% of patients in the GPB and placebo arms, respectively, experiencing events). Enrollment was stopped at a prespecified date (October 31, 2011), by which time 178 patients had been enrolled. The statistical analysis of the primary endpoint was conducted using a Cochran-Mantel-Haenszel (CMH) test stratified by country. Per the prespecified analysis plan, efficacy analyses were to be performed using the per-protocol population only if the sample size differed from the ITT population by more than 10%. A post-hoc sensitivity analysis of patients with HE events adjusted for Child-Pugh classification was performed.

Prespecified secondary endpoints, including time to first HE event and total HE events, were assessed by country-stratified Cox's proportional hazards and Poisson's regression analysis, respectively. Logistic regression analysis was used to analyze the relationship of HE events to blood ammonia at baseline or during the study, assessed as time-normalized area under the curve (TNAUC).

Results

Study Population. From June 1, 2010 to October 31, 2011, 178 patients were enrolled (Fig. 1) from a total of 51 centers, including 35 in the United States, 7 in the Ukraine, and 9 in Russia. The average number of patients recruited per site was 3.7, with a range of 0-9. Screen failures (n = 98) were predominately the result of exclusionary laboratory findings and comorbidities (Fig. 1). All 178 patients were included in the ITT and safety analyses summarized below. A per-protocol efficacy analysis was not performed because the difference from the ITT population did not differ by more than 10%. The majority of demographic and baseline disease characteristics, including baseline use of lactulose and rifaximin, were similar between the two treatment groups (Table 1). More

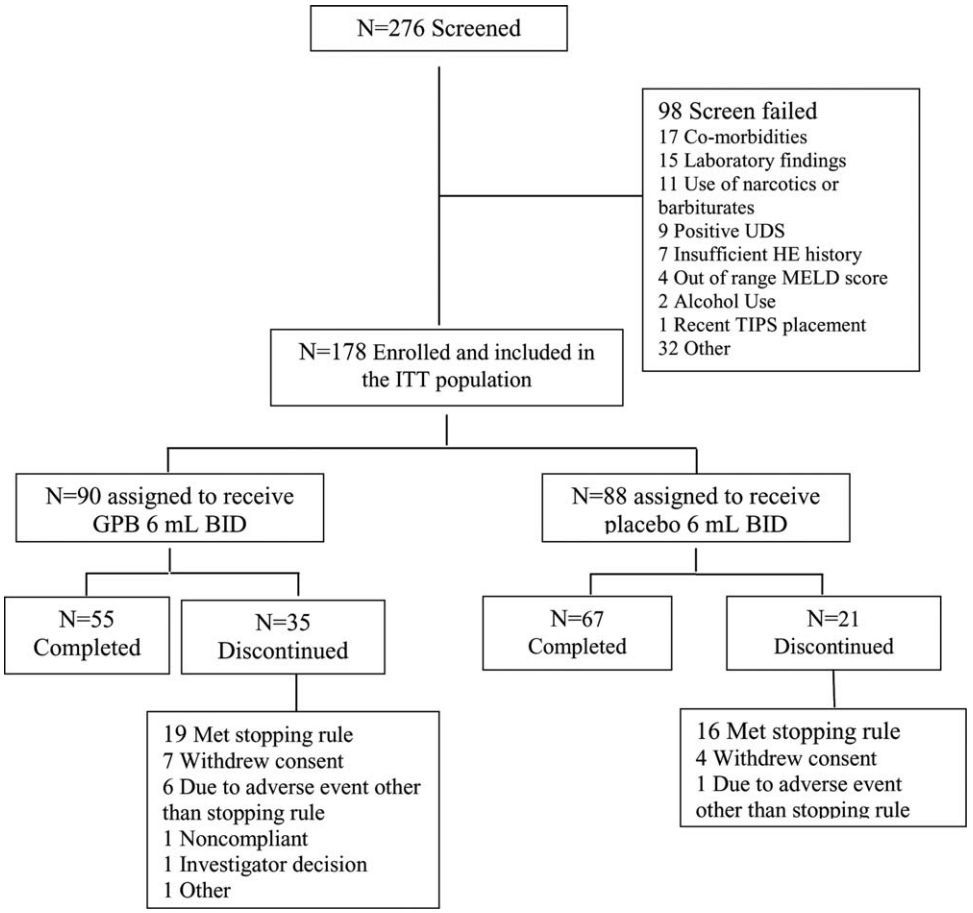

Fig. 1. Disposition of patients. The flow chart indicates the disposition of the 276 patients screened, including the most common reasons for screen failure and the most common reasons for discontinuation among the 178 patients enrolled.

Child-Pugh class C patients were randomized to GPB than to placebo (21 versus 8, respectively), and fewer men were randomized to GPB than to placebo (45 versus 59; Table 1). Only U.S. patients were using rifaximin at baseline.

Compliance. Compliance with study drug was >90% in both treatment groups. Lactulose use did not differ between the two treatment arms, either at baseline or during the study. Most early terminations (19 for GPB and 16 for placebo) were the result of predefined study stopping rules (Fig. 1; Supporting Table 1). Other reasons for study discontinuation were more frequent in the GPB group (Supporting Table 1), but were consistent with those expected for this patient population. The difference in the proportion of patients completing the study was largely the result of the imbalance in randomization of the Child-Pugh class C patients, 69% of whom exited prematurely, regardless of treatment.

HE Events. The study met its prespecified primary endpoint. In the ITT population, a lower proportion of patients in the GPB group than in the placebo group experienced an HE event (21% versus 36%, respectively; $P = 0.02$; Table 2). Treatment effect was also significant when analyzed as the time to first HE event (hazard ratio [HR; 95% confidence interval; CI] = 0.56 [0.32, 0.99] in the ITT population; $P < 0.05$, Fig. 2), when analyzed among patients taking lactulose at baseline (22% versus 45%; $P < 0.01$) and in patients experiencing the more severe (WH $\geq$2) events (18% versus 31%; $P = 0.04$), which accounted for approximately 80% of events in both treatment arms. Compared with Child-Pugh class A/B patients, a greater percentage of Child-Pugh class C patients experienced an event (38% versus 28%), and the treatment effect remained significant when adjusted for the imbalance in Child-Pugh classification between arms ($P < 0.05$).

The total number of HE events was also lower in the GPB arm (35 versus 57 in the placebo arm; $p = 0.04$; Fig. 3; Table 2), and fewer patients experienced dose interruptions resulting from an HE event in the GPB arm (3 patients with 4 total interruptions versus 15 patients with 27 total interruptions; $P < 0.01$). Factors precipitating HE events were most commonly listed as unknown or other, followed by dehydration, infection, constipation, excess dietary protein, and use of sedatives.

Table 1. Patient Demographics and Baseline Characteristics*

Demographics/Characteristics	GPB (N = 90)	Placebo (N = 88)
Age, years		
Mean (SD)	53.8 (8.9)	55.4 (8.8)
Median	55	56
Minimum/maximum	23/69	26/77
Sex, n (%)*		
Female	45 (50)	29 (33)
Male	45 (50)	59 (67)
Race, n (%)		
White	83 (92)	80 (91)
Country, n (%)		
United States	44 (49)	44 (50)
Russia	26 (29)	24 (27)
Ukraine	20 (22)	20 (23)
Qualifying HE events, n (%)/events†		
WH 2	57 (63.3)/134	57 (64.8)/137
WH 3	32 (35.6)/59	29 (33.0)/45
WH 4	1 (1.1)/1	2 (2.3)/5
Causes of qualifying HE events, n (%)‡		
Constipation	16 (17.8)	10 (11.4)
Dehydration	14 (15.6)	8 (9.1)
Excess dietary protein	12 (13.3)	16 (18.2)
Infection	10 (11.1)	9 (10.2)
Sedatives	4 (4.4)	0
Other	9 (10)	14 (15.9)
Unknown	70 (77.8)	67 (76.1)
Duration of remission at entry, months		
Mean (SD)	1.4 (0.8)	1.3 (0.8)
Median	1.2	1.2
Child-Pugh classification, n (%)*		
A	37 (41)	29 (33)
B	32 (36)	50 (58)
C	21 (23)	8 (9)
History of complications		
Ascites	39 (43)	36 (41)
Esophageal varices§	35 (39)	38 (43)
Hepatocellular carcinoma	0	0
Lactulose use at baseline (mL/day)	n = 72	n = 65
Mean (SD)	58 (46)	57 (35)
Median	45.00	45.00
Minimum/maximum	5.0/266.0	15.0/160.0
Rifaximin use at study entry (mg/day)	n = 30	n = 29
Mean (SD)	1,060 (190)	1,100 (185)
Median	1,100	1,100
Minimum/maximum	550/1,200	400/1,650
MELD score		
Mean (SD)	12.6 (3.7)	12.3 (3.8)
Median	12.0	12.0
Minimum/maximum	6/21	5/26
HE grade at study entry, n (%)		
0	82 (91)	79 (92)
1	8 (9)	7 (8)
Ammonia (μmol/L)		
Mean (SD)	48 ±35	54 ±34
Abnormal values, %	58	60
Asterixis grade at study entry, n (%)		
0	74 (82)	66 (76)
1	10 (11)	15 (17)
2	3 (3)	5 (6)
3	3 (3)	(1)

*Significantly different by Fisher's exact tests (categorical).

†Subjects reporting more than one HE event are counted only once using the maximum grade; qualifying HE events are counted in each reported grade.

‡Subjects could be counted in more than one category.

§Includes varices with and without bleeding.

As compared with the overall population, treatment effect was more pronounced among the 119 patients not on rifaximin at entry. There was a significant difference in favor of the GPB arm in the proportion of patients experiencing any HE event (10% versus 32.2%; $P < 0.01$; the time to the first HE event: HR [95% CI] of 0.29 [0.12, 0.73]; $P = 0.01$; Fig. 2), the proportion of patients who experienced WH $\geq$2 events (5% versus 25%; $P < 0.01$; HR [95% CI] of 0.18 [0.05, 0.63]; $P = 0.01$), and the number of total HE events (7 versus 31; $P < 0.001$; Fig. 3). Treatment effect among patients not on rifaximin at baseline was similar, regardless of geography. There were 69% fewer events among patients on GPB versus placebo in the United States and 68% fewer in Eastern Europe.

Among the 59 patients taking rifaximin at entry, there was no difference between treatment arms in patients experiencing an HE event, time to event (Table 2; Fig. 2), or total events. A total of 69 patients received rifaximin at some time during the study (Table 2; Fig. 3). Of the 10 subjects who received rifaximin subsequent to an on-study event, 9 were in the placebo group, and 2 of those 9 subjects went on to have subsequent events.

HE Hospitalizations. Among patients randomized to GPB, there were fewer patients hospitalized (10% versus 16%), fewer total hospitalizations (13 versus 25), and fewer total hospital days (66 versus 134; Table 2). These differences were not statistically significantly different.

Plasma Ammonia. Baseline fasting plasma ammonia levels were similar in the two study arms, assessed either as mean or the percentage of abnormal values (Table 1). However, during the study, ammonia levels were significantly lower in patients treated with GPB, assessed as TNAUC (46 versus 58 μmol/L*week; $P = 0.04$) or least-squares mean (38 versus 47 μmol/L; $P = 0.002$), as were the mean maximum postbaseline values (62 versus 76 μmol/L; $P = 0.04$). Ammonia values were nonsignificantly higher among patients on rifaximin than patients not on rifaximin, both at baseline (mean-= 70 versus 43 μmol/L) and during the study assessed as TNAUC (Table 2). Baseline ammonia was higher among patients who subsequently experienced an HE event on study, as compared to those who did not (Fig. 4). There was a highly significant correlation between the odds of a patient experiencing an HE event and that patient's ammonia level assessed either at baseline ($P = 0.01$) or during the study (TNAUC; $P = 0.01$).

Daily Assessments. The percentage of patients with a CHESS score $\geq$3, which generally correlates with a WH score of 2,[16] was significantly lower in the

Table 2. HE Events, HE-Related Hospitalizations, and Blood Ammonia*

	GPB (%)	Placebo (%)	*P* Value
All patients (N = 178)	90	88	
Percent (#) of patients with an HE event (primary analysis)	21 (19)	36 (32)	0.020
Percent (#) of patients with an event (WH ≥2)	18 (16)	31 (27)	0.040
HR (± 95% CI) based on time-to-event analysis, GPB relative to placebo	0.56 (0.32, 0.99)		0.047
Total HE events	35	57	0.040†
Patients reporting HE hospitalization	10	16	0.230
HE hospitalizations	13	25	0.060†
HE hospital days	66	134	NS
Patients with CHESS score ≥3	13 (14)	27 (31)	0.015
Ammonia (TNAUC; μmol/L × week)	46	58	0.040
Patients not on rifaximin at study entry (N = 119)	60	59	
Percent (#) of patients with an HE event	10 (6)	32 (19)	0.003
Percent (#) of patients with an HE events, WH ≥2	5 (3)	25 (15)	0.002
Total HE events	7	31	<0.001†
HE hospitalizations	2	5	0.300†
HE hospital days	9	44	NS
Patients with CHESS score ≥3	4 (7)	12 (20)	0.020
Ammonia (TNAUC; μmol/L × week)	36	43	0.080
Patients taking rifaximin at study entry (N = 59)	30	29	
Percent (#) of patients with an HE event	43 (13)	45 (13)	0.900
Percent (#) of patients with an event (WH ≥2)	43 (13)	41 (12)	0.900
Total HE events	28	26	0.800†
HE hospitalizations	11	20	0.100†
HE hospital days	57	90	NS
Patients with CHESS score ≥3	9 (30)	15 (52)	0.200
Ammonia (TNAUC; μmol/L × week)	67	91	0.100
Patients on rifaximin at study entry or after first event (N = 69)	31	38	
Percent (#) of patients with an HE event	42 (13)	58 (22)	0.200
Total HE Events	28	42	0.600†

*All analyses based on ITT population.
†Statistical analysis pertains to event rate.
Abbreviation: NS, not statistically significant.

GPB arm both overall (14% versus 31%; $P = 0.02$) and in patients not using rifaximin at study entry (7% versus 20%; $P = 0.02$).

Pharmacokinetics. Plasma metabolite levels among patients on GPB did not change over time. Mean ± standard deviation (SD) fasting values ranged from 10.6 ± 24.7 at day 7 to 18.2 ± 31.0 μg/mL at study exit for PBA, from 43 ± 76.3 to 40.9 ± 87.0 μg/mL for PAA, and from 30.0 ± 39.1 to 26.8 ± 29.9 for PAGN. Among approximately 580 PAA measurements, only one value (532 μg/mL) was in the range (499-1,285 μg/mL) reportedly associated with reversible AEs (e.g. nausea, vomiting, headache, and somnolence) in phase I cancer studies involving intravenous PAA administration.[17,18] Mean ± SD conversion of PBA administered as GPB to urinary PAGN was 52% ± 28%.

Safety. The frequency and types of AEs were similar in the two treatment arms and consistent with those expected in a study population with clinically decompensated cirrhosis (Table 3). Serious AEs (SAEs) and study drug discontinuations resulting from AEs were slightly more frequent in the GPB group. Discontinuations resulting from meeting predefined study stopping rules were similar in the two treatment groups and were typically for laboratory stopping rules (most commonly a 5-point increase in MELD score or a hemoglobin level <8 g/dL or level requiring transfusion; Supporting Table 1). There were 2 deaths in the GPB arm and 1 in the placebo arm. All 3 deaths were considered unrelated to study drug and were the result of causes consistent with advanced liver disease (GI bleeding, kidney failure, and hepatic insufficiency in 1 patient each). Four patients underwent LT, including 3 in the GPB arm and 1 in the placebo arm.

There were no treatment-related effects on liver biochemical tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin, international normalized ratio (INR), or MELD (Table 4). Similarly, no treatment-related differences were observed in hematology or chemistry studies or in ECG findings.

Discussion

GPB decreased HE events in patients with cirrhosis, assessed either as the proportion of patients with at

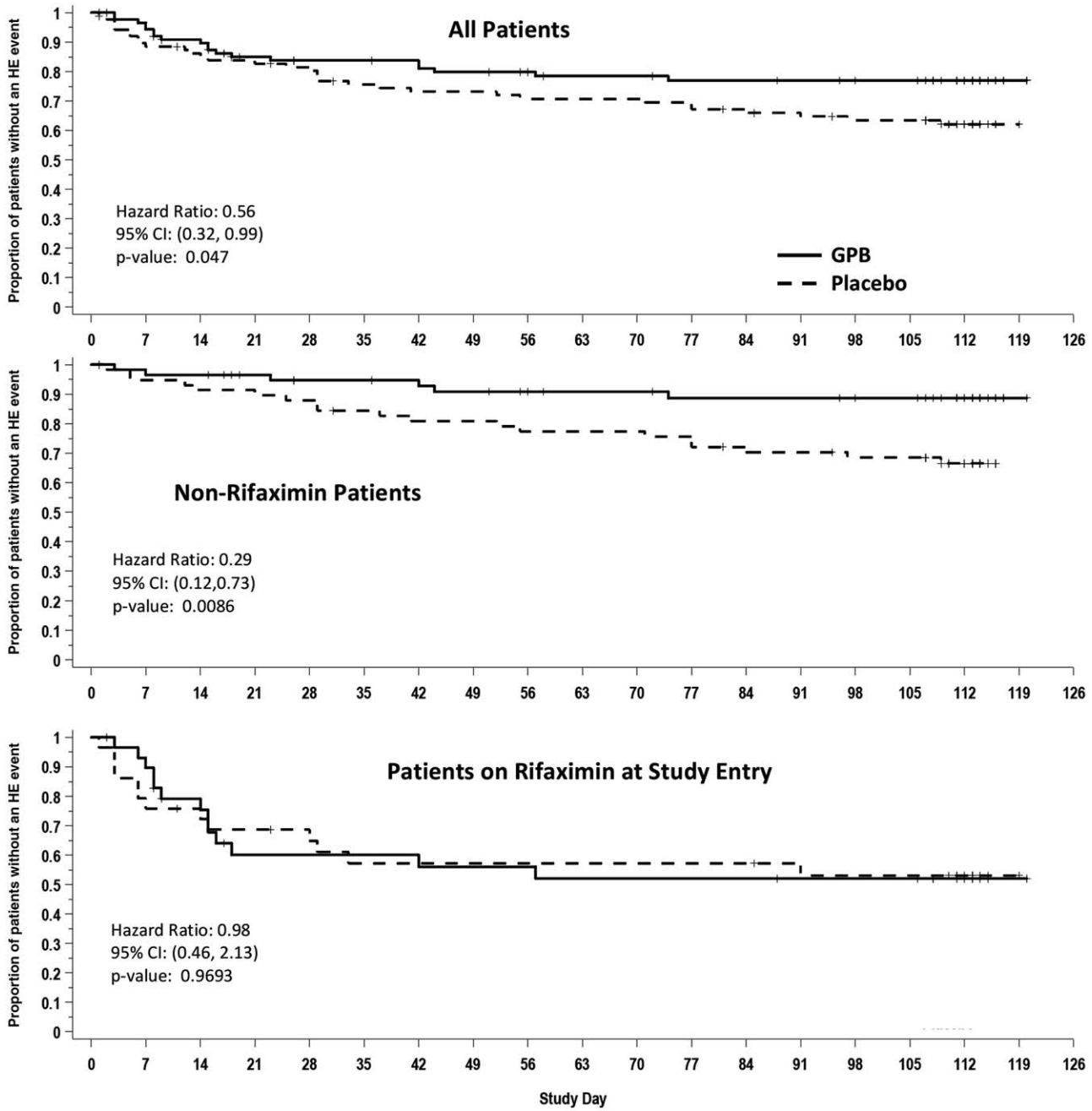

Fig. 2. Time to HE event. The time to the first HE event over time is depicted for all patients (top panel; n = 178), in patients not on rifaximin at baseline (middle panel; n = 119), and in patients on rifaximin at baseline (bottom panel; n = 59).

least one event or the total number of events. A significant treatment effect was also present when analyzed as time to HE event and was stronger when restricted to the more severe WH grade $\geq$2 events, which accounted for over 80% of all events in both treatment arms. Patients randomized to GPB were also significantly less likely to experience HE event-related interruptions in study drug treatment and tended to experience less-severe HE events.

Among the 119 patients not on rifaximin at baseline, GPB treatment was similarly associated with a significant reduction both in the proportion of patients with at least one HE event as well as total events. Treatment effect compared favorably with that reported for rifaximin in a similar patient population (HR for time to event = 0.29 versus 0.42 reported for rifaximin)[1] and was even stronger when restricted to WH grade $\geq$2 events (HR = 0.18). Although the proportion of patients with at least one HE event was similar in the two treatment arms among the 59 patients taking rifaximin at baseline, there was a nonsignificant difference in

Table 3. AEs and SAEs

Organ System (Preferred Term)	GPB (N = 90) n (%)	Placebo (N = 88) n (%)
Any AE	71 (79)	67 (76)
AEs reported in at least 5% of patients		
GI disorders	36 (40)	33 (38)
Nausea	11 (12)	13 (15)
Diarrhea	8 (9)	9 (10)
Abdominal pain	5 (6)	5 (6)
Vomiting	2 (2)	8 (9)
Constipation	4 (4)	5 (6)
Ascites	5 (6)	0
General disorders and administration-site conditions	26 (29)	21 (24)
Edema peripheral	13 (14)	7 (8)
Fatigue	6 (7)	7 (8)
Pyrexia	5 (6)	3 (3)
Investigations	25 (28)	15 (17)
AST increased	10 (11)	5 (6)
ALT increased	8 (9)	4 (5)
WBC count decreased	5 (6)	2 (2)
Nervous system disorders	21 (23)	17 (19)
Headache	8 (9)	5 (6)
Infections	18 (20)	13 (15)
Metabolism and nutrition disorders	14 (16)	8 (9)
Musculoskeletal and connective tissue disorders	13 (14)	7 (8)
Back pain	5 (6)	3 (3)
Psychiatric disorders	10 (11)	7 (8)
Respiratory, thoracic, and mediastinal disorders	9 (10)	8 (9)
Dyspnea	5 (6)	3 (3)
Blood and lymphatic system disorders	10 (11)	6 (7)
Skin and subcutaneous tissue disorders	9 (10)	7 (8)
Hepatobiliary disorders	3 (3)	10 (11)
Injury, poisoning, and procedural complications	6 (7)	5 (6)
Vascular disorders	4 (4)	6 (7)
Cardiac disorders	2 (2)	6 (7)
Any AE reported as possibly or probably related	35 (38.9)	31 (35.2)
AEs reported as possibly or probably related in at least 5% of patients		
GI disorders	22 (24.4)	16 (18.2)
Nausea	8 (8.9)	6 (6.8)
Diarrhea	6 (6.7)	4 (4.5)
General disorders and administration-site conditions	6 (6.7)	6 (6.8)
Fatigue	4 (4.4)	5 (5.7)
Investigations		
AST increased	6 (6.7)	3 (3.4)
ALT increased	5 (5.6)	3 (3.4)
Nervous system disorders	9 (10.0)	8 (9.1)
Headache	5 (5.6)	4 (4.5)
Any SAE	20 (22)	12 (14)
SAEs Reported in at least 3% of patients		
GI disorders	7 (8)	1 (1)
GI hemorrhage	4 (4)	0
Infections	3 (3)	3 (3)
Hepatobiliary disorders	1 (1)	4 (5)
Metabolism and nutrition disorders	3 (3)	2 (2)

WBC, white blood cell.

favor of fewer total HE events on the GPB arm for the 69 patients who ever received rifaximin.

GPB significantly lowered plasma ammonia and correlated strongly with HE events when assessed either at baseline or during the study. The finding that patients on rifaximin tended to have higher ammonia levels was unexpected in light of a preliminary report that rifaximin lowered blood ammonia by approximately 5% among HE patients.[9] Our results may reflect rifaximin use as a marker of more-severe disease.

Among patients not on rifaximin at baseline, the ammonia-lowering effect of GPB was similar to that reported in the open-label safety and dose-finding

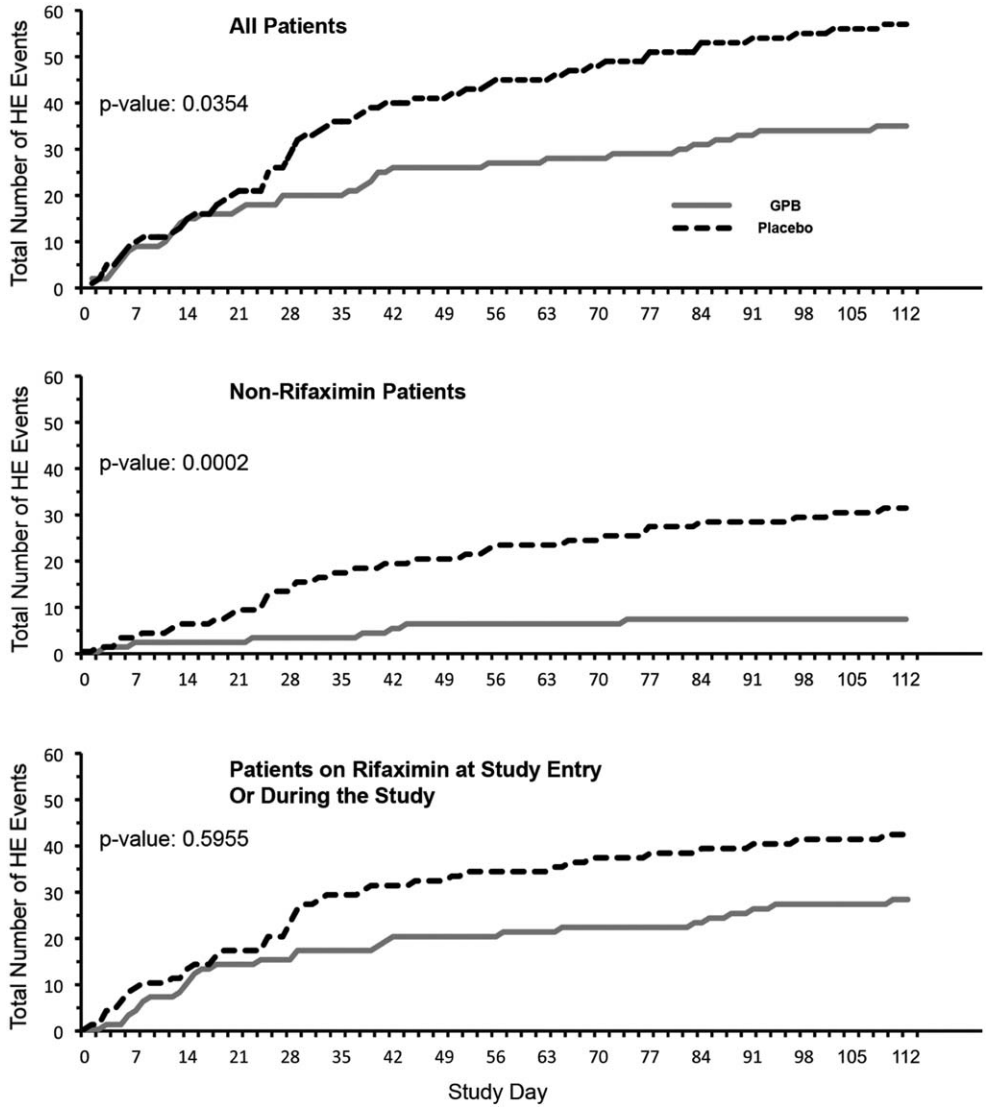

Fig. 3. Cumulative HE events. Cumulative HE events over time are shown for all patients (n = 178), patients not on rifaximin at baseline (middle panel; n = 119), and for patients on rifaximin either at baseline or who were put on rifaximin during the study (bottom panel; n = 69). The bottom panel includes 9 patients not on rifaximin at study entry randomized to placebo who began rifaximin treatment after an on-study HE event and 1 patient randomized to GPB who received rifaximin after the last dose of GPB.

study.[15] Although ammonia levels were also lowered by GPB among patients taking rifaximin, ammonia remained substantially elevated. This finding may help explain the similar rate of HE events among active versus placebo arms in this subgroup.

The number and percentage of patients in the two treatment arms who experienced AEs was similar, and the types of AEs were consistent with those anticipated in the study population. The higher proportion of SAEs, certain laboratory abnormalities (e.g., elevated INR), and patients who withdrew from the study in the GPB arm, as compared to placebo, may be explained by the higher number of Child-Pugh class C patients in the GPB group. Child-Pugh class C patients, by definition, have worse liver function, as reflected by INR, bilirubin, and albumin, as well as more-severe HE and ascites. Among all Child-Pugh class C patients, 48% experienced SAEs and 69% failed to complete the study, irrespective of treatment. There did not appear to be any drug-related differences in biochemical tests, including chemistry, hematology, and liver tests such as transaminases, or MELD score.

Pharmacokinetics findings indicated that circulating levels of PAA were generally well below those reportedly associated with reversible AEs in cancer patients.[17,18] Moreover, the proportion of the administered phenylbutyrate that is metabolized to urinary PAGN is similar to that in urea-cycle disorder patients,[11-13] indicating that patients with cirrhosis and HE can effectively metabolize GPB and utilize its waste nitrogen removal capacity.

We recognize limitations of this phase II study. First, the study enrolled 178 patients, rather than the 186 calculated to be necessary to detect a 50% treatment effect and therefore was slightly underpowered (78% versus 80%). Fortunately, the study still met its

Table 4. Liver, Hematological Tests, and MELD in Relation to Treatment

	GPB (N = 90)		Placebo (N = 88)	
Mean (SD)	**Result**	**Change**	**Result**	**Change**
ALT, U/L				
Baseline	48.8 (41.1)		43.2 (32.5)	
Final visit	52.0 (57.6)	0.2 (27.94)	46.9 (43.65	5.4 (45.3
AST, U/L				
Baseline	64.4 (36.8)		67.6 (53.1)	
Final visit	68.0 (53.4)	4.4 (34.1)	65.0 (49.1)	1.1 (43.7)
ALP, U/L				
Baseline	181.1 (115.0)		180.9 (143.7)	
Final visit	172.6 (87.8)	13.1 (88.2)	164.6 (84.0)	19.9 (75.9)
Albumin, g/L				
Baseline	30.6 (10.42)		30.8 (9.69)	
Final visit	31.6 (9.26)	0.04 (4.33)	30.4 (9.28)	−0.5 (3.79)
Total bilirubin, μmol/L				
Baseline	31.2 (18.44)		30.7 (20.31)	
Final visit	30.6 (21.38)	2.7 (17.81)	33.5 (23.39)	2.9 (13.61)
INR				
Baseline	1.4 (0.37)		1.4 (0.29)	
Final visit	1.4 (0.36)	0.1 (0.30)	1.4 (0.37)	−0.01 (0.26)
Creatinine, μmol/L				
Baseline	80.0 (34.60)		79.2 (38.40)	
Final visit	77.6 (32.67)	−2.1 (18.65)	81.0 (50.55)	1.2 (26.23)
MELD score				
Baseline	12.6 (3.69)		12.3 (3.77)	
Final visit	12.3 (4.84)	0.2 (3.23)	12.8 (4.59)	0.4 (2.57)
Hemoglobin, g/L				
Baseline	113.6 (26.37)		112.2 (29.66)	
Final visit	107.3 (31.24)	−4.3 (11.59)	112.6 (31.94)	0.3 (10.92)
White blood cell count, $\times 10^3/\mu L$				
Baseline	5.8 (2.45)		5.5 (1.95)	
Final visit	5.5 (2.88)	−0.1 (2.07)	5.8 (2.23)	0.3 (1.71)
Platelet count, $\times 10^3/\mu L$				
Baseline	116.7 (59.32)		126.5 (61.94)	
Final visit	113.2 (60.19)	−4.4 (38.63)	120.7 (57.24)	−5.4 (36.65)

prespecified objective. Second, more patients in the GPB than in the placebo arm exited before study completion, a finding likely resulting from the fact that more Child-Pugh class C patients were randomized to GPB. This could have a confounding effect on interpretation, in that Child-Pugh class C patients were more likely to experience HE events, which could bias the results against a treatment effect, whereas the greater proportion of early terminators might bias the results toward fewer patients with events in the GPB arm. However, the fact that the treatment effect remained significant when analyzed using time to event survival methodology, which accounts for dropouts, as well as when adjusting for Child-Pugh classification imbalance between study arms, suggests a statistically robust treatment effect of GPB. The inclusion of HE events defined as an increase ≥ 1 in both WH score and asterixis grades, if baseline WH was 0, in the definition of an HE event was based on a previous study with rifaximin and on consultation with the FDA, rather than the current definition of overt HE.[1,7] However, HE events so defined accounted for less than 20% of all HE events, and the treatment effect remained statistically significant when defined as only overt HE (i.e., only including events WH ≥ 2. Last, the restriction of the rifaximin subgroup to only those who had experienced at least one event after 4 weeks of treatment likely skewed this subpopulation to those with the most refractory disease.

In summary, the results demonstrate that GPB reduced the likelihood of HE events in patients with preexisting HE, and we conclude, therefore, that it deserves further study as a potential therapeutic for these patients. The results further suggest that elevated blood ammonia plays an important role in the pathogenesis of recurrent overt HE.

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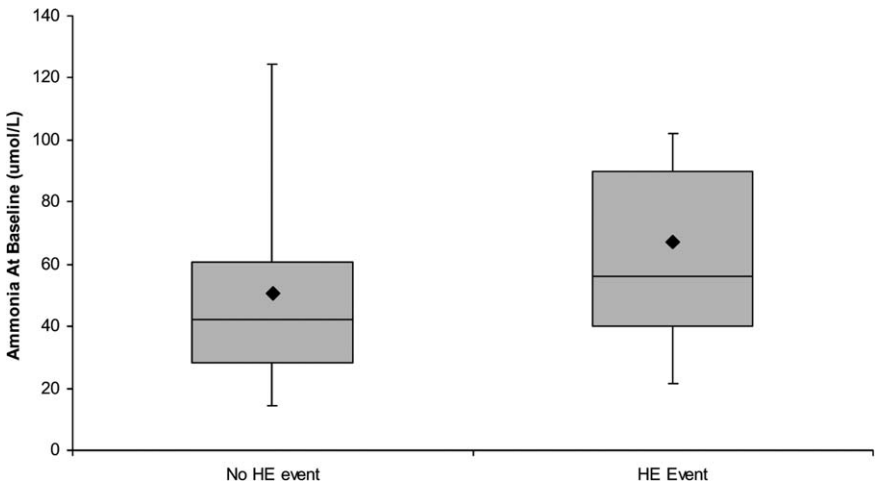

Fig. 4. Baseline ammonia in relation to HE events. The relationship between baseline ammonia and HE events that occurred on study (mean [+], median [horizontal line], 25%-75% [box] and 5%-95% [whiskers]) is shown.

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Appendix

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