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Randomised clinical trial: emricasan versus placebo significantly decreases ALT and caspase 3/7 activation in subjects with non-alcoholic fatty liver disease

Mitchell Shiffman[1] | Bradley Freilich[2] | Raj Vuppalanchi[3] | Kymberly Watt[4] | Jean L. Chan[5] | Al Spada[5] | David T. Hagerty[5] | Eugene Schiff[6]

[1]Liver Institute of Virginia, Bon Secours Health System, Richmond and Newport News, Virginia

[2]Kansas City Research Institute, Kansas City, Missouri

[3]Division of Gastroenterology and Hepatology, Indiana University School of Medicine, Indianapolis, Indiana

[4]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota

[5]Conatus Pharmaceuticals Inc., San Diego, California

[6]Schiff Center for Liver Diseases, University of Miami Miller School of Medicine, Miami, Florida

Correspondence
David T. Hagerty, Conatus Pharmaceuticals Inc., San Diego, CA.
Email: dhagerty@conatuspharma.com

Funding information
Conatus Pharmaceuticals, Inc.

Summary

Background: Lipotoxicity leading to excessive caspase-mediated apoptosis and inflammation is believed to drive liver damage in NAFLD. Emricasan is a pan-caspase inhibitor that decreased serum ALT and apoptotic and inflammatory markers in subjects with chronic hepatitis.

Aims: To assess whether 28 days of emricasan would reduce elevated levels of serum ALT, AST, cleaved cytokeratin-18, full-length cytokeratin-18, and caspase 3/7 in subjects with NAFLD and raised aminotransferases.

Methods: Double-blind, placebo-controlled, office-practice study assessed the efficacy, safety, and tolerability of emricasan in subjects with NAFLD and ALT levels ≥1.5 x ULN during screening. Subjects were randomised to emricasan 25 mg twice daily or matching placebo. Subjects with cirrhosis and other causes for raised aminotransferases were excluded. The primary endpoint was the change in ALT at day 28 in the emricasan group vs placebo.

Results: 38 subjects were randomised, 19 each to emricasan or placebo. Baseline disease factors were well balanced except for lower median ALT values in emricasan subjects. Three subjects randomised to placebo discontinued prior to day 28. ALT values decreased significantly in emricasan-treated subjects vs placebo at days 7 ($P < 0.0001$) and 28 ($P = 0.02$). cCK18 (day 7), fICK18 (days 7 and 28), and caspase 3/7 (day 7) were also significantly decreased in emricasan-treated subjects vs placebo. Emricasan treatment was generally safe and well tolerated.

Conclusions: Emricasan decreased ALT and biomarkers in subjects with NAFLD and raised aminotransferases after 28 days. These results support the further development of emricasan in patients with NAFLD. Trial registration: ClinicalTrials.gov, Identifier: NCT02077374.

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1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the US with a prevalence estimated to be 15%-30%.[1–3] Approximately 10%-20% of patients with NAFLD progress to non-alcoholic steatohepatitis (NASH) and 10%-20% of those patients will eventually develop cirrhosis.[4] Steatohepatitis with fibrosis is an important histologic finding that identifies a subgroup of patients likely to progress to cirrhosis.[5,6]

Caspases are a family of intracellular cysteine proteases that mediate apoptosis and inflammation through the processing and activation of pro-inflammatory cytokines such as IL-1β, IL-18, and IL-33.[7] Cellular injury activates caspases which cleave a number of cell proteins, including cytokeratin 18 (CK18). Liver aminotransferases associated with inflammation (ALT, AST) and biomarkers associated with caspase activation and apoptosis, such as cleaved cytokeratin 18 (cCK18), are usually elevated in patients with NASH.[8,9] The magnitude of apoptosis, as measured by levels of cCK18 in serum, correlates with the fibrosis stage in NASH patients.[10] In addition, therapeutic studies in NASH patients using pioglitazone and vitamin E revealed that decreases in ALT and cCK18 were associated with improvements in resolution of NASH, steatosis, lobular inflammation, hepatocyte ballooning, and fibrosis stage.[11,12] Thus, there is reason to believe that excessive caspase-mediated apoptosis and inflammation are clinically important drivers of progressive liver disease in NASH and that caspase inhibition may be able to halt or resolve liver damage in NASH.

Human studies have shown that emricasan lowers ALT and has anti-apoptotic and anti-inflammatory effects in patients with HCV hepatitis and elevated aminotransferases, as well as in patients with cirrhosis of different etiologies.[13–16] The present study assessed whether emricasan could lower ALT and biomarkers of hepatocyte injury, apoptosis, and inflammation in subjects with NAFLD and elevated aminotransferases.

2 | MATERIALS AND METHODS

2.1 | Study oversight

The Sponsor, Conatus Pharmaceuticals Inc, designed the trial. Data were collected by the investigators in outpatient clinics and analysed by the Sponsor; the results were reviewed by the Sponsor and the authors. All authors participated in the writing of the manuscript and approved the draft that was submitted for publication. The trial was conducted in accordance with the provisions of the International Conference on Harmonisation Guidelines for Good Clinical Practice and the Declaration of Helsinki. The protocol was reviewed and approved by Western IRB or the Institutional Review Board at each investigational site.

2.2 | Study protocol

This was a placebo-controlled, multicentre, double-blind, randomised trial in subjects with NAFLD/NASH and elevated alanine aminotransferase values (ALT) conducted in an office practice setting. Subjects were enrolled between March 2014 and February 2015.

There was a 28-day double-blind treatment phase and 28-day follow-up phase. Subjects were required to have two ALT values ≥1.5 times the upper limit of normal (40 IU/L) on at least two occasions during screening. NAFLD was diagnosed by the investigator on the basis of clinical characteristics and had to be confirmed by either an imaging test (ultrasound, CT scan, or MRI) or liver biopsy within 6 months of screening. In addition, other causes of chronic liver disease had to be excluded. Additional exclusions included hepatocellular carcinoma, inflammatory bowel disease, suspected systemic lupus erythematosus, or rheumatoid arthritis. Subjects who planned to make a significant lifestyle change to their diet or exercise regimen during the study were also to be excluded.

Emricasan (Conatus Pharmaceuticals Inc, San Diego, CA, USA) 25 mg twice daily was studied because that was the lowest dose that maximally inhibited a panel of four biomarkers (ALT, AST, caspase 3/7, and cCK18 in subjects with hepatitis C [unpublished data]).

2.3 | Blinding and randomisation

The Sponsor, investigational staff, and subjects were blinded to the treatment assignment throughout the study. Subjects were randomised 1:1 to emricasan or matching placebo using a validated, verified central randomisation program.

2.4 | Measures of clinical efficacy

The primary efficacy endpoint was the reduction in ALT at day-28 compared to baseline in subjects treated with emricasan or placebo. Secondary efficacy endpoints included the change from baseline to day-28 in AST, caspase 3/7, cCK18, and fICK18.

Clinical and biomarker measurements were performed by Lab Connect LLC, Johnson City, TN. CK18 is a major cytoplasmic intermediate filament protein in hepatocytes and epithelial cells that is cleaved by caspases during apoptosis and cell death. Full-length CK18 and caspase-cleaved CK18 were quantified in sera using enzyme-linked immunosorbent assays detecting the M65 epitopes (M65 EpiDeath, VLVbio, Sundbyberg, Sweden; measures both full-length and cleaved CK18, reference range: 63-311 U/L) and the M30 epitope (M30 Apoptosense, VLVbio, Sundbyberg, Sweden; measures cleaved CK18 only, reference range: 80-102 U/L), respectively. Caspase 3/7 activity (Caspase-Glo 3/7, Promega, Madison, WI, USA; reference range: 1429-3908 relative light units) was measured in sera and detects the executioner caspases 3 and 7.

2.5 | Safety assessments

All adverse events were coded by the Medical Dictionary for Regulatory Activities. Treatment-emergent AEs and serious AEs were summarised by system organ class and preferred term, and stratified by intensity and relationship to study drug.

Laboratory assessments included serum chemistries, hematology, urinalysis, and changes in haemoglobin A1c and HOMA-IR Changes

from baseline in heart rate, QTc interval, PR interval, and QRS interval were also assessed.

2.6 | Statistical analysis

An estimation approach was applied for the sample size calculation. With a sample size of 20 in each treatment arm, a two-sided 95% CI based on the t-distribution for the difference of two means would have an interval that would extend no more than 29.2 U/L from the observed difference in means with 90% power, assuming that the common standard deviation for changes from baseline in ALT was 40 U/L.[17] If 16 subjects per group completed the study, the interval would extend no more than ±33.5 U/L.

The full analysis set included all subjects who were randomised and received at least one dose of study drug. Efficacy analyses were performed according to the intent to treat principle. Efficacy analyses were conducted on the full analysis set unless noted. Missing values at day-28 were imputed using the last observation on-study.

The safety analysis set included all subjects who took at least one dose of study drug during the study. Safety analyses were based on the treatment received. All safety analyses were conducted on the safety analysis set.

All parameters were analysed descriptively and included the number of subjects, mean, standard deviation, median, minimum, maximum, 25th percentile, and 75th percentile. Categorical variables were presented by frequency counts and percentages. Log transformations used the natural logarithmic transformation.

The primary study endpoint compared the median change from baseline in ALT at day 28 in the emricasan group compared to placebo. Prespecified secondary endpoints for cCK18, flCK18, and caspase 3/7, compared the difference between treatment groups, as well as the change from baseline within each treatment group, at days 7 and 28. The primary treatment comparison at day 28 between the emricasan and placebo groups used the non-parametric Wilcoxon-Mann-Whitney test. An analysis of covariance (ANCOVA) on log-transformed data adjusting for baseline was performed as a sensitivity analysis. The median differences between treatment groups were tested using Wilcoxon-Mann-Whitney tests and the corresponding 95% confidence intervals (CIs) were presented using the Hodges-Lehmann estimator. For the natural log-transformed endpoints, least square mean differences between treatment groups were obtained by ANCOVA using treatment group and baseline value as terms in the model, and the corresponding 95% CIs were back transformed to provide an estimate of the ratio (emricasan/placebo).

3 | RESULTS

3.1 | Demographics and baseline disease characteristics

Baseline demographics and disease characteristics for each treatment arm were generally balanced (Table 1). More subjects in the emricasan group were in the age category <44 years (47.4% vs 21.1%), and fewer in the category 45-64 years (42.1% vs 63.2%), compared to the placebo group. Weight (241 lbs. vs 228 lbs.) and BMI (35.0 and 32.6) were slightly higher in the emricasan group while more subjects were diagnosed with hypertension (47.4% vs 63.2%) and dyslipidaemia (47.4% vs 68.4%) in the placebo group. Subjects were predominantly male, Caucasian, and under 65 years old.

3.2 | Disposition

Subject disposition is shown in Figure S1 (CONSORT 2010). Thirty-eight subjects were randomised and treated with placebo or emricasan (19 subjects each). Thirty-six subjects completed the day 28 visit and

TABLE 1 Demographics and baseline disease characteristics

	Placebo (N = 19)	Emricasan (N = 19)
Gender, n (%)		
Male	12 (63.2)	12 (63.2)
Female	7 (36.8)	7 (36.8)
Race, n (%)		
Caucasian	17 (89.5)	17 (89.5)
Non-caucasian	2 (10.5)	2 (10.5)
Age (years), n (%)		
<44	4 (21.1)	9 (47.4)
45-64	12 (63.2)	8 (42.1)
≥65	3 (15.8)	2 (10.5)
Weight (pounds), Mean (SD)		
Males	228 (42.8)	241 (44.9)
Females	190 (30.9)	212 (56.5)
BMI (kg/m^2), Mean (SD)		
Males	32.8 (5.5)	33.8 (5.1)
Females	32.2 (3.0)	37.2 (8.6)
Hypertension, n (%)	12 (63.2)	9 (47.4)
Dyslipidaemia[a]	13 (68.4)	9 (47.4)
Diabetes mellitus[b]	9 (47.4)	8 (42.1)
ALT, Median (range)	92.7 (42.3-226.7)	65.5 (46.3-238.9)
AST, Median (range)	44.3 (23.3-158.8)	43.8 (24.5-101.1)
cCK18, Median (range)	416.0 (152-3811)	586.0 (281-1140)
flCK18, Median (range)	879.0 (431-5547)	812.0 (317-1708)
Caspase 3/7, Median (range)	1356 (300-2138)	1190 (316-2478)
HOMA-IR, Median (range)	5.60 (0.9-65.5)	5.10 (1.3-67.9)
HbA1c, Median (range)	6.00 (5.1-9.8)	5.40 (4.7-7.5)

[a]Includes hypercholesterolaemia, hypertriglyceridaemia, or dyslipidaemia.
[b]Includes diabetes mellitus, impaired glucose tolerance, or pre-diabetes mellitus.
BMI, body mass index; SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; cCK18, cleaved cytokeratin 18; flCK18, full-length cytokeratin 18; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HbA1c, hemoglobin A1 c.

two placebo-treated subjects prematurely discontinued from the study prior to the day 28 visit. The reasons for discontinuation were "violation of inclusion or exclusion criteria" (one subject started metformin treatment on day 2) and "other" (increased work-related travel, one subject). The first subject entered the study in March 2014 and the last subject completed the study in February 2015.

3.3 | Changes in ALT

The primary study endpoint was the median change from baseline in ALT at day 28 in the emricasan group vs placebo. Median ALT values at baseline and on study days 7, 28, and 56 (off treatment) are shown in Figure 1A. Median day 7 ALT values decreased −8.7 IU/L in the placebo group and −36.7 IU/L in the emricasan group (95% CI [−51.8, −22.8]; $P < 0.0001$ compared to placebo). At day 28, median ALT values decreased −9.4 IU/L in the placebo group and −25.8 IU/L in the emricasan group (95% CI [−30.7, −2.5]; $P = 0.02$ compared to placebo). ALT values returned towards baseline by day 56 without evidence of overshoot. There were two subjects in the emricasan group who were non-compliant, one of whom missed ~25% of doses and another who missed >20% of doses and had undetectable levels of both parent drug and long-lasting metabolites at day 28. When those two subjects were excluded, ALT values on day 7 and 28 were more similar (32.3 and 37.5 U/L, respectively).

A sensitivity analysis (Wilcoxon-Mann-Whitney test, per-protocol population) confirmed the primary analysis and showed a statistically significant difference in favour of emricasan ($P = 0.017$) at day 28. An ANCOVA sensitivity analysis on log-transformed ALT, using the full analysis set, also showed a statistically significant reduction in ALT in the emricasan group vs placebo (ratio of 0.6524; $P = 0.0006$) at day 28.

Individual subject changes in ALT were plotted. This analysis excluded the two non-compliant subjects in the emricasan group. All other subjects, including eight subjects with borderline compliance who missed 10%-20% of doses, were included in the analysis. Every subject treated with emricasan had a decrease in ALT at days 7 and 28 (Figure 2A). At day 7, 11/17 (64.7%) of subjects treated with emricasan achieved an ALT value within the normal range (upper limit of lab normal: 40 IU/L). At day 28, 9/17 (52.9%) of emricasan-treated subjects had ALT values that remained within the normal range and two additional subjects had values of 40.3 and 41.1 IU/L. Responses at the day 28 visit remained less than baseline in all subjects, although some subjects had an increase in ALT values compared to day 7. Changes in ALT for individual subjects treated with placebo are shown in Figure 2B. In the placebo-treated subjects, 2/19 subjects (10.5%) at day 7 had ALT values ≤40 IU/L. At day 28, 3/19 placebo-treated subjects (15.8) had ALT values <40 IU/L.

3.4 | Changes in AST

Median AST values at baseline were relatively lower compared to ALT (44.3 and 43.8 in the placebo and emricasan subjects, respectively) and are shown on study days 7, 28, and 56 (off treatment) in Figure 1B. Median day 7 AST values increased +1.5 IU/L in the placebo group and decreased −20.1 IU/L in the emricasan group (95% CI [−36.65, −15.4]; $P = 0.0002$ compared to placebo). At day 28, median AST values decreased −5.2 IU/L in the placebo group and −6.7 IU/L in the emricasan group (95% CI [−14.5, 11.2]; $P = 0.8724$).

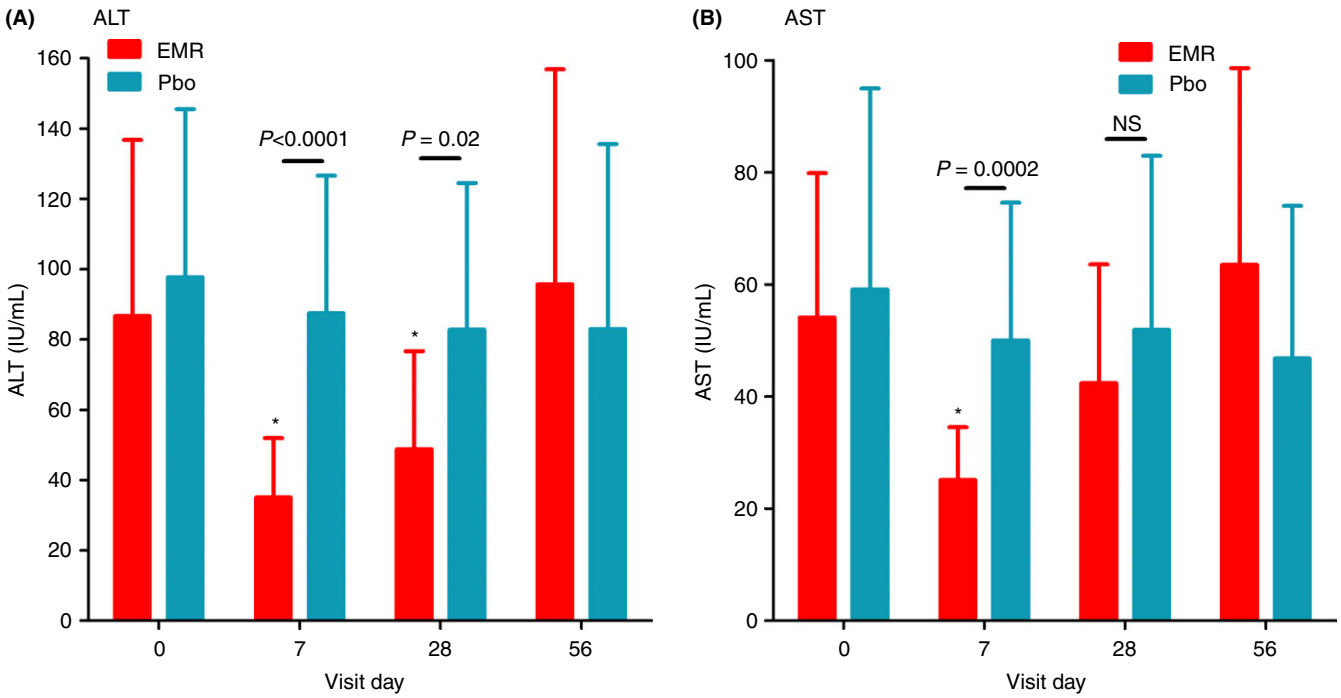

FIGURE 1 Baseline and on-treatment (days 7 and 28) values of ALT (mean, panel A) and AST (mean, panel B). On-treatment values statistically different ($P < 0.05$) from placebo are indicated and the error bars are the standard deviation (SD) values

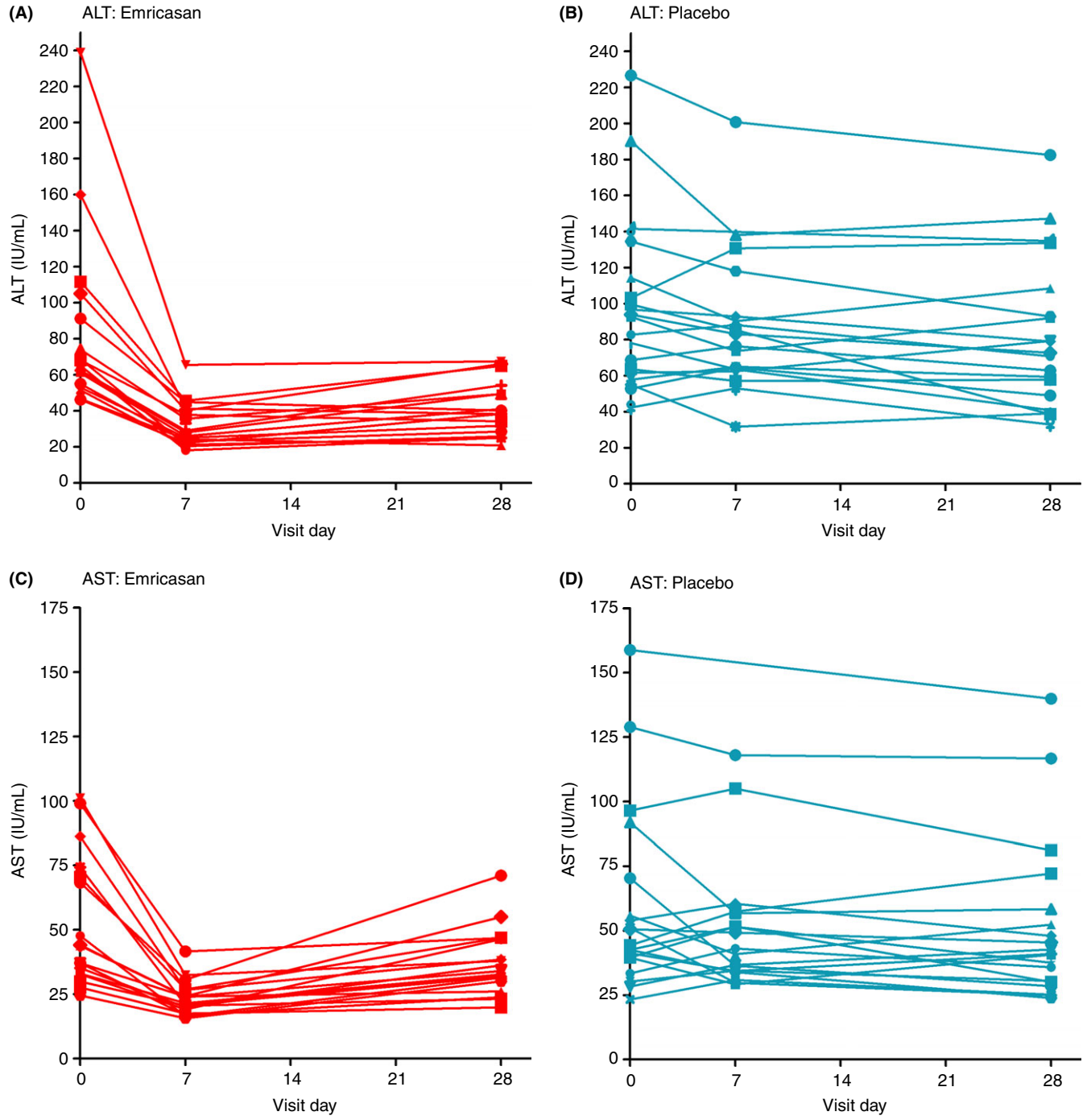

FIGURE 2 Individual subjects treated with emricasan or placebo. A, ALT values, emricasan treatment. B, ALT values, placebo. C, AST values, emricasan treatment. D, AST values, placebo

AST values returned towards baseline by day 56 without evidence of overshoot.

Individual subject responses were also examined for AST. This analysis also excluded the two non-compliant subjects. Every subject treated with emricasan had a lower AST value at day 7, and all but one subject normalised their AST value at day 7 (Figure 2C). There was more variability in the AST response at day 28 in the emricasan-treated subjects when compared to ALT. Sixteen out of seventeen emricasan-treated subjects (94.1%) had an AST value ≤ULN at day 7, while 9/18 (50%) had AST values ≤ULN at day 28. In the placebo-treated subjects (Figure 2D), 3/19 (15.8%) and 6/19 (31.6%) of placebo-treated subjects had an AST value ≤ULN at days 7 and 28, respectively.

3.5 | Changes in biomarkers of excessive caspase activation

The prespecified analysis plan stated that cCK18, fICK18, and caspase 3/7 would be analysed two ways: as the change from

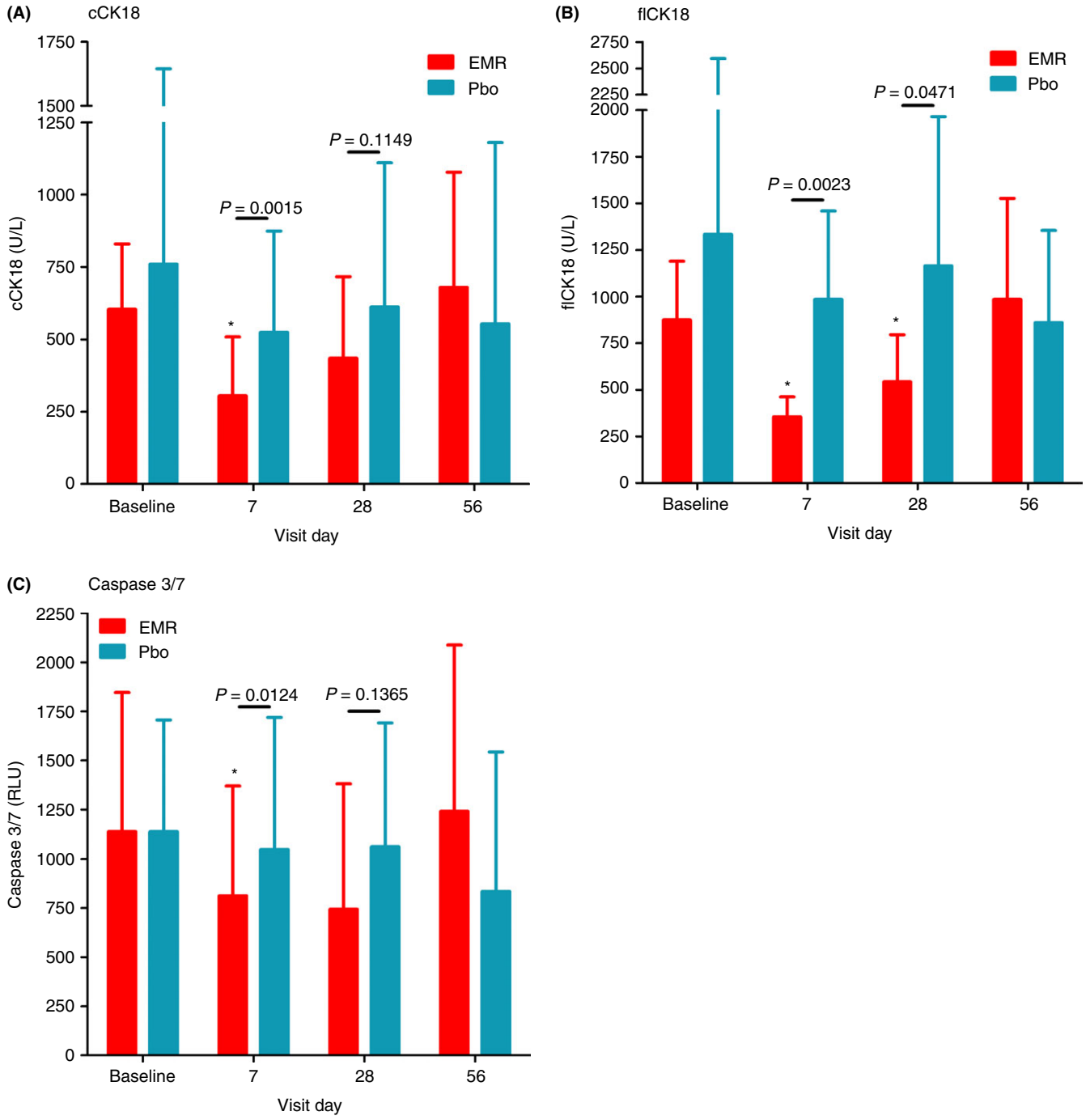

FIGURE 3 Baseline and on-treatment (days 7 and 28) values of cCK18 (mean, panel A), flCK18 (mean, panel B), and caspase-3/7 (median, panel C). On-treatment values statistically different ($P < 0.05$) from placebo are indicated and the error bars are the standard deviation (SD) values

baseline within each treatment group and the difference between treatment groups at days 7 and 28. All three biomarkers of excessive caspase activation were elevated at baseline and showed significant decreases with emricasan treatment while placebo treatment showed no significant change in any of the biomarkers. Figure 3 shows the median values of cCK18 (Figure 3A), flCK18 (Figure 3B), and caspase 3/7 (Figure 3C) at baseline and on days 7, 28, and 56.

Baseline values of cCK18 were elevated compared to values previously observed in three studies in which healthy volunteers were treated with emricasan (median values in each study of 240, 161.5 and 223.5 U/L, unpublished data). Subjects treated with placebo had a median baseline cCK18 value of 416.0 U/L which increased by +4.5 U/L at day 7 ($P = 0.8401$). Subjects treated with emricasan had a median baseline cCK18 value of 586.0 U/L which decreased by −284.0 U/L at day 7 ($P < 0.0001$ compared to baseline, $P = 0.0015$

vs placebo). At day 28, placebo-treated subjects had an increased cCK18 value relative to baseline (+14.0; $P = 0.7983$) and emricasan-treated subjects had decreased cCK18 values relative to baseline (−183.0 U/L; $P = 0.0033$) but the emricasan group was not significantly different from placebo ($P = 0.1149$).

Similar results were observed for fICK18 (Figure 3B). Baseline values of fICK18 were elevated compared to values previously observed in three studies in which healthy volunteers were treated with emricasan (median values in each study of 334, 284, and 427.5 U/L, unpublished data). Subjects treated with placebo had a median baseline fICK18 value of 879.0 U/L which increased by +32.5 U/L at day 7 ($P = 0.9661$). Subjects treated with emricasan had a median baseline fICK18 value of 812.0 U/L which decreased by −534.5 U/L at day 7 ($P < 0.0001$ compared to baseline, $P = 0.0023$ vs placebo). At day 28, placebo-treated subjects had an increased fICK18 value relative to baseline (+4.0 U/L, $P = 1.0000$) and emricasan-treated subjects had decreased fICK18 values (−296.0 U/L). The day 28 value in the emricasan-treated subjects was significantly different from baseline ($P = 0.0006$) and significantly different from placebo ($P = 0.0471$).

The results for caspase 3/7 activity are shown in Figure 3C. Baseline caspase 3/7 values were at the upper range of values previously observed in three studies in which healthy volunteers were treated with emricasan (median values in each study of 919.5, 963.5, and 1290 RLU, unpublished data). Subjects treated with placebo had a median baseline caspase 3/7 value of 1356 RLU which decreased by −52.5 RLU at day 7 ($P = 0.2247$). Subjects treated with emricasan had a median baseline caspase value of 1190 RLU which decreased by −228.5 RLU at day 7 ($P < 0.0001$ compared to baseline, $P = 0.0124$ vs placebo). At day 28, placebo-treated subjects had an increased caspase 3/7 value relative to baseline (+68.0 RLU, $P = 0.7680$) and emricasan-treated subjects had decreased caspase 3/7 values (−287.0 RLU). The day 28 value in the emricasan-treated subjects was significantly different from baseline ($P = 0.0108$) but not significantly different from placebo (95% CI [−590, 63]; $P = 0.1365$).

Values for all three biomarkers returned towards baseline values after emricasan treatment was stopped for 28 days (day 56 visit).

3.6 | Potential effect of weight change on efficacy measures

Any subject who planned to make a significant lifestyle change to their diet and exercise regimens during the study was to be excluded. Nevertheless, three subjects (one randomised to placebo, two randomised to emricasan) had a weight change of greater than five pounds (−9 lbs. in the placebo subject, and −18 and +14 lbs. in the emricasan subjects) between screening and day 28. To assess the potential effect of weight change on the study conclusions, these three subjects were excluded from analysis and the changes in ALT, AST, cCK18, fICK18, and caspase 3/7 between the emricasan and placebo groups at day 28 were again compared. The decreases in ALT and fICK18 at day 28 remained significant in the subjects treated with emricasan compared to placebo. The non-significant difference between the emricasan and placebo groups for cCK18 at day 28 became significant ($P = 0.0477$) when those three subjects were excluded. Excluding those three subjects had essentially no effect on the p values for AST and caspase 3/7 at day 28. Overall, change in weight did not have a major confounding effect upon the overall study results.

3.7 | Changes in other chemistry values

There were no significant differences between treatment groups in serum values of alkaline phosphatase, gamma glutamyl transpeptidase, amylase, or lactate dehydrogenase at days 7 or 28. There were no significant differences between treatment groups in lipid parameters, including total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, or triglyceride values at day 28 (not measured on day 7). There were no significant differences between treatment groups at day 28 in measures of glucose tolerance or control, including insulin levels, haemoglobin A1c, serum glucose, and HOMA-IR (not measured on day 7). Circulating inflammatory markers were not measured.

3.8 | Analysis of adverse events

Emricasan treatment was generally well tolerated. Table 2 shows an overview of AEs. The number of AEs reported per group, number of subjects reporting any AE, and number of subjects with serious, moderate, and severe AEs was similar between the placebo and emricasan groups (Table 2). Most AEs were mild. Two subjects in each group reported moderate AEs. No severe AEs were reported. Most AEs were reported by only one subject. The only events reported by two subjects were frequent bowel movements, chest pain, and headache in the emricasan arm, and the urinary tract infection in the placebo arm (Table 3). One AE (dry mouth) in a placebo subject was considered by the investigator as possibly related to blinded treatment. No deaths were reported during the study.

One subject in each group reported a serious AE. Neither was felt to be related to study medication (Table 2). A placebo-treated

TABLE 2 Overview of adverse events (AEs)

	Placebo (N = 19)	Emricasan (N = 19)
Number of AEs	20	23
Number (%) of serious AEs	1[a] (5.3)	1[b] (5.3)
Number (%) of subjects with any AE	8 (42.1)	10 (52.6)
Number (%) of subjects with related AE	1 (5.3)	–
Number (%) of subjects with moderate AE	2 (10.5)	2 (10.5)
Number (%) of subjects with severe AE	0	0
Number (%) of subjects with AE leading to interruption of study medication	–	1 (5.3)

[a]Gastrointestinal haemorrhage.
[b]Cellulitis.

TABLE 3 Most frequent adverse events (AEs) by system organ class and preferred term

System organ class Preferred term	Placebo (N = 19)	Emricasan (N = 19)
Blood and lymphatic system	1 (5.3)	–
Endocrine	–	1 (5.3)
Hyperthyroidism		
Gastrointestinal	5 (26.3)	3 (15/8)
Frequent bowel movements		2 (10.5)
General disorders and administration site conditions	1 (5.3)	3 (15.8)
Chest pain	–	2 (10.5)
Immune system	–	1 (5.3)
Hypersensitivity		
Infections and infestations	2 (10.5)	4 (21.1)
Bronchitis	1 (5.3)	–
Cellulitis	–	1 (5.3)
Pharyngitis streptococcal	–	1 (5.3)
Urinary tract infection	2 (10.5)	1 (5.3)
Viral upper respiratory tract infection	–	1 (5.3)
Musculoskeletal and connective tissue disorder	1 (5.3)	1 (5.3)
Groin pain		1 (5.3)
Pain in extremity	1 (5.3)	
Nervous system	–	3 (15.8)
Headache	–	2 (10.5)
Renal and urinary disorders	–	1 (5.3)
Renal failure acute		
Respiratory, thoracic, and mediastinal disorders	2 (10.5)	–
Cough		
Nasal congestion		
Skin and subcutaneous tissue disorders	1 (5.3)	–
Psoriasis		
Total	8 (42.1)	10 (52.6)

subject reported a GI haemorrhage and an emricasan-treated morbidly obese diabetic subject had lower extremity cellulitis.

There were no adverse effects of emricasan upon any laboratory tests, serum lipids, glucose, vital signs, or EKG parameters.

4 | DISCUSSION

This proof-of-concept study provides evidence that emricasan lowers ALT and inhibits excessive caspase activation in subjects with NASH and elevated aminotransferases. Both total levels of cytokeratin-18 (fICK18) as well as cleaved cytokeratin-18 (cCK18) were reduced by emricasan. Circulating levels of the executioner caspases –3 and –7 were also reduced. Of note, cCK18, fICK18, and caspase-3/7 activity were reduced towards, but not below, median values observed in healthy volunteer studies. This is consistent with previously published data from healthy volunteers treated with a single 25 mg dose or 25 mg twice-daily of emricasan for 10 days where no effect on cCK18, fICK18, or caspase-3/7 was observed.[18] Thus, there appears to be a basal level of normal apoptosis that is not affected by emricasan, perhaps since OATP1B1 and 1B3 expression is primarily limited to hepatocytes and enterocytes,[19] and emricasan exhibits relatively poor passive diffusion across cell membranes (unpublished data).

One important observation in this study is that serum ALT levels decreased to below 40 IU/L in approximately 65% of emricasan-treated patients by the day 7 visit and the response was maintained in most subjects through the final day 28 visit. Normalisation of ALT values in NASH patients has been associated with improvement in liver histology. The 96-week PIVENS study[20] that compared vitamin E, which is believed to mitigate hepatocyte oxidative stress, and pioglitazone to placebo showed that both vitamin E and pioglitazone reduced ALT levels, steatosis, and lobular inflammation. A subsequent post-hoc analysis of the PIVENS data showed that if ALT levels were elevated at baseline, a decrease of at least 30%-40 IU/L or less predicted improvement in most histologic liver disease measures.[21] Other studies have also shown that decreases in ALT are usually associated with improvement in histological activity in NASH patients.[22] Thus, proof-of-concept for ALT reduction with emricasan in setting of excessive caspase activation warrants further investigation.

Caspase inhibition is a novel approach to treating NASH that is believed to modulate the hepatocyte response to toxic levels of lipids. Excessive hepatocyte apoptosis has been repeatedly observed in patients with NASH[9,10] and lipotoxicity is an important mechanism of hepatocyte injury that leads to caspase activation, apoptosis, inflammation, and fibrosis in NASH.[23] Mice genetically deficient in either caspase-1, –3, or –8 are resistant to diet-induced NASH.[24,25] Caspase activation following lipotoxic and other forms of liver injury is one of the important pathways that results in hepatocyte apoptosis, inflammation, and fibrosis.[23,26]

Caspase inhibition with emricasan was previously found to decrease apoptosis and return elevated liver aminotransferases towards normal in patients with hepatitis C infection.[13,14] Two recent studies in patients with cirrhosis of different etiologies (including NAFLD, hepatitis C, and alcohol use) also showed small decreases in ALT and AST after treatment with emricasan for 28 days or up to 6 months. In the 28 day uncontrolled cirrhosis study, all subjects were compensated and the decrease in ALT relative to baseline was statistically significant.[15] In the 6-month cirrhosis study (placebo-controlled for 3 months), nearly all subjects were decompensated, mean baseline ALT values were well-within the normal range, and the observed treatment effect on ALT vs placebo at 3 months was small and not significant.[16] The present study extends those findings to patients with non-cirrhotic NAFLD by showing that pan-caspase inhibition with emricasan improved elevated aminotransferases and decreased markers of excessive caspase activation over 28 days of treatment. Serum ALT levels were elevated at

baseline and decreased to below 40 IU/L in approximately 65% of emricasan-treated patients by the day 7 visit and the response was maintained in most subjects through the final day 28 visit. Improvement in other biomarkers associated with liver inflammation (AST) and excessive caspase activity (caspase 3/7 activity and cCK18 and fICK18 levels) was also observed. In this study, baseline levels of cCK18 and fICK18 were 2- to 3-fold higher than those observed in the emricasan cirrhosis studies,[15,16] and decreases in the emricasan-treated subjects were clear. Conversely, baseline levels of caspase 3/7 were 2- to 3-fold higher in the cirrhosis studies compared to this study, and decreases in caspase 3/7 in those studies were more obvious with emricasan treatment. In general, it is easier to observe decreases in a biomarker that is elevated compared to when the biomarker is near-normal. It is possible that higher levels of the executioner caspases 3/7 observed in the cirrhosis studies resulted in lower levels of cCK18 and fICK8 due to a lower hepatocyte cell mass (and CK18 substrate) in cirrhosis subjects. Conversely, the lower levels of caspases 3/7 observed in this study resulted in higher levels of cCK18 and fICK18, potentially due to a greater hepatocyte mass (and CK18 substrate). Complicating interpretation of these observations in subjects with normal vs impaired liver function, is that production and clearance rates influence steady-state serum levels, and those rates for cCK18, fICK18, and caspase 3/7 are poorly understood in both groups of subjects.

Dietary modifications, exercise, and bariatric surgery with resultant weight loss have all been shown in some studies to improve NAFLD or NASH.[27,28] However, diet and exercise alone are frequently not successful and surgery is not always an option. Treating insulin resistance with insulin-sensitizing therapies or thiazolidinediones (eg, pioglitazone) may improve insulin sensitivity, hepatic fat content, and resolve NASH, but may not improve fibrosis.[18–21]

Vitamin E is an anti-oxidant that is postulated to improve the hepatocellular response to oxidative stress. Pilot studies showed that vitamin E treatment could lower elevated aminotransferase levels.[29,30] The larger PIVENS study that compared vitamin E and pioglitazone to placebo following 96 weeks of treatment[20] showed that while both vitamin E and pioglitazone reduced ALT levels, steatosis, and lobular inflammation, neither showed a statistically significant effect upon fibrosis (P = 0.24 and 0.12, respectively). A subsequent post-hoc analysis of the PIVENS data showed that if ALT levels were elevated at baseline, a decrease of at least 30% to 40 IU/L or less predicted improvement in most histologic liver disease measures but not fibrosis.[21] Other studies have also shown that decreases in ALT are usually associated with improvement in histological activity in NASH patients.[22]

Emricasan treatment was generally safe and well-tolerated in this 28 day study. Future studies with larger numbers of subjects and longer duration are needed to better define the safety and efficacy profile of emricasan in patients with NASH fibrosis. Additional safety information is available from studies that were recently completed in which patients with cirrhosis were treated for 28 days[15] and up to 6 months.[16] Twenty-three patients were treated with emricasan in the 28 day cirrhosis study. Only one patient reported a serious adverse event and the most common adverse events (≥5% of patients) were fatigue, headache, edema, dehydration, diarrhea, constipation, and nausea. In the 6 month cirrhosis study (placebo-controlled for 3 months), serious adverse events and adverse events leading to discontinuation were balanced between treatment groups. Adverse events were also generally balanced between treatment groups with the most common adverse events (≥5% of patients) being headache, nausea, hepatic encephalopathy, vomiting, fatigue, abdominal pain, arthralgia, and urinary tract infection.

While the study met the primary endpoint for ALT reduction at day 28, it was not prospectively sized to detect treatment differences for other biomarkers such as cCK18, fICK18, or caspase 3/7. While statistically significant differences were noted at day 7 (cCK18 and fICK18) and day 28 (fICK18 and caspase 3/7) there was no adjustment of p values for multiplicity and these effects will need confirmation in future studies. In addition, while reduction in ALT has been a reliable predictor for improvement in NASH histology, future studies are needed to assess whether emricasan will improve liver histology in NASH.

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AUTHORSHIP

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ORCID

David T. Hagerty http://orcid.org/0000-0002-1837-3935

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.

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