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# Emricasan Improves Liver Function in Patients With Cirrhosis and High Model for End-Stage Liver Disease Scores Compared With Placebo

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**BACKGROUND & AIMS:** **Caspase-mediated apoptosis and inflammation contribute to progression of liver disease. Emricasan is a pan-caspase inhibitor that reduced serum markers of apoptosis and liver inflammation in patients with hepatitis C and non-alcoholic steatohepatitis (NASH).**

**METHODS:** **We performed a multicenter study of 86 patients with cirrhosis (Child-Pugh class A or B; mean score, 6.9; 38% with alcohol-associated cirrhosis, 29% with HCV-associated cirrhosis, and 23% with NASH) and model for end-stage liver disease (MELD) scores of 11–18 (mean, 12.8). Patients were randomly assigned to groups given placebo (N = 42) or Emricasan (25 mg, N = 44), twice daily for 3 months; subjects then received open-label Emricasan (25 mg) twice-daily for 3 months. The primary endpoint was the change from baseline in serum levels of cleaved keratin 18 (CK-18) at month 3.**

**RESULTS:** **Seventy-four patients completed the 3-month study period (40 given Emricasan and 34 given placebo); 69 patients received open-label Emricasan for 3 months afterward. At the 3-month timepoint, Emricasan significantly reduced mean MELD ($P = .003$) and Child-Pugh ($P = .003$) scores in subjects with high MELD scores (15 or more), compared with placebo, with significant reductions in INR (95% CI, −0.2882 to −0.0866) and total bilirubin (95% CI, −1.5069 to −0.0823) vs placebo. There were no significant differences between Emricasan and placebo groups in mean MELD ($P = .466$) or Child-Pugh ($P = .124$) scores overall at 3 months compared to placebo. Of patients with high MELD scores, 6/9 given Emricasan (67%) had a reduction of 2 points or more at month 3, compared with 2/10 given placebo (20%). Serum levels of full-length CK-18 ($P = .02$) and caspase 3/7 ($P < .001$), but not cleaved CK-18 ($P = .092$), decreased significantly at 3 months in the Emricasan vs placebo group. Emricasan was well tolerated, and adverse events were balanced between groups. Emricasan's effects were generally maintained or increased after 6 months of treatment.**

**CONCLUSIONS:** **In a randomized trial of patients with cirrhosis, we found 3 months treatment with Emricasan to improve liver function, compared with placebo, reducing MELD and Child-Pugh scores, INR, and total bilirubin in patients with MELD scores ≥15. ClinicalTrials.gov no: NCT02230670.**

*Keywords:* IDN-6556; Cell Death; Biomarker; CK-18; CASP3; CASP7.

***Abbreviations used in this paper:*** **AE, adverse event; ALT, alanine aminotransferase; CK-18, keratin 18; cCK-18, cleaved keratin-18; flCK-18, full-length keratin-18; HCV, hepatitis C virus; INR, international normalized ratio; LSM, least square mean; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis.**



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Many chronic, progressive liver diseases are associated with excessive hepatocyte apoptosis, inflammation, and fibrosis. Apoptosis is markedly increased in patients with nonalcoholic steatohepatitis (NASH),[1,2] hepatitis C,[3] hepatitis B,[4] and alcoholic liver disease.[2] Excessive apoptosis leads to cell repair, inflammation, fibrosis, and ultimately cirrhosis.

Caspases are a family of 11 intracellular cysteine proteases that mediate apoptosis and regulate inflammatory and immune responses to dying cells.[5,6] Cellular injury from lipotoxicity, endoplasmic reticulum stress, and viral replication activate caspases. Executioner caspases[5] (caspases 3, 6, and 7) cleave many cell proteins, including keratin 18 (CK-18), and mediate the production of proinflammatory, profibrotic hepatic microvesicles,[7] which interact with hepatic stellate/myofibroblasts and sinusoidal endothelial cells[7] leading to activation, migration, and profibrotic gene expression.[8] Inflammatory caspases[5] (eg, caspases 1, 4, and 5) process interleukin-1 family members to activated forms[6] and initiator caspases[5] (caspases 2, 8, 9, and 10) play an important role in priming the NLRP3 inflammasome and producing interleukin-1$\beta$. Caspase inhibition could therefore lead to beneficial effects on liver inflammation and fibrosis and portal hypertension.

Emricasan (IDN-6556) is an oral pan-caspase inhibitor that decreased apoptosis, inflammation, and fibrosis in animal models of liver injury including NASH[9] and $CCl_4$-induced cirrhosis.[10] Emricasan also decreased excessive caspase activity and alanine aminotransferase (ALT) in subjects with hepatitis C[11,12] and nonalcoholic fatty liver disease.[13]

This study assessed whether Emricasan treatment might improve caspase-related biomarkers, hepatic function, Model for End-Stage Liver Disease (MELD), and Child-Pugh scores in Child-Pugh class A or B cirrhosis patients with MELD scores 11–18.

### What You Need to Know

#### Background

Emricasan is a pan-caspase inhibitor that decreases serum apoptotic and inflammatory liver markers. This was a randomized, blinded study of emricasan versus placebo in subjects with Child-Pugh class A or B cirrhosis and MELD scores 11–18.

#### Findings

Emricasan treatment improved MELD and Child-Pugh scores in high-MELD ($\geq$15) subjects after 3 months of treatment due to improvements in INR and total bilirubin.

#### Implications for patient care

Larger studies in patients with single etiologies of cirrhosis will be needed to better characterize the safety profile of emricasan and the potential clinical benefit of emricasan treatment.

## Materials and Methods

### *Study Oversight*

Conatus Pharmaceuticals Inc designed the trial. Data were collected by the investigators and analyzed by the sponsor. Authors had access to the study data, participated in writing the manuscript, and approved the final manuscript. The trial was conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice. The protocol was approved by the institutional human research review committee at each site.

### *Subjects*

Subjects were Child-Pugh class A or B with clinical, radiologic, or biochemical evidence of cirrhosis and classified as compensated or decompensated by medical history. MELD scores were 11–18 during screening. Male and female subjects of childbearing potential used 2 reliable forms of contraception.

Exclusions included autoimmune hepatitis, active inflammatory bowel disease, hepatitis B–infected subjects on treatment for less than 3 months, and hepatitis C–infected subjects planning to receive anti–hepatitis C virus (HCV) treatment during the study. Other exclusions were Child-Pugh class C, international normalized ratio (INR) $\geq$2.5, platelets $\leq 20 \times 10^9$/L, hepatic encephalopathy $\geq$grade III, serum creatinine $\geq$2 mg/dL, alcohol consumption >21 oz/week for males or 14 oz/week for females, variceal hemorrhage within 3 months of screening, and uncontrolled ascites.

### *Study Protocol*

This study had 2 treatment periods of 3 months: an initial randomized, double-blind, placebo-controlled phase, and an open-label phase. All subjects provided informed consent. Subjects were assessed monthly and 28 days after stopping treatment. Emricasan, 25 mg twice a day, was studied because that was the lowest dose that maximally inhibited 4 biomarkers (ALT, aspartate aminotransferase, caspase 3/7, and cleaved CK-18 [cCK-18]) in subjects with hepatitis C (unpublished data). Subjects received standard-of-care treatment for cirrhosis, including adjustment of medications to treat ascites or hepatic encephalopathy. New or worsening complications of cirrhosis were to be reported as adverse events (AEs).

### *Measures of Clinical Efficacy*

The primary endpoint was the change from baseline in cCK-18/M30 at Month 3. cCK-18/M30 is a cleavage

fragment of CK-18 produced by the action of executioner caspases, such as caspase 3/6/7.[14] Secondary clinical endpoints included changes in total bilirubin, INR, albumin, MELD score, and Child-Pugh status. Secondary biomarker endpoints included changes in ALT, caspase 3/7 activity, and full-length CK-18 (flCK-18/M65, which measures both intact and cCK-18). Clinical and biomarker measurements were performed by PPD Global Central Labs, LLC (Highland Heights, KY). cCK-18/M30 (Apoptosense), flCK-18/M65 (EpiDeath), and caspase 3/7 activity (Caspase-Glo 3/7) were measured in sera (laboratory reference ranges: 80–102 U/L, 63–211 U/L, and 1429–3908 relative light units, respectively). However, in 3 previous studies where 31 healthy subjects were treated with Emricasan, group mean values between 178.1 and 280.5 U/L were observed for cCK-18, 332.0 and 401.1 U/L for flCK-18, and 1173.0 and 1293.4 relative light units for caspase 3/7 (unpublished data).

### Safety Assessments

Safety assessments included collection of AEs, clinical examination, vital signs, laboratory tests, and electrocardiograms. The severity of AEs and relation to study medication treatment was collected. An independent monitoring committee evaluated unblinded data regularly.

### Blinding and Randomization

The sponsor, investigational staff, and subjects were blinded to the original treatment for the entire 6 months of treatment. Subjects were randomized 1:1 to Emricasan or placebo for the initial 3 months using a validated, verified randomization program.

### Statistical Analysis

Seventy-two evaluable subjects provided 90% power to reject the null hypothesis of no treatment difference at a statistical significance level (2-sided) of 0.05 if the true mean difference in log-transformed cCK-18/M30 was −0.331 with a common standard deviation of 0.417. Those parameters were estimated from a previous study in subjects without cirrhosis with active hepatitis C and an ALT or aspartate aminotransferase $\geq$1.5 times the upper limit of normal during screening.[12]

Efficacy analyses were conducted on the full analysis set, which consisted of all randomized subjects who received at least 1 dose of treatment. Efficacy analyses used the intention-to-treat principle. Missing values at Month 3 were imputed using the last observation on-study. The statistical analysis plan was finalized before study unblinding and identified predefined efficacy subgroups: subjects with MELD scores $\geq$15 at baseline, and disease etiology subgroups HCV, NASH, and alcohol/other.

Safety analyses were conducted according to the treatment subjects received. No adjustments for multiplicity were made.

The mean difference between Emricasan and placebo in log transformed cCK-18/M30 at Month 3 was tested using the least square means (LSM) 2-sided 0.05 statistical significance level including treatment as a factor, adjusted for baseline. The LSM estimate of the difference and corresponding 95% confidence intervals was back transformed to provide the relative changes between Emricasan, 25 mg, and placebo. The LSM primary analysis model included treatment group and adjusted for baseline value and treatment group. The LSM secondary analysis model for other endpoints included treatment group and adjusted for baseline differences in MELD category ($<$15; $\geq$15), etiology, and treatment group interaction terms.

## Results

### Disposition

A total of 140 subjects were screened, 87 subjects were randomized, and 86 were treated with placebo (N = 42) or Emricasan (N = 44). Eight placebo and 4 Emricasan subjects withdrew during the blinded phase. One Child-Pugh class C subject was inadvertently randomized and withdrawn. Four subjects with MELD scores $<$11 and 1 with a MELD score of 19 were randomized because of errors. The low MELD score subjects continued in the study but the subject with the MELD score of 19 was discontinued. Detailed disposition information is shown in Supplementary Figure 1.

### Baseline Demographics and Liver Disease Characteristics

Baseline demographics and liver disease characteristics are shown in Table 1. The patient population was heterogeneous in terms of cirrhosis etiology with more HCV cirrhosis patients in the placebo group and more "alcohol/other" patients in the Emricasan group. Baseline MELD scores were 8–19 with more Child-Pugh class A patients in the Emricasan group (50.0% vs 35.7% in placebo group) and more Child-Pugh class B patients in the placebo group (61.9% vs 50.0% in Emricasan group), although the Child-Pugh scores were the same (6.9 in both groups). By history, 90.7% of patients were decompensated, including variceal hemorrhage (12.8%), ascites (52.3%), and hepatic encephalopathy (68.6%).

Baseline demographics in the prespecified high-MELD (score $\geq$15) and NASH cirrhosis subgroups were relatively balanced. Supplementary Table 1 shows the baseline liver function in all subgroups, including the low-MELD ($\leq$14) and alcohol/other subgroups.

**Table 1.** Baseline Demographics and Liver Disease Characteristics

| | All patients | | MELD ≥15 | | NASH patients | |
|---|---|---|---|---|---|---|
| | Placebo (N = 42) | Emricasan (N = 44) | Placebo (n = 10) | Emricasan (n = 9) | Placebo (n = 9) | Emricasan (n = 11) |
| Age | 57.5 ± 8.75 | 58.5 ± 8.15 | 57.4 ± 7.88 | 58.3 ± 9.14 | 62.3 ± 6.00 | 59.9 ± 8.92 |
| | | | Number (%) of patients | | | |
| Gender | | | | | | |
| Male | 30 (71.4) | 24 (54.5) | 9 (90.0) | 6 (66.7) | 7 (77.8) | 4 (36.4) |
| Female | 12 (28.6) | 20 (45.5) | 1 (10.0) | 3 (33.3) | 2 (22.2) | 7 (63.6) |
| Race | | | | | | |
| White | 37 (88.1) | 39 (88.6) | 9 (90.0) | 8 (88.9) | 7 (77.8) | 11 (100) |
| Black/ African American | 2 (4.8) | 4 (9.1) | 0 (0) | 1 (11.1) | 0 (0) | 0 (0) |
| Unknown | 1 (2.4) | 1 (2.3) | 1 (10.0) | 0 (0) | 0 (0) | 0 (0) |
| Asian | 1 (2.4) | 0 (0) | 0 (0) | 0 (0) | 1 (11.1) | 0 (0) |
| Multiracial | 1 (2.4) | 0 (0) | 0 (0) | 0 (0) | 1 (11.1) | 0 (0) |
| Cirrhosis etiology | | | | | | |
| Alcohol/other | 17 (40.5) | 24 (54.5) | 4 (40.0) | 6 (66.7) | 0 (0) | 0 (0) |
| HCV | 16 (38.1) | 9 (20.5) | 4 (40.0) | 2 (22.2) | 0 (0) | 0 (0) |
| NASH | 9 (21.4) | 11 (25.0) | 2 (20.0) | 1 (11.1) | 9 (100) | 11 (100) |
| MELD | | | | | | |
| ≤14 | 32 (76.2) | 35 (79.5) | 0 (0) | 0 (0) | 7 (77.8) | 10 (90.9) |
| ≥15 | 10 (23.8) | 9 (20.5) | 10 (100) | 9 (100) | 2 (22.2) | 1 (9.1) |
| Score | 12.9 ± 2.6 | 12.8 ± 2.1 | 16.3 ± 1.3 | 16.0 ± 1.1 | 12.8 ± 2.1 | 13.0 ± 2.1 |
| Child-Pugh | | | | | | |
| Class A | 15 (35.7) | 22 (50.0) | 1 (10.0) | 1 (11.1) | 4 (44.4) | 3 (27.3) |
| Class B | 26 (61.9) | 22 (50.0) | 8 (80.0) | 8 (88.9) | 5 (55.6) | 8 (72.7) |
| Class C | 1 (2.4) | 0 (0) | 1 (10.0) | 0 (0) | 0 (0) | 0 (0) |
| Score | 6.9 ± 1.2 | 6.9 ± 1.3 | 8.2 ± 1.0 | 7.8 ± 1.0 | 6.9 ± 1.1 | 7.3 ± 1.2 |
| INR | 1.31 ± 0.18 | 1.33 ± 0.17 | 1.45 ± 0.17 | 1.54 ± 0.19 | 1.27 ± 0.23 | 1.25 ± 0.14 |
| Total bilirubin, *mg/dL* | 2.59 ± 1.49 | 2.25 ± 1.12 | 4.30 ± 1.94 | 3.17 ± 1.59 | 2.26 ± 1.10 | 2.68 ± 1.53 |
| Albumin, *g/dL* | 3.48 ± 0.54 | 3.46 ± 0.41 | 3.19 ± 0.46 | 3.41 ± 0.24 | 3.60 ± 0.46 | 3.39 ± 0.39 |
| Serum creatinine, *mg/dL* | 0.74 ± 0.30 | 0.81 ± 0.31 | 0.74 ± 0.37 | 0.79 ± 0.36 | 0.79 ± 0.49 | 0.85 ± 0.36 |
| ALT, *U/mL* | 30.1 ± 17.5 | 31.7 ± 14.9 | 33.0 ± 21.8 | 27.7 ± 12.8 | 29.3 ± 13.1 | 36.3 ± 19.3 |
| AST, *U/mL* | 50.0 ± 28.1 | 54.3 ± 22.0 | 61.2 ± 35.8 | 49.7 ± 17.7 | 43.2 ± 15.4 | 58.4 ± 26.1 |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis.

### *Liver Function at 3 Months in the High Model for End-Stage Liver Disease and Nonalcoholic Steatohepatitis Subgroups*

There were no differences between treatments in total bilirubin, INR, or serum albumin at 3 months in the overall population but there were significant differences in prespecified subgroups. Total bilirubin and INR improved at 3 months in the high-MELD subjects treated with Emricasan (−0.79 mg/dL and −0.19, respectively, vs placebo) (Table 2). The 95% confidence intervals for the treatment differences in total bilirubin (−1.5069 to −0.0823) and INR (−0.2882 to −0.0866) excluded 0 and were statistically significant at 3 months (Figures 1*A* and *B*, respectively). Improvements in total bilirubin and INR were significant by Month 2 (Figure 2*A*) and Month 1, respectively (Figure 2*B*).

The NASH subgroup treated with Emricasan showed a nonsignificant trend toward greater improvement in total bilirubin versus placebo at 3 months (Figure 1*A*) with a difference of −0.40 mg/dL (Table 2). Total bilirubin values remained stable between baseline and Month 3 in the Emricasan-treated subjects, whereas placebo-treated subjects had increasing bilirubin values (Figure 2*C*). INR values remained stable in Emricasan subjects and increased in placebo subjects (Figure 2*D*) and the treatment difference at Month 3 (−0.13; Table 2) was significantly lower versus placebo (Figure 1*B*).

There was no effect of Emricasan treatment on serum albumin at 3 months (+0.04 g/dL, high-MELD subgroup; −0.06 g/dL, NASH subgroup).

There was no confounding effect of weight loss in the NASH cirrhosis subgroup where body mass index at 3 months increased in the Emricasan group (+0.83) and was stable in the placebo group (0.00).

### *Consistency of Functional Improvement Across Etiologies at 3 Months*

Emricasan treatment was associated with consistent, directional changes in most efficacy measures, observed across different etiologies of cirrhosis (Table 2). Although the magnitude of the treatment effect varied by etiology, directional changes in efficacy were similar in all etiologies irrespective of baseline MELD score.

**Table 2.** Treatment Difference Between Placebo and Emricasan at 3 Months in High-MELD and Etiology Subgroups

| | MELD ≥15 | NASH cirrhosis | HCV | Alcohol/other |
|---|---|---|---|---|
| | Placebo: n = 10<br>Emricasan: n = 9 | Placebo: n = 9<br>Emricasan: n = 11 | Placebo: n = 16<br>Emricasan: n = 9 | Placebo: n = 17<br>Emricasan: n = 24 |
| MELD score (LSM) | –2.2[a] | –1.6[a] | –0.6 | –0.7 |
| Child-Pugh score (LSM) | –1.3[a] | –1.0[a] | –0.3 | –0.8[a] |
| INR (LSM) | –0.19[a] | –0.13[a] | –0.05 | –0.10[a] |
| Total bilirubin, *mg/dL* (LSM) | –0.79[a] | –0.40 | –0.43 | –0.45 |
| Albumin, *g/dL* (LSM) | 0.04 | –0.06 | –0.06 | 0.03 |
| Serum creatinine, *mg/dL* (LSM) | 0.03 | –0.07 | 0.01 | 0.10[a] |
| ALT, *U/mL* (median) | –3.0 | –2.0 | –6.0 | –3.0 |
| AST, *U/mL* (median) | –5.5 | –3.0 | –12.0 | –3.0 |
| Caspase 3/7 (% relative change to placebo) | –56%[a] | –52%[a] | –53%[a] | –52%[a] |
| fICK-18 (% relative change to placebo) | –19% | –4% | –26%[a] | –17%[a] |
| cCK-18 (% relative change to placebo) | –26% | –9% | –15% | –28%[a] |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; cCK-18, cleaved keratin-18; fICK-18, full-length keratin-18; HCV, hepatitis C virus; INR, international normalized ratio; LSM, least square mean; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis.
[a]Statistically significant ($P < .05$) treatment differences unadjusted for multiplicity.

### *Model for End-Stage Liver Disease Score at 3 Months*

Emricasan treatment improved MELD scores in the high-MELD and NASH cirrhosis subgroups versus placebo at 3 months ($P = .003$ and .029, respectively) (Figure 1*C*). The low-MELD subgroup showed no meaningful change. The treatment difference at 3 months in the high-MELD subgroup (–2.2; Table 2) was caused by MELD score increase in the placebo group (+0.6) and

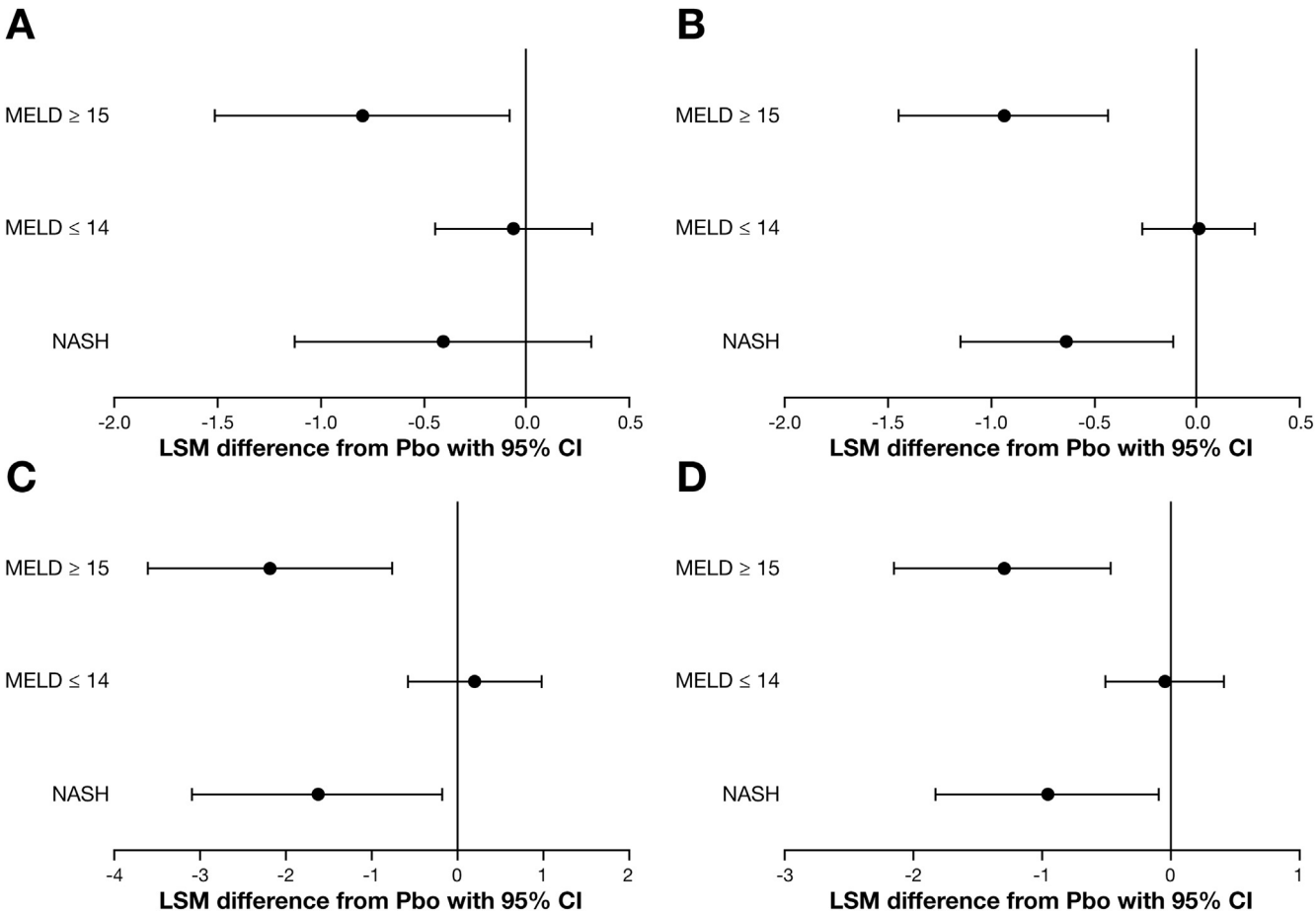


**Figure 1.** The LSM treatment difference with 95% confidence intervals in (*A*) total bilirubin, (*B*) INR, (*C*) MELD score, and (*D*) Child-Pugh score for the high-MELD (≥15), low-MELD (<14), and NASH cirrhosis subgroups after 3 months of treatment CI, confidence interval; Pbo, placebo.

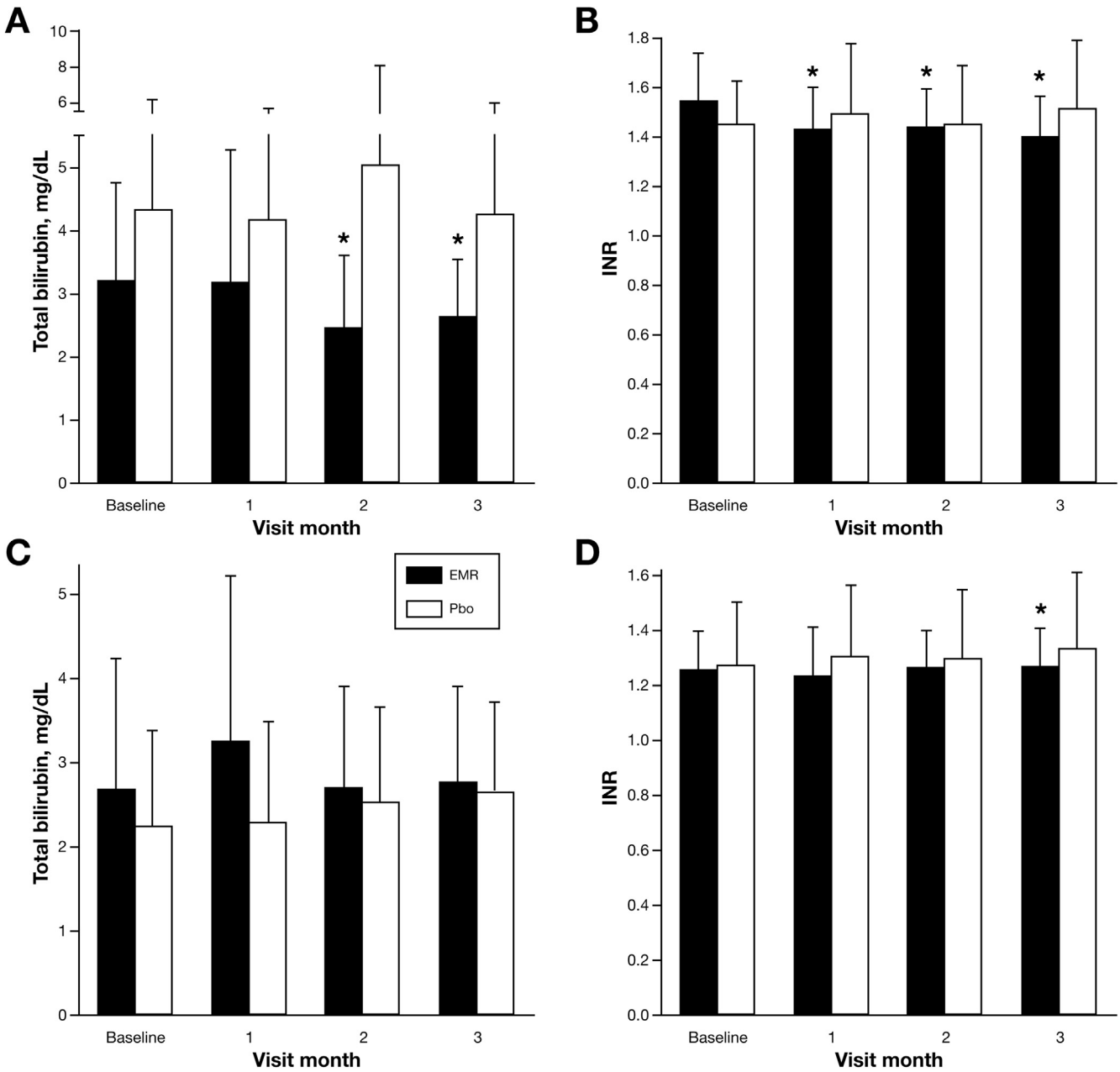


**Figure 2.** Mean (SD) changes in total bilirubin and INR after 1, 2, and 3 months of treatment in the high-MELD and NASH cirrhosis subgroups. (*A*) High-MELD subgroup change in total bilirubin. (*B*) High-MELD subgroup change in INR. (*C*) NASH cirrhosis subgroup change in total bilirubin. (*D*) NASH cirrhosis subgroup change in INR. Emricasan group in *black bars* and placebo group in *white bars*. Statistically significant differences between treatment groups in change from baseline are indicated by an *asterisk*. EMR, Emricasan; Pbo, placebo.

improvement in the Emricasan group (-1.6). The NASH cirrhosis subgroup had a significant treatment difference at 3 months (–1.6, Figure 1*C* and Table 2) because of MELD score decrease in the Emricasan group (-0.46) and increase in the placebo group (+1.17).

The serum creatinine was stable at 3 months in both subgroups (change: –0.01 to 0.1 mg/dL), indicating that the MELD score difference was driven by improvement in INR and total bilirubin.

MELD score changes for each subject in the high-MELD and NASH cirrhosis subgroups are shown in Supplementary Figure 2.

### *Child-Pugh Score at 3 Months*

There was no difference between groups in the Child-Pugh score (–0.2 vs +0.1) but the prespecified high-MELD and NASH cirrhosis subgroups had statistically significant improvement (Figure 1*D*). Both Emricasan-treated subgroups had a significant treatment difference from placebo in Child-Pugh score (–1.3, $P = .003$ and –1.0, $P = .030$, respectively). The Child-Pugh score treatment difference for the high-MELD subgroup was caused by increase in the placebo group (+1.06) and decline in the Emricasan group (–0.25). The low-MELD

subgroup showed no meaningful change. In the NASH subgroup the Child-Pugh score increased +0.8 in the placebo group and decreased −0.2 in the Emricasan group (Table 2).

Child-Pugh score changes for each subject in the high-MELD and NASH cirrhosis subgroups are shown in Supplementary Figure 3.

### Change in Cleaved Keratin-18 and Other Biomarkers at 3 Months

Mean baseline values of cCK-18 were 486.0 (placebo, N = 44) and 385.2 (Emricasan, N = 44). Mean baseline caspase 3/7 activity (2795.0, placebo; 2923.2, Emricasan) and flCK-18 (673.1, placebo; 859.3, Emricasan) were also elevated. ALT values were within the normal range (Table 1).

The primary analyses adjusted treatment groups for baseline biomarker value and treatment group. In the primary endpoint analysis for cCK-18, Emricasan reduced cCK-18 −13% relative to placebo at 3 months (Figure 3*A*) but statistical significance was not achieved ($P = .092$; 2-sided), although the changes at 1 and 2 months were significant. Caspase 3/7 activity (Figure 3*B*; −45.5% vs +8.8%) and flCK-18 (Figure 3*C*; −18.0% vs −3.0%) were also reduced by Emricasan relative to placebo at 3 months and statistical significance was achieved for both ($P < .001$ and $P = .020$, respectively), and at the 1- and 2-month visits. Ad hoc analyses that additionally adjusted for baseline differences in MELD score and cirrhosis etiology achieved statistical significance for all 3 biomarkers at each study visit.

Mean ALT values decreased relative to baseline but were not statistically significant versus placebo (Figure 3*D*). Within each treatment group there was no clear relationship between change in platelet count and change in total bilirubin, INR, MELD, or Child-Pugh score.

### Results at 6 Months

Because there was no control group for the second 3 months of treatment, the changes in efficacy measures following 6 months of Emricasan treatment were referenced to baseline (Supplementary Table 2).

The high-MELD subgroup either stabilized or continued to improve between 3 and 6 months. The MELD score (−1.6 to −2.8) and INR (−0.14 to −0.21) at 3 and 6 months showed significant improvement, whereas the Child-Pugh score, total bilirubin, and albumin were essentially stable between months 3 and 6 (Supplementary Table 2).

The NASH cirrhosis subgroup during the second 3 months of treatment showed stabilization or improvement relative to baseline, and improvement relative to Month 3, in MELD scores, Child-Pugh scores, INR, total bilirubin, and albumin (Supplementary Table 2).

### Adverse Events

An overview of AEs at 3 months is shown in Table 3. There were no deaths. Serious AEs were reported by 13.6% and 11.9% of subjects in the Emricasan and placebo group, respectively. The proportion of subjects with moderate AEs (38.6% and 28.6%), severe AEs (11.4% and 9.5%), and AEs leading to study discontinuation (6.8% and 4.8%) were similar between the Emricasan and placebo groups.

The frequency of new or worsening decompensation events was similar in both treatment groups at 3 months (Table 3). More placebo subjects had new or worsening ascites (7.1% vs 2.3%); more Emricasan subjects had new or worsening hepatic encephalopathy (4.8% vs 11.4%). All Emricasan-treated subjects who reported hepatic encephalopathy had a prior history of hepatic encephalopathy and were taking either lactulose alone (1/5), rifaximin alone (1/5), or lactulose plus rifaximin (3/5). Hepatic encephalopathy in both groups was generally precipitated by intercurrent illness, noncompliance with lactulose or rifaximin, or resolved despite continued treatment with study medication. One subject randomized to placebo was found to have recurrent hepatocellular carcinoma at the 3-month visit, before treatment with Emricasan.

There was no difference between treatment groups for any laboratory evaluation except for measures that were related to the pharmacodynamic effect of Emricasan (eg, ALT, aspartate aminotransferase, total bilirubin, and INR). There was no effect on red blood cells, white blood cells, or platelet counts. There were no differences in systolic and diastolic blood pressure, heart rate, or electrocardiogram parameters.

## Discussion

This study suggests that 3–6 months of Emricasan treatment may improve hepatic function relative to placebo in patients with cirrhosis of different etiologies, with greater effects in subjects with MELD scores ≥15 and NASH as the etiology of cirrhosis. Following 6 months of treatment, Emricasan improved total bilirubin (−0.65 mg/dL), INR (−0.21), albumin (+0.1), MELD scores (−2.8), and Child-Pugh scores (−0.7) in subjects with baseline MELD scores ≥15.

Chronic progressive liver diseases, such as NASH,[1] hepatitis C,[3] hepatitis B,[4] and alcoholic liver disease,[2] are all associated with excessive caspase activation and hepatocyte apoptosis. It was therefore interesting to compare the improvement in liver function observed with treating the result of hepatocyte injury (caspase activation) with directly treating the cause of injury. Belli et al[15] studied the improvement in hepatic function following treatment of HCV infection with direct-acting antivirals in 102 decompensated viremic patients with cirrhosis listed for transplant.[16] Treatment with

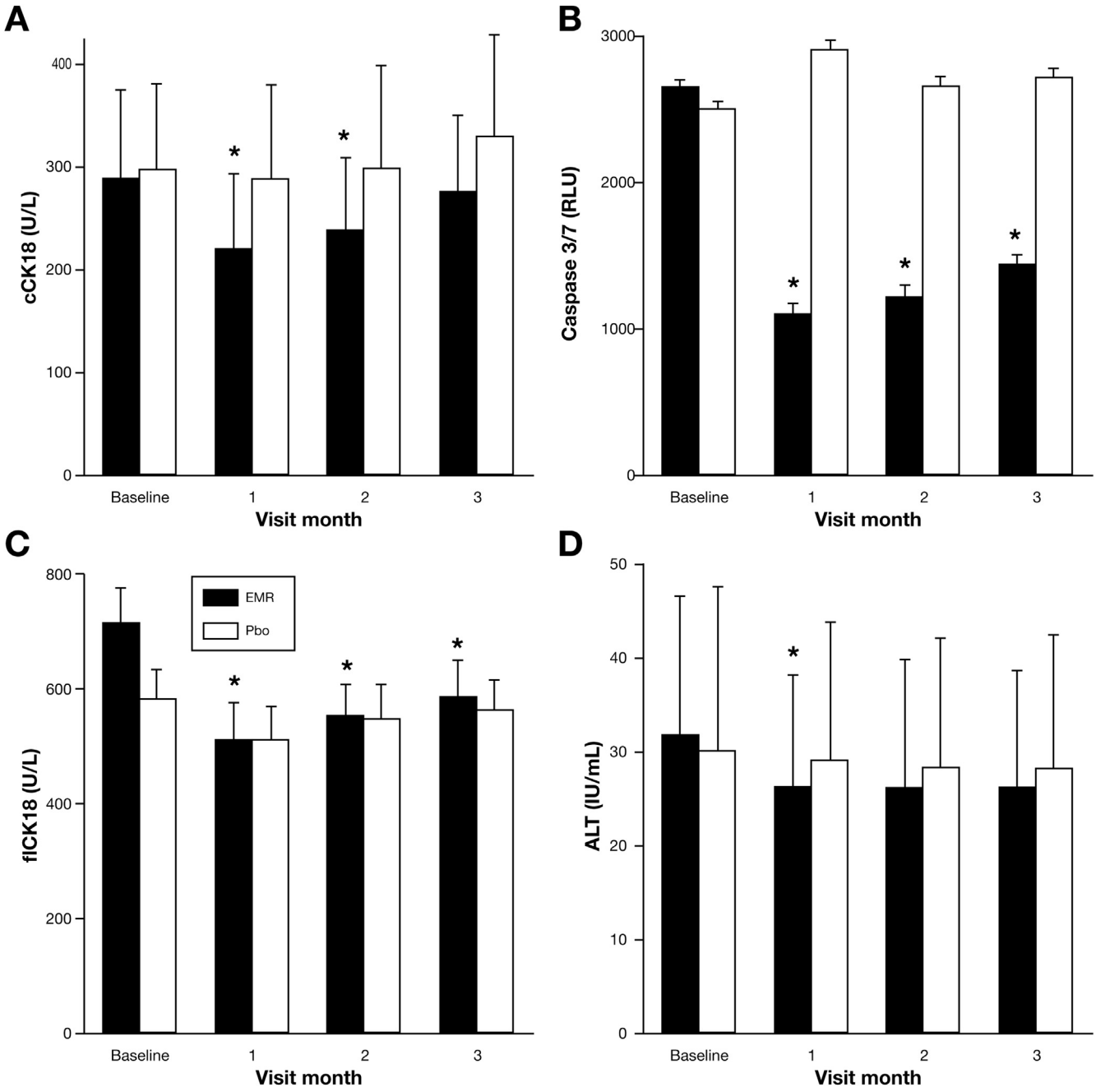


**Figure 3.** Baseline and Month 1, 2, and 3 values of cCK-18, caspase 3/7, fICK-18, and ALT in all subjects treated with Emricasan (*black bars*) or placebo (*white bars*). (*A*) Geometric mean cCK-18 values, U/L (primary endpoint). (*B*) Geometric mean caspase 3/7 activity, relative light units. (*C*) Geometric mean fICK-18 values, U/L. (*D*) Mean ALT values, IU/mL. Statistically significant differences between treatment groups (primary analysis) in change from baseline are indicated by an *asterisk*. Pbo, placebo; RLU, relative light unit.

direct-acting antivirals improved total bilirubin (−0.395 mg/dL), albumin (+0.3 g/dL), MELD score (−1) and Child-Pugh score (−1) but not INR 24 weeks after completing treatment.[16] It was interesting that treating both the cause of HCV cirrhosis and the caspase activation that resulted from different kinds of hepatocyte injury resulted in similar degrees of functional improvement after 24 weeks.

The results from this human study showing that Emricasan improved hepatic function in subjects with cirrhosis are supported by preclinical studies in the rat $CCl_4$ cirrhosis model where Emricasan decreased intrahepatic vascular resistance, reduced inflammation, improved sinusoidal cell/hepatic stellate cell phenotype and microcirculatory function, and caused fibrosis regression.[10]

One explanation for the apparently different responses in the high-MELD versus low-MELD subgroup is that the total bilirubin and INR values were much higher in the high-MELD versus low-MELD subgroup and it was

**Table 3.** Summary of AEs and AEs Reported in 3 or More Subjects

| Overview of adverse events during 3-month double-blind phase | | |
|---|---|---|
| Evaluable subjects | Placebo (N = 42) n (%) | Emricasan (N = 44) n (%) |
| Subjects with AEs | 30 (71.4) | 34 (77.3) |
| Subjects with SAEs | 5 (11.9) | 6 (13.6) |
| Subjects with moderate AEs | 12 (28.6) | 17 (38.6) |
| Subjects with severe AEs | 4 (9.5) | 5 (11.4) |
| Subjects with AEs leading to discontinuation | 2 (4.8) | 3 (6.8) |
| Preferred term | Number (%) of subjects | |
| | Placebo | Emricasan |
| Headache | 3 (7.1) | 7 (15.9) |
| Nausea | 4 (9.5) | 7 (15.9) |
| Hepatic encephalopathy | 2 (4.8) | 5 (11.4) |
| Vomiting | 1 (2.4) | 5 (11.4) |
| Fatigue | 6 (14.3) | 4 (9.1) |
| Abdominal pain | 3 (7.1) | 3 (6.8) |
| Arthralgia | — | 3 (6.8) |
| Urinary tract infection | 1 (2.4) | 3 (6.8) |
| Edema peripheral | 4 (9.5) | 1 (2.3) |
| Ascites | 3 (7.1) | 1 (2.3) |
| Muscle spasms | 3 (7.1) | — |
| New or worsening decompensation events | 6 (14.3) | 7 (15.9) |
| New or worsening ascites | 3 (7.1) | 1 (2.3) |
| New or worsening hepatic encephalopathy | 2 (4.8) | 5 (11.4) |
| New or recurrent variceal bleeding | — | — |
| Other | 1 (2.4) | 1 (2.4) |

AE, adverse event; SAE, serious adverse event.

therefore easier to detect improvement in liver function. Baseline liver function in the NASH subgroup was more similar to the low-MELD subgroup. MELD and Child-Pugh scores, and total bilirubin and prothrombin time, increase in a greater-than-linear manner as liver function deteriorates.[16,17] Heterogeneity between treatment groups, particularly with regard to etiology and Child-Pugh class, and the small number of subjects may have obscured a potential treatment effect. Analyses that minimized the potential effect of heterogeneity by analyzing subjects with single etiologies of cirrhosis did suggest that heterogeneity was a factor because those analyses showed consistent trends for improvement in each subgroup were observed regardless of baseline MELD score.

Although Emricasan treatment was associated with decreases in cCK-18, fICK-18, and the executioner caspase 3/7, there was no apparent correlation between biomarker and clinical responses. Assessment of the effect of Emricasan on inflammatory or initiator caspases may lead to better correlation with specific measures of liver function.

Emricasan may have other beneficial effects in patients with cirrhosis. A pilot study in compensated, Child-Pugh class A subjects with cirrhosis suggested that Emricasan improved severe portal hypertension (hepatic venous pressure gradient >12 mm Hg) within 28 days.[18] The ability of Emricasan to potentially improve the 2 critical components of end-stage liver disease, portal hypertension and liver function, is important. Although HCV infection can be successfully treated in patients with compensated and decompensated cirrhosis,[19,20] regression of cirrhosis and improvement in portal hypertension may take years and patients who achieve a sustained viral response but continue to have portal hypertension remain at risk for decompensation and death.[21,22] A medication that improves both liver function and portal hypertension is greatly needed.

Emricasan treatment was generally well-tolerated in this study. AEs were frequent in both treatment groups with small differences in the frequencies of AEs between groups. AEs were those typically observed in patients with decompensated cirrhosis. Larger and longer studies are necessary to better understand the safety and tolerability profile of Emricasan in subjects with cirrhosis.

This study has limitations. Because of limited safety experience in subjects with cirrhosis, the number of subjects and length of treatment was limited and only a single dose level of Emricasan was studied. Higher and lower doses will be assessed in future studies to select an optimal clinical dose. Patients will also need to be treated for longer than 3–6 months. Although improvement in hepatic inflammation may occur relatively rapidly, improvement in fibrosis will take longer. The multiple etiologies of cirrhosis in a single study increased heterogeneity and may have obscured the treatment effect, as suggested by the single-etiology efficacy analyses where treatment effects were observed regardless of baseline MELD score. The magnitude of improvement in clinical measures, such as total bilirubin, INR, MELD, or Child-Pugh scores, must be confirmed in future studies.

In conclusion, this study comparing treatment with Emricasan with placebo in largely decompensated patients with cirrhosis showed significant improvement of hepatic function in subjects with baseline MELD scores ≥15 and stabilization of function in subjects with NASH cirrhosis. Future single-etiology studies will better characterize the safety profile of Emricasan and better estimate the magnitude of the treatment benefit.

## 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.2018.06.012.

## References

1. Wieckowska A, Zein NN, Yerian LM, et al. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology 2006;44:27–33.

2. Feldstein AE, Gores GJ. Apoptosis in alcoholic and nonalcoholic steatohepatitis. Front Biosci 2005;10:3093–3099.
3. Bantel H, Lugering A, Heidemann J, et al. Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. Hepatology 2004; 40:1078–1087.
4. Bae CB, Kim SS, Ahn SJ, et al. Caspase-cleaved fragments of cytokeratin-18 as a marker of inflammatory activity in chronic hepatitis B virus infection. J Clin Virol 2013;58:641–646.
5. Galluzzi L, Lopez-Soto A, Kumar S, et al. Caspases connect cell-death signaling to organismal homeostasis. Immunity 2016; 44:221–231.
6. Afonina IS, Muller C, Martin SJ, et al. Proteolytic processing of interleukin-1 family cytokines: variations on a common theme. Immunity 2015;42:991–1004.
7. Lemoinne S, Thabut D, Housset C, et al. The emerging roles of microvesicles in liver diseases. Nat Rev Gastroenterol Hepatol 2014;11:350–361.
8. Wang R, Ding Q, Yaqoob U, et al. Exosome adherence and internalization by hepatic stellate cells triggers sphingosine 1-phosphate-dependent migration. J Biol Chem 2015; 290:30684–30696.
9. Barreyro FJ, Holod S, Finocchietto PV, et al. The pan-caspase inhibitor Emricasan (IDN-6556) decreases liver injury and fibrosis in a murine model of non-alcoholic steatohepatitis. Liver Int 2015;35:953–966.
10. Gracia-Sancho J, Contreras PC, Vila S, et al. The pan caspase inhibitor Emricasan improves the hepatic microcirculatory dysfunction of $CCL_4$-cirrhotic rats leading to portal hypertension amelioration and cirrhosis regression. Hepatology 2016; 64(Suppl 1):1043A.
11. Pockros PJ, Jeffers L, Afdhal N, et al. Final results of a double-blind, placebo-controlled trial of the antifibrotic efficacy of interferon-gamma1b in chronic hepatitis C patients with advanced fibrosis or cirrhosis. Hepatology 2007;45:569–578.
12. Shiffman ML, Pockros P, McHutchison JG, et al. Clinical trial: the efficacy and safety of oral PF-03491390, a pancaspase inhibitor: a randomized placebo-controlled study in patients with chronic hepatitis C. Aliment Pharmacol Ther 2010;31:969–978.
13. Shiffman M, Freilich B, Vuppalanchi R, et al. A placebo-controlled, multicenter, double-blind, randomised trial of Emricasan in subjects with non-alcoholic fatty liver disease (NAFLD) and raised transaminases. J Hepatol 2015;62:S263.
14. Caulin C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. J Cell Biol 1997;138:1379–1394.
15. Belli LS, Berenguer M, Cortesi PA, et al. Delisting of liver transplant candidates with chronic hepatitic C after viral eradication: A European study. J Hepatol 2016;65:524–531.
16. Festi D, Capodicasa S, Sandri L, et al. Measurement of hepatic functional mass by means of 13C-methacetin and 13C-phenylalanine breath tests in chronic liver disease: comparison with Child-Pugh score and serum bile acid levels. World J Gastroenterol 2005;11:142–148.
17. Sheng QS, Lang R, He Q, et al. Indocyanine green clearance test and model for end-stage liver disease score of patients with liver cirrhosis. Hepatobiliary Pancreat Dis Int 2009; 8:46–49.
18. Garcia-Tsao G, Fuchs M, Shiffman M, et al. Emricasan (IDN-6556) administered orally for 28 days lowers portal pressure in patients with compensated cirrhosis and severe portal hypertension. Hepatology 2015;62(Suppl 6):1382A.
19. Curry MP. Direct acting antivirals for decompensated cirrhosis. Efficacy and safety are now established. J Hepatol 2016; 64:1206–1207.
20. Manne V, Akhtar E, Saab S. Cirrhosis regression in patients with viral hepatitis B and C: a systematic review. J Clin Gastroenterol 2014;48:76–84.
21. Lens S, Rincon D, Garcia-Retortillo M, et al. Association between severe portal hypertension and risk of liver decompensation in patients with hepatitis C, regardless of response to antiviral therapy. Clin Gastroenterol Hepatol 2015; 13:1846–1853.
22. Mandorfer M. Sustained virologic response to interferon-free therapies ameliorates HCV induced portal hypertension. J Hepatol 2016;65:692–699.



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**Conflicts of interest**
These authors disclose the following: Catherine T. Frenette is a consultant to Bayer, Gilead, Intercept, BTG, Wako, and Conatus; is a member of the speaker's bureau for Bayer, Bristol-Myers Squibb, Gilead, Merck, Salix, Intercept, and Abbvie; receives research support from Bayer, Genfit, and Conatus; and is an advisory board member for Eisai. Mitchell L. Shiffman has received research grants from Conatus, Abbvie, Bristol-Myers Squibb, CymaBay, Exalenz, Galectin, Genfit, Gilead, Intercept, Immuron, Merck, NGMBio, Novartis, and Shire; has consulted for Abbvie, Bristol-Myers Squibb, Gilead, Intercept, Merck, OptumRx, and Salix; and is on the speaker's panel for Abbvie, Bayer, Bristol-Myers Squibb, Daiichi Sankyo, Gilead, Intercept, and Merck. R. Todd Frederick receives research support from Conatus, Gilead, Ocera, Mallinckrodt, and Sequana; and is a consultant for Gilead, Ocera, Abbvie, and Mallinckrodt. Raymond A. Rubin receives research support from Gilead, Intercept, Abbvie, and Mallinckrodt; and has received honoraria from Gilead. Jason T. Cheng receives research support from Roche, Salix, Conatus, Cepheid, and Prometheus. Matt Cave receives research support from Conatus, Intercept, diaPharma, and Abbvie; is a member of the speaker's bureau for Abbvie, Gilead, Merck, and Intercept; and is a consultant for Abbvie and Gilead. Nikolaos Pyrsopoulos receives research support from Conatus, Genfit, Gilead, Hologic, Prometheus, Intercept, Zydus, Vital Therapies, Abbvie, and Merck; and is an advisory board member for Gilead, Vital Therapies, Abbvie, and Merck. James M. Robinson is an employee of Conatus Pharmaceuticals, Inc. Mason Yamashita is an employee of Conatus Pharmaceuticals, Inc. Alfred P. Spada is an employee of Conatus Pharmaceuticals, Inc. Jean L. Chan is an employee of Conatus Pharmaceuticals, Inc. David T. Hagerty is an employee of Conatus Pharmaceuticals, Inc. The remaining authors disclose no conflicts.


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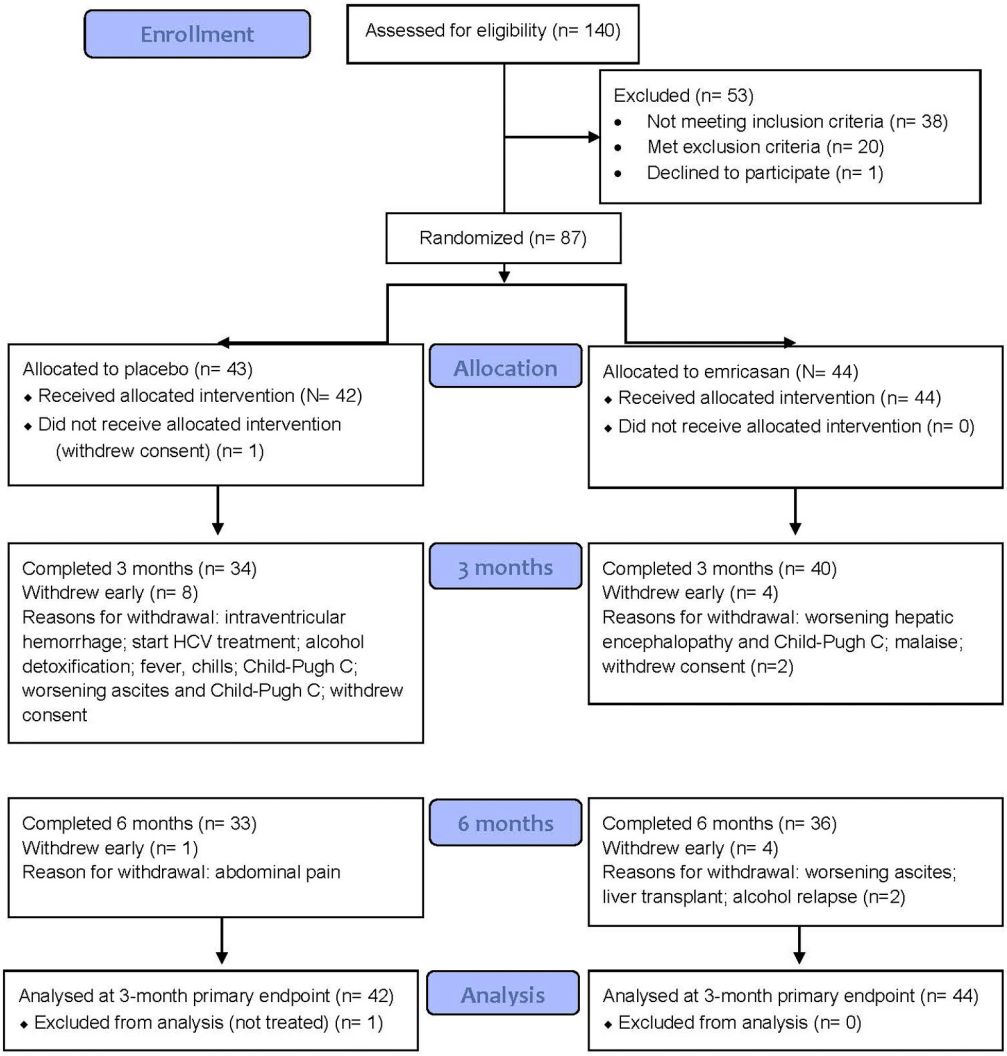
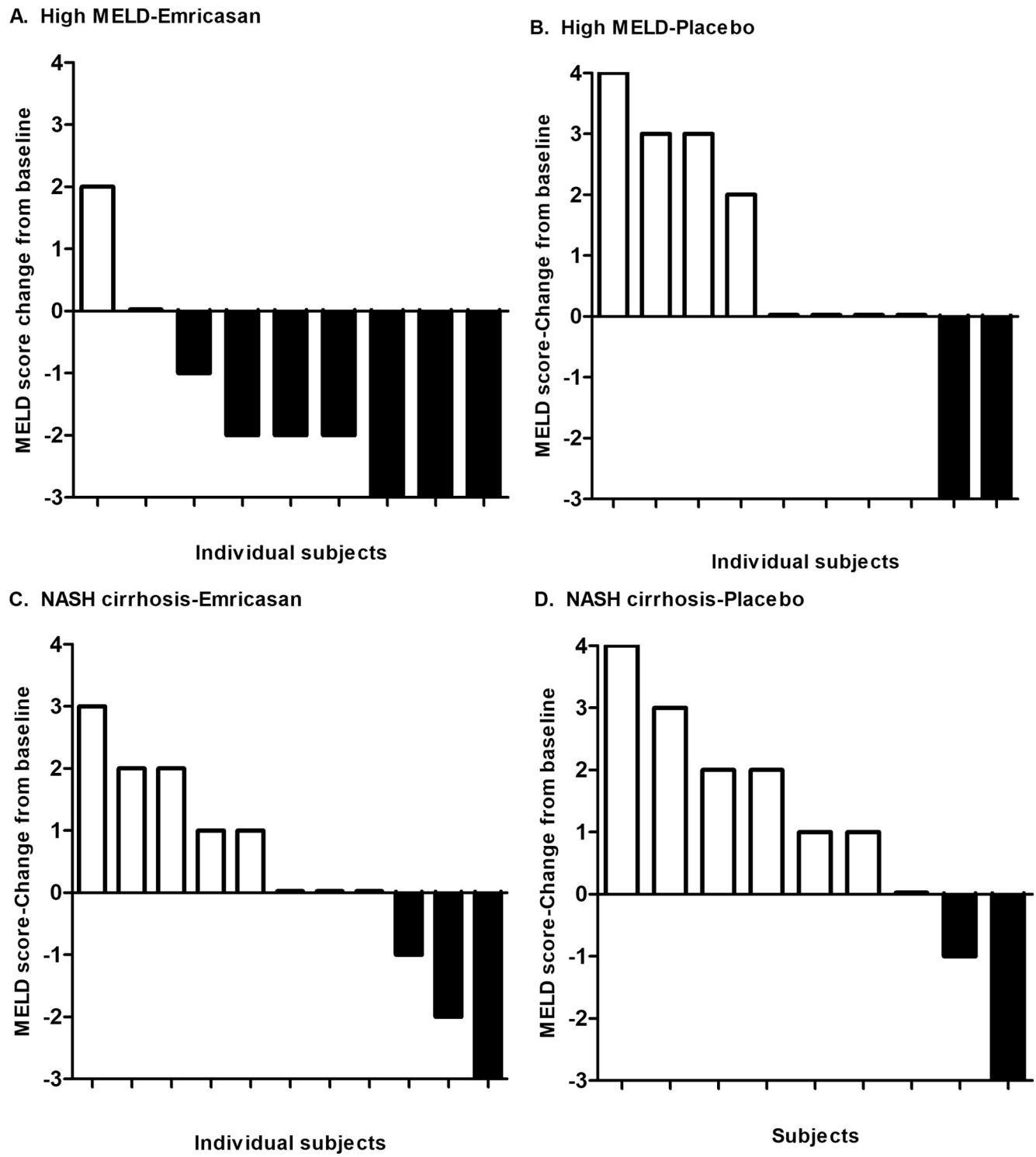
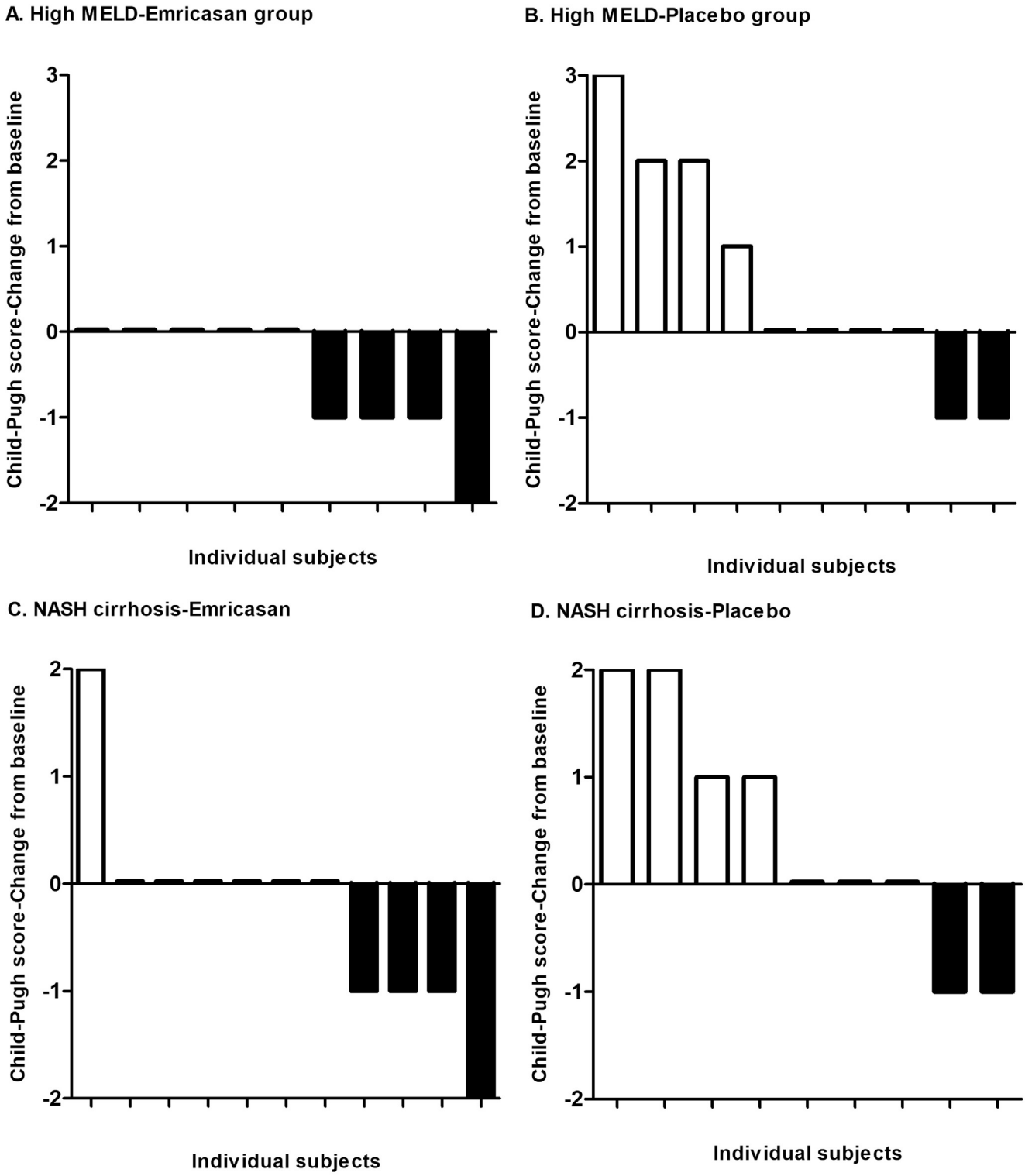



**Supplementary Figure 1.** CONSORT flowchart showing the disposition and reasons for discontinuation of the subjects.




**Supplementary Figure 2.** Individual subject changes in MELD score for the (*A*) high-MELD Emricasan subgroup, (*B*) high-MELD placebo subgroup, (*C*) NASH cirrhosis-Emricasan subgroup, and (*D*) NASH cirrhosis-placebo subgroup. Increases in MELD score at the 3-month visit are shown in *white bars* and decreases in MELD score are shown in *black bars*. Seven of 9 high-MELD subjects treated with Emricasan improved MELD scores, 1 of 9 had no change, and 1 of 9 increased at 3 months. Most placebo-treated subjects had stable (4/10) or increased MELD scores (4/10), with only 2 of 10 having improved MELD scores. A total of 67% (6/9) of high-MELD Emricasan patients had $\geq$2-point decrease in MELD at Month 3 versus 20% (2/10) of the placebo group. In the NASH cirrhosis subgroup, individual subject responses were consistent with less progression in the Emricasan-treated subjects (*C* and *D*) in whom 3 of 11 improved MELD scores, 3 of 11 remained stable, and 5 of 11 worsened compared with the placebo subjects in whom 2 of 9 improved, 1 of 9 remained stable, and 6 of 9 worsened.




**Supplementary Figure 3.** Individual subject changes in Child-Pugh score in the High-MELD and NASH subgroups at Month 3. (*A*) High-MELD subgroup treated with Emricasan. (*B*) High-MELD subgroup treated with placebo. (*C*) NASH subgroup treated with Emricasan. (*D*) NASH subgroup treated with placebo. In the high-MELD subjects treated with Emricasan for 3 months, 4 of 9 subjects improved Child-Pugh scores, 5 of 9 had no change, and none increased. Placebo-treated subjects demonstrated stability or worsening of disease, with 4 of 10 having a stable Child-Pugh score, 4 of 10 showing an increased Child-Pugh score, and 2 of 10 having an improved Child-Pugh score. A total of 44% of the high-MELD Emricasan group had a decrease in the Child-Pugh score versus 20% of placebo-treated patients. The NASH cirrhosis subgroup also had a Child-Pugh score pattern of response that favored Emricasan. Emricasan treatment resulted in 4 of 11 subjects improving Child-Pugh scores, 6 of 11 having stable scores, and 1 of 11 having a worse score. Placebo treatment resulted in 2 of 9 improving the Child-Pugh score, 3 of 9 having a stable score, and 4 of 9 worsening.

**Supplementary Table 1.** Baseline Liver Function in Key Subgroups

| | MELD ≤14 | | MELD ≥15 | | NASH patients | | Alcohol/other | |
|---|---|---|---|---|---|---|---|---|
| | Placebo (n = 32) Mean ± SD | EMR (n = 35) Mean ± SD | Placebo (n = 10) Mean ± SD | EMR (n = 9) Mean ± SD | Placebo (n = 9) Mean ± SD | EMR (n = 11) Mean ± SD | Placebo (n = 17) Mean ± SD | EMR (n = 24) Mean ± SD |
| INR | 1.27 ± 0.16 | 1.27 ± 0.12 | 1.45 ± 0.17 | 1.54 ± 0.19 | 1.27 ± 0.23 | 1.25 ± 0.14 | 1.33 ± 0.19 | 1.36 ± 0.16 |
| Total bilirubin, *mg/dL* | 2.05 ± 0.78 | 2.01 ± 0.84 | 4.30 ± 1.94 | 3.17 ± 1.59 | 2.26 ± 1.10 | 2.68 ± 1.53 | 2.73 ± 1.53 | 2.21 ± 1.03 |
| Albumin, *g/dL* | 3.57 ± 0.53 | 3.47 ± 0.44 | 3.19 ± 0.46 | 3.41 ± 0.24 | 3.60 ± 0.46 | 3.39 ± 0.39 | 3.44 ± 0.45 | 3.46 ± 0.43 |

EMR, Emricasan; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis; SD, standard deviation.

**Supplementary Table 2.** Change From Baseline in Efficacy Measures in the High-MELD and NASH Cirrhosis Subgroups Who Were Treated With Emricasan for 6 Months

| | MELD ≥15 subgroup (n = 9) | | | NASH patients (n = 11) | | |
|---|---|---|---|---|---|---|
| | Baseline | M3 Change | M6 Change | Baseline | M3 Change | M6 Change |
| MELD | 16.0 | –1.6[a] | –2.8[a] | 13.0 | 0.3 | 0.1 |
| Child-Pugh | 7.8 | –0.6 | –0.7 | 7.3 | –0.3 | –0.1 |
| Total bilirubin | 3.17 | –0.55 | –0.65 | 2.68 | 0.09 | –0.03 |
| INR | 1.54 | –0.14[a] | –0.21[a] | 1.25 | 0.01 | 0.00 |
| Albumin | 3.41 | 0.07 | 0.10 | 3.39 | 0.05 | 0.03 |

INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; NASH, nonalcoholic steatohepatitis.
[a]Statistically significant ($P < .05$) paired comparison with baseline unadjusted for multiplicity.