A Placebo-Controlled, Multicenter, Double-Blind, Phase 2 Randomized Trial of the Pan-Caspase Inhibitor Emricasan in Patients with Acutely Decompensated Cirrhosis

Authors: Gautam Mehta[1], Sam Rousell[2], Gary Burgess[3], Mark Morris[2], Gavin Wright[4], Stuart McPherson[5], Catherine Frenette[6], Matthew Cave[7], David T. Hagerty[8], Alfred Spada[8], Rajiv Jalan[1]

Affiliations:

1. Institute for Liver and Digestive Health, Royal Free Campus, UCL, London, UK.
2. Clinical Outcomes Solutions, Folkestone, UK (formerly affiliated with Conatus Pharmaceuticals Inc.).
3. Vectura Group PLC, Chippenham, UK (formerly affiliated with Conatus Pharmaceuticals Inc.).
4. Department of Gastroenterology, Basildon and Thurrock University Hospitals NHS Foundation Trust, Basildon, UK.
5. Liver Unit, The Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK.
6. Department of Organ Transplantation, Scripps Green Hospital, San Diego, USA.
7. Department of Medicine, University of Louisville School of Medicine, Louisville, USA.
8. Conatus Pharmaceuticals Inc., San Diego, USA.

Keywords: Cirrhosis; Apoptosis; Cell death; Liver failure.

Word count: 3284 (excluding title page, abstract, acknowledgements, references, tables and figures).

Number of figures: 3. **Number of tables**: 5.

Abstract

Background: Cirrhosis and acute-on-chronic liver failure (ACLF) are associated with systemic inflammation, and caspase-mediated hepatocyte cell death. Emricasan is a novel, pan-caspase inhibitor. Aims of this study were to assess the pharmacokinetics, pharmacodynamics, safety and clinical outcomes of Emricasan in acute decompensation (AD) of cirrhosis.

Methods: This was a phase 2, multicentre, double-blind, randomized trial. The primary objective was to evaluate the pharmacokinetics, pharmacodynamics and safety of Emricasan in patients with cirrhosis presenting with AD and organ failure. AD was defined as an acute decompensating event ≤6 weeks' duration. Patients were randomised proportionately to Emricasan 5mg bid, Emricasan 25mg bid, Emricasan 50mg bid or placebo. Treatment was continued to 28 days, or voluntary discontinuation.

Participants: Twenty-three subjects were randomized, of whom 21 were dosed (placebo n=4; 5mg n=5; 25mg n=7; 50mg n=5). Pharmacokinetic data showed 5mg dose was associated with low plasma levels (<50ng/ml), and 25mg and 50mg doses showed comparable pharmacokinetic profiles. Therefore, for analysis of secondary endpoints, placebo and 5mg groups were merged into a 'placebo/low-dose' group, and 25mg and 50mg groups were merged into a 'high-dose' group. Five deaths occurred amongst the 21 patients, all due to progression of liver disease (2 in placebo/low-dose, 3 in high-dose). No statistically significant changes from baseline MELD score or CLIF-C ACLF score were noted between placebo/low-dose and high-dose groups at day 7 (MELD -1 vs -1, CLIF-C ACLF 0.7 vs 0.8). An initial reduction in cleaved keratin M30 fragment was noted between placebo/low-dose and high-dose groups (Percent relative change: day 2: -11.6 vs -42.6, p=0.017, day 4: -3.5 vs -38.9 p=0.017) although this did not persist to day 7 (-3.1 vs -20.8, p=0.342).

Interpretation: This study demonstrates that Emricasan is safe and well tolerated in advanced liver disease. However, this study fails to provide proof-of-concept support for caspase inhibition as a treatment strategy for ACLF.

Trial registration: EudraCT 2012-004245-33

Funding: Conatus Pharmaceuticals Inc.

Introduction

The natural history of cirrhosis is characterised by progression to episodes of acute decompensation (AD) of liver function(1). Most patients with cirrhosis and AD are treated successfully in the majority of cases. However, about 30% of these patients develop hepatic and/or extra-hepatic organ failure that progresses in about 20% to multi-organ failure and death(2). When this occurs rapidly, within a period of weeks, the condition is referred to as acute-on-chronic liver failure (ACLF). The 170,000 cirrhosis deaths in Europe each year are largely due to ACLF and the condition costs $3Bn in the USA and £50K per survivor in the UK(3). There are as yet no specific therapies for ACLF.

The pathobiology of ACLF is characterized by hepatic and systemic inflammation, and progressive, unrelenting hepatocyte injury and death(4, 5). Approaches targeting systemic inflammation have been tried, such as anti-TNF therapies, although these have led to negative outcomes suggesting that alternative approaches are required(6).

Apoptosis is a highly regulated form of or programmed cell death. In response to injury or inflammation, hepatocytes can undergo apoptosis via an extrinsic pathway activated by death ligands, Fas, and tumour necrosis factor- related apoptosis-inducing ligand (TRAIL), or an intrinsic pathway activated by intracellular stress of membrane-bound organelles, such as lysosomes, endoplasmic reticulum and mitochondria(7-10). Both pathways of apoptosis converge on the caspases (cysteine aspartyl proteases), which play an essential role in the initiation, execution and regulation of apoptosis(11).

Previous work has demonstrated that apoptosis is a key pathway of cell death in patients with ACLF. Fragmented chromatin and caspase-dependent cleaved keratin 18 are both terminal end-products of the apoptotic pathway(12). Adebayo et al have shown that serum levels of cleaved keratin 18 (M30 fragment) are significantly elevated in patients with ACLF compared to patients with AD alone, and correlate with disease severity(13). Immunohistochemistry of liver tissue also demonstrates apoptotic M30-positive hepatocytes in patients with severe ACLF. Similarly, Cao et al have demonstrated elevated levels of fragmented chromatin in the ACLF compared with the AD group(14). These data

support the central role of hepatocyte apoptosis in the progression of ACLF, and provide the rationale for therapeutic targeting of apoptosis in ACLF.

Caspases provide a druggable target for the inhibition of apoptosis in liver disease. As such, caspase inhibitors have been shown to decrease liver injury in rodent models of acute liver failure, fatty liver disease, cholestatic liver injury and alcohol-induced liver injury(15-20).

Emricasan (IDN-6556) is a novel, orally active, pan-caspase protease inhibitor. Emricasan has been studied in eight phase 1 studies and eight phase 2 studies involving over 650 patients, providing initial safety data and supporting the dose range of 5-50mg used in phase 2 studies in patients with chronic liver disease(21, 22). The aims of this study were to assess the pharmacokinetics, pharmacodynamics, safety and clinical outcomes of Emricasan in patients with cirrhosis and a rapid deterioration of liver function associated with organ failure.

Patients and Methods

Study IDN-6556-02 (ClinicalTrials.gov NCT01937130) was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. Written informed consent was obtained from all patients before enrolment, in agreement with approved protocols from research ethics committees (North West-Haydock Research Ethics Committee, reference 13/NW/0464).

Study design: This was a phase 2, multicentre, double-blind, randomized trial was conducted in 10 sites in the United States and United Kingdom. The primary objective was to evaluate the pharmacokinetics, pharmacodynamics and safety of emricasan, orally administered for 28 days in patients with cirrhosis and a rapid deterioration of liver function associated with organ failure (figure 1).

Selection of doses: The relationship between emricasan dose and biomarker responses had been thoroughly characterized in subjects with active HCV hepatitis and normal hepatic function(21,22). However, the dose-biomarker response relationship had not been characterized in subjects with

impaired hepatic function. The initial studies in subjects with HCV hepatitis and normal function assessed the effect of emricasan upon a panel of 4 biomarkers (caspase 3/7, M30, ALT and AST) at oral doses ranging from 0.5 mg BID up to 200 mg BID. Doses as low as 0.5 mg BID dose were pharmacodynamically active, decreasing M30 and ALT by nearly 50%, but had less effect upon AST and caspase 3/7. Doses greater than 50 mg BID did not appear to have any greater reduction in the 4 biomarkers compared to the 50 mg BID dose. Thus, doses of 5, 25 and 50 mg BID were selected for this study in patients with severe hepatic impairment.

Sample size: The sample size was based around a simulation exercise of 1000 simulated outcomes, which were used to predict the dose required for a set target exposure. A sample size of 15 subjects per group (60 total) would have resulted in >80% of simulated outcomes recommending the correct dose to take forward to a follow-on ACLF efficacy study, if the exposure and pharmacodynamic effects in cirrhotic subjects from this study were similar to that observed in subjects with active HCV hepatitis and normal hepatic function previously studied (data modelled from Pockros et al.(21) and Shiffman et al.(22)).

Inclusion criteria: Eligible patients included those 18 years of age or older, with stable compensated or decompensated cirrhosis, presenting with an acute deterioration of liver function and associated organ failure. Cirrhosis was diagnosed by clinical, radiological and/or histological means. An acute deterioration of liver function was defined as an acute decompensating event or illness of ≤ 6 weeks' duration, on the background of cirrhosis (see definitions below).

Exclusion criteria: recent hospital admission (within 4 weeks) for a complication of cirrhosis, greater than two-organ failure, HIV infection, uncontrolled bacterial infection, pre-existing chronic kidney disease, autoimmune liver disease, active malignancy aside from hepatocellular carcinoma, need for mechanical ventilation, inability to obtain consent, and haemodynamic instability (including use of inotropes, aside from terlipressin for hepatorenal syndrome).

Definitions: Criteria for organ failure used in this study were:

Liver: bilirubin ≥ 12.0 mg/dL

Kidney: creatinine ≥ 2.0 mg/dL

Cerebral: hepatic encephalopathy West Haven grade III or IV

Coagulation: INR ≥ 1.7

In terms of organ failure, eligibility for this study was on the basis of one of the following: (i) renal failure (defined as creatinine ≥ 2.0 to ≤ 3.4 mg/dL); (ii) other single organ failure with a) renal impairment (defined as an increase in creatinine of > 0.3 mg/dL from an established baseline level) and/or b) hepatic encephalopathy grade I or II; (iii) two organ failures. As noted above, patients with respiratory failure requiring mechanical ventilation, and circulatory failure requiring inotropic support, were excluded. Additionally, MELD score and CLIFC-ACLF score were calculated as previously described(23, 24).

Treatment and Randomisation: The Sponsor, investigational staff and subjects were all blinded to the treatment group assignment. Patients were randomised proportionately into four groups: emricasan 5mg bid, emricasan 25mg bid, emricasan 50mg bid and matching placebo bid through the study's Interactive Web Response System. The randomization schedule was generated using a validated randomization program and verified for accuracy using quality control procedures. Treatment was continued for 28 days in all four groups. Randomisation was stratified on the basis of a positive alcohol history, and also prior steroid use. A positive alcohol history was defined as ongoing alcohol use up until the time of entry into the study. Treatment was continued for 28 days unless the patient voluntarily discontinued treatment.

Haematology, Biochemistry and Biomarkers: Routine haematology and biochemistry parameters were monitored, as well as blood tests for pharmacokinetic and biomarker evaluations. Pharmacokinetic studies were performed at day 1 and day 4 following initiation, and serum biomarkers of apoptosis (cleaved keratin 18 (M30 Apoptosense®; Peviva, West Chester, OH, USA and full-length keratin 18 (M65®; Peviva, West Chester OH, USA) were also collected at days 1, 2, 4 and 7. Follow-up visit was scheduled at 4 weeks following the end of treatment, and telephone contact at 12 and 24 weeks.

Statistics: Statistical analyses of the change from Baseline in M30, M65, M30/M65 ratio, MELD and CLIF-C ACLF score were conducted using an analysis of covariance (ANCOVA) adjusted for baseline. Given the distribution of data, M30 and M65 were log-transformed prior to analysis and results were back-transformed to provide a relative percent change from baseline. No adjustments for multiple comparisons were made. Associations between changes in cell death biomarkers and efficacy endpoints were assessed using Pearson's correlation coefficient.

The decision to pool the placebo and 5 mg emricasan for purposes of efficacy analyses was made after the premature termination of the study and unblinding. The goal of pooling those groups was to increase the number of subjects for summary purposes. The justification for pooling was that the 5 mg emricasan dose had no detectable pharmacodynamic effect on the biomarkers that were measured (see Results).

Results

Patients: Between 22 January 2014 and 17 September 2014, 23 subjects were randomized into this study, of whom 21 were dosed (placebo n=4; emricasan 5 mg bid n=5; emricasan 25 mg bid n=7; emricasan 50 mg bid n=5) at 10 sites. The study was stopped once adequate pharmacokinetic samples had been obtained since the study was slow to recruit. The CONSORT diagram is presented in figure 2. Baseline characteristics of these patients by individual randomized treatment group is presented in Supplementary Table 1.

Emricasan pharmacokinetics: A summary of the key pharmacokinetic data is presented in Table 1. The geometric means of the emricasan $AUC_{0\text{-}8}$, C_{max}, and $AUC_{0\text{-}last}$ increased in an approximately dose-proportional manner between the 5 mg and 50 mg doses on day 1 and day 4. No plasma accumulation was apparent in any of the treatment arms on day 4 compared to day 1. Generally, lower between-subject variability was observed in all PK parameters in the lower dose treatment arms than in the 50 mg arm, with coefficients of variation (CVs) ranging from 28% to 48% in the IDN-6556 5 mg and 25 mg groups, and from 99% to 258% in the IDN-6556 50 mg group.

Biomarker responses: Changes in the keratin fragment M30 (mean+/-SD) and caspase 3/7 (mean+/-

SD) are shown in Figure 3. In this study, changes in caspase 3/7 activity (Figure 3b), rather than M30 levels (Figure 3a), were a more sensitive indicator of emricasan pharmacodynamic activity. The placebo and 5 mg groups had no significant effect upon M30 levels on any study day, although there was a trend towards reduction in M30 with the 25mg and 50mg doses between days 2 and 4. Caspase 3/7 activity also showed a non-significant trend in reduction with the 25mg and 50mg doses by the day 2 visit and throughout the initial 7 days of the study, while the placebo and 5mg dose had no effect (Figure 3b). Since the 5 mg emricasan group had no detectable pharmacodynamic effect, the placebo and 5 mg groups were merged into a 'placebo/low-dose' group, and the 25mg and 50mg groups were merged into a 'high-dose' group. A statistically significant decrease in log-transformed M30 at day 2 and day 4 was noted between the placebo/low-dose and high-dose groups. These results were back-transformed to provide percent relative change from Baseline (Day 2: -11.6 vs -42.6, p=0.017, Day 4: -3.5 vs -38.9 p=0.017). The direction of treatment effect remained in favour of the high-dose group at Day 7; however this was not statistically significant (-3.17 vs -20.8, p=0.342). No statistically significant differences between the high-dose and placebo/low dose were seen for the change from baseline in log-transformed M65 at any time point up to day 7. No consistent correlations were seen across timepoints in associations between changes in cell death biomarkers, caspase 3/7 activity and efficacy endpoints.

Patient Outcomes: As evident in the CONSORT diagram (Figure 2), of the 21 subjects dosed, 7 completed 28 days of administration. Of the remaining 14 patients, 3 died as a consequence of their liver disease and 6 were lost to follow-up. The patient baseline characteristics, summarised by the placebo/low-dose and high-dose groups, are presented in Tables 2 and 3. No significant differences in demographics were noted between the two groups. All patients, bar one, had alcohol-related liver disease (ARLD). The majority of patients (81%) had ACLF present at baseline. Alcohol was identified as a precipitant for the rapid deterioration in liver function in all cases aside from two (one in the low dose group and one in the high dose group). Bacterial infection was identified as a further precipitant in 29% of cases. No significant differences in severity of liver disease at baseline were noted, as assessed by MELD score or CLIF-C ACLF score (where ACLF was present).

Patient disposition and mortality are presented in Figure 2. A total of 5 deaths occurred amongst the 21 patients dosed during the study period, although 2 deaths were after completion of the 28-day treatment

period. All deaths were due to progression of liver disease – 2 in the placebo/low-dose group and 3 in the high-dose group. For the remaining secondary efficacy endpoints, data was analysed up to the day 7 time point, in view of the attrition of patient numbers at day 28.

Changes in MELD score and CLIF-C ACLF score are presented in Table 4. No statistically significant differences in these parameters were noted between the placebo/low-dose and high-dose groups at day 7 (MELD -1 vs -1, CLIF-C ACLF 0.7 vs 0.8). Similarly, no specific changes in liver, kidney, brain or coagulation function were noted at day 7, by CLIF-C ACLF score parameter.

Safety: As demonstrated in Figure 2, there were a total of 10 deaths across all treatment groups. There were 5 on-study deaths, 2 of which were during the one-month follow-up period following the full course of treatment, and a further 5 were registered via serious adverse event (SAE) reporting following discontinuation or study completion. All of these deaths were attributed to progressive liver disease.

Adverse events (AEs) were reported by 17 of the 21 patients, of whom 13 patients reported SAEs. None of the SAEs was determined to be treatment-related. The only AEs deemed to be treatment-related were nausea and vomiting which were reported by one placebo subject. The AEs and SAEs are presented in Table 5.

Discussion

Excessive cell death has been demonstrated to play a fundamental role in the progression of ACLF. Hepatocyte apoptosis has been shown to lead to Kupffer cell and hepatic stellate cell activation, and in pre-clinical models, apoptosis has been shown to promote inflammation and fibrogenesis(25-27). Caspase inhibitors have been shown to protect from liver injury in a rodent model of acute liver failure, and to attenuate inflammation and fibrosis in rodent models of cholestatic, alcohol and fatty liver disease(15, 16, 19). This study represents the first use of the caspase inhibitor emricasan in patients with decompensated cirrhosis.

The primary objective of this study was to evaluate the pharmacokinetics, pharmacodynamics and safety of emricasan in this population. All emricasan doses led to >10-fold higher levels of exposure

than observed in previous studies of either healthy volunteers or non-cirrhotic subjects with active HCV hepatitis, at the same doses, likely as consequence of porto-systemic shunting and associated bypass of first-pass hepatic uptake. Despite this, none of the reported adverse events were deemed to be treatment related, although, as discussed above, there was no attrition of subjects at later time points. The patient deaths and adverse events reported generally reflected the very severe liver disease in the study population.

From a pharmacodynamic perspective, the 5 mg dose had negligible biologic effects upon the biomarkers measured (M30, M65 and caspase 3/7) in this study, and as a consequence, the placebo and 5mg dose groups were merged for the analysis of secondary efficacy endpoints. However, it is important to note that in non-cirrhotic subjects with active hepatitis C infection and presumably normal hepatic function, the 5 mg dose resulted in C_{max} concentrations that were over 10-fold lower than observed in this study and yet decreased M30 levels ~50% (Study A8491010, unpublished data). The 5 mg dose has also been shown to significantly decrease ALT levels[21,22]. The failure to see significant pharmacodynamic effects of the 5 mg dose in this study was not related to inadequate circulating drug concentrations but likely due to porto-systemic shunting and severe hepatocellular functional impairment. These factors, either separately or collectively, could result in low, sub-therapeutic hepatocyte levels of emricasan particularly since emricasan is a high first-pass extraction drug.

Statistically significant reductions in the keratin fragments M30 were noted at day 2 and day 4 following therapy when the 25 mg and 50 mg doses were analysed together, compared with the combined placebo and 5 mg group. However, this reduction was not sustained to 7 days or beyond. Potential contributing reasons for the failure to achieve statistical significance include insufficient power of the study due to the low recruitment of subjects, high attrition rates, poor compliance, and consequent shortened duration of follow-up for analysis.

Despite the reduction in apoptosis markers, the results of this study failed to provide proof-of-concept support for the hypothesis of targeting apoptosis to prevent progression of ACLF. Although both the 25 and 50 mg doses of emricasan decreased caspase 3/7 activity, proving that although emricasan was able to inhibit its biological target, only the 50 mg dose clearly decreased M30 levels, a marker of apoptosis, and there were no significant changes in MELD score, CLIF-C ACLF score or CLIF-C organ function score at 7 days. There are several possible reasons for this, including the above-noted comments of an

underpowered study population, shortened duration of analysis and sub-therapeutic hepatic drug levels due to porto-systemic shunting. However, a further possibility is the complexity of modes of cell death involved in the progression of liver injury in ACLF. Aside from apoptosis, other forms of hepatocyte cell death have been described in ACLF, including autophagy, necroptosis and pyroptosis(28). The relative contribution of these, or predominant mode of cell death with different mechanisms of liver injury, remains to be established. Moreover, it remains unclear as to whether apoptosis is causal in the progression of ACLF or is an adaptive response to liver injury. In an open-label study, Frenette et al have recently reported that emricasan significantly decreased cleaved keratin levels and serum ALT levels in a population of patients with cirrhosis after six months of treatment at the 25 mg bid dose(29). Moreover, the subgroup of patients with a baseline MELD scores $\geq$15 had a significant improvement in MELD score, bilirubin and INR over the 3-month study period. Therefore, further, studies are warranted to address the efficacy of emricasan in advanced cirrhosis and ACLF. Amongst patients with hepatitis B related ACLF, levels of the M30 apoptosis marker were higher in survivors than non-survivors, suggesting that apoptosis may also be an adaptive response to liver injury, possibly involved in liver regeneration(30).

There are several weaknesses with this study. Only 23 patients were recruited from the planned study population of 60 subjects on the basis of the difficult enrolment. Additionally, the population was predominantly alcohol-related liver disease, thus limiting the applicability of the findings of this study to other aetiologies of cirrhosis. The follow-up data from this cohort was also limited, and less than half of the subjects completed 28 days of follow-up, hence limited conclusions can be drawn regarding longer-term secondary endpoints addressing the efficacy of emricasan in ACLF. Despite these limitations, the study conclusions are likely to be valid.

In conclusion, this study demonstrates that emricasan is safe and well-tolerated in advanced liver disease, although hepatic drug levels may be sub-therapeutic due to porto-systemic shunting. Although reductions in caspase 3/7 activity and M30 component of the cytokeratin fragments were detected, this was not associated with any clinical benefit in this small study. The efficacy of emricasan in advanced cirrhosis and ACLF was not confirmed. Further mechanistic work is required to delineate the role of other modes of cell death, such as necroptosis and pyroptosis, in the progression of ACLF.

Acknowledgements:

We would like to acknowledge the contribution of other co-investigators: Dr Omar Massoud, Birmingham, AL; Dr John Greer, Manchester; Dr Peter Isaacs, Blackpool; Dr Andrew Fowell, Portsmouth; Dr Peter Collins, Bristol; Dr Stephen Ryder, Nottingham.

Declaration of Potential Conflicts of Interest:

The study was sponsored and funded by Conatus Pharmaceuticals. GM received fees for preparing the manuscript from Conatus. RJ has received speaker fees or consultancy from Ocera, Yaqrit and Sequana. SM has received speaker fees or consultancy from Abbvie, BMS, Gilead, MSD, and Novartis. CF has received consultancy from Conatus. MC has received speaker fees from Gilead, Merck, Intercept and Abbvie. AS and DH are current employees of Conatus. GB and MM are previous employees of Conatus and own stock in the company. SR was previously a paid consultant of Conatus. GW has no disclosures.

References:

1. Jalan R, Williams R. Acute-on-chronic liver failure: pathophysiological basis of therapeutic options. *Blood Purification* 2002; 20(3): 252-61.
2. Jalan R, Gines P, Olson JC, et al. Acute-on chronic liver failure. *Journal of Hepatology* 2012; 57(6): 1336-48.
3. Shawcross DL, Austin MJ, Abeles RD, et al. The impact of organ dysfunction in cirrhosis: survival at a cost? *Journal of Hepatology* 2012; 56(5): 1054-62.
4. Moreau R, Jalan R, Gines P, et al. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; 144(7): 1426-37, 37 e1-9.
5. Arroyo V, Moreau R, Jalan R, Gines P, Study E-CCC. Acute-on-chronic liver failure: A new syndrome that will re-classify cirrhosis. *Journal of Hepatology* 2015; 62(1 Suppl): S131-43.
6. Naveau S, Chollet-Martin S, Dharancy S, et al. A double-blind randomized controlled trial of infliximab associated with prednisolone in acute alcoholic hepatitis. *Hepatology* 2004; 39(5): 1390-7.
7. Feldstein AE, Canbay A, Angulo P, et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. *Gastroenterology* 2003; 125(2): 437-43.
8. Feldstein AE, Canbay A, Guicciardi ME, Higuchi H, Bronk SF, Gores GJ. Diet associated hepatic steatosis sensitizes to Fas mediated liver injury in mice. *Journal of Hepatology* 2003; 39(6): 978-83.
9. Volkmann X, Fischer U, Bahr MJ, et al. Increased hepatotoxicity of tumor necrosis factor-related apoptosis-inducing ligand in diseased human liver. *Hepatology* 2007; 46(5): 1498-508.
10. Wang D, Wei Y, Pagliassotti MJ. Saturated fatty acids promote endoplasmic reticulum stress and liver injury in rats with hepatic steatosis. *Endocrinology* 2006; 147(2): 943-51.
11. Li J, Yuan J. Caspases in apoptosis and beyond. *Oncogene* 2008; 27(48): 6194-206.
12. Caulin C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. *The Journal of Cell Biology* 1997; 138(6): 1379-94.
13. Adebayo D, Morabito V, Andreola F, et al. Mechanism of cell death in acute-on-chronic liver failure: a clinico-pathologic-biomarker study. *Liver International* 2015; 35(12): 2564-74.
14. Cao Z, Wang Y, Chen L, et al. Association between the extent of hepatocyte death and types of precipitating event in acute on chronic liver failure. *Journal of Hepatology* 2016; 64(2).

15. Canbay A, Feldstein A, Baskin-Bey E, Bronk SF, Gores GJ. The caspase inhibitor IDN-6556 attenuates hepatic injury and fibrosis in the bile duct ligated mouse. *The Journal of Pharmacology and Experimental Therapeutics* 2004; 308(3): 1191-6.
16. Ueno Y, Ohmi T, Yamamoto M, et al. Orally-administered caspase inhibitor PF-03491390 is retained in the liver for prolonged periods with low systemic exposure, exerting a hepatoprotective effect against alpha-fas-induced liver injury in a mouse model. *Journal of Pharmacological Sciences* 2007; 105(2): 201-5.
17. Deaciuc II, D'Souza NB, Fortunato F, Hill DB, Sarphie TG, McClain CJ. Alcohol-induced sinusoidal endothelial cell dysfunction in the mouse is associated with exacerbated liver apoptosis and can be reversed by caspase inhibition. *Hepatology Research* 2001; 19(1): 85-97.
18. Witek RP, Stone WC, Karaca FG, et al. Pan-caspase inhibitor VX-166 reduces fibrosis in an animal model of nonalcoholic steatohepatitis. *Hepatology* 2009; 50(5): 1421-30.
19. Anstee QM, Concas D, Kudo H, et al. Impact of pan-caspase inhibition in animal models of established steatosis and non-alcoholic steatohepatitis. *Journal of Hepatology* 2010; 53(3): 542-50.
20. Barreyro FJ, Holod S, Finocchietto PV, Camino AM, Aquino JB, Avagnina A, et al. The pan-caspase inhibitor Emricasan (IDN-6556) decreases liver injury and fibrosis in a murine model of non-alcoholic steatohepatitis. *Liver International*. 2015;35(3):953-66.
21. Pockros PJ, Schiff ER, Shiffman ML, McHutchison JG, Gish RG, Afdhal NH, et al. Oral IDN-6556, an antiapoptotic caspase inhibitor, may lower aminotransferase activity in patients with chronic hepatitis C. *Hepatology.* 2007;46(2):324-9.
22. Shiffman ML, Pockros P, McHutchison JG, Schiff ER, Morris M, Burgess G. Clinical trial: the efficacy and safety of oral PF-03491390, a pancaspase inhibitor - a randomized placebo-controlled study in patients with chronic hepatitis C. *Alimentary Pharmacology and Therapeutics.* 2010;31(9):969-78.
23. Kamath PS, Wiesner RH, Malinchoc M, Kremers W, Therneau TM, Kosberg CL, et al. A model to predict survival in patients with end-stage liver disease. *Hepatology.* 2001;33(2):464-70.
24. Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Gines P, et al. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *Journal of Hepatology.* 2014;61(5):1038-47.

25. Breitkopf K, Godoy P, Ciuclan L, Singer MV, Dooley S. TGF-beta/Smad signaling in the injured liver. *Zeitschrift Fur Gastroenterologie.* 2006;44(1):57-66.
26. Lee UE, Friedman SL. Mechanisms of hepatic fibrogenesis. *Best Practice & Research Clinical Gastroenterology*. 2011;25(2):195-206.
27. Liu C, Tao Q, Sun M, Wu JZ, Yang W, Jian P, et al. Kupffer cells are associated with apoptosis, inflammation and fibrotic effects in hepatic fibrosis in rats. *Labarotory Investigation.* 2010;90(12):1805-16.
28. Eguchi A, Wree A, Feldstein AE. Biomarkers of liver cell death. *Journal of Hepatology*. 2014;60(5):1063-74.
29. Frenette C. Emricasan (IDN-6556) orally for three months in patients with cirrhosis and MELD scores 11-18 improves clinical parameters of cirrhosis in patients with baseline MELD score >15. *Journal of Hepatology.* 2016;64(2).
30. Zheng SJ, Liu S, Liu M, McCrae MA, Li JF, Han YP, et al. Prognostic value of M30/M65 for outcome of hepatitis B virus-related acute-on-chronic liver failure. *World Journal of Gastroenterology*. 2014;20(9):2403-11.

Table 1. Pharmacokinetic parameters

Visit	Parameter	Emricasan 5 mg		Emricasan 25 mg		Emricasan 50 mg	
		N	Geo. Mean (CV%)	N	Geo. Mean (CV%)	N	Geo. Mean (CV%)
Day 1	AUC_{0-8} (ng h/mL)	5	275 (44)	5	1164 (45)	5	2441 (97)
	C_{max} (ng/mL)	5	84.4 (44)	7	330 (48)	5	696 (117)
	t_{max} (h)	5	2.71 (65)	7	2.98 (89)	5	2.65 (119)
	$t_{1/2}$ (h)	3	2.96 (89)	5	2.11 (33)	2	-
Day 4	AUC_{0-8} (ng h/mL)	4	224 (37)	5	1908 (33)	4	2362 (315)
	C_{max} (ng/mL)	5	40.3 (32)	5	587 (34)	4	686 (194)
	t_{max} (h)	4	2.57 (146)	5	2.17 (85)	4	1.72 (1.08)
	$t_{1/2}$ (h)	1	-	3	1.95 (7)	2	2.99 (150)

Table 2: Baseline Characteristics of Organ Failures (according to CLIF-SOFA score)

Organ	Low Dose (Placebo, 5mg) N=9		High Dose (25, 50mg) N=11	
	n	%	n	%
Liver (≥ 12.0 mg/dL)	7	77.8%	9	81.8%
Kidney (≥2.0 mg/dL or renal replacement)	0	0%	1	9.1%
Brain (HE grade III or IV)	0	0%	0	0%
Coagulation (INR >2.5 or platelet count <20x10^9/L)	5	83.3%	3	27.3%
Circulation (vasopressor use)	1	11.1%	0	0%
Respiratory (PaO/FiO_2≤200 or SpO_2/FiO_2≤214)	0	0%	0	0%

Table 3: Changes from baseline in biomarkers M30, M65 and M30/65, and in MELD and CLIF-C ACLF score

	Placebo / 5mg			25mg / 50mg			p-value
	n	**Median**	**Range**	**n**	**Median**	**Range**	
cCK18 (M30) % change							
Day 2	7	-7.4	-40.0 / 18.3	11	-48.8	-70.7 / -4.4	0.017*
Day 4	7	0	-43.5 /41.4	11	-41.9	-72.9 / -5.6	0.017*
Day 7	8	-1.2	-43.6 / 75.8	11	-17.1	-66.3 / 97.4	0.342
flCK18 (M65) % change							
Day 2	7	-9.7	-41.5 / 9.9	11	-31.0	-69.4 / 15.6	0.166
Day 4	7	-4.7	-41.6 / 25.6	11	-25.4	-76.5 / 182.7	0.997
Day 7	8	-3.7	-37.5 / 55.7	11	1.0	-60.7 / 164.6	0.430
cCK18/flCK18 (M30/M65)							
Day 2	7	0.01	-0.12 / 0.06	11	-0.13	-0.26 / 0.57	0.624
Day 4	7	0.04	-0.03 / 0.09	11	-0.08	-0.80 / 0.03	0.004*
Day 7	8	-0.02	-0.07 / 0.09	11	-0.10	-0.75 / 0.87	0.427
MELD							
Day 2	5	0	-5 / 3	11	0	-8 / 4	0.563
Day 4	6	-2	-3 / 2	9	-2	-5 / 6	0.933
Day 7	7	-1	-7 / 9	9	-1	-4 / 3	0.767
CLIF-C ACLF Score†							
Day 2	3	-0.06	-0.11 / 2.28	6	0.62	-1.83 / 3.12	0.966
Day 4	3	1.55	-2.90 / 2.44	8	1.67	-4.07 / 4.54	0.871
Day 7	5	-1.47	-4.00 / 5.28	6	0.87	-2.99 / 7.30	0.466

(*$p<0.05$, †where ACLF is present at baseline).

Due to the distribution of the data for M30 and M65 the statistical analyses are based upon the change from baseline in log-transformed data rather than percentage change from baseline.

Table 4: Changes from baseline in M30, M65, M30/65, MELD, and CLIF-C ACLF score

	Placebo / 5mg (N=9)			25mg / 50mg (N=12)			
	n	**Median (Range)**	**LSMean %Relative Change from Baseline (95% CI)**	**n**	**Median (Range)**	**LSMean %Relative Change from Baseline (95% CI)**	**p-value**
cCK18 (M30)							
Day 2	7	425 (284 – 1999)	-11.6 (-32.0, +14.9)	11	560 (179 – 2162)	-42.6% (-53.4%, -29.3%)	0.017*
Day 4	7	717 (297 – 2162)	-3.5 (-27.3, +28.2)	11	516 (237 – 2016)	-38.9 (-51.2, -23.4)	0.017*
Day 7	8	546.5 (278 – 2162)	-3.1 (-30.3, 34.9)	11	903 (203 – 2985)	-20.8 (-40.2, +4.9)	0.342
flCK18 (M65)							
Day 2	7	1195 (531-3145)	-11.1 (-30.6, +14.1)	11	1945 (157 – 4365)	-28.6 (-41.4, -13.1)	0.166
Day 4	7	1237 (548 – 2664)	-14.1 (-34.8, +13.1)	11	1646 (662 – 4566)	-14.1 (-31.0, +7.0)	0.997
Day 7	8	1249 (614 – 3098)	+8.1 (-31.2, +22.8)	11	2555 (695 - 6999)	+6.5 (-16.7, +36.1)	0.430
	n	**Mean (95% CI)**	**LSMean in Change from Baseline (95% CI)**	**n**	**Mean (95% CI)**	**LSMean in Change from Baseline (95% CI)**	**p-value**
cCK18/flCK18 ratio (M30/M65)							
Day 2	7	0.51 (0.39, 0.62)	-0.01 (-0.17, 0.16)	11	0.51 (0.21, 0.81)	-0.06 (-0.19, 0.07)	0.624
Day 4	7	0.61 (0.47, 0.75)	0.04 (-0.06, 0.14)	11	0.39 (0.28, 0.50)	-0.16 (-0.24, -0.08)	0.004*
Day 7	8	0.54 (0.42, 0.66)	-0.01 (-0.19, 0.18)	11	0.45 (0.25, 0.64)	-0.10 (-0.25, 0.06)	0.427
MELD							
Day 2	5	27.8 (23.6, 32.0)	-1.0 (-3.8, 1.8)	11	29.4 (26.8, 31.9)	-0.1 (-2.0, 1.8)	0.563
Day 4	6	26.8 (23.6, 30.0)	-1.1 (-3.2, 0.9)	10	27.5 (23.4, 31.6)	-1.2 (-2.9, 0.4)	0.933
Day 7	7	28.6 (22.9, 34.2)	-0.2 (-3.6, 3.1)	10	28.5 (24.5, 32.5)	-0.8 (-3.8, 2.1)	0.767
CLIF-C ACLF Score							
Day 2	3	43.8 (22.2, 65.5)	0.7 (-2.3, 3.7)	8	45.9 (40.1, 51.7)	0.3 (-1.6, 2.2)	0.796
Day 4	4	45.6 (35.1, 56.2)	0.6 (-3.5, 4.6)	10	45.3 (41.2, 49.4)	0.6 (-1.8, 2.9)	0.998
Day 7	6	46.4 (40.1, 52.8)	0.3 (-3.3, 3.8)	8	45.6 (38.2, 52.9)	0.9 (-2.4, 4.1)	0.785

(*$p<0.05$)

Due to the distribution of the data for M30 and M65 the statistical analyses are based upon the change from baseline in log-transformed data rather than percentage change from baseline, and results were back transformed to percent relative change. LSMean=Least-squares mean from ANCOVA model adjusting for baseline value.

Table 5: Treatment-Related and All Causality Adverse Events, including Serious Adverse Events

		Placebo (N=4)		5mg BID (N=5)		25mg BID (N=7)		50mg BID (N=5)		All Subjects (N=21)	
System organ class	**Preferred term**	**AE**	**SAE**	**AE**	**SAE**	**AE**	**SAE**	**AE**	**SAE**	**AE**	**SAE**
Treatment-Related											
Gastro intestinal disorders	Nausea	1 (25.0%)	0	0	0	0	0	0	0	0	1 (4.8%)
	Vomiting	1 (25.0%)	0	0	0	0	0	0	0	0	1 (4.8%)
All Causalities											
Gastrointestinal disorders	Abdominal pain	0	0	1 (20.0%)	0	2 (28.6%)	1 (14.3%)	0	0	3 (14.3%)	1 (4.8%)
	Ascites	1 (25.0%)	0	3 (60.0%)	0	3 (42.9%)	1 (14.3%)	0	0	7 (33.3%)	0
	Constipation	0	0	1 (20.0%)	0	2 (28.6%)	0	1 (20.0%)	0	4 (19.0%)	0
	Gastrointestinal haemorrhage	0	0	1 (20.0%)	1 (20.0%)	0	0	2 (40.0%)	2 (40.0%)	3 (14.3%)	3 (14.3%)
	Nausea	3 (75.0%)	0	0	0	3 (42.9%)	0	1 (20.0%)	0	7 (33.3%)	0
	Vomiting	1 (25.0%)	1 (25.0%)	2 (40.0%)	0	1 (14.3%)	0	1 (20.0%)	0	5 (23.8%)	1 (4.8%)
General disorders an administration site conditions	Multiple-organ failure	1 (25.0%)	1 (25.0%)	1 (20.0%)	1 (20.0%)	1 (14.3%)	1 (14.3%)	0	0	3 (14.3%)	3 (14.3%)
Hepatobiliary disorders	Hepatorenal syndrome	0	0	1 (20.0%)	0	2 (28.6%)	1 (14.3%)	0	0	3 (14.3%)	1 (4.8%)
Metabolism and nutrition disorders	Hypokalaemia	1 (25.0%)	0	0	0	0	0	2 (40.0%)	0	3 (14.3%)	0
Nervous system disorders	Hepatic encephalopathy	0	0	2 (40.0%)	1 (20.0%)	1 (14.3%)	0	0	0	3 (14.3%)	1 (4.8%)
Respiratory, thoracic and mediastinal disorders	Dyspnoea	2 (50.0%)	1 (25.0%)	0	0	1 (14.3%)	0	0	0	3 (14.3%)	1 (4.8%)

Supplementary Table 1: Demography and Baseline Characteristics of Laboratory Data and Hepatic Biomarkers by Individual Randomized Treatment Groups

Parameter	Placebo (N=4)		5 mg BID (N=5)		25 mg BID (N=7)		50 mg BID (N=5)	
	n	%	n	%	n	%	n	%
Male	2	50%	3	60%	5	71.4%	3	60%
Etiology of cirrhosis								
Alcohol	4	100%	5	100%	6	85.7%	5	100%
Hepatitis C	0	0%	0	0%	1	14.3%	2	40%
ACLF present at baseline	3	75%	4	80%	6	85.7%	4	80%
Precipitating event for acute deterioration								
Bacterial Infection	1	33.3%	2	50%	3	50%	0	0%
Alcohol	3	100%	3	75%	5	83.3%	5	100%
GI haemorrhage	0	0%	0	0%	1	16.7%	0	0%
HE Grade								
0	0	0%	3	60%	2	28.6%	3	60%
1	3	75%	2	40%	4	57.1%	2	40%
2	1	25%	0	0%	1	14.3%	0	0%
3/4	0	0	0	0%	0	0%	0	0%
Ascites	4	100%	4	80%	7	100%	5	100%

	n	Median	Range	n	Median	Range	n	Median	Range	n	Median	Range
Age	4	53.5	34–59	5	49	41 – 60	7	50	30 - 58	5	61	42 - 71

CLIF-C ACLF score	4	46.7	34.2 - 53.9	4	46.7	46.4 - 48.8	6	43.9	35.9 - 53.6	4	43.1	40.8 - 56.4
MELD	4	26	19 – 31	5	29	27 – 33	6	29.5	28 - 34	5	23	22 - 36
Laboratory Data												
Creatinine	4	0.83	0.64-0.92	5	0.73	0.46 – 1.23	7	0.84	0.50 – 1.93	5	0.77	0.48 – 2.09
Bilirubin	4	15.77	4.7-21.0	5	14.7	11.3 - 33.4	7	23.3	14.1 – 34.7	5	17.7	2.2 – 33.2
INR	4	2.41	1.77-3.30	5	3.15	1.92 – 3.90	6	2.42	2.10 – 3.12	5	2.09	1.90 – 3.81
Platelet Count	4	67	39-133	3	60	25 – 274	5	119	57 - 155	4	144	29 - 210
Sodium	4	129	110-138	5	136	133 - 139	7	131	126 - 144	5	134	126 - 139
ALT	4	41.9	21.3-53.7	5	24.2	21.3 – 49.4	7	43.2	20.3 – 115.9	5	43.6	10.0 – 61.5
AST	4	124.5	65.5-201.7	5	69.7	42.0 – 152.9	7	89.8	74.8 – 251.7	5	100.5	23.2 – 189.1
Hepatic Biomarkers												
cCK18 (M30)	4	1085.5	240-3593	5	703	302 - 2162	7	1392	330 - 3593	5	919	395 – 1851
flCK18 (M65)	4	1887.5	507-3808	5	1345	876 - 2862	7	2819	277 - 3762	5	1946	705 – 6999
cCK18/flCK18 (M30/M65)	4	0.587	0.344-0.944	5	0.523	0.345-0.774	7	0.679	0.244 – 1.191	5	0.423	0.216 – 0.560
Caspase 3/7	4	3631.5	2730 - 5331	5	4418	2516 - 6692	7	3967	2615 – 6884	5	4304	1135 – 5405

Figure Legends:

Figure 1. Study design outline.

Figure 2. CONSORT diagram of trial participants.

†Two subjects met an exclusion criterion that was considered a major protocol deviation, but were randomized and included in all analyses as determined during a BDRM.

*Reasons include: investigator decision to not continue with trial, and subject started on dialysis due to deteriorating renal function.

**One patient had severe encephalopathy and was unable to swallow trial medications.

***One patient had a severe upper GI bleed and another was unable to swallow trial medications.

Admin. = administrative, AE = adverse event, BDRM = blind data review meeting, *bd* = twice daily, dec. = decision, FU = follow-up, invest. = investigator, N = number of subjects.

Figure 3a. Change in cCK18 (M30) by treatment group. (Values are mean +/- SD)

Figure 3b. Change in Caspase 3/7 by treatment group. (Values are mean +/- SD)