

**Introduction:**

Emricasan (IDN-6556, PF-03491390) is a potent irreversible pan-caspase inhibitor. To date, emricasan has been studied in more than 550 subjects and has exhibited a safety profile similar to placebo. Emricasan is currently in three Phase 2 clinical trials. Here we report the effects of emricasan in patients with acute-on-chronic liver failure (ACLF).

**Background:**

Caspases play a central role in the processes of apoptosis and inflammation. As such, caspases are attractive targets for the treatment of a variety of liver diseases<sup>1</sup>.

ACLF is a syndrome characterized by the acute deterioration of liver function in patients with compensated or decompensated, but stable cirrhosis. It is commonly precipitated by an acute event and associated with failure in the function of extra-hepatic organs<sup>2,3</sup>. The lack of liver detoxification, and metabolic and regulatory dysfunction, together with an altered immune response, lead to life-threatening complications, such as renal failure, increased susceptibility to infection, hepatic coma and systemic hemodynamic dysfunction<sup>3,4</sup>. In recent years, the recognition of clinically different patterns of liver failure associated with cirrhosis has led to the demarcation of two different entities: 'end-stage' and 'acute-on-chronic' liver failure<sup>3,5</sup>. Although substantial overlap exists between these entities in terms of clinical presentation, the main differences between them are the potential for recovery, the presence or absence of a precipitating event and the substantial evolution towards multi-organ failure.

Current goals of treatment for ACLF are to prevent further deterioration in liver function, reverse precipitating factors, and support failing organs. Liver transplantation is required in selected subjects. Left untreated, the in-hospital mortality of these subjects is reported to be greater than 50%, and mean length of hospitalization is 13.8 days, with repeat hospitalizations for ACLF reaching even higher rates of mortality<sup>6</sup>.

An almost constant feature in cirrhotic subjects with ACLF is the rapid deterioration in liver function. Although the exact mechanisms remain unclear, massive liver cell necrosis may not be predominant. There is evidence that serum markers of caspase-driven apoptosis, such as cleaved CK18, are raised in these subjects<sup>7,8</sup>. Death receptors and ligands, Fas/FasL and TNF- $\alpha$ , that induce apoptosis via activation of caspases are also up-regulated in ACLF<sup>9,10</sup>.

**Methods:**

This placebo-controlled, multicentre study (UK and USA) enrolled subjects with compensated or stable decompensated liver cirrhosis presenting with ACLF defined as a decompensating event or illness (including but not limited to alcohol use, GI haemorrhage, etc.) of  $\leq 6$  weeks duration on a background of chronic liver disease. The primary objective was to evaluate the pharmacokinetics and pharmacodynamics of emricasan 5, 25, and 50 mg BID orally administered for 28 days. Secondary objectives included assessment of safety, evaluation of clinical outcomes and recommendation of a dose(s) of emricasan for a follow-on efficacy study in ACLF.

Randomization was stratified based on presence or absence of alcohol use. If a subject had an alcohol history, then a further stratification was made based on prior steroid use or not to ensure that subjects were proportionally distributed between treatment groups.

Serial (pre-dose, 0.5, 1, 2, 3, 4, 5 and 8 hr.) pharmacokinetic (PK) samples were taken at Day 1 and Day 4 (if still hospitalized), and a single PK sample was taken at Day 28/End of Treatment. In select subjects, a 12 hour PK sample was also collected on Day 1 and Day 4. The PK parameters assessed were comprised of C<sub>max</sub>, AUC<sub>inf</sub>, AUC<sub>0-8</sub>, AUC<sub>last</sub>, T<sub>max</sub>, and t<sub>1/2</sub>. Serum biomarker variables assessed included cCK18/M30, fICK18/M65, and Caspase 3/7.

**Results:**

Twenty-three subjects with ACLF were randomised in the study, 21 were dosed. Baseline characteristics were well matched between treatment groups. The majority of subjects were white, male, within the 45-64 years age range, and categorized as Child-Pugh C. Table 1 shows the number of subjects by treatment group who reached/completed each study visit. The ability of such ill subjects to complete the study was a concern and subject disposition was reflective of the patient population (mainly alcoholic liver disease) enrolled into the study. Of the 21 subjects dosed, only 7 were dosed through to the end of study on Day 28. Nine of the 21 subjects were known to be alive at Day 28 with 6 subjects lost to follow up and 6 subjects dying with complications of their liver

disease (see below for details). This further reflected the complexity of the patient population and underlying disease severity.

**Table 1: Number of Subjects who Reached/Completed each Study Visit**

Visit		Placebo	IDN-6556		
		BID	5mg BID	25 mg BID	50 mg BID
		N	N	N	N
Baseline	Day 1	4 (100%)	5 (100%)	7 (100%)	5 (100%)
	Day 2	3 (75%)	5 (100%)	7 (100%)	5 (100%)
Double-Blind Phase	Day 4	2 (50%)	5 (100%)	6 (86%)	5 (100%)
	Day 7	3 (75%)	5 (100%)	6 (86%)	5 (100%)
	Day 14	2 (50%)	3 (60%)	5 (71%)	3 (60%)
	Day 21	2 (50%)	2 (40%)	3 (43%)	2 (40%)
	ET/EOT*	3 (75%)	0	4 (57%)	2 (40%)
Follow-Up	Day 56	1 (25%)	0	2 (29%)	1 (20%)

\* Seven subjects (2 on placebo, 3 on 25mg BID and 2 on 50 mg BID) were dosed until End of Treatment (EOT) on Day 28. Subjects who discontinued prior to Day 28 were required to have an Early Termination (ET) visit.

Table 2 summarizes the main PK parameters from the study. Exposure was much greater in this population than anticipated. The geometric mean AUC<sub>0-8</sub> in the 5 mg BID, 25 mg BID and 50 mg BID treatment group was 275 ng·h/mL, 1164 ng·h/mL and 2441 ng·h/mL, respectively. Geometric mean AUC<sub>0-8</sub> and C<sub>max</sub> on Day 1 was approximately 4 times higher in the 25 mg BID group and 8-9 times higher in the 50 mg BID group compared to the 5 mg BID group.

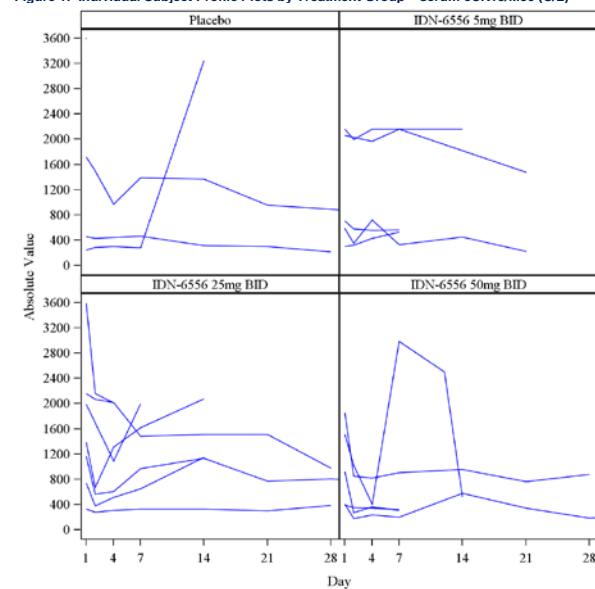
**Table 2: Summary Pharmacokinetics of Emricasan in ACLF Subjects**

Visit	Parameter	IDN-6556 5 mg BID		IDN-6556 25 mg BID		IDN-6556 50 mg BID	
		N	Geo. Mean (CV)*	N	Geo. Mean (CV)*	N	Geo. Mean (CV)*
Day 1	AUC <sub>0-8</sub> (ng h/mL)	5	275 (44)	5	1164 (45)	5	2441 (87)
	C <sub>max</sub> (ng/mL)	5	84.4 (44)	7	330 (48)	5	696 (117)
	T <sub>max</sub> (h)	5	2.71 (65)	7	2.98 (89)	5	2.65 (119)
	t <sub>1/2</sub> (h)	3	2.96 (89)	5	2.11 (33)	2	-
Day 4	AUC <sub>0-8</sub> (ng h/mL)	4	224 (37)	5	1908 (33)	4	2362 (315)
	C <sub>max</sub> (ng/mL)	5	40.3 (32)	5	587 (34)	4	686 (194)
	T <sub>max</sub> (h)	4	2.57 (146)	5	2.17 (85)	4	1.72 (108)
	t <sub>1/2</sub> (h)	1	-	3	1.95 (7)	2	2.99 (150)

\* Geometric Mean (Coefficient of Variation %)

Emricasan exposure levels were higher than those observed in previous studies in healthier patient populations. One explanation for this was that all subjects had significant portal hypertension. Emricasan has a high first-pass uptake into the liver but patients with portal hypertension have underlying porto-systemic shunting which may reduce first pass hepatic uptake and result in higher systemic exposure of the compound. Concomitant medications may also have increased emricasan exposures. All subjects were on a large number of concomitant medications, some of which were potential OATPB inhibitors. In vitro profiling studies previously showed that emricasan was taken up into cells via OATPB. The implication of the co-administration of concomitant medications commonly used in this patient population probably warrants further exploration.

**Figure 1: Individual Subject Profile Plots by Treatment Group – serum cCK18/M30 (U/L)**



Literature reports suggest increasing levels of cCK18/M30 were associated with increasing hepatic disease severity and that the higher the cCK18/M30 level, the worse the patient outcome. This study confirmed that ACLF patients had elevated levels of all 3 biomarkers (cCK18/M30, fICK18/M65, Caspase 3/7) at Baseline. Similarly, in patients who survived to 28 days, cCK18/M30 generally decreased by at least 30% suggesting that this may be a useful clinical biomarker in this disease area. Subjects who had an increase in cCK18/M30 during the course of the study experienced a new complication, e.g. infection, active haemorrhage, encephalopathy, organ failure, etc. In this study, no early cCK18/M30 response was evident in subjects randomised to either placebo or 5 mg emricasan BID. In the 25 mg BID treatment group, the majority of subjects had a reduction of at least 30% in cCK18/M30 within the first 24 hours post dose. However, this initial reduction was not sustained for the full dosing period. Subjects randomised to the 50 mg BID dose of emricasan had an early response of  $\geq 30\%$  in cCK18/M30 and, in the majority of subjects, this initial reduction was sustained for the full dosing period. Patterns of change in Caspase 3/7 and fICK18/M65 were generally similar to those observed in cCK18/M30.

Evaluation of clinical outcomes was severely hampered by the low patient numbers randomised in the study and further adversely impacted by the small number of subjects who completed 28 days of treatment. As an ad hoc analysis, Conatus consulted an expert hepatologist with extensive clinical expertise in ACLF to establish objective criteria and determine clinical responses on a case-by-case basis. The expert was blinded to study drug assignment at the time of evaluation.

A subject was defined as a Clinical Responder if they met any of the following criteria:

- Survival to Day 28 or last known follow-up;
- Laboratory response parameters evaluated at Day 28 or last known follow-up:
  - CLIF-SOFA Score: Improvement by at least 2 points from Baseline
  - MELD Score: Improvement by at least 5 points from Baseline
  - Total Bilirubin: Improvement by approximately 50% from Baseline

**Table 3: Clinical Responder Analysis**

Criteria	Placebo BID	IDN-6556		
		5mg BID	25 mg BID	50 mg BID
Survival at Day 28 or last known follow-up	2	3	3	3
CLIF-SOFA Score (improvement by 2 points from Baseline)	1	3	2	1
MELD Score (improvement by 5 points from Baseline)	1	2	3	2
Total Bilirubin (improvement by approx. 50% from Baseline)	2	3	2	1
Number of Responders	2	3	3	3

Improvements in MELD and CLIF-SOFA Scores were primarily driven by improvements in Bilirubin levels. Subjects who survived to Day 28 or last known follow-up all had  $\geq 30\%$  reduction in cCK18/M30, with the exception of one subject in the 25 mg BID dose group who experienced a 17% increase.

Although subjects in this study had higher systemic exposures of emricasan than in previous studies where oral administration was employed, the adverse event profile reported generally reflected the study population and the severity of hepatic disease. Of note, these included worsening of ascites and/or hepatic encephalopathy, multi-organ failure and esophageal variceal haemorrhage. The 6 deaths reported prior to the end of the dosing period on Day 28 were also all attributed to complications of severe liver disease. Other adverse events reported in the study, and the severity thereof, were generally similar to those reported in previous studies.

**Conclusions:**

- Of the 21 subjects who received study treatment, 7 subjects completed Day 28 of the study
- A total of 10 deaths across all treatment groups (2, 3, 3 and 2 deaths in placebo, 5, 25 and 50 mg BID groups, respectively) occurred prior to last known follow-up. By Day 28 there were a total of 6 deaths (2 in placebo, 1, 1 and 2 deaths in the 5, 25 and 50 mg BID groups, respectively)
- Seventeen of the 21 subjects randomised experienced adverse events, of which 13 reported serious adverse events. None of these serious adverse events were considered to be treatment related by the investigator
- Emricasan blood levels at all doses studied were increased in subjects with ACLF compared to previous patient populations studied

- Increased blood levels of emricasan were likely a consequence of porto-systemic shunting but may also have been due to concomitant medication
- Key biomarkers of inflammation and caspase activity (cCK18/M30, fICK18/M65, Caspase 3/7) were implicated in the ACLF disease process, as well as disease severity, and appeared to be associated with patient outcome
  - Baseline values of cCK18/M30 and Caspase 3/7 were significantly higher in the ACLF population compared with previous populations studied
  - Increases in cCK18/M30 levels appeared to be associated with an adverse clinical event in ACLF subjects
- Oral emricasan 25 and 50 mg BID reduced cCK18/M30, fICK18/M65 and Caspase 3/7 levels within 24 hours post administration by at least 30% in most subjects
- Only oral emricasan 50 mg BID resulted in sustained reductions in cCK18/M30 over the entire dosing period in the majority of subjects
- The combined PK and PD data suggested that higher doses of oral emricasan may be required in subjects with ACLF in order to achieve sustained reductions of key biomarkers of inflammation and apoptosis
- All clinical responders (except one on 25 mg BID) experienced at least a 30% reduction in cCK18/M30
- Further confirmatory studies are required to assess the utility of emricasan in subjects with ACLF

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