Rapid and statistically significant reduction of markers of apoptosis and cell death in subjects with mild, moderate and severe hepatic impairment following a single dose of the pan-caspase inhibitor, emricasan

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Introduction:

Emricasan (IDN-6556, PF-03491390) is a potent irreversible pan-caspase inhibitor with the ability to rapidly reduce elevated levels of serum ALT, AST and caspase mediated cleavage of cytokeratin-18 in HCV infected patients.[1,2,3] To date, emricasan has been studied in more than 550 individuals and has exhibited a safety profile similar to placebo.

Emricasan is currently in five Phase 2 clinical trials, including three trials in patients with liver cirrhosis. Here we report the effect of the emricasan on measures of serum caspase activity and apoptosis in subjects with mild, moderate and severe hepatic impairment, classified according to Child-Pugh criteria, and a cohort of matched healthy subjects.

Background:

Caspases play a central role in the processes of apoptosis and inflammation. As such, caspases are attractive targets for the treatment of variety liver diseases.[4] In liver disease, chronically elevated apoptosis results in the accumulation of apoptotic cells as well as the release of apoptotic bodies and other subcellular fragments such as microvesicles.[4,5] These cellular end-products of apoptosis, which contain a wide variety of biologically active substances, are engulfed by neighboring tissue and promote disease pathology.[6] Inhibition of caspases may therefore reduce the disease-driven loss of hepatocytes and production of apoptotic bodies and microparticles that promote disease progression, Figure 1.

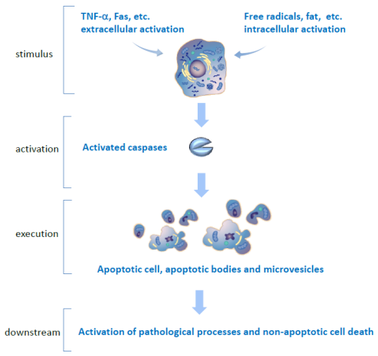

Figure 1: Hepatocyte cell death leads to amplification of disease pathology

Caspase mediated apoptosis is driven by the enzymatic action of caspase 3 and 7 on a wide variety of cellular substrates. One target substrate of caspases is the intermediate filament protein, cytokeratin-18 (CK18). Caspase cleavage of CK18 yields a protein fragment, cCK18, which is generally recognized as a specific mechanistic biomarker of apoptosis. Elevated serum levels of cCK18 have been associated with severity in a variety of liver diseases.[7] In patients with cirrhosis, cCK18 has been reported to increase progressively with disease severity[8]. A recent prospective study of 238 cirrhotic patients reported that serum cCK18 markers of apoptosis were associated with stage of disease and overall survival in patients with cirrhosis[9].

Elevated serum levels of full-length CK18, (flCK18), are also associated with liver disease. Full-length CK18 is released following cell wall rupture, as occurs in necrosis, and as such is considered a mechanism independent, generic biomarker of overall cell death. Serum levels of cCK18 and flCK18 are measured by the specific ELISA based monoclonal antibody assays, M30-Apoptosense® ELISA, and M65® ELISA (VLVbio), respectively. Caspase 3/7 activity was measured using Caspase-Glo® 3/7 (Promega).

Methods:

A total of 28 subjects with hepatic impairment and 8 matched healthy controls were enrolled in an open label study to evaluate the pharmacokinetics and pharmacodynamics effect of emricasan. Twelve subjects classified as mild (Child-Pugh A), eight as moderate (Child-Pugh B) and 8 severe (Child-Pugh C) were enrolled in the study. Eight healthy control subjects were matched to severe subjects based on demographic characteristics.

On Study Day 1, a pre-dose blood sample was collected followed by administration of a single 50 mg dose of emricasan to all subjects. Eight blood samples were then collected over a 12 hour period post-dose with further samples collected at 24 and 48 hours post-dose.

Results:

Consistent with literature reports, baseline serum levels of cCK18 were elevated in subjects with advanced liver disease. In addition, serum titers of cCK18 increased along with the progression of disease stage, Figure 2. These data suggest that elevated levels of apoptosis in these subjects may contribute to disease progression and pathology in patients with advanced liver disease.

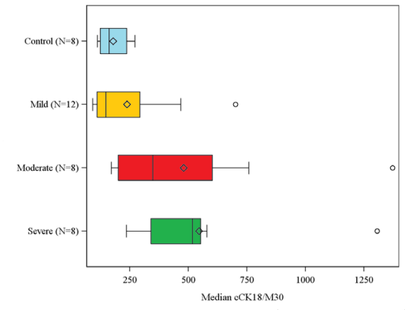

Figure 2: Baseline levels of caspase cleaved cytokeratin-18 as determined by M30-Apoptosense® ELISA. Mean and median levels

Similar to cCK18, baseline levels of caspase 3/7 were elevated relative to control subjects, Table 1. Caspase levels were rapidly and consistently reduced and in all subjects with liver disease following a single 50 mg oral dose of emricasan, Figure 3. A maximal response was observed approximately 4 hours post-dose. Importantly, caspase 3/7 activity quickly approached levels observed in the cohort of matched healthy subjects.

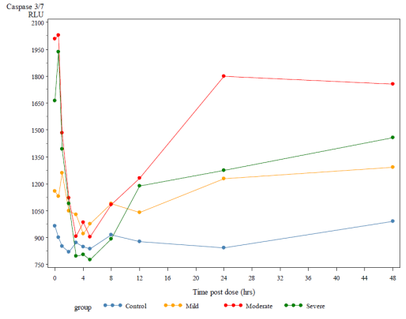

Figure 3: Median of serum levels of caspase 3/7 enzymatic activity following a single 50 mg oral dose of emricasan as determined by Caspase- Glo® 3/7 assay (Promega).

Serum levels of cCK18 were rapidly reduced in all subjects with liver disease following a single 50 mg oral dose, suggesting that, in addition to decreasing levels of caspase 3/7, emricasan can effectively reduce apoptosis in these subjects. It is particularly noteworthy that this marker was reduced in all subjects with hepatic impairment while no effect was observed in any healthy subject, Figure 4.

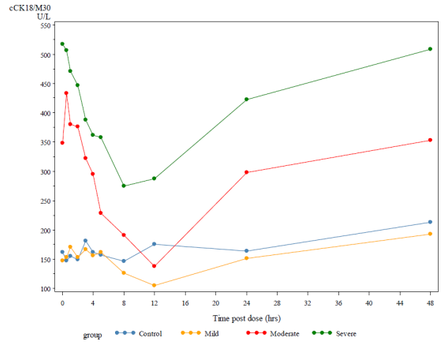

Figure 4: Median serum levels of caspase cleaved cytokeratin-18 in hepatically impaired and matched healthy subjects following a single 50 mg oral dose of emricasan.

A mechanism-independent biomarker of overall cell death, flCK18, was also rapidly reduced in subjects with hepatic impairment. This observation suggests that emricasan may effectively reduce non-apoptotic cell death as well as of apoptotic cell death in patients with liver disease, Figure 5.

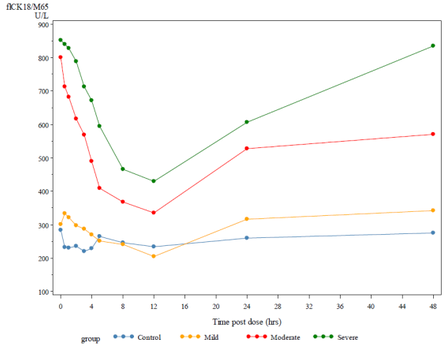

Figure 5: Median serum levels of full-length cytokeratin-18 in hepatically impaired and matched healthy subjects following a single 50 mg oral dose of emricasan.

The collective data and observations presented above suggest that the pan-caspase inhibitor, emricasan, can significantly and effectively reduce elevated levels of caspase activity, apoptosis and, more generally, cell death in patients with advanced liver disease. These attributes may translate into clinical benefit in patients with advanced stages of liver disease. Summary statistics of these data are presented in Table 1.

	Median Caspase 3/7 Activity (RLU)			Median cleaved cytokeratin 18 (M 30) (U/L)			Median full length cytokeratin 18 (M 65) (U/L)		
Group (n)	Baseline (RLU)	Change in median @ 4 hours post dose Absolute (Relative)	P-Value*	Baseline (U/L)	Change in median @ 12 hours post dose Absolute (Relative)	P-Value*	Baseline (U/L)	Change in median @ 12 hours post dose Absolute (Relative)	P-Value*
Control (8)	963.5	-174.0 (-18%)	0.1094	161.5	29.5 (+17%)	0.3125	284.0	7.0 (+4.7%)	1.0000
Mild (12)	1158.5	-262.5 (-23.8%)	0.0049	147.5	-37.5 (-26.9%)	0.0005	301.0	-64.5 (-24.5%)	0.0024
Moderate (8)	2006	-1035.5 (-52.9%)	0.0078	348.0	-166.0 (-49.9%)	0.0156	801	-532.5 (-60.6 %)	0.0156
Severe (8)	1622.5	-881.0 (-56.5%)	0.0156	517	-172.0 (-36.2%)	0.0078	851.0	-340.5 (-38.4%)	0.0078

* Wilcoxon sign rank test

Table 1: Effect of emricasan on caspase activity and biomarkers of cell death and apoptosis in subjects with hepatic impairment and matched healthy controls

The pharmacokinetic information derived from this trial was used to support the design of ongoing trials in patients with cirrhosis. These data will be presented, along with pharmacokinetic data from additional trials in patients with organ impairment.

Conclusions:

- Caspase activity and apoptosis are elevated in subjects with advanced liver disease and may play a role in disease pathology and progression.
- Following a single 50 mg oral dose, emricasan rapidly reduced caspase enzymatic activity, serum markers of apoptosis (M30) and overall cell death (M65) in all subjects with hepatic impairment.
- These reductions were statistically significant
- Emricasan may provide clinical benefit to patients with advanced stages of liver disease.

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