Inhibition of caspase activity with emricasan in HCV patients: potential implications for chronic dosing and long term safety

Alfred P. Spada*, Patricia Contreras, Gary C. Burgess
Conatus Pharmaceuticals, San Diego, California

Introduction:

The progression of chronic liver disease is often associated with an increased rate of apoptosis of hepatocytes. The inability to efficiently remove pro-inflammatory apoptotic cell debris results in a state of persistent inflammation in the liver. Caspase inhibition represents a promising approach to reduce excessive apoptosis and accompanying progressive liver damage associated with a variety of liver diseases. Emricasan (IDN-6556, PF-03491390) is a potent irreversible pan-caspase inhibitor that has demonstrated the ability to rapidly reduce elevated levels of serum ALT and AST in HCV infected patients in phase 1 and phase 2 clinical trials. Emricasan also effectively reduced markers of mechanism related activity, including caspase enzymatic activity and caspase 3/7 mediated cleavage of cytokeratin-18, a well-accepted specific marker of apoptosis.

Background:

A significant concern however surrounding inhibition of caspase activity relates to the potential to interfere with normal homeostatic mechanisms and theoretically the promotion of tumorgenesis. Here we report the effect of the emricasan on measures of serum caspase activity in a 12-week dose response study conducted in patients with HCV. The design and results of this phase 2 clinical trial were recently published.[1] Briefly, emricasan was well tolerated and produced durable reductions in transaminase levels during the course of the trial without affecting HCV viral load. In addition, an analysis of various markers of inflammation and fibrosis was also published in abstract form.[2]

Caspase mediated apoptosis is driven by the enzymatic action of caspase 3 and 7 on a wide variety of cellular substrates. As shown in Figure1, both of these caspases exist in an inactive pro-caspase form that requires cleavage by either caspase 8 or caspase 9 to release active caspase 3 and 7 to initiate the apoptotic program. One substrate of caspase 3/7 is the filament protein cytokeratin-18. Caspase mediated cleavage of cytokeratin-18 has been extensively studied and is well recognized to be associated with cellular apoptosis. Serum levels of caspase-cleaved CK-18 has been associated with severity in a variety of disease conditions including HCV[3] and NASH.[4] Circulating levels of caspase-cleaved CK-18 in healthy volunteers are also well documented and are likely reflective of on-going normal cellular homeostasis.

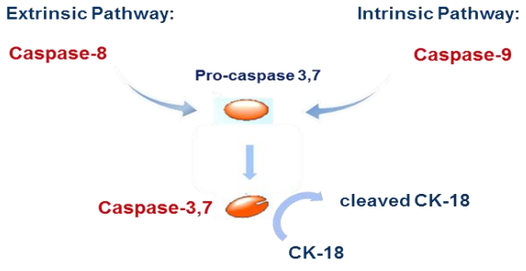

Figure 1: Caspase activation pathways and caspase cleavage of cytokeratin 18

Methods:

204 HCV patients who either previously failed to achieve SVR or were intolerant to therapy were randomized to receive placebo, 5 mg, 25 mg or 50 mg of emricasan twice daily for 12 weeks. At week 10, in patients whose transaminase levels had not normalized, the dose of emricasan was doubled through week 12.

At day 1 and week 10 study visits, pre-dose and post-dose blood samples were drawn and analyzed for drug levels as well as against a panel of biomarkers.[2]

Two specific and complementary serum based assays were employed to directly evaluate the effect of emricasan on caspase activity and apoptosis in this clinical study. The first utilized a direct and specific assay to detect caspase 3/7 enzymatic activity. This *ex vivo* assay measures a luminescent signal generated by the action of caspase 3/7 on a synthetic substrate. The intensity of the signal is proportional to the concentration of caspase 3/7 activity in the serum sample. While this assay is a direct measure of caspase 3/7 activity, as shown in Figure 1, it also serves as a surrogate indicator of intact up-stream caspase 8/9 activity.

An ELISA based monoclonal antibody assay that captures a caspase-cleaved cytokeratin-18 was employed as a second assay in this study, (M30-Apoptosense ELISA). The specific epitope generated by caspase cleavage of cytokeratin-18 is well accepted to be associated with the physiological process of apoptosis. Therefore, serum titers of this cleavage fragment provide a direct readout of the extent of ongoing apoptosis as well as a measure of functional caspase activity on a physiological substrate.

Results:

The absolute mean and median values and percent reduction from baseline of cleaved CK-18 and caspase 3/7 activity across the dose groups is shown in Table 1. There was no change in either analyte in the

Table 1: Mean and Median Changes in Serum CK-18 and Caspase 3/7 Enzymatic Activity

			Cleaved CK 18 U/L (M30)			Caspase 3/7 activity (RLU)		
Dose (BID)	(n)	Value	Baseline	Week 10	% Reduction	Baseline	Week 10	% Reduction
0	46	Mean	348	306	5±38	646	612	11± 45
		Median	198	205	0	512	588	0
5	51	Mean	382	242	-26 ±28	720	461	-30 ±33
		Median	296	199	-33	572	404	-30
25	47	Mean	393	250	-27 ±33	696	389	-37±30
		Median	334	169	-49	553	326	-41
50	46	Mean	519	328	-33 ±32	768	295	-65±17
		Median	380	207	- 46	638	241	-62

placebo cohort. This is constant with no observed change in transaminase levels in this group.[1] Approximately 26 - 33 percent reduction in mean levels of cleaved CK-18 from baseline was observed across the 3 active dose groups at week 10. Approximately 30 - 65 percent reduction in mean levels of caspase 3/7 enzymatic activity from baseline was observed across the 3 active dose groups at week 10.

The reduction in caspase 3/7 enzymatic activity was rapid statistically significant and durable from the initial dose through week 10, Figure 2.

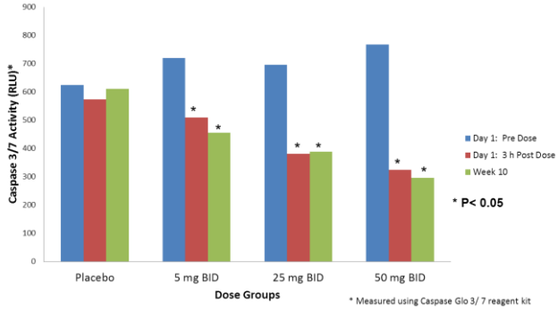

Figure 2: Mean reduction of caspase 3/7 enzymatic activity as determined by luminescent assay

Three hours following the first dose on Day 1 caspase 3/7 activity was significantly reduced, regardless of active dose, when compared with pre-dose levels. The level of reduction observed after 3 hours on day 1 was not statistically different than that observed at 3 hours post-dose on week 10.

Levels of cleaved CK-18 were also tended to be reduced following the first dose on Day 1, Figure 3. All active dose levels achieved statistically significant reductions from baseline values at week 10. Although not shown, both pre- and post-dose levels of CK-18 were measured at the week 10 study visit. The pre-dose levels correspond to trough levels of emricasan while the post-dose levels represent peak blood levels of emricasan. There was no statistical difference between the pre- and post-dose reductions in cleaved CK-18 at week 10, suggesting that a steady state reduction in caspase mediated apoptosis was achieved.

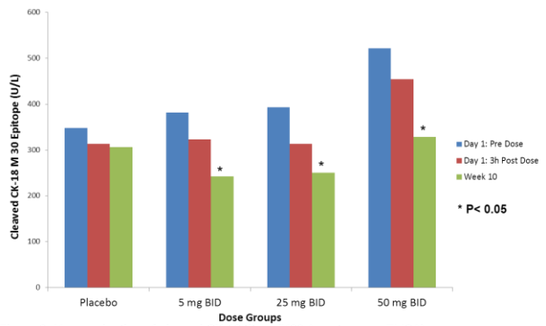

Figure 3: Mean reduction of cleaved CK-18 titers (M30 Apoptosense ELISA)

Regardless of dose level, serum levels of CK-18 approached titers typically observed in healthy volunteers; which have been reported to range between 100 – 250 U/L.[3,4] This observation suggests that pan-caspase inhibition by emricasan can reduce, but not abolish, caspase mediated apoptosis, Figure 3.

An analysis of individual patients across all dose groups whose baseline titers of cleaved CK-18 ranged from 100 – 250 revealed that emricasan had little to no effect on cleaved CK-18 levels, Figure 4. Between 10 – 15 patients per cohort fell into this category. This observation suggests that

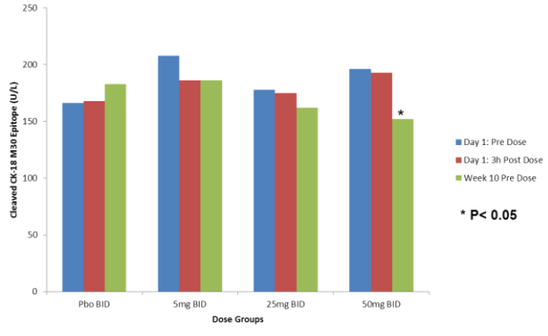

Figure 4: Minimal change in cleaved CK-18 in HCV patients with near normal baseline titers

emricasan may not reduce or interfere with the normal homeostatic process of apoptosis.

Drug levels of emricasan increase in a dose proportional manner and do not accumulate over time, Figure 5. Patients samples used to determine concentrations of emricasan were taken on Day 1 and week 10 at the same time that samples were collected for caspase 3/7 and cleaved CK-18. Therefore increasing levels of emricasan over a 10-fold dose range with proportional increases in exposure levels did not abolish caspase 3/7 activity nor did it reduce titers of cleaved CK-18 below normal titers.

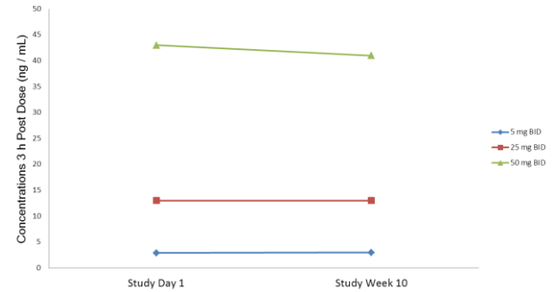

Figure 5: Mean serum concentration of emricasan in HCV patients at Day 1 and Week 10 (3 h post-dose)

The data and observations presented above suggest that the pan-caspase inhibitor, emricasan, can effectively reduce elevated levels of caspase activity and apoptosis but appears to have minimal, if any, impact on homeostatic levels of apoptosis as measured by these assays. This may have beneficial implications on the potential of chronic dosing and long term safety of emricasan.

Conclusions:

- Statistically significant reductions in caspase 3/7 activity and caspase cleaved cytokeratin-18 were rapidly achieved and maintained throughout the duration of the study.
- Neither caspase enzymatic activity nor cleaved CK-18 was abolished regardless of dose.
- Elevated titers of cleaved CK-18, a surrogate of apoptosis, were reduced to levels approaching those reported in healthy volunteers.
- Minimal to no reductions of cleaved CK-18 were observed in patients with near normal baseline titers of cleaved CK-18.
- Observed dose proportional exposure of emricasan with no evidence of drug accumulation.
- Pharmacodynamic responses with respect to reduction in transaminases, caspase enzymatic activity and cleaved cytokeratin-18 remained durable throughout the course of the trial.
- These observations suggest that apoptosis and caspase activity remain functionally intact and thus may minimize concerns regarding safety.

Reference:

1. Schiffman 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.
2. Burgess G, Colman P, Engmann E, et al. PF-03491390 inhibits liver fibrosis in patients with chronic hepatitis C infection via suppression of pro-apoptotic caspase activation. Hepatology 2007; 46, 818, (abstract 1307).
3. Jazwinski AB, Thompson AJ, Clark PJ, et al. J Viral Hepatitis 2012; 19, 278-282.
4. Joka D, Wahl K, Moeller S, et al. Hepatology 2012; 55, 455-464.

* aspada@conatuspharma.com