Physiologically normal levels of apoptosis in healthy volunteers are not reduced by the pan-caspase inhibitor, emricasan

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Introduction:

Emricasan (IDN-6556, PF-03491390) is a potent irreversible pan-caspase inhibitor that has demonstrated the ability to reduce elevated levels of serum ALT and AST in HCV infected patients in Phase 1 and Phase 2 clinical trials.[1,2] These effects were durable throughout the duration of dosing and emricasan has been well-tolerated in all clinical trials. Emricasan also rapidly 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.[3] Emricasan is currently in a Phase 2 clinical trial in patients with acute-on-chronic liver failure as well as studies in acute alcoholic hepatitis.[4] Emricasan is scheduled to enter a 2 - year Phase 3 trial in patients with liver fibrosis due to recurrent HCV infection post-orthotopic liver transplant.

Background:

Inhibition of caspase activity has long been recognized as a potentially attractive approach for the treatment of variety liver diseases. However, a longstanding concern associated with the presumed potential of the mechanism to disrupt normal homeostatic mechanisms and promote tumorgenesis, has understandably hampered development in this field. Numerous recent studies however have clearly demonstrated that excessive apoptosis can directly lead to hepatocellular carcinoma by driving compensatory proliferation that leads to an accumulation of transforming genetic mutations.[5] Elegant studies employing tissue specific genetic ablation of caspase activity have also recently demonstrated benefit in preventing tumor development in models of hepatocellular carcinoma.[6] Caspases are now recognized to govern, network and integrate with other pathways that control the fate of a cell well beyond the classical understanding of the biology associated with this class of enzymes. Paradoxically, it has also been shown that the addition of small molecule caspase inhibitors can in fact improve the activity of cytotoxic agents in models of cancer.[7] Some cancers have even been shown to require caspase activity for their survival.[8] Thus, the biology caspases is considerably more complex than previously appreciated and their inhibition can give rise to apparently paradoxical results when viewed from the standpoint of our classical understanding of the role of these enzymes. Here we report the effect of the emricasan on measures of serum caspase activity in healthy human volunteers. We believe that this is the first report to describe the effect of a potent pan-caspase inhibitor on measures of caspase activity and apoptosis in healthy volunteers.

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[9] and NASH.[10] Circulating levels of caspase-cleaved CK-18 in healthy volunteers are also well documented and are likely reflective of on-going normal cellular homeostasis.

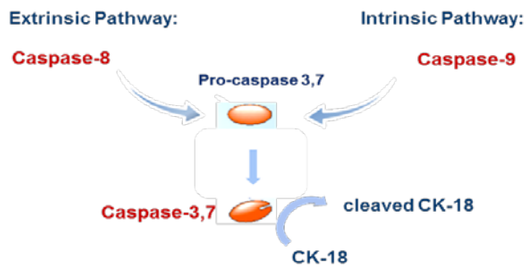

Figure 1: Caspase activation pathways and caspase cleavage of cytokeratin 18

We previously reported that emricasan rapidly reduced levels of both caspase 3/ 7 enzymatic activity and caspase cleaved CK-18 in chronic HCV patients.[3] Statistically significant reductions from baseline values were very rapidly achieved for both analytes at 3 hours following the first dose of emricasan on Day 1, Figures 2 and 3. In addition the effect on both analytes was durable throughout the dosing period of this study in HCV patients. We also reported that regardless of dose, caspase cleaved cytokeratin-18 titers were reduced to within ranges typically observed in healthy volunteers. In addition, caspase enzymatic activity was reduced but not abolished. Together, these observations demonstrate that emricasan can rapidly and predictably reduce both caspase enzymatic activity and apoptosis but, regardless of dose, does not abolish either.

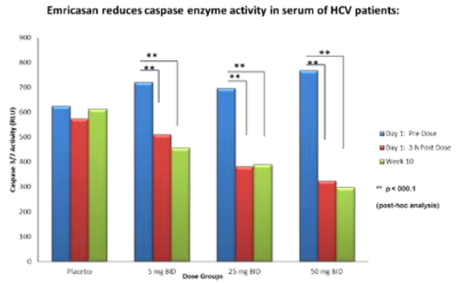

Figure 2: Mean reduction of caspase 3/7 enzymatic activity as determined by luminescent assay

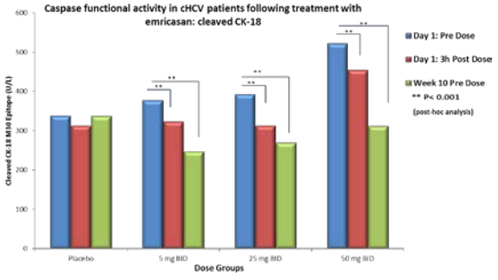

Figure 3: Mean reduction of caspase cleaved cytokeratin 18 as determined by M30 (Apoptosense ELISA)

Methods:

A total of 15 healthy male and female volunteers were enrolled in an open label pharmacokinetic interaction study between emricasan and cyclosporine and to evaluate the effect of emricasan on markers of apoptosis in healthy volunteers. A dose of 25 mg BID of emricasan was chosen for this trial based upon PK / PD modeling studies conducted to support dose selection for the upcoming Phase 3 clinical trial.

The ELISA based monoclonal antibody assay (M30-Apoptosense ELISA, PREVIA) was employed in this study for the determination of serum titers of caspase-cleaved cytokeratin-18.

This pharmacokinetic interaction study was divided into two Treatment Periods. Treatment Period 1, (Study Days 1 – 9) and Treatment Period 2 (Study Days 17 – 24). A 7-day washout period, (Study Days 10 – 16), was inserted between the two Treatment Periods. This study also included a follow up period from Days 25 -33.

Serum levels of caspase-cleaved cytokeratin-18 were measured on Study Days 1, 17-20, 22 and 24 – 27.

On study Day 1, a pre-dose blood sample was collected and, following a single 25 mg dose of emricasan, blood samples were collected serially over a period of 12 hours. The dosing schedule during the remainder of Treatment Period 1 was designed to assess pharmacokinetic interactions between emricasan and cyclosporine.

On study Days 17 – 26, emricasan was administered 25 mg BID. On study Day 24 a single dose of cyclosporine co-administered with emricasan.
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 caspase-mediated apoptosis as well as a measure of functional caspase activity on a physiological substrate.

Results:

The mean pre-dose serum titer of caspase-cleaved cytokeratin 18 on study Day 1 in this cohort of healthy male and female volunteers was 275 U/L. Pre-dose levels and serial monitoring of serum titers of caspase-cleaved cytokeratin-18 following administration of emricasan was conducted on study Days 1, 17 and 24, Figure 4. As can be seen, emricasan had no significant effect on serum titers of caspase cleaved cytokeratin-18 at any time point in these healthy volunteers. This is in sharp contrast to the rapid and statistically significant reduction of serum levels of caspase-cleaved cytokeratin-18 at this dose in chronic HCV patients.

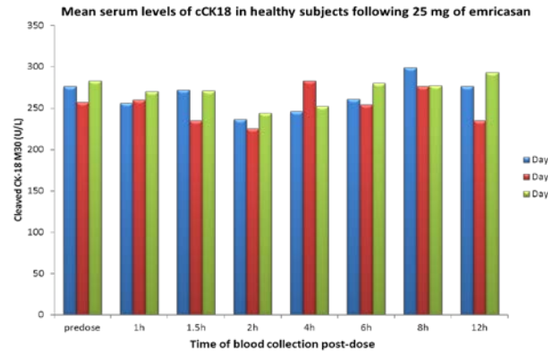

Figure 4: Mean serum levels of cCK18 in healthy subjects following serial blood draws post-oral dosing with emricasan

Additionally, pre-dose titers of cleaved cytokeratin-18 we unchanged throughout the course of this study, regardless of the dosing protocol, Figure5.

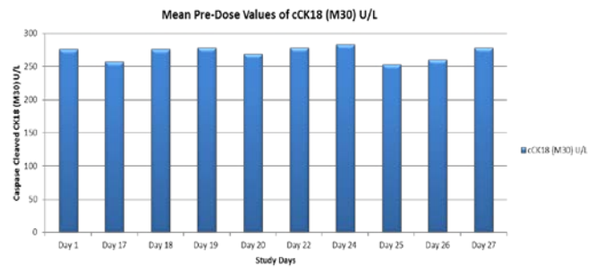

Figure 5: Mean pre-dose serum levels of caspase-cleaved cytokeratin-18 in healthy subjects

The data and observations presented above suggest that the pan-caspase inhibitor, emricasan, can effectively reduce elevated levels of caspase activity and apoptosis in patients with liver disease, but appears to have minimal, if any, impact on homeostatic levels of apoptosis as measured in healthy volunteers. This may have important implications about the potential of chronic dosing and long term safety of emricasan.

We believe that this is the first publication to describe the effect of pan-caspase inhibition on markers of caspase activity and apoptosis in healthy individuals. These data provide important new insight and increase our understanding of the effect of small molecule pan-caspase inhibitors in man. These observations are also consistent with the large pre-clinical database of safety information along with the clinical experience with emricasan, both of which are remarkably devoid of theoretically anticipated mechanism-driven side effects.

Conclusions:

- In healthy volunteers, the potent small molecule pan-caspase inhibitor emricasan does not reduce serum levels of caspase-cleaved cytokeratin-18, a well described marker of caspase activity and apoptosis.
- This observation suggest that the on-going caspase dependent processes in healthy individuals are largely unaffected by emricasan.
- These results suggest that normal levels of apoptosis and caspase activity remain functionally intact following administration of emricasan, thereby decreasing the potential of safety signals specifically associated with caspase inhibition.

References:

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