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 in serum sample. While this is a direct measure of caspase 3/7 activity, as shown in Figure 1, it also serves as a surrogate measure of the potential to interfere with normal homeostasis of apoptosis. An ELISA based monoclonal antibody assay that captures a caspase 3/7 cleaved cytokeratin-18 was employed as a second assay in this study, (M50-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 are shown in Table 1. There was no change in either analyte in the placebo cohort. This is constant with no observed change in transaminase levels in this group. 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.

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 maintained at the week 10 study visit. The pre-dose levels correspond to trough levels of emricasan while the post-dose values 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.

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 seen in healthy volunteers.
• Minimal to no reductions of cleaved CK-18 were observed in patients randomized to placebo.
• Observed dose proportional exposure of emricasan with no evidence of drug-drug interactions.
• 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:

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.

The data and observations presented above suggest that the pan-caspase inhibition by emricasan is associated with significant levels of apoptosis 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.

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