Title: **PAN-CASPASE INHIBITION PROTECTS AGAINST FIBROTIC NASH INDUCED BY CHOLINE DEFICIENT AMINO ACID DEFINED DIET (CDAA)**

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Text: Hepatocyte cell death is a key feature of nonalcoholic steohepatitis (NASH). Emerging data suggest that inhibition of caspases may be an attractive therapeutic approach for patients with NASH. Our **aim** was to test the hypothesis that the pan-caspase inhibitor IDN-6556 reduces hepatocellular apoptosis and fibrosis in a pathophysiological relevant murine model of human NASH.

Methods: C57BL/6 mice, aged 6 to 8 weeks at the beginning of the study, were fed either a choline deficient amino acid-defined (CDAA), a choline-sufficient amino acid-defined (CSAA) or a low-fat chow diet for 20 weeks. At 16 weeks the mice fed the CDAA diet were treated with 3 mg/kg/day of IDN-6556 (n=12) or with a placebo (n=12), via gavage, for 5 weeks. After 5 weeks the mice were sacrificed and their livers and blood were collected. Hepatocellular damage, fibrosis and inflammatory activity were assessed by liver histopathology, hepatic triglyceride (TG) quantification, serum ALT levels, and immunoblotting. Markers of hepatic stellate cell (HSC) activation including alpha-smooth muscle actin (alpha-SMA), collagen 1-alpha (COL1A1), and transforming growth factor-Beta (TGF-beta) were determined by real time qPCR.

Results: Treated mice showed improved liver histology with significant reduction in fibrosis, determined by morphometric analysis of collagen using Sirius red staining (Treated 4.81 ± 1.18 vs. Placebo 8.58 ± 2.12 percentage of fibrotic area, p = 0.037) when compared to placebo mice. Mice in the treatment group also demonstrated improved insulin sensitivity compared to placebo mice (0.112 ± 0.0041 vs. 0.0084 ± 0.0029 ng/ml, $P = 4.1 \times 10^{-4}$). Insulin function assessment via homeostatic model assessment (HOMA) displayed ameliorated insulin sensitivity in treated mice compared to placebo mice (P = 0.048). However, ALT, AST, TG, and FFA levels were similar in drug treated and placebo treated mice. In addition, we examined the role of the pan-caspase inhibitor in hepatic apoptosis. Liver mitochondrial fractions from drug treated mice were immunoblotted and showed reduced expressions of the pro-apoptotic proteins, Bid and Bax compared to placebo mice.

In conclusion, we have demonstrated that the pan-caspase inhibitor IDN-6556 improved insulin sensitivity and attenuated hepatic fibrosis and apoptosis in mice with CDAA diet-induced NASH.