Title: **SAFETY AND EFFICACY OF THE PAN-CASPASE INHIBITOR IDN-6556 ON THE TREATMENT OF NONALCOHOLIC FATTY LIVER AND INSULIN RESISTANCE**

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Text: Hepatocellular and extrahepatic cell death are key features of obesity-associated fatty liver disease (NAFLD) and metabolic syndrome. Emerging data suggest that inhibition of caspases may be an attractive therapeutic approach for patients with these conditions. Our **aim** was to test the hypothesis that the pan-caspase inhibitor IDN-6556 will improve hepatic steatosis and metabolic abnormalities commonly observed with NAFLD, such as insulin resistance and dyslipidemia.

Methods: C57BL/6 mice, aged 6 to 8 weeks at the beginning of the study, were fed either a high fat (HFAT) "western" diet or a low fat control diet for 20 weeks. During the last 5 weeks mice fed the HFAT died were treated with 3 mg/kg/day of IDN-6556 (n=12) or with a placebo (n=12), via gavage.Mice were then sacrificed and their livers, adipose tissue (epididymal) and blood were collected. Liver and adipose tissue inflammation and cell death was assessed by histopathology, TUNEL assay, immunoblotting, F4/80 immunohistochemistry, and real time qPCR for pro-inflammatory cytokines (IL-1beta, TNF-alpha, and IL-6). Liver injury was further determined by serum ALT levels and alpha-smooth muscle actin (alpha-SMA), collagen 1-alpha (COL1A1), and transforming growth factor-Beta (TGF-beta) by real time qPCR.

Results: Infiltration of pro-inflammatory macrophages into epididymal white adipose tissue (eWAT), assayed by immunohistochemistry staining for $F4/80^+$ crown-like structures, was reduced in treated mice compared to placebo mice. Despite the similarity in body weight, treated mice showed a decrease in terminal blood glucose levels compared to placebo mice ($P = 0.0037$). Interestingly, IDN-6556 treatment resulted in improved insulin function as measured by plasma insulin ELISA versus placebo (0.23 ± 0.089 vs. 0.97 ± 0.26ng/ml, $P = 0.023$). We examined the inhibition of caspase proteins by IDN-6556 in the eWAT and saw significant reduction in apoptosis in the treated versus the placebo group ($P = 0.0072$). In addition, IDN-6556 also caused decreased hepatic steatosis in treated mice compared to placebo mice. We concluded that IDN-6556 treatment ameliorated insulin resistance in HFD-fed mice and attenuated inflammation as measured by diminished levels of adipocyte macrophage infiltration. Furthermore, we observed that the pan-caspase inhibitor reduced apoptosis in the eWAT and improved hepatic steatosis in diet-induced obesity. In conclusion, are study demonstrates that the pan-caspase inhibitor IDN-6556 protects adipocyte cell death associated with obesity and improves insulin sensitivity and

hepatic steatosis