Mitochondrial Uncoupler GEN-3026 (HU-6) Successfully Treats NASH in DIAMOND™ Mouse Model

R. CAFFREY[1], J. MARIONEAUX[1], P. SANTHEKADUR[1], P. BEDOSSA[1], F. PORTELL[2], S. EATON[2], J. DENNIS[2], I. ONYANGO[2], O. KHAN[2], S. KHAN[2] AND A. SANYAL[3]

1 Sanyal Biotechnology, Norfolk, Virginia, U.S.A. 2 Gencia Biotech, Charlottesville, Virginia, U.S.A. 3 VCU, Richmond Virginia, U.S.A

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INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is caused by caloric overconsumption in humans. Excess lipids accumulate in the liver and lead to cirrhosis, liver cancer and death. Sanyal Biotechnology has developed a proprietary isogenic mouse strain, DIAMOND™ (Diet Induced Animal Model of Non-Alcoholic fatty liver Disease), mice which develop NAFLD, NASH, Fibrosis and HCC in response to a high-fat high-sugar Western Diet. The mice become insulin resistant, obese, and dyslipidemic and the model recapitulates the key pathological changes seen in humans with progressive NASH, thus serving as an excellent animal model of NASH.

The increased consumption of the western diet leads to an excess amount of metabolic substrate. Typically, substrate is transported to mitochondria where the processes of beta oxidation and the TCA cycle oxidize fat carbons to CO_2. When excess substrate is transported to liver mitochondria, incomplete beta oxidation and reduced TCA cycle flux fail to oxidize fat carbons to CO_2 and excess fat is deposited in the liver. Lipid accumulation sets the stage for the increased reactive oxygen species (ROS), inflammatory signaling and pro-fibrotic metabolism in the liver of NASH.

Gencia has developed a novel uncoupler, GEN-3026 (HU6), that diverts fat carbons from lipid droplet formation and increases substrate utilization via mitochondrial beta oxidation and Krebs Cycle. This uncoupler has a large therapeutic safety window in rodents, demonstrates a significant reduction in liver fat, and an improvement of key markers of NASH progression.

AIMS

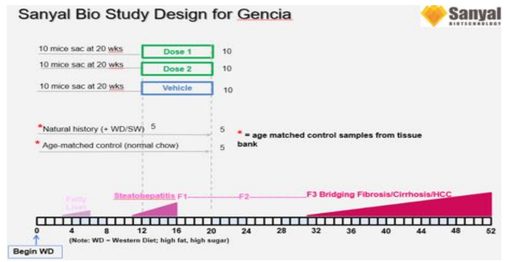
(1) To determine the efficacy of the liver-targeted mitochondrial uncoupler, GEN-3026 (HU-6) in preventing the progression of NASH

(2) To determine an effective dose of HU-6 capable of reducing steatosis in a relevant *in vivo* model of NASH

METHODS

In this study design, mice were grouped into three groups: 1 mg/kg HU-6 (Low Dose), 5 mg/kg HU-6 (High Dose), and vehicle control (VC). The WD/SW positive (PC) and NC/NW negative (NC) serum controls were age-matched samples of liver and serum aliquots from Sanyal Biotechnology's tissue bank. Mice were raised for 12 weeks on diet, corresponding to a baseline NASH with mild fibrosis in the WD/SW groups. Treatment groups were then orally gavaged once daily with aqueous vehicle or drug in vehicle for 8 weeks while continuing on Western Diet. At 20 weeks, the mice were euthanized and samples collected for serum analysis (AST, ALT, ALP, Total Cholesterol) and histopathology assessment (liver embedded in FFPE blocks and resulting slides stained with H&E and Sirius Red) . The liver histology included H&E and Sirius Red staining. 3-5 slides per mouse per stain type are cut and sent to Dr. Pierre Bedossa, MD, Ph.D, Director of Department of Pathology at Hospital Beaujon (Paris, France) who performed staining, scoring and histopathology interpretation.

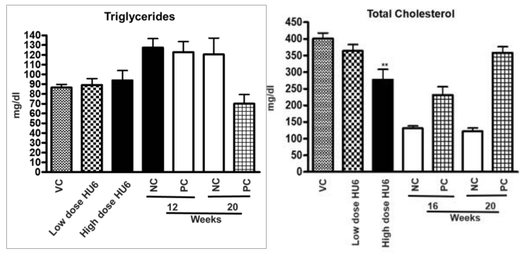
EXPERIMENTAL DESIGN



RESULTS

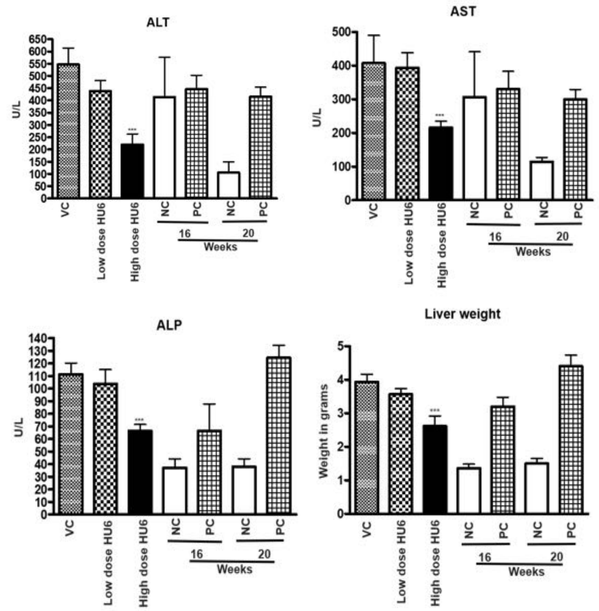
- Steatosis was significantly lower after eight weeks of 5mpk GEN-3026 (HU-6) QD PO treatment (percent and grade)
- ALT, ASP and ALP were significantly improved
- Ballooning was significantly improved
- Lobular inflammation was significantly reduced
- Cholesterol levels were significantly reduced
- Liver weight was significantly reduced, more closely approximating normal healthy liver
- Bodyweight was significantly lower in treated animals.
- SAF and NAS scores were significantly reduced
- Progression to NASH was avoided in all but one animal with GEN-3026 (HU6) treatment. All vehicle control animals had progressed to NASH after 20 weeks

GEN-3026 (HU-6) Reduces Total Cholesterol



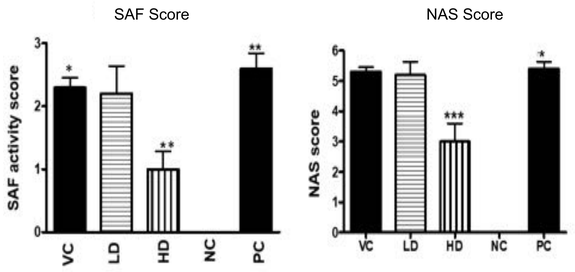
No significant difference in serum triglycerides was observed between HU-6 treated animals and vehicle control, thus ruling out a direct effect of HU-6 on triglyceride homeostasis. Treatment lead to a significant reduction in cholesterol levels which may contribute to reduced liver weight

GEN-3026 (HU-6) improves liver function after eight weeks treatment in DIAMOND™ Mice



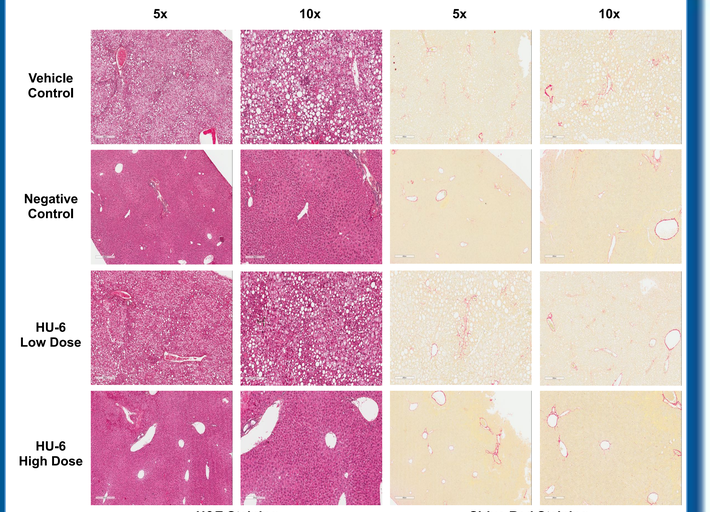
In mice, ALT is the best measure of liver function and elevation typically indicates liver damage. The significant decrease between vehicle control and 5mpk HU-6 treatment suggests that HU-6 attenuates liver damage in the model.

SAF and NAS are Significantly Improved



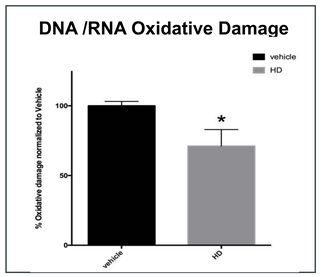
Steatosis Activity and Fibrosis (SAF) is the combination of steatosis, activity (ballooning and inflammation) and fibrosis measures. NASH Activity Score (NAS) is the sum of steatosis, ballooning and lobular inflammation scores and is used as a measure of disease severity.

H&E and Sirius Red of FFPE Liver sections



GEN-3026 (HU-6) reduces Oxidative Damage

Both DNA and RNA damage by oxidative stress has been observed in NASH patients. Diamond mice faithfully reproduce this phenotype at 20 weeks. DNA/RNA damage are significantly reduced after eight weeks of treatment with GEN-3026 (HU-6) (5mpk).



CONCLUSIONS

GEN-3026 (HU-6) successfully met the primary study endpoint of treating and preventing progression of NASH. Significant improvements in body and liver weight, serum LFTs and lipids, and liver pathology were observed in the 5mg/kg dose group. GEN-3026 (HU-6) warrants further clinical development and additional studies at higher dose groups.

CONTACT INFORMATION

Gencia Biotech: frportell@genciabiotech.com

Sanyal Biotechnology: info@sanyalbio.com