Abstract # P4

Subcutaneous Administration of a Novel Glycomimetic Compound (GMI-1600) with Functional Antagonism to Galectin-3 Inhibits the Progression of NASH through the Attenuation of Fibrosis and Hepatocyte Ballooning

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Abstract

Galectin-3 (Gal-3), a β-galactoside binding protein, is implicated in the progression of non-alcoholic fatty liver disease (NAFLD) including non-alcoholic steatohepatitis (NASH). In the current investigation we assessed the impact of a novel galectin-3 glycomimetic antagonist, GMI-1600, administered subcutaneously on the progression of NASH. NASH was established in streptozotocin-treated male C57BL/6 mice fed a high fat diet beginning at 4 weeks of age. At six weeks of age mice were randomized (n=8/group) and treated daily for 3 or 6 weeks with 40 μg/kg GMI-1600 or vehicle control. At study conclusion (12 weeks of age) mice were necropsied and blinded-samples were analyzed using clinical, biochemical and histological determinants, with an emphasis on NAFLD score (NAS) and fibrotic area proximal to the central vein. Image analysis demonstrated treatment with GMI-1600 resulted in a marked decrease in the fibrotic area with statistical significance achieved following 6 weeks of administration compared with vehicle alone (68% decrease in fibrosis, p<0.001). This reduction in hepatic fibrosis following treatment with GMI-1600 was coincident with a decreasing trend in plasma galectin-3 level, but not the liver-associated fibroblast pool as assessed by phenotypic markers. Treatment with GMI-1600 also showed a statistically significant decrease in NAS after 6 weeks of administration compared with vehicle alone (p<0.05). The improvement in NAS was attributable to a reduction in hepatocyte degeneration and ballooning. Consistent with this histological finding GMI-1600 affected a decreasing trend in plasma ALT levels. Since hepatocyte ballooning and liver fibrosis are hallmarks in disease progression of NASH, we conclude that the subcutaneous administration of 40 μg/kg GMI-1600 improved NASH pathology through the inhibition of hepatocyte damage and hepatic fibrosis as mediated by galectin-3 antagonism.

Background

- NASH is an aggressive form of fatty liver disease, which is marked by liver inflammation and progression to cirrhosis/fibrosis and liver failure.
- Galectin-3 is linked to hepatic disease involving cirrhosis/fibrosis:
  - Galectin-3 is elevated in alcoholic and non-alcoholic cirrhosis and in toxic hepatitis
  - Galectin-3 is a prognostic biomarker of hepatocellular carcinoma, a known complication of liver cirrhosis
- GMI-1600 is a novel glycomimetic that binds to the carbohydrate recognition domain of Galectin-3 and prevents its interaction with Galectin-3 binding proteins

Results

Figure 1. Specificity of GMI-1600 Antagonism to Galectin-3

The IC50 of GMI-1600 for inhibition of human Galectin-3, -1 or -9 binding to Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-PAA-biotin polymer was determined by ELISA. Fixed concentrations of galectin-3 (2 μg/mL), galectin-1 (1 μg/mL), or galectin-9 (0.2 μg/mL) were incubated with GMI-1600 (20-2000 μM) and transferred to polymer coated plates. The wells were incubated for 90 minutes and the resulting absorbance due to streptavidin/biotin interaction was assessed. The IC50 (nM) of GMI-1600 for inhibition of galectin binding to the polymer was determined for each galectin.

	Galectin-3	Galectin-9	Galectin-1
GMI-1600	30	100	1880

Results (cont.)

Figure 2. Experimental Design and Treatment Schedule

NASH was induced in C57BL/6J male newborn mice by a single subcutaneous injection of streptozotocin solution 2 days after birth and feeding with a high fat diet after four weeks of age and provided to the mice ad libitum. At study week 6, mice were randomized (n=8/group) and treatment with vehicle or GMI-1600 (40 μg/kg) were administered subcutaneously in a volume corresponding to 5 mL/kg body weight. Telmisartan was included as a positive control for the NASH model and administered at a dose of 10 mg/kg once daily. Mice were sacrificed at study week 6 to obtain baseline parameters and at study conclusion (week 12). All animals used in the study were housed and cared for in accordance with the Japanese Pharmacological Society Guidelines for Animal Use. The animal study protocols used in this study were approved by the animal use ethics committee at the Stelic Institute.

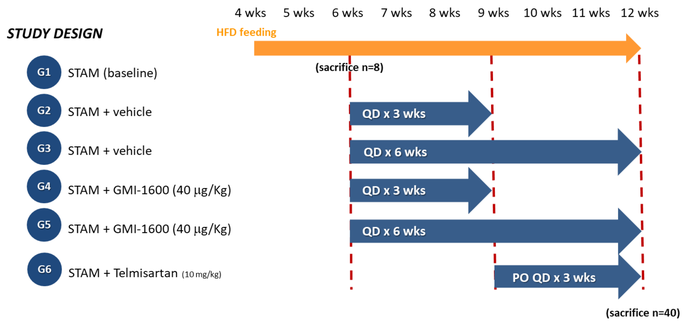
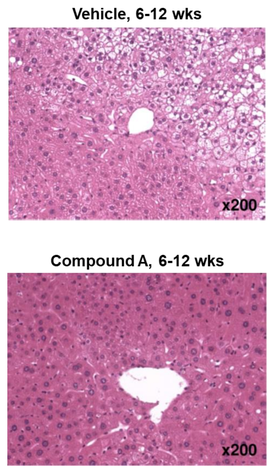
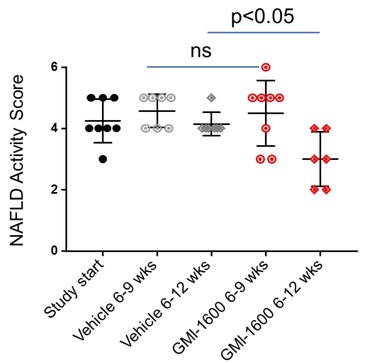


Figure 3. NAFLD Activity Score (NAS) is Significantly Decreased following GMI-1600 Administration

NAFLD Activity Score (NAS) was calculated according to previously described criteria. Scoring using NAS was performed by a pathologist blinded to treatment groups using the three criteria of steatosis, hepatocyte ballooning, and lobular inflammation. These individual scores were summed to give the NAS for each animal. Differences between the vehicle groups and the GMI-1600 treatment groups were assessed by one way ANOVA followed by Bonferroni Multiple Comparison Test. *P* values <0.05 were considered significant and results expressed as mean ± SD.





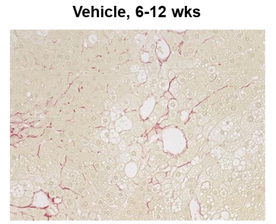
Summary (Figure 3)

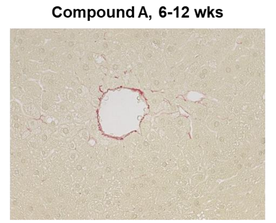
- Treatment with GMI-1600 showed a statistically significant decrease in NAS after 6 weeks of administration compared with vehicle alone (p<0.05)
- The improvement in NAS was principally attributed to a reduction in hepatocyte degeneration and ballooning
- Consistent with this histological finding GMI-1600 affected a decreasing trend in plasma ALT levels (data not shown)

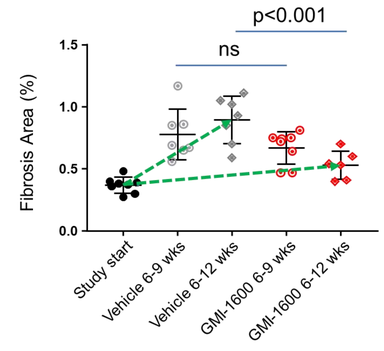
Results (cont.)

Figure 4. Quantification of collagen area.

For quantitative analysis of the fibrosis area, bright field images of Sirius red-stained sections were captured around the central vein using a digital camera at 200-fold magnification, and the positive areas in 5 fields/section were imaged using ImageJ software. Differences between the vehicle groups and the GMI-1600 treatment groups were determined as described above.







Summary (Figure 4)

- Image analysis demonstrated treatment with GMI-1600 resulted in a marked decrease in the fibrotic area with statistical significance achieved following 6 weeks of administration compared with vehicle alone (68% decrease in fibrosis, p<0.001)
- Comparison with the extent of fibrosis present at study start, demonstrates that GMI-1600 modified the progression of hepatic disease
- This reduction in hepatic fibrosis following treatment with GMI-1600 was coincident with a decreasing trend in plasma galectin-3 level (data not shown)

Conclusions

- Subcutaneous administration of GMI-1600, a Galectin-3 antagonist, in the STAM mouse model of NASH, improved progression of disease via inhibition of hepatocyte ballooning and periportal fibrotic development
- Additional glycomimetic antagonists targeting the CRD of Galectin-3 with improved potency and specificity showing oral bioavailability are being developed and will be assessed for therapeutic activity

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