A3907, a Novel Inhibitor of Bile Acid Transport in the Intestine and Kidney, Improves Markers of Metabolic and Hepatic Pathology, and Reduces Nonalcoholic Fatty Liver Disease Activity Score and Fibrosis Stage in a Diet-Induced and Biopsy-Confirmed Mouse Model of Nonalcoholic Steatohepatitis

Peter Åkerblad[1], Pål Lundin[1], Michael Feigh[2], Jacob Nøhr-Meldgaard[2], Patrick Horn[1], Jan P. Mattsson[1], Per-Göran Gillberg[1], Arun J Sanyal[3]

[1]Albireo Pharma, Inc., Boston, MA, USA; [2]Gubra Aps, Hørsholm, Denmark; [3]Division of Gastroenterology, Virginia Commonwealth University, Richmond, VA, USA

INTRODUCTION

- Bile acids are signalling molecules involved in lipid, glucose, and energy homeostasis[1]
- Because elevated bile acid levels are associated with nonalcoholic steatohepatitis (NASH), which is characterized by steatosis, inflammation, fibrosis, and loss of hepatic function, inhibition of bile acid transporters is a potential therapeutic option to treat NASH[2,3]
- A3907 is a selective apical sodium-dependent bile acid transporter (ASBT) inhibitor with oral bioavailability
- As a result, A3907 can inhibit ASBT in the intestine and kidney, with the potential to increase elimination of bile acids by both fecal and urinary excretion
- The objective of these preclinical studies was to characterize the effects of A3907 in Gubra Amylin NASH (GAN) diet–induced ob/ob mice with biopsy-confirmed NASH with fibrosis

METHODS

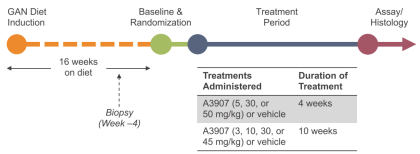
Animals

- Leptin-deficient ob/ob mice were pre-fed a GAN diet high in fat, fructose, and cholesterol for a total of 16 weeks prior to study start (**Figure 1**)
- Four weeks prior to A3907 treatment, all animals underwent liver biopsy for histological confirmation of liver pathology (defined as steatosis score ≥2 and fibrosis stage ≥1)

Treatments

- After randomization, A3907 (3−50 mg/kg) or vehicle was given by oral gavage once daily for either 4 or 10 weeks (**Figure 1**)

Figure 1. Study Design, Dosing, and Duration of Treatment in GAN Diet–Induced ob/ob Mouse Models of NASH



Treatments Administered	Duration of Treatment
A3907 (5, 30, or 50 mg/kg) or vehicle	4 weeks
A3907 (3, 10, 30, or 45 mg/kg) or vehicle	10 weeks

GAN, Gubra Amylin NASH; NASH, nonalcoholic steatohepatitis.

Assessments

- Metabolic, biochemical, histological, and gene expression analyses were conducted
  - Specific assays included measure of plasma and liver parameters, histomorphometry, RNAseq, immunohistochemistry, and histopathological scoring for Nonalcoholic Fatty Liver Disease (NAFLD) Activity Score and fibrosis stage (pre- vs post-treatment)

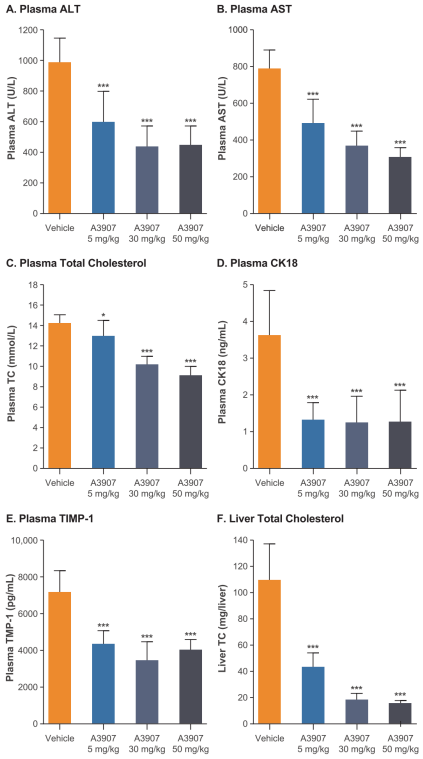
RESULTS

Short-Term Treatment Effects

- With 4 weeks of A3907 treatment, plasma levels of transaminases, total cholesterol, and markers of cell damage and fibrosis (cytokeratin-18 [CK18] and tissue inhibitor of metalloproteinase-1 [TIMP-1]) were significantly reduced (**Figures 2A–2F**)
- A3907 also reduced liver weight: in animals treated with vehicle control for 4 weeks, the mean liver weight was 6.7 g; in animals treated with A3907 at doses of 5, 30, or 50 mg/kg, mean liver weight was 5.0, 4.9, and 5.7 g, respectively

RESULTS (CONT'D)

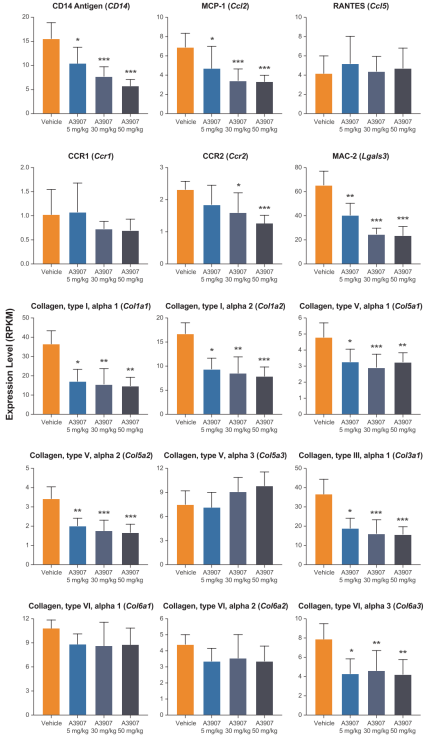
Figure 2. Mean (SD) Effects on Biochemical and Hepatic Parameters After 4 Weeks of A3907 Treatment



Mean (SD) values depicted for n=6–10 animals per group.
*P<0.05, ***P<0.001 compared with vehicle, Dunnett's test one-factor linear model.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK18, cytokeratin 18; TC, total cholesterol; TIMP 1, tissue inhibitor of metalloprotease-1.

- Liver RNAseq analysis indicated that A3907 modulated pathways involved in monocyte recruitment and stellate cell activation (**Figure 3**), as well as pathways involved in cholesterol metabolism and bile acid transport (data not shown)

Figure 3. Select Gene Expression Changes With 4 Weeks of A3907 Treatment

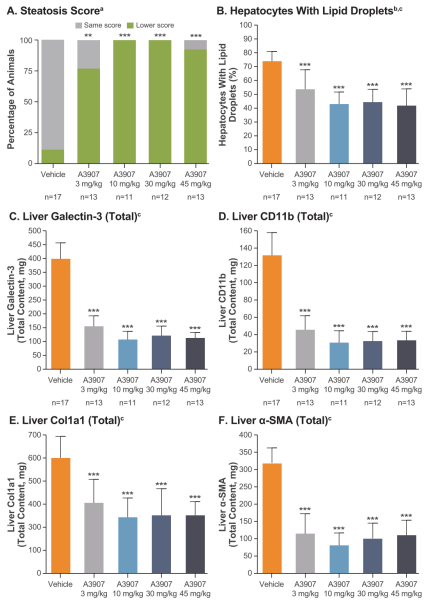


Mean (SD) values depicted for n=6–8 animals per group.
*P<0.05, **P<0.01, ***P<0.001 compared with vehicle after correction for gene-wise multiple testing.
CCR, C-C motif chemokine receptor; CD, cluster of differentiation; COL, collagen; MAC, macrophage-associated lectin; MCP, monocyte chemoattractant protein; RANTES, regulated upon activation, normal T cell expressed and presumably secreted; RPKM, reads per kilobase per million.

Longer-Term Treatment Effects

- Detailed histological assessments performed after 10 weeks of A3907 treatment revealed that liver steatosis score (pre- vs post-treatment) and the percentage of hepatocytes with lipid droplets were reduced in response to A3907 (**Figures 4A** and **4B**)
- In addition, histological markers of inflammation (eg, galectin-3, CD11b) were significantly reduced by A3907 treatment (**Figures 4C** and **4D**), and histomorphometry analyses revealed that A3907 also reduced liver collagen 1a1 and α-smooth muscle actin levels (**Figures 4E** and **4F**)

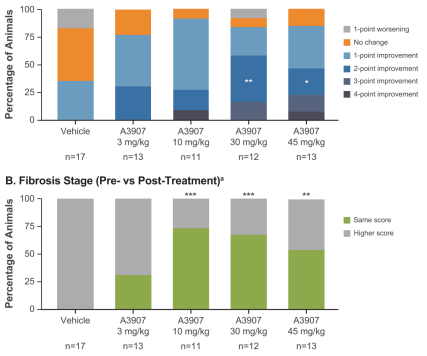
Figure 4. Histological Findings After 10 Weeks of Treatment With A3907



[a]Pre-treatment versus post-treatment score. The percentage of animals with a higher score (worsening), or the same or a lower score (improvement) is indicated by the heights of the bars. Significance determined using a one-sided Fisher's exact test with Bonferroni correction. **P<0.01, ***P<0.001 compared with vehicle.
[b]Determined by deep learning–based image analysis.
[c]Significance determined using Dunnett's test one-factor linear model. ***P<0.001 compared with vehicle.
Panels B–F depict mean (SD) values.
α-SMA, alpha smooth muscle actin; CD, cluster of differentiation; Col1a1, collagen, type 1, alpha 1.

- After 10 weeks, liver weight was 6.7 g in animals treated with vehicle and 4.8, 4.3, 4.4, and 4.5 g in animals treated with A3907 at doses of 3, 10, 30, or 45 mg/kg, respectively
- Importantly, A3907 reduced the NAFLD Activity Score by ≥2 points in >50% and >40% of animals at 30 and 45 mg/kg, respectively (*P*<0.01 and *P*<0.05 vs vehicle, respectively; **Figure 5A**) and prevented fibrosis stage progression in >50% of animals at doses of 10, 30, and 45 mg/kg (*P*<0.001, *P*<0.001, and *P*<0.01 vs vehicle, respectively; **Figure 5B**)

Figure 5. NAFLD Activity Score and Fibrosis Stage After 10 Weeks of Treatment With A3907



[a]Significance determined using a one-sided Fisher's exact test with Bonferroni correction. *P<0.05, **P<0.01, ***P<0.001 compared with vehicle.
NAFLD, nonalcoholic fatty liver disease.

CONCLUSIONS

- In the GAN diet–induced, ob/ob mouse model of biopsy-confirmed NASH with fibrosis, A3907 treatment produced profound improvement in key plasma and liver histological markers of metabolic-associated NASH
- Importantly, A3907 treatment improved clinically relevant histopathological scoring and demonstrated no worsening of fibrosis
- These findings introduce combined renal/intestinal ASBT inhibition, and A3907 treatment in particular, as a novel intervention for NASH and fibrotic progression

REFERENCES

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AUTHOR DISCLOSURES

P. Åkerblad, J.P. Mattsson, P.-G. Gillberg, and P. Horn are employees of and own stock/stock options in Albireo. P. Lundin is a consultant for Albireo. M. Feigh and J. Nøhr-Meldgaard are employees of Gubra. A.J. Sanyal is President of Sanyal Biotechnology and has stock options in Genfit, Akarna, Tiziana, Indalo, Durect Inversago, and Galmed. He has served as a consultant to Astra Zeneca, Nitto Denko, Conatus, Nimbus, Salix, Tobira, Takeda, Jannsen, Gilead, Terns, Birdrock, Merck, Valeant, Boehringer-Ingelheim, Bristol Myers Squibb, Lilly, Hemoshear, Zafgen, Novartis, Novo Nordisk, Pfizer, Exhalenz, and Genfit. He has been an unpaid consultant to Intercept, Echosens, Immuron, Galectin, Fractyl, Syntlogic, Affimune, Chemomab, Zydus, Nordic Bioscience, Albireo, Prosciento, and Surrozen. His institution has received grant support from Gilead, Salix, Tobira, Bristol Myers Squibb, Shire, Intercept, Merck, Astra Zeneca, Mallinckrodt, Cumberland, and Novartis. He receives royalties from Elsevier and UptoDate.

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