1813-P

Coadministration of a GLP-1/Glucagon Receptor Agonist With an FXR Agonist and ACC Inhibitor Reverses Nonalcoholic Steatohepatitis in Diet-Induced and Biopsy-Confirmed Mice

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Introduction

- Cilofexor (CILO) is a farnesoid X receptor (FXR) agonist in clinical development for nonalcoholic steatohepatitis (NASH), and demonstrates dose-dependent reductions in liver fat in patients with NASH and F2–3 fibrosis[1]; the additional benefit of combining CILO and firsocostat (FIR) vs monotherapy is currently being evaluated in patients with F3–4 fibrosis due to NASH (NCT03449446; results pending)
- FIR is a liver-targeted acetyl-coenzyme A carboxylase (ACC) 1/2 inhibitor that dose-dependently reduces liver fat (by magnetic resonance imaging–proton density fat fraction), and improves markers of liver injury in patients with NASH and F2–3 fibrosis[2-4]; in preclinical models, ACC inhibition via tool FIR analog GS-834356 (ACC inhibitor [ACCi]) reduced liver fat and fibrosis[5,6]
- The combined effects of CILO and FIR, and the glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide (SEMA) on liver fat and biomarkers of liver injury and fibrosis are also being evaluated in patients with nonalcoholic fatty liver disease (NAFLD; NCT03987074)
- The preclinical effects of CILO/ACCi/SEMA have been evaluated in the amylin liver (AMLN) transfat diet-induced obese mouse model of NASH (DIO-NASH)[7]
- Dual GLP-1 and glucagon receptor (GLP1:GcG) agonists have been reported to reduce liver fat in patients with type 2 diabetes[8]; however, the benefit of additional therapies is unknown

Objective

- To explore the effects of 12 wk of treatment with GLP1:GcG and/or CILO and/or ACCi on metabolic and liver pathology in AMLN DIO-NASH

Methods

AMLN DIO-NASH Study Design

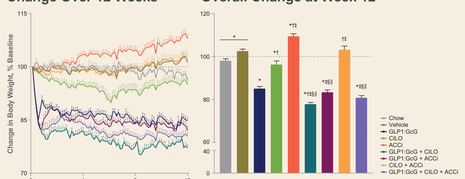
n	Group	po Test Agent	sc Test Agent
6	Chow	Vehicle	Vehicle
16	Vehicle	Vehicle	Vehicle
15	GLP1:GcG	Vehicle	GLP1:GcG 0.015 mg/kg
16	CILO	CILO 30 mg/kg	Vehicle
16	ACCi	ACCi 5 mg/kg	Vehicle
15	GLP1:GcG + CILO	CILO 30 mg/kg	GLP1:GcG 0.015 mg/kg
16	GLP1:GcG + ACCi	ACCi 5 mg/kg	GLP1:GcG 0.015 mg/kg
16	CILO + ACCi	CILO 30 mg/kg, ACCi 5 mg/kg	Vehicle
16	GLP:GcG + CILO + ACCi	CILO 30 mg/kg, ACCi 5 mg/kg	GLP1:GcG 0.015 mg/kg

- Male C57BL/6JRj mice (JANVIER LABS, Le Genest-Saint-Isle, France) were fed either a standard chow diet (Altromin 1324, Brogaarden ApS, Lynge, Denmark) or AMLN diet (D09100301, Research Diets, Inc., New Brunswick, NJ) from age ~5 wk
- After 29 wk on AMLN diet, mice were subjected to liver biopsy,[9] and randomized to test groups based on baseline liver steatosis (score ≥2) and fibrosis (stage ≥1)
- All mice were dosed qd po with either vehicle (0.5% sodium carboxymethylcellulose, 1% ethanol, 98.5% 50 mM tris buffer) or test agent, and sc with either vehicle (0.007% polysorbate 20, 50 mM sodium phosphate, 70 mM sodium chloride) or test agent, both at 5 mL/kg, for 12 wk
- Body weight was measured daily; blood was collected via tail vein during Week 12 after 4-h fast for measurement of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG), and total cholesterol (TC; cobas c 501 module, Roche Diagnostics, Indianapolis, IN)
- Mice were euthanized at the end of the study via isoflurane anesthesia in nonfasted state; liver tissue was collected, weighed, and subjected to TG and TC measurement or histologic analyses
- Histology and immunohistochemistry (IHC) were performed using published protocols[1]
  - Hemotoxylin and eosin (H&E) staining was used to determine clinically derived NAFLD Activity Score (NAS; sum of scores of 0–3 for steatosis and lobular inflammation, and 0–2 for ballooning); individual changes (pre to post) were evaluated by unbiased artificial intelligence–assisted Gubra Histopathological Objective Scoring Technique and confirmed by a blinded pathologist
  - Recombinant anti-α-smooth muscle actin (α-SMA) antibody (EPR5638 [ab124964], Abcam plc, Cambridge, MA), goat anti-type I collagen (Col1a1)–UNLB (Cat. No.1310-01, SouthernBiotech, Birmingham, AL), and cluster of differentiation molecule 11b (CD11b; antimelatonin receptor 1B/MTNR1B antibody [ab13357], Abcam) were detected using standard IHC procedures, and quantified using VIS Image Analysis Software (Visiopharm, Hoersholm, Denmark)
- Data are expressed as mean ± standard error of mean
- Statistical analyses:
  - Statistical significance was determined by 2-tailed unpaired Student's t-test (chow vs NASH vehicle groups) or 1-way analysis of variance (ANOVA) with Tukey post-hoc test (excluding chow group)
  - For NAS and fibrosis responder rate comparisons, Fisher exact test adjusted for multiple comparisons was performed using SAS 9.4 (SAS Institute Inc., Cary, NC)
  - All other graphs and analyses were generated using Prism 8.1.2 for Windows (GraphPad Software, San Diego, CA)

Results

Body Weight

Change Over 12 Weeks



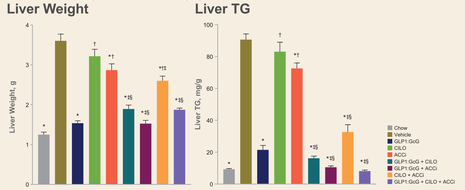
Overall Change at Week 12

*p <0.05 vs vehicle or as indicated between groups; †p <0.05 vs GLP1:GcG; ‡p <0.05 vs CILO; §p <0.05 vs ACCi; ||p <0.05 vs CILO + ACCi.

- Body weight was significantly reduced (15%) by GLP1:GcG treatment
- Further body weight loss was observed (22%) in GLP1:GcG + CILO group, but not with addition of ACCi or in triple-combination group

Liver Weight and Triglyceride Content at Week 12

Liver Weight



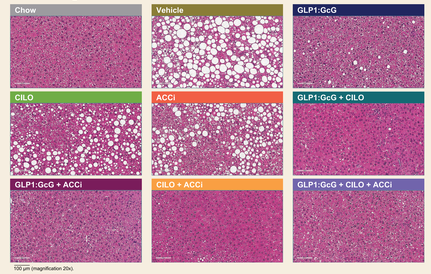
Liver TG

*p <0.05 vs vehicle; †p <0.05 vs GLP1:GcG; ‡p <0.05 vs CILO; §p <0.05 vs ACCi.

- AMLN DIO-NASH demonstrated increased liver weight and TG content
- Liver weight and TG were significantly reduced by GLP1:GcG
- Addition of ACCi and triple therapy reduced hepatic TG levels to those of chow controls

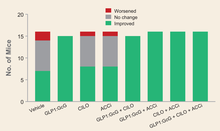
NAFLD Activity

H&E Staining of Terminal Liver Sections

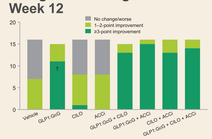


Change in NAS*

NAS Outcome at Week 12



Categorical Change in NAS at Week 12

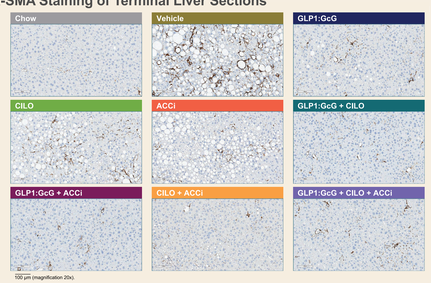


*Numbers inside circles represent no. of mice per score; †p <0.001 vs vehicle.

- AMLN DIO-NASH demonstrated increased NAS, primarily driven by steatosis and inflammation
- GLP1:GcG treatment significantly reduced NAS, which was minimally enhanced by addition of CILO or ACCi
- CILO + ACCi combination similarly improved NAS
- Improvement in NAS by ≥3 points required >1 pharmacology

α-SMA as Marker of Fibrogenesis

α-SMA Staining of Terminal Liver Sections



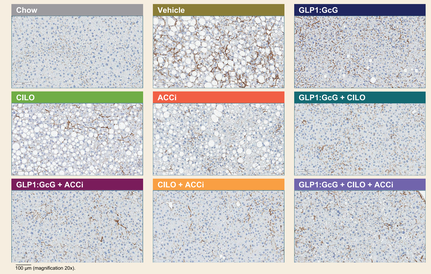
Morphometric Image Analysis Quantitation of α-SMA

*p <0.05 vs vehicle; †p <0.05 vs CILO; ‡p <0.05 vs ACCi.

- AMLN DIO-NASH demonstrated increased α-SMA deposition
- All groups significantly reduced α-SMA % fractional area vs vehicle
- GLP1:GcG was the most effective treatment; there was no additional benefit with dual-combination or triple therapy

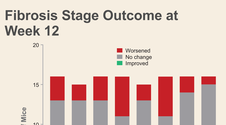
Fibrosis

Col1a1 IHC of Terminal Liver Sections

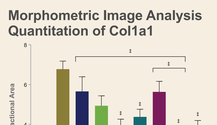


Change in Fibrosis Stage*

Fibrosis Stage Outcome at Week 12



Morphometric Image Analysis Quantitation of Col1a1

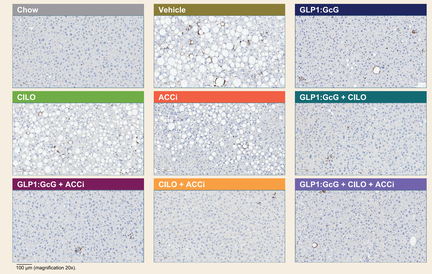


*Numbers inside circles represent no. of mice per stage; †p <0.05 vs GLP1:GcG; ‡p <0.05 vs vehicle or as indicated.

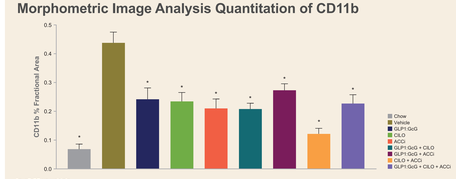
- AMLN DIO-NASH demonstrated intrahepatic Col1a1 formation and clinically derived fibrosis staging
- GLP1:GcG treatment did not significantly reduce Col1a1 % fractional area or lead to significant number of mice with improved fibrosis (n=1/15 [7%])
- Both CILO + ACCi and triple-combination therapy led to highest numbers of animals with improved fibrosis (n=5/16 [31%] and n=7/16 [44%], respectively), and the greatest reductions in Col1a1 % fractional area

CD11b

CD11b IHC of Terminal Liver Sections



Morphometric Image Analysis Quantitation of CD11b



*p <0.05 vs vehicle.

- AMLN DIO-NASH demonstrated increased CD11b deposition as a marker for liver inflammatory macrophage infiltration
- All groups significantly reduced CD11b vs vehicle

Terminal Plasma Biochemical Analyses at 12 Weeks

	Chow	Vehicle	GLP1:GcG	CILO	ACCi	GLP1:GcG + CILO	GLP1:GcG + ACCi	CILO + ACCi	GLP1:GcG + CILO + ACCi
ALT, U/L	33 ± 4	350 ± 33*	50 ± 3†	297 ± 42‡	118 ± $15^{\S}$	141 ± $37^{\S}$	49 ± $6^{\S\|}$	60 ± $6^{\S\|}$	100 ± $11^{\S\|}$
AST, U/L	67 ± 9	343 ± 31*	153 ± $12^{\S}$	359 ± $46^{\S\|}$	147 ± $13^{\S}$	392 ± $91^{\S\|\#}$	149 ± $18^{\S\|\S}$	106 ± $8^{\S\|}$	311 ± $54^{\S}$
TG, mg/dL	61 ± 5	40 ± 3*	37 ± 2	30 ± $1^{\S}$	44 ± $2^{\S}$	30 ± $2^{\S\|}$	51 ± $2^{\S\|}$	43 ± $4^{\S\|}$	39 ± 4
TC, mg/dL	99 ± 3	307 ± 14*	109 ± $5^{\S\|}$	163 ± $12^{\S\|\#}$	231 ± $6^{\S\|\S}$	92 ± $5^{\S\|\#}$	102 ± $7^{\S\|}$	132 ± $6^{\S\|}$	91 ± $6^{\S\|\#}$

*p <0.05 vs chow group (t-test); †p <0.05 vs vehicle; ‡p <0.05 vs GLP1:GcG; §p <0.05 vs CILO; ||p <0.05 vs ACCi (1-way ANOVA, Tukey's post-hoc test); #p <0.05 vs CILO + ACCi.

- AMLN DIO-NASH exhibited increased plasma levels of ALT, AST, and TC, with TG being below chow levels
- GLP1:GcG and ACCi significantly reduced ALT, which was not further decreased by addition of other agents
- Plasma TG was slightly elevated in ACCi groups and lower in CILO groups
- Plasma TC was elevated by diet and reduced in all test groups

Conclusions

- GLP1:GcG effectively reduced hepatic lipids, and the addition of CILO and ACCi led to greater reductions in NAS and fibrosis stage in AMLN DIO-NASH
- Whereas CILO and ACCi exhibited modest efficacy as monotherapy, the combination of CILO + ACCi significantly improved NASH and fibrosis in the absence of weight loss
- The effects of combination pharmacology on histologic markers of fibrosis approached the maximal effective therapeutic utility of the model
- Assessment of fibrosis endpoints demonstrated superior effect of combination vs monotherapy groups
- These data support the development of combination approaches for NASH

References: 1. Patel K, et al. Hepatology 2020 (in press); **2.** Harrison S, et al. J Hepatol 2018;68:S583; **3.** Lawitz EJ, et al. Clin Gastroenterol Hepatol 2018;16:1983-91; **4.** Loomba R, et al. Gastroenterology 2018;155:1463-73; **5.** Bates J, et al. J Hepatol 2018;68:S399-400; **6.** Goedeke L, et al. Hepatology 2018;68:2197-211; **7.** Norlin J, et al. ADA 2020, poster 1810-P; **8.** Ambery P, et al. Lancet 2018;391:2607-18; **9.** Oldham S, et al. J Vis Exp 2019:e59130.

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