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ABSTRACT BOOK

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are prone to steatosis-associated H4K16 acetylation and gamma H2AX.
Results: Liver triglyceride levels were more than double those of control animals after 6 months of high-fat diet in rats and 8 weeks of high-fructose diet in mice. Transcriptome and histone analysis in livers of rodent models of MAFLD confirmed that steatosis is associated with genome-wide loss of transcriptional repression and increased histone acetylation. *In vitro*, steatosis was associated with relaxed chromatin across the genome and increased gamma H2AX. ChIP-seq revealed that steatosis-associated gamma H2AX was enriched at highly expressed genes in pathways such as fat metabolism, histone acetylation and oxidative stress. Furthermore, known HCC tumour suppressor genes had increases in H4K16 acetylation and gamma H2AX. Finally, steatosis-associated gamma H2AX was reversed through inhibition of acetyl-CoA anabolism.
Conclusion: Our data point to metabolism-driven genome-wide epigenetic change being a new pathological mechanism in liver steatosis which promotes cellular dysfunction and carcinogenesis.

OS-1746
Cilofexor and firsocostat treatment is associated with widespread changes in the hepatic transcriptome in NASH patients with advanced fibrosis

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Background and aims: In the phase 2b ATLAS study, the combination of the ACC inhibitor firsocostat (FIR) and the FXR agonist cilofexor (CILO) led to histologic improvement in patients with advanced fibrosis (F3-F4) due to NASH. We investigated hepatic transcriptomic changes with treatment and associations with histologic responses and serum biomarkers.
Method: FFPE tissue blocks of liver biopsies were obtained from NASH patients with F3-F4 fibrosis in the ATLAS trial (NCT03449446). Fibrosis stage and the NAFLD Activity Score (NAS) were assessed at baseline (BL) and Week 48 (W48) by a central pathologist. Extracted RNA was used to quantify gene expression by RNA-seq. We evaluated gene expression and transcriptome pathway activity (ssGSEA score) at BL and summarized longitudinal changes by treatment group at W48. Transcriptomic pathways were from the Hallmark collection (Broad Institute). Pearson correlations between changes in transcriptome pathways and serum biomarkers were calculated. p values were adjusted using the Benjamini-Hochberg procedure to control the FDR at 0.1.
Results: Compared to BL, CILO+FIR treatment (n = 41) for 48 weeks was associated with significant up-regulation of 1015 genes and down-regulation of 320 genes, whereas no changes were observed with placebo (n = 20). Placebo-adjusted transcriptome pathway analysis demonstrated 20 significantly affected pathways with CILO+FIR, of which 8 were influenced by CILO treatment, 6 with FIR, and 3 were shared across treatment groups (Figure). The most up-regulated genomic pathway associated with CILO+FIR treatment was related to oxidative phosphorylation, whereas statistically significant changes in pathways or individual genes related to fibrosis (e.g., *COL1A1*, *COL1A2*, *TIMP1*, *ACTA2*, *TGFB*, and *PDGF*) were not observed. Integration of pathway analysis and serum biomarkers revealed significant inverse associations between changes in the bile acid metabolism pathway and serum total bile acids (r = –0.42), chenodeoxycholic acid (r = –0.41), and primary bile acids (r = –0.41) (all $p < 0.05$). Changes in the TNF-a signaling pathway, which was reduced by the combination of CILO+FIR, were associated with changes in the NAS (r = 0.39; $p < 0.05$) and fibrosis stage (r = 0.35; $p = 0.1$).

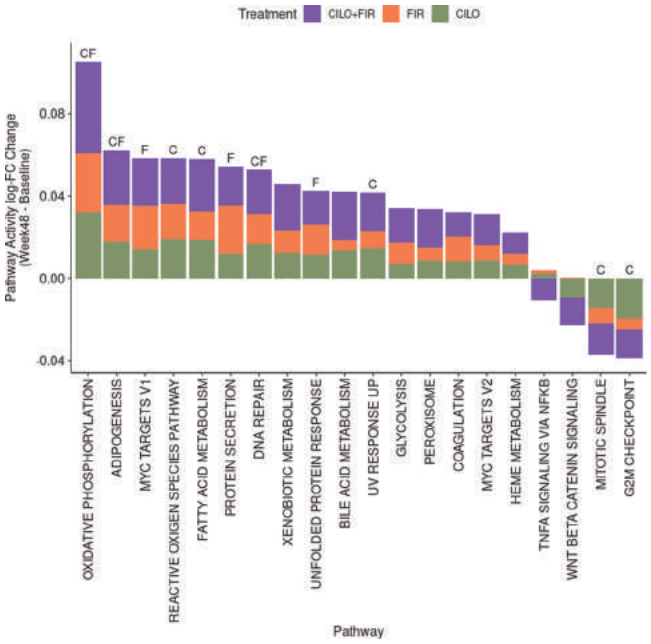

Figure: Each bar, representing log-fold- change (FC) for the pathway activity score, is divided to show the log-FC observed in each group. All depicted pathways are significantly affected by CILO+FIR treatment. Pathways that are also affected by the monotherapies are indicated with C (CILO) and/or F (FIR).

Conclusion: In NASH patients with advanced fibrosis, the combination of CILO+FIR led to widespread changes in hepatic gene expression and transcriptomic pathways. Significant associations were observed between changes in gene expression pathways and bile acid metabolism and inflammation with serum bile acids and changes in the NAS score, respectively.

OS-2450
First in class, orally active Toll-like receptor signaling inhibitor mosedipimod (PLAG) attenuates molecular, biochemical and histological features of non-alcoholic steatohepatitis (NASH) in vitro and in vivo

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Background and aims: There are no currently approved pharmacotherapies for non-alcoholic steatohepatitis (NASH). There is increasing evidence of an important role of the Toll-like receptors (TLRs) in initiation of the fibro-inflammatory cascade in NASH. Attenuating TLR signaling represents an attractive pharmacological strategy to prevent and reverse hepatic fibrosis. The acetylated diacylglycerol 1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol (PLAG, chemical name mosedipimod), is an orally active synthetic mono-acetyl-diglyceride that has been shown to attenuate TLR signaling.
Method: We studied the effects of mosedipimod on molecular, metabolic and histologic facets of NASH in two nutrient-based murine models: a high-fat, high-fructose (HFHF) model of steatosis and inflammation, and the STAM™ model of fibrosing NASH. Effects

of PLAG on palmitic acid-induced TLR4 signaling were also studied *in vitro*. In the STAM™ model, effects of PLAG were compared to two agents in advanced clinical trials, obeticholic acid (OCA) and resmetirom (MGL-3196).

Results: Mosedipimod significantly mitigated HFHF diet-induced hepatic steatosis, reducing lipogenesis-associated signaling, as measured by mRNA expression levels of ChREBP, SREBP-1c and FAS ($p < 0.05–0.001$), indicating attenuation of *de novo* lipogenesis by mosedipimod. Mosedipimod also reduced surface level expression of TLR4 and decreased TNF-α, IL-6 and MIP-2 levels ($p < 0.05–0.001$). Mosedipimod treatment significantly decreased hepatic inflammation (as measured by F4/80), NAFLD activity score (NAS) and fibrosis (Sirius red surface area %, see figure) on endo of treatment liver biopsies ($p < 0.05–0.001$). PLAG demonstrated similar effects to OCA and resmetirom in reducing hepatic steatosis, inflammation, NAS and fibrosis compared with the vehicle group.

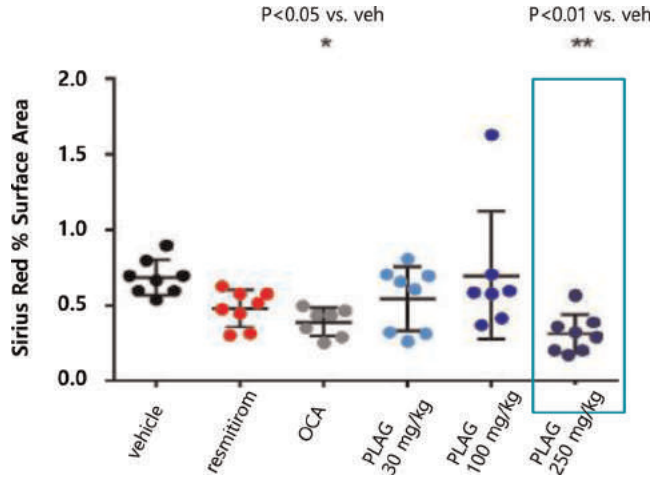

Figure:

Conclusion: Mosedipimod/PLAG mitigates the HFHF diet-induced hepatic injury and inflammatory cytokine production by modulating TLR4-dependent signaling pathways. Mosedipimod also prevents the histological and metabolic effects of NASH to a degree comparable to OCA and resmetirom in a widely utilized preclinical model. Taken together these data identify mosedipimod/PLAG, through a TLR4 signaling dependent mechanism, as a potential therapy for NASH that merits clinical investigation.

OS-2831

Pro-inflammatory hepatic progenitor cell response is associated with progression of human non-alcoholic fatty liver disease

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Background and aims: Activation of hepatic progenitor cells (HPCs), observed as ductular reaction, in response to parenchymal tissue damage has been associated with advanced non-alcoholic fatty liver disease (NAFLD) and portal infiltrating immune cells. Yet, the role of HPCs in the progression of NAFLD is still unclear. This study aims to investigate the biological relevance and prognostic value of HPCs in steatohepatitis (NASH) development and fibrosis progression.

Method: A total of 279 patients were included in this study. HPCs and matching hepatocytes were isolated from end-stage NAFLD using laser microdissection and processed for RNA sequencing (RNAseq). Gene signature clustering was performed on a RNAseq cohort of 206 NAFLD patients representing the entire NAFLD spectrum. Cell of origin was determined using publicly available single cell RNAseq and targets were validated using cyclic multiplex immunofluorescence and functionally assessed using ex-vivo human liver slices. In a subgroup of NASH F2/F3 patients with a baseline and a follow-up biopsy at least one year apart, HPC immunophenotype was correlated with clinico-pathological prognostic features. The histological semi-quantitative NASH CRN system was used to score NAFLD biopsies.

Results: High-throughput RNAseq of the HPC niche in end-stage NASH F4 identified 3, 282 differentially expressed genes correlating to a strong pro-inflammatory immune response and tissue remodelling (genes such as *CCR2*, *CCL2*, *CD44*, *SPP1*, *CXCL6*, *S100A6*). Interestingly, this HPC signature stratified the 206 NAFLD patients into distinct clusters characterised by a gradual increase in the presence of NASH, disease activity score (ballooning and inflammation), fibrosis stage and AST levels. Integrated single cell RNAseq analysis and protein level validation showed that HPCs themselves express pro-inflammatory cytokines such as CCL2 and SPP1. Moreover, ex-vivo HPC expansion in human liver slices resulted in an increased release of CCL2 and SPP1. Moreover, comparing baseline and follow-up liver biopsies, the presence of HPCs in the parenchyma at baseline was significantly associated with progression of at least 1 fibrosis stage.

Conclusion: Pro-inflammatory HPCs are associated with disease activity and inflammation, and predict fibrosis progression in NAFLD patients. Further assessment of the HPC phenotype may prove important for both stratifying patients at risk at baseline and for the design of targeted therapies to patients with high likelihood of disease progression.