**SAT014**

**Identification of novel biomarkers and therapeutic targets for steatohepatitis and advanced fibrosis in patients with non-alcoholic fatty liver disease (NAFLD): an in silico analysis**

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**Background and Aims:** To integrate multiple gene expression datasets from liver biopsies to search for novel biomarkers and/or therapeutic targets for advanced fibrosis and steatohepatitis in NAFLD patients.

**Method:** Data was obtained from Gene Expression Omnibus or other public repositories and integrated using R studio. Expression data was quantile normalized using RMA (oligo package version 1.480.0) and BrainArray (version 23.0.0) probe/gene mappings to increase precision and accuracy. Probes that were not present in all arrays were excluded from the analysis. After RMA pre-processing, array data with a different provenance (from different studies/platforms) were cross-platform normalized (aka merged) using Combat method (insilicoMerging package version 1.14.0) to remove batch effects. The search for biomarkers and therapeutic candidates of NAFLD was performed using data from six cohorts (N=317) by pairwise comparisons between healthy individuals (n=82) or patients with simple steatosis (n=96) with individuals with NASH (NAS score ≥5) (n=145). The search for advanced fibrosis markers was performed using exclusively individuals diagnosed with NAFLD (3 cohorts, N = 188) that had either mild (F0-F1, n = 152) or advanced (F3-F4, n = 36) fibrosis according to Kleiner Score. Changes in gene expression were considered significant when the FDR corrected p-value was ≤ 0.05 and the Fold Change (FC) ≥ 1.4. The behaviour of genes identified was further explored in two additional gene expression datasets that compared: 1) human primary hepatocytes exposed or not to satiated (palmitate) or unsaturated (oleate) fatty acids, alone or in combination. TGF-beta was used as a positive control for hepato-cellular injury and stellate cell activation. To assess therapeutic efficacy against steatosis, chips were treated for two days after initiating steatosis (therapeutic), or co-treated (prophylactic) with a liver-targeted analogue of firsocostat, a known inhibitor of acetyl-CoA carboxylase (ACC - i). Morphological evaluation of the hepatocytes and AdipoRed™ staining was used to evaluate steatosis. Quantification of triglycerides released in the media was used to evaluate lipid removal, and alpha-SMA staining was used to assess stellate cell activation.

**Results:** We demonstrated induction of steatosis in hepatocytes in a concentration-dependent manner following continuous exposure to oleate, palmitate, or in combination. Withdrawal of fatty acids significantly diminished the steatotic phenotype as well as levels of triglycerides released in accordance with relevant human in vivo data. Administration of TGF - beta resulted in increased stellate cell activation, hepato-cellular injury, and lipid accumulation compared to the vehicle controls. Chips treated with the ACC - i demonstrated a concentration-dependent reduction in lipid accumulation in both the therapeutic and prophylactic paradigms when compared to steatosis induced controls.

**Conclusion:** In this study we provide preliminary data supporting the potential application of the Liver-Chip for modelling NAFLD-like phenotypes and conducting human-relevant therapeutic efficacy assessment using clinically relevant endpoints.

**SAT016**

**AKR-001, an engineered Fe-FGF21 variant, directly modulates human liver and adipose tissue physiology, exerting beneficial metabolic, anti-inflammatory, and anti-fibrotic effects without FGFR4 agonism**

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**Background and Aims:** Non-alcoholic steatohepatitis (NASH) is a progressive liver disease with complex etiology. Pathophysiology results from the accumulation of liver fat due to increased adipose tissue lipolysis, excess calories directed to the liver, and increased hepatic de novo lipogenesis. Accumulating fat drives lipotoxicity, endoplasmic reticulum stress, and oxidative stress in hepatocytes, leading to apoptosis, inflammation, and fibrosis. FGFR2 acts as both an endocrine and a paracrine hormone, integrating whole body responses to nutritional changes and protecting cells against various stressors. To understand the potential for AKR-001, a clinical-stage FGFR2 analog, to mitigate each of the core processes underlying NASH pathology and progression, its activity has been profiled in different human ex vivo tissue models relevant to NASH pathology described
Various endocrine FGF analogs have been profiled using 2D and 3D ex vivo models. The biological activity of different endocrine FGF analogs was characterized using transcriptomics and candidate protein expression analysis. We used a functional culture 3D liver model to understand direct hepatic effects of FGF21 activity on steatotic, inflammatory, and fibrotic readouts. Results: FGF21 and AKR-001 suppress TGF-β-induced fibrogenic effects in a human hepatic stellate cell line. In 3D liver microtissues consisting of primary hepatocytes and various nonparenchymal cells, FGF21 and AKR-001, but not FGF19, suppress DNL and triglyceride accumulation. In this 3D human liver cell co-culture model, FGF21 and AKR-001 also suppress inflammatory activation by LPS to a greater extent than FGF19, while also suppressing fibrogenesis. Unbiased transcriptome profiling of these 3D liver microtissues, and of human adipocytes differentiated in vitro, demonstrated that AKR-001 recapitulates FGF21’s regulation of key metabolic pathways supporting its potential for use as a therapeutic in NASH patients.

Conclusion: FGF21 and AKR-001 exert direct actions in human adipocytes, hepatocytes, and liver non-parenchymal cells consistent with suppression of steatosis, inflammatory activation, and fibrogenesis. These actions appear to be mediated by FGF21’s canonical receptors FGFR1c/2c/3c, since agonism of FGF4 does not seem to contribute additional efficacy in these tissues. This supports the appealing pharmacology of AKR-001, which has been reported to have favorable effects on markers of glycemic control and lipid metabolism and to be well-tolerated in humans.

SAT017 Propionate intervention attenuates NASH while negatively affecting cognition
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Background and Aims: There is an increasing interest to elucidate the health effects of short-chain fatty acids (SCFAs) on metabolism, obesity, and brain function. Obesity is often associated with the development of non-alcoholic steatohepatitis (NASH) and cognitive impairment. We herein investigated potential health effects of the SCFA propionic acid (PA) on NASH development and brain function including cognition and behaviour readouts.

Method: During 17 weeks of run-in, LDR−/− mice received either high-fat diet (HFD) to establish obesity or chow as control. Obese mice were matched into groups (n = 15/group) and treated with propionic acid (PA+HFD), or a reference fatty acid (caproic acid; CA+HFD), or HFD without supplements (HFD). Cognitive and behavioral effects, as well as metabolic and inflammatory risk factors, were assessed prior to and after 12 weeks of treatment. At endpoint, liver, adipose and brain tissue were histologically and biochemically analyzed.

Results: PA, but not reference CA, reduced body weight and this effect was independent of food intake. PA also reduced fasting insulin levels and plasma cholesterol levels relative to the start of intervention. In addition, PA reduced total and subcutaneous fat mass, but did not affect VAT inflammation. Histopathological analysis of the liver demonstrated that PA reduced macrovesicular steatosis, hypertrophy and inflammation. Consistent herewith, PA reduced the inflammatory marker serum amyloid A and lowered the hepatic collagen content. PA treatment did not affect behavior in the open field test but mice showed impaired spatial memory, i.e. the latency to find the platform in the Morris water maze was increased. In line with these findings, we observed alterations in tissue integrity and gene expression in the hippocampus, a brain region important in memory consolidation. The reference fatty acid CA exerted no effects on the above readouts.

Conclusion: PA treatment during obesity had favorable metabolic effects, reducing body weight gain, improving metabolic risk factors and reducing the development of NASH and associated fibrosis. Simultaneously, PA had detrimental effects on the brain, reducing synaptogenesis signaling and affecting spatial memory. Altogether, the results from this study indicate that while the beneficial metabolic effects of PA treatment seem promising, it can also have negative effects on brain functioning and cognition, and should therefore be treated with caution.

SAT018 A shortcut from non-alcoholic fatty liver disease to HCC: c-Myc, a promising theranostic target
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Background and Aims: Non-Alcoholic Fatty Liver Disease (NAFLD) has rapidly risen as one of the leading etiologies for HCC and represents a large societal and health problem. Many factors are responsible for the high risk of NAFLD-related HCC development. Lately, oncogenes have been suggested to be determinant; however, their role still remains unknown.

Here we analysed the impact of the proto-oncogene c-Myc in the development of murine NAFLD and NAFLD-associated HCC.

Method: Transgenic mice bearing overexpression of c-Myc in hepatocytes (alb-MYC+) were studied at baseline conditions (36 weeks, 1 year) as well as after application of Western diet (WD).

Results: Mild obesity (Fig.1A), spontaneous hyperlipidaemia, glucose intolerance and insulin resistance were characteristic of 36 week-old mice. Alb-MYC+ mice exhibited profound spontaneous changes at 36 weeks: (A) hypertrophy of eWAT cells; (B) macrovesicular steatosis and (C) significant collagen accumulation in liver; (D) Multiple tumour nodules in alb-MYC+ mice livers after 10 months of WD feeding.

Results: Mild obesity (Fig.1A), spontaneous hyperlipidaemia, glucose intolerance and insulin resistance were characteristic of 36 week-old mice.