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ABSTRACT
BOOK

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[1]
REGENERATE: A PHASE 3, DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED MULTICENTER STUDY OF OBETICHOLIC ACID THERAPY FOR NONALCOHOLIC STEATOHEPATITIS

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Abstract Category: Clinical Trial Design

Background: Nonalcoholic Steatohepatitis (NASH) is a slowly progressive chronic liver disease without approved therapies. Patients with NASH and fibrosis are at high risk of increased mortality. Obeticholic Acid (OCA) is a selective and potent farnesoid X receptor (FXR) agonist, that has been shown to improve liver histology, including NAFLD activity score (NAS) and fibrosis, in a Phase 2 clinical trial (FLINT). Furthermore in FLINT, OCA treated patients had significant improvements in select liver biochemistries, markers of inflammation, and select cardiometabolic parameters. The ongoing, randomized, global, Phase 3 study REGENERATE, will further evaluate the effect of OCA on liver histology and clinical outcomes in patients with biopsy-confirmed NASH with stage 2-3 fibrosis.

Methods: 2065 patients will be randomized 1:1:1 to 10 mg OCA, 25 mg OCA or placebo each added to standard of care. An interim analysis at 18 months will evaluate the effect of OCA on liver histology. Total study duration is driven by time required to accrue a total of 264 outcome events and is estimated to be ~6 years. Safety assessments will include adverse events (AEs), adjudicated cardiovascular events, and hepatic events as well as laboratory assessments. The effect of OCA on NASH and fibrosis severity will also be assessed by multiple noninvasive methods (FIB-4, APRI, transient elastography, magnetic resonance elastography, etc.).

Analysis: The co-primary liver histology endpoints at 18 months include: (I) improvement in fibrosis by ≥1 stage with no worsening of NASH and (II) resolution of NASH with no worsening in fibrosis stage. Further, confirmation of clinical benefit of OCA will be assessed at the end of the study by comparing the time to first occurrence of any of the following adjudicated events: histological progression to cirrhosis; uncontrolled ascites; hospitalization for: variceal bleed, hepatic encephalopathy or spontaneous bacterial peritonitis; hepatocellular carcinoma; liver transplant or eligibility for liver transplant (defined by model for end stage liver disease (MELD) score ≥15); and death.

Conclusions: REGENERATE is the first pivotal study in NASH, designed in conjunction with FDA and meant to support approval of OCA for NASH with fibrosis. This robust Phase 3 study is designed to evaluate the effect of OCA on liver histology and effects on progression to cirrhosis, liver-related clinical outcomes and mortality.

Previously Presented at: EASL 2016, EASL Monothematic: NASH Beyond the Acronym, Swiss Society of Gastroenterology Annual Meeting 2016, UEGW 2016.

[2]
A DISEASE SEVERITY INDEX (DSI) FROM THE HEPQUANT®-SHUNT TEST IS REPRODUCIBLE AND QUANTIFIES HEPATIC IMPAIRMENT IN PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS (NASH), CHRONIC HEPATITIS C (CHC), AND PRIMARY SCLEROSING CHOLANGITIS (PSC)

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Abstract Category: Diagnostic procedures NASH/liver fibrosis

Background/Aim: The dual cholate test (HepQuant®-SHUNT) yields a disease severity index, DSI, that quantifies global liver function and physiology. Herein, we define the performance and reproducibility of DSI across a spectrum of chronic liver diseases, including Non-Alcoholic SteatoHepatitis (NASH), Chronic Hepatitis C (CHC), and Primary Sclerosing Cholangitis (PSC).

Patients: 16 healthy controls, 16 patients with CHC (8 with METAVIR fibrosis stage F0 to F2, 8 with F3 or F4), 16 patients with NASH (8 with BRUNT/KLEINER fibrosis stage F0 to F2, 8 with F3 or F4), and 46 patients with a wide clinical spectrum of PSC were studied. CHC and NASH patients with cirrhosis had compensated disease.

Methods: Controls, HCV, and NASH cases were tested 3 times, and PSC cases twice. Hepatic filtration rates (HFRs) were defined from clearances of cholic acid-24-13C, 20 mg intravenously (systemic), and cholic acid-2,2,4,4-d4, 40 mg orally (portal). Clearances were calculated from labeled cholate serum concentrations at baseline and 5, 20, 45, 60, and 90 minutes after simultaneous IV cholic acid-24-13C and oral cholic acid-2,2,4,4-d4 administration. DSI was calculated from HFRs: DSI = 10.86 x SQRT $[(\text{Log}_e\ 51.69 - \text{Log}_e\ \text{Portal HFR})^2 + (\text{Log}_e\ 10.72 - \text{Log}_e\ \text{Systemic HFR})^2]$

Results: The means ± SDs of DSIs for NASH (16.8 ± 3.4), HCV (18.9 ± 6.0), and PSC (18.2 ± 7.4) were higher than for controls (9.8 ± 3.3) ($p < 0.001$) and correlated with fibrosis stage in NASH and HCV. The average deviation from the mean of replicates was 0.94 ± 0.86 DSI units. Intra-class correlations for DSI were > 0.90.

Conclusion, DSI quantifies hepatic impairment and is reproducible over a broad spectrum of etiologies of liver disease, stages of fibrosis, and clinical severity. The minimally invasive HepQuant®- SHUNT test could be useful for defining severity and monitoring progression of chronic liver diseases, including Non-Alcoholic SteatoHepatitis (NASH), Chronic Hepatitis C (CHC), and Primary Sclerosing Cholangitis (PSC).

[3]
STAGING FIBROSIS AND EXCLUDING ADVANCED FIBROSIS IN PATIENTS WITH NAFLD: COMPARISON OF NON-INVASIVE MARKERS IN AN INTERIM ANALYSIS FROM A PROSPECTIVE MULTICENTRE STUDY

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Background/aim: Hepatic fibrosis is a major determinant of clinical outcomes in non-alcoholic fatty liver disease (NAFLD) and there remains a clear need to establish the accuracy of non-invasive markers of fibrosis. This study aims to prospectively compare the diagnostic performance and ability to exclude advanced fibrosis of the following non-invasive tests in NAFLD: FibroScan, FibroMeter V, FibroMeter NAFLD, FibroMeter VCTE, NAFLD Fibrosis score (NFS), Fib4, APRI, BARD and AST/ALT ratio.

Methods: Patients with suspected NAFLD prospectively underwent FibroScan examination and blood sampling within 2 weeks of a standard of care liver biopsy (LB) between March 2014 and January 2016 at seven UK centres. LB were staged in a blinded manner by two expert pathologists according to the NASH CRN system. Diagnostic performance was assessed in terms of area under the ROC curves (AUC). Ability to exclude advanced fibrosis was assessed using published cut-offs except for FibroMeter (FM), for which cut-offs have not yet been published. Cut-offs for FM were determined that maximized the Youden index.

Results: 155 patients (57% male, median age 54 [IQR 20] years, median BMI 33.2 [8.1] kg/m²) had a complete dataset for analysis. Fibrosis distribution was: F0: 23%, F1: 25%, F2: 21%, F3: 25%, F4: 6%. 43% of the patients had a NAS score ≥5. Performance of the tests is presented in the Table 1.

Table 1. Performance of non-invasive fibrosis scores.

	Performance to stage fibrosis (AUC *[95%CI]*)			Ability to exclude F≥3	
	F≥2	F≥3	F=4	Cutoff	PPV / NPV
FibroScan	**0.79** *[0.72-0.86]*	**0.84** *[0.77-0.90]**	**0.92** *[0.85-0.98]*	9.6†[3]	0.65 / 0.87
FMVCTE	**0.80** *[0.73-0.87]*	**0.89** *[0.83-0.95]*	**0.93** *[0.87-1.00]*	0.41‡	**0.67 / 0.93**
FMV	**0.75** *[0.67-0.82]**	**0.83** *[0.76-0.90]**	**0.89** *[0.79-0.99]*	0.40‡	0.55 / 0.91
FMNAFLD	**0.74** *[0.66-0.82]**	**0.81** *[0.74-0.89]**	**0.85** *[0.74-0.96]**	0.33‡	0.59 / 0.89
Fib4	**0.72** *[0.64-0.80]**	**0.81** *[0.73-0.88]**	**0.87** *[0.79-0.95]**	-1.445†[4] 0.676†[4]	0.51 / 0.90 0.64 / 0.72
NFS	**0.70** *[0.61-0.78]**	**0.78** *[0.70-0.85]**	**0.80** *[0.68-0.92]**	1.30†[4] 3.25†[4]	0.61 / 0.87 0.67 / 0.71
APRI	**0.71** *[0.62-0.79]**	**0.71** *[0.63-0.80]**	**0.82** *[0.74-0.90]**	1†[4]	0.54 / 0.70
AST/ALT	**0.59** *[0.50-0.68]**	**0.69** *[0.60-0.78]**	**0.77** *[0.61-0.93]**	0.8†[4] 1†[4]	0.46 / 0.79 0.50 / 0.74
BARD	**0.65** *[0.57-0.74]**	**0.69** *[0.61-0.78]**	**0.73** *[0.63-0.84]**	2†[4]	0.41 / 0.84

AUC significantly different FMVCTE (*), unilateral Delong test for AUC comparison
Cutoffs were either as published (†) or established by maximizing Youden index (‡)

Conclusion: FibroMeter VCTE, which combines biochemical parameters with liver stiffness measured by FibroScan, has the highest performance characteristics with positive and negative predictive values of 67 and 93% respectively at confirming or excluding ≥F3 fibrosis**.**

[4 - ORAL WINNER]
ASSESSMENT OF SERUM LEVELS OF CHITINASE-3-LIKE PROTEIN 1 (CHI3L1) IMPROVES IDENTIFICATION OF THE NASH PATIENTS AT RISK WHO SHOULD BE TREATED

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Background and aims: The management of the NASH epidemic is a global health priority. However, the identification of NASH patients is hampered by the requirement to perform a liver biopsy to make the diagnosis. The goal of this study was to identify additional biomarkers that could improve a recently reported simple, rapid, reliable, non-invasive diagnostic test (Sanyal et al., J. Hepatol. 2016, vol. 64, S717) for the detection of NASH patients to be treated, ie NASH patients with a NAFLD Activity Score (NAS) of 4 or more and a fibrosis grade of 2 or 3.

Methods: Data and samples from the 274 biopsy proven NASH patients included in the GOLDEN505 phase IIb trial with elafibranor were used for this study. This patient cohort is well-characterized, including a complete anthropometric and biochemical data set, centralized biopsy reading, and covers a wide spectrum of NASH disease activity and severity. The original data set, which contained more than 100 variables plus 9 different circulating miRNAs assessed at baseline, was completed with the measurements of Chitinase-3-like protein 1 levels (CHI3L1, also called YKL-40) in serum samples. Two independent biostatistical approaches (Median and Bootstrap approaches) were used to generate thousands of cohorts from the initial patient population in order to assure the translatability of the results to the global NAFLD/NASH population. For both approaches, a stepwise logistic regression method was applied to select the most frequent variables included in the algorithms.

Results: Interestingly, none of the variables included in the data set were correlated with CHI3L1 levels, showing the unique pattern of this parameter in NASH patients. The two parallel biostatistical approaches independently identified the same 6 variables: miR-200a, miR-34a, A2M, HbA1c, P3NP and CHI3L1. The Area Under the Receiver Operating Characteristic (ROC) Curves (AUC) were 0.84 for both approaches. Conclusion: The inclusion of circulating levels of CHI3L1 as a new variable to both the Median and the Bootstrap algorithms clearly enhances the performances of the 2 methods leading to a better identification of the NASH patients at risk who should be treated. These 2 algorithms showed also significantly better accuracies than the existing scoring systems such as the NAFLD Fibrosis Score, the ELF test, the Fibrotest, the Fibrometer and FIB-4. (no table selected)

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[5]
CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF PATIENTS WITH NASH AND HIGH INFLAMMATORY ACTIVITY ON LIVER HISTOLOGY

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Background: Inflammation is a key driver of disease progression in NASH. Inflammation is commonly defined by liver histology and has been linked to disease progression. However, liver histology is limited by availability and sampling variability. Therefore clinical characteristics and novel serum biomarker that characterize patients with high inflammatory activity and at risk of disease progression have urgently to be defined.

Methods: We explored a German cohort of 130 patients with histologically defined NAFLD and identified patients with a high degree of inflammation (Kleiner lobular inflammation score ≥2) or no inflammation (Kleiner lobular inflammation score = 0) on liver histology. These groups were characterized clinically and by determination of experimental cytokines (interleukin-8, interleukin-10, interleukin-1b, interleukin-12p70, interleukin-6 and tumour necrosis factor alpha) using Cytometric Bead Array (CBA).

Results: Overall 19 patients were included into this analysis that exhibited high (n=10) or no (n=9) inflammation on liver histology. Median age at date of biopsy in patients with high inflammatory activity was 52.6 years and 51.3 years in patients with no inflammatory activity All patients in the high inflammatory group had significant fibrosis (stage 2-4, mean Kleiner/SAF fibrosis stage of 2.57) and 8 out of 9 patients had at least ballooning grade I on histology. In contrast 9 out of 10 patients with no inflammatory activity showed mild or no fibrosis (stage 0-1) and no ballooning. Mean NAS-Score was 5.3 vs. 1.2.. Both groups showed elevated levels of AST (60.25 vs. 45 iu/L), ALT (76.375 vs. 69.1 iu/L) and gGT (82 vs. 191.8 iu/L). Commonly used inflammatory markers including CRP (5.67 vs. 2.90 mg/dl), Ferritin (195.0 vs. 161.46 ug/L) and IgG (14.77 vs. 11.09 g/L) or thrombocytes (228.3 vs. 238.7 10^9/L) were not different. Next cytokines were determined by CBA: interleukin-8 was significantly higher in the group with high histological inflammatory activity (23.56 vs. 9.91, p=0.05), while no significant differences were observed for Interleukin-10, interleukin-1b, interleukin-12p70, interleukin-6 and tumour necrosis factor alpha.

Conclusion: Patients with high inflammatory activity on liver histology are not identified by standard laboratory test but do exhibit increased levels of interleukin-8. Thus cytokines could potentially be used to stratify and identify patients non-invasively and guide further diagnostics. NASH and HCV. The average deviation from the mean of replicates was 0.94 ± 0.86 DSI units. Intra-class correlations for DSI were > 0.90.

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[6]
THE BREATHID 13C-METHACETIN BREATH TEST (MBT) CORRELATES WELL WITH DEGREE OF LIVER DISEASE SEVERITY: A META-ANALYSIS OF 1992 TESTS IN 1549 SUBJECTS

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Abstract Category: Diagnostic procedures NASH/liver fibrosis

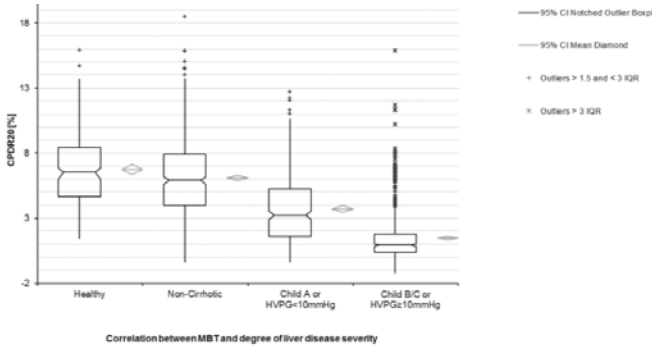
Background: The ^{13}C-Methacetin Breath Test (MBT) is a non-invasive real-time molecular correlation spectroscopy assay. Using the Exalenz BreathID® system, MBT measures $^{13}CO2$ in expired breath produced by hepatic CYP450 metabolism of ingested non-radioactive ^{13}C isotope-labelled Methacetin, an acetaminophen precursor. MBT is a non-invasive point of care test, non-operator or patient dependent, not affected by BMI, etiology, ascites or implantables and not requiring patient cooperation. MBT has been extensively studied to assess liver disease severity, usually defined by histology. This study assessed MBT as a measure of metabolic liver function.

Aim: To assess the correlation between metabolic function as measured by MBT to the degree of severity of liver disease in patients with chronic liver disease (CLD).

Methods: 1375 patients with various degrees of liver disease and etiologies from multiple studies throughout the world and 174 healthy volunteers underwent a total of 1992 MBTs using 75mg of 13C-Methacetin oral solution including 443 follow up visits. Healthy volunteers were defined based on liver profile and ultrasound; 628 patients were defined non-cirrhotic by liver biopsy; 364 patients were defined as cirrhotic by liver biopsy or based on clinical and laboratory findings with Child A and/or HVPG<10mmHg; and 383 patients were defined as advanced cirrhotic based on clinical and laboratory findings with Child B/C and/or HVPG≥10mmHg.

Results: MBT Cumulative PDR20 (CPDR20) correlates with the degree of CLD severity with r=-0.69 (p<0.0001). Liver reserve is maintained in non-cirrhotics, as can be seen in the high overlap of CPDR20 between non-cirrhotic patients and healthy volunteers (9% reduction in mean metabolic function). The mean metabolic function markedly declined between non-cirrhotic and cirrhotic as well as between cirrhotic and advanced cirrhotic with 40% and 60% reduction respectively. MBT differentiated non-cirrhotic from all cirrhotic stages combined (AUC=0.86, 95% CI: 0.85-0.88; p<0.0001).

Conclusions: MBT is well correlated with the degree of severity of liver disease. As expected, liver reserve is maintained in non-cirrhotic patients while metabolic function declined gradually in correlation to disease progression. Thus, MBT may be a valuable non-invasive surrogate for the assessment of hepatic reserve and can be used for monitoring of disease progression.



This Abstract has been presented at the AASLD 2016 Conference.

[7]
DIAGNOSING NASH PATIENTS AND THEIR RISK OF VARICES AND DECOMPENSATION BY A GLOBAL MEASURE OF LIVER FUNCTION, THE HEPQUANT®-SHUNT TEST

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Background/Aim: Currently NASH is diagnosed by liver histology. Because NASH biopsies are subject to sampling error and 40% variability in staging (Ratziu, et al, 2005), assessing risk for portal hypertension, varices, and liver decompensation is problematic. FibroScan may be inaccurate and subject to interference by steatosis (Durango, et al, 2013), and HVPG is invasive and not used routinely. HepQuant®-SHUNT is a minimally-invasive test that provides a global measure of liver function, the disease severity index (DSI). The goal of this pilot study was to determine if DSI could diagnose NASH and assess the risk for varices and decompensation.

Methods: The study comprised 81 subjects, of whom 50 were healthy controls, (30 normal weight (BMI 18.5-25), 16 overweight (BMI 25-30), and 4 obese (BMI>30)). In addition there were 16 NASH patients from the University of Colorado Denver and 15 NASH patients from Baylor University Medical Center Dallas. Of these, 27 had biopsy-diagnosed NASH, and 4 had cryptogenic cirrhosis, concurrent obesity, and presumed late stage NASH. Patients had a range of Brunt-Kleiner fibrosis stages, F1 (N=4), F2 (N=4), F3 (N=5), and F4 (cirrhosis, N=18). Clinical manifestations of NASH disease severity were captured from patient histories and included endoscopy findings (small, medium, or large varices) and any clinical decompensation events (ascites, encephalopathy, variceal bleed, or jaundice). The HQ-SHUNT test involves serum sampling prior to, and at 5, 20, 45, 60, and 90 minutes after simultaneous administration of IV cholic acid-24-^{13}C and oral cholic acid-2,2,4,4-d4. Clearances of labeled cholates, measured by LCMS of serum samples, were used to calculate a disease severity index (DSI). The ability of DSI to diagnose NASH and to assess the risk for varices and decompensation was evaluated by ROC analyses (c-statistic and the sensitivity, specificity, PPV, NPV at the optimum cutoff defined by the maximum Youden Index) and logistic regression.

Results: The HQ-SHUNT DSI could differentiate NASH patients from healthy control subjects, even overweight and obese controls (ROC c-statistic 0.94, optimum cutoff DSI >16.5, sensitivity 84%, specificity 98%, PPV 96%, NPV 91%, Youden Index 0.82). Within the NASH cohort, DSI could identify patients at risk of any varices (ROC c-statistic 0.87, optimum cutoff DSI >21.2, sensitivity 86%, specificity 82%, PPV 80%, NPV 88%, Youden Index 0.68), those at risk of medium/large varices (ROC c-statistic 0.93, optimum cutoff DSI >28.0, sensitivity 89%, specificity 91%, PPV 80%, NPV 95%, Youden Index 0.80), and those at risk of decompensation (ROC c-statistic 0.99, optimum cutoff DSI >28.0, sensitivity 100%, specificity 95%, PPV 90%, NPV 100%, Youden Index 0.95). By logistic regression, DSI was a highly significant predictor for diagnosing NASH, and also DSI could significantly predict the risk of any varices, medium/large varices, and decompensation. The DSI cutoffs for 50% probability for any varices (DSI 24.7) and for medium/large varices (DSI 30.4) were similar to the DSI cutoffs found in a previous study of varices risk in 217 HCV patients (DSI 24.8 and 33.2, respectively).

Conclusions: This pilot data suggests that the HepQuant®-SHUNT test could be a minimally-invasive alternative to biopsy for the diagnosis of NASH. DSI could be a measurement for assessing risk for any varices, for medium/large varices, and for liver decompensation in NASH.

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[8]
NON-INVASIVE MULTIPARAMETRIC MRI(LIVERMULTISCAN™) EFFECTIVELY EXCLUDES NASH AND LIVER FIBROSIS IN AT RISK PATIENTS

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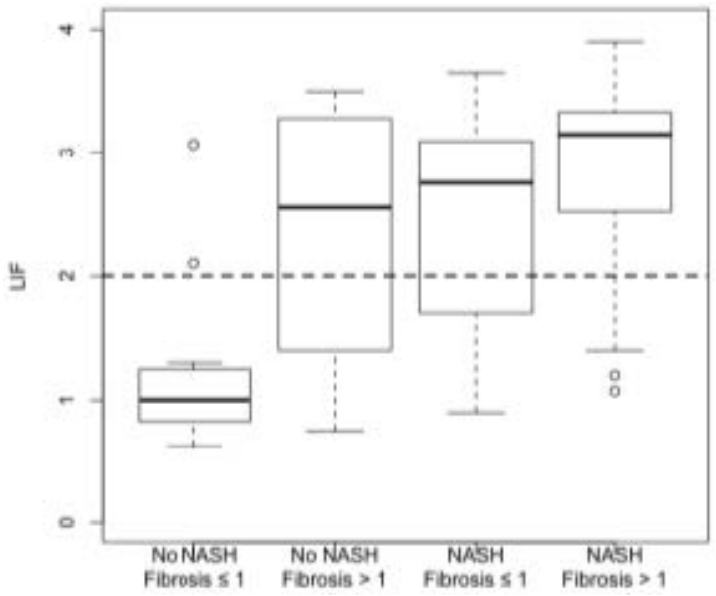
Abstract Category: Diagnostic procedures NASH/liver fibrosis

Aim: To characterise the performance of the MRI-derived Liver Inflammation and Fibrosis (LIF) score in discriminating between patients with and without NASH and significant fibrosis (Kleiner-Brunt > 1). LIF > 2 has been shown to predict clinical outcomes in a general hepatology outpatient clinic population, here we examine patients with suspected NASH only, to see if LIF can enrich a population for NASH with significant fibrosis.

Method: This is a pooled analysis of two prospective clinical trials with a total of 120 patients, comparing multiparametric MRI to liver biopsy in patients with NAFLD. One cohort comprised 50 patients from two centres (Birmingham and Edinburgh), who were booked for non-targeted liver biopsy for any indication and had a histologically confirmed diagnosis of NAFLD. The other cohort comprised 70 patients from a single centre (Oxford), with NAFLD, suspected NAFLD, or who were to undergo bariatric surgery. Iron-corrected T1 (cT1) maps were acquired with Liver*MultiScan* and used to calculate the LIF score. For all patients, MRI was performed prior to biopsy. Histologists were blinded to the MRI results, and MRI operators and analysts were blinded to the patients' clinical status. Biopsies were graded for steatosis, inflammation, ballooning, staged for fibrosis, and NASH status determined.

Results: A LIF cut-off of 2, as previously described, gives a high NPV to rule out patients with NASH and significant fibrosis. 29 patients had LIF < 2; 24 of these did not have both NASH and significant fibrosis, giving an NPV of 0.83. Alternatively, for identifying patients with any disease, 89 of the 91 patients with LIF ≥ 2 had NASH and/or significant fibrosis.



	No NASH; no significant fibrosis	No NASH; significant fibrosis	NASH; no significant fibrosis	NASH; significant fibrosis
LIF < 2	11	5	8	5
LIF >= 2	2	10	20	59

Conclusions: LIF can rule out NASH and significant fibrosis with a high NPV. This enables clinicians to stratify patients with suspected NASH, including identifying those who would not benefit from a liver biopsy. This should improve healthcare delivery and also enrich recruitment for clinical trials that rely on biopsy-proven NASH.

[9 - ORAL WINNER]
13C-METHACETIN BREATH TEST TO ASSESS PRESENCE OF CLINICALLY SIGNIFICANT PORTAL HYPERTENSION: A NOVEL TOOL FOR THE MANAGEMENT OF PATIENTS WITH COMPENSATED ADVANCED CHRONIC LIVER DISEASES

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Background: In patients with compensated advanced chronic liver disease (cACLD), i.e. patients with advanced liver fibrosis/compensated cirrhosis, a Hepatic Venous Pressure Gradient (HVPG) ≥10mmHg is defined as Clinically Significant Portal Hypertension (CSPH), and is associated with an increased risk of varices, ascites, variceal hemorrhage, hepatic encephalopathy, hepatocellular carcinoma (HCC) and poor outcome after HCC resection. However, HVPG is invasive, not universally available, inconvenient for serial use and requires expertise and experience. The 13C-Methacetin Breath Test (MBT) using Exalenz' BreathID© system, is a non-invasive real-time molecular correlation spectroscopy system that measures the abundance of 13CO2 in expired breath exclusively produced by hepatic CYP450 metabolism of ingested non-radioactive 13C-labeled Methacetin. MBT has been shown to reflect degree of liver impairment.

Aim: To investigate if MBT can assess CSPH in patients with cACLD.

Methods: MBT, HVPG and clinical variables (demographics, etiology, blood tests, and treatments) were collected from 205 patients with cACLD who had routine measurement of HVPG. A total of 77 patients with hepatic decompensation, portal vein thrombosis, variceal bleeding, HCC direct anti-HCV therapy with recent SVR, or missing data were excluded from the analysis. The relationship between collected parameters and HVPG was analyzed by logistic regression modeling.

Results: Analysis was conducted on 128 patients (62.5% males) with 61.7% having CSPH. Average age was 60.3 years (±9.99). Etiology of cACLD was 62% HCV, 13% NASH, 11% ASH, 3% HBV and 12% others, including HIV/HBV or HCV co-infections. The developed model detected CSPH with an AUROC of 0.91, p<.0001. Selecting two cutoff points in the model with 90% sensitivity and 89% specificity, CSPH could be ruled in or ruled out in 74% of these patients (with 93% PPV and 85% NPV). Applying the same model for the detection of portal hypertension (PH; HVPG ≥6mmHg in 81%), the AUROC was 0.92, p<.0001. Selecting two new cutoff points with 92% sensitivity and 92% specificity, PH could be ruled in or ruled out in 80% of these patients (with 99% PPV and 61% NPV). When combining all the cutoffs there are only 3.1% of the patients that have no PH or CSPH classification by the MBT.

Conclusions: MBT non-invasively detects CSPH at point-of-care with high sensitivity and specificity. MBT may serve as a useful non-operator, non-etiology dependent tool in the clinical follow-up of patients with cACLD.

This Abstract has been presented at the AASLD 2016 Conference.

[10]
NOVEL FIBROSCAN-BASED SCORE TO DIAGNOSE NASH AND ITS SEVERITY IN A MULTI-CENTRE UK COHORT OF PATIENTS WITH SUSPECTED NAFLD

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Abstract Category: Diagnostic procedures NASH/liver fibrosis

Background/aims: Reliable non-invasive biomarkers are needed for the diagnosis and monitoring of patients with non-alcoholic steatohepatitis (NASH). Our study set out to determine the performance of a score based on Fibroscan measurements (liver stiffness and controlled attenuation parameter linked to steatosis) to detect patients with NASH.

Methods: Patients with suspected NAFLD prospectively underwent FibroScan examination within 2 weeks of a standard of care liver biopsy (LB) between March 2014 and January 2016 at 7 UK centers. LB were read in a blinded manner by two expert pathologists. NASH was diagnosed using the FLIP algorithm. NASH severity was assessed using the NAS score. The cohort was split randomly into a training (80%) and validation set (20%) to develop a score to diagnose NASH and its severity. Sample splitting was repeated 100 times. Eventually one model was selected and tested on an external validation cohort that consisted of 47 NAFLD patients from a single liver centre in France. Patients there underwent FibroScan examination within 1 day of LB, which were read by the same pathologists.

Results: 174 patients with BMI <40 kg/m^2 were studied. The following patients were excluded for the score development: LB not interpretable/ diagnostic of NAFLD (n=18), FibroScan not feasible (n=1), FibroScan unreliable according to Boursier's criteria (n=10). Patients had a median BMI of 32.9 [IQR=6.9] kg/m^2 and age of 54 [21] years. 58% were male, 74% had a NAS score ≥3 and 58% had NASH. The external validation cohort had a median BMI of 30.0 [8.0] kg/m^2 and age of 53 [22] years. 67% were male, 82% had a NAS score ≥3 and 71% had NASH. Performance of the scores is shown in the Table 1.

Table 1. NASH scores performance.

		Training Set (N=116)	Validation set (N=28)	External validation (N=43)
Repeated split sample	NAS≥3	AUC=0.85±0.02	AUC=0.85±0.07	AUC=0.89±0.00
	NASH (FLIP)	AUC=0.85±0.02	AUC=0.83±0.08	AUC=0.85±0.01
Selected scores	NAS≥3	**AUC=0.85** C=0.45, CC=0.83	**AUC=0.87** CC=0.86	**AUC=0.89** CC=0.86
	NASH (FLIP)	**AUC=0.84** C=0.67, CC=0.76	**AUC=0.88** CC=0.82	**AUC=0.84** CC=0.79

AUC: area under the ROC curve.
CC: correctly classified patients, cut-off (C) maximizing negative + positive predictive values.

Conclusion: A novel score based on FibroScan Elastography and CAP was able to correctly classify almost 80% of patients with/without NASH as well as correctly staging severity in 86%. This has promise as a non-invasive marker for detecting/staging inflammation in patients with NASH.

[11 - ORAL WINNER]
GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONIST POTENTLY ATTENUATES STEATOHEPATITIS AND LIVER FIBROSIS BY REGULATING LIVER MACROPHAGE INFILTRATION, ACTIVATION AND POLARIZATION

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Abstract Category: Experimental/basic science, NAFLD/NASH, non-humans

Background and aims: Glucagon-like peptide-1 (GLP-1) improves insulin sensitivity via enhanced glucose-dependent insulin secretion, inhibition of glucagon release, and delayed gastric emptying following its release into the circulation from the gut. We aimed to explore the utility of the long-acting GLP-1 receptor agonist Bydureon (BY) to address both inflammation and fibrosis in models of NASH (nonalcoholic steatohepatitis) and biliary fibrosis.

Methods: BY was administered twice weekly by subcutaneous injection of 0.4 or 2 mg/kg to Mdr2$^{-/-}$ mice, and to C57BL/6 mice fed a methionine and choline deficient (MCD) diet for 4 weeks. Inflammation, fibrosis, steatohepatitis and especially macrophage function and polarization were assessed.

Results: In both the MCD and Mdr2$^{-/-}$ model, BY significantly decreased serum liver enzymes, liver collagen content, and fibrosis and inflammation related transcripts and protein levels (e.g., Col1a1, α-Sma, CD68, CCL3, and TNFα), while it increased the (anti-inflammatory) macrophage markers Arg1 and Ym1. BY treatment also reduced the IHC expression of collagen type III, CD68, F4/80 and caspase3. In FACS analysis MCD livers showed no difference in CD11c$^+$ cells in all the groups, while the total percentage of F4/80$^+$ macrophages was significantly decreased on BY treatment, indicating a direct effect of BY on macrophages but no significant impact on hepatic dendritic cell (DC) populations. Moreover, BY induced a significant decrease of liver CD11b$^+$Ly6C^{hi} inflammatory/ fibrogenic myeloid cells and accompanied by a massive increase of hepatic CD11b$^+$Ly6C^{lo} cells. BY also reduced the CD68$^+$ total macrophages and increased anti-inflammatory Ym1$^+$ macrophages in both epididymal tissue and liver. Overall BY treatment reduced phosphorylation JNK, ERK and phosphorylated- ERK1/2 (p-ERK) levels.

Conclusions: We show that GLP-1 exerts a direct beneficial effect on liver macrophages which is accompanied by significant effects on inflammation, fibrosis and steatosis in models of biliary fibrosis and hepatic lipoapoptosis/ NASH. GLP-1/GLP-1R signaling correlates with JNK/ ERK1/2 activation and macrophage (M2-type) polarization. Our data support further clinical evaluation of the utility of GLP-1R agonists for the treatment of patients with NASH and liver fibrosis.

Presented in modified form at DDW2016.

[12 - ORAL WINNER]
PNPLA3 148M LIPID DROPLET LOCALIZATION IS ESSENTIAL TO PNPLA3 148M-DEPENDENT LIPID ACCUMULATION

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Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease in western countries and has emerged as a major public health issue worldwide. A single nucleotide polymorphism in residue 148 (I148M, rs738409) of patatin-like phospholipase-3 (PNPLA3) is the strongest genetic determinant of NAFLD and NASH but the mechanism remains largely unknown. In order to gain insight into the mechanism(s) by which PNPLA3 148M increases NAFLD risk, we investigated the consequences of targeted PNPLA3 148M lipid droplet (LD) localization to cellular lipid content and examined the impact of PNPLA3 148M on lipid remodeling dynamics *in vitro* and *in vivo*. We generated human hepatocyte stable cells lines (Huh7) overexpressing various mutants of GFP-tagged-hPNPLA3 (148I, 148M and the catalytically dead, S47A) and quantified cellular lipid content and PNPLA3 localization by high resolution imaging. In addition, we generated a double mutant to impair PNPLA3 148M localization to the LD (hPNPLA3 148M-195L) and generated mutants with an added sequence to drive hPNPLA3 148M localization to the ER (ER-hPNPLA3 148M) or to the mitochondria (mito-hPNPLA3 148M). We demonstrated that overexpressed hPNPLA3 148M and hPNPLA3 S47A colocalized to lipid droplets and increased Huh7 cell lipid droplet content. As expected, hPNPLA3 LD localization was impaired in hPNPLA3 148M-195L double mutant. Furthermore, hPNPLA3 localized to the expected subcellular compartments in mito-hPNPLA3 148M and in ER-hPNPLA3 148M overexpressing cell lines, respectively. Significantly, hPNPLA3 148M LD localization was necessary to increase hepatocyte cellular lipid content as hPNPLA3 148M-195L, mito-hPNPLA3 148M and ER-hPNPLA3 148M overexpressing cell lines were similar to control cells or hPNPLA3 148I overexpressing cell lines with respect to lipid content. Additionally, we investigated PNPLA3 148I- and 148M-specific hepatic lipidomic signatures *in vitro* and *in vivo* using siRNA knock down and adenovirus-mediated overexpression systems. Murine PNPLA3 (mPNPLA3) 148I knock down was successful in reducing endogenous expression 60-80% using three independent *in vivo* siRNA sequences in mice 3 days post injection under sucrose diet fed conditions. Adenovirus mediated overexpression of hPNPLA3 148I reduced hepatic triglyceride content 3 and 7 days post administration compared to adenovirus control. *In vivo* overexpression of hPNPLA3 148M increased hepatic triglyceride content compared to adenovirus control by 2.2-fold indicating a dominant lipid enrichment function in 7 days in the absence of diet intervention. Targeted lipidomic analysis was performed on hepatocyte cell lines overexpressing hPNPLA3 148I and 148M, and further in mouse liver under mPNPLA3 148I knock down and hPNPLA3 148I and 148M overexpressing adenoviral systems. Selective lipid species were significantly enriched upon hPNPLA3 148I knock down and decreased the overexpression systems (both *in vitro* and *in vivo*), pointing to possible endogenous PNPLA3 substrates. Altogether our results demonstrate that PNPLA3 148M lipid droplet localization is essential to the increased lipid phenotype *in vitro*, and furthermore highlights potential endogenous PNPLA3 substrates. While targeting PNPLA3 148M offers a potential therapeutic target for NAFLD, further understanding of PNPLA3 148I and 148M biological function, mechanism, and selective lipid signatures is required.

[13 - ORAL WINNER]
A SHORT-TERM HIGH CARBOHYDRATE DIET INDUCES ACUTE LIVER INJURY, ENHANCED DE NOVO LIPOGENESIS, INFLAMMATION, FIBROSIS AND M2 MACROPHAGE POLARIZATION

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Background/Aim: Macro- and micro-nutrients affect the development of NAFLD/NASH, but the role of the relative contribution of defined dietary carbohydrates vs fats is understudied. We therefore assessed the effect of different dietary fat:carbohydrate ratios on a murine NAFLD/NASH development.

Methods: Female 6 wk old C57BL/6 mice (n=10/group) were fed Western transfat diets with different fat:carbohydrate ratios (10:68, 30:50, 59:26, kJ%; hydrogenated coconut oil, calories adjusted with corn-starch, plus 2% (w/w) cholesterol and additional fructose-sucrose 12.6%: 55:45 in drinking water) for 8 and 12 weeks. An isocaloric diet (65 kJ% carbohydrates; 13 kJ% fat from soybean oil) was used as control. At sacrifice, insulin resistance, serum liver function tests, lipid accumulation (Sudan III stain), inflammation, fibrosis, and transcripts related to lipogenic metabolism, hypoxia, inflammation and fibrogenesis were analyzed.

Results: There were no significant differences in body and liver weights at 8 weeks, with a significant increase only in the 59 kJ% fat group at 12 weeks. HOMA-IR was increased in proportion to dietary fat content at 8 and 12 weeks ($p< 0.05$). Interestingly, liver collagen content and fibrosis related COL1A1, ACTA2, TGFB1, TIMP1, and MMP2 transcripts were significantly upregulated in the 10:68 kJ% group at 8 weeks as well as the 59:26 kJ% group at 12 weeks. Compared to moderate increases of serum parameters of liver injury (AST and LDH) and total cholesterol in the high fat diet (59:26 kJ%) group at 12 weeks, all parameters (AST, ALP, ALT, LDH, triglycerides and total cholesterol) were significantly elevated in the high carbohydrate (10:68 kJ%) group at 8 weeks. Of note, steatosis and NAS scores were also significantly increased in the high carbohydrate groups at 8 weeks but decreased at 12 weeks. At 8 weeks, steatosis in the high carbohydrate group was accompanied by upregulated expression of lipogenic markers (PGC1A, SREBP1C, ACC1, FAS and FGF21) and inflammation (IL6 and TNFA). Markers of M2 macrophage polarization (MCP1, IL4, ARG1, MRC1, IL13RA1 and IL13RA2) were also significantly-upregulated in the high carb group at 8 weeks vs all other groups and time points.

Conclusions: A high carbohydrate diet with a low fat content as well as a high fat diet favour hepatic steatosis and profibrotic (M2 polarized) inflammation which may promote the development of fibrotic NASH. Importantly, with a high carbohydrate but not with a high fat diet metabolic adaptation appears to occur after 8 weeks, with attenuation of inflammation and fibrogenesis, possibly induced by a prominent M2 macrophage polarization

This abstract has been presented at AASLD the Liver meeting 2016

[14]
PPARδ AGONIST SELADELPAR REVERSES NASH AND DECREASES FIBROSIS IN DIABETIC OBESE MICE

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Background: Lipotoxicity associated with insulin resistance and diabetes is central to the pathogenesis of non-alcoholic steatohepatitis (NASH), leading to a strong inflammatory component that drives the resultant liver fibrosis and its potential to progress to cirrhosis. Seladelpar (MBX-8025) is a selective PPARδ _agonist with anti-inflammatory effects that improves atherogenic dyslipidemia in rodents and humans. We tested whether seladelpar could improve insulin resistance, decrease lipotoxic hepatic lipids, reverse NASH and decrease fibrosis in an obese diabetic mouse model.

Methods: From 4 weeks of age, female Alms1 mutant (foz/foz) and wildtype (Wt) mice were fed an atherogenic diet throughout the 28-week study. At Week 16, groups (8-12 mice) were randomized to receive seladelpar (10 mg/kg in 1% methylcellulose vehicle) or vehicle by gavage for 8 weeks. We performed intraperitoneal glucose tolerance testing (IpGTT) at Week 27, and sacrifice at Week 28.

Results: Seladelpar had insignificant effects on body or liver weight, but normalized hyperglycemia, hypercholesterolemia, hyperinsulinemia and glucose disposal (by IpGTT) in foz/foz mice. After 16 weeks of atherogenic dietary intake, serum ALT ranged from 300-600 U/L in vehicle-treated foz/foz mice; seladelpar reduced this by ~50% (P<.05). In foz/foz mice, hepatic lipid fractions showed increased free cholesterol, diacylglycerol, saturated fatty acid and triglyceride content seladelpar normalized serum lipids, and corrected all hepatic lipid pools. Treatment with seladelpar was associated with abolition of hepatocyte ballooning, substantial reduction of steatosis, and reduced liver inflammation with a striking decrease in the number of macrophage crown-like structures; the average non-alcoholic fatty liver disease (NAFLD) Activity Score (NAS) for all vehicle treated foz/foz mice was 6.9 (range 6-7) with all animals (8/8) showing definite NASH. In contrast, seladelpar reversed NASH in all foz/foz mice (NAS 3.13; P<.05), with only residual simple steatosis being present. Seladelpar also inhibited liver fibrosis as quantified by collagen densitometry and decreases in fibrosis-associated mRNA transcripts.

Conclusions: The selective PPARδ _agonist seladelpar improves glucose tolerance and reverses diabetes, atherogenic dyslipidemia, hepatic lipid storage (including lipotoxic lipids), and NASH pathology in atherogenic diet-fed obsese diabetic mice. Seladepar acts independently of weight reduction, appears to counter lipotoxicity related to insulin resistance, with accompanying reductions in liver inflammation and fibrosis. Seladelpar provides a novel mechanism-based therapeutic agent with the potential to treat NASH

Disclosures: This study was supported by a grant from CymaBay. CAM and Y-JC are employees of CymaBay. FH and NCT received research support from CymaBay. HW, VB, ARM, MMY, WGH, GNI and GCF have no disclosures.

[15 - ORAL WINNER]
INHIBITION OF SPHINGOSINE 1-PHOSPHATE SIGNALING BY FTY720 AMELIORATES MURINE NONALCOHOLIC STEATOHEPATITIS

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Abstract Category: Experimental/basic science, NAFLD/NASH, non-humans

Nonalcoholic steatohepatitis (NASH) is the most prevalent chronic liver disease in the Western world with no regulatory agency approved pharmacologic therapy. NASH is a lipotoxic disorder, wherein pro-inflammatory effects of accumulated lipids, such as ceramide and its derivative sphingosine 1-phosphate (S1P), contribute to macrophage-associated liver injury and inflammation. FTY720 (fingolimod), a small molecule, orally available, S1P antagonist, approved for the treatment of relapsing multiple sclerosis due to its immune-modulatory properties, has not been studied in NASH. Therefore, we **hypothesized** that FTY720 would ameliorate NASH by inhibiting S1P induced proinflammatory monocyte chemotaxis. The **aim** of this study was to assess the therapeutic efficacy of FTY720 in reversing established NASH.

Methods: 12 week old C57Bl/6 male mice fed a high saturated **F**at, **F**ructose, and **C**holesterol (FFC) diet or standard chow for 24 weeks were treated with either FTY720 (1mg/kg) or saline daily by intraperitoneal injections starting at week 22 of the feeding study for 14 days. Upon completion body weight, liver mass, liver tissue and blood were obtained for analyses.

Results: Saline treated FFC-fed mice (control group) recapitulated human NASH including the cardinal features of hepatocyte steatosis, ballooning, and fibrosis. FTY720-treated FFC-fed mice (treatment group) demonstrated significant improvement in hepatocyte ballooning and liver fibrosis compared with the control group; this was reflected in an improved NAFLD activity score ($p<0.05$). Serum ALT was 268 ± 40 U/L in the control group compared with 144 ± 9 U/L in the treatment group ($p<0.01$). Macrophage accumulation was significantly reduced in the treatment group by immunohistochemistry for activated macrophage marker Mac-2, and confirmed by quantitative PCR (qPCR). Monocyte attracting chemokines, MCP-1, and MIP-1alpha were significantly reduced in the treatment group. Consistent with this, there was a reduction in the hepatic accumulation of Ly6C expressing macrophages-likely due to a reduction in the recruitment of Ly6C expressing pro-inflammatory monocytes to the liver. Collagen accumulation measured by picro-sirius red staining, and liver mRNA expression of alpha-smooth muscle actin and metalloproteinase inhibitor-1 were significantly reduced in the treatment group in keeping with the reduction in liver injury and inflammation. Body mass and liver mass remained unchanged between the two groups.

Conclusions: FFC-diet induced NASH is significantly ameliorated by 2 week treatment with FTY720. FTY720 should be further explored for possible therapeutic use in human NASH patients.

[16 - ORAL WINNER]
TARGETING MITOCHONDRIAL PYRUVATE METABOLISM TO AMELIORATE HEPATIC FIBROSIS IN A MOUSE MODEL OF NASH

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Background/Aim: Nonalcoholic steatohepatitis (NASH) is highly prevalent in obese individuals and resultantly, is emerging as an important public health problem. Insulin-sensitizing thiazolidinediones have shown promise for treating NASH, but their use has raised concerns due to dose-limiting side effects. Recently, we have shown that a novel insulin sensitizer, MSDC-0602, binds and inhibits the mitochondrial pyruvate carrier (MPC). MPC1 and MPC2 proteins comprise the MPC complex, which mediates a key step in intermediary metabolism. Given the known connection between insulin resistance and NASH, we evaluated the efficacy of the insulin-sensitizing MSDC-0602 or genetic deletion of MPC2 in hepatocytes to treat a diet-induced mouse model of NASH.

Methods: Mice were fed either a low fat (LF) control diet or a high trans-fat, fructose, and cholesterol (HTF-C) containing diet to induce NASH. A subset of mice was fed plain HTF-C diet then switched to HTF-C diet containing 330ppm MSDC-0602. Hepatic stellate cells (HSCs) were isolated via collagenase perfusion of livers, and purified by density gradient centrifugation. Plasma exosomes were enriched by treating 500μL of plasma with ExoQuick, diluted in PBS, and used to treat 6-well plates of HSCs for 24 hours 2-3 days post HSC isolation.

Results: In an initial study, mice were fed plain HTF-C diet for 4 weeks and switched to HTF-C-containing MSDC-0602 for 12 weeks. Hepatic steatosis was increased by HTF-C diet compared to control and this was not attenuated by MSDC-0602. However, treatment with this compound improved plasma ALT/AST concentrations, which were increased by HTF-C diet. MSDC-0602 significantly decreased liver gene expression markers of hepatic stellate cell activation and fibrotic scar formation, which were also induced by HTF-C diet. In a second experiment, to determine if MSDC-0602 could reverse existing hepatic injury caused by HTF-C, mice were switched to HTF-C containing MSDC-0602 for 3 weeks after 16 weeks on HTF-C diet. MSDC-0602 again improved plasma ALT/AST concentrations and histologic and molecular markers of stellate cell activation and fibrosis, suggesting that MSDC-0602 can reverse pre-existing hepatic fibrosis. Liver-specific MPC2 knockout (LS-Mpc2-/-) mice also exhibited lower plasma ALT concentrations and fibrotic gene expression on the HTF-C diet. Consistent with MPC2 mediating the beneficial effects of MSDC-0602 in liver, treatment with this compound produced no further effect on liver injury markers in LS-Mpc2-/- mice. Isolated HSCs displayed decreased RNA expression for fibrotic collagen and extracellular matrix regulating enzymes if directly treated with MSDC-0602, or if treated with plasma exosomes from MSDC-0602 treated mice, or LS-Mpc2-/- mice, compared to plasma exosomes from WT mice fed HTF-C diet.

Conclusions: These data demonstrate that targeting the MPC in liver by both direct inhibition of HSC fibrogenesis or by affecting a paracrine signal from hepatocytes is a viable option to improve fibrosis and treat NASH.
*Similar abstract was presented as a poster at the 2016 AASLD Liver Meeting.

[17 - ORAL WINNER]
LXR INVERSE AGONISTS DEMONSTRATE LIVER LIPID LOWERING EFFECTS THROUGH MULTIPLE MECHANISMS IN RODENT MODELS OF NASH AND IN HUMAN HEPATOCYTES

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Abstract Category: Experimental/basic science, NAFLD/NASH, non-humans

Background/aims: Several mechanisms are currently evaluated as potential pharmacotherapies for the spectrum of non-alcoholic fatty liver disease (NAFLD), including modulators of nuclear receptors such as PPARa/g/d or FXR. Activation of Liver X Receptor (LXR) in the liver by potent, synthetic agonists is known to result in severe steatosis and hypertriglyceridemia in various animal models. Thus, we have designed and synthesized LXR inverse agonists with the aim to inhibit LXRs pro-steatotic transcriptional activity. The pharmacologic effects of these compounds were evaluated in human hepatocytes and in mouse and rat models of NAFLD .

Methods/Results: Compounds PX-L493 and PX-L603 were characterized in cellular reporter assays as inverse agonists of LXRalpha and beta [EC_{50} for LXR(α/β) in NCoR recruitment mammalian 2-hybrid assay = PX-L493 (5.3/1.4 nM); PX-L603 (966/326 nM)]. In human primary hepatocytes addition of LXR inverse agonists dose-dependently reduced the hepatocyte lipid load. Furthermore, the PX compounds demonstrated an inhibition of *de novo* lipogenesis in this cellular model as evidenced by [1,2-^{13}C]-acetate incorporation and mass isotopomer distribution analysis (MIDA). For an *in vivo* NAFLD model, Zucker (fa/fa) rats or, alternatively, C57 mice, were maintained on a Survit-type high fat diet with 1 % cholesterol (HFD) for two weeks. Thereafter they were either administered the LXR inverse agonist PX-L603 at 10 mg/kg p.o., PX-L493 at 10 mg/kg (rats only) by i.p. injection, or vehicle for another 2 weeks (rats) or 4 weeks (mice) on the same diet. Analysis of the hepatic lipid content yielded a significant reduction of triglycerides as well as of total cholesterol in the livers of animals treated with PX-L603 or PX-L493 compared to vehicle treatment. Gene expression analysis of candidate genes in the liver by qRT-PCR showed that the LXR inverse agonists repressed known LXR target genes involved in fatty acid synthesis, uptake and triglyceride storage or export such as *Scd-1*, *Fas*, *Angptl-4*, *Cd36* and others. Of special note, treatment of C57 mice on HFD with PX-L603 yielded a potent reduction of *Pnpla3* mRNA, a gene that harbors a polymorphism which was clearly associated with human NASH . Pharmacokinetic analysis of these LXR compounds in plasma and different tissues incl. liver demonstrated a strong hepatotropism which is a necessity to avoid LXR-antagonism related side effects in tissues other than the liver.

Conclusion: We have demonstrated that reducing LXRs transcriptional activity in human hepatocytes or in the liver of Zucker (fa/fa) rats or C57 mice by synthetic inverse agonists yields clear anti-steatotic effects through multiple mechanisms. This suggests that inhibition of the LXR pathway in the liver is a useful novel approach for a pharmacotherapy of NAFLD.

Disclosures: Claus Kremoser - Consulting: Gilead Sciences; Management Position: Phenex Pharmaceuticals AG; Stock Shareholder: Phenex Pharmaceuticals AG

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[18 - ORAL WINNER]
THE EFFECT OF LIRAGLUTIDE, ELAFIBRANOR AND OBETICHOLIC ACID ON NAFLD ACTIVITY SCORE AND FIBROSIS STAGE IN A DIET-INDUCED OBESE MOUSE MODEL OF NASH

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Abstract Category: Experimental/basic science, NAFLD/NASH, non-humans

Aim: To determine metabolic and histopathological effects of the GLP-1 analogue, liraglutide, the peroxisome proliferator activated receptor (PPAR) α/δ agonist, elafibranor, and the farnesoid x receptor agonist, obeticholic acid (OCA), in a diet-induced obese and biopsy-confirmed mouse model of nonalcoholic steatohepatitis (NASH).

Methods: Male C57BL/6 mice were fed the AMLN diet (40% trans-fat, 20% fructose and 2% cholesterol) (DIO-NASH) or chow (LEAN-CHOW) for a total of 26 weeks. After the diet-induction period, a liver pre-biopsy was obtained under isoflurane anesthesia. Only biopsy-confirmed steatotic and fibrotic animals (steatosis score ≥2; Fibrosis Stage ≥1) were included and stratified into treatment groups: DIO-NASH Vehicle (PO, QD), DIO-NASH Liraglutide (0.2 mg/kg, SC, BID), DIO-NASH Elafibranor (30 mg/kg, PO, QD), DIO-NASH OCA (30 mg/kg, PO, QD), and LEAN-CHOW Vehicle (PO, QD). Animals were treated for 8 weeks. At termination, blood samples were collected for plasma liver enzymes (alanine/aspartate aminotransferases; ALT/AST) and lipids (total cholesterol; TC, triglycerides; TG). Furthermore, liver post-biopsies and plasma samples were obtained for histological and biochemical analysis. Primary endpoints included a blinded histological evaluation of NAFLD Activity Score (steatosis, inflammation, ballooning degeneration) including Fibrosis Stage and biochemical assessment of hepatic lipid content (TC/TG).

Results: Liraglutide and elafibranor induced a weight loss of approximately 10%. whereas OCA treatment did not influence body weight. Interestingly, OCA and liraglutide reduced liver weight by 26 and 37% respectively, whereas elafibranor increased liver size. Only liraglutide reduced plasma levels of ALT/AST. In contrast OCA improved plasma TC. Notably, all treatments improved hepatosteatosis by reducing liver TG/TC content. In addition, OCA, liraglutide and elafibranor reduced NAFLD Activity Score – predominantly by reducing the steatosis component. Only elafibranor reduced liver Fibrosis Stage.

Conclusions: Pharmacological intervention with OCA, liraglutide and elafibranor induced a diverse metabolic profile. Irrespectively, all treatments exerted an anti-steatotic action and, importantly, improved liver histopathology by reducing NAFLD Activity Score. In addition, elafibranor exerted anti-fibrotic effects and improved liver Fibrosis Stage.

Data has not been presented elsewhere

[19 - ORAL WINNER]
DIGOXIN PROTECTS FROM STERILE INFLAMMATION IN THE LIVER BY TARGETING PYRUVATE KINASE M2 (PKM2) PROMOTED HIF-1 TRANSACTIVATION

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Abstract Category: Experimental/basic science, NAFLD/NASH, non-humans

Background: Sterile inflammation after tissue damage is a ubiquitous immune response, and occurs with highest amplitude in the liver. This has major clinical consequences for alcoholic and non-alcoholic steatohepatitis (ASH and NASH) with both lacking effective therapies. Key requirements for sustained sterile inflammation are high degree of cellular oxidative stress and the activation of HIF-1_ pathway. The cardiac glycoside digoxin was identified as potent suppressor of HIF-1_, but the mechanism for this, and for hepatic protection is not well defined.

Aim: To assess whether digoxin has therapeutic effects in NASH and ASH in mice, and investigate the molecular mechanisms in both mouse and human cells.

Methods: C57BL/6J male mice were placed on a 45% high fat diet (HFD) for 11weeks with and without digoxin (ip 1, 0.2 and 0.05 mg/kg twice a week). Digoxin 1mg/kg ip daily in mice results in the therapeutic serum levels achieved in humans (0.5-2 ng/ml). Plasma ALT, liver histology, leukocytes profiling, mitochondrial ROS, and gene transcriptome microarrays were analyzed. The chronic plus binge model of ASH was performed. The identification of digoxin interacting protein(s) in maintaining cellular redox homeostasis and suppressing HIF1_ activation were investigated by proteomics, RT-PCR, reporter luciferase and ChIP-PCR assay.

Results: Digoxin dose-dependently reduced histological injury, neutrophilic infiltrate, inflammasome activation and serum ALT values in both co-treatment (starting digoxin same time with HFD, ALT, 417 +/- 398 U/L in HFD vs 91 +/- 73 U/L in HFD+DIG, P< 0.001), and post-treatment (starting digoxin after 4 weeks HFD, neutrophil 24.6% in HFD vs 14.3% in HFD+DIG; monocytes 31.6% in HFD vs 19.1% in HFD+DIG; ALT, 400 +/- 130 U/L in HFD vs 80 +/- 17 U/L in HFD+DIG, P< 0.001) without a reduction in food intake. A low dose of digoxin (0.05 mg/kg) also shows significant protective effects again injury oxidative stress and sterile inflammation in both NASH and ALD models. The microarray transcriptome analysis revealed that digoxin treatment resulted in the significant down-regulation of ROS metabolism, antioxidant and HIF1_ signaling pathway gene expression from HFD liver tissues. A broad mass spectrometry-based proteomic screening revealed that digoxin binds pyruvate kinase M2 (PKM2), and independent of PKM2 kinase activity results in chromatin remodeling and down-regulation of HIF-1_ transactivation.

Conclusions: Our data identify PKM2 as a novel mediator and therapeutic target for regulating liver sterile inflammation, and demonstrate the protective role of digoxin from ASH and NASH.

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[20]
THE MOLECULAR PROGRESSION OF NONALCOHOLIC FATTY LIVER DISEASE

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Background: Nonalcoholic fatty liver disease (NAFLD), especially its aggressive form nonalcoholic steatohepatitis (NASH), can progress to cirrhosis. Changes in the hepatic transcriptome during disease progression have not been fully characterized and limit the development of precision medicine-based therapeutics.

Aim: To model the molecular progression of NAFLD.

Approach: Isogenic C57Bl6J/S129 mice were fed a high fat-high sugar diet (WD) for 8, 24 and 52 wks (n=4-5/group). This diet-induced animal model of NAFLD (DIAMOND) closely mimics progressive human NAFLD in terms of biochemical, histological parameters and transcriptome. The liver transcriptome of WD-mice was analyzed using Illumina microarrays (Mouse WG-6 2.0) and compared to that of control chow diet (CD)-fed mice at similar time points. Following quantile normalization, 2 groups differences were evaluated by 2-way ANOVA. Biological enrichment among differentially expressed genes (false discovery rate <0.1) was evaluated using Ingenuity Pathway Analysis.

Results: Compared to control CD-fed mice, 3136 genes were differentially expressed in WD-fed mice across the different time points. All WD-fed mice had NAFL at 8 wks; the top enriched pathways at 8 wks included eIF2 signaling, unfolded protein response, mitochondrial dysfunction, oxidative stress and acute phase response ($p<0.001$). Lipid metabolism pathways, including FXR/RXR activation, fatty acid synthesis, mitochondrial/peroxisomal fatty acid oxidation and sterol biosynthesis were also significantly changed, as were amino acid metabolism, protein synthesis, activation of cell death/turnover, tissue differentiation and oncogenic signaling. By 24 wks, the histology had progressed to NASH with F0-F2 fibrosis. While the early pathway changes persisted, they had decreased in magnitude/significance; and IL-1/inhibition of RXR, granulocyte diapedesis/adhesion, Fc macrophage activation, prothrombin activation and hepatic stellate cell activation represented the principal changes in the transcriptomic signature ($p<0.001$). By 52 wks, mice had florid NASH with F3 fibrosis and the transcriptomic signature was dominated by Fc macrophage activation, cell death and turnover and activation of cancer-related networks ($p<0.001$).

Conclusions: The molecular progression of NAFLD involves a metabolic perturbation which triggers subsequent cell stress and inflammation driving cell death and turnover. Over time, inflammation and fibrogenic pathways become dominant while in advanced disease an inflammatory-oncogenic profile dominates. Tissue regenerative signatures are prominent early and decrease by the time advanced disease is present.

[21]
DISEASE PROGRESSION IN A DIET-INDUCED OBESE MOUSE MODEL OF NON-ALCOHOLIC STEATOHEPATITIS (NASH)

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Abstract Category: Experimental/basic science, NAFLD/NASH, non-humans

Aim: The Gubra AMLN diet-induced obese NASH model is a well characterized mouse model of liver steatosis and fibrosis for preclinical efficacy studies. Here we aimed to investigate the progression of NASH by assessing metabolic parameters, lipid metabolism, and liver specific histopathological and molecular markers during a 50-week diet induction period.

Methods: Male C57Bl/6J mice (5 weeks of age) were offered *ad libitum* access to a diet high in trans-fat (40%), fructose (22%) and cholesterol (2%) (the AMLN diet). Following 0, 1, 5, 10, 15, 20, 25, 30, 40 and 50 weeks on the diet subgroups of animals were terminated for histopathological evaluation of NAFLD activity score, as well as fibrosis stage. RNAseq analyses of key pathways were obtained and supported by biochemical analysis of liver cholesterol, triglycerides and hydroxyproline levels, as well as plasma analyses of alanine aminotransferase and aspartate aminotransferase.

Results: Plasma and liver biochemistry revealed a gradual increase in all investigated parameters starting with steatosis, followed by inflammation and ending with a fibrosis development reminiscent of human NASH. All components of the NAFLD activity score increased gradually throughout the study period, with fibrosis induction being apparent from week 20 and onwards. Interestingly, steatosis evolved from initial microvesicular around 5 weeks into the diet-period to a pronounced grade 3 macrovesicular steatosis. The prevalence of inflammation steadily increased over the 50 weeks first occurring around week 10 on the diet, whereas ballooning degeneration was observed only in late time points and only to a low extent similar to other rodent models of NASH. Total RNAseq analysis with a focus on pathways key to NASH development revealed a striking dysregulation of genes known to be involved in hepatic lipid handling, inflammation and macrophage recruitment and collagen deposition. Notably, genes involved in macrophage recruitment, such as MCP-1 and CCR1/2, as well as macrophage markers as CD68 and MAC-2, provided strong mechanistic insight into the pronounced and sudden onset of inflammation. Furthermore, the expression of key genes in fibrosis development, such as collagens, TIMPs and MMPs, allowed us to chart a detailed map of the temporal dynamics underlying fibrosis development in the Gubra AMLN diet-induced obese NASH model.

Conclusions: We demonstrate that the AMLN diet induces an instant effect on liver metabolism, and continues to increase in severity throughout the diet induction period. The highly detailed characterization provides and unprecedented temporal understanding of NASH development at both the whole animal, histological and molecular level, and implies that the Gubra-AMLN NASH model can be used as a translational NASH model.

Data has not been presented elsewhere.

[22]
EFFECTS OF BMS-986036 (PEGYLATED FIBROBLAST GROWTH FACTOR 21) ON HEPATIC STEATOSIS AND FIBROSIS IN A MOUSE MODEL OF NONALCOHOLIC STEATOHEPATITIS

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Background: Fibroblast growth factor 21 (FGF21), a non-mitogenic hormone, is an important regulator of glucose and lipid metabolism. FGF21 analogs improve insulin sensitivity and lipid profiles, which contribute to non-alcoholic steatohepatitis (NASH) pathogenesis, in preclinical models as well as in obese humans with type 2 diabetes. BMS-986036 is a pegylated recombinant form of human FGF21 with an extended elimination half-life and duration of action. In this study, the effects of BMS-986036 treatment were evaluated in the Stelic mouse model (STAM™) of NASH and liver fibrosis.

Methods: C57BL/6 mouse pups were given a single injection of streptozotocin two days after birth to induce diabetes and were subsequently placed on a high-fat (57%) diet at 4 weeks of age. In the Stelic model, mice develop histological correlates of NASH by 7 weeks of age and liver fibrosis by week 9. Twice-weekly (BIW) subcutaneous administration of 3 mg/kg BMS-986036 was initiated in 9-week old NASH mice and was continued for 6 weeks. Analyses were conducted on serum and liver lobes prepared from mice that were sacrificed 72 h after the last treatment.

Results: BMS-986036 conferred a survival benefit; 8 of 20 mice in the Vehicle group died during the in-life phase and 0 of 20 in the BMS-986036 group. When compared with Vehicle, 6 weeks of BIW treatment with 3 mg/kg BMS-986036 resulted in statistically significant changes in liver-to-body weight ratio (-40%), blood glucose (-37%), plasma ALT (-53%), liver triglycerides (-50%), liver cholesterol (-51%), and serum adiponectin (+67%). Based on histologic analyses, treatment with BMS-986036 resulted in a mean reduction vs. Vehicle of 3.6 units in the NAFLD Activity Score (NAS; $P< 0.001$) with reductions in all 3 components of the NAS, a 61% decrease in oil red-positive area (hepatic steatosis; $P< 0.001$), and a 34% decrease in Sirius red-positive area (liver fibrosis; $P < 0.05$).

Conclusion: Six weeks of treatment with BMS-986036 improved survival, increased serum adiponectin and decreased hepatic steatosis, NAS, and fibrosis in the Stelic mouse model of NASH and liver fibrosis.

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[23]
NUTRITIONAL WHEAT AMYLASE TRYPSIN INHIBITORS, ACTIVATORS OF INTESTINAL TOLL LIKE RECEPTOR 4, EXACERBATE NON-ALCOHOLIC STEATOHEPATITIS IN HIGH FAT DIET FED MICE

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Background and Aims: Non-alcoholic steatohepatitis (NASH) is now ranked the most prevalent liver disease worldwide with an escalating demand for liver transplantation over the next decade. Apart from life style changes and pharmacological strategies, specific (micro-) nutrients may play an important role in NASH pathogenesis. A possible candidate is the family of what amylase trypsin inhibitors (ATIs) that represent 3% of wheat protein. ATIs are highly resistant to digestive proteolysis and activate intestinal innate immunity via toll like receptor 4 (TLR4) on monocytes, macrophages and dendritic cells (Junker Y et al, J Exp Med 2012). We therefore assessed the effect of nutritional ATIs equivalent in quantity to human average daily wheat consumption on the severity of diet induced NASH in mice.

Methods and Results: Male C57BI/6J mice received a carbohydrate and protein (zein from corn, 22.1.% of weight) defined low or high fat diet (HFD, 53KJ% vs 13 KJ% of calories as saturated fats), with or without 30% of the zein being isocalorically replaced by crude wheat gluten (which contains approx. 0.1g ATIs per 10g gluten, G+ATI), or 0.7% of the zein replaced by purified ATIs for 8 weeks. At sacrifice blood, liver and peripheral adipose tissues were collected for biochemical and histological analysis. Tissues were quantified for lipid content, inflammation and fibrosis, and inflammation and fibrosis related transcript levels were quantified by qPCR. Macrophage subsets were quantified by IHC. Compared to the HFD alone, mice fed the HFD/G+ATI or the HFD/ATI diets gained 10% and 15% more weight, respectively (34.87±0.8 vs. 38.188±0.4 and 40.1g±0.5) and displayed significantly higher serum triglycerides and liver, epididymal, mesenteric and inguinal fat. The intraperitoneal glucose tolerance test (IPGTT) revealed a significantly higher glucose intolerance in mice on the HFD/ATI containing diets than on the HFD alone. Moreover, compared to the HFD alone, mice on the HFD/G+ATI and HFD/ATI diets had a significantly higher histological NAS score, accompanied by elevated transcript levels of CD68 (total macrophages), IL-6 and TNF-alpha, whereas alternative macrophage (and putatively anti-inflammatory) transcripts (ARG1 and Ym-1) were decreased. This was confirmed histologically via elevated CD68+ liver macrophages and myeloperoxidase (MPO)+ cells, and decreased numbers of YM-1^+ M2 liver macrophages in the HFD/G+ATI and HFD/ATI fed animals. Furthermore, HFD/ATI fed mice had not only developed significantly enlarged epidydimal adipocytes and $CD68^+$ crown like structures (as an indication of adipose tissue inflammation) compared to the HFD controls and a significant increase in adipose tissue gene expression of inflammatory markers such as CD68, IL-6 and IL-1β.

Conclusions: Our study clearly implicates dietary wheat ATIs as proinflammatory nutritional drivers of NAFLD/NASH. This effect occurs at a daily intake that is comparable to average human consumption of wheat products and occurs despite the lack of any additional caloric value of the added ATIs.

Presented in modified form at AASLD2016.

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[24]
GLIPTINS MITIGATE INFLAMMATION, FIBROSIS AND VASCULAR DYSFUNCTION IN MODELS OF NON-ALCOHOLIC STEATOHEPATITIS AND LIVER FIBROSIS VIA ALTERNATIVE MACROPHAGE ACTIVATION

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Abstract Category: Experimental/basic science, NAFLD/NASH, non-humans

Background and Aims: Non-alcoholic steatohepatitis (NASH) is characterized by steatosis, panlobular inflammation, liver fibrosis and increased cardiovascular mortality. Dipeptidyl peptidase-4 (DPP-4) inhibitors (gliptins) are indirect glucagon like peptide 1 (GLP-1) agonists with antidiabetic, anti-inflammatory and anti-obesogenic activity, used for the treatment of type 2 diabetes. Their potential and underlying mechanisms to treat metabolic liver inflammation and fibrosis as well as the associated vascular dysfunction remain to be explored.

Methods: Linagliptin or Sitagliptin were administered orally for 4-6 weeks to C57BL/6 mice fed a methionine and choline deficient (MCD) or sufficient (MCS) diet for 8 weeks. Biliary fibrotic $Mdr2^{-/-}$ mice received increasing doses of Linagliptin for 4 weeks. Serum, liver and aorta were thoroughly analyzed for inflammation, oxidative stress and fibrosis, and the aorta was examined for vascular function.

Results: Gliptin treatment of MCD mice mitigated or corrected hepatic steatosis, fibrosis, inflammation, hepatocyte apoptosis, systemic oxidative stress and vascular dysfunction in MCD mice, and attenuated inflammation and fibrosis in $Mdr2^{-/-}$ mice. Gliptins prominently suppressed monocyte-macrophage infiltration and shifted their polarization towards an anti-inflammatory M2 phenotype.

Conclusions: Gliptins decrease overall oxidative stress, steatosis, apoptosis, inflammation, and vascular dysfunction in a mouse model of NASH, and exert mild direct anti-fibrotic properties via induction of alternative macrophage polarization. These cells provide a mechanistic link between the liver and cardiovascular complications in NASH. Gliptins qualify as adjunctive drugs for the treatment of NASH, the prevention of the associated cardiovascular complications and liver fibrosis.

Presented in modified form at DDW2016.

[25]
LOSS OF AMPK ACTIVITY IMPAIRS AUTOPHAGY AND RESULTS IN LIVER FIBROSIS AND HEPATOCELLULAR CARCINOMA IN VIVO

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Background/Aim: A major complication of non-alcoholic fatty liver disease (**NAFLD**) is the development of hepatocellular carcinoma (**HCC**). NAFLD-associated HCC often occurs in the absence of cirrhosis, thereby delaying diagnosis. The use of metformin, a 5'AMP-activated kinase (**AMPK**) activator, is associated with a reduced risk of developing HCC in population-based studies. Our goal was to determine the mechanisms behind this protective effect.

Methods: Livers from young (3 months old) and aged (12-17 months old) male (n=9) and female (n=15) AMPKγ1 KO mice (**KO**) were evaluated and compared to control (n=9) and heterozygous (n=11) mice. qPCR, immunoblotting, hydroxyproline, and immuno-histochemical stains were utilized to examine for liver fibrosis and HCC development. Flow cytometry, immunoblots and electron microscopy were used to detect changes in autophagy.

Results: AMPKγ1 deletion resulted in equivalent loss of the catalytic AMPKα subunit in male and female mice. Increased fibrosis manifested by a 5-fold increase in hydroxyproline occurred in male, but not female, AMPKγ1 KO mice. Consistent with these findings, α-smooth muscle actin (α-SMA) labeling and TGFβ expression was pronounced in male but not female KO mice. Cirrhosis or bridging fibrosis (0/24) was not apparent in any mice. AMPK activity reciprocally regulates autophagy and the mTOR pathways. Loss of AMPKγ1 led to loss of inhibition through the mTOR pathway. In contrast, rate limiting steps in autophagosome formation were significantly decreased, while p62 accumulation, a marker of impaired autophagy and driver of hepatocarcinogenesis, was pronounced in KO livers. AMPK couples cell cycle control with the metabolic state of the cell. Loss of AMPK led to decreased levels of the active form of tumor suppressor p53 in male KO mice. HCC developed in 21% (5/24) of aged AMPKγ1 KO mice, compared to none (0/9) in WT controls and AMPKγ1 heterozygous (0/11) mice, $p<0.05$ (Fischer's Exact Test). Male AMPKγ1 KO mice developed HCC (4/9) more frequently than females (1/15). HCC were characterized by nuclear β-catenin labeling, loss of reticulin staining, and peri-tumor iron overload.

Conclusion: Loss of AMPK activity in AMPKγ1 KO mice impairs normal autophagy responses, tumor suppressive mechanisms, and results in liver fibrosis and sporadic HCC formation. These findings provide a mechanistic explanation for the protective effects of metformin on HCC development.

[26]
A NOVEL AND HIGHLY POTENT FXR AGONIST EDP-305 SUPPRESSES LIVER INJURY AND FIBROSIS IN A MURINE MODEL OF STEATOHEPATITIS

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Background: Farnesoid X receptor (FXR) agonism is a promising strategy to treat chronic liver diseases such as non-alcoholic steatohepatitis (NASH). EDP-305 is a novel and potent FXR agonist with a single-digit nanomolar affinity *in vitro*. It is highly selective for FXR with no/minimal cross-reactivity to the G protein-coupled bile acid receptor 1 (TGR5) or other nuclear receptors. Herein we report therapeutic efficacy of EDP-305 in mice with steatohepatitis and fibrosis, in direct comparison with the first-in-class FXR agonist, obeticholic acid (OCA).

Methods: Steatohepatitis was induced in C57Bl/6 mice with a methionine-choline deficient diet (MCD). All treatments were administered between 4 weeks (steatohepatitis with incipient fibrosis) and 8 weeks (advanced steatohepatitis with advanced fibrosis) on MCD (n=8-12/group). Two doses of EDP-305 (10 and 30 mg/kg) or vehicle were administered via daily oral gavage. A parallel group received OCA (30mg/kg/day *p.o.*) as a comparator. Liver injury and progression of liver fibrosis were evaluated by serum chemistry, histology, and biochemical determination of collagen.

Results: No apparent adverse effects of treatments were noted during the study. Serum levels of transaminases ALT and AST were both significantly decreased (by 62% and 37%, respectively) in MCD-fed mice receiving 30mg/kg EDP-305 compared to vehicle controls. Mice receiving low dose 10 mg/kg EDP-305 and obeticholic acid (30mg/kg) showed a clear trend towards lower ALT/AST levels compared to vehicle control, but these changes did not reach statistical significance. Total bilirubin levels were not affected by any of the treatments. EDP-305 at both doses (10 and 30 mg/kg) had a profound inhibitory effect on liver fibrosis progression, with up to 70% reduction in hepatic collagen deposition ($p<0.05$, ANOVA) as determined biochemically via hydroxyproline measurement. Histologically, MCD-fed control mice developed the advanced perisinusoidal fibrosis ("chicken wire") characteristic of NASH. Treatment with EDP-305 was associated with markedly reduced periosinusoidal fibrosis compared to placebo group. OCA (30 mg/kg) did not have an appreciable effect on hepatic hydroxyproline levels and connective tissue histology.

Conclusions: Treatment with the novel FXR agonist EDP-305 potently improved pre-established liver injury and hepatic fibrosis (assessed biochemically and histologically) in an MCD-induced model of steatohepatitis in mice. By all studied parameters of liver injury and fibrosis, EDP-305 outperformed the first in class FXR agonist, obeticholic acid.

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[27]
PNPLA3 OVEREXPRESSION RESULTS IN REDUCTION OF PROTEINS PREDISPOSING TO FIBROSIS

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Abstract Category: Pathogenesis, translational science, NAFLD/NASH, liver fibrosis, humans

Background and aim: Non-alcoholic fatty liver disease (NAFLD) is a growing health problem in Western Countries. NALFD is characterized by increased liver fat content and inflammation resulting in liver fibrosis and failure. Hepatic stellate cells (HSCs) once activated play a key role in fibrogenesis in the liver. PNPLA3 I148M is a common genetic variant robustly associated with liver fibrosis but the mechanisms underlying this association remain obscure. The aim of this work was to examine the role of PNPLA3 148I wild type and 148M mutant protein in hepatic stellate cells.

Methods: Primary hepatic stellate cells (pHSCs and human HSCs *ex vivo*) and immortalized human HSCs (LX-2) were used. pHSCs were incubated with transforming growth factor-beta (TGF-β) (10 ng/mL) or platelet-derived growth factor (PDGF) (10 ng/mL) in presence or absence of PNPLA3 siRNA. Western blotting was performed to assess the PNPLA3 levels and oil red O (ORO) staining was performed to measure intracellular lipid droplet content. LX-2 stably over-expressing PNPLA3 148I wild type or 148M cell lines were generated. Intracellular lipid droplet content was measured by ORO staining. Cells and medium fractions were collected after incubation with and without retinol 10 μM/palmitic acid 100 μM and analyzed by western blotting.

Results: We found that PNPLA3 is upregulated by TGF-β. Furthermore, retinol release from human HSCs *ex vivo* is lower in cells with the PNPLA3 148M compared with 148I wild type protein. Overexpression of PNPLA3 148I wild type but not 148M mutant induces a reduction in the secretion of matrix metalloproteinase 2 (MMP2) and tissue inhibitors of metalloproteinases 1 and 2 (TIMP1 and TIMP2), enzymes involved in extracellular matrix remodeling and this reduction is mediated by retinoid metabolism.

Conclusion: In conclusion we show for the first time a role of PNPLA3 in HSC activation in response to fibrogenic stimuli. Moreover PNPLA3 may have a role in protecting against liver fibrosis by inducing a specific signature of proteins secreted by HSC and involved in extra cellular matrix remodeling.

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[28]
ADENOSINE DEAMINASE 2 EXPRESSION IN PORTAL MACROPHAGES IS ASSOCIATED WITH INFLAMMATION AND LIVER FIBROSIS IN NONALCOHOLIC FATTY LIVER DISEASE

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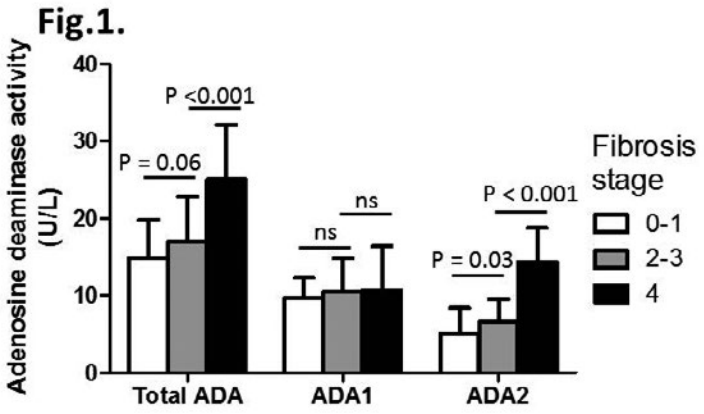
Abstract Category: Pathogenesis, translational science, NAFLD/NASH, liver fibrosis, humans

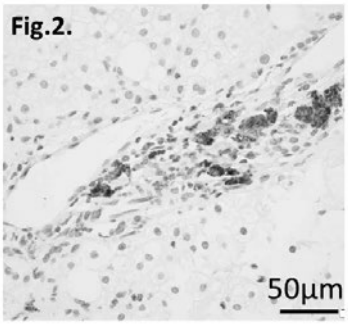
Background/Aim: Adenosine deaminase 2 (ADA2), also known as cat-eye chromosome region 1 (CECR1), is one of the two adenosine deaminases in humans that catalyze the conversion of adenosine to inosine. Recent studies demonstrate that loss-of-function mutations in ADA2 promote a proinflammatory phenotype switch of macrophages and result in vasculopathy and early-onset stroke. As both adenosine and its antagonist caffeine have been shown to impact liver fibrosis, we aim to understand whether ADA2, an adenosine-converting enzyme is involved in the pathogenesis of fibrosis in nonalcoholic fatty liver disease (NAFLD).

Methods: We measured total ADA and ADA2-specific activity in the serum among 100 patients with biopsy proven NAFLD from a patient registry at a tertiary referral liver center. The expression of ADA2 in the liver was determined by immunohistochemistry on formalin-fixed liver biopsy samples (n=91), and quantitated using histological scores. Serum ADA2 activity and the ADA2 expression in the liver were correlated with NAFLD activity score, the stage of liver fibrosis and clinical features of NAFLD.

Results: The serum activity of ADA2, but not ADA1, correlates with the stage of fibrosis determined by liver biopsy (Fig.1.). Clinically, serum ADA2 activity are also positively associated with age and diabetes after adjustment for stage of liver fibrosis. In human liver, ADA2 expression was observed in macrophages in the portal area (Fig.2.). The number of ADA2-positive macrophages per portal triad on histology correlates with the degree of steatohepatitis and the stage of liver fibrosis, although this correlation disappears in cirrhosis.

Conclusion: ADA2-positive macrophages in the portal area correlate with inflammation and liver fibrosis in non-cirrhotic patients with NAFLD. ADA2 may be implicated in the pathogenesis of liver fibrosis in NAFLD. ADA2 may represent a drug target, whilst serum ADA2 level may be a potential biomarker for disease monitoring and prognostication.





[30 - ORAL WINNER] TRANSCRIPTOMIC SIGNATURE IN RODENT NASH BUT NOT NAFL MODELS IS SIMILAR TO THAT IN THE LIVERS OF NASH PATIENTS WITH MODERATE-TO-SEVERE DISEASE

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Abstract Category: Pathogenesis, translational science, NAFLD/NASH, liver fibrosis, humans

Background: Non-alcoholic Steatohepatitis (NASH) will develop in a substantial proportion of people with non-alcoholic fatty liver disease (NAFLD). Currently, there is no approved therapy for NASH. Academic research and drug discovery dedicated to NASH treatment have a need for models that represent the severe NASH pathology. The aim of our study is to compare the hepatic gene expression signature identified in moderate-to-severe NASH patients with that of three rodent NASH models, that were previously used to validate the efficacy of Elafibranor, a dual PPARα/δ agonist, currently being evaluated in a phase 3 study RESOLVE-IT.

Methods: Gene expression in all samples was established by Affymetrix technology. The differential analysis was performed using R program and the Limma package. Each probeset was annotated using Ingenuity Pathways Analysis (IPA). The human NASH signature was then compared to the signature that was identified in NASH and NAFL models. Rodent models were classified as either NASH or NAFL by histology.

Results: Transcriptomic analysis in this study has identified three major gene clusters in livers of patients with moderate-to-severe NASH that represent genes involved in (1) liver remodeling and repair, (2) immune system response and (3) selected anabolic and catabolic reactions. A significant overlap of the hepatic gene expression signature was observed between the human moderate-to-severe NASH dataset and the datasets from the rodent NASH models. The overlap between the human NASH signature and the rodent NAFL models was less significant. Elafibranor treatment prevented NASH development in rodent models as evaluated by histology and had a profound effect on human NASH-associated gene expression in these models. The expression of the genes that are associated with such physiological pathways as EMT, ECM remodeling, immune system function and response to infection were most affected by Elafibranor treatment.

Conclusion: A significant overlap of the hepatic gene expression signature was observed between the human moderate-to-severe NASH dataset and the datasets from the three rodent NASH models. Elabrinanor had a profound effect on gene expression within the moderate-to-severe NASH signature in rodents. We speculate that a similar set of genes is regulated by elafibranor in human NASH patients, which is consistent with the efficacy of elafibranor on ballooning and inflammation (NASH resolution) in the Ph2b GOLDEN505 study.

[31 - ORAL WINNER]
VLX103, A FIRST-IN-CLASS, INVESTIGATIONAL HEPATOSELECTIVE THERAPEUTIC STRATEGY FOR THE TREATMENT OF INFLAMMATORY LIVER DISEASES

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Abstract Category: Pharmacology

Background/Aims: Intestinal dysbiosis is an important determinant of metabolic syndrome and chronic liver disease, such as Non-Alcoholic Fatty Liver Disease (NAFLD), Alcoholic or Non-Alcoholic Steatohepatitis (ASH or NASH), and even cirrhosis. Dysbiosis results in intestinal inflammation, increased gut permeability and subsequent translocation of LPS (lipopolysaccharide: endotoxin) into the portal circulation and the liver. Chronically elevated portal and hepatic LPS exposure triggers CD14/TLR4-dependant pro-inflammatory, pro-fibrogenic and pro-steatotic signaling pathways in the liver. Reduction of the LPS burden in animal models is associated with reduction of this response. Consequently, LPS neutralization is proposed as a therapeutic intervention in human liver disease, and justifies the development of VLX103, a new hepatoselective LPS neutralizer.

Methods: The oral administration of VLX103 confers a unique hepatoselective biodistribution profile to the drug, very different from the IV route. This minimizes systemic exposure to non hepatic organs and therefore optimizes safety. In order to study the pharmacological, pharmacokinetic and clinical profile of VLX103, a preclinical and clinical drug development program is being carried out.

Results: VLX103 binds LPS *in vitro* more efficiently than Polymixin B, with a binding affinity around 0.12 μM. Several *in vivo* animal studies also confirmed that pentamidine can prevent the hepatoxic effects of LPS. VLX103 has shown strong biochemical and histological evidence of hepatoprotection, along with improved survival (19-37 fold) in the murine Galactosamine/LPS model. In this fulminant acute liver injury model, VLX103 blocked liver injury even 2 and 3 hours post-injury, when damage was at peak. Moreover, oral VLX103 significantly reduced ALT, hepatic triglycerides contents and bodyweight in the High Fat Diet NAFLD/NASH mouse model, when given only the last 2-3 weeks of the dietary period. On the other hand, the NIAAA Alcoholic Hepatitis mouse model was used to evaluate the therapeutic potential of VLX103 in ASH. In this experiment, plasma AST and ALT were significantly decreased by 50% vs control, and hepatic triglycerides by 34%. Moreover, TNF-alpha, Interferon gamma, IL-6 and chemokine (C-C motif) ligand were significantly reduced by 69 to 81%. Thus, VLX103 had beneficial effects on the complete triad of abnormalities in ALD: steatosis, injury and inflammation. Hepatoselectivity was confirmed clinically in liver disease patients, in a double blind, placebo controlled, randomized, dose ascending Phase I study. Hepatic tissue pentamidine concentrations were more than 1,000-fold higher than plasma. Safety and tolerance of VLX103 was also assessed, and showed a Maximum Tolerated Dose of 900 mg QD with GI adverse events being the only clinically significant observations reported, and only at the higher doses of the drug. Currently, VLX103 is also being studied in moderate ASH subjects, in collaboration with the NIAAA, and will be explored in severe ASH as well as non-cirrhotic NASH.

Conclusion: VLX103 is safe and hepatoselective within a wide range of human doses. Its unique LPS neutralization ability provides an opportunity to treat inflammatory liver diseases such as NASH and ASH in a different way compared to metabolic dysregulation correction. Moreover, since VLX103 addresses the endotoxemic component of liver disease, it may be useful in conditions where LPS translocation is enhanced by cofactors such as alcohol, in particular in overlapping NASH/ASH (BASH).

Only partial data from this abstract was presented at AASLD 2015 and 2016.

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[32]
VOLIXIBAT, A MINIMALLY ABSORBED, ORAL, APICAL SODIUM-DEPENDENT BILE ACID TRANSPORTER INHIBITOR, INCREASES BILE ACID EXCRETION, REDUCES SERUM LIPIDS, AND IS SAFE AND TOLERABLE IN OVERWEIGHT AND OBESE SUBJECTS, A POPULATION CHARACTERISTIC OF NONALCOHOLIC STEATOHEPATITIS

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Abstract Category: Pharmacology

Background/Aim: Abnormal cholesterol metabolism and accumulation of toxic free cholesterol in hepatocytes may result in hepatic inflammation and fibrosis, and is a potential factor contributing to the pathogenesis of nonalcoholic steatohepatitis (NASH). Accordingly, removal of cholesterol from the liver is a treatment approach that could decrease and possibly reverse damage. Volixibat (SHP626, formerly LUM002) blocks bile acid (BA) reabsorption by inhibiting the apical sodium-dependent bile acid transporter (ASBT) in the terminal ileum. Consequently, BAs are excreted in the feces forcing the liver to synthesize new BA from cholesterol in the liver and serum. It is hypothesized that inhibition of BA reuptake could lead to therapeutically beneficial metabolic, anti-inflammatory, anti-steatotic, and anti-fibrotic effects in NASH. This study aimed to assess the safety, tolerability, pharmacodynamics (PD) and pharmacokinetics (PK) of volixibat administered for 12 days.

Methods: In this double-blind, randomized, placebo (PBO)-controlled, dose-finding, phase 1 study, overweight or obese men and non-childbearing women (18–65 years old) were randomized to seven cohorts of varying volixibat dose (2–80 mg) and regimen (once daily [QD], twice daily [BID] or titration). Evaluations included PD assessments (fecal BA and serum 7α-hydroxy-4-cholesten-3-one [C4] concentration – a marker of synthesis of BA from cholesterol), PK, stool hardness (Bristol Stool Chart [BSC]), safety and tolerability.

Results: 84 subjects were randomized to volixibat (n = 63) or PBO (n = 21). Consistent with the minimal absorption of volixibat, PK could not be calculated. Mean (± SD) daily fecal BA excretion was higher in subjects receiving volixibat (930.61 ± 468.965 μmol) than in those receiving

PBO (224.75 ± 195.403 μmol). Maximal inhibition of BA reabsorption occurred at volixibat doses ≥ 20 mg QD; 10 mg QD was about two thirds of maximal effect, and 5 mg BID was about one third greater than 10 mg QD and comparable to 20 mg QD. On the final day of dosing (Day 12), mean serum C4 concentration was higher with volixibat than PBO. Median (range) reduction of 0.70 (–2.8 to 0.4) mmol/L in total cholesterol and of 0.6990 (–3.341 to 0.570) mmol/L in low-density lipoprotein cholesterol was observed with volixibat. While, overall, volixibat was considered to be safe and well-tolerated, the frequency of bowel movements increased from an overall median of 1 daily evacuation (range: 0–4) pre-dose to 2 evacuations (range: 0–8) during treatment, and was not dose-dependent. Proportionately more stool samples were rated Type 6 or 7 (BSC) with volixibat than PBO. There were no serious adverse events; all subjects completed the trial.

Conclusions: This dose-finding study supports further investigation of volixibat in patients with NASH.

Abstract previously presented as a poster at The Liver Meeting® 2016, 67th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), 11–15 November, Boston, MA, USA. Associated abstract details: Palmer M *et al*. *Hepatology* 2016;64(S1):574A (Abstract 1139), published by John Wiley & Sons, Inc., Hoboken, NJ, USA © 2016 AASLD.

[33]
A PHASE 1 STUDY OF BMS-986036 (PEGYLATED FGF21) IN HEALTHY OBESE SUBJECTS

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Abstract Category: Therapeutic trials NASH/liver fibrosis

Background: Fibroblast growth factor 21 (FGF21), a non-mitogenic hormone, is an important regulator of glucose and lipid metabolism. FGF21 analogs improve insulin sensitivity and lipid profiles, which contribute to nonalcoholic steatohepatitis (NASH) pathogenesis, in preclinical models as well as in obese humans with type 2 diabetes. BMS-986036 is a pegylated recombinant human FGF21 with an extended elimination half-life, enabling daily and weekly regimens to be studied.
Methods: 96 healthy obese (BMI 30-40 kg/m²) subjects were randomized 3:1 to subcutaneous (SC) BMS-986036 or placebo (PBO) in a 16-arm single ascending dose (SAD)/ multiple ascending dose (MAD) single-center study with primary endpoints of safety, tolerability, PK and pharmacodynamics. SAD subjects received doses of 0.3, 1, 3, 10, 30, or 60 mg of BMS-986036 or PBO. MAD subjects received BMS-986036 0.3 mg QD, 1 mg QD, 3 mg QD, 10 mg QD, 30 mg QD, or PBO for 14 d; one arm received BMS-986036 21 mg QD for 8 d. Subjects were in-house from D-2 until D15 (SAD) or D22 (MAD) and received an identical diet 48 h prior to D1 and D16. RNA expression was analyzed from D-1 and D16 (MAD) abdominal adipose tissue biopsies, and pathway analysis was conducted. Metabolomic profiling was performed on D1, D8 and D16 (MAD) serum.

Results: Study subjects had a mean age of 41.8 y and mean BMI of 34.1 kg/m². 83% of participants were men and 41% were Hispanic. There were no deaths, SAEs, discontinuations due to AEs or dose-related changes in AEs. Among MAD subjects who received BMS-986036, the most common AE was injection site erythema (22.2%). All treatment-related AEs were mild. BMS-986036 showed linear PK; the average elimination T1/2 was approximately 24 h. Accumulation was 2-3 fold with QD dosing and negligible with QW dosing. 14 days of BMS-986036 was associated with dose-dependent improvements in body weight, insulin sensitivity, triglycerides, lipids and adiponectin (Table 1), as well as increased expression of genes involved in mitochondrial oxidative phosphorylation and metabolites associated with branched chain amino acid catabolism.

Conclusions: Single and multiple SC doses of BMS-986036 QD for up to 14 days or QW up to 8 days in healthy subjects were well tolerated and associated with dose-dependent improvements in body weight, insulin resistance, triglycerides, LDL and adiponectin. Gene expression and metabolomics analyses suggest that BMS-986036 may improve mitochondrial function and amino acid homeostasis, which may be perturbed in NASH. These beneficial effects on key drivers of NASH pathogenesis support evaluation of BMS-986036 in Phase 2 studies of NASH.

D15 Adiponectin-% change from D1						
PBO	0.3mg QD	1mg QD	3mg QD	21mg QW	10mg QD	30mg QD
-34.9	-22.9	-9.1	-2.7	0.3	19.4	41.7

[34 - ORAL WINNER]
A PHASE 2 STUDY OF BMS-986036 (PEGYLATED FGF21) IN OBESE ADULTS WITH TYPE 2 DIABETES AND A HIGH PREVALENCE OF FATTY LIVER

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Abstract Category: Therapeutic trials NASH/liver fibrosis

Background: Fibroblast growth factor 21 (FGF21), a non-mitogenic hormone, is an important regulator of glucose and lipid metabolism. FGF21 analogs improve insulin sensitivity and lipid profiles, which contribute to nonalcoholic steatohepatitis (NASH) pathogenesis, in preclinical models as well as in obese humans with type 2 diabetes mellitus (T2DM). BMS-986036 is a pegylated recombinant human FGF21 with an extended elimination T1/2. T2DM is a risk factor for non-alcoholic fatty liver disease (NAFLD), NASH and fibrosis. This study aimed to determine if BMS-986036 is an effective treatment for T2DM and its metabolic complications, which are highly associated with NASH pathogenesis.

Methods: In this randomized, placebo (PBO)-controlled Phase 2 trial, 120 obese (BMI 30-50 kg/m²) subjects with T2DM on diet and exercise alone or with metformin received subcutaneous (SC) BMS-986036 1 mg QD, 5 mg QD, 20 mg QD, or 20 mg QW vs. PBO for 12 wks. Primary endpoints (EP) were safety, tolerability and HbA1c; secondary EP included body weight, insulin sensitivity (composite insulin sensitivity index, CISI) and pharmacokinetics (PK). Exploratory EP included circulating triglycerides, LDL, HDL, adiponectin and Pro-C3 (N-terminal type Ill collagen propeptide), a biomarker associated with hepatic fibrosis.

Results: At baseline, mean age was 56 years, female 53%, white 79%, Hispanic 25%, mean weight 96.2 kg, mean BMI 34.8², mean HbA1c 7.6%, metformin use 47%, statin use 44%. 100/104 (86.2%) had fatty liver index ≥60, indicating a high prevalence of fatty liver. PK was linear and Ctrough was stable during dosing. BMS-986036 did not meaningfully change HbA1c or body weight. BMS-986036 improved CISI (20 mg vs PBO, p=0.03) and led to dose dependent improvements in triglycerides, LDL and HDL, as well as increases in serum adiponectin (Table 1) and decreases in serum Pro-C3. There were no deaths or discontinuations due to SAEs. No AEs appeared to be dose dependent, although diarrhea (14.6% vs 4.2%), nausea (6.3% vs 0%) and dyspepsia (6.3% vs 0%) were more frequent for BMS-986036 vs PBO. 95.1% of AEs were mild or moderate. No subjects had laboratory-confirmed hypoglycemia and injection site reactions were infrequent (4.2%).

Conclusions: BMS-986036 improved insulin sensitivity, triglycerides, HDL and LDL in T2DM patients with a high prevalence of fatty liver. BMS-986036 addresses metabolic derangements that may contribute to NASH pathogenesis. Furthermore, improvements in adiponectin and Pro-C3 suggest that BMS-986036 may have an independent antifibrotic effect. Results support further clinical trials in NASH.

Wk12 Adiponectin-% Change from D1				
PBO	1mg QD	20mg QW	5mg QD	20mg QD
3.9	24.2	25.2	31.4	39.7

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[35]
ALT AS A NON-INVASIVE BIOMARKER OF HISTOLOGICAL RESPONSE TO PHARMACOTHERAPY IN NASH PATIENTS: INSIGHTS FROM THE ELAFIBRANOR GOLDEN505 TRIAL

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Abstract Category: Therapeutic trials NASH/liver fibrosis

Background and aims: While ALT imperfectly predicts histological severity, a relation between ALT changes and histological response (HR) in treated NASH patients (pts) has been suggested. If confirmed, this will help predict treatment efficacy without the need for a control liver biopsy. We assessed ALT and histological changes in the 1-year GOLDEN505 randomized trial of elafibranor (ELA).

Methods: Pts treated with placebo (PBO, N=77) or ELA 120mg (N=77) were included. HR was defined as resolution of NASH without fibrosis worsening. All completers (N=154) and NAS≥4 (NAFLD activity score) completers (N=129), both with high baseline ALT (N=75, ALThigh: ALT>1.5 ULN) and low baseline ALT (N=79, ALTlow: ALT≤1.5 ULN) were analyzed for effects on ALT and HR. HR was also assessed in pts with ALT decrease (N=99) or with stable or increased ALT (N=53) at end-of-treatment.

Results: At baseline, median ALT was higher with increasing NAS: 41 IU/ml in NAS=3 to 63 IU/ml in NAS=6 (p<0.001). ALThigh had more severe histological lesions than ALTlow (NAS=5.4±1.2 vs 4.6±1.2, p<0.001). When considering baseline ALT, the HR rate was higher with ELA than with PBO in ALThigh pts (22% vs 11%, respectively, OR=11.6, p<0.001) while in ALTlow pts the difference was lower: 22% vs 18%, OR=1.54, p=0.5. This was confirmed in NAS≥4 pts (ALThigh: 22% ELA vs 8% PBO, OR = 16.8, p<0.001; ALTlow: 19% ELA vs 15% PBO, OR=1.97, p=0.4). Compared to PBO, ELA reduced ALT both in ALThigh and in ALTlow (LSmean±SE was -9.9± 10.1% and -13.9±12.3 % respectively). When considering ALT changes on treatment, the HR rate was higher in pts with an ALT decline than in those with stable or increasing ALT (26% (26/99) vs 4% (2/53), p<0,001). Also, ALT changes were larger in HR (N=28) than in non responders (N=126):-29±23% vs +1±57%; p<0.001. In both ELA and PBO, a progressive decrease in ALT was observed in HR but not in non-responders. In ELA, ALT reduction was higher in HR (N=17) than in non-responders (N=60):-34±23% vs -3±67, p<0.05. Similarly, in PBO, ALT reduction was higher in HR than in non-responders (N=66):-20±21% vs +5±47%, p<0.05. In pts with declining ALT, ELA had higher HR rates than PBO (30% vs 22%, OR = 2.05, p<0.145) with a stronger difference for NAS≥4: 30% vs 16%, OR = 3.21, p<0.05).

Conclusion: A decline in ALT is associated with histological improvement, particularly on active pharmacotherapy. Pts who resolve NASH have the strongest time-dependent reduction in ALT. A higher baseline ALT is associated with more active disease and better response of HR to ELA over PBO. Although ALT reduction is not an absolute predictor of HR it may provide important insight on treatment effect.

[36 - ORAL WINNER]
IMPROVEMENT IN NASH HISTOLOGICAL ACTIVITY HIGHLY CORRELATES WITH FIBROSIS REGRESSION

Vlad Ratziu[1,2], Sven Francque[3], Stephen Harrison[4], Quentin M. Anstee[5], Pierre Bedossa[6], John Brozek[7], Dean W. Hum[7], Sophie Megnien[7], Alice Roudot[7], Rémy Hanf[7], Bart Staels[8], Arun J. Sanyal[9]

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Abstract Category: Therapeutic trials NASH/liver fibrosis

Introduction: A NASH treatment should protect from long-term progression to cirrhosis and its complications by suppressing the underlying cause of fibrogenesis. Whether changes in individual histological features of NASH alter fibrosis progression remains to be determined.

Methods: All completers of the 1-year GOLDEN-505, elafibranor vs. placebo trial (N=237) were analyzed. Biopsies were scored by the NASH-CRN classification at baseline and end-of-treatment (EOT). At inclusion all pts had scores ≥1 for steatosis, lobular inflammation and hepatocyte ballooning. Pts were grouped by changes between EOT and inclusion for steatosis (from -3 to +2), lobular inflammation (from -2 to +2) or ballooning (from -2 to +1) scores. For each group, the percentage of patients experiencing an improvement or a worsening (>1 stage) in fibrosis stage was calculated. Associations between changes in scores and fibrosis evolution were assessed by the Fisher exact test.

Results: Changes in both lobular inflammation and ballooning were highly and positively correlated with changes in fibrosis ($p<0.001$ and $p=0.04$, respectively). Among pts with a 2 point score reduction in inflammation, 67% improved fibrosis (0% worsened); in contrast, if inflammation progressed by >1 point, 56% of pts worsened fibrosis and only 6% improved. For ballooning changes, a 2 point reduction in score resulted in 71% fibrosis improvement (0% worsening); conversely, a 1 point increase resulted in 35% fibrosis worsening and only 26% improvement. In contrast, there was no association between changes in steatosis scores and changes in fibrosis: 25% improved and 0% worsened for a change ≤-2; 45% improved and 18% worsened for a change ≥1. An activity index defined as the sum of lobular inflammation and ballooning scores shows a positive linear relationship with mean changes in fibrosis score (R2=0.95). Similar results were obtained when considering placebo and elafibranor-treated patients separately or when considering only patients with NAS≥4 and F≥2 at inclusion.

Conclusion: Improvement in NASH activity and regression of fibrosis are highly correlated supporting the concept that resolution of NASH can reverse fibrosis progression and is reasonably likely to predict long term clinical benefit. (no table selected).

[37 - ORAL WINNER]
CHOLESTEROL CRYSTALS TRIGGER INFLAMMASOME ACTIVATION AND MARKS THE TRANSITION FROM SIMPLE STEATOSIS TO STEATOHEPATITIS IN HUMANS AND MICE WITH NON-ALCOHOLIC STEATOHEPATITIS

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Abstract Category: Pathogenesis, translational science, NAFLD/NASH, liver fibrosis, humans

Non-alcoholic fatty liver disease (NAFLD) is an emerging medical problem and is characterized by a spectrum of liver disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH). The triggers of inflammation and factors that facilitate the transition from simple steatosis to steatohepatitis are largely unknown. Immune responses are key to inflammation. Inflammasomes are multiprotein oligomers that lead to production of interleukin (IL)-1 and -18 in myeloid immune cells, which contributes to perpetuation of inflammation.

Here we assessed the role of cholesterol and the inflammasome activation in the liver of humans with fatty liver disease (n=21), C57Bl6 mice who developed steatohepatitis after prolonged feeding high-fat diet, and in vitro cultured mouse hepatocytes model cell line Hepa 1-6 after stimulation with cholesterol crystals. Frozen liver tissue was analyzed for unesterified free cholesterol by staining with filipin. Liver architecture was analyzed by light (H&E stain) and electron microscopy. Gene expression was assessed by PCR.

We identified free cholesterol, detected by fillipin staining and strong birefringence under polarized light, in the liver of humans with NASH, C57Bl6 mice who developed steatohepatitis after prolonged feeding high-fat diet, and in vitro cultured mouse hepatocytes/their model cell line Hepa 1-6 after stimulation with cholesterol crystals; no such findings were identified in the livers of normal control humans, control-diet-fed animals or control Hepa 1-6. Electron microscopy identified crown-like crystal-enriched structures in livers of human patients with high NAS score (5-6) but not in those with low NAFLD activity score (NAS) score (1-3) or in controls without steatohepatitis. The expression of NLRP3, CASP1, AIM2, Caspase-1, Pannexin-1, PYCARD, and IL1B RNA, suggestive of inflammasome activation, was increased in liver of humans with NAFLD and C57Bl6 mice with dietary fat-induced steatohepatitis compared to normal humans and control diet-fed mice, respectively. Livers of humans with low NAFLD activity score (NAS) score (1-3) had lower levels of inflammasome markers RNA compared to those with high NAS score (5-6). Exposure to crystalized, but not uncrystalized, cholesterol led to increase in expression of inflammasome genes in Hepa 1-6. The degree of increase in expression of inflammasome genes was augmented when peripheral blood mononuclear cells were added to the crystalized cholesterol-exposed Hepa 1-6 cells, and further augmented when the co-culture was stimulated with bacterial lipopolysaccharide (LPS). The cholesterol crystal-triggered inflammasome activation in Hepa 1-6 cells was blocked by ROS scavengers, ATP depletion, BAPTA-AM, and culture in calcium-depleted media, suggesting a role for these pathways.

Our data suggest that inflammasome activation, likely triggered by crystalized cholesterol, marks the transition from steatosis to non-alcoholic steatohepatitis. These findings suggest potential translational value of inflammasome blockade with intent to stop progression of in NAFLD/NASH.

[38 - ORAL WINNER]
PREDICTING THE DEGREE OF LIVER-BIOPSY-CONFIRMED STEATOSIS AND NASH USING TRANSIENT ELASTOGRAPHY AND MRI-BASED IN ADULT PATIENTS WITH SUSPECTED NAFLD

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Background/Aim: The ongoing prospective NASH prevalence study aims: 1) to assess the prevalence and severity of NASH; 2) to evaluate the diagnostic performance of FibroScan- (FS) and MRI-based methods in predicting the degree of hepatic fibrosis and steatosis as compared to liver biopsy; 3) to investigate the ability of these imaging modalities to discriminate patients with NASH from those with non-NASH.

Methods: Medical beneficiaries (18y - 80y) from the San Antonio region that were referred for routine colon cancer screening and did not have a prior history of liver disease, NAFLD or alcohol abuse were requested to participate in the study. FibroScan data (Echosens, Paris, France) were analyzed for the Liver Stiffness measurement (FS-LSM) and Controlled Attenuation Parameter (FS-CAP). MRI data (Liver Multiscan or LMS, Perspectum Diagnostics, Oxford, UK) were analyzed for proton density fat fraction (LMS-PDFF) and liver inflammation fibrosis score (LMS-LIF). Patients with an FS-LSM ≥7 kPa, MRE-LSM ≥3 kPa, LMS-PDFF ≥5%, or LMS-LIF ≥ 2 underwent a liver biopsy which were read by a single pathologist. Steatosis and fibrosis were scored according to Brunt and NASH was defined by the presence of lobular inflammation, steatosis > 5% and ballooning.

Results: To date, 388 patients underwent imaging of which 172 completed liver biopsy. Patients that received a liver biopsy had more fibrosis and steatosis compared to patients that did not. The prevalence of biopsy-confirmed NASH within enrolled patients was 7,7% (30/388 patients). There was a mean LMS-PDFF of 4.38%, 8.64%, 17.04 and 26.17% for grade 0, 1, 2 and 3 steatosis, respectively. Those who did not qualify for biopsy had an average LMS-PDFF of 3.68%. All five groups were found to be significantly different in LMS-PDFF % (Kruskal-Wallis p<0.001). LMS-PDFF was found to be sensitive to identification of steatosis (≥ 5%) with an AUC of 0.896. Similarly, there was a mean FS-CAP of 276.8 dB/m, 313.8 dB/m, 358.8 dB/m, and 350.3 dB/m, for grade 0, 1, 2 and 3 steatosis. Those without liver biopsy had a mean FS-CAP of 270.9. All groups, except grade 2 versus 3, were significantly different (Kruskal-Wallis, p<0.01). FS-CAP values of <240 dB/m excluded steatosis with a sensitivity of 96% and a CAP of >350 dB/m confirmed a diagnosis of NAFLD with a specificity of 98% as measured by LMS-PDFF.

Conclusions: Using several novel imaging markers**,** this study confirms the high prevalence of NASH in adult patients in the USA. LMS-PDFF is excellent, and FS-CAP is very good at predicting the grade of hepatic steatosis in NAFLD patients.

The results of this abstract will reflect an update of those presented at AASLD 2016.