Wrap-up session:
Metabolic and alcohol related liver disease

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Number of abstracts per category

- Alcoholic liver disease
- NAFLD: Clinical aspects
- NAFLD: Diagnostics
- NAFLD: Experimental
- NAFLD: Therapy
Alcohol: pathogenesis
Distinct functions for RIP3 and MLKL
In murine models of ALD vs NAFL/NASH

Ethanol-induced liver injury:
• RIP3 drives injury independently of MLKL

High fat diet-induced liver injury
• MLKL drives injury independently RIP3
Alcohol: clinical aspects
Health Survey for England 2016
• Cross sectional survey of households
• Designed to be representative of the general population of England
• 7,826 adults of whom 3,791 (48%) provided a blood sample
• 89.2% of sample had at least one risk factor for liver disease
• 28.4% had two or more risk factors (alcohol>14 units/wk (112g/wk), BMI≥25, waist circumference high/very high, diabetes)
• 13% of population are drinking above recommended alcohol (but not high risk alcohol) AND are obese or overweight
  • we call this group at risk of BAFLD = Both Alcohol And Fatty Liver Disease)
  • This group is often missed in clinical referral pathways from primary to secondary care
Proportion of the general population with high liver blood tests and fibrosis scores

- 10.9% had high ALT or AST (approx. half previous estimates)
- AST:ALT ratio high in >70% of general population
- Awareness of risk and testing for liver disease was low
Fibroscan (LS) predicts long-term survival in a 10-year prospective cohort of heavy drinkers

**Aim:** Establish long-term survival data in a 10-year prospective cohort of heavy Caucasian drinkers primarily presenting for alcohol detoxification

- 675 patients with mean alcohol consumption 186.5 g/d
- Mean duration of heavy drinking was 14.3 years
- 106 patients (15.7%) died during the observation period
Fibroscan (LS) predicts long-term survival in a 10-year prospective cohort of heavy drinkers

The cause of death could be clarified in 42 patients (39%) and it was liver-related in 16 (38%).
LS was an independent predictor of death in multivariate analysis (next to age, ALP, and albumin)
AUROC 0.72 at 14 kPa cutoff

<table>
<thead>
<tr>
<th></th>
<th>&lt;6 kPa</th>
<th>6–12.5 kPa</th>
<th>&gt;12.5 kPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-year survival rate</td>
<td>94%</td>
<td>88%</td>
<td>74%</td>
</tr>
<tr>
<td>5-year survival rate</td>
<td>90%</td>
<td>78%</td>
<td>64%</td>
</tr>
</tbody>
</table>

LS monitoring should be mandatory for surveillance of drinkers
Serum bile acid profiles distinguish severe alcoholic hepatitis from decompensated cirrhosis

**Aim:** determine a new non-invasive test that would distinguish SAH acute decompensation of alcohol-related cirrhosis

- SAH patients had biopsy-proven steatohepatitis with MDF ≥32
- Serum BAs measured by mass spectrometry in two cohorts (89 and 105 patients)

<table>
<thead>
<tr>
<th></th>
<th>Full BA profile AUROC</th>
<th>GCA AUROC</th>
<th>TCA AUROC</th>
<th>Bilirubin AUROC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exploratory cohort</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AUROC</td>
<td>0.93</td>
<td>0.90</td>
<td>0.87</td>
<td>0.79</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.87–0.99</td>
<td>0.83–0.97</td>
<td>0.77–0.97</td>
<td>0.67–0.91</td>
</tr>
<tr>
<td><strong>Validation Cohort</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUROC</td>
<td>0.93</td>
<td>0.85</td>
<td>0.83</td>
<td>0.65</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.88–0.98</td>
<td>0.77–0.92</td>
<td>0.74–0.92</td>
<td>0.54–0.76</td>
</tr>
</tbody>
</table>

The entire BA profile and individual BAs of GCA and TCA are promising non-invasive biomarkers for SAH, and may reduce the need for liver biopsy.
Baseline neutrophil-to-lymphocyte ratio in alcoholic hepatitis

**Aim:** To assess the role of neutrophil-to-lymphocyte ratio (NLR), which reflects sepsis and inflammation, in the prognosis of alcoholic hepatitis

- 789 patients in the STOPAH trial
- Prevalent and incident infections treated prior to randomization. Infections developing after inclusion were recorded
- Prevalent and incident AKI was recorded.

<table>
<thead>
<tr>
<th>Incident AKI</th>
<th>Present (n=67)</th>
<th>Absent (n=403)</th>
<th>NLR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident AKI</td>
<td>7.5 (6.4, 8.7)</td>
<td>6.0 (5.6, 6.4)</td>
<td>p=0.0056</td>
<td></td>
</tr>
<tr>
<td>Infection by Day 7</td>
<td>Present (n=94)</td>
<td>7.8 (6.3, 9.2)</td>
<td>p=0.035</td>
<td></td>
</tr>
<tr>
<td>Absent (n=695)</td>
<td>6.1 (5.8, 6.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection by Day 28</td>
<td>Present (n=185)</td>
<td>7.1 (6.3, 8.0)</td>
<td>p=0.025</td>
<td></td>
</tr>
<tr>
<td>Absent (n=604)</td>
<td>6.1 (5.6, 6.5)</td>
<td></td>
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</tbody>
</table>
Baseline neutrophil-to-lymphocyte ratio in alcoholic hepatitis

Risk of developing infection and incident AKI after prednisolone treatment greater if NLR $>8$ vs $\leq 8$:

High NLR associates with prevalent AKI and infection in alcoholic hepatitis. A Lille response to prednisolone is more likely if NLR $\geq 5$, but development of infection or AKI after prednisolone treatment is greater if NLR $>8$. 

\[ p=0.01; \text{OR 1.86 (1.16, 2.99)} \]
NAFLD: basic science and pathogenesis
Western diet triggers a unique inflammatory phenotype of myeloid leukocytes in bone marrow and liver.

Liver
Ly6G\textsuperscript{neg} non-lymphoid CD45\textsuperscript{pos} cells

MoMF = Monocyte derived macrophages

MoMF I
MoMF II
MoMF III

PS-004 Hundermarkt

The diet-induced inflammatory polarization of monocytes and myeloid precursors is already stably imprinted in the bone marrow and determines pathogenic responses driving NAFLD in the liver.
Targets for NASH therapy derived from genetic studies
Hepatic MBOAT7 silencing by i.p. ASO induces microvesicular steatosis *in vivo*

Scramble MPO MBOAT7

H&E (200X)

*P < .005 vs Scramble

Intrahepatic TG (%)

MBOAT7 protein (AU)
Hypothesis of mechanism: Post-prandial state/ hyperinsulinemia/MBOAT7 risk allele carriers

- **Co-A**
- **MBOAT7**
- **Arachidonoyl-CoA**
- **TG**
- **FATP1**
- **FABP1**
- **PI3P**
- **De novo lipogenesis**
- **Saturated-PI**
- **Free Arachidonic Acid**
- **Eicosanoids**
- **Inflammation**
The miR-34a/SIRT1:AMPK pathway is activated in the human NAFLD muscle

Targeting of the miR-34a/SIRT1:AMPK pathway may benefit the liver and the muscle improving whole body metabolism.
Although diet reversal induced metabolic and histological normalization in HFHFD-fed mice, alterations in hepatic Th17, VAT Tc, and VAT Treg cells were not reversed within 12 weeks.

This finding challenges our current understanding of reversibility of NAFLD-related inflammation upon lifestyle modification.
The glucocorticoid antagonist ST-001 prevents fibrosis development in the DIAMOND mouse model

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Fibrosis (NASH CRN)</th>
<th>Perisinusoidal fibrosis (0–2)</th>
<th>NAS</th>
<th>SAF activity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>0.67</td>
<td>1.00</td>
<td>4.56</td>
<td>1.78</td>
</tr>
<tr>
<td>High dose</td>
<td>0.00 p=0.0006</td>
<td>0.10 p=0.0069</td>
<td>4.10</td>
<td>1.1</td>
</tr>
<tr>
<td>Low dose</td>
<td>0.00 p=0.0010</td>
<td>0.11 p=0.0110</td>
<td>4.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

While ST-001’s effect on NASH development is inconclusive, improvements in measures of fibrosis and NAFLD were demonstrated.
NAFLD: clinical aspects
Non-alcoholic fatty liver disease in young adults

A previous cross-sectional analysis of the ALSPAC cohort in late teens identified a NAFLD prevalence of 2.5% by ultrasound criteria.

This study aimed to identify the prevalence of NAFLD in this cohort, now young adults, using TE to measure fibrosis and steatosis with CAP (4,021 participants, exclusion excessive daily alcohol intake).

- ALT, AST, and GGT all rose with F score and CAP score
- CAP associated with F score
- BMI rose with rising F score and CAP score

Using TE, 1 in 5 patients had steatosis and 1 in 40 had fibrosis at 24 years, an increase on the previous estimate within the same cohort 6 years prior. Greater public health awareness of NAFLD is needed in young adults in the UK.
Dietary risk factors for non-alcoholic fatty liver disease by cirrhosis status

**Aim:** examine dietary factors in NAFLD by cirrhosis status in the US Multiethnic Cohort, a large prospective study with >215,000 participants in Hawaii and California

- Nested case-control study
- NAFLD cases identified using Medicare claims
- Diet assessed at baseline via a validated quantitative food frequency questionnaire

2,974 NAFLD cases (518 with cirrhosis; 2,456 non-cirrhotic) and 29,474 matched controls

- Red meat, processed red meat, poultry, and cholesterol independently associate with risk of NAFLD
- Dietary fiber is a protective factor
- Red meat and cholesterol also associated with NAFLD-related cirrhosis
NAFLD, alcohol drinking habits and genetics predict progression

**Aim:** analyze risk factors for the development of advanced liver disease in the general population with NAFLD

- Data from national health surveys: FINRISK 1992–2012 and Health 2000
- Linkage with national registers for hospitalization, death, and cancer
- NAFLD defined as a fatty liver index >30 + alcohol use ≤20g/d (women)/≤30g/d (men)

- 6,462 NAFLD subjects, 58 liver events
- 43% rise in risk of liver events per each additional alcohol drink/day
- Potential misclassification negligible based on validation against CDT

Alcohol **drinking** habits and **genetics** (TM6SF2, PNPLA3) are important co-factors in the progression of NAFLD
Longitudinal prognostic value of the most common NITs for fibrosis

- 918 patients underwent a liver biopsy for suspicion of NAFLD in main referral tertiary centres in Italy and the United Kingdom
- The following scores were calculated: NAFLD Fibrosis Score (NFS), APRI, FIB-4, BAAT and BARD

- Liver-related events (ascites, varices or encephalopathy) → 75
- HCC → 15
- Cardiovascular events → 91

- NFS and FIB4 → prediction of liver events and HCC
- BAAT and BARD → prediction of CV events
- Values of NFS, FIB4, APRI and BARD are associated with poor survival
PS-201
External validation in NAFLD cohorts of the FibroScan-based FAST score combining liver stiffness, controlled attenuation parameter and AST to identify patients with active NASH (NAS ≥ 4) and significant fibrosis (F ≥ 2)

PS-202
Genome-wide association studies of abdominal MRI scans identifies loci associated with liver fat and liver iron in the UK Biobank
Gut-microbiome signature as a biomarker of NAFLD-cirrhosis

Total n= 203 participants
Gut-microbiome 16S rRNA sequencing

<table>
<thead>
<tr>
<th>Probands</th>
<th>Non-NAFLD controls</th>
<th>n=54</th>
<th>Probands with NAFLD without AF</th>
<th>n=18</th>
<th>Probands NAFLD-cirrhosis</th>
<th>n=26</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st degree relative</td>
<td>n= 44</td>
<td></td>
<td>1st degree relative</td>
<td>n= 17</td>
<td>1st degree relative</td>
<td>n = 37</td>
</tr>
</tbody>
</table>

1. Familial gut-microbiome similarity driven by shared housing

2. Gut-microbiome derived signature of NAFLD-cirrhosis: 27 bacterial features and age, sex and BMI

High diagnostic accuracy for the detection of NAFLD-cirrhosis in NAFLD
Objective: to develop an open-access, interactive tool for patients and providers that help them understand the risk of long-term outcomes associated with NAFLD/NASH

Secondary objective: create awareness about adverse consequences of NAFLD/NASH
NAFLD/NASH patients in Italy have a high comorbidity burden. Those with AdvLD have significantly higher costs. Early identification and effective management are needed to minimize disease progression and resource utilization.
NASH: therapy
Data from some ongoing studies

Friedman et al., Nat Med 2018
SAT-357: Tropifexor, a farnesoid X receptor agonist for the treatment of non-alcoholic steatohepatitis: Interim results based on baseline body mass index from first two parts of Phase 2b study FLIGHT-FXR

PS-108: NGM313, a novel activator of beta-Klotho/FGFR1c: A single dose significantly reduces steatosis (liver fat by MRI-PDFF), inflammation (ALT, AST) and fibrogenic activity (Pro-C3) in NAFLD subjects (FGF21)

PS-111: Six month interim results of MSDC-0602 K in a large phase 2b NASH study demonstrate significant improvement in liver enzymes and glycemic control (TZDs)

LBP-20: VK2809, a Novel Liver-Directed Thyroid Receptor Beta Agonist, Significantly Reduces Liver Fat with Both Low and High Doses in Patients with Non-Alcoholic Fatty Liver Disease: A Phase 2 Randomized, Placebo-Controlled Trial

LBP-04: Investigation of synbiotic treatment in non-alcoholic fatty liver disease (NAFLD): Results of the INSYTE study
LBP-10: A structurally engineered fatty acid, icosabutate, rapidly normalises elevated plasma ALT and gamma-glutamyl transferase (GGT) concentrations in a study population at high risk of NAFLD/NASH

PS-109: Partial inhibition of de novo lipogenesis with the acetyl-CoA carboxylase inhibitor PF-05221304 does not increase circulating triglycerides in humans and is sufficient to lower steatosis in rats

PS-110: Ketohexokinase inhibitor PF-06835919 administered for 6 weeks reduces whole liver fat as measured by magnetic resonance imaging-proton density fat fraction in subjects with non-alcoholic fatty liver disease (fructose, DNL)

PS-106: An international, randomized, placebo-controlled phase 2 trial demonstrates novel effects of DGAT2 antisense inhibition in reducing steatosis (TG synthesis)
Lubiprostone for NAFLD: The LUBIPRONZE Phase 2 study

**Aim:** Double-blind, placebo-controlled, randomized, Phase 2 trial to determine whether lubiprostone (LUB) improves gut permeability in NAFLD patients, resulting in reduction of ALT

- Parameters of gut permeability were significantly lower in LUB vs. placebo
- ≥15% MRI-PDFF reduction significantly higher in LUB vs. placebo (*Figure C*)
- LUB24 had a higher rate of AEs (33%) vs. placebo (7%, p=0.0025) and LUB12 (7%)

Manipulating gut permeability may be a promising novel approach in NAFLD
REGENERATE: a Phase 3 trial of obeticholic acid (OCA) for NASH

- In the Phase 2b FLINT study, the potent FXR agonist OCA 25 mg for 72 weeks improved fibrosis and other histological features of NASH
- Month 18 interim analysis of REGENERATE evaluated OCA on liver histology in NASH patients with F2/F3 fibrosis

Study success was defined as achievement of one of these two primary endpoints:

- Fibrosis improvement by ≥1 Stage with No Worsening of NASH
- NASH Resolution with No Worsening of Fibrosis
• OCA 25 mg QD met the primary endpoint of improvement in liver fibrosis with no worsening of NASH

• Although the additional primary endpoint of NASH resolution with no worsening of fibrosis was not met, resolution of NASH based on the overall pathologist’s assessment was more frequent with OCA 25 mg
REGENERATE: a Phase 3 trial of obeticholic acid (OCA) for NASH

- 65.6% in OCA 25 mg normalized ALT vs. 37.3% in placebo
- **Safety**: Increase in LDL-C with both doses of OCA, with an eventual decrease over time
- **Treatment emergent AEs**: Pruritus was reported by 51% of patients with OCA 25 mg vs. 19% in the placebo group
- **Treatment discontinuation** was similar in the three groups. More patients in the OCA 25 mg group (9%) discontinued due to pruritus.

First successful phase 3 trial in patients with NASH
Study is ongoing to confirm benefit on clinically relevant outcomes
Regression in fibrosis following a 12-week aerobic exercise intervention

- **Aim**: Investigate the effects of a 12-week exercise intervention (EI) on hepatic fibrosis in individuals with biopsy-proven NAFLD
- Assessments at baseline (T0), after EI (T1), and 12 weeks after T1 (T2)*
- Liver biopsy, transient elastography, cardiorespiratory fitness (VO2max), physical activity levels, and anthropometry
- EI group: 2 supervised and ≤3 unsupervised sessions per week, increasing intensity (45–75% heart rate reserve) and duration (24–45 minutes), for 12 weeks. Control group: 3 physical assessments

25 individuals (16 exercise, 9 controls)

58% of individuals demonstrated fibrosis regression at T1, despite only 3/12 achieving ≥5% weight loss

By VTCE, stiffness reduction was not maintained at T2
Endoscopic duodenal mucosal resurfacing in type 2 diabetes

**Aim:** Evaluate effect of DMR on glycaemia, hepatic fat, and mechanistic endpoints

Putative role of duodenal mucosal hyperplasia in metabolic disease

- **Nutrient-induced stem cell division**
- **Duodenal mucosal hyperplasia**
- **Insulin resistance syndrome**

High fat + sugar diets $\rightarrow$ Duodenal mucosal hyperplasia $\rightarrow$ Insulin resistance syndrome

Can reversal of hyperplasia alone reverse/ameliorate insulin resistance?

DMR: REVITA single catheter

Schematic of DMR

Multicenter study with early open-label cohort (training purposes, n=24) and randomized double-blind cohort (n=108)
Endoscopic duodenal mucosal resurfacing in type 2 diabetes

Favorable safety/tolerability profile
Promising potential treatment for T2D and NAFLD/NASH.

ALT (U/L): -8.5 ± 2.17 (p<0.001)

Absolute MRI-PDFF: -7.0 ± 1.6 (p<0.001)

Relative (-35.8% ± 7.8, p<0.001) fat fraction reduction
FFA
Adipokine imbalance
Inflammatory cytokines

Bacterial products
Bile acids
Hormones
Specific nutrients

Inflammatory cells
Kupffer cells
HSC
Fibrosis

ROS
JNK
NF-kB
ER stress

Myokines

Modified from Rosselli et al., Curr Pharm Des 2014
Patients with NASH cirrhosis and BL HVPG ≥12 mmHg randomized 1:1:1:1 to **emricasan (pan-caspase inhibitor)**, 5, 25, 50 mg or placebo orally twice daily for 48 wks

- Primary endpoint: Follow-up HVPG at Wk 24
- 263 subjects randomized at 59 US/EU sites → central HVPG reading

<table>
<thead>
<tr>
<th>Mean change from baseline at Wk 24</th>
<th>Emricasan 5 mg N=65</th>
<th>Emricasan 25 mg N=65</th>
<th>Emricasan 50 mg N=66</th>
<th>Placebo N=67</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVPG (Overall)</td>
<td>-0.6; p=0.96</td>
<td>-0.8; p=0.79</td>
<td>-1.0; p=0.65</td>
<td>-0.4</td>
</tr>
<tr>
<td>HVPG (compensated, HVPG ≥16 mmHg)</td>
<td>-1.6; p=0.01</td>
<td>-1.7; p&lt;0.01</td>
<td>-1.5; p=0.02</td>
<td>+0.5</td>
</tr>
</tbody>
</table>

Primary endpoint was not met. Data suggest that emricasan for 24 wks reduced portal pressure in compensated NASH cirrhosis with higher BL HVPG
In memoriam…

Valerio Nobili, 1966 - 2019