MAFLD

NASH ASH vs. MAFLD: Diagnostic and Treatment Implications

• 2020

• Robert G Gish MD
  Robert Gish Consultants LLC
Terms and Definitions

• NAFLD
  – Nonalcoholic Fatty Liver Disease

• MAFLD
  – Metabolic Associated Fatty Liver Disease

• NASH
  – NonAlcoholic SteatoHepatitis

• Steatosis
  – Fatty changes

• Hepatitis
  – Inflammation

• ASH, AALD, AUD, AH
  – Alcohol induced steatohepatitis, Alcohol Associated Liver Disease, Alcohol Use Disorder, Alcoholic Hepatitis,

• CASH  combined ASH + NASH
Figure 1. Venn diagram illustrating the spectrum of fatty liver diseases and the overlap and distinction of the 2 main entities, namely, metabolic dysfunction predominant and alcohol predominant fatty liver. An updated and more appropriate nomenclature and classification system is required to reflect the nuances of disease etiology within the spectrum of fatty liver disease. The abbreviations are used to merely illustrate the various subgroups: MPFL, metabolic dysfunction predominant fatty liver; APFL, alcohol predominant fatty liver; MPFL/A and MPFL/N: metabolic dysfunction predominant fatty liver with and without alcohol intake that is anything more than ceremonial; and APFL/M and APFL/N: alcoholic predominant fatty liver with metabolic dysfunction or with no metabolic dysfunction.
The changing scenario of Fatty Liver Disease (FLD) classification

Valenti & Pelusi, Liver Int 2020
See: Eslam Gastroenterology 2020, JHEP 2020
ALD and MAFLD rates increasing compared to other risk factors for CLD
Moderate Alcohol Use in Nonalcoholic Fatty Liver Disease

Beneficial effects of moderate alcohol use on cardiovascular mortality well recognised. Cardiovascular mortality is the most common cause of death in MAFLD.

Do patients with MALFD benefit from moderate alcohol use?

**NO CLEAR SAFETY ZONE**

<table>
<thead>
<tr>
<th>Author</th>
<th>Study Size</th>
<th>Location</th>
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<td>331/582</td>
<td>USA</td>
<td>Reduced NASH and fibrosis</td>
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<td>Kwon</td>
<td>52/77</td>
<td>USA</td>
<td>Reduced fibrosis</td>
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<td>Dixon</td>
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<td>Australia</td>
<td>Reduced NASH</td>
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<tr>
<td>Cotrim</td>
<td>75/132</td>
<td>Brazil</td>
<td>Increased NASH</td>
</tr>
<tr>
<td>Ekstedt</td>
<td>65/71</td>
<td>Scandinavia</td>
<td>Fibrosis with heavy episodic alcohol</td>
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<tr>
<td>Ascha</td>
<td>58/195</td>
<td>USA</td>
<td>Increased HCC if cirrhosis</td>
</tr>
</tbody>
</table>
A Disturbing Evolutionary Development
“when you lose weight, you can extend your life by _____ years”
When MAFLD is seen Globally it is associated with Cal intake
The MAFLD Continuum

Normal Liver

Steatosis
“NAFL”
Fatty liver without inflammation or hepatocyte ballooning

Steatohepatitis
“NASH”
Fatty liver with inflammation and hepatocyte ballooning

Cirrhosis
Increasing fibrosis leading to cirrhosis, hepatocellular carcinoma

Worldwide prevalence:
~ 25%
1.50% to 6.45%

Metabolic Syndrome and Its Hepatic Manifestation

Abdominal obesity
Glucose intolerance/insulin resistance
Hypertension
Atherogenic dyslipidemia
Proinflammatory/prothrombotic state

Diabetes
CVD
MAFLD
NASH

National Cholesterol Educational Program (NCEP), Adult Treatment Panel (ATP) III; 2001.
Pathogenesis of MAFLD NASH
The Multi-hit Hypothesis

The Metabolic Syndrome

Insulin Resistance

1st hit
- Normal Liver

2nd hit
- Steatosis

3rd hit
- NASH

- Cytokines
- Adipokines
- Oxidative stress
- Apoptotic pathways
- Others

Fibrosis
Pathogenesis of Nonalcoholic Steatohepatitis: An Overview

Gopanandan Peeranathany, Xavier Revolo, and Harmesh Malhi

FIG. 1. Metabolic interorgan crosstalk in NAFLD. This illustration depicts interorgan crosstalk in NAFL on the left and NASH on the right. Hepatic NEFAs are predominantly derived from three sources: lipolysis in adipose tissue, dietary lipid absorption, and DNL from carbohydrates in the liver. These NEFAs are stored in the liver as TG-rich lipid droplets leading to hepatic steatosis or may be exported out of the liver as very low-density lipoprotein to adipose tissue. Bile acids from the liver are key regulators of the gut-liver axis. Several mediators orchestrate the inflammatory milieu in the liver that results in NASH and fibrosis. Lipotoxic lipid species lead to hepatic stress and subsequent release of EVs, cytokines, chemokines, and DAMPs from liver cells. This results in recruitment of immune cells from the bone marrow. Bile acids from the liver, PAMPs from the gut, and adipokines from adipose tissues also influence various steps in this process. Abbreviations: LD, lipid droplet; VLDL, very low-density lipoprotein.
Prevalence of Self-Reported Obesity Among U.S. Adults by State and Territory, BRFSS, 2014

Prevalence estimates reflect BRFSS methodological changes started in 2011. These estimates should not be compared to prevalence estimates before 2011.

Source: Behavioral Risk Factor Surveillance System, CDC.
NASH Prevalence Among Ethnic Groups

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<tr>
<th>Ethnicity</th>
<th>Prevalence (%)</th>
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<tr>
<td>Overall</td>
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<tr>
<td>Hispanic</td>
<td>19.4</td>
<td>14/72</td>
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<td>Caucasian</td>
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<td>20/205</td>
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<td>African American</td>
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<td>5/37</td>
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<tr>
<td>Other</td>
<td>6.7</td>
<td>1/14</td>
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p=0.03

Williams, Gastroenterology 2011
## Risk Factors for MAFLD

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<th>Major Co-morbidities</th>
<th>Emerging Associations</th>
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<tr>
<td>Type 2 Diabetes</td>
<td>Hypothyroidism</td>
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<tr>
<td>Dyslipidemia</td>
<td>Sleep Apnea</td>
</tr>
<tr>
<td>Obesity</td>
<td>Hypopituitarism</td>
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<tr>
<td>Metabolic Syndrome</td>
<td>Polycystic Ovary Syndrome</td>
</tr>
<tr>
<td>HTN</td>
<td>Increased waist circumference in Lean Nash</td>
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</table>

IS MAFLD Progressive?

Consequences of MAFLD

Normal → Simple Steatosis → NASH → Cirrhosis → HCC

- Simple Steatosis: 15%
- NASH: 7%
- Cirrhosis: 7%
- HCC: 7%

By itself: Non-progressive

References:
Types of Fatty Liver Disease

**NASH**
- Over 10 years
- 10%-20%

**Steatosis alone (NAFL)**
- By itself

**Cirrhosis**
- Benign
The association between liver fibrosis and cognitive impairment in type 2 diabetes: a cross-sectional sub-study of the South London Diabetes (SOUL-D) cohort

Lisa Kuriakose1, Calum D. Moulton1, Anna Rokakis1, Mussarat Rahim2, Khalida Ismail3, Michael J. Heneghan2
1. Department of psychological Medicine, King’s College London, UK;
2. Department of Hepatology, King’s College Hospital NHS Foundation Trust

BACKGROUND
Type 2 diabetes and fatty liver disease are respective risk factors for cognitive impairment. However, their comorbidity in relation to cognitive impairment is poorly studied. For the first time, we tested whether comorbid liver fibrosis is associated with cognitive impairment in people with type 2 diabetes.

METHODS
Design and setting: This is a cross-sectional study nested within the South London Diabetes (SOUL-D) cohort, which is a population-based, multi-ethnic cohort of 17335 people recruited at diagnosis of type 2 diabetes from South London primary care centres between 2008-2012. Subjects were followed up 8-10 years later for the current study, such that confounding by duration of diabetes was minimized.

Predictor variable: Between 8-10 years after diagnosis of type 2 diabetes, we recruited a subset of 101 people to undergo transient elastography (Fibroscan) of the liver. We defined significant (F2) fibrosis using a standard cut off 27kPa. By recruiting all patients at 8-10 years after diagnosis of type 2 diabetes, confounding by duration of diabetes was minimized.

Outcomes: We assessed cognitive function using the multi-domain Rey-Osterreith Complex Figure test, a neuropsychological assessment in which patients reproduce a complicated line drawing under three conditions: 1) by copying; 2) by immediate recall without reference to the figure; and 3) by recall after a 30-minute delay. This assess a range of cognitive domains including visuospatial ability, attention, concentration, executive function and visual memory.

Statistical analysis: We used linear regression models to test the association between liver fibrosis and cognitive performance on each condition. Results were adjusted for age, sex, ethnicity, HbA1c, body mass index and current depressive symptoms using the Patient Health Questionnaire-9.

RESULTS
Of 149 people invited to participate, 101 consented for both Fibroscan and cognitive assessment. The mean age was 63.6 (10.0) years, mean HbA1c 60.0 (19.2) mmol/mol, mean BMI 31.0 (5.8) kg/m² and mean type 2 diabetes duration 9.0 (0.5) years. Of those recruited, 51.5% were female and 51.5% were of non-white ethnicity. On Fibroscan, 27 patients (26.7%) had significant fibrosis. After adjustment for confounders including depression and glycaemic control, liver fibrosis was associated with impaired performance on the copy condition (β = -5.47 [95% CI -8.34, -2.60], p<0.001), immediate recall condition (β = -6.30 [95% CI -10.30, -2.30], p=0.003) and delayed recall condition (β = -4.51 [95% CI -8.27, -0.74], p=0.02).

LIMITATIONS: The study was limited by its cross-sectional design and sample size. Although assessing multiple cognitive domains, the cognitive assessment did not include domains such as verbal memory and social cognition. No non-diabetes control group was recruited, such that the respective impacts of diabetes and liver fibrosis on cognition could not be assessed.

CONCLUSION: In this primary care population with type 2 diabetes, there is preliminary evidence of impaired cognitive performance across multiple domains in those with comorbid liver fibrosis. Longitudinal replication of these findings on a larger scale is needed, including the assessment of potentially modifiable targets such as insulin resistance.

Figure 1: The Rey-Osterreith Complex Figure
Each of the 18 segments is scored 0-2, providing a range of scores between 0-36. The test is carried out under 3 conditions:
1. Copy condition – subject copies directly from the figure.
2. Immediate recall condition – immediately after the copy condition, subject is asked to redraw figure without reference to figure. Subject is not warned in advance of this.
3. Delayed recall condition – subject asked to redraw figure 30 minutes later without reference to figure.

Figure 2: Example of Rey-Osterreith Complex Figure
Subject shows good performance on the copy condition (left) but very poor performance on the delayed recall condition (right), reflecting severe deficits in visual memory.

Figure 3: Parallel Lines
Outcomes in MAFL-D

<table>
<thead>
<tr>
<th>Overall Mortality</th>
<th>Surrogates</th>
<th># Studies</th>
<th>OR [95% CI]</th>
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<tbody>
<tr>
<td></td>
<td>• NAFLD vs. General Population</td>
<td>8 studies</td>
<td>1.57 [1.18-2.10]</td>
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</table>

<table>
<thead>
<tr>
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<th>Surrogates</th>
<th># Studies</th>
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<td></td>
<td>• ALT as a surrogate</td>
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<td></td>
<td>• GGT as a surrogate</td>
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<td></td>
<td>• Imaging as a surrogate</td>
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<td>2.05 [1.81-2.31]</td>
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<table>
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<th># Studies</th>
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<td>• GGT as a surrogate</td>
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<td></td>
<td>• Imaging as a surrogate</td>
<td>3 studies</td>
<td>3.51 [2.28-5.41]</td>
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Fibrosis Drives Outcomes in NAFLD
Follow-up of 209 Patients – AFIP, 2010

Liver-related mortality

NASH is the Fastest Growing Cause of HCC Among Liver Diseases

Younossi et al, *J Clin Gastro Hep*, 2018
## Liver-Related and Overall Mortality in Patients with NAFLD

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Follow-up (mean, yrs)</th>
<th>Liver-related mortality</th>
<th>Overall mortality</th>
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<td><strong>Simple Steatosis</strong></td>
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<td>9</td>
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<td>Ekstedt et al (2006)</td>
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<td>Rafiq et al (2009)</td>
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<td>Matteoni et al (1999)</td>
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<td>Ekstedt et al (2006)</td>
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<td>Rafiq et al (2009)</td>
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<td>Hui et al (2003)</td>
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<td>Sanyal et al (2006)</td>
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Lomonaco & Cusi, 2012
Estimated fructose intake and weight trends in the U.S.

Fructose

• Dietary Carbohydrates can be converted to fat in the liver

• Fructose (alone or as part of sucrose) drives lipogenesis and promotes MAFLD

• Epidemiologic studies, clinical trials, and animal studies show that excess carbohydrate consumption contributes to MAFLD

• High fructose consumption depletes hepatic ATP and impairs recovery from ATP depletion mitochondrial toxin

Abdelmalek M, Hepatology 2012
FIG. 2. Molecular pathways of palmitate-induced lipotoxicity in hepatocytes. Palmitate activates the extrinsic death receptor-mediated pathway of apoptosis and also activates the intrinsic pathway of apoptosis. Lysosomal permeabilization leads to the release of the protease caspase 8. Lipotoxic ER stress leads to up-regulation of the proapoptotic transcription factor CHOP. The stress-induced kinase JNK and CHOP induce the death receptor TRAIL-R2 and the proapoptotic Bcl-2 family proteins PUMA and Bim. PUMA and Bim are also up-regulated by palmitate-induced autophagic degradation of Keap1. Palmitate decreases the expression of antiapoptotic proteins Mcl-1 and Bcl-XL. TRAIL-R2 can undergo ligand-independent oligomerization, cleavage-induced activation of caspase 8, Bid cleavage to tBid, and activation of Bax. Oligomeric Bax results in mitochondrial outer membrane permeabilization, release of cytochrome c, activation of effector caspases, and apoptosis. Abbreviations: BAX, B-cell lymphoma 2-like protein 4; Bcl-XL, B-cell lymphoma-extra large; Bim, B-cell lymphoma 2-like protein 11; Keap1, Kelch-likeECH-associated protein 1; Mcl-1, induced myeloid leukemia cell differentiation protein; MOMP, major outer membrane protein; tBid, truncated p15 BID.
The intestinal microbiome modulates insulin resistance and metabolism
The NASH WD/CCl$_4$ Model Alters the Microbiome – Taxonomic analysis

- Relative abundance
- Absolute abundance

Unpublished – Josh Borgerding and Jeremiah Faith; See AASLD Poster
Genetic Modifiers of NAFLD Risk & Progression

Genetic Modifiers of Insulin Sensitivity/Resistance
e.g. IRS-1, ENPP1, GCKR, PPARG, TCF7L2, SLC2A1

Genetic Modifiers of Fatty Acid Flux & Triglyceride Levels
e.g. SLC27A5, LIPN1, MTTP, PEMT, ADIPOQ, ADIPOR2, ApoC3, TCF7L2, ApoE, NR1I2/PXR, PPARG, FNDCS5, FADS1, PNPLA3, TM6SF2, MBOAT7, HSD17B13

Genetic Modifiers of Oxidative Stress
e.g. HFE, SOD2, GCLC, MRP2 (ABCC2), MTHFR

Genetic Modifiers of Endotoxin Response
e.g. TLR4, CD14

Genetic Modifiers of Cytokine Activity
e.g. TNF, sTNF-R2, FDF1T1, IL6

Genetic Modifiers of Fibrogenesis
e.g. AGT, ATGR1, KLF6, TGFb1, COL13A1, CDKN1A, PNPLA3, TM6SF2, MBOAT7, HSD17B13

Genes: Genetics of NAFLD Risk & Progression
Clinical Features of NAFL/NASH

**Symptoms:**
- Variable
- Vague (fatigue, malaise, RUQ discomfort)
- Mostly absent

**Signs:**
- Hepatomegaly common
- Splenomegaly in some
- Portal hypertension unusual

**Labs:**
- Increased AST, ALT typical
- ± increased Alk. Phos., GGT
- Increased cholesterol, triglycerides common
- Increased glucose common
- Viral markers (-)
- Autoantibodies (-) **+ low titer ASMA and ANA**
- Iron studies abnormal sometimes

**Imaging:**
- Fatty liver
Work up of patients with NAFLD

- Imaging to establish the presence of steatosis
- Meticulous alcohol and medication history
- Exclusion of co-existing or competing etiologies
- Auto-antibodies and hyperferritennemia are common
- Fasting lipid profile and measures of insulin resistance
- Liver biopsy to establish the presence of NASH
Imaging Techniques for Evaluating Hepatic Steatosis

Ultrasound, liver, full abdomen
Elastography
  VCTE (Fibroscan), 2D, PointSW, ARFI, MRE
Computed tomography
Magnetic resonance imaging
  Percent fat calculations
  Estimated protein-density fat-fraction MR-PDFF
Magnetic resonance elastography
Controlled attenuation parameter
  For fat using FibroScan Machine
How to establish diagnosis of MAFLD and identify patients with NASH?

- Patients with MAFLD or NASH are generally “asymptomatic” = many nonspecific symptoms
- Clinical presentations cannot distinguish NASH
- Current radiologic modalities are unable to distinguish NASH or accurately detect fibrosis
- Non-invasive biomarkers are not established (getting closer)
- Therefore, in 2017, liver biopsy remains “the imperfect gold standard” to diagnosis and stage NASH
Clinical Factors that are different between Isolated Fatty Liver and NASH

<table>
<thead>
<tr>
<th>Clinical Variable</th>
<th>Not NASH (n=89)</th>
<th>NASH (n=40)</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>BMI</td>
<td>31.7 (5.3)</td>
<td>34.4 (5.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>Fasting Insulin</td>
<td>14 (8.4)</td>
<td>23.2 (13)</td>
<td>&lt;0.0005</td>
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<tr>
<td>ALT (U/L)</td>
<td>36.2 (15.7)</td>
<td>50.9 (19.6)</td>
<td>&lt;0.0005</td>
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<td>AST (U/L)</td>
<td>25.6 (7.4)</td>
<td>36.3 (13.1)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>49.2 (15.7)</td>
<td>44.3 (9)</td>
<td>0.03</td>
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<tr>
<td>Adiponectin (ng/mL)</td>
<td>11028 (13078)</td>
<td>7815 (4811)</td>
<td>0.02</td>
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<tr>
<td>hsCRP (ng/mL)</td>
<td>5355 (5537)</td>
<td>7351 (6397)</td>
<td>0.04</td>
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<td>CK-18 (U/L)</td>
<td>210.3 (118)</td>
<td>307.1 (233.1)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Williams CD, Gastroenterology 2011
MAFLD: sonographic evidence

- Bright liver
- Echotexture increased compared to kidney
- Vascular blurring
CT scan: fatty liver

Noncon CT on left vessels are bright
Treatment of NASH

The Metabolic Syndrome

- Weight Loss
- Insulin Sensitizing Agents
- Lipid Lowering Agents
- Farnesoid-X Receptor Agonist

Antioxidants Vit E
- Cytokines
- Adipokines
- Oxidative stress
- Others

1st hit: Normal Liver
1st hit: Steatosis
2nd hit: NASH
3rd hit: Fibrosis

- Insulin Resistance
- Cytokines
- Adipokines
- Oxidative stress
- Others

Insulin Sensitizing Agents
# Treatment: Weight Loss

<table>
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<tr>
<th>Study</th>
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<th>Duration (months)</th>
<th>Design</th>
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<th>Histology</th>
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<td>Huang</td>
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<td>+</td>
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<td>Andersen</td>
<td>41</td>
<td>Diet</td>
<td>4-23</td>
<td>Open label</td>
<td>+</td>
<td>+/-*</td>
</tr>
<tr>
<td>Kugelmas</td>
<td>8</td>
<td>Diet/Ex</td>
<td>3</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Ueno</td>
<td>15</td>
<td>Diet/Ex</td>
<td>3</td>
<td>Open label</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Zhu</td>
<td>34</td>
<td>Diet/Ex</td>
<td>12</td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Suzuki</td>
<td>348</td>
<td>Diet/Ex</td>
<td>12-24</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Harrison</td>
<td>10</td>
<td>Orlistat</td>
<td></td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sabuncu</td>
<td>13/12</td>
<td>Sibutramine/Orlistat</td>
<td>6</td>
<td>Open label</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Luyckx</td>
<td>69</td>
<td>Surgery</td>
<td>27</td>
<td>Case series</td>
<td>+</td>
<td>+/-*</td>
</tr>
<tr>
<td>Silverman</td>
<td>91</td>
<td>Surgery</td>
<td>2-61</td>
<td>Case series</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Kral</td>
<td>104</td>
<td>Surgery</td>
<td>41</td>
<td>Case series</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dixon</td>
<td>36</td>
<td>Surgery</td>
<td>26</td>
<td>Case series</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Weight Loss Works

31 Patients
Randomized, controlled trial

40% in intervention group lost 10% body weight vs 0% in control group

72% vs 30% achieved study endpoint

Weight loss of ≥ 7% associated with improvement in all parameters of NASH except fibrosis (need 10% for fibrosis)

Promrat K, Hepatology 2010;51:121-129
Non-alcoholic fatty liver disease
Weight loss works

• 36 patients with obesity underwent paired liver biopsies at time of laparoscopic gastric banding and 24 months later

• Mean weight loss 34 kg

• Histologic improvements in steatosis, inflammation, and fibrosis
  – Only 4 fulfilled criteria for NASH at second biopsy (24 at entry)
  – 18 had improvement in fibrosis by 2 stages

<table>
<thead>
<tr>
<th>Weight Loss</th>
<th>Outcome Among Patients Achieving Weight Loss</th>
<th>Patients Sustaining Weight Loss at 1 Yr[^1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 10%^[1]</td>
<td>Fibrosis regression (45% of patients[^1])</td>
<td>&lt; 10%</td>
</tr>
<tr>
<td>≥ 7%^[1]</td>
<td>NASH resolution (64% to 90% of patients)*</td>
<td>18%</td>
</tr>
<tr>
<td>≥ 5%^[1-3]</td>
<td>Ballooning/inflammation improvement</td>
<td>30%</td>
</tr>
<tr>
<td>≥ 3%^[1-4]</td>
<td>Steatosis improvement</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

[^1]: Depending on degree of weight loss.

Significant Improvement in histology following bariatric surgery

1st biopsy

2nd biopsy at 8.5 months

BACKGROUND & AIMS
• Bariatric surgery is associated with a reduced overall mortality as well as improvement in histological features of NAFLD
  – However, it is unclear whether this translates to a reduced incidence of clinically relevant liver-related outcomes such as HCC or decompensated cirrhosis in an unselected population
• AIM: to evaluate whether bariatric surgery is associated with fewer clinically relevant outcomes compared to standard obesity treatment

METHODS
• Main outcome
  – Composite endpoint of ICD-based diagnoses
    • Cirrhosis, decompensation events, HCC or death from liver disease†
  – Patients were classified as having alcohol-related cirrhosis with any ICD-coding for AUD or alcohol-related liver disease during follow-up
• Cox regression used to estimate HRs for severe liver disease
  – Adjusted for age, sex and baseline AST/ALT ratio

Swedish Obese Subjects study* (matched cohort study)

Bariatric surgery (n=2,010)

Conservative obesity management (n=2,037)

125 patients excluded

Pre-existing liver disease other than NAFLD

*Recruitment between 1987 and 2001;
†Ascertained by linkage to Swedish national registers until the end of 2016
Hagström H, et al. DILC 2020; LBP10

Bariatric surgery is not associated with a reduction in risk of severe liver disease in comparison to standard obesity treatment in 3,922 subjects

Swedish Obese Subjects study* (matched cohort study)

Bariatric surgery (n=2,010)

Conservative obesity management (n=2,037)

125 patients excluded

Pre-existing liver disease other than NAFLD
Bariatric surgery is not associated with a reduction in risk of severe liver disease in comparison to standard obesity treatment in 3,922 subjects

**RESULTS**

- Baseline characteristics in surgery group
  - Mean age: 47.1 years
  - Sex: 28.3% males
  - Mean BMI: 42.4 kg/m²
  - Type 2 diabetes: 17.1%
- During median follow-up of 21 years*
  - No difference in risk of severe liver disease (p=0.48)
    - Surgery group: 44 cases (2.3%)
    - Conservative management: 47 cases (2.4%)
- Regression analysis
  - No reduced risk of severe liver disease in the surgery group vs the conservative obesity management group†

**CONCLUSION** Liver-related outcomes were rare in this controlled study of obese subjects exposed to bariatric surgery. We found no association between bariatric surgery and reduced incidence of severe liver disease as compared with standard obesity treatment.

*Results were consistent across pre-defined sensitivity analyses

Hagström H, et al. DILC 2020; LBP10
Lifestyle Modification Program

• Assessed benefits of dietician led lifestyle modification for 12 months
  – Weekly meetings x 4 month, then monthly x 8
  – Moderate carbohydrate, low fat, low glycemic index
    • Emphasis on fruits and vegetables
  – Exercise: moderate intensity for 30 minutes 3-5 days/week
    • Increased to daily
• 154 Patients Enrolled
• Primary Endpoint
  – Remission of MAFLD: IHTG of < 5% by MRS
• 64% in intervention group resolved MAFLD
• 20% in control group resolved MAFLD

Wong VW, J Hepatol 2013
Percentage of weight loss from baseline to month 12

<table>
<thead>
<tr>
<th>Degree of weight loss and resolution of MAFLD by hepatic TG content</th>
<th>% patients with resolution of MAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of weight loss from baseline to month 12</td>
<td></td>
</tr>
<tr>
<td>&lt;3.0%</td>
<td>13</td>
</tr>
<tr>
<td>3.0-4.9%</td>
<td>41</td>
</tr>
<tr>
<td>5.0-6.9%</td>
<td>50</td>
</tr>
<tr>
<td>7.0-9.9%</td>
<td>60</td>
</tr>
<tr>
<td>≥10.0%</td>
<td>97</td>
</tr>
</tbody>
</table>

n = 72  22  10  20  30

Wong VW, J Hepatol 2013
Ketogenic diet cures NAFLD


- After 48 hours of caloric deficit, carbohydrate restriction induces a 3-fold greater liver fat loss than fat restriction (Kirk E et al. Gastroenterology 2009)

- Hypocaloric carbohydrate restriction decreases liver fat by 30% in just 6 days (Sevastianova K et al. Am J Clin Nutr 2011)
  - Despite plasma NEFA, the main substrate of liver fat, increased
  - The intrahepatic fate of NEFA during KD has remained unclear
Summary –
Intrahepatic metabolism during ketogenic diet

Glycogen ↓

Glucose ↓ → Insulin ↓

TG hydrolysis

NEFA ↑

Acetyl CoA ↑

Redox ↑

$V_{CS}$ ↓

OAA

Citrate

$CO_2$ ←
Exercise

• A recent large, cross-sectional study assessed the relationship between meeting/exceeding US national guidelines for physical activity and MAFLD severity
  – Self-reported
  – 813 patients
  – Divided into 3 exercise categories based on time spent in activity and metabolic equivalents (METS):
    • Inactive (54%)
    • Moderate (20%): >150 min/week; Activities with MET values 3-5.9
    • Vigorous (26%): >75 min/week: Activities with MET values >6

Kistler KD, Am J Gastroenterol 2011;106:460-468
Exercise

• Vigorous exercise associated with decreased adjusted odds of having NASH
  – OR: 0.65 (0.43-0.98)
• Doubling recommended time spent in vigorous exercise (>150 min/week), associated with decreased adjusted odds of advanced fibrosis
  – OR: 0.53 (0.29-0.97)

Younger age, higher education, higher income, lower BMI and no diabetes

Kistler KD, Am J Gastroenterol 2011;106:460-468
Exercise

• Optimal Intensity
  – Goal is to maintain a lifestyle change
  – Moderate exercise, burning ~400 kcal/session
    – 3 times/week
    – Improves insulin resistance
    – Overall energy expenditure achieved per work-out more important than intensity
      » Training at 60% VO2max as effective as 80% VO2max
  – Weight loss
    – Need to work out for longer period of time

Ryan AS, Aging Health, 2010
Sleep

10 overweight adults assigned to sleep 8.5 vs 5.5 hours each night for 14 days

Moderate caloric restriction

Lost same amount of weight (~6.6 pounds)

Sleep curtailment decreased proportion of weight lost as fat by 55% and increased loss of fat-free mass by 60%

Nedeltcheva AV, Ann Intern Med 2010
## Treatment: Lipid Lowering Agents

<table>
<thead>
<tr>
<th>Study</th>
<th>Design (mts)</th>
<th>Meds</th>
<th>N</th>
<th>ALT</th>
<th>Hist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laurin</td>
<td>Open label (12)</td>
<td>Clofibrate</td>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basaranoglu</td>
<td>RCT (1)</td>
<td>Gemfibrozil</td>
<td>46</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Horlander</td>
<td>Open label (12)</td>
<td>Atrovastatin</td>
<td>7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kiyici</td>
<td>Open label (6)</td>
<td>Atrovastatin</td>
<td>27</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Hatzitolios</td>
<td>Open label (6)</td>
<td>Atrovastatin</td>
<td></td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Gomez-Dominguez</td>
<td>Open label (12)</td>
<td>Atrovastatin</td>
<td>25</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Rallidis</td>
<td>Open label (7)</td>
<td>Pravastatin</td>
<td>5</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Merat</td>
<td>RCT (6)</td>
<td>Probucol</td>
<td>30</td>
<td>+</td>
<td>NA</td>
</tr>
</tbody>
</table>

Statins are safe, improve ALT, not histology
## Treatment: Insulin Sensitizing Agents

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Drug</th>
<th>Duration (months)</th>
<th>Design</th>
<th>ALT</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caldwell</td>
<td>10</td>
<td>Troglitazone</td>
<td>3-6</td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acosta</td>
<td>8</td>
<td>Pioglitazone</td>
<td>2-12</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Shadid</td>
<td>5</td>
<td>Pioglitazone</td>
<td>4.5</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Sanyal</td>
<td>21</td>
<td>Pioglitazone + Vit E</td>
<td>6</td>
<td>RCT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Promrat</td>
<td>18</td>
<td>Pioglitazone</td>
<td>12</td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tetri</td>
<td>30</td>
<td>Rosiglitazone</td>
<td>12</td>
<td>Open label</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Belfort</td>
<td>55</td>
<td>Pioglitazone ± Diet</td>
<td>6</td>
<td>RCT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Marchesini</td>
<td>14</td>
<td>Metformin</td>
<td>4</td>
<td>Open label</td>
<td>+</td>
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<tr>
<td>Nair</td>
<td>15</td>
<td>Metformin</td>
<td>12</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
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<tr>
<td>Bugianesi</td>
<td>55</td>
<td>Metformin</td>
<td>6</td>
<td>RCT</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Uygun</td>
<td>17</td>
<td>Metformin</td>
<td>6</td>
<td>RCT</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Duseja</td>
<td>7</td>
<td>Metformin</td>
<td>6</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td>Schwimmer</td>
<td>10</td>
<td>Metformin</td>
<td>6</td>
<td>Open label</td>
<td>+</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Pioglitazone and Vitamin E
PIVENS Trial

NO Pioglitazone for NASH

Pros
• Insulin sensitivity
• ALT
• Steatosis
• Inflammation
• ? Ballooning

Cons
• Weight gain (2−4.7 kg)
• Cardiac toxicity
• Fracture risk
• ? Bladder cancer

Meta-analysis of 19 trials (16,390 patients) with T2DM, pioglitazone
• Death, MI, or CVA: 4.4% of pioglitazone vs 5.7% of control ($P = 0.005$)
• More CHF in pioglitazone (2.3%) vs control (1.8%) ($P = .002$), no effect on mortality

Abbreviations: ALT, alanine aminotransferase; CHF, congestive heart failure; CVA, cerebrovascular accident; MI, myocardial infarction; NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus.

Courtesy of Mary Rinella, MD.
Summary of PIVENS findings

- Vitamin E effective over placebo for NASH
- Pioglitazone improved, IR, ALT, steatosis and inflammation, but not 1° outcome
- Only 34% (Pio) and 43% (Vit E) had histological response, neither improved fibrosis
- Cannot generalize to diabetics or cirrhotics
Why not empirically treat suspected MAFLD with vitamin E?

- 70-75% have MAFLD, most isolated steatosis
- 50% of patients don’t respond to Vitamin E
  - liver enzymes are not reliable to assess quiescence or progression
- The long-term safety remains unknown
- Prostate poorly diff cancer risk? (absolute increase 1.6 per 1000 person yrs) if use synthetic Vitamin E, more aggressive pathology, not seen with natural Vit E
Obeticholic acid

- Semi-synthetic bile acid derivative
- Farnesoid-X Receptor (FXR) agonist

Adorini L, Drug Dis Today 2012;17:988-997
## Phase 3 REGENERATE Trial of Obeticholic Acid

### NASH pts with stage 2/3 fibrosis

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Placebo (N=311)</th>
<th>OCA, 10 mg (n=312)</th>
<th>OCA, 25 mg (n=308)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis improvement (≥ 1 stage) with no NASH worsening</td>
<td>11.9%</td>
<td>17.6%</td>
<td>23.1%</td>
</tr>
<tr>
<td>p=0.0446</td>
<td>p=0.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITT plus Stage 1 included</td>
<td>10.6% (n=407)</td>
<td>15.7% (n=407)</td>
<td>21.0% (n=404)</td>
</tr>
<tr>
<td>p=0.0286</td>
<td>p &lt; 0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NASH resolution with no worsening of fibrosis</td>
<td>8%</td>
<td>11.2%</td>
<td>11.7%</td>
</tr>
<tr>
<td>p=0.1814</td>
<td>p=0.1268</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITT plus Stage 1 included</td>
<td>7.9% (n=407)</td>
<td>11.3% (n=407)</td>
<td>14.9% (n=404)</td>
</tr>
<tr>
<td>p=0.0903</td>
<td>p=0.0013</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Younossi et al, *Lancet* online, 2019
**REGENERATE Trial – Adverse events**

- Overall frequency of AEs similar across all arms

- **Dose related pruritis:**
  - 19% in placebo (drug d/c’d in <1%)
  - 28% in OCA 10 mg (drug d/c’d in <1%)
  - 51% in OCA 25 mg (drug d/c’d in 9%)

- **Increased LDL cholesterol:**
  - Peak increase of 22.6 mg/dL at 4 weeks, then returned to baseline by 18 months
  - Low and equal rate of CV events (1-2%)
NASH: Metabolic Agents in Phase 3 Clinical Development

**AGENT** | **MoA (TARGET)** | **TRIAL, PATIENTS AND ENDPOINT(S)** | **METABOLIC AGENTS**
---|---|---|---
Elafibranor | Lipotoxicity/oxidative stress (PPARα/δ agonist) | RESOLVE-IT (n=2000*, fibrosis stage 1–3) – PRO: JAN 2020, FRO: DEC 2021 | NASH resolution without worsening of fibrosis
| | | > Long-term composite of all-cause mortality, cirrhosis and liver-related events |
Obeticholic Acid (Ocaliva) | Lipotoxicity/oxidative stress (FXR agonist) | REVERSE (n=540*, compensated cirrhosis) – Q3 2020 | Fibrosis improvement ≥1 stage without NASH worsening
| | | > Fibrosis improvement ≥1 stage without NASH worsening |
| | | REGENERATE (n=2065*, fibrosis stage 1–3) – PRO: FEB 2019, final Completion: OCT 2022 | Fibrosis improvement ≥1 stage without NASH worsening
| | | > NASH resolution without fibrosis worsening / All-cause mortality and liver-related events |
Resmetirom (MGL-3196) | Lipotoxicity (THR-β agonist) | MAESTRO-NASH (n=2000*, fibrosis stage 2–3) – PRO: JUN 2021, final Completion: MAR 2024 | NASH resolution without worsening of fibrosis
| | | ARMOR (NASH and fibrosis) – PRO: JUN 2022, final Completion: DEC 2024 | Histological endpoint at 52 weeks
| | | > Composite of progression to cirrhosis, liver-related clinical outcomes and all-cause mortality |
Aramchol | Fatty acid synthesis (SCD1 inhibitor) | | |

**PRIMARY and FINAL READOUT**

| 2019 | 2020 | 2021 | 2022 | 2023 | 2024 | 2025 | 2026 | 2027 | 2028 |
---|---|---|---|---|---|---|---|---|---|


Courtesy of Stephen Harrison
NASH: Anti-inflammatory and anti-fibrotic agents in Phase 3 clinical development

**ANTI-INFLAMMATORY AGENTS**

<table>
<thead>
<tr>
<th>AGENT</th>
<th>MoA (TARGET)</th>
<th>TRIAL, PATIENTS AND ENDPOINT(S)</th>
</tr>
</thead>
</table>
| Selonsertib | Apoptosis/necrosis (ASK1 inhibitor) | STELLAR-4 (n=883, compensated cirrhosis) – Q1 2019\(^1\)  
Fibrosis improvement ≥1 stage without NASH worsening  
Event-free survival |
| Belapectin (GR-MD-02) | Fibrosis (Galectin-3 inhibitor) | NASH-RX (n=500*, compensated NASH cirrhosis) – Q4 2022\(^3\)  
NASH resolution without worsening of fibrosis |
| Cenicriviroc | Inflammation/fibrosis (CCR2/5 antagonist) | AURORA (n=2000*, fibrosis stage 2–3) – PRO: OCT 2021, final Completion: 2028\(^4\)  
Fibrosis improvement ≥1 stage without NASH worsening  
Composite of progression to cirrhosis, liver-related clinical outcomes and all-cause mortality |

**ANTI-FIBROTIC AGENTS**

*Planned. PRO, Primary Readout; FRO, Final Readout; ASK, apoptosis signal-regulating kinase; CCR, CC chemokine receptor; PPAR, peroxisome proliferator-activated receptor; FXR, farnesoid X receptor; THR, thyroid hormone receptor. 1. ClinicalTrials.gov. NCT03053063; 2. ClinicalTrials.gov. NCT03053050; 4. ClinicalTrials.gov. NCT02704403;*

Courtesy of Stephen Harrison
Cirrhosis Is More Than A Single Stage

<table>
<thead>
<tr>
<th>Histological</th>
<th>Clinical</th>
<th>Symptoms</th>
<th>Sub-stage</th>
<th>Hemodynamic (HVG, mmHg)</th>
<th>Biological</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1-F3</td>
<td>Non-cirrhotic</td>
<td>None</td>
<td>-</td>
<td>Fibrogenesis and angiogenesis</td>
<td></td>
</tr>
<tr>
<td>F4 Cirrhosis</td>
<td>Compensated</td>
<td>None (No varices)</td>
<td>Stage 1</td>
<td>Scar and x-linking</td>
<td></td>
</tr>
<tr>
<td>F4 Cirrhosis</td>
<td>Compensated</td>
<td>None (Varices)</td>
<td>Stage 2</td>
<td>Thick (acellular scare and nodules)</td>
<td></td>
</tr>
<tr>
<td>F4 Cirrhosis</td>
<td>Decompensated</td>
<td>Ascites VH, encephalopathy</td>
<td>Stage 3</td>
<td>Insoluble scar</td>
<td></td>
</tr>
</tbody>
</table>

- F1-F3 range: ≤6
- Stage 1 range: >6<br></noscript>≤10
- Stage 2 range: >10<br></noscript>≤12
- Stage 3 range: >12
"Point of No return"?
Altered Sinusoidal Flow and Angiogenesis in Portal HT

Ongoing Antifibrotic Trials in Cirrhotic Patients

1. Intercept REVERSE trial – FXR agonist
2. Genfit RESOLVE-IT trial – PPARα,δ agonist
3. Galectin Therapeutics - GR-MD-02 – Galectin antagonist
4. BMS Pegbelfermin – Pegylated FGF21
5. NGM – recombinant, non-mitogenic FGF19
6. BMS / Nitto – Liposomal siRNA to HSP-47
7. Novartis / Allergan – FXR agonist (tropifexor) plus CCR2/CCR5 antagonists (cenicriviroc)
Sequence of Liver Fibrosis Resolution

1. Termination of Chronic Injury
2. Inflammation Resolved
3. Loss of Activated Myofibroblasts
4. ECM Degradation

Specialized Pro-Resolving Mediators:
- Lipoxins
- Resolvins
- Protectins
- Maresins

Based on Musso et al, Trends Pharm Sci, 2018
Glucagon-Like Peptide-1 Analogue: Liraglutide

- **GLP-1**
  - Controls serum glucose
    - Induces insulin secretion
    - Reduces glucagon secretion
  - Induces weight loss, suppression of appetite and delayed gastric emptying

Phase 2 (n=52) (UK, 4 sites)

Double-blind, placebo-controlled
Histologic evidence of definite NASH (steatosis >5%, hepatocyte ballooning, lobular inflammation)
Liver biopsy within 6 months of entry
Stable type 2 diabetes allowed
No Child-Pugh B/C cirrhosis

LEAN: Liraglutide Efficacy and Action in NASH.
Patients stratified by diabetes status.
Primary endpoint (week 72, ITT):
Improvement in liver histology without worsening of fibrosis.
Improvement: disappearance of hepatocellular ballooning.
Worsening of fibrosis: any increase in Kleiner fibrosis stage.
Examples of NASH Treatments in Phase II or III Investigations

**Steatohepatitis (NASH)**
- Targets related to insulin resistance and/or lipid metabolism
- PPAR\(\gamma\): Pioglitazone
- GLP-1: Liraglutide, semaglutide
- ACC: GS-0976, PF-05221304
- SCD1: Aramchol
- FGF21: BMS-986036
- THR-\(\beta\): MGL-3196, VK2809
- PPAR\(\alpha/\delta\): Elafibranor
- PPAR\(\alpha/\delta/\gamma\): Lanifibranor
- FXR: OCA, GS-9674, tropifexor
- FGF19: NGM282
- MPC: MSDC-0602K
- CCR2/5: Cenicriviroc (inflammatory target but affects fibrosis)
- ASK1: Selonsertib (cell death target but affects fibrosis)
- P2X7R: SGM-1019
- Caspase: Emricasan, FAIL
- Galectin: GR-MD-02

**Cirrhosis**
- Targets related to fibrogenesis and collagen turnover

Some agents have multiple targets

**MAFLD Bold = phase III**

Slide credit: clinicaloptions.com
Hep-EVs in fatty liver diseases.

Summary of EVs associated to the progression of NAFLD/NASH and ALD/ASH.

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</table>

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ALD, alcoholic liver disease; ASH, alcoholic steatohepatitis; EV, extracellular vesicle; MP, microparticle; EC, endothelial cell; HSC, hepatic stellate cells; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; VEGF-A, vascular endothelial growth factor A; CCN2, pro-fibrogenic connective tissue growth factor; CD40L, CD40 ligand; miR, microRNA.

Eguchi et al., Liver Research, 2018
Proteases Regulate all Major Biological Pathways of NASH

Lipogenesis
- FGF21
- IGFBPs
- MMP-11
- CTSD (IC)

Inflammation
- CCLs
- IL1b
- IL-8
- TNFα
- MMP-2
- CTSD (IC)
- MMP-11
- CTSA (IC)
- MMP-9
- GSK3β
- LAMP2

Fibrosis
- Integrins
- Elastin
- Collagen I, II, III, IV
- Integrins
- Laminin
- Vitronectin
- MMPs
- MMP-9
- MMP-12
- MMP-2
- CTSD (IC)

Cell Proliferation
- TGFβ
- HGF
- PDGF-D
- FGFs
- VEGF
- MMP-2
- CTSD (IC)

Representative Proteases
- Secreted
- Membrane-bound
- FAP
- MMP-14
- Furin (IC)
- ST14
- FAP
- MMP-8
- ADAMTS2
- Furin (IC)
- ST14
- FAP
- ADAMTS2
- Furin (IC)

Cells
- Hepatocyte
- Fibroblast
- Stellate cells
- Kupffer cells
- Hepatocyte
- Fibroblast
- Stellate cells
- Neutrophil
- Kupffer cells
- Kupffer cells
- Stellate cells
Mechanisms and Therapy in NASH - Summary

1. We are at ‘the end of the beginning’ in understanding and treating NASH: ie, the disease/unmet need are defined, first drug approval likely.

2. Recent mechanistic insights include: a) the importance of the microbiome; b) cellular heterogeneity, based on single cell seq; c) identification of potential lipotoxic drivers through genetics and animal models; d) the emergence of IL-11 as a therapeutic target.

3. Diagnosis of NASH is moving away from biopsy as quickly as possible, but not soon enough!

4. Although there will likely be an approved therapy in 2020, response rates and efficacy need improvement – combination therapies may be essential.
Summary

• MAFLD is part of a systemic inflammatory process and other diseases (cardiovascular) are associated
• More prevalent than previously estimated
  – Hispanics and diabetics at particular risk
• Biopsy is required for research studies not practical in clinical practice, steatosis benign from hepatic point of view, NASH can progress to cirrhosis
• Smoking and excess alcohol are bad, but coffee and sleep likely good
Summary

- Weight loss goal of 10% is best for immediate first level histopathology improvement.
- Moderate exercise may not be enough to effect change in NASH. Vigorous exercise for >150 min/week ideal.
- Vitamin E may be considered for patients with proven NASH with caveats (use natural Vit E).
- Statins are safe and effective for lipid disorders, not NASH.
- Bariatric surgery is an expensive solution.
- Dietician visits for all patients.
- Consider weight loss contract.
- Fructose free diet.
- Vegetarian diet preferred.
- Mediterranean diet optimal.
- Stop use of plastic water bottles, containers, bisPhenols.
Acknowledgments

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