Fatty liver: Terminology

• **ALD**: Alcoholic Liver Disease: Significant alcohol consumption*

• **NAFLD**: Non-Alcoholic Fatty Liver Disease: steatosis without hepatocyte injury

• **NASH**: Non-Alcoholic Steatohepatitis: steatosis with inflammation, hepatocyte injury with or without fibrosis

*Sanyal, et al *Hepatology* 2011
Metabolic associated liver disease (MAFLD) Histopathology

Alcohol-like liver disease in individuals who do not consume excessive alcohol*

**Histologic spectrum of liver damage**

- **NAFLD** - fatty liver (steatosis)
- **NASH** – fatty liver + increased hepatocyte death (steatohepatitis)
- **Cirrhosis** – regenerative nodules + fibrosis

*<25 gr/day male; <15 gr/day female*
• Few symptoms / signs of liver disease
• Benign course?
  <10% evolves into cirrhosis
Risk factor for cirrhosis in HCV & ALD
Decreases efficacy of HCV anti-viral therapy

The Liver Disease Spectrum

- NAFLD

- NASH

• Subtle symptoms common
• More severe metabolic syndrome
• Inflammatory cytokine excess
• ~30% advance to fibrosis
• May promote HCC
NAFLD and NASH epidemiology

Nonalcoholic fatty liver disease (NAFLD) affects around one-fourth of the general population worldwide.

Nonalcoholic steatohepatitis (NASH), the active form of NAFLD, characterized by histological lobular inflammation and hepatocyte ballooning, is associated with faster fibrosis progression and affects around 1.5%–6.5% of the general population.
The prevalence of NAFLD among children in Western countries is 3-10%, rising up to 40-70% among obese children. Moreover, pediatric NAFLD increased from about 3% a decade ago to 5% today, with a male-to-female ratio of 2:1.
NAFLD and NASH epidemiology

NAFLD is frequently associated with metabolic comorbidities, such as obesity (51%), type 2 diabetes (22%), hyperlipidemia (69%) hypertension (39%), and metabolic syndrome (42%).

NAFLD is becoming a major cause of liver disease–related morbidity (eg, cirrhosis, end-stage liver disease, hepatocellular carcinoma, and liver transplantation).
NAFLD and NASH epidemiology

The most common cause of death in patients with NAFLD is cardiovascular disease.

It is estimated that liver-specific mortality and overall mortality among patients with NAFLD are 0.77 and 11.77 per 1000 person-years,
And 15.44 and 25.56 per 1000 person-years among patients with NASH.

# Diagnostic criteria for metabolic syndrome

*Metabolic syndrome is diagnosed by the presence of 2 or more of these parameters.*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impaired glucose tolerance</td>
<td>Fasting blood glucose level $\geq 110$ mg/dL</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>$\geq 130/85$ mm Hg</td>
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<tr>
<td>Elevated triglyceride levels</td>
<td>$&gt;250$ mg/dL</td>
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<tr>
<td>Low high-density lipoprotein level</td>
<td>$&lt;40$ mg/dL for men; $&lt;50$ mg/dL for women</td>
</tr>
<tr>
<td>Abdominal obesity Waist:</td>
<td>$&gt;102$ cm for men; $&gt;88$ cm for women</td>
</tr>
</tbody>
</table>
NAFLD vs MAFLD

The consensus group has suggested an acronym (MAFLD) that we believe more accurately reflects current knowledge of fatty liver diseases associated with metabolic dysfunction that should replace NAFLD/NASH.
Metabolic Associated Fatty Liver Disease: A Heterogeneous Phenotype
We now recognize that metabolic fatty liver disease is a phenotype with complex and disparate causes; the current terminology (NAFLD) represents an umbrella term for the multiple underlying subtypes.

The final vote favored Metabolic Associated Fatty Liver ± Disease (MAFL/MAFLD) (supported by 72.4% of participants). The second preference, Metabolic Fatty Liver ± Disease (MEFL/MEFLD), was supported by 17.2%

Thus, the panel suggests we eliminate the term “NAFLD” from the lexicon and replace it with metabolic associated fatty liver “MAFLD.”

The term MAFLD represents the overarching umbrella of the common disease we treat and will have multiple subphenotypes, reflecting the dominant driver of disease.

Obviously, many, if not most, patients will have overlapping contributions from other and distinct liver diseases that range from alcohol (regardless the amount) to viral hepatitis.

The natural history of these latter groups is likely very different from those with pure metabolic dysfunction.
NAFLD vs MAFLD

Table 1. Statements of the Consensus Panel

Nomenclature and definition of metabolic associated fatty liver disease (MAFLD)
- We suggest that the nomenclature of NAFLD should be updated to MAFLD.
- The diagnosis of MAFLD should be based on the presence of metabolic dysfunction not the absence of other conditions.
- MAFLD can coexist with other liver diseases.
- A reference to alcohol should not be included in the MAFLD acronym.
- Patients with both MAFLD and a contribution from alcohol to their liver disease represent a large and important group that requires further investigation and characterization.

MAFLD heterogeneity
- MAFLD is a heterogeneous entity.
- Appropriate patient stratification must be considered when noninvasive fibrosis scores are developed and in clinical trial design.
- Studies are required to map the landscape of MAFLD and to precisely define subtypes of the disease.

Clinical trials for MAFLD
- Detailed patient stratification and tailoring clinical trial inclusion criteria based on drivers of disease will likely yield more informative and meaningful results.
- Innovative designs for clinical trials and personalized combination therapy approaches will likely be required to overcome the challenges of disease heterogeneity and for optimal clinical efficacy.

MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease
MAFLD: Risk factors

- Metabolic syndrome
  - Diabetes
  - Obesity
  - Dyslipidaemia
  - Arterial hypertension

- SNPs
  - PNPLA3
  - KLF6
  - TM6SF2

- Gender
- Ethnicity
- Family history

- Intrauterine environment
- Early infant feeding

- Diet
  - High saturated FA intake
  - High cholesterol intake
  - High fructose intake
  - High carbohydrate intake
  - Low polyunsaturated FA intake
  - Low antioxidant, zinc and fibre intake

- Sedentary lifestyle

NAFLD

NAFL  NASH  Cirrhosis  HCC
MAFLD: Risk factors

Epigenetics
- Liver and circulating microRNAs
- DNA and histone modifications
- Dietary sedentary behaviours
- Intrauterine factors

Genetics
- Predisposing genetic variants in PNPLA3, TM6SF2, GCKR, MBOAT7
- Protective genetic variants in PPP1R3B
- Predisposing genetic variants in PNPLA3, IFNL4
- Protective genetic variants in HSD17B13, MERTK

patatin-like phospholipase domain-containing 3 (PNPLA3)
MAFLD genetic factors

At least 5 common variants in different genes have been associated with NAFLD, PNPLA3, transmembrane 6 superfamily member 2 (TM6SF2), glucokinase regulator (GCKR), MBOAT7, and hydroxysteroid 17-beta dehydrogenase-13 (HSD17B13)
The rs738409 C > G variant in patatin-like phospholipase domain-containing 3 (PNPLA3)—encoding for the amino acid substitution I148M—has been identified as a major determinant of hepatic fat content. Different PNPLA3 gene alleles have been shown to either confer susceptibility (rs738409[G], encoding I148M, Hispanics), or protection from NAFLD (rs6006460[T], encoding S453I, African-American populations). Importantly, the presence of the mutant I148M seems to increase NAFLD risk, specifically in the context of body weight gain. Human studies have suggested that the PNPLA3-I148M allele remodels liver TAG by transferring polyunsaturated FA (PUFA) from diacylglycerol (DAG) species to feed phosphatidylcholine synthesis. By contrast, the I148 wild type protein hydrolyses triglycerides from lipid droplets and therefore reduces their size.
MAFLD genetic factors

The rs738409 C > G variant in patatin-like phospholipase domain-containing 3 (PNPLA3)—encoding for the amino acid substitution I148M—has been identified as a major determinant of hepatic fat content.

Figure 1. The patatin-like phospholipase domain-containing 3 (PNPLA3) 148M variant favors triglyceride (TG) accumulation upon carbohydrate feeding. The reduced capacity of subjects with
Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis
MAFLD genetic factors

FIGURE 2  Worldwide prevalence of NASH and PNPLA3 I148M genotypes approximated from their frequencies in patients with NAFLD.
MAFLD genetic factors

At least 5 common variants in different genes have been associated with NAFLD, PNPLA3,

<table>
<thead>
<tr>
<th>Reference</th>
<th>Participants</th>
<th>Interventions</th>
<th>Study design</th>
<th>Main liver-related outcome</th>
<th>PNPLA3 A31148M association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shen et al&lt;sup&gt;150&lt;/sup&gt;</td>
<td>Adults with NAFLD (N = 154)</td>
<td>12-mo dietician-led programme or standard care</td>
<td>Parallel-group</td>
<td>Greater decrease in liver fat&lt;sup&gt;a&lt;/sup&gt; than standard care</td>
<td>Increased reduction in liver fat&lt;sup&gt;a&lt;/sup&gt; in the intervention group</td>
</tr>
<tr>
<td>Krawczyk et al&lt;sup&gt;151&lt;/sup&gt;</td>
<td>Adults with suspected NAFLD (N = 143)</td>
<td>4-mo dietician-led programme</td>
<td>Prospective, observational</td>
<td>Significant decrease in liver fat&lt;sup&gt;b&lt;/sup&gt; from baseline</td>
<td>None</td>
</tr>
<tr>
<td>Krawczyk et al&lt;sup&gt;152&lt;/sup&gt;</td>
<td>Adults with obesity (N = 84)</td>
<td>Bariatric surgery</td>
<td>Prospective, observational</td>
<td>Decrease from baseline in liver fat&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Increased reduction in liver fat&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Palmer et al&lt;sup&gt;61&lt;/sup&gt;</td>
<td>Adults with obesity (N = 3473)</td>
<td>Bariatric surgery</td>
<td>Parallel-group</td>
<td>Reduced BMI and serum triglycerides in surgery group only&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Increased reduction in BMI and serum triglycerides</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; NAFLD, non-alcoholic fatty liver disease.

<sup>a</sup>Magnetic resonance spectroscopy.
<sup>b</sup>Ultrasonography.
<sup>c</sup>Magnetic resonance imaging proton density fat fraction.
<sup>d</sup>Liver fat not assessed.
De novo lipogenesis (DNL) is considered an important driver of NAFLD despite relatively low absolute levels of DNL in humans compared to rodents. Hyperinsulinemia in metabolic syndrome leads to excessive DNL via activation of the liver X receptor alpha-sterol regulatory element-binding protein 1c (LXRα-SREBP1c) cascade. In addition, nutrient signaling through mechanistic targeting of rapamycin complex 1 (mTORC1) by amino acids and carbohydrate response element-binding protein (ChREBP) by glucose and fructose can further stimulate this pathway.
Figure: Molecular pathogenesis of NAFLD and NASH

NAFLD = non-alcoholic fatty liver disease. NASH = non-alcoholic steatohepatitis. PAMPs = pathogen-associated molecular patterns. DAMPs = damage-associated molecular patterns.
Drug discovery and treatment paradigms in nonalcoholic steatohepatitis
NAFLD-NASH: two hits model
Serum PIINP levels → Increased fibrosis involving type III collagen matrix
Serum PRO-C3 levels → Lipid globules (steatosis)

Collagen-based diagnostic scores for advanced fibrosis

NASH

Increased ethanol
Altered levels of SCFAs

HCC

LPS

TLR4

Increased LPS

Unhealthy calorie-dense, fibre-poor diet

Gut microbiota dysbiosis leading to increased LPS and altered levels of SCFAs and gut hormones

Poor colonic epithelial health and integrity and loss of tight junction cohesion

Stellate and Kupffer cell activation and angiogenesis

Release of platelet-derived growth factors, cytokines and chemokines

Antiplatelet agents

Activated platelets
Altered gut microbiota

Expanded and inflamed visceral adipose tissue

NAFLD and NASH

- Insulin resistance and related disorders (e.g. atherogenic dyslipidaemia, hypertension, dysglycaemia and nonesterified fatty acids)
- Pro-inflammatory factors (e.g. IL-1β, IL-6, TNF and other cytokines)
- Vasoactive and thrombogenic molecules (e.g. TGFβ, fibrogen and plasminogen activator inhibitor 1)

- Coronary atherosclerosis
- Cardiac dysfunction and hypertrophy
- Cardiac arrhythmias and conduction defects
  Cardiac autonomic changes and electrical remodelling
NAFLD: risk factors for progression

- Middle age
- Over-weight or obese
  - Viral hepatitis
  - Iron overload
- Drugs
  - Rapid weight loss
  - Starvation/refeeding syndrome
  - Reye’s syndrome
- Auto-immune disease
- Malnutrition
- Abetalipoproteinemia
- Overgrowth of bacteria in small intestines
- TPN
- Acute fatty liver of pregnancy
- Hereditary
Risk factor: drugs

- Estrogens
- Tamoxifen
- Valproic acid
- Methotrexate
- Isoniazide
- Corticosteroids
- Vitamin A
- L-Asparaginase
- Amiodarone
- Calcium channel blockers
- Nucleoside analogues
- HAART (Anti-retroviral therapy)
MAFLD diagnosis

- Staging of steatosis
- Staging inflammation
- Staging fibrosis
MAFLD: common findings

- **Symptoms**
  - Malaise, fatigue, upper right abdomen discomfort
  - Snore, disturbed sleep, nocturnal apnea

- **Physical exam**
  - Abdominal obesity
  - Enlarged liver
  - RUQ tenderness on palpation

- **Biochemistry**
  - Elevate levels
    - AST/ALT
    - γGT
    - Total cholesterol, triglycerides, LDL
    - Glucose

- **Sonography**
  - Epatomegaly and liver steatosis
# MAFLD histopathology

<table>
<thead>
<tr>
<th>NAFLD activity score</th>
<th>NASH fibrosis stage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Steatosis</strong></td>
<td></td>
</tr>
<tr>
<td>&lt; 5%: 0</td>
<td>Stage 0</td>
</tr>
<tr>
<td>5–33%: 1</td>
<td>No fibrosis</td>
</tr>
<tr>
<td>34–66%: 2</td>
<td></td>
</tr>
<tr>
<td>&gt; 66%: 3</td>
<td></td>
</tr>
<tr>
<td><strong>Lobular inflammation</strong></td>
<td></td>
</tr>
<tr>
<td>None: 0</td>
<td>Stage 1</td>
</tr>
<tr>
<td>&lt; 2: 1</td>
<td>Zone 3 perisinusoidal fibrosis</td>
</tr>
<tr>
<td>2–4: 3</td>
<td>• Mild – 1a</td>
</tr>
<tr>
<td>&gt; 4: 4</td>
<td>• Moderate – 1b</td>
</tr>
<tr>
<td><strong>Ballooning of hepatocytes</strong></td>
<td></td>
</tr>
<tr>
<td>None: 0</td>
<td>• Portal/periportal – 1c</td>
</tr>
<tr>
<td>Few ballooned: 1</td>
<td></td>
</tr>
<tr>
<td>Many ballooned: 2</td>
<td></td>
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<tr>
<td><strong>NAS score (0–8)</strong></td>
<td><strong>Stage 2</strong></td>
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<tr>
<td>&lt; 3: not NASH</td>
<td>Perisinusoidal and portal/periportal fibrosis</td>
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<tr>
<td>≧ 5: NASH</td>
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<td><strong>Stage 3</strong></td>
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<td></td>
<td>Bridging fibrosis</td>
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<td><strong>Stage 4</strong></td>
</tr>
<tr>
<td></td>
<td>Cirrhosis</td>
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### Table 1: Available Serum Biomarkers for Diagnosing Steatosis or for Staging Fibrosis in Patients With Nonalcoholic Fatty Liver Disease

<table>
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<tr>
<th>Index (ref)</th>
<th>Items, n</th>
<th>Age</th>
<th>Sex</th>
<th>BMI</th>
<th>Diabetes</th>
<th>Platelet count</th>
<th>AST level</th>
<th>ALT level</th>
<th>AST/ALT ratio</th>
<th>GGT level</th>
<th>TG level</th>
<th>Other components</th>
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<td>Waist circumference</td>
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<td>A2M, ApoA1, haptoglobin, T bilirubin, cholesterol, and glucose</td>
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<tr>
<td>LAP</td>
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<td></td>
<td>X</td>
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<td>Waist circumference</td>
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<td>ION</td>
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<td>Waist-to-hip ratio (male yes; female no), and HOMA</td>
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</tbody>
</table>

A2M, α2-macroglobulin; APOA1, apolipoprotein A1; FLI, fatty liver index; GGT, γ-glutamyltransferase; HSI, Hepatic Steatosis Index; ION, Index of NASH; LAP, lipid accumulation product; NAFLD-LFS, NAFLD liver fat score; NFS, NAFLD fibrosis score.
Fatty liver: Clinical evaluation

Diagnostic images
- US (bright liver grade 0-3)

Score 0: Normal echogenicity
- Slight, diffuse increase of fine echoes in liver parenchyma, with normal visualization of diaphragm and intrahepatic vessel borders

Score 1: Moderate, diffuse increase of echoes with a beam attenuation in the deeper part of the parenchyma

Score 2: Marked increase of echoes with poor or no visualization of vessel borders, diaphragm and posterior part of the liver
MAFLD staging

Diagnosis and Grading of Steatosis: Controlled attenuation parameter (CAP) is a novel method for the non-invasive assessment of steatosis, which measures the increased attenuation of ultrasound waves when travelling through steatotic hepatic tissue, compared to normal liver.

- **Figure 1:** Controlled attenuation parameter (CAP) and the FibroScan

- **Figure 2:** Pediatric (S), standard (M) and XL probes

Example cases:

a) Male patient aged 34 years suffering from CHB with steatosis score 117 dB/m that equals S0, fibrosis 4.9 KPa that equals F0, BMI 25.38 serum cholesterol 98 mg/dl, Triglycerides 101 mg/dl, LDL 44 mg/dl, HDL 34 mg/dl, fasting blood glucose 80 mg/dl, fasting insulin 11 μU/mL and HOMA-IR 2.1 μU/mL.

b) Female patient aged 46 years suffering from CHB with steatosis score 313 dB/m that equals S3, fibrosis 4 KPa that equals F0, BMI 29.9, serum cholesterol 209 mg/dl, Triglycerides 158 mg/dl, LDL 129 mg/dl, HDL 34 mg/dl, fasting blood glucose 89 mg/dl, fasting insulin 28 μU/mL and HOMA-IR 6 μU/mL.
450 patients with suspicion of NAFLD prospectively recruited

Underwent liver biopsy within 2 weeks of FibroScan (M or XL probe according to the automatic probe recommendation tool)

Results and conclusions

- CAP for steatosis (S<1):
  - > AUC = 0.97 (0.92-0.99)
- CAP for advanced fibrosis (F>3):
  - > AUC = 0.80 (0.75-0.84)
- LSM for cirrhosis (F=4):
  - > AUC = 0.89 (0.84-0.93)

Steatosis or probe type had no impact on LSM (multivariable analysis)

CAP and LSM by FibroScan are reliable biomarkers to non-invasively assess liver steatosis and fibrosis respectively in NAFLD
MAFLD staging

**Diagnosis and Grading of Steatosis: Magnetic Resonance Imaging Proton-Density Fat Traction**

MRS has been employed in several large epidemiologic studies, and now with the development of MRI-PDFF, it has been more widely utilized in epidemiologic studies to classify presence of hepatic steatosis as well as to quantify the amount of liver fat.
Figure 1: Severe hepatic steatosis without fibrosis (80% steatosis on histologic analysis; PDFF, 24.4%) in a 28-year-old man. Bottom row shows histologic biopsy result from the liver (stained with hematoxylin-eosin a). On PDFF images (top two rows), liver appears more hyperintense on grayscale images and lighter blue on colored images as the degree of steatosis increases. IP, in-phase; OP, out-of-phase; c-PDFF, colored (rainbow) PDFF c-R2*, colored (hot iron) R2* map.

Idilman IS. Published Online: June 01, 2013 https://doi.org/10.1148/radiol.13121360
Diagnosis of Nonalcoholic Steatohepatitis: Serum Biomarkers

In summary, none of the currently available serum marker are able to differentiate NASH from simple steatosis with high sensitivity and specificity, however, their diagnostic accuracy can be improved by combining different approaches.
Staging of Liver Fibrosis
Serum Biomarkers
The diagnostic performances of serum biomarkers have already been summarized in several and, therefore, will not be detailed here. Briefly, as for non patented tests, a recent meta-analysis (based on 64 studies in 13,046 NAFLD patients) comparing BARD, APRI, FIB-4, and NAFLD fibrosis score (NFS) for diagnosing advanced fibrosis reported summary AUROCS of 0.76, 0.77, 0.84, and 0.84, respectively.
# NAFLD/NASH fibrosis score*

<table>
<thead>
<tr>
<th>Non-invasive Scores of NAFLD</th>
<th>NAFLD Fibrosis Score (NFS) *</th>
<th>FIB-4 **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AST</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ALT</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Platelet count</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>BMI</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Impaired fasting glucose or diabetes</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* NFS:
  [gihep.com/calculators/hepatology/nafld-fibrosis-score](http://gihep.com/calculators/hepatology/nafld-fibrosis-score)

** FIB-4:
  [www.mdcalc.com/fibrosis-4-fib-4-index-liver-fibrosis](http://www.mdcalc.com/fibrosis-4-fib-4-index-liver-fibrosis)
  [gihep.com/calculators/hepatology/fibrosis-4-score](http://gihep.com/calculators/hepatology/fibrosis-4-score)
NAFLD/NASH fibrosis score*

1. NAFLD typically develops based on various metabolic disorders such as obesity, diabetes mellitus, and dyslipidemia;
2. the prognosis and outcome of the patients with advanced liver fibrosis are predominantly determined by the liver disease-related clinical events, including hepatic failure and hepatocellular carcinoma.
3. Therefore, physicians are required to accurately differentiate NASH from NAFLD and evaluate the severity of liver fibrosis in order to determine the prognosis and optimal treatment.


- < -1.455: predictor of absence of significant fibrosis (F0-F2 fibrosis)
- ≤ -1.455 to ≤ 0.675: indeterminate score
- > 0.675: predictor of presence of significant fibrosis (F3-F4 fibrosis)
MAFLD staging

Shear wave elastography relies on the displacement of tissues induced by a force, either external pressure or the radiation force from a focused ultrasound beam. The displacement of tissues induces elastic **shear waves**, which propagate and are detected by the ultrasound transducer.
NAFLD/NASH and fibrosis evaluation Elastography

**SCORING CARD**

**CORRELATION BETWEEN LIVER STIFFNESS (kPa) & FIBROSIS STAGE**

- **Hepatitis B**
- **HCV-HIV co-infection**
- **Hepatitis C recurrence after liver transplantation**
- **Hepatitis C**
- **Chronic cholestatic diseases**
- **Alcohol**
- **NAFLD**

*According to liver scome Transient elastography (FibroScan) v. de Veldhuis, J. Veugelers, Gastroenterologie Clin Bio (2008) 32, 58-67


FibroScan®, a reliable tool in hepatology
Patients with suspected NAFLD

1st line: primary health care

Rule-out/in advanced fibrosis
FIB-4 or NAFLD fibrosis score

- FIB-4 < 1.3
  - NFS < -1.455
  - Low risk

- FIB-4 ≥ 1.3
  - NFS ≥ -1.455
  - Intermediate to high risk

Attempt lifestyle modifications and exercise

No further assessment, repeat evaluation at 1 year?

2nd line: referral center

Rule-out/in advanced fibrosis
Transient elastography

- LSM < 8 kPa
  - Low risk

- LSM ≥ 8 kPa
  - Intermediate to high risk
  - Consider liver biopsy

Attempt lifestyle modifications and exercise
Vitamin E / pioglitazone?

- Consider repeat evaluation (1 year)
- Eligible for therapeutic trial? Monitoring with MRE?
- Screen for OV and HCC if cirrhosis

Search for other causes of liver disease (alcohol, HBV, HCV)

Failure (XL probe) 3.0%—6.7%

Consider MRE, 2D SWE, or ARFI according to local availability
**NASPGHAN guideline**
- Lifestyle modifications to improve diet and increase physical activity are recommended as the first-line treatment
- Avoidance of sugar-sweetened beverages
- Increasing moderate to high-intensity physical activity and limiting screen time activities to <2 hours per day
- No currently available medications or supplements are recommended to treat NAFLD because none has been proven to benefit the majority of patients with NAFLD

**AASLD guidance**
- Intensive lifestyle modifications should be the first-line treatment
- Metformin at 500 mg twice daily should not be prescribed as a NASH-specific therapy
- Vitamin E can be used to treat paediatric NASH, but risks and benefits should be discussed with each patient given that long-term safety of high-dose vitamin E in children is unknown

---

**Current treatments**

**NAFLD**

**NASH**

**Fibrosis (F2–F3)**

**New therapies**

- Medications with positive metabolic effects
  - Omega 3 FAs
  - High-dose metformin?
  - Probiotics

- Medications with anti-inflammatory effects
  - Vitamin E
  - Elafibranor
  - Obeticholic acid

- Medications with anti-fibrotic effects
  - Cenicriviroc
  - Selonsertib
  - Combination therapy?

---

**Lifestyle modifications for all children with the NAFLD spectrum**
**NASH Therapy: low risk patients**

- Structured programmes aimed at lifestyle changes towards healthy diet and habitual physical activity are advisable in NAFLD (C2)

- Patients without NASH or fibrosis should only receive counselling for healthy diet and physical activity and no pharmacotherapy for their liver condition (B2)

- In overweight/obese NAFLD, a 7–10% weight loss is the target of most lifestyle interventions, and results in improvement of liver enzymes and histology (B1)

- Dietary recommendations should consider energy restriction and exclusion of NAFLD-promoting components (processed food, and food and beverages high in added fructose. The macronutrient composition should be adjusted according to the Mediterranean diet (B1)

- Both aerobic exercise and resistance training effectively reduce liver fat. The choice of training should be tailored based on patients’ preferences to be maintained in the long-term (B2)
NASH management low risk patients
Lifestyle Interventions
Mediterranean diet (MD) vs Low fat vs High carbohydrates diet (LF/HCD)

NASH management low risk

Weight loss by lower caloric intake and increased physical exercise led to improvement in biopsy

3-5% weight loss improves steatosis but more is needed to improve inflammation

9.3% weight loss: improvement in steatosis, necrosis, and inflammation; not fibrosis
NASH Therapy

Antioxidants

Vitamin E (α-tocopherol)
NASH management low risk

Vitamin E 800 IU/day improves liver histology in NASH patients but not fibrosis (PIVEN study)

Table 3. Changes in Serum Biochemical Levels, Metabolic Factors, and Quality of Life from Baseline to 96 Weeks.†

<table>
<thead>
<tr>
<th>Variable</th>
<th>Change from Baseline</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (N=74)</td>
<td>Vitamin E (N=78)</td>
</tr>
<tr>
<td>Serum enzymes and bilirubin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase (U/liter)</td>
<td>-20.1</td>
<td>-37.0</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/liter)</td>
<td>-3.8</td>
<td>-21.3</td>
</tr>
<tr>
<td>γ-Glutamyltransferase (U/liter)</td>
<td>-4.0</td>
<td>-14.0</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/liter)</td>
<td>-3.8</td>
<td>-9.3</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>Lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>-6.7</td>
<td>-0.6</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>-9.6</td>
<td>-13.6</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>-1.9</td>
<td>-0.9</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>-5.8</td>
<td>-12.0</td>
</tr>
<tr>
<td>Metabolic factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting serum glucose (mg/dl)</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Insulin resistance (mU/ml)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.2</td>
<td>-0.4</td>
</tr>
<tr>
<td>Body composition (% fat)</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>Quality of life</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-36 score, physical component</td>
<td>-0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>SF-36 score, mental component</td>
<td>0.4</td>
<td>-0.5</td>
</tr>
</tbody>
</table>

NASH management low risk

Vitamin E 800 IU/day improves liver histology in NASH patients but not fibrosis

NASH management low risk

Vitamin E 800 IU/day improves liver histology in NASH patients but not fibrosis

Table 2. Primary Outcome and Changes in Histologic Features of the Liver after 96 Weeks of Treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo</th>
<th>Vitamin E</th>
<th>Pioglitazone</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary outcome&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of subjects randomly assigned</td>
<td>83</td>
<td>83</td>
<td>80</td>
<td>0.001</td>
</tr>
<tr>
<td>Subjects with improvement (%)</td>
<td>19</td>
<td>43</td>
<td>34</td>
<td>0.04</td>
</tr>
<tr>
<td>Changes from baseline in histologic features</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of subjects with biopsy specimens at baseline and 96 wk</td>
<td>72</td>
<td>80</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Steatosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects with improvement (%)</td>
<td>31</td>
<td>54</td>
<td>69</td>
<td>0.005</td>
</tr>
<tr>
<td>Mean change in score</td>
<td>−0.1</td>
<td>−0.7</td>
<td>−0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lobular inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects with improvement (%)</td>
<td>35</td>
<td>54</td>
<td>60</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean change in score</td>
<td>−0.2</td>
<td>−0.6</td>
<td>−0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hepatocellular ballooning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects with improvement (%)</td>
<td>29</td>
<td>50</td>
<td>44</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean change in score</td>
<td>−0.2</td>
<td>−0.5</td>
<td>−0.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Total NAFLD activity score (mean change)</td>
<td>−0.5</td>
<td>−1.9</td>
<td>−1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrosis&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects with improvement (%)</td>
<td>31</td>
<td>41</td>
<td>44</td>
<td>0.24</td>
</tr>
<tr>
<td>Mean change in score</td>
<td>−0.1</td>
<td>−0.3</td>
<td>−0.4</td>
<td>0.19</td>
</tr>
<tr>
<td>Resolution of definite nonalcoholic steatohepatitis (% of subjects)</td>
<td>21</td>
<td>36</td>
<td>47</td>
<td>0.05</td>
</tr>
</tbody>
</table>

NASH management low risk

Vitamin E 800 IU/day improves liver steatosis at histology in NASH patients

Not recommended to treat NASH in those with other chronic liver diseases, diabetics, those with NASH cirrhosis or cryptogenic cirrhosis, NAFLD without biopsy
NASH management

- **Ursodeoxycholic acid**
  - No histologic benefit

- **Omega-3 fatty acids**
  - Effective in treating hypertriglyceridemia in pts with NAFLD
  - Evidence for treatment of NASH inconclusive to date
  - Large multi-center trial on-going now
NASH management low risk

Improving insulin sensitivity—weight reduction

Weight loss (bariatric) surgery may be beneficial for patients with morbid obesity; again, this should be considered early, as most programs will decline such surgery for patients who are already cirrhotic. Limited studies have reported a dramatic improvement in liver disease, as well as other complications of metabolic syndrome/insulin resistance, following successful bariatric surgery.
NASH management intermediate/high risk

**Insulin sensitizing agents**

Drugs targeting insulin resistance, such as thiazolidinediones and metformin, are approved for diabetes therapy but not for NAFLD/NASH, and should be considered experimental

- **Metformin**
  - reduction in IR and enzymes,
  - no improvement in histology

- **Thiazolidinediones (PIVENS)**
  - Pioglitazone causes weight gain, but improvement in hepatocellular injury. Pioglitazone can be used to treat certain patients with biopsy-proven NASH who do not have DM but long term safety and efficacy has not been established
NASH management: intermediate high risk

Metformin

Current information indicates that metformin improves liver function, HOMA-IR and BMI to some extent, but not histological response in NAFLD patients.
Statins

- CVD common cause of death for NAFLD and NASH
- Stratify risks and treat accordingly
- No RCTs with histological end points using statins to treat NASH

NASH management: intermediate high risk

**Treatment of metabolic conditions**

- Proper control of diabetes, hyperlipidemia, and cardiovascular risks is recommended. Studies with atorvastatin and pravastatin have shown improvement in histology in patients with NASH.

- **NAFLD patients with dyslipidemia should be treated with statins.** Patients with underlying liver disease do not seem to have any additional risk of statin toxicity. Serious hepatotoxicity from statins is rare

NASH therapy

Statins for the treatment of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis

✓ Preliminary studies have shown that statins may possibly improve hepatic histology in patients with underlying NAFLD. CVD common cause of death for NAFLD and NASH. Stratify risks and treat accordingly

✓ No convincing histological data are available,

✓ At present, treatment with statins to cure liver disease in patients with NAFLD is not recommended.

✓ New RCTs of adequate size and duration are required to assess efficacy of statins for the treatment of NAFLD.
NASH therapy: recommendations

- Pharmacotherapy should be reserved for patients with NASH, particularly for those with significant fibrosis (stage F2 and higher). Patients with less severe disease, but at high risk of disease progression (i.e. with diabetes, MetS, persistently increased ALT, high necroinflammation) could also be candidates to prevent disease progression (B1)

- While no firm recommendations can be made, pioglitazone (most efficacy data, but off-label outside T2DM) or vitamin E (better safety and tolerability in the short-term) or their combination could be used for NASH (B2)

- The optimal duration of therapy is unknown; in patients with increased ALT at baseline, treatment should be stopped if there is no reduction in aminotransferases after 6 months of therapy; in patients with normal ALT at baseline, no recommendations can be made (C2)

- Statins may be confidently used to reduce LDL-cholesterol and prevent cardiovascular risk, with no benefits or harm on liver disease. Similarly n-3 polyunsaturated fatty acids reduce both plasma and liver lipids, but there are no data to support their use specifically for NASH (B1)
NASH management: what’s next
<table>
<thead>
<tr>
<th>Drug</th>
<th>Developer</th>
<th>Mode of action</th>
<th>Highest phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obeticholic acid (OCA/Ocaliva)</td>
<td>Intercept Pharmaceuticals/Sumitomo Dainippon Pharma</td>
<td>FXR agonist and semi-synthetic bile acid analogue</td>
<td>Phase III</td>
</tr>
<tr>
<td>Elafibranor (GF-505)</td>
<td>Genfit</td>
<td>PPAR α/δ agonant</td>
<td>Phase III</td>
</tr>
<tr>
<td>Aramchol</td>
<td>Galmed Pharmaceuticals</td>
<td>Synthetic fatty acid–bile acid conjugate</td>
<td>Phase II/III</td>
</tr>
<tr>
<td>Emricasan (IDN-6556)</td>
<td>Conatus Pharmaceuticals</td>
<td>Caspase inhibitor</td>
<td>Phase II</td>
</tr>
<tr>
<td>Simtuzumab (GS-6624)</td>
<td>Gilead Sciences</td>
<td>Anti-LOXL12 monoclonal antibody</td>
<td>Phase IIb</td>
</tr>
<tr>
<td>GR-MD-02</td>
<td>Galectin Therapeutics</td>
<td>Galectin 3 inhibitor</td>
<td>Phase II</td>
</tr>
<tr>
<td>GS-4997</td>
<td>Gilead Sciences</td>
<td>MAPK5 inhibitor</td>
<td>Phase II</td>
</tr>
<tr>
<td>Liraglutide (Victoza/Saxenda)</td>
<td>Novo Nordisk</td>
<td>GLP1R agonist</td>
<td>Phase II</td>
</tr>
<tr>
<td>Cenicriviroc (TBR-652)</td>
<td>Allergan (formerly Tobira Therapeutics)</td>
<td>Dual CCR2 and CCR5 antagonist</td>
<td>Phase II</td>
</tr>
<tr>
<td>BMS-986036</td>
<td>Bristol-Myers Squibb</td>
<td>FGF21 agonist</td>
<td>Phase II</td>
</tr>
<tr>
<td>Tipelukast</td>
<td>MediciNova</td>
<td>LTD4 receptor antagonist</td>
<td>Phase II</td>
</tr>
<tr>
<td>ARI 3037MO</td>
<td>Arisaph Pharmaceuticals</td>
<td>Niacin analogue</td>
<td>Phase II</td>
</tr>
<tr>
<td>Volixibat (SHP 626)</td>
<td>Shire/Sanofi</td>
<td>ASBT inhibitor</td>
<td>Phase II</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Molecules</td>
<td>Furthest developmental stage for NASH treatment</td>
<td>Refs or clinical trial identifiers</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------</td>
<td>-----------------------------------------------</td>
<td>----------------------------------</td>
</tr>
</tbody>
</table>
| **FXR activators**  | • Semi-synthetic bile acid: OCA (INT-747)  
• Synthetic non-steroidal isoxazoles: GW4064 and Px-104  
• Natural polyphenol: EGCG | Phase III  
Phase IIa (Px-104)  
Preclinical | 16  
ClinicalTrials.gov identifier: NCT0199910 |
| **PXR activators**  | • Natural compounds: carpin, santonin and isokobusone | Preclinical | 22 |
| **PPAR\(\alpha\) and PPAR\(\delta\) activators** | • Synthetic agonists: K-877, GFT505, GW501516 and L-165041 | Phase IIb (GFT-505) | 57, NCT01694849 |
| **PPAR\(\alpha\) and PPAR\(\gamma\) activators** | • Synthetic agonist: saroglitazar | Phase IIa | ClinicalTrials Registry India identifier: CTRI/2010/091/00010 |
| **SREBP2 and/or miR-33a inhibitors** | • Natural antioxidants: proanthocyanidins, resveratrol and curcumin  
• Synthetic anti-miR-33a oligonucleotides | Preclinical | 71,75,76 |
| **Ceramide depletion** | • FIASMAs: TCAs (amitriptyline and desipramine), SSRIs (fluoxetine and sertraline) and calcium channel blockers (amlodipine and verapamil)  
• SPT inhibitors: myriocin and FTY-720 | Preclinical | 85,91–93 |
| **DGAT1 inhibitors** | • Pradigstatt | Phase IIa | 99 |
| **DGAT2 inhibitors** | • Niacin  
• Plant-derived: mangiferin  
• Synthetic: indolyl acrylamide derivatives and pyrrolo[2,3-b]pyridine derivatives (H2-003 and H2-005) | Preclinical | 98,109,101 |
<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
<th>Phase</th>
<th>ID(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRF2 activators</td>
<td>Electrophilic compounds: Natural: sulforaphane, resveratrol, curcumin, EGCG and dimethyl fumarate; Synthetic: diisothiocyanates (oltipraz, anethole diisothiocyanate) and bardoxolone methyl</td>
<td>Phase Ila (oltipraz)</td>
<td>NCT01373554</td>
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<tr>
<td></td>
<td>Non-electrophilic compounds: Natural: berberine</td>
<td>Preclinical</td>
<td>118,123</td>
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<tr>
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<td>Synthetic: NK-252, tetrahydrosoquinoline, MG-132 and tert-butylhydroquinone</td>
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<tr>
<td>Natural antioxidants</td>
<td>Resveratrol</td>
<td>Phase Ila</td>
<td>130–134</td>
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<td></td>
<td>Quercetin</td>
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<td>139,140</td>
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<tr>
<td>FGF21 analogues</td>
<td>PEGylated FGF21 and Fc-FGF21(RG)</td>
<td>Phase Ila (LY2405319)</td>
<td>152</td>
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<tr>
<td></td>
<td>FGF21-mimetic monoclonal antibody (mimAb1)</td>
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<td></td>
<td>FGF21 analogue LY2405319</td>
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<tr>
<td>AMPK activators</td>
<td>Natural: monascin, ankaflavin, quercetin, berberin and curcumin</td>
<td>Phase Ila (oltipraz)</td>
<td>NCT01373554</td>
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<tr>
<td>mTORC1 and mTORC2 inhibitors</td>
<td>mTORC1 and mTORC2 inhibitors: rapamycin and AZD3147</td>
<td>Preclinical</td>
<td>172–174,166</td>
</tr>
<tr>
<td></td>
<td>mTORC1 inhibitors: Z1001, rottlerin and XL388</td>
<td></td>
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<tr>
<td>Inflammasome inhibitors</td>
<td>Ethyl pyruvate, phenylmethimazole, eritoran, apyrase, A438079, etheno-NAD, auranofin, NMDA agonists, isoleucine, and GS-9450</td>
<td>Phase Ila (auranofin)</td>
<td>201</td>
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<tr>
<td>Chemokine antagonists</td>
<td>Dual CCR2 and CCR5 antagonists: cenicriviroc</td>
<td>Phase Iib</td>
<td>NCT02217475</td>
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<td></td>
<td>CCR5 antagonists: Met-CCL5 and maraviroc</td>
<td>Preclinical</td>
<td>221,222</td>
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<td></td>
<td>CXCR3 antagonist: NIBR2130</td>
<td>Preclinical</td>
<td>207</td>
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<tr>
<td>RvD1 analogues</td>
<td>BDA-RvD1 and lipo-RvD1</td>
<td>Preclinical</td>
<td>243,246</td>
</tr>
<tr>
<td>Galectin 3 inhibitors</td>
<td>GM-CT-01 (galactomannan)</td>
<td>Phase I</td>
<td>265</td>
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<tr>
<td></td>
<td>GR-MD-02 (galactoarabinotetraosylgalacturonan)</td>
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<tr>
<td>LOXL2 inhibitors</td>
<td>Simtuzumab</td>
<td>Phase Iib</td>
<td>NCT01672866, NCT01672879</td>
</tr>
<tr>
<td>Leukotriene receptor antagonists</td>
<td>MN-001 (tipelukast)</td>
<td>FDA-approved for a Phase IIa trial (see Further Information)</td>
<td>NA</td>
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</table>