


# Non-Alcoholic Steatohepatitis: Limited Available Treatment Options but Promising Drugs in Development and Recent Progress Towards a Regulatory Approval Pathway

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**Abstract** The prevalence of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) is increasing world-wide in parallel to the increase of the obesity epidemic. Insulin resistance (IR) and the accumulation of triglyceride-derived toxic lipid metabolites play a key role in its pathogenesis. Multiple biomarkers are being evaluated for the non-invasive diagnosis of NASH. However, a percutaneous liver biopsy is still the gold standard method; the minimal diagnostic criteria include the presence of >5 % macrovesicular steatosis, inflammation, and liver cell ballooning. Several pharmaceutical agents have been evaluated for the treatment of NASH; however, no single therapy has been approved so far. Due to the increasing prevalence and the health burden, there is a high need to develop therapeutic strategies for patients with NASH targeting both those with early-stage disease as well as those with advanced liver fibrosis. There are unique challenges in the design of studies for these target populations. Collaborative efforts of health authorities, medical disease experts, and the pharmaceutical industry are ongoing to align options for a registrational pathway. Several companies pursuing different mechanisms of action are nearing the end of phase II

with their candidates. This manuscript reviews those compounds with a variety of mode of actions that have been evaluated and/or are currently being tested with the goal of achieving a NAFLD/NASH indication.

## Key Points

Prevalence of steatohepatitis is increasing worldwide. Patients with obesity, type 2 diabetes (T2DM), and insulin resistance are specifically affected.

There is no approved drug for the treatment of NASH but there are a wide variety of compounds with different modes of actions currently in clinical development.

The ideal treatment is expected, in the short term, to reduce liver inflammation and fibrosis, and improve insulin sensitivity and metabolic complications; however, in the long term, a benefit in reducing cardiovascular and hepatic outcomes will need to be demonstrated.

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## 1 Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined by the presence of hepatic accumulation of triglycerides in the hepatocytes in the absence of significant alcohol intake, viral infection, or any other specific etiology of liver disease. It represents a histopathologic spectrum ranging from steatosis alone to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. NAFLD may lead to liver failure or hepatocellular carcinoma. NASH is currently the most common cause of

liver disease in the West, but it is important to note that cardiovascular disease is the single most important cause of mortality in this patient population [1, 2].

NAFLD is closely associated with obesity and insulin resistance and its prevalence has risen rapidly in parallel with the dramatic global rise in levels of obesity and diabetes mellitus [3]. It has been suggested that NAFLD represents the hepatic manifestation of the metabolic syndrome [4].

NAFLD is a major potential threat to public health. It affects at least 30 % of the general population and is present in more than 60 % of obese subjects. Patients with a diagnosis of NAFLD have been shown to have a significantly higher risk of diabetes, cardiovascular disease, and overall and liver-related mortality when compared with an age- and sex-matched general population [2]. Cirrhosis due to NASH is now the third most common cause of liver transplantation in the USA [5].

There are many uncertainties in the diagnostic approaches, evaluation, and management of NASH. The formal diagnosis still requires a liver biopsy, a procedure that is invasive, somewhat painful, and may be associated with life-threatening complications due to the potential for trauma and bleeding complications that occur in some individuals. Additionally, it has several limitations as a surrogate marker of clinical outcomes. For instance, it enables an evaluation of only a small sample of the liver parenchyma, which may not be representative of the pathology in the rest of the liver tissue [6]. Due to these limitations, the high cost, and the lack of effective treatment options, only a minority (less than 25 %) of academic gastroenterologists and hepatologists in the USA routinely perform liver biopsies in patients with presumed NASH [7]. The lack of accurate, reproducible, and easily applied methods has been a major limitation not only in the clinical management of NASH patients but also for research.

Although several drugs with different targets have shown efficacy in clinical trials of various designs, there are currently no approved therapies for NASH. A major impediment to therapeutic advances to improve outcomes in NAFLD and NASH is the long natural history of the disease (i.e., it can take decades for NAFLD to progress to NASH, and subsequently for NASH to become symptomatic and to potentially lead to cirrhosis and death). Therefore, the critical need guiding drug development for NAFLD/NASH is to identify viable surrogates that are predictive of those outcomes. There are ongoing efforts among members of the scientific community, global regulatory agencies, and the pharmaceutical industry to agree on the best path forward to determine and validate the appropriate markers for NASH diagnosis that can be used to evaluate efficacious and safe therapies to treat patients with NASH.

The objective of this review is to summarize the magnitude of the health burden and the current state of NAFLD/NASH diagnosis, to discuss the available data for several compounds that have completed clinical trials in NAFLD/NASH (including those currently in clinical development aiming for a NAFLD/NASH indication), and to discuss the challenges and potential future paths for development.

## 2 Prevalence and Natural History

Excess liver fat is now extremely common, consistent with the increasing prevalence of the metabolic syndrome linked to the global epidemic of obesity. It has been estimated that more than 30 % of adults in the USA and other Western countries have NAFLD [8].

The reported prevalence of NAFLD varies depending on the methodology used and the population studied. Most of the studies in the general population are based on liver ultrasound (US) or liver enzymes, with liver biopsy mostly restricted to subjects at high risk and magnetic resonance imaging (MRI) and spectroscopy (MRS), typically only used in clinical research settings.

Population-based studies in the USA estimate that the prevalence of NAFLD ranges between 17 and 46 % in the general population [9]. In the Dallas Heart Study [10], when assessed by MRS, the prevalence of NAFLD was 31 %. Most recently, the prevalence of NAFLD was 46 % in a multi-ethnic group of patients based on ultrasonography. Interestingly, NASH was confirmed in a subset of 12.2 % of this total cohort [11].

The prevalence can be higher in certain populations. There is a very high prevalence of NAFLD in obese subjects and among patients with T2DM. In patients with severe obesity undergoing bariatric surgery, the prevalence of NAFLD can exceed 90 % [12]. The prevalence of NAFLD in patients with T2DM has been estimated to be between 60 and 70 % [9]. In addition, T2DM worsens liver disease, although the underlying mechanisms remain unclear [13]. Several studies have reported that the presence of T2DM is associated with a two- to four-fold increase in serious liver disease, cirrhosis, and hepatocellular carcinoma [14].

High serum triglyceride levels and low serum HDL levels are also very common in patients with NAFLD. The prevalence of NAFLD in individuals with dyslipidemia attending lipid clinics was estimated to be 50 % [15].

Ethnic variation has also been suggested to influence the phenotype of patients with NAFLD. It has been suggested that Hispanics (predominantly of Mexican origin) are at particular risk for NAFLD and tend to have a more aggressive disease course [16]. However, Kallwitz et al.

[17] found no significant differences in hepatic steatosis, NASH, or liver fibrosis ( $\geq$ F2) between morbidly obese Hispanic and non-Hispanic White patients receiving bariatric surgery. It has been reported that when Hispanics and Caucasians are matched for adiposity, the severity of NASH is similar [18], suggesting that ethnic differences might be a reflection of the differences in metabolic risk of Hispanics in these studies.

NAFLD has also recently been recognized among children. In the USA, the prevalence of elevated plasma aminotransferase levels in adolescents was reported between 3.5 and 12.4 %, higher in males relative to females and the highest among Mexican American boys [19]. This likely underestimates the true prevalence of NAFLD in children, as many patients with NAFLD have normal liver enzymes.

The natural history of progression from NAFLD to NASH remains unclear. While most patients with simple hepatic steatosis are likely to have a benign and non-progressive course, about 12–40 % of patients with fatty liver will progress to NASH. Approximately 15–20 % of patients with NASH will subsequently develop liver fibrosis and cirrhosis. It has also been reported that a fair proportion of patients may progress from liver steatosis to advanced fibrosis [20, 21]. NAFLD patients with progressive fibrosis have been reported to be more insulin resistant and significantly more likely to have a weight gain exceeding 5 kg [21]. Patients with NASH are also at increased risk of hepatocellular carcinoma, even in the absence of cirrhosis [22], with an approximately threefold increase in liver-related mortality [23]. In a 33-year follow-up study, fibrosis stage was found to be the strongest predictor for disease-specific mortality [24]. Patients with NAFLD and type 2 diabetes are especially at risk for mortality due to hepatic complications [23]. However, most patients with NAFLD will not have a progressive disease and some patients can spontaneously improve. Among 359 individuals in the NASH clinical research network (CRN) database with two or more biopsies separated by a mean of 4.4 years, 128 cases showed fibrotic progression and 103 showed regression [25]. This bidirectional nature of the disease adds difficulty in the interpretation of data in clinical trials. The challenge remains in the early identification of those individuals that will progress to NASH and cirrhosis.

### 3 Pathogenesis

Insulin resistance (IR) plays a major role in the pathogenesis of NAFLD and is considered a key factor in the initiation and perpetuation of NASH [26, 27]. A “two-hit” process has been proposed [28]. The first “hit” involves

accumulation of triglycerides in the hepatocytes, which is closely associated with central obesity and insulin resistance. IR leads to enhanced lipolysis which in turn increases circulating free fatty acids and their uptake by the liver. An increased delivery of free fatty acids to the liver is combined with impaired hepatic fatty acid metabolism. On the other hand, the accumulation of lipid molecules in the liver exacerbates insulin resistance by interfering with the tyrosine phosphorylation and signalling potential of cellular insulin receptor substrates.

The factors involved in determining the progression from steatosis to steatohepatitis and fibrosis are less well understood. The accumulation of fat in the liver appears to have several cellular and metabolic effects, including: upregulation of hepatocyte apoptosis, mitochondrial dysfunction with increase in reactive oxygen species (ROS) that leads to lipid peroxidation of cell membranes, and induction of pro-inflammatory genes such as TNF $\alpha$  (tumor necrosis factor alpha) and COX-2 (cyclooxygenase-2), which also induce additional inflammatory mediators with pro-fibrotic effects. On the other hand, the increased secretion of adipocytokines (leptin, resistin) and pro-inflammatory markers (TNF $\alpha$ ) from the adipose tissue in the setting of insulin resistance, and the decreased levels of anti-inflammatory cytokines such as adiponectin, also produced by adipocytes, facilitates a net balance that leads to apoptosis, necroinflammation, and fibrosis in the hepatocytes [29]. Lipid released from damaged hepatocytes may also result in mechanical and/or inflammatory cell-mediated occlusion of hepatic venules, leading to parenchymal collapse and fibrosis [30]. Immune responses to lipid peroxidation products may also be involved in the disease progression [3]. There is also an activation of profibrogenic cytokines, such as interleukin-10 and transforming growth factor- $\beta$ , which in turn are regulated by other factors including leptin and neurotransmitters such as noradrenaline.

### 4 Diagnosis and Current Status of Non-Invasive Methods

The diagnosis of NAFLD requires a demonstration of excess liver fat by imaging or histology with exclusion of: (1) significant alcohol consumption, (2) competing etiologies for hepatic steatosis (e.g., hepatitis C, medications, parenteral nutrition, Wilson’s disease, severe malnutrition, lipodystrophy, etc.), and (3) coexisting causes for chronic liver disease (e.g., hemochromatosis, autoimmune liver disease, chronic viral hepatitis). Although alcoholic liver disease and NAFLD have similar pathological spectra, from simple hepatic steatosis to steatohepatitis and liver cirrhosis, the clinical characteristics of these two diseases

differ [31]. Unfortunately, self-report questionnaires often underestimate patients' actual alcohol consumption. Several recently developed biochemical measures (e.g., ethyl glucuronide, ethyl sulfate, phosphatidyl ethanol, and carbohydrate-deficient transferrin) can provide additional information on a patient's use of alcohol [32].

NAFLD is frequently underdiagnosed, as most of the time it is asymptomatic and patients usually have normal liver aminotransferases [33, 34]; thus, clinicians often do not suspect the potential presence of NAFLD. Liver biopsy is still the gold standard method for the diagnosis, grading (severity of ongoing injury) and staging (degree of progression to cirrhosis) of the disease [35]. The minimal criteria for the diagnosis of steatohepatitis include the presence of >5 % macrovesicular steatosis, inflammation, and liver cell ballooning, typically with a predominantly centrilobular distribution in adults. The staging and grading of liver disease was a concept introduced in the mid-1990s. This early proposed grading scheme for steatohepatitis (mild, moderate, or marked) was a composite of four separate features: steatosis, ballooning injury, lobular inflammation, and portal inflammation [36]. More recently, the pathologists in the NASH clinical research network validated an updated scoring system that can be used to assess histologic change in studies of both adults and children with NAFLD/NASH (Table 1) [37]. The NAFLD activity score (NAS) is an un-weighted composite of steatosis, inflammation, and ballooning scores. It is a useful tool to quantify disease activity and assess changes in clinical trials. However, the diagnosis of NASH is defined

by the presence and pattern of specific histologic abnormalities and the NAS has not been validated as a marker for likelihood of disease progression (e.g., cirrhosis, mortality) and/or response to therapy. A score of 5 or more is associated with a greater likelihood of having NASH. However, a  $NAS \geq 5$  does not confirm NASH. For instance, in an evaluation of 976 liver biopsies with a  $NAS \geq 5$  of 976 adults in the NASH clinical research network (CRN) database, 86 % had NASH and 3 % did not have steatohepatitis (SH). Only 75 % of biopsies with definite SH had a  $NAS \geq 5$ , whereas 28 % of borderline SH and 7 % of "not SH" biopsies had  $NAS \geq 5$  [38]. The use of NAS is currently limited to clinical trial settings. Clinically important differences have been reported between community general pathologists and expert hepatologists in assessing NAFLD using the NASH CRN scoring system, and more studies are needed to investigate its suitability for community-based clinical practice [39].

One of the most pressing challenges in this field is the lack of a validated, non-invasive set of tests to diagnose NASH, and liver fibrosis in patients with NASH. Sensitivity and specificity of some of these tests to identify NASH patients are described in Table 2.

Mild elevations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), usually in the range of 1.5- to threefold above the upper limit of normal, in the absence of other diagnoses strongly suggests NASH. However, it is also essential to note that approximately two-thirds of patients have normal aminotransferase levels at any given time [6], grade, and stage of their disease. Use

**Table 1** Non-alcoholic steatohepatitis (NASH) clinical research network (CRN) scoring system describes the non-alcoholic fatty liver disease activity score (NAS) and the fibrosis score (disease stage). The NAS represents the sum of scores for steatosis, lobular inflammation, and ballooning, and ranges from 0 to 8. The total score for the fibrosis stage (below) ranges from 0 to 4

Steatosis		Lobular inflammation		Ballooning		Total
Degree	Description (% hepatocytes)	Degree	Description	Degree	Description	
0	<5	0	0/20× <sup>a</sup>	0	0	
1	5–33	1	<2 foci/200×	1	Few/inapparent	
2	34–66	2	2–4 foci/200×	2	Easily noted/many	
3	>67	3	>4 foci/200×			
NAS score	0–3			0–2		0–8
Stage		Fibrosis location				
1A	Zone 3, perisinusoidal, delicate					
1B	Zone 3, perisinusoidal, dense					
1C	Portal, periportal only					
2	Zone 3, perisinusoidal + portal, periportal only					
3	Bridging fibrosis					
4	Cirrhosis					

<sup>a</sup> Optical field

**Table 2** Accuracy of some biomarkers and diagnostic panels for non-alcoholic steatohepatitis (NASH) (inflammation, oxidative stress, apoptosis) and advanced liver fibrosis (bottom). A wide range of biomarkers and panels have been tested. In general, studies are small and need further validation. The sensitivity, specificity, and area under the receiver operating characteristic (AUROC) curves vary depending on the defined cut-off point

Test	AUROC (%)	Cut-off value	Sensitivity (%)	Specificity (%)	PPV	NPV
TNF $\alpha$ mRNA [117]	0.685	100 ng/mL	66.7	74.1		
IL-6 [118]	0.817	4.6 pg/mL	58.1	100		
hsCRP [119]	0.906	3.5	82	88		
Ferritin [120]	0.82	240 ng/mL	91	70		
	0.732	196	64.2	76.5		
Adiponectin [121]	0.765	<4 $\mu$ g/mL	68	79		
CK-18	0.93 [122]	395 U/L	85.7	99		
	0.83 <sup>a</sup> [45]	225 U/L	70	82	84	73
		250 U/L	60	93	95	69
		300 U/L	53	100	100	67
	0.84 <sup>b</sup> [68]	121–479	78	87		
	95 % CI 64–92					
Hyaluronic acid [123, 124]	0.797	43 ng/mL			97.1	
NAFLD diagnostic panel (diabetes, gender, BMI, TG, CK-18) [47]	0.81	0.221	91.2	47.4	60.8	85.7
	0.85	0.3641	79.4	73.7	73	80
		0.6183	44.1	92.1	83.3	64.8
		$\leq 1.455^c$	90	60		
		$>0.676^d$	67	97		
Hyaluronic acid [125]	0.975	148.8 ng/mL	97.5	95.7		
FIB4 index (age, ALT, AST, platelet count) [125, 126]	0.802	<1.30	74	71	43	90
ELF (HA, TIMP1, PIIINP) [51, 68]	0.90	0.3576	80	90	71	94
	0.76 <sup>e</sup>	–0.2070	61	80		
	0.82 <sup>f</sup>	–0.1068	70	80		
	0.90 <sup>g</sup>	0.3576	80	90		
Fibrotest (a2macroglobuline, haptoglobin, GGT, tot BB, ApoA1) [127]	0.81	0.3	77	77	54	90
		0.7	15	98	73	76
NAFLD fibrosis score (age, hyperglycemia, BMI, platelet count, albumin, AAR) [9, 16, 68]	0.84	$\leq 1.455$	82	77	56	93
	0.85 <sup>h</sup>	$>0.676$	51	98	90	85
	95 % CI 0.81–0.90	$\leq 1.455$	90 (86–95)	60 (56–65)		
		$>0.676$	64 (59–70)	97 (94–99)		

PPV positive predictive value, PNV negative predictive value, CK-18 cytokeratin 18, BMI body mass index, TG fasting triglycerides, ALT alanine aminotransferase, AST aspartate aminotransferase, ELF enhanced liver fibrosis panel, HA hyaluronic acid, TIMP1 tissue inhibitor of metalloproteinase 1, PIIINP amino-terminal propeptide of type III collagen, AAR AST/ALT ratio

<sup>a</sup> Accuracy for differentiating patients with NASH from those with simple steatosis with three different cut points: 225, 250, and 300 U/L. For every 50-U/L increase in the plasma level of CK-18, the likelihood of having NASH increased 30 % [OR (95 % CI) 1.3 (1.1–1.4)]. CK-18 fragment levels were significantly higher in patients with fibrosis as compared to those without fibrosis [45]

<sup>b</sup> Summary estimates of nine studies [68]

<sup>c</sup> To exclude advanced fibrosis

<sup>d</sup> To identify the presence of advanced fibrosis

<sup>e</sup> 95 % CI 0.69–0.83 to identify any fibrosis (stage 1–4)

<sup>f</sup> 95 % CI 0.75–0.88 to identify  $\geq$ F2

<sup>g</sup> 95 % CI 0.84–0.96 to identify  $\geq$ F3

<sup>h</sup> Summary estimates of 13 studies. To identify  $\geq$ F3

of the recently proposed modified normal ALT thresholds has provided improved sensitivity to identify patients who are “at risk” for chronic liver disease with an acceptable

trade-off in specificity [40]. However, simply documenting elevations in liver enzymes is still unreliable for the diagnosis and monitoring of the disease activity.



The presence of the metabolic syndrome is a strong predictor of steatohepatitis in patients with NAFLD [41]. In fact, a confirmed diagnosis of the metabolic syndrome has been suggested to be used to best identify patients with persistently abnormal liver biochemistries who would benefit diagnostically and prognostically from a liver biopsy [41].

Markers of inflammation like  $\text{TNF}\alpha$ , interleukin-6, high-sensitivity C-reactive protein, monocyte chemoattractant protein-1, pentraxin 3, and ferritin, among others, have been reported to be elevated in patients with NASH. However, most of these studies were small and did not provide a cut-off value. Their potential for differentiating a diagnosis of NASH from fatty liver has not been fully elucidated [42, 43].

Oxidative stress has been recognized as an important mechanism in the pathogenesis of NASH. However, circulating markers known to be associated with a variety of oxidation pathways were investigated for use in NASH diagnosis, but they failed to show robust and consistent results. One possible explanation for these findings is that the serum or plasma measurement of oxidative markers may not necessarily reflect the activity of different oxidation pathways in the liver [42, 43].

Currently, the most promising biomarker in the circulation for the diagnosis of NASH is represented by serum cytokeratin-18 (CK-18) levels, which is associated with the degree of hepatocyte apoptosis. Circulating levels of CK-18 fragments have been investigated extensively for the presence of steatohepatitis in patients with NAFLD. Sensitivity and specificity values have varied across studies depending on the diagnostic “cut-off level” used. CK-18 fragments have been reported to be significantly lower for NAFL than for biopsy-proven borderline or definite NASH [44]. However, there is considerable variability in the suggested cut-offs and their respective diagnostic accuracy among studies [45]. A few studies suggested that CK-18 fragments may have a better performance for the diagnosis of NASH when combined with other tests (e.g., liver attenuation on computed tomography (CT) scan, fibroblast growth factor 21, etc.) [43].

Several other biomarkers have been evaluated in NAFLD/NASH populations: serum adiponectin is lower in patients with NASH than in those with simple steatosis. Soluble FAS (sFAS) is a death receptor from the TNF receptor family that has been implicated in apoptosis and is upregulated in NASH in animal models. An apoptosis panel combining CK-18 with sFAS was found to have greater accuracy than either alone [46].

Different algorithms have been proposed for the recognition of NASH using non-invasive techniques (Table 2). Many of these include components of the metabolic syndrome in their formula. For instance, the NAFLD diagnostic

panel includes body mass index (BMI), fasting triglycerides, gender, the presence of diabetes, and CK-18 [47].

A non-invasive and cost-effective marker of hepatic fibrosis would be extremely valuable to detect NASH patients that may progress to cirrhosis (Table 2). Current non-invasive methods for assessing fibrosis range from serum biomarker assays to advanced imaging techniques. One of the biggest challenges to developing non-invasive tests is the lack of a reliable gold standard, since even the percutaneous liver biopsy has a poor diagnostic performance. In a study using a blinded evaluation of two cores of liver sampled at the same biopsy session, only about half of the time the cores were assessed as having the same stage of fibrosis. Additionally, potential regional variability may cause meaningful sampling variability [48]. This variability compromises the ability of the liver biopsy to serve as a reliable gold standard to which the non-invasive tests and biomarkers are compared.

Several non-invasive methods have been evaluated as predictors of advanced fibrosis in patients with NAFLD. Constituents of the extracellular matrix [hyaluronic acid (HA), tissue inhibitors of metalloproteinase-1 (TIMP-1), procollagen type III N-terminal peptide (PIIINP), type IV collagen 7S] domain seem to have “acceptable” (Table 2) sensitivity and specificity for the detection of NASH although not without controversy. Several diagnostic panels have been developed for the prediction of significant fibrosis. The NAFLD fibrosis score was developed from a large cohort ( $n = 773$ ) and is calculated from six variables: age, hyperglycemia, BMI, platelet count, albumin, and AST/ALT ratio [49]. The “BAAT” scoring system [50] is calculated based on the following parameters: age, BMI, triglycerides, and ALT. The ELF panel consists of plasma levels of three matrix turnover proteins (HA, TIMP-1, and PIIINP). The liver fibrosis panel adds the age to these three serum biomarkers [51]. Different cut-off values have been proposed. In general, the higher the cut-off value, the higher the specificity, but at the expense of losing sensitivity.

#### 4.1 Imaging

Liver US is currently the imaging technique of choice in clinical practice for the diagnosis of NAFLD, given its wide availability and relatively low cost. Compared with ultrasonography, however, CT scanning, MRI, and MRS, all have a better sensitivity and specificity in detecting fatty liver. Both MRS and MRI have been proven to accurately measure hepatic fat in both adults and children, and are increasingly being used in clinical research [52, 53]. Hepatic fat content  $>5.5\%$  is consistent with the diagnosis of hepatic steatosis [10].

Tests that employ measurement of stiffness as a biomarker of hepatic fibrosis include magnetic resonance

elastography [54] and US-based transient elastography [55]. The rationale for these studies is that the collagen deposition associated with fibrosis produces a lattice-like framework that imparts rigidity to the pressure compliance of the liver. Transient elastography (TE) has been validated as a measure of fibrosis across a wide spectrum of chronic liver disease (hepatitis B and hepatitis C) and has overall a good accuracy. It has the advantage of being quick, easy to learn, and well tolerated by patients [55]. The new XL probes provide comparable diagnostic accuracy to the standard probe and enable the examination of obese patients [56]. A detailed description of all these available methods is beyond the scope of this article and can be found elsewhere [56–58].

In patients with advanced liver disease, hepatic venous pressure gradient (HVPG) assessment is the best-validated predictor of eventual decompensation [57]. It provides an indirect measure of portal venous pressure [58]. HVPG has been shown to correlate with different outcomes [59, 60] and has been proposed as a potential surrogate endpoint that might be considered reasonably likely to predict clinical outcome to support an accelerated approval [61]. For instance, in patients with cirrhosis but no varices (all with an HVPG >5 mmHg), HVPG has been shown to be the best predictor of the development of varices. Inversely, in patients with compensated cirrhosis (without varices), a decrease in HVPG >10 % is associated with a significantly lower incidence of varices. In a study performed in post-transplant hepatitis C patients treated with antiviral therapy, HVPG decreased significantly in those who had an improvement in fibrosis stage, remained stable in those in whom fibrosis remained stable, and worsened in those in whom fibrosis progressed. Since the HVPG test is invasive and requires skilled practitioners, it is not widely used.

## 5 Available Treatment Options and Drugs in Development Pipelines

Currently, there is no approved therapy for NAFLD/NASH. Treatment strategies may be grouped into those that address weight loss, improve insulin sensitivity and/or are antidiabetics, reduce lipids, are antioxidants, or target the liver.

### 5.1 Weight Loss

#### 5.1.1 Lifestyle Intervention

Lifestyle modification is the first step for the treatment of the metabolic comorbidities in NAFLD and, at present, it is the standard of care to treat NAFLD itself. Many studies indicate that lifestyle modification may reduce aminotransferases and improve hepatic steatosis when measured

by either US [62, 63] or MRI and MRS [64, 65]. In the LOOK AHEAD trial, a 12-month, randomized controlled trial investigating the long-term health impact of an intensive lifestyle intervention (ILI) in overweight or obese adults with type 2 diabetes, a total of 96 subjects completed an MRS substudy to quantify hepatic steatosis (Fatty Liver Ancillary Study). After 12 months, the participants assigned to ILI ( $n = 46$ ) lost more weight ( $-8.5$  vs.  $-0.05$  %;  $p < 0.01$ ) and had a greater decline in steatosis than those assigned to the standard diet arm (SDA) ( $-50.8$  vs.  $-22.8$  %;  $p < 0.04$ ). At 12 months, 26 % of SDA participants and 3 % (one of 31) of ILI participants without NAFLD at baseline developed NAFLD ( $p < 0.05$ ) [66].

Several studies using a variety of interventions, either by diet alone or in combination with different exercise prescriptions [64, 67], have consistently reported a significant reduction in liver fat by an average of ~40 % (ranging from 20 to 80 %). In general, the degree of hepatic fat reduction was proportional to the intensity of the lifestyle intervention [68]. Loss of at least 3–5 % of body weight appears necessary to improve steatosis, but a greater weight loss (up to 10 %) seems to be needed to improve necroinflammation [41].

#### 5.1.2 Antiobesity Drugs

A few small studies suggest that weight loss induced by medications such as orlistat [69], sibutramine [70], or cannabinoid-1 (CB1) antagonists [71] may lead to an improvement in steatosis and reduction in ALT levels. However, there is very little evidence from controlled clinical trials to support the hypothesis that either orlistat or sibutramine improve NAFLD in the short term and there are currently no long-term data available regarding the effect of these medications on liver-related outcomes. The endocannabinoid system has emerged as a pivotal mediator of acute and chronic liver injury. Unfortunately, the CB1 antagonist rimonabant, initially approved for the management of overweight and related cardiometabolic risks, was withdrawn because of an alarming rate of adverse effects affecting mood. Attempts to avoid potential psychiatric adverse effects of drugs in this class has prompted the development of peripherally-restricted CB1 antagonists with limited brain penetrance. The efficacy of several of these compounds has been validated in preclinical models of NAFLD where beneficial effects on fibrosis have been observed [72].

#### 5.1.3 Bariatric Surgery

Data suggest that most obese patients undergoing bariatric surgery have NAFLD [73]. A recent review of 15 studies of 766 paired liver biopsies in patients undergoing bariatric surgery reported that steatosis resolved in 91.6 % (95 % CI

82.4–97.5), steatohepatitis improved in 81.3 % (95 % CI 61.9–94.9), and fibrosis in 65.5 % (95 % CI 38.2–88.1) of cases [68]. In a 5-year prospective study of bariatric surgery in 381 subjects, steatosis, ballooning, and NAS improved significantly, NASH resolved in 48 % of cases. However, fibrosis worsened slightly but significantly. Most of the improvement occurred within 1 year, and, interestingly, it was the persistence of insulin resistance at 1 year, rather than the degree of weight loss, that predicted the lack of a histologic response at 5 years [74].

Though some evidence suggest that steatosis, steatohepatitis, and fibrosis may significantly improve or even completely resolve after bariatric surgery, a Cochrane review concluded that the lack of randomized clinical trials prevents a definitive assessment of the benefits and harms of bariatric surgery as a therapeutic approach for patients with NASH [75].

## 5.2 Insulin-Sensitizing Agents

### 5.2.1 Thiazolidinediones

Given the role of insulin resistance in the pathogenesis of NASH, insulin sensitizers such as PPAR- $\gamma$  agonists have been extensively tested. Several pilot studies examining the effect of glitazones on NAFLD and NASH have reported favorable results, with improvement in both liver function tests and liver histology [76–78]. Rosiglitazone has been proven to improve serum aminotransferase levels and hepatic steatosis, but not inflammation or fibrosis [79]. In a phase II, double-blind, placebo-controlled, 24-month study, pioglitazone significantly improved aminotransferase levels, steatosis, ballooning, and inflammation in patients with NASH who had impaired glucose tolerance or T2DM. The NAS improved with pioglitazone in 73 % compared with 24 % of placebo-treated patients ( $p < 0.001$ ) and there was a trend toward improvement in fibrosis [76]. The PIVENS [78] (pioglitazone vs. vitamin E vs. placebo for the treatment of non-diabetic patients with non-alcoholic steatohepatitis) study was a large multicenter, randomized, 96-week clinical trial that randomized 247 non-diabetic patients with biopsy-confirmed NASH to pioglitazone (30 mg/day), vitamin E (800 IU/day), or placebo for 24 months. The primary endpoint was an improvement in the composite of NAS  $\geq 2$  points with at least a 1-point improvement in hepatocellular ballooning and a 1-point improvement in either the lobular inflammation or steatosis score, and no increase in the fibrosis score. This was achieved in 19 % of subjects in the placebo group compared with 34 % in the pioglitazone group ( $p = 0.04$ , NS) (pre-specified alpha of 0.025). In the vitamin E group, 43 % of the patients had improvements in NASH as defined by the primary endpoint ( $p < 0.01$ ).

Although pioglitazone did not meet the pre-specified significance level for the primary outcome, it was associated with highly significant reductions in the individual variables of steatosis, inflammation, and hepatocellular ballooning, as well as with improvements in insulin resistance and liver-enzyme levels. It also led to the resolution of steatohepatitis in a significant proportion of subjects. One possible reason for the failure to achieve the primary outcome with pioglitazone therapy is that more subjects in the pioglitazone group than in the vitamin E and placebo groups were classified as not having had ballooning at baseline (a reduction in ballooning was one of the criteria for the primary outcome and thus these subjects were classified as non-responders). Additionally, more subjects in the pioglitazone group did not have a post-treatment liver biopsy (therefore considered by default as non-responders). Patients in the vitamin E group did achieve a significant improvement in SH as defined by the primary endpoint. When vitamin E and pioglitazone patients were matched for baseline histology (i.e., ballooning), no statistical differences between both treatments were observed.

The Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association [80] suggest that pioglitazone can be used to treat steatohepatitis in non-diabetic patients with biopsy-proven NASH. However, pioglitazone is not approved for the treatment of NASH, data in diabetics is scarce, and long-term safety and efficacy of pioglitazone in patients with NASH has not been established.

### 5.2.2 Metformin

Several studies have investigated the effect of metformin on aminotransferases and liver histology in patients with NASH. Although some small, open-label studies suggested that metformin reduced insulin resistance and aminotransferases, a randomized control trial of metformin versus placebo with similar dietary and exercise interventions in both groups failed to show major benefit for metformin on hepatic insulin sensitivity, aminotransferases, or liver histology. Metformin is thus not recommended as a specific treatment for liver disease in adults with NASH primarily because it has no significant effect on liver histology [80].

### 5.2.3 Incretin Mimetics

Long-term exenatide administration has been associated with decreased liver triglyceride content in obese mouse models [81, 82]. In an open-label, uncontrolled clinical trial using exenatide to assess drug safety in patients with diabetes, patients were noted to have had improved AST and insulin sensitivity over the 3.5-year follow-up period



[83]. In addition, those with elevated ALT at baseline had a significant reduction in ALT, and 41 % experienced a normalization of ALT levels with treatment, independent of weight loss. In a 12-month prospective study in patients with type 2 diabetes, both pioglitazone and the combination of pioglitazone and exenatide [85] led to significant reductions in liver fat and ALT, but the combination therapy was superior (−60 %) compared to pioglitazone alone (−40 %). Importantly, no significant change in weight was observed in the combination group, suggesting that there could be a direct effect of exenatide on liver steatosis independent of metabolic improvements that may have resulted from weight loss.

From animal data, benefits are not limited to GLP-1R agonists, which are given at pharmacologic doses, but also in models with DPP4 deficiency or inhibition. Administration of sitagliptin to mice on a linoleic acid and sucrose diet decreases liver triglycerides and the histologic grade of hepatic steatosis [86]. In a recently published study, a 4-month treatment with sitagliptin in 30 patients with T2DM and US diagnoses NAFLD resulted in significant decreases in AST, ALT, and gamma-GTP levels [87]. However, the potential benefits of dipeptidyl peptidase IV (DPP-IV) inhibitors on NASH are still preliminary and long-term randomized clinical trials are warranted.

### 5.3 Compounds that Reduce Lipids

#### 5.3.1 Statins

The antioxidant and anti-inflammatory properties, the frequent coexistence of NAFLD and dyslipidemia, and the increased cardiovascular risk of these patients make statins an attractive therapeutic tool in NAFLD. Strong data support the use of statins to reduce cardiovascular disease in patients with dyslipidemia [88]. Though there is reluctance to use statins in patients with suspected or established chronic liver disease, including NAFLD and NASH, there is no evidence that patients with NAFLD and NASH are at higher risk for serious liver injury from statins than those without liver disease [41]. However, data on statin efficacy in NAFLD are sparse. In a pilot trial in which 16 participants with biopsy-proven NASH were randomized to receive simvastatin 40 mg or placebo for 12 months, no statistically significant improvement in the aminotransferase level was seen in the simvastatin group compared with the placebo group. Liver histology was not significantly affected by simvastatin [89]. In a post-hoc analysis of the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) cardiovascular outcomes study, statins significantly improved liver biochemistries and cardiovascular outcomes in patients with elevated liver enzymes likely due to NAFLD [90].

Given the lack of evidence to show that patients with NAFLD and NASH are at increased risk for serious drug-induced liver injury from statins, they can be used to treat dyslipidemia in this patient population [41]. However, until randomized clinical trials with histologic endpoints prove their efficacy, statins should not be used to specifically treat NASH [41].

#### 5.3.2 Omega-3 Fatty Acids

Omega-3 fatty acids have several potential mechanisms of action leading to hypothesize a potential beneficial effect in patients with NAFLD. The most important one is the alteration in the hepatic gene expression, thereby switching intracellular metabolism from lipogenesis and storage to fatty acid oxidation and catabolism. There is also evidence that they improve insulin sensitivity, are anti-inflammatory, and reduce TNF $\alpha$  levels, thus offering several potential therapeutic mechanisms.

Animal studies have shown a reduction in hepatic steatosis, improved insulin sensitivity, and reduced inflammation and oxidative stress. In humans, preliminary clinical trials have confirmed this potential, reporting a reduction in hepatic steatosis on imaging, increased insulin sensitivity, and improved serum liver function tests [91]. However, most of these trials have been open label. Data from a randomized, double-blind, 12-month clinical trial with a pure eicosapentanoic acid compound (Epadel, EPA-E) in subjects with biopsy-confirmed NASH (NAS  $\geq$ 4, with minimum scores of 1 for steatosis and inflammation, along with either ballooning or at least stage 1a fibrosis) have recently been published [92]. Subjects were randomly assigned to placebo, low-dose EPA-E (1800 mg/day), or high-dose EPA-E (2700 mg/day). The primary efficacy endpoint was achieving a NAS  $<$ 3, without worsening of fibrosis, or a decrease in NAS by  $\geq$ 2 with a contribution from more than one parameter, without worsening of fibrosis, after 1 year of the last dose of EPA-E. Epadel did not demonstrate any improvement of any of the histologic features of NASH. One potential explanation for the negative results of this trial is that the dosage of EPA-E was not high enough for the enrolled USA population. The dosing for this trial was selected on the basis of existing data for its efficacy for dyslipidemia in a Japanese population. Supporting this hypothesis is the fact that the observed effect on serum triglycerides in this trial seemed to be less than what has been seen in Japan. Additionally, the placebo response rate in this trial was higher than that reported previously in other studies. Randomized studies to demonstrate if other omega-3 fatty acids (e.g., docosahexaenoic acid, DHA) are efficacious in improving features of NASH are warranted.

## 5.4 Antioxidation

### 5.4.1 Vitamin E

Oxidative stress is considered to be a key mechanism in the second hit leading to hepatocellular injury and disease progression. Vitamin E has antioxidant properties and has been investigated as a potential treatment for NASH. Early small studies of vitamin E for short duration in patients with NAFLD/NASH reported inconsistent results [93, 94]. However, in PIVENS [78], a significant difference in the response to the primary histologic endpoint (an improvement of >2 points in the NAS, with at least 1 point improvement in hepatocellular ballooning and 1 point in either the lobular inflammation or steatosis score, with no worsening of fibrosis) was observed in patients receiving vitamin E compared with placebo-treated patients (43 vs. 19 %;  $p = 0.001$ ). However, some data suggest potential safety concerns with the long-term use of vitamin E. A meta-analysis from 11 trials that tested the effect of vitamin E supplementation in humans reported that high-dose vitamin E supplementation (4400 U/day) was associated with an increase in all-cause mortality [95].

In children with NAFLD, neither vitamin E nor metformin was superior to placebo in attaining the primary outcome of sustained reduction in ALT level in the TONIC study [96]. However, children treated with vitamin E (who had biopsy-proven NASH or borderline NASH) had significant improvements in secondary histologic outcomes with vitamin E. Those children who showed an improvement over placebo were those who had hepatocellular ballooning degeneration on their initial biopsies.

In summary, lifestyle intervention remains the cornerstone of treatment in NAFLD. However, it is well recognized that lifestyle changes in diet and exercise are difficult to achieve and maintain in the long term. Guidelines recommend that pioglitazone and vitamin E can be used to treat steatohepatitis in non-diabetic patients with biopsy-proven NASH in a spite of inconclusive data about their long-term safety [41]. Nevertheless, none of these therapies have been approved for the improvement of NASH features in these patients and there is a huge unmet medical need to get an FDA/EMA approved therapy for this disease. Ongoing clinical trials may expand our current understanding of the disease and provide hope for finding safer and more effective agents in the future.

## 6 Emerging Pharmacological Agents: What are the Best Endpoints?

Due to the long natural history of the disease, the improvement in hard endpoints [e.g., reduction in the development of liver-related outcomes (cirrhosis, variceal bleeds, ascites, hepatocellular carcinoma) and mortality] may not be feasible in a

2-year pivotal trial. Development of cirrhosis may be a suitable endpoint in these studies. In spite of the limitations of the liver biopsy, there is evidence that cirrhosis on histology is predictive of clinical outcomes. Reversal of NASH or a decrease in disease activity (NAS) are likely to reflect a decrease in risk of progression to cirrhosis and thus mortality. This should be combined with either improvement or lack of progression in the fibrosis score to ensure that true disease reversal is captured by this endpoint. However, it is important to note the limitations of the NAS (e.g. it has not been validated as a marker for likelihood of disease progression and/or response to therapy and the relative impact of improvement of steatosis vs. inflammation vs. ballooning is not clear). In any case, it will require longterm post-approval follow-up to demonstrate that treatment prevents cirrhosis and clinical outcomes (e.g., progression to cirrhosis, mortality, liver-related outcomes) [61]. Additional objectives should include: changes in cardiovascular risk profile, quality-of-life measures, assessments of healthcare resource utilization, clinical symptoms (especially fatigue), and safety.

In early proof of concept trials, it is not practically feasible and potentially unethical to perform multiple liver biopsies within a short time-frame (6–24 weeks). Given these considerations, the primary objectives of these early trials can be to demonstrate proof of mechanism of action, define safety, and gather preliminary efficacy data. A reduction in liver fat has been consistently associated with improvement of steatosis and inflammation. Therefore, reduction in liver fat as assessed by MR technology can be a suitable endpoint in early trials [35]. A composite endpoint including reduction of hepatic steatosis and decrease in ALT may also be considered since this enzyme is a traditional marker of liver injury. It is also advisable to get additional biomarkers of liver injury, e.g., CK-18, to add further confidence to the results [61]. Changes in insulin sensitivity and oxidative stress, anthropometric parameters, and changes in components of the metabolic syndrome may provide further information about a clinically meaningful benefit of the new compound.

The combined efforts of regulatory agencies, the pharmaceutical industry, and academia in supporting the development of potential therapeutic options are evident with the several compounds with a variety of mode of actions that are currently being tested aiming a NAFLD/NASH indication (Table 3).

## 7 Available Clinical Data on Emerging Compounds

### 7.1 Obeticholic Acid (OCA)

Obeticholic acid, a derivative of chenodeoxycholic acid, is a selective Farnesoid X receptor (FXR) agonist [97]. FXR,

**Table 3** Ongoing, recently completed and planned trials in non-alcoholic steatohepatitis (NASH) populations

Name/company	Mode of action (MOA)	Phase: ongoing/planned trial	Study design
Obeticholic acid (INT-747)/ Intercept	Farnesoid X receptor (FXR) agonist see text	Phase III trial (planned) to start by IH 2015 [128]	A 72-week randomized, double-blind, placebo-controlled trial is planned to start in Q3 2015. Patients will be randomized 1:1:1 to placebo, 10 or 25 mg of OCA. Co-primary endpoints: (1) Proportion of OCA-treated patients relative to placebo achieving at least one stage of liver fibrosis improvement with no worsening NASH. (2) Proportion of OCA-treated patients relative to placebo achieving NASH resolution with no worsening of liver fibrosis. The trial will include a pre-planned interim histology analysis after 72 weeks of treatment in approximately 1400 patients which is intended to serve as the basis for seeking USA and international marketing approvals of OCA for the treatment of NASH patients with liver fibrosis. The REGENERATE trial will enrol approximately 2500 patients at 250 sites
Cysteamine bitartrate (RP 103)/Raptor Pharmaceutical Corp	Lysosomal cysteine transporter: it lowers intra-lysosomal cysteine, with antioxidant effects	Phase IIb (ongoing). Results expected by 1Q2015 <sup>a</sup>	A 52-week phase IIb trial (CyNCh) in 160 pediatric participants <sup>a</sup> is currently ongoing. Primary objective: improvement in NASH assessed as changes in NAS at week 52
GF505/Genfit	Dual PPAR $\alpha$ $\delta$ agonist see text	Phase IIb completed in March 2015 [129] Phase III (planned)	A multicenter, randomized, double-blind, placebo-controlled 52-week study to evaluate the efficacy and safety of GF505 in patients with NASH and a NAS $\geq 3$ has recently been completed. Primary objective: Percentage of responders defined by the disappearance of steatohepatitis without worsening of fibrosis [129]
Simtuzumab/Gilead	Humanized monoclonal antibody with an immunoglobulin IgG4 isotype directed against human lysyl oxidase-like 2 (LOXL2)	Phase IIb (ongoing). Data from the 48-week endpoint are expected by mid-2015. Final data expected by 2019 <sup>b</sup>	A phase IIb, randomized, double-blind, placebo-controlled 96-week trial in 225 subjects with compensated cirrhosis secondary to NASH is currently ongoing. The primary objective of this trial is to assess regression in morphometric quantitative collagen on liver biopsy after a 96-week period of once-weekly subcutaneous injections of 75 or 125 mg of simtuzumab vs. placebo in patients with advanced liver fibrosis but not cirrhosis secondary to NASH <sup>b</sup>
Aramchol/Galmed	Inhibition of the stearyl coenzyme A desaturase 1 (SCD1) activity, a key enzyme that modulates fatty acid metabolism in the liver [102]	Phase II (ongoing) <sup>c</sup>	Ongoing phase II trial to evaluate the safety and efficacy (assessed as changes in liver fat with MRS) of two Aramchol doses (400 mg and 600 mg) relative to placebo, once daily for 52 weeks in overweight or obese pre diabetics or T2DM patients with NASH <sup>c</sup>
Liraglutide/Novo Nordisk	GLP1 agonist see text	Phase II completed <sup>d</sup>	LEAN is a recently completed clinical trial to evaluate whether a 48-week treatment with 1.8 mg liraglutide improves liver histology in overweight patients with NASH with or without diabetes <sup>d</sup>
Emricasan/Conatus	Pan-caspase inhibitor see text	Phase II completed	A 28-day placebo-controlled, multicenter, double-blind, randomized trial in subjects with non-alcoholic fatty liver disease and raised transaminases has been completed in the first quarter 2015

Table 3 continued

Name/company	Mode of action (MOA)	Phase: ongoing/planned trial	Study design
Cenicriviroc/Tobira Therapeutics	Immunomodulator and dual inhibitor of chemokine receptors CCR2 and CCR5 (important players in the trafficking of monocytes/macrophages and other cell types)	Phase II (ongoing) <sup>e</sup> . Data is expected by end of 2016–2017	The primary objective of the ongoing (CENTAUR) trial is to evaluate improvement in NAS (defined by a reduction of at least 2 points with at least a 1-point improvement in more than one category) and resolution of NASH without worsening of liver fibrosis after 1 and 2 years of treatment with cenicriviroc (150 mg) as compared to placebo in adult subjects with NASH and liver fibrosis. <sup>e</sup>  There is another ongoing study to assess the effects of cenicriviroc on insulin sensitivity in subjects with prediabetes and suspected NAFLD (ORION) <sup>f</sup>
PX 104/Phenex Gilead	FXR agonist	Phase II (ongoing) estimated date of completion Sept 2015 <sup>f</sup>	Ongoing open-label 28-day study aiming to assess the safety and efficacy of Px 104 (5 mg) assessed as changes in liver fat <sup>g</sup>

NAS NASH activity score, NAFLD non-alcoholic steatohepatitis

<sup>a</sup> ClinicalTrials.gov Identifier: NCT 01529268

<sup>b</sup> ClinicalTrials.gov Identifier: NCT01672866

<sup>c</sup> ClinicalTrials.gov Identifier: NCT02279524

<sup>d</sup> ClinicalTrials.gov Identifier: NCT01237119

<sup>e</sup> ClinicalTrials.gov Identifier: NCT02217475

<sup>f</sup> ClinicalTrials.gov Identifier: NCT02330549

<sup>g</sup> ClinicalTrials.gov Identifier: NCT01999101

a member of the nuclear receptor superfamily, is mainly expressed in liver, intestine, kidney, and, to a lesser extent, in adipose tissue. It regulates a variety of target genes involved in the control of bile acids, lipid, and glucose homeostasis, as well as genes affecting the regulation of immune responses. FXR controls glucose metabolism through regulation of gluconeogenesis and glycogenolysis in the liver, and through regulation of peripheral insulin sensitivity in skeletal muscle and adipose tissue. Treatment with OCA has been shown to increase insulin sensitivity, regulate glucose metabolism and lipid metabolism, and exert anti-inflammatory properties along with marked antifibrotic effects in preclinical models.

In a phase IIa [97] randomized, placebo-controlled, 6-week study in 23 patients with T2DM and presumed NAFLD (high liver enzymes, enlarged liver by imaging, or histologic diagnosis of NAFLD on prior biopsy), OCA significantly improved insulin sensitivity (assessed as the change post- vs. pre-treatment in the glucose infusion rate during low- and high-dose insulin infusion periods). There was a small but significant weight loss in the OCA relative to the placebo arm.

The FLINT [98] trial was a randomized, placebo-controlled, 72-week study in 282 subjects with confirmed NASH and NAS  $\geq 4$  with at least 1 point from each component. The primary outcome measure was a decrease in NAS by at least 2 points without worsening of fibrosis from baseline. A planned interim analysis of the primary outcome showed a significant improved efficacy of OCA (25 mg) relative to placebo and supported a decision not to do end-of-treatment biopsies in 64 patients. A total of 50 (45 %) of 110 patients in the OCA group had improved liver histology compared with 23 (21 %) of 109 patients in the placebo group ( $p = 0.0002$ ). More patients assigned to OCA compared with placebo had improvement in fibrosis, hepatocellular ballooning, steatosis, and lobular inflammation. However, the proportion of patients with resolution of NASH was not statistically higher in patients treated with OCA compared with placebo (22 [22 %] of 102 vs. 13 [13 %] of 98;  $p = 0.08$ ). Serum ALT and AST concentrations were significantly reduced in the OCA relative to the placebo arm. By contrast, serum alkaline phosphatase levels increased, and gamma glutamyl transferase (GGT) concentrations decreased. These changes in liver enzyme concentrations reversed after OCA was stopped. Compared with placebo, treatment with OCA was also associated with higher concentrations of total serum cholesterol and LDL cholesterol, and a decrease in HDL cholesterol. Other adverse events were generally mild to moderate in severity and were similar in the two groups for all symptoms except pruritus, which was reported more frequently and was more severe in patients on OCA relative to placebo (23 vs. 6 %). Future studies will need to provide confirmatory data about

the effects of OCA in improving markers of NAFLD as well as its impact on liver-related outcomes. Additionally, these studies will need to address the potential consequences of lipid changes on cardiovascular outcomes.

## 7.2 Cysteamine Bitartrate

Cysteamine bitartrate (RP 103) is an aminothioliol antioxidant approved for the treatment of cystinosis. In a 24-week pilot open-label, phase IIa clinical trial in 13 children with biopsy-confirmed diagnosis of moderate-to-severe NAFLD and baseline ALT and AST  $\geq 2$ ULN, seven (64 %) subjects achieved the primary objective of a reduction  $>50$  % from baseline values in ALT and AST levels at week 24. There was also a significant reduction in mean ALT ( $p = 0.002$ ) and AST ( $p = 0.007$ ) without a significant change in mean BMI. No significant change in fasting insulin levels compared with baseline were observed either at 24 weeks or after 24 weeks of safety follow-up after drug discontinuation, suggesting that the improvement in ALT and AST was an insulin-independent response. Mean CK-18 fragment levels decreased 43 % from baseline. Following the 24-week cysteamine therapy, the mean plasma adiponectin levels increased by 31 % compared with the mean baseline levels. Superoxide dismutase values increased by 25 % after the 24-week cysteamine therapy (indicating antioxidant activity) and returned to baseline levels at week 48 [99].

## 7.3 GFT505

GFT505 is dual peroxisome proliferator-activated receptor alpha/delta (PPAR- $\alpha/\delta$ ) agonist. PPAR $\alpha$  is highly expressed in the liver, where it controls genes involved in lipid and lipoprotein metabolism. PPAR $\delta$  is widely expressed and plays a critical role in mitochondrial function, fatty acid oxidation, and insulin sensitivity in mice. In preclinical models of NAFLD/NASH and liver fibrosis, GFT505 demonstrated liver-protective effects on steatosis, inflammation, and fibrosis. In addition, GFT505 improved liver dysfunction markers, decreased hepatic lipid accumulation, and inhibited proinflammatory (interleukin-1 beta, TNF $\alpha$ ) and profibrotic (transforming growth factor beta, tissue inhibitor of metalloproteinase 2, collagen type I, alpha 1, and collagen type I, alpha 2) gene expression.

In phase II studies in abdominally obese patients with either combined dyslipidemia or prediabetes, a 1-month treatment with GFT505 (80 mg/day) significantly improved lipid and glucose homeostasis. Additionally, GFT505 treatment decreased GGT, ALT, and ALP levels [102]. A randomized, placebo-controlled, three-arm (placebo, 80 mg, and 120 mg) phase IIb (GOLDEN) trial that evaluated 274 subjects with biopsy-confirmed NASH and a



NAS  $\geq 3$  has been completed recently. The primary outcome of improvement in NASH without worsening of fibrosis was not achieved. One potential explanation was the unexpected rate of resolution of NASH in patients randomized to placebo (NAS of 3, placebo response rate  $>57\%$ ). In the subanalysis of the population of NASH patients with an initial NAS score of  $\geq 4$  ( $N = 120$ ), GFT505 at 120 mg/day led to a significant improvement on both the primary endpoint (29 vs. 5 % for placebo;  $p = 0.01$ ) and on the lowering of the NAS score by at least two points (48 vs. 21 % for placebo;  $p = 0.02$ ). GFT505 led to a significant reduction in LDL-cholesterol ( $-9.28$  mg/dL,  $p < 0.001$  vs. placebo) and a significant increase in HDL-cholesterol ( $+4.25$  mg/dL,  $p < 0.01$  vs. placebo). HbA1c levels were significantly reduced in diabetic patients ( $-0.46\%$ ,  $p < 0.05$  vs. placebo). The safety assessment of this 1-year study demonstrated a favorable safety profile. Weight remained stable, and no signal for edema was observed. A mild dose-dependent increase in creatinine was noted ( $<5\%$ ; GFT505 120 mg vs. placebo). The most common adverse events were of a gastrointestinal nature and of mild intensity [100].

#### 7.4 Simtuzumab

Simtuzumab is a humanized monoclonal antibody against lysyl oxidase-like 2 (LOXL2), an enzyme that in humans is encoded by the LOXL2 gene. It is essential to the biogenesis of connective tissue, encoding an extracellular copper-dependent amine oxidase that catalyzes the first step in the formation of cross-links in collagens and elastin, a key component in the core regulatory pathway of fibrogenesis.

In 20 patients with liver fibrosis of diverse etiologies, simtuzumab (up to 10 mg/kg infused over 1 h) every 2 weeks (three infusions) appeared to be well tolerated. The most frequently reported adverse events were abdominal pain, fatigue, musculoskeletal pain, and headache. While simtuzumab is being developed as an antifibrotic agent, an acute reduction in transaminases was observed suggesting a potential anti-inflammatory effect in addition to the antifibrotic effect [101]. A PhIIb in patients with compensated cirrhosis secondary to NASH is currently ongoing (Table 3).

#### 7.5 Aramchol

Aramchol is a conjugate of two natural components, cholic acid and arachidic acid. Aramchol inhibits the activity of stearoyl coenzyme A desaturase 1 (SCD1) in the liver. The physiologic effects of SCD1 inhibition are: decreased synthesis of fatty acids, resulting in a decrease in storage triglycerides and other esters of fatty acids. This reduces

liver fat (including triglycerides and free fatty acids), and results in an improvement in insulin resistance and anti-atherogenic effect in animal studies. In a randomized, double-blind, placebo-controlled trial of 60 patients with biopsy-confirmed NAFLD (six with NASH), patients were given aramchol (100 or 300 mg) or placebo once daily for 3 months. No serious or drug-related adverse events were observed in the 58 patients who completed the study. Over 3 months, liver fat content decreased by 12.57–22.14 % in patients given 300 mg/day aramchol, but increased by 6.39–36.27 % in the placebo group ( $p = 0.02$ ). Liver fat content decreased in the 100-mg aramchol group, by 2.89–28.22 %, but this change was non-significant. [102]. There is an ongoing phase II trial in overweight/obese patients with pre-diabetes or T2DM and NASH (Table 3).

#### 7.6 Liraglutide

Liraglutide is a long-acting glucagon-like peptide-1 receptor agonist approved for the treatment of diabetes and, most recently, for the treatment of obesity in adults with related co-morbidity. A meta-analysis from six randomized clinical trials comprised in the “Liraglutide Effect and Action in Diabetes” (LEAD) program and including several thousand patients with NAFLD [84], subjects treated with liraglutide (1.8 mg/day for 26 weeks) showed a reduction in ALT and hepatic steatosis at CT evaluation, as well as in NAFLD fibrosis score. In a substudy of a randomized phase II study (subgroup of NASH patients), liraglutide (1.8 mg) significantly reduced weight, waist circumference, HbA1c, fasting glucose, LDL, and liver enzymes versus placebo. Liraglutide significantly reduced circulating NEFA in the fasting state, low-dose and high-dose insulin states, and significantly reduced adipose tissue lipolysis. Liraglutide significantly improved serum markers of adipose inflammation, e.g., leptin and adiponectin [103].

In the Liraglutide Efficacy and Action in NASH (LEAN) trial overweight patients with biopsy-confirmed NASH were randomized (1:1) to receive 48-week treatment with once-daily, subcutaneous injections of either 1.8 mg liraglutide or liraglutide-placebo (control). The primary outcome measure was improvement in liver histology, defined as resolution of definite NASH and no worsening in fibrosis. A total of nine (39 %) of 23 patients on liraglutide had resolution of definite NASH compared to two (9 %) of 22 patients on placebo ( $p = 0.019$ ). Only two (9 %) patients on liraglutide had worsening of fibrosis compared to eight (36 %) on placebo ( $p = 0.026$ ). As expected, liraglutide led to weight loss ( $-5.3$  vs.  $-0.6$  kg,  $p = 0.001$ ) and lower fasting glucose ( $-1.0$  vs.  $-0.7$  mmol,  $p = 0.005$ ) compared to placebo. Reductions in ALT ( $-27$  vs.  $-10$ ,  $p = 0.126$ ) and HbA1c ( $-0.5$  vs.  $-0.03\%$ ,  $p = 0.07$ ) were also seen with liraglutide, albeit

not significant versus placebo. Liraglutide was well tolerated with only two (8 %) of 26 patients withdrawing from treatment due to drug-related gastrointestinal (nausea, diarrhoea) side effects [104].

### 7.7 Emricasan

Emricasan is a potent irreversible pan-caspase inhibitor. Caspases play a central role in the processes of apoptosis and inflammation. They are responsible for executing apoptotic pathways, or programmed cell death, and for activation of cytokines such as IL-1 $\beta$  and IL-18. Both caspase-mediated apoptosis and inflammation have been shown to play important roles in the development and progression of NASH and NAFLD, leading to the hypothesis that inhibition of caspases may have a significant therapeutic benefit for the treatment of NAFLD/NASH. Emricasan reduced steatosis, inflammation, apoptosis, and fibrosis in preclinical models. Human studies have demonstrated that emricasan can lower serum transaminases after intravenous or oral administration. In a recently completed randomized, placebo-controlled, 28-day phase II study in subjects with NAFLD and elevated ALT, emricasan led to statistically significant reductions in ALT and CK-18 (approximately 30 % relative to placebo) at day 28. Emricasan was generally well tolerated in the study and no changes were reported in weight, cholesterol, HDL, LDL, or triglycerides in the study in either the emricasan or placebo arms [105].

### 7.8 Cenicriviroc

It is an immunomodulator and dual inhibitor of chemokine receptors CCR2 and CCR5 (important players in the trafficking of monocytes/macrophages and other cell types). In a post-hoc analysis in patients with HIV, treatment with cenicriviroc was associated with improvements in AST to platelet ratio and FIB-4 scores, and correlations were observed between changes in AST to platelet ratio and FIB-4 scores and sCD14 levels at week 48 [106]. There are two ongoing phase II studies in patients with NAFLD (Table 3).

### 7.9 Remogliflozin

Remogliflozin is an SGLT2 inhibitor shown to reduce HbA1c in type 2 diabetics. Remogliflozin has been shown to improve insulin sensitivity in subjects with type 2 diabetes. Post-hoc analysis in a 12-week trial in diabetics showed an approximate 40 % reduction in ALT levels in subjects with elevated values at Baseline. Additionally, remogliflozin has been reported to have anti-oxidant activity as measured by the oxygen radical antioxidant capacity (ORAC) assay and serum markers of oxidative stress in animal models of steatohepatitis [107].

## 8 Strategic Considerations in Developing Drugs to Treat Non-Alcoholic Steatohepatitis

As described above, there is much uncertainty on the optimal endpoints to determine the efficacy of drugs in the treatment of NASH. The hope is that with the development of validated biomarkers, we will be able to move away from biopsy and histopathology. There are also many questions remaining on the natural history of NASH, and why patients progress to this condition from NAFLD. Fortunately, the last decade has seen regulatory agencies implement several initiatives to expedite the development of drugs for serious conditions such as NASH—and these initiatives are especially applicable in cases where there is an unmet medical need. For example, the US Food and Drug Administration (FDA) has developed several pathways to expedite drug development for unmet medical needs [108], and the European Medicines Agency (EMA) has created similar mechanisms known collectively as “adaptive pathways” [109]. Often, these accelerated development pathways to approve a drug for marketing are referred to as conditional approvals—since they are usually “conditional” upon post-marketing studies. However, the overall goal with all of the accelerated development pathways is to quickly bring useful drugs to patients where an unmet medical need exists. A summary of these accelerated development pathways is shown in Table 4 and a

**Table 4** Examples of regulatory pathways to accelerate marketing approvals for life-saving therapies

Accelerated development pathway (location; year of introduction)	Comment
Accelerated approval (USA 1992)	Shortened clinical development time
Priority review (USA 1992)	Shortened marketing application review time (6 months)
Fast track (USA 1997)	Shortened clinical development time. Rolling review of marketing application
Breakthrough therapy (USA 2012)	Shortened clinical development time
Approval under exceptional circumstances (EU 1993)	Shortened clinical development time
Conditional marketing authorization (EU 2005)	Shortened clinical development time

more in-depth discussion is provided in a recent review [110]. These approaches are particularly relevant to NASH, and may reduce the time to marketing approval and reimbursement. Indeed, one company in the NASH drug development space has already received a breakthrough designation for an investigational compound [111]. This designation guarantees a shortened clinical development plan and substantial FDA engagement during the development of the product. Consequently, it seems reasonable to assume that regulatory agencies will be receptive to expedited approvals for drugs to treat or prevent NASH.

Of course, pathways to accelerate drug development cannot exist without modernizing clinical trial methodology. A number of guidances on new clinical development methodologies have been published by regulatory agencies in recent years. These guidances demonstrate the need for innovation and flexibility in developing products for conditions with a major unmet medical need, such as NASH. Examples of these clinical trial guidances include adaptive designs [112] and enrichment strategies to support marketing approvals [113]. A recent review provides additional information on these clinical trial methodologies in greater depth [114].

All of the above regulatory tools are likely to be incorporated into the first successful marketing application for the treatment of NASH. As mentioned, several companies are in the process of completing phase II studies and moving toward phase III registration studies. These companies are now in discussions with regulatory agencies. A recent industry forum on NASH suggested that surrogate endpoints might include reversal of NASH as measured by histologic improvement, histologic resolution, or improvements in fibrosis; with post-approval trials looking at clinical outcomes such as liver transplant-free survival or progression to cirrhosis [115]. However, in the final analysis, we remain in a trial-and-error scenario with regard to the registration pathway for a drug in the treatment of NASH. It could be several years before we resolve the best surrogate markers and clinical outcome endpoints.

Finally, with uncertainty in clinical endpoints and trial design, the interaction between a wide range of stakeholders is extremely important to explore ways to optimize development pathways. Such stakeholders include regulatory agencies (such as FDA and EMA), the biotech/pharmaceutical industry, health-technology assessment bodies, physicians, researchers, and patients. While all of these stakeholders are important for successful drug development, the interactions between sponsors and the regulatory agencies cannot be overestimated. For example, the success rates for drug approvals by regulatory agencies are increased substantially when the drug developers take advantage of meetings with regulatory agencies [116]. The quality of the interaction between sponsor and regulatory agency will be

especially important in developing drugs for NASH—not only to ensure selection of adequate endpoints, but to also accelerate development using expedited pathways.

## 9 Conclusions and Future Directions

NAFLD is the most common cause of chronic liver disease in the Western world today. With rising levels of obesity and T2DM, its prevalence will increase in the future, and cause considerable morbidity and mortality. Despite considerable research and multiple clinical trials, at present no single pharmacologic agent has achieved a clinically meaningful benefit/risk profile to warrant regulatory approval for marketing. The combined efforts of academia, pharmaceutical industry, and regulatory agencies will eventually bring the first approved therapy within a few years. The ideal drug will need to address not only the liver complications but prevent cardiovascular death, the main cause of mortality in this patient population. There is a wide variety of compounds with a different mode of actions currently in clinical development. It is most likely that a multifaceted combination therapy will be needed. The ideal treatment will lead, in the short term, to a reduction in liver inflammation and fibrosis, and an improvement in insulin sensitivity and metabolic complications, but in the long term will need to reduce cardiovascular and liver outcomes.

### Compliance with Ethical Standards

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