Mouse models of nonalcoholic steatohepatitis in preclinical drug development

Henrik H. Hansen, Michael Feigh, Sanne S. Veidal, Kristoffer T. Rigbolt, Niels Vrang and Keld Fosgerau

Gubra Aps, Hørsholm Kongevej 11b, Hørsholm DK-2970, Denmark

Nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease in the Western world. NAFLD is a complex spectrum of liver diseases ranging from benign hepatic steatosis to its more aggressive necroinflammatory manifestation, nonalcoholic steatohepatitis (NASH). NASH pathogenesis is multifactorial and risk factors are almost identical to those of the metabolic syndrome. This has prompted substantial efforts to identify novel drug therapies for correcting underlying metabolic deficits, and to prevent or alleviate hepatic fibrosis in NASH. Available mouse models of NASH address different aspects of the disease, have varying clinical translatability, and, therefore, also show different utility in drug discovery.

Introduction

The prevalence of NAFLD is rapidly increasing worldwide and it is now the most common liver disorder in the Western world [1]. Obesity, type 2 diabetes (T2D), hyperlipidemia, and hypertension are highly prevalent in individuals with NAFLD and, therefore, NAFLD risk factors are almost identical to the constituents of the metabolic syndrome [2,3]. NAFLD is a complex spectrum of liver diseases ranging from benign, usually asymptomatic, steatosis to the more aggressive necroinflammatory form, nonalcoholic steatohepatitis (NASH). NASH is characterized by varying degrees of steatosis, cytoskeletal damage (hepatocellular ballooning), and lobular inflammation with or without fibrosis [4]. Although not all patients with NAFLD develop liver-related complications, patients with NASH are at increased risk of developing hepatic fibrosis, which can progress to cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease [5,6]. As a consequence, NASH is currently the second indication for orthotopic liver transplantation, and it is projected that NASH will become the leading indication for liver transplantation within developing countries by 2020 [7]. To date, no evidence-based drug therapy has been approved for NASH management and, because therapeutic advances have been slow, NASH is classified as a medical condition with high unmet therapeutic need.

To facilitate the development of novel diagnostic and therapeutic interventions in NASH, a plethora of animal models have been used to identify molecular targets that are involved in the onset and progression of NASH. In view of recent advances in the understanding of the pathogenesis of NASH and progress in the clinical development of anti-NASH compounds, here we discuss the advantages and limitations of current *in vivo* mouse models of NASH.

NASH pathogenesis

Current NAFLD treatment focuses on reducing metabolic risk factors, with lifestyle intervention being the mainstay therapy; however, this approach is often inefficient because of long periods of dieting and weight cycling [8]. Recently, several breakthroughs have been made in the understanding of NASH pathogenesis, which is now known to be multifactorial, implicating several pathways in disease onset and progression. The pathogenesis of NASH was originally interpreted with a 'dual-hit' hypothesis, where steatosis ('first hit'), resulting from increased lipolysis and lipogenesis (accentuated by insulin resistance), predisposes to the initiation of NASH through downstream ('second hit') proinflammatory mediators [9]. Today, more complex 'multiple-hit'

Corresponding author: Hansen, H.H. (hbh@gubra.dk)

hypotheses have been proposed with the aim to explain how fatty acids and their metabolites promote NASH through multiple sequential or parallel cytotoxic pathways. In general, most recent hypotheses involve fatty acid-mediated lipotoxicity, which exhausts hepatocyte adaptive and regenerative responses, enabling accumulating oxidative stress to trigger hepatocyte necroinflammation, scar tissue formation (fibrosis), and disruption of hepatic cytoarchitecture, which can ultimately progress to cirrhosis and HCC [10,11]. A recent meta-analysis study of microarray data sets from rodent activated hepatic stellate cells (HSCs, principal collagen-producing cells) underlined the complexity in fibrogenesis signaling pathways and suggested several novel candidate genes potentially serving as biomarkers or therapeutic targets for fibrotic NASH [12]. NASH-specific pathways and druggable targets are also likely to be expanded in detail by 'omics' approaches (gut metagenomics, plasma metabolomics, and liver transcriptomics), which are increasingly applied in NASH research [13–15].

There is evidence for concurrent immune imbalances in NASH. Although the immune signaling pathways involved are incompletely understood, activation of hepatic resident Kupffer cells (specialized macrophages) and neutrophils, in addition to the recruitment of other innate immune cells, is an important effector of parenchymal inflammation in NASH [16]. Recent research on the potential role of the adaptive immune system in NASH has focused on proinflammatory T cells, including T helper (Th)-17 cells, which are the primary producers of the IL-17 family of proinflammatory cytokines [17]. Given that IL-17 receptors are ubiquitously expressed in the liver (including by hepatocytes, Kupffer cells, and HSCs), dysregulated IL-17 secretion could lead to the mobilization of several deleterious cell signaling pathways [18,19]. These cell types also express other receptor families that have been implicated in NASH immunopathology, including Toll-like receptors (TLRs [20]) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs [21]). NLRs have received special attention because they are recognized as inflammasome sensory molecules. Metabolic inflammation triggered by the inflammasome (multiprotein complexes that assemble upon the sensing of danger signals and initiate the release of potent proinflammatory cytokines and chemokines) is suggested to link the metabolic syndrome and NAFLD [22], and could have an important role in the transition to fibrotic NASH [23].

Gut microbial imbalances, bacterial translocation, and maladaptive host responses ('gut dysbiosis') are emerging as important contributing factors in the pathogenesis of obesity-related disorders, including NASH. The gut microbiota also has a critical role in bile acid metabolism, and might thereby indirectly modulate farnesoid X receptor (FXR) function, which is an important therapeutic target for NASH (see below). Gut dysbiosis causes gut dysmotility and inflammation. Importantly, dysbiosis can also lead to increased gut permeability to dietary factors and bacterial immunogens, thereby increasing hepatic exposure to injurious stimuli that promote hepatic inflammation and fibrogenesis. Compositional changes in the gut microbiome, reduced intestinal barrier function, translocated bacterial proinflammatory products, and associated inflammasome activation have been reported in NAFLD, and multiple studies in mouse models of NASH have supported these findings [24]. However, most of the current evidence in this field comes from animal experiments, and further human studies are needed to determine whether gut dysbiosis translate into NASH pathology, and whether gut microbiome alterations precede and precipitate NASH, or simply reflect secondary adaptive responses to the dysmetabolic features of the disease.

Clinical development of anti-NASH drug therapies

The current understanding of NASH pathogenesis has led to broad efforts to target several features of the disease, alone or in combination, even in the absence of liver-guided therapies. Therefore, drug development in NASH is a rapidly changing field. A considerable number of single modality therapies are in various stages of clinical development, and it is expected that combination therapies will also soon be targeted. Most investigational new drugs have a hepatic metabolic target, engineered to reduce hepatic fat accumulation, inflammation, insulin resistance, or mitochondrial dysfunction. In addition, several emerging medical therapies are directly interfering with fibrosis pathways aiming to decrease hepatic fibrosis progression [25]. It is advantageous that drug therapies in NASH also induce weight loss, because successful weight management (≥5–10% weight loss) per se improves liver histology in NASH [26]. Current drug targets under clinical investigation are summarized in Fig. 1.

Given that there are no benchmark standard endpoints that can be followed in lieu of histology, liver histology remains the main outcome variable for clinical trials. Liver biopsy is applied to confirm (or exclude) the diagnosis and stage of NASH, which also provides a rational basis for evaluation of treatment efficacy upon completion of the trial [4]. Several histological scoring systems have been developed for monitoring histopathological changes in NASH, including the NAFLD activity score (NAS) and steatosis–activity–fibrosis (SAF) systems [27,28]. The NAS system is widely used in clinical trials and grades the severity of macrovesicular and/or microvesicular steatosis, hepatocellular ballooning, and lobular inflammation on liver biopsies. Fibrosis is not included in NAS because it is a sign of the disease stage rather than of the grade of injury; hence, a separate semiquantitative scoring system is utilized for fibrosis stage monitoring [27]. The disease scoring and staging systems are semiquantitative and only consider changes in hepatic tissue architecture, which could narrow the window of treatment efficacy. Consequently, there is an increasing consensus that quantitative histology is required to fully conclude on treatment outcome [29].

The extent of liver fibrosis, rather than of NASH, is the major driver for cardiovascular co-morbidity, malignancy, and mortality in NASH [30]. Therefore, antifibrotic therapeutics have gained considerable focus in NASH drug discovery. Current antifibrotic strategies include reducing the primary disease, improving hepatocyte integrity, suppressing hepatic inflammation, downregulating HSC activation, or promoting extracellular matrix degradation (reviewed in [23]). Several of these strategies are approached by emerging immunotherapies for NASH and other fibrotic liver diseases [31]. Although there are currently no universal regulatory approval pathways for drug development in NASH, there is an emerging consensus that NASH resolution with halted progression or improvement of liver fibrosis stage are tangible primary endpoints in most clinical trials [32,33]. From a regulatory perspective,
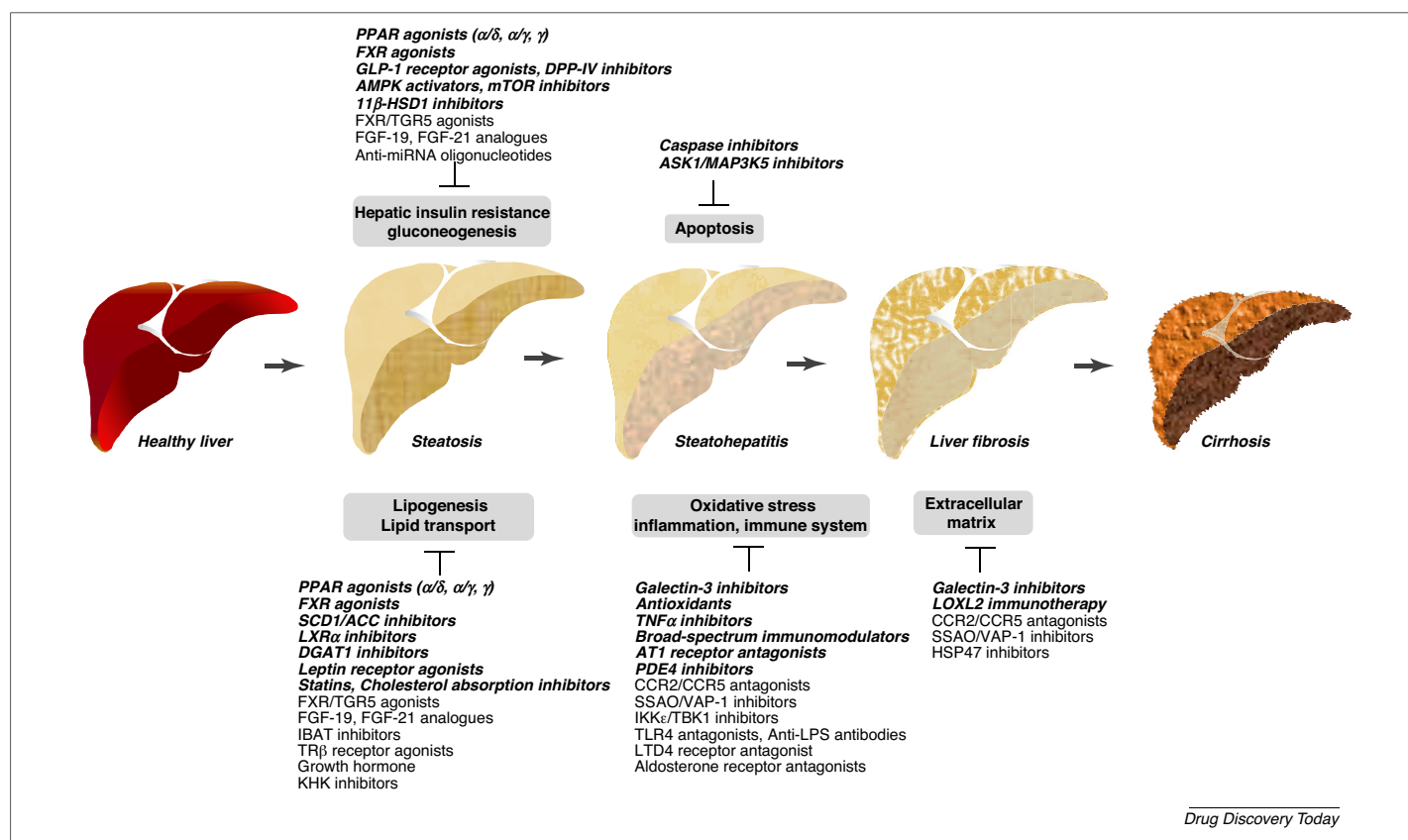
FIGURE 1

Hepatic drug classes in current (recruitment/active phase) or recently concluded clinical trials for NASH (source: ClinicalTrials.gov). Drug classes representing drugs with completed Phase II trials are indicated in bold italics. For abbreviations, see Table 1.

current pivotal clinical trials for precirrhotic NASH will likely need to demonstrate a decreased rate of progression to cirrhosis, which will require long-term extension trials [32].

In summary, an ideal drug candidate for NASH should reduce key clinical endpoints (i.e., steatosis, hepatic inflammation, and liver cell injury) and have antifibrotic effects, while also correcting underlying metabolic derangements, such as hepatic insulin resistance and obesity (Fig. 1). In this regard, most advanced clinical trials in NASH have indicated improved NASH with no worsening (but not reversal) of hepatic fibrosis on liver biopsies, but not all compounds have shown additional beneficial effects on insulin resistance and body weight [34–36].

Animal models of NASH

In drug discovery, an applicable animal model of NASH should enable the assessment of test compound pharmacodynamics with an emphasis on the key metabolic, biochemical, and histological parameters mentioned above. Considering the array of rodent models of NASH reported over the past decade, the models are essentially distinguished by their ability to mimic the etiology and/or natural history (obesogenic dietary models) or histopathology (nutrient-deficient dietary models or chemically induced models). Also, genetic models (monogenetic or polygenetic) are widely used in NASH research. Consequently, available animal models of NASH have different utility and clinical translatability.

The human NAS system (see above) is largely reproducible in NAFLD mouse models [37] and, therefore, has been increasingly applied in the preclinical assessment of liver histological responses to test compounds. In general, the NAS system is well suited for this purpose, although there is not a complete overlap in NASH pathology between humans and rodents. For example, distinct hepatocyte ballooning is often absent or marginal in rodent NAFLD/NASH models [37] and, therefore, composite NAS in experimental NASH models is largely determined by the grade of hepatic steatosis and inflammation. This also indicates that currently available mouse models of NASH are not optimal for evaluating drug effects on hepatocyte degeneration and, thus, measures should also include apoptotic markers.

Murine models constitute the bulk of research in preclinical NASH pathology, and a subset of mouse models exhibits good clinical translatability. Thus, here we discuss selected mouse models to demonstrate the diversity of the attempts to establish in vivo models that recapitulate the etiology, natural history, histopathology, and disease progression. We focus on murine NASH models used for test of pharmacological agents, as listed in Tables 2–4.

Obesogenic dietary models

The primary driver of NAFLD is overnutrition and a sedentary lifestyle leading to increased weight and, ultimately, obesity. The strong association between NAFLD and obesity has spurred the development of various diet-induced obesity (DIO) models aimed at mimicking the etiology and natural history of NASH. However, there are significant mouse interstrain differences in the susceptibility to NASH when fed an obesogenic and/or atherogenic diet.

Reviews • POST SCREEN

C57BL/6 mice exhibit high sensitivity to obesogenic diets and, therefore, are the most common mouse strain used in experimental NASH (Table 2). For example, C57BL/6 mice are significantly more prone to develop diet-induced hepatic necroinflammation and fibrosis compared with BALB/c and C3H/HeN mice [38,39].

The diet formulas are varied to induce different degrees of adiposity (40–70% fat calories, i.e. high-fat diet) and dyslipidemia (0.1–2.0% cholesterol, i.e. atherogenic diet). A major limitation with this approach is that animals fed these diets, regardless of the dieting periods used (≥20–30 weeks), typically develop dyslipidemia, fatty liver, and mild NASH without appreciable fibrosis. Thus, such dietary models of NASH can only be used for the characterization of potential drug effects on body weight, hepatic steatosis, and (to some degree) inflammatory markers [40,41]. Therefore, different attempts have been made to add dietary factors that would amplify NASH and trigger a robust fibrotic response without significantly compromising the nutrient balance. For example, composite diets are often supplemented with fructose or sucrose ('Western diets') to promote hepatic insulin resistance with more pronounced weight gain and dyslipidemia. Even though these diets elicit more marked steatohepatitis and inflammation, only inconspicuous and mild-stage (perisinusoidal) fibrosis has been reported with these diet modifications [42]. Thus, standard Western diet formulas are suboptimal for preclinical NASH research, and only a few compounds have so far been reported to be profiled in these DIO mouse models of NASH [43–45].

To circumvent this limitation, a different concept has recently been introduced with the use of a dietary lipid composition that more closely reflects a prototypic fast-food diet. Accordingly, an 'American Lifestyle-Induced Obesity Syndrome' (ALIOS) mouse model of NASH was developed by Tetri and colleagues [46], and subsequently refined for NASH research by Amylin Pharmaceuticals and other research laboratories [47–49] (now termed 'Amylin Liver NASH model', abbreviated AMLN). Affected C57BL/6 mice on a AMLN diet ('AMLN mice') develop marked steatosis, moderate lobular inflammation, and mild-stage hepatocellular ballooning within 26–30 weeks of dieting. Notably, the addition of cholesterol (2%) and trans-fatty acids (45% of total fat amount) to the diet are critical factors for steatohepatitis to progress to mild–moderate fibrosis in AMLN mice [47–49].

In addition, an inbred isogenic C57BL/6J x 129S1/SvImJ mouse strain (termed DIAMOND) with age-dependent onset of NASH and fibrosis has been developed [50]. DIAMOND mice are kept on a prototypical Western diet (with 0.1% cholesterol), but affected mice nevertheless developed robust NASH and mild fibrosis at week 16–22. Bridging fibrosis (stage 3) was observed in almost all mice at week 52. Moreover, a major proportion of DIAMOND mice also showed HCC development at week 32–52. In comparison, the parent strains fed the same high-fat diet exhibited either similar (C57BL/6J) or slightly reduced NAS (129S1/SvImJ, because of a lower steatosis grade). Fibrosis was also lower (129S1/SvImJ) or almost absent (C57BL/6J), and both parent strains showed no histological evidence of HCC development. Although no pharmacological intervention has so far been reported in DIAMOND mice, this model could be applicable for the determination of drug treatment efficacy in NASH with or without co-morbid hepatocellular malignancy.

As also seen in the clinic, the NASH phenotype varies in rodent models of the disease, (i.e., have unpredictable onset, occurs at varying rates, and shows different severity). Accordingly, available data on Western diet-based NASH models indicate that mouse cohorts represent all stages of NAFLD for any dieting period ≥20 weeks, and a significant proportion of up to 30% of the animals fail to develop steatohepatitis and fibrosis [38,47,51]. This poses a challenge when designing preclinical NASH studies sensitive enough to consistently detect treatment effects. For example, the heterogeneity in the disease stage potentially limits the conclusiveness in pharmacological studies because of unintentional large variability in control group histopathology or responsiveness in treatment groups (e.g., compounds with anticipated fibrosis-preventive effects will only be efficient in nonfibrotic animals). Given the lack of diagnostic circulating biomarkers for NASH staging, such inherent problematics are usually not considered in preclinical studies, and increasing group sizes might not be sufficient to prevent false positive or negative study outcomes. With reference to standard clinical practice, biopsy-confirmation procedures have therefore recently been applied to AMLN mice for staging of baseline liver pathology to equalize NASH severity in the experimental groups [47,48] and allow for within-subject comparisons during the course of drug treatment [43,52,53].

Nutrient-deficient dietary models

To account for the insufficient hepatic fibrosis response to most hypercaloric diets, nutrient-deficient diets have been applied with the aim to provide an additional 'second hit' on hepatic metabolism. Nutrient-deficient diets are either low or devoid of certain essential nutrients, such as methionine (an essential amino acid and important methyl donor) and/or choline [precursor for de novo phosphatidylcholine synthesis and hepatocyte export of triglycerides via very-low-density lipoprotein (VLDL) packaging]. In addition, nutrient-deficient diets can be made even less lipotrope by replacing dietary proteins with equivalent amounts of L-amino acids. This approach has resulted in various diet types, such as choline-deficient (CD), methionine-deficient (MD), methionine-choline deficient (MCD), and semisynthetic (choline-deficient, L-amino acid-defined (CDAA); moderately low in methionine)) diets, which are commonly used in preclinical NASH research. The diets can vary in fat content (usually 20% fat kcal), and sucrose levels are typically high (45–60% carbohydrate kcal). The main advantage to using nutrient-deficient diets is the induction of NASH histological features, including mild to moderate fibrosis, within a shorter feeding period than with obesogenic diets.

These nonphysiological dietary manipulations promote NASH with different severity. For example, MD or CD feeding alone results in steatohepatitis, but only the MD diet is able to induce mild hepatocellular injury [54]. The NASH-inducing properties of the CD diet can be enhanced by a higher dietary fat content (60% fat kcal, CDHF model), which was recently reported to promote steatosis, inflammation, and moderate pericellular fibrosis after 8 weeks of dieting [55]. In comparison, MCD mice develop hepatic macrovesicular steatosis and infiltration of inflammatory cells after 1–3 weeks of dieting and robust perisinusoidal fibrosis occurred from week 5–7; thus, these mice have been frequently used to study the short-term effects of pharmacological treatments (Table 2). The degree of hepatic fibrosis in MCD mice appears to

vary across research laboratories, which could be the result of different mouse strains used, diet composition, and housing conditions. The CDAA diet is a variant of the MCD diet, because it contains reduced dietary methionine levels. Mice on CDAA develop macrovesicular steatosis and unspecific lobular inflammation starting from week 3, with an onset of fibrogenesis at week 6–9. This progresses to mild–moderate fibrosis stage around week 21 and HCC develops with a high incidence at week 44 [56].

A disadvantage with the MCD and CDAA diets is the induction of hypophagia and hypercatabolism resulting in significant body-weight loss with a proportional loss of liver mass. By contrast, CD mice display normal body weight [54,55]. The lack of obesogenic effects of the nutrient-deficient diets prevents any insulin-resistant phenotype, which could be a disadvantage if the mode of action of the test compound involves improvement of insulin function. The hypercatabolic state is particular evident for MCD-fed mice, where body-weight loss of 20–40% can occur during the feeding period [57]. In general, the catabolic profile limits the clinical translatability, which should be considered when interpreting data on nutrient-deficient diet models. Attempts have been made to reduce the catabolic impact of the nutrient-deficient diets by introducing less methionine deficiency. Although such diet modifications result in less pronounced weight loss or are weight neutral [56,58], compounds inducing weight loss are generally not feasible to test in nutrient-deficient dietary models of NASH. Hence, the principal hypercatabolic phenotype limits the utility of nutrient-deficient NASH models to only evaluate drugs directly targeting the liver for probing efficacy on hepatic injury and regeneration. Therefore, to consider aspects of the metabolic syndrome, drug effects in MCD and CDAA models should be confirmed in DIO NASH models.

Chemically induced models

Chemically induced parenchymal liver damage and fibrosis is specifically used for studying mechanisms of hepatic fibrosis progression and regression. Fibrosis in these models eventually progresses to liver cirrhosis and HCC with a very high incidence. Typical liver-targeted chemotoxins used are carbon tetrachloride (CCl_4), thioacetamide (TAA), and streptozotocin (STZ). The hepatotoxic mechanisms of CCl_4 and TAA are not fully understood, but involve hepatocyte uptake and conversion of CCl_4 and TAA to reactive metabolites causing oxidative necroinflammation and excessive activation and proliferation of collagen-producing HSCs [59]. By contrast, STZ is particularly toxic to pancreatic β cells, leading to progressive loss of insulin production, but STZ can also have hyperglycemia-independent direct hepatotoxic effects [60]. The fibrosis induction period varies among chemotoxin models but is short (1–8 weeks), depending on the relevant dosing regimen and fibrosis severity in the experimental setting. Whereas CCl_4 and TTA (with or without high-fat dieting) are used in adult mice, STZ is administered to neonatal mice (STAM model) [61]. STAM mice develop manifest NASH at 8 weeks, which progresses to fibrosis at 12 weeks, and eventually develop HCC at a rate of nearly 100% in males [61]. A recent lipidomics study revealed distinct changes in the hepatic lipid profile at different stages of NASH progression in STAM mice [62]. The STAM model has been used to study anti-NASH effects of several compounds (Table 3).

Similar to nutrient-deficient diets, hepatic chemotoxins cause weight loss in mice and, thus, do not mirror the etiology and natural history of NASH. As a result, chemotoxin-induced fibrosis models are mainly used in initial in vivo proof-of-concept studies on antifibrotic therapies (Table 3).

Genetic models

The large variety of mono- and polygenetic mouse models available for NAFLD research has been reviewed elsewhere [63]. Here, we highlight genetic models that most closely replicate the disease spectrum of the metabolic syndrome (Table 4). A major advantage with these genetic models is a generally more severe disease phenotype and development of diet-induced NASH within a shorter timeframe, compared with corresponding wild-type DIO mouse models.

ob/ob mice

Given that leptin deficiency is reported to protect against liver fibrosis, it has been interpreted that hyperphagic and obese ob/ob mice (homozygous for a spontaneous Lep^{ob} point mutation in the gene encoding leptin) can be used to study treatment effects on steatosis, but are less applicable for testing antifibrotics [64]. Therefore, secondary hepatotoxic insults, such as MCD diet, CCl_4, or TAA, have been applied with the aim to trigger fibrosis during the progression of steatohepatitis in ob/ob mice, but none of these combinations have provided an improved model of NASH. In contrast, ob/ob mice are consistently fibrosis prone when cholesterol (2%) and trans-fatty acids (45% of total fat amount) are added to the high-caloric diet (i.e., AMLN diet) [43,48,65]. ob/ob mice on AMLN diet (termed 'ob/ob AMLN mice') develop steatohepatitis and fibrosis within a shorter timeframe ($\leq$12 weeks) compared with wild-type C57BL/6 mice (AMLN mice) fed the same diet ($\geq$26 weeks, see also 'Obesogenic diet models' above) [43,48,66]. Liver biopsy-confirmed histology was recently reported applied to ob/ob AMLN mice, which unequivocally confirmed marked steatohepatitis and consistent development of liver fibrosis with all severity grades represented in the cohort [48]. Compared with AMLN mice, ob/ob AMLN mice display a more severe NASH phenotype, reflected by higher liver triglyceride and cholesterol levels, higher liver hydroxyproline content, increased fibrosis stage, and the presence of bridging fibrosis [48]. The more marked hepatopathology in ob/ob AMLN mice makes this model well suited for testing the anti-NASH efficacy of various compound classes [43,53,65,67] (Table 4).

db/db mice

The db/db mouse is homozygous for a spontaneous diabetic mutation in the gene encoding the leptin receptor ($Lepr^{db}$). In general, the liver histology is rather similar, but less pronounced compared with that of ob/ob mice [68]. Depending on the diet formulation and feeding period, db/db mice develop micro- and macrovesicular hepatic steatosis as well as moderate necroinflammation. As for ob/ob mice, db/db mice maintained on a high-caloric diet do not present consistent histological evidence of fibrosis, and attempts to provide a suitable 'second hit' (e.g., MCD diet or diethylnitrosamine) have resulted in combination models that have been used for the characterization of antifibrotics [69–71]. However, because db/db mice do not display the whole spectrum of human NASH histopathology, secondary nonphysiological stimuli are necessary to induce fibrosis. Moreover, db/db mice are reported to show

TABLE 1

Drug classes in current (recruitment/active phase) or recently concluded clinical trials for NASH[a]

Drug class	Abbreviation	Compounds	Refs (to clinical data)
Acetyl-CoA carboxylase inhibitors	ACC inhibitors	GS-0976/NDI-010976	[87]
Aldosterone receptor antagonists		MT-3995	
AMP-activated protein kinase activators	AMPK activators	Metformin, NS-0200	[88]
Anti-lipopolysaccharide antibodies	Anti-LPS antibodies	IMM124E	
Anti-microRNA oligonucleotides		RG-125/AZD4076	
Antioxidants		Vitamin E, cysteamine	[89,90]
Apoptosis signal-regulating kinase-1/ mitogen-activated protein kinase-5 inhibitors	ASK1/MAP3K5 inhibitors	Selonsertib/GS-4997	[91]
Angiotensin II receptor type 1 antagonists	AT1 receptor antagonists	Losartan	[92,93]
11-beta-hydroxysteroid dehydrogenase inhibitors	11β-HSD1 inhibitors	RO5093151	[94]
Broad-spectrum immunomodulators		Pentoxifylline	[95]
Caspase inhibitors		Emricasan/IDN-6556, GS-9450/LB84451	[96,97]
Cholesterol absorption inhibitors		Ezetimibe	[98]
CC-chemokine receptor 2/5 inhibitors	CCR2/CCR5 inhibitors	Cenicriviroc	[99,100]
Diacylglycerol acyltransferase-1 inhibitors	DGAT1 inhibitors	Pradigastat/LCQ908	
Dipeptidyl peptidase-IV inhibitors	DPP-IV inhibitors	Sitagliptin, vildagliptin	[101]
Fibroblast growth factor-19 agonists	FGF-19 agonists	NGM282	
Fibroblast growth factor-21 agonists	FGF-21 agonists	BMS-986036, PF-05231023	
Farnesoid X receptor agonists	FXR agonists	Obeticholic acid/INT-747, GS-9674/Px104, LJN452, EDP-305	[34]
Farnesoid X receptor/transmembrane G protein-coupled receptor-5 dual agonists	FXR/TGR5 agonists	INT-767	
Galectin-3 inhibitors		GR-MD-02	[102]
Glucagon-like peptide-1 analogues	GLP-1 analogues	Liraglutide, semaglutide, exenatide	[36,103]
Growth hormone receptor agonists		Growth hormone	
Heat shock protein 47 inhibitors	HSP47 inhibitors	ND-L02-s0201	
Ileal bile acid transporter inhibitors	IBAT inhibitors	Volixibat/SHP626	
IkappaB kinase-epsilon/TANK-binding kinase-1 dual inhibitors	IKKε/TBK1 dual inhibitors	Amlexanox	
Ketohexokinase inhibitors	KHK inhibitors	PF-06835919	
Leptin receptor agonists		Leptin, metreleptin	[104]
Lysyl oxidase like 2 enzyme antibodies	LOXL2 antibodies	Simtuzumab/GS-6624	
Leukotriene D4 receptor antagonists	LTD4 antagonists	Tipelukast/MN-001	
Liver X receptor-α receptor antagonists	LXRα antagonists	Oltipraz	[105]
Mechanistic target of rapamycin protein inhibitors	mTOR inhibitors	MSDC-0602K	
Phosphodiesterase cyclic nucleotide type 4 inhibitors	PDE4 inhibitors	ASP9831, Roflumilast	[106]
Peroxisome proliferator-activator receptor agonists	PPAR agonists	Elafibranor/GFT-505 pioglitazone, rosiglitazone, fenofibrate, saroglitazar/ZYH1, IVA337	[35,89,93,107]
Stearoyl CoA desaturase-1 inhibitors	SCD1 inhibitors	Aramchol	[108]
Sodium-glucose transporter-2 inhibitors	SGLT-2 inhibitors	Ipragliflozin, dapagliflozin, empagliflozin	[88,109]
Semicarbazide-sensitive amine oxidase/vascular adhesion protein-1 inhibitors	SSAO/VAP-1 inhibitors	PXS4728A	
Statins		Rosuvastatin, atorvastatin, pitavastatin	[110]
Thyroid β receptor agonists	TRb receptor agonists	VK2809, MGL-3196	
Toll-like receptor 4 antagonists	TLR4 antagonists	Nalmafene/JKB-121	
Tumor necrosis factor-alpha inhibitors	TNFα inhibitors	VLX103	

[a] Source: ClinicalTrials.gov; see Fig. 1 in the main text for graphical overview of drug targets.

TABLE 2

Obesogenic and nutrient-deficient dietary models of NASH in C57BL/6 mice[a]

Dietary model	Obesity	Dyslipidemia	Liver enzymes	Hepatomegaly	NASH	Fibrosis	HCC	Compounds tested in model	Induction period (weeks)	Comments on model	Refs
HF-HC	+	+	+	+	+	Very mild	–	Ezetimibe, sildenafil, leucine, metformin	6–42	Few mice show slight fibrosis	[40,41]
HF-FRUC	+	+	+	+	+	Mild–moderate	–	Liraglutide, BAR502	14-–18	Trans-fat in diet	[44,111]
HF-HC-FRUC	+	+	+	+	+	Mild	–	AC3174, elafibranor, obeticholic acid, liraglutide, YH25724, ipragliflozin, APD668	20–30	Biopsy-confirmed NASH and fibrosis; *trans*-fat in diet	[43,52,53, 66,112]
HF-SUCR	+	+	+	?	+	?	–	Fexaramine, G49, atglistatin	10	Combined with partial hepatectomy	[45]
HF-HC-SUCR	+	+	?	?	+	?		Obeticholic acid	24	Very mild NASH	[51]
MCD	–	–	+	+	+	Moderate	–	Wy-14,643, pentoxifylline, G49, YH25724, rosiglitazone, bezafibrate, GW501516, sitagliptin, MCC950, olaparib, WAY-362450	5–8	Weight loss, may be combined with partial hepatectomy	[45,52, 113,128]
CDAA	–	+	+	+	+	Mild–moderate	+	rFGF-1	3–6	Weight loss	[114]

[a] Abbreviations: FRUC, high fructose diet; HC, high-cholesterol diet; HCC, hepatocellular carcinoma; HF, high-fat diet; MCD, methionine- and choline-deficient diet; SUCR, high-sucrose diet; +, induction; −, no induction; ?, not determined/not reported.

TABLE 3

Chemotoxin-induced hepatic fibrosis models in mice[a]

Chemotoxin model	Obesity	Dyslipidemia	Liver enzymes	Hepatomegaly	NASH	Fibrosis	HCC	Compounds tested in model	Induction period (weeks)	Comments on model	Refs
CCl$_4$	–	+	+	+	+	Marked	+	Sorafenib, BAR502, cilostazol, brivanib, obeticholic acid	0.5–8	Dose-dependent fibrosis; weight loss; HCC after ≥12 weeks	[44,115,129]
TAA	–	?	+	+	+	Marked	+	Sorafenib, brivanib	4–8	Dose-dependent fibrosis; weight loss; HCC after ≥40 weeks	[115,116]
STZ + HF	–	+	+	+	+	Marked	+	Empagliflozin, linagliptin, telmisartan, cenicriviroc, ezetimibe, rosuvastatin, fenofibrate	2–16	Neonatal STZ model; early-onset diabetes; weight loss; HCC after ≥16 weeks on diet	[116–118]

[a] Abbreviations: CCl$_4$, carbon tetrachloride; HCC, hepatocellular carcinoma; HF, high-fat diet; STZ, streptozotocin; TAA, thioacetamide; +, induction; −, no induction;?, not determined/not reported.

TABLE 4

Monogenetic models of NASH[a]

Genetic model	Diet	Obesity	Dyslipidemia	Liver enzymes	Hepatomegaly	NASH	Fibrosis	HCC	Compounds tested in model	NASH induction period (weeks)	Comments on model	Refs
ob/ob	Chow	+	+	?	+	+	–	–	rFGF1	8–12	Moderate NASH	[114]
	HF-HC-FRUC	+	+	+	+	+	Moderate	–	AC3174, elafibranor, obeticholic acid, INT-767, liraglutide, SR9238	6–12	Biopsy-confirmed NASH and fibrosis; *trans*-fat in diet; fibrosis onset ≤12 weeks	[43,53, 65,67]
	MCD	–	+	+	+	+	Mild	–	LY2405319	10	Weight loss; fibrosis onset ≥8 weeks	[119]
db/db	MCD	–	+	+	+	+	Mild	–	Exendin-4, elafibranor (GFT505)	4–8	Fasting hyperglycemia; fibrosis onset 7–14 weeks	[69,70]
	Chow + DEN	+	+	?	?	+	Marked	+	Pitavastatin, metformin	14–36	Fasting hyperglycemia; fibrosis onset 16–20 weeks	[71]
foz/foz	HF-HC	+	+	+	+	+	Moderate	+	Obeticholic acid, Wy 14,643, ezetimibe, atorvastatin, SR141716A	16–28	Early-onset of fasting hyperglycemia; fibrosis onset ≥16 weeks	[51,74]
ApoE^{−/−}	HF-HC	+	+	+	+	+	Moderate	?	Simvastatin	7	Fibrosis onset ≥4–5 months of age	[77,120]
LDLr^{−/−}	HF-HC	+	+	+	+	+	Moderate	–	Rosiglitazone	12	Fibrosis onset ≥12 months of age	[78,121]
FLS	Chow	–	+	+	+	+	Mild	(–)	Fenofibrate, ezetimibe	13–20	Inbred strain; normoglycemia; fibrosis onset ≥24 weeks	[76,122, 123]
FLS-ob/ob	Chow	+	+	+	+	+	Moderate	+	Sitagliptin, aliskiren, ambrisentan, irbesartan	20–24	Inbred strain; spontaneous NASH; glucosuria; fibrosis onset ≥24 weeks	[76,1 24–127]

spontaneous reversal of steatohepatitis [68]. Therefore, db/db mice might have limited utility in NASH drug discovery.

foz/foz mice

foz/foz ('fat Aussie') mice carry an 11-base pair truncating mutation in the Alström gene product (Alms1), and were genetically engineered by researchers at the Australian National University Medical School, Canberra Hospital [72]. The exact function of the ALMS1 protein is unknown, but might include a role in the intracellular transport of lipid cargo. The rare human homolog causes the Alström syndrome, a childhood obesity syndrome complicated by T2D, premature cardiovascular disease, and cirrhosis. Similarly, foz/foz mice are hyperphagic and display essential characteristics of the metabolic syndrome, including obesity, fasting hyperglycemia, insulin resistance, dyslipidemia, and hypertension. The attractiveness of using foz/foz mice (on a high-fat atherogenic diet) in NASH research is the spontaneous development of significant fatty liver (extreme hepatic triglyceride accumulation), steatohepatitis (severe steatosis, moderate hepatocyte ballooning, and reproducible necroinflammation) with appreciable pericellular fibrosis after 24 weeks of dieting [73,74]. Thus, foz/foz and ob/ob mice have several NASH phenotypic commonalities, but foz/foz mice exhibit a different lipid deposition profile. Therefore, foz/foz mice are becoming increasingly relevant in experimental NASH pharmacology research (Table 4).

Fatty liver Shionogi mice

Polygenetic fatty liver Shionogi (FLS) lean mice were originally bred by Shionogi & Co. (Shiga, Japan), and develop spontaneous insulin resistance, hypertriglyceridemia, and steatohepatitis under normal environmental conditions [75]. Hepatic fibrosis is modest in FLS mice [38,76], and only lipid-lowering compounds have so far been tested in this model (Table 4). To provide a more robust fibrotic NASH model, a mixed genetic variant of the ob/ob mouse model was recently developed at Tottori University (Yonago, Japan) by backcross mating of ob/ob mice with FLS mice. The resulting phenotype of FLS-ob/ob mice combines the characteristics of both genetic models, and the mice therefore develop obesity, diabetes, severe hepatic steatosis, necroinflammation, age-dependent progression of pericellular fibrosis, and (to some degree) spontaneous tumorigenesis [76]. FLS-ob/ob mice have been increasingly used in the characterization of potential anti-NASH compounds (Table 4).

Genetic models of impaired lipoprotein function

Assembly, secretion, and transport of VLDL represents a major route for intrahepatic disposition of triglycerides. High serum levels of VLDL and LDL subclasses are linked to hepatic accumulation of cholesterol and lipids, which are considered contributing factors for hepatocellular injury in NASH. Several genetic mouse models of impaired lipoprotein function are applicable for NASH research, including Apolipoprotein E (ApoE$^{-/-}$) and LDL-receptor (LDL$^{-/-}$)-deficient mice. ApoE$^{-/-}$ mice fed a high-fat/cholesterol (1.25%) diet show slightly increased levels of fasting glucose, but the major phenotypic characteristic is marked dyslipidemia, including hypertriglyceridemia, increased serum VLDL levels, and hepatic cholesterol accumulation [77]. In contrast to chow-fed ApoE$^{-/-}$ mice, ApoE$^{-/-}$ mice maintained on the high-fat/cholesterol diet develop marked hepatic steatosis, inflammation, hepatocyte ballooning, HSC activation, and appreciable collagen deposition [77]. LDL$^{-/-}$ mice fed a high-fat/high-carbohydrate/

low cholesterol (0.2%) diet develop a NASH phenotypic profile similar to ApoE$^{-/-}$ mice, although at an older age [78]. The major advantage of using these models in NASH research is the more marked dyslipidemic profile, compared with DIO NASH models in wild-type mice. Modifications of these genetic models have been used to accelerate NASH and fibrosis progression, such as by introducing nutrient-deficient diets. On a related note, a transgenic mouse model of human-like lipoprotein metabolism (APOE*3-Leiden.CETP mice) has recently been applied in preclinical NASH research [79,80].

Surgery-based models: bile duct ligation

Bile acids are ligands for the FXR, Takeda G-protein-coupled receptor 5 (TGR5, also termed GPBAR1 and GPCR19), and pregnane X receptors (PXR), which are involved in diverse metabolic functions, including regulation of glucose and lipid homeostasis, and energy expenditure, as well as prevention of intestinal bacterial overgrowth [81]. However, accumulation of bile acids is detrimental to liver function. Hepatic accumulation of bile acids promotes acute oxidative stress, necroinflammation, and apoptosis, leading to fibrosis that eventually progresses to cirrhosis and end-stage liver failure [82]. It was recently reported that patients with NAFLD show alterations in bile acids homeostasis [83], and FXR/TGR5 receptor function has been subject to intense research in NASH pathology and represent an important antifibrotic drug target [82] (Fig. 1, Table 1). Surgical manipulation of bile acid circulation has been introduced as method for fast-onset and robust induction of experimental hepatic fibrosis. For example, common bile duct ligation (BDL) is a model of obstructive cholestasis (extrahepatic biliary obstruction) in which impaired bile flow leads to hepatic accumulation of bile acids and cholestatic liver injury. BDL mice are an emerging tool in preclinical NASH research [84,85]. In addition, a range of nonsurgical models of biliary fibrosis in mice is available, including diet-induced cholestatic liver injury, chemically induced cholangitis, as well as genetic models [86].

Concluding remarks

The ideal model of NASH should faithfully replicate the multifactorial disease mechanisms, while also being reproducible and efficient. Regardless of the approaches currently used to mimic NASH in mice, none of the present models fulfill all requirements for an ideal model. Therefore, selection of the relevant NASH model must be based on prior knowledge of the individual drug target, and it is recommended that at least two individual NASH models should be used for the preclinical characterization of anti-NASH drugs. Given the marked interest in the clinical development of drugs with antifibrotic efficacy, obese NASH mouse models with consistent histology-proven fibrosis have relatively good clinical translatability and, thus, are highly applicable for preclinical drug testing in NASH.

Acknowledgments

Reviews • POST SCREEN

References

1 Bellentani, S. (2017) The epidemiology of non-alcoholic fatty liver disease. *Liver Int.* 37, 81–84

2 Younossi, Z.M. *et al.* (2016) Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 64, 73–84

3 Tilg, H. *et al.* (2016) NAFLD and diabetes mellitus. *Nat. Rev. Gastroenterol. Hepatol.* 14, 32–42

4 Bedossa, P. (2017) Pathology of non-alcoholic fatty liver disease. *Liver Int.* 37, 85–89

5 White, D.L. *et al.* (2012) Association between nonalcoholic fatty liver disease and risk for hepatocellular cancer, based on systematic review. *Clin. Gastroenterol. Hepatol.* 10, 1342–1359

6 Singh, S. *et al.* (2015) Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin. Gastroenterol. Hepatol.* 13, 643–654

7 Agopian, V.G. *et al.* (2012) Liver transplantation for nonalcoholic steatohepatitis. *Ann. Surg.* 256, 624–633

8 Marchesini, G. *et al.* (2016) Diet, weight loss, and liver health in nonalcoholic fatty liver disease: pathophysiology, evidence, and practice. *Hepatology* 63, 2032–2043

9 Day, C.P. and James, O.F. (1998) Steatohepatitis: a tale of two 'hits'? *Gastroenterology* 114, 842–845

10 Rosso, N. *et al.* (2014) Translational approaches: from fatty liver to non-alcoholic steatohepatitis. *World J. Gastroenterol.* 20, 9038–9049

11 Berlanga, A. *et al.* (2014) Molecular pathways in non-alcoholic fatty liver disease. *Clin. Exp. Gastroenterol.* 7, 221–239

12 Fagone, P. *et al.* (2015) Identification of novel targets for the diagnosis and treatment of liver fibrosis. *Int. J. Mol. Med.* 36, 747–752

13 Teufel, A. *et al.* (2016) Comparison of gene expression patterns between mouse models of nonalcoholic fatty liver disease and liver tissues from patients. *Gastroenterology* 151, 513–525

14 Boursier, J. *et al.* (2016) The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 63, 764–775

15 Tokushige, K. *et al.* (2013) Serum metabolomic profile and potential biomarkers for severity of fibrosis in nonalcoholic fatty liver disease. *J. Gastroenterol.* 48, 1392–1400

16 Ganz, M. and Szabo, G. (2013) Immune and inflammatory pathways in NASH. *Hepatol. Int.* 7 (Suppl. 2), 771–781

17 Peverill, W. *et al.* (2014) Evolving concepts in the pathogenesis of NASH: beyond steatosis and inflammation. *Int. J. Mol. Sci.* 15, 8591–8638

18 Rau, M. *et al.* (2016) Progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis is marked by a higher frequency of Th17 cells in the liver and an increased Th17/resting regulatory T cell ratio in peripheral blood and in the liver. *J. Immunol.* 196, 97–105

19 Giles, D.A. *et al.* (2015) IL-17 axis driven inflammation in non-alcoholic fatty liver disease progression. *Curr. Drug Targets* 16, 1315–1523

20 Sharifnia, T. *et al.* (2015) Hepatic TLR4 signaling in obese NAFLD. *Am. J. Physiol. Gastrointest. Liver Physiol.* 309, G270–G278

21 Csak, T. *et al.* (2011) Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. *Hepatology* 54, 133–144

22 Szabo, G. and Petrasek, J. (2015) Inflammasome activation and function in liver disease. *Nat. Rev. Gastroenterol. Hepatol.* 12, 387–400

23 Fagone, P. *et al.* (2016) Emerging therapeutic targets for the treatment of hepatic fibrosis. *Drug Discov. Today* 21, 369–375

24 Leung, C. *et al.* (2016) The role of the gut microbiota in NAFLD. *Nat. Rev. Gastroenterol. Hepatol.* 13, 412–425

25 Musso, G. *et al.* (2016) Non-alcoholic steatohepatitis: emerging molecular targets and therapeutic strategies. *Nat. Rev. Drug Discov.* 15, 249–274

26 Patel, N.S. *et al.* (2015) Effect of weight loss on magnetic resonance imaging estimation of liver fat and volume in patients with nonalcoholic steatohepatitis. *Clin. Gastroenterol. Hepatol.* 13, 561–568

27 Kleiner, D. *et al.* (2005) Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 41, 1313–1321

28 Bedossa, P. *et al.* (2012) Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology* 56, 1751–1759

29 Bedossa, P. and Patel, K. (2016) Biopsy and noninvasive methods to assess progression of nonalcoholic fatty liver disease. *Gastroenterology* 150, 1811–1822

30 Angulo, P. *et al.* (2015) Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 149, 389–397

31 Tacke, F. (2017) Targeting hepatic macrophages to treat liver diseases. *J. Hepatol.* 66, 1300–1312

32 Sanyal, A.J. *et al.* (2015) Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop. *Hepatology* 61, 1392–1405

33 Ratziu, V. *et al.* (2010) A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J. Hepatol.* 53, 372–384

34 Neuschwander-Tetri, B.A. *et al.* (2015) Farnesoid X. nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 385, 956–965

35 Ratziu, V. *et al.* (2016) Elafibranor, an agonist of the peroxisome proliferator-activated receptor-α and -δ, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. *Gastroenterology* 150, 1147–1159

36 Armstrong, M.J. *et al.* (2016) Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 387, 679–690

37 Liang, W. *et al.* (2014) Establishment of a general NAFLD scoring system for rodent models and comparison to human liver pathology. *PLoS One* 9, e115922

38 Farrell, G.C. *et al.* (2014) Strain dependence of diet-induced NASH and liver fibrosis in obese mice is linked to diabetes and inflammatory phenotype. *Liver Int.* 34, 1084–1093

39 Yamazaki, Y. *et al.* (2008) Interstrain differences in susceptibility to non-alcoholic steatohepatitis. *J. Gastroenterol. Hepatol.* 23, 276–282

40 Zheng, S. *et al.* (2008) Ezetimibe improves high fat and cholesterol diet-induced non-alcoholic fatty liver disease in mice. *Eur. J. Pharmacol.* 584, 118–124

41 Bruckbauer, A. *et al.* (2016) A combination of leucine, metformin, and sildenafil treats nonalcoholic fatty liver disease and steatohepatitis in mice. *Int. J. Hepatol.* 2016, 1–16

42 Sanches, S.C.L. *et al.* (2015) Nonalcoholic steatohepatitis: a search for factual animal models. *BioMed Res. Int.* 2015, 1–13

43 Trevaskis, J.L. *et al.* (2012) Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. *AJP Gastrointest. Liver Physiol.* 302, G762–G772

44 Carino, A. *et al.* (2017) BAR502, a dual FXR and GPBAR1 agonist, promotes browning of white adipose tissue and reverses liver steatosis and fibrosis. *Sci. Rep.* 7, 42801

45 Valdecantos, M.P. *et al.* (2017) A novel glucagon-like peptide 1/glucagon receptor dual agonist improves steatohepatitis and liver regeneration in mice. *Hepatology* 65, 950–968

46 Tetri, L.H. *et al.* (2008) Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. *AJP Gastrointest. Liver Physiol.* 295, G987–G995

47 Clapper, J.R. *et al.* (2013) Diet-induced mouse model of fatty liver disease and nonalcoholic steatohepatitis reflecting clinical disease progression and methods of assessment. *AJP Gastrointest. Liver Physiol.* 305, G483–G495

48 Kristiansen, M.N.B. *et al.* (2016) Obese diet-induced mouse models of nonalcoholic steatohepatitis-tracking disease by liver biopsy. *World J. Hepatol.* 8, 673–684

49 Mells, J.E. *et al.* (2015) Saturated fat and cholesterol are critical to inducing murine metabolic syndrome with robust nonalcoholic steatohepatitis. *J. Nutr. Biochem.* 26, 285–292

50 Asgharpour, A. *et al.* (2016) A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. *J. Hepatol.* 65, 579–588

51 Haczeyni, F. *et al.* (2017) Obeticholic acid improves adipose morphometry and inflammation and reduces steatosis in dietary but not metabolic obesity in mice. *Obesity* 25, 155–165

52 Kim, J.H. *et al.* (2017) YH25724, a novel long-acting GLP-1/FGF21 dual agonist improves hepatic steatosis, inflammation and fibrosis in nonalcoholic steatohepatitis (NASH) animal models. *J. Hepatol.* 66, S16–S17

53 Feigh, M. *et al.* (2017) Comparative metabolic and hepatic effects of liraglutide, elafibranor and obeticholic acid in diet-induced and genetically obese mouse models of biopsy-confirmed NASH. *The 2nd NASH Summit*, Boston

54 Caballero, F. *et al.* (2010) Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial s-adenosyl-l-methionine and GSH. *J. Biol. Chem.* 285, 18528–18536

55 Honda, T. *et al.* (2017) Branched-chain amino acids alleviate hepatic steatosis and liver injury in choline-deficient high-fat diet induced NASH mice. *Metabolism* 69, 177–187

56 Matsumoto, M. *et al.* (2013) An improved mouse model that rapidly develops fibrosis in non-alcoholic steatohepatitis. *Int. J. Exp. Pathol.* 94, 93–103

57 Koppe, S.W.P. *et al.* (2004) Pentoxifylline attenuates steatohepatitis induced by the methionine choline deficient diet. *J. Hepatol.* 41, 592–598

58 Chiba, T. et al. (2016) Evaluation of methionine content in a high-fat and choline-deficient diet on body weight gain and the development of non-alcoholic steatohepatitis in mice. PLoS One 11, e0164191

59 Kim, Y.O. et al. (2017) Optimized mouse models for liver fibrosis. Methods Mol. Biol. 1559, 279–296

60 Bolzán, A.D. and Bianchi, M.S. (2002) Genotoxicity of streptozotocin. Mutat. Res. 512, 121–134

61 Fujii, M. et al. (2013) A murine model for non-alcoholic steatohepatitis showing evidence of association between diabetes and hepatocellular carcinoma. Med. Mol. Morphol. 46, 141–152

62 Saito, K. et al. (2015) Characterization of hepatic lipid profiles in a mouse model with nonalcoholic steatohepatitis and subsequent fibrosis. Sci. Rep. 5, 12466

63 Mann, J.P. et al. (2016) How useful are monogenic rodent models for the study of human non-alcoholic fatty liver disease? Front Endocrinol. 7, 145

64 Leclercq, I.A. et al. (2002) Leptin is essential for the hepatic fibrogenic response to chronic liver injury. J. Hepatol. 37, 206–213

65 Griffett, K. et al. (2015) The LXR inverse agonist SR. 9238 suppresses fibrosis in a model of non-alcoholic steatohepatitis. Mol. Metab. 4, 353–357

66 Honda, Y. et al. (2016) The selective SGLT2 inhibitor ipragliflozin has a therapeutic effect on nonalcoholic steatohepatitis in mice. PLoS One 11, e0146337

67 Roth, J. et al. (2016) The FXR/TGR5 dual agonist INT-767 reduces NAFLD activity score and fibrosis stage and improves plasma and hepatic lipid profiles in the GUBRA-AMLN mouse model of diet-induced and biopsy-confirmed nonalcoholic steatohepatitis. p. 144401, AASLD LiverLearning®

68 Trak-Smayra, V. et al. (2011) Pathology of the liver in obese and diabetic ob/ob and db/db mice fed a standard or high-calorie diet. Int. J. Exp. Pathol. 92, 413–421

69 Staels, B. et al. (2013) Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Hepatology 58, 1941–1952

70 Yamamoto, T. et al. (2016) Glucagon-like peptide-1 analogue prevents nonalcoholic steatohepatitis in non-obese mice. World J. Gastroenterol. 22, 2512–2523

71 Ohno, T. et al. (2015) Metformin suppresses diethylnitrosamine-induced liver tumorigenesis in obese and diabetic C57BL/KsJ- + Leprdb/ + Leprdb mice. PLoS One 10, e0124081

72 Arsov, T. et al. (2006) Fat Aussie—a new Alström Syndrome mouse showing a critical role for ALMS1 in obesity, diabetes, and spermatogenesis. Mol. Endocrinol. 20, 1610–1622

73 Van Rooyen, D.M. et al. (2013) Pharmacological cholesterol lowering reverses fibrotic NASH in obese, diabetic mice with metabolic syndrome. J. Hepatol. 59, 144–152

74 Bell-Anderson, K.S. et al. (2011) Coordinated improvement in glucose tolerance, liver steatosis and obesity-associated inflammation by cannabinoid 1 receptor antagonism in fat Aussie mice. Int. J. Obes. 35, 1539–1548

75 Soga, M. et al. (1999) The FLS mouse: a new inbred strain with spontaneous fatty liver. Lab. Anim. Sci. 49, 269–275

76 Sugihara, T. et al. (2013) Fatty liver Shionogi- ob/ob mouse: a new candidate for a non-alcoholic steatohepatitis model. Hepatol. Res. 43, 547–556

77 Schierwagen, R. et al. (2015) Seven weeks of Western diet in apolipoprotein-E-deficient mice induce metabolic syndrome and non-alcoholic steatohepatitis with liver fibrosis. Sci. Rep. 5, 12931

78 Bieghs, V. et al. (2012) LDL receptor knock-out mice are a physiological model particularly vulnerable to study the onset of inflammation in non-alcoholic fatty liver disease. PLoS One 7, e30668

79 Liang, W. et al. (2015) Salsalate attenuates diet induced non-alcoholic steatohepatitis in mice by decreasing lipogenic and inflammatory processes. Br. J. Pharmacol. 172, 5293–5305

80 Wang, Y. et al. (2014) Exendin-4 decreases liver inflammation and atherosclerosis development simultaneously by reducing macrophage infiltration. Br. J. Pharmacol. 171, 723–734

81 Vítek, L. and Haluzíkm, M. (2016) The role of bile acids in metabolic regulation. J. Endocrinol. 228, R85–R96

82 Yuan, L. and Bambha, K. (2015) Bile acid receptors and nonalcoholic fatty liver disease. World J. Hepatol. 7, 2811–2818

83 Ferslew, B.C. et al. (2015) Altered bile acid metabolome in patients with nonalcoholic steatohepatitis. Dig. Dis. Sci. 60, 3318–3328

84 Kluwe, J. et al. (2010) Modulation of hepatic fibrosis by c-Jun-N-terminal kinase inhibition. Gastroenterology 138, 347–359

85 Wang, X. et al. (2017) A20 attenuates liver fibrosis in NAFLD and inhibits inflammation responses. Inflammation 40, 840–848

86 Delire, B. et al. (2015) Animal models for fibrotic liver diseases: what we have, what we need, and what is under development. J. Clin. Transl. Hepatol. 3, 53–66

87 Stiede, K. et al. (2017) Acetyl-CoA carboxylase inhibition reduces de novo lipogenesis in overweight male subjects: A randomized, double-blind, crossover study. Hepatology http://dx.doi.org/10.1002/hep.29246 Published online May 3, 2017

88 Tang, W. et al. (2016) Comparative efficacy of anti-diabetic agents on nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a systematic review and meta-analysis of randomized and non-randomized studies. Diabetes Metab. Res. Rev. 32, 200–216

89 Sanyal, A.J. et al. (2010) Pioglitazone, Vitamin E, or placebo for nonalcoholic steatohepatitis. N. Engl. J. Med. 362, 1675–1685

90 Schwimmer, J.B. et al. (2016) In children with nonalcoholic fatty liver disease, cysteamine bitartrate delayed release improves liver enzymes but does not reduce disease activity scores. Gastroenterology 151, 1141–1154

91 Loomba, R. et al. (2016) GS-4997, an inhibitor of apoptosis signal-regulating kinase (ASK1), alone or in combination with simtuzumab for the treatment of nonalcoholic steatohepatitis (NASH): A randomized, Phase 2 trial. Hepatology 64, 1119A

92 McPherson, S. et al. (2017) A randomised controlled trial of losartan as an anti-fibrotic agent in non-alcoholic steatohepatitis. PLoS One 12, e0175717

93 Torres, D.M. et al. (2011) Rosiglitazone versus rosiglitazone and metformin versus rosiglitazone and losartan in the treatment of nonalcoholic steatohepatitis in humans: a 12-month randomized, prospective, open-label trial. Hepatology 54, 1631–1639

94 Stefan, N. et al. (2014) Inhibition of 11β-HSD1 with RO5093151 for non-alcoholic fatty liver disease: a multicentre, randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2, 406–416

95 Zeng, T. et al. (2014) Pentoxifylline for the treatment of nonalcoholic fatty liver disease. Eur. J. Gastroenterol. Hepatol. 26, 646–653

96 Shiffman, M. et al. (2015) A placebo-controlled, multicenter, double-blind, randomised trial of emricasan in subjects with non-alcoholic fatty liver disease (NAFLD) and raised transaminases. J. Hepatol. 62, S282

97 Ratziu, V. et al. (2012) A phase 2, randomized, double-blind, placebo-controlled study of GS-9450 in subjects with nonalcoholic steatohepatitis. Hepatology 55, 419–428

98 Nakade, Y. et al. (2017) Ezetimibe for the treatment of non-alcoholic fatty liver disease: A meta-analysis. Hepatol. Res. http://dx.doi.org/10.1111/hepr.12887 Published online March 3, 2017

99 Friedman, S. et al. (2016) Efficacy and safety study of cenicriviroc for the treatment of non-alcoholic steatohepatitis in adult subjects with liver fibrosis: CENTAUR Phase 2b study design. Contemp. Clin. Trials 47, 356–365

100 Sanyal, A. et al. (2016) Cenicriviroc versus placebo for the treatment of nonalcoholic steatohepatitis with liver fibrosis: results from the Year 1 primary analysis of the Phase 2b CENTAUR study. Hepatology 64, 1118

101 Cui, J. et al. (2016) Sitagliptin vs: placebo for non-alcoholic fatty liver disease: A randomized controlled trial. J. Hepatol. 65, 369–376

102 Harrison, S.A. et al. (2016) Randomised clinical study: GR-MD-02, a galectin-3 inhibitor, vs. placebo in patients having non-alcoholic steatohepatitis with advanced fibrosis. Aliment Pharmacol. Ther. 44, 1183–1198

103 Armstrong, M.J. et al. (2016) Glucagon-like peptide 1 decreases lipotoxicity in non-alcoholic steatohepatitis. J. Hepatol. 64, 399–408

104 Safar Zadeh, E. et al. (2013) The liver diseases of lipodystrophy: the long-term effect of leptin treatment. J. Hepatol. 59, 131–137

105 Kim, W. et al. (2017) Randomised clinical trial: the efficacy and safety of oltipraz, a liver X receptor alpha-inhibitory dithiolethione in patients with non-alcoholic fatty liver disease. Aliment Pharmacol. Ther. 45, 1073–1083

106 Ratziu, V. et al. (2014) Lack of efficacy of an inhibitor of PDE4 in Phase 1 and 2 trials of patients with nonalcoholic steatohepatitis. Clin. Gastroenterol. Hepatol. 12, 1724–1730

107 Kostapanos, M.S. (2013) Current role of fenofibrate in the prevention and management of non-alcoholic fatty liver disease. World J. Hepatol. 5, 470–478

108 Safadi, R. et al. (2014) The fatty acid-bile acid conjugate aramchol reduces liver fat content in patients with nonalcoholic fatty liver disease. Clin. Gastroenterol. Hepatol. 12, 2085–2091

109 Ohki, T. et al. (2016) Effectiveness of ipragliflozin, a sodium-glucose co-transporter 2 inhibitor, as a second-line treatment for non-alcoholic fatty liver disease patients with Type 2 diabetes mellitus who do not respond to incretin-based therapies including glucagon-like peptide-1 analogs and dipeptidyl peptidase-4 inhibitors. Clin. Drug Investig. 36, 313–319

110 Kargiotis, K. et al. (2015) Resolution of non-alcoholic steatohepatitis by rosuvastatin monotherapy in patients with metabolic syndrome. World J. Gastroenterol. 21, 7860–7868

111 Rahman, K. et al. (2016) C/EBP homologous protein modulates liraglutide-mediated attenuation of non-alcoholic steatohepatitis. Lab. Invest. 96, 895–908

112 Bahirat, U.A. et al. (2017) APD668, a G protein-coupled receptor 119 agonist improves fat tolerance and attenuates fatty liver in high-trans fat diet induced steatohepatitis model in C57BL/6 mice. Eur. J. Pharmacol. 801, 35–45

Reviews • POST SCREEN

113 Ip, E. *et al*. (2004) Administration of the potent PPARa agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. *Hepatology* 39, 1286–1296

114 Liu, W. *et al*. (2016) Effective treatment of steatosis and steatohepatitis by fibroblast growth factor 1 in mouse models of nonalcoholic fatty liver disease. *Proc. Natl. Acad. Sci.* 113, 2288–2293

115 Nakamura, I. *et al*. (2014) Brivanib attenuates hepatic fibrosis *in vivo* and stellate cell activation *In vitro* by inhibition of FGF, VEGF and PDGF signaling. *PLoS One* 9, e92273

116 Lefebvre, E. *et al*. (2016) Antifibrotic effects of the Dual CCR2/CCR5 antagonist cenicriviroc in animal models of liver and kidney fibrosis. *PLoS One* 11, e0158156

117 Klein, T. *et al*. (2014) Linagliptin alleviates hepatic steatosis and inflammation in a mouse model of non-alcoholic steatohepatitis. *Med. Mol. Morphol.* 47, 137–149

118 Orime, K. *et al*. (2016) Lipid-lowering agents inhibit hepatic steatosis in a non-alcoholic steatohepatitis-derived hepatocellular carcinoma mouse model. *Eur. J. Pharmacol.* 772, 22–32

119 Lee, J.H. *et al*. (2016) An engineered FGF21 variant, LY2405319, can prevent non-alcoholic steatohepatitis by enhancing hepatic mitochondrial function. *Am. J. Transl. Res.* 8, 4750–4763

120 Schierwagen, R. *et al*. (2016) Statins improve NASH via inhibition of RhoA and Ras. *Am. J. Physiol. − Gastrointest. Liver Physiol.* 311, G724–G733

121 Gupte, A.A. *et al*. (2010) Rosiglitazone attenuates age- and diet-associated nonalcoholic steatohepatitis in male low-density lipoprotein receptor knockout mice. *Hepatology* 52, 2001–2011

122 Wang, X. *et al*. (2014) Novel effect of ezetimibe to inhibit the development of non-alcoholic fatty liver disease in Fatty Liver Shionogi mouse. *Hepatol. Res.* 44, 102–113

123 Harano, Y. *et al*. (2006) Fenofibrate, a peroxisome proliferator-activated receptor alpha agonist, reduces hepatic steatosis and lipid peroxidation in fatty liver Shionogi mice with hereditary fatty liver. *Liver Int.* 26, 613–620

124 Onoyama, T. *et al*. (2015) Therapeutic effects of the dipeptidyl peptidase-IV inhibitor, sitagliptin, on non-alcoholic steatohepatitis in FLS-ob/ob male mice. *Mol. Med. Rep.* 12, 6895–6902

125 Kishina, M. *et al*. (2014) Therapeutic effects of the direct renin inhibitor, aliskiren, on non-alcoholic steatohepatitis in fatty liver Shionogi *ob/ob* male mice. *Hepatol. Res.* 44, 888–896

126 Okamoto, T. *et al*. (2016) Antifibrotic effects of ambrisentan, an endothelin-A receptor antagonist, in a non-alcoholic steatohepatitis mouse model. *World J. Hepatol.* 8, 933–941

127 Koda, M. *et al*. (2012) Therapeutic effects of angiotensin II type 1 receptor blocker, irbesartan, on non-alcoholic steatohepatitis using FLS-ob/ob male mice. *Int. J. Mol. Med.* 30, 107–113

128 Zhang, S. *et al*. (2009) Farnesoid X receptor agonist WAY-362450 attenuates liver inflammation and fibrosis in murine model of non-alcoholic steatohepatitis. *J. Hepatol.* 51, 380–388

129 Zhang, D.G. *et al*. (2017) Obeticholic acid protects against carbon tetrachloride-induced acute liver injury and inflammation. *Toxicol. Appl. Pharmacol.* 314, 39–47