Non-alcoholic fatty liver disease - opportunities for personalized treatment and drug development

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ABSTRACT

Introduction: Non-alcoholic fatty liver disease (NAFLD) constitutes a highly prevalent liver disorder whose rise in prevalence is closely connected to the growing rates of obesity, dyslipidemia, and type 2 diabetes. Importantly, kinetics and likelihood of NAFLD onset and its progression to non-alcoholic steatohepatitis (NASH) and fibrosis differs considerably between individuals. In recent years, the understanding of NAFLD pathogenesis has increased substantially and a multitude of factors, genetic predispositions, molecular signatures or NAFLD-related liver injury and comorbidities have been identified.

Areas Covered: This article summarizes inter-individual differences in NAFLD, including genetic variations, epigenetic and metabolic alterations and differences in the microbiome. We also discuss how these features might be leveraged for treatment personalization.

Expert opinion: The complexity and heterogeneity of NAFLD provides considerable challenges for drug developers and has resulted in numerous costly project failures. We expect that increased knowledge and appreciation of patient-specific factors will facilitate better patient stratification and identification of those individuals that benefit most from a given therapeutic strategy. Furthermore, we anticipate that pathophysiologically relevant in vivo and ex vivo disease models as well as large-scale chemogenomic projects hold promise to drastically improve NAFLD drug development to complement lifestyle and surgical interventions with pharmacological approaches.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined by the accumulation of triglycerides within liver cells that is not due to excessive alcohol intake (>30 g/d for men and >20 g/d for women), the use of medications known to induce steatosis, and other concurrent chronic liver diseases [1]. In this regard, NAFLD can be regarded as a disease of fat utilization, either because of excessive fat influx to the liver or due to decreased hepatic beta-oxidation. NAFLD describes a spectrum of liver disorders from benign steatosis to inflammatory non-alcoholic steatohepatitis (NASH) and hepatic fibrosis. Particularly the latter constitutes the strongest predictor of both general and disease-specific mortality in NAFLD patients [2] and NAFLD has surpassed hepatitis C as etiology of end-stage liver disease that requires orthologous transplantation as of 2019 (Figure 1A).

Most NAFLD patients also feature other metabolic conditions, including insulin resistance, type 2 diabetes (T2D), hypertension, abdominal obesity and dyslipidemia, and, as such, the most common cause of deaths in NAFLD patients, independent of other comorbidities is cardiovascular diseases [5,6]. However, NAFLD can also manifest in patients without metabolic syndrome. As evident from this short synopsis, NAFLD is a complex and nuanced disease, characterized by an interplay of many pathways and patient-specific risk factors.

1.1. Epidemiology of NAFLD

In the general adult population, the reported prevalence of NAFLD varies, depending on the demographic of the study population and the choice of diagnostic tools. Using imaging modalities, such as computerized tomography, ultrasonography, or magnetic resonance imaging, the global prevalence of NAFLD in adults was estimated to be around 25%, with the highest regional prevalence in South America (31%) and the Middle East (32%) (Figure 1B). In the US, France, Germany, Italy and the UK combined, this accounts for about 116 million people with NAFLD [7]. While the prevalence of NASH in the general population has not been determined directly, among individuals with NAFLD the prevalence of NASH is approximately 21% [3]. Combined, these results suggest that the prevalence of NASH in the general population can be estimated to be around 5%.

In addition to geographic factors, NAFLD prevalence has been shown to also vary by ethnicity as reported by various studies from the US showing that frequencies were highest among Hispanic individuals (45%), whereas prevalence in African-Americans was considerably lower (24%; Figure 1C). Besides ethnogeographic differences, there are also substantial differences between cities and rural areas as evidenced by a Chinese study showing that NAFLD was overall 30-times more frequent in urban regions than in the countryside [8].
Combined, these epidemiological data suggest that NAFLD risk is likely impacted by a multitude of both genetic and environmental factors.

1.2. Clinical and economic burden of NAFLD and NASH

As a major cause of chronic liver disease, NAFLD is a major health concern for decision makers and stakeholders worldwide that is predicted to further increase in prevalence. Specifically, using real-world surveillance data it was estimated that the prevalence of NAFLD will rise from 25% in 2015 to 34% by 2030, which is paralleled by an increase in NASH cases by 63%, from 17 to 25 million, as well as a rise in liver-related mortality by 180% [4]. Average total annual costs per NASH patient in Europe and the US was estimated at €2,763 (USD 3,160), €4,917 (USD 5,620), and €5,509 (USD 6,302) for the direct medical, direct non-medical, and indirect cost categories, respectively, resulting in an estimated economic burden of NASH of €30 billion (assuming NASH prevalence in the general population of 1.5% to 3.5%) to €98 billion (NASH prevalence of 5% to 9%) [9].

2. The molecular pathogenesis of NAFLD

2.1. Steatosis

Accumulation of triglycerides in hepatocytes constitutes the first step in the pathogenesis of NAFLD. Steatosis results from an imbalance between fatty acid uptake, de novo lipogenesis and triglyceride utilization, which is commonly linked to insulin resistance in liver and adipose tissue [10]. Mechanistically, dysfunctional insulin signaling favors the excessive degradation of triglycerides stored in the adipose tissue into free fatty acids, which in turn are actively imported into hepatocytes by CD36, FATP2, and FATP5 [11]. Particularly, overexpression of CD36 in the liver of mice increased hepatic steatosis and was sufficient to recapitulate the hepatic effects of diet-induced obesity [12]. In patients, CD36 expression is correlated with extent of steatosis [13], jointly suggesting that CD36 constitutes an important factor in the molecular pathogenesis of hepatic steatosis. In addition to fatty acid uptake, hepatic steatosis is associated with an increase of de novo lipogenesis. At the molecular level, activation of the lipogenic master regulator SREBP1c and its target enzymes, such as acetyl-CoA carboxylase (ACC) and stearyl-CoA desaturase (SCD1) contribute to the development of steatosis [14]. Furthermore, hepatic triglyceride accumulation has been attributed to increased signaling via the thyroid stimulating hormone (TSH) – thyroid hormone receptor β (THRB) axis, which is believed to increase lipogenesis via activation of SREBP1c [15].

However, while lipogenesis is increased around threefold in NAFLD patients [16], uptake of non-esterified fatty acids from adipose tissue lipolysis nevertheless accounts for the majority (59%±9.9%) of hepatic lipids in obese NAFLD patients with fasting hypertriglyceridemia and hyperinsulinemia, whereas de novo lipogenesis and diet only contribute 26% and 15%, respectively [17]. Among the nutritional factors, particularly fructose precipitates hepatic steatosis through induction of hepatic lipogenesis, as well as reduction of beta-oxidation [18]. Notably, genetic ablation of both fructokinase A and fructokinase C in mice protects from fructose-induced fatty liver, suggesting a direct link between fructose activation and triglyceride accumulation [19].

Hepatic steatosis also disrupts hepatic calcium handling [20]. Specifically, insulin resistance results in reduced activation of insulin-sensitive InsP3R channels [21,22]. Furthermore, steatosis impacts the stoichiometry between phosphatidylcholine and phosphatidylethanolamine, which alters endoplasmic reticulum (ER) membrane fluidity and impairs the functionality of the ER calcium pump SERCA [23]. Combined, these molecular events result in reduced calcium levels in the ER of hepatocytes, resulting in lower activity of calcium-dependent chaperones and the activation of a cascade of ER overload with misfolded proteins, unfolded protein response (UPR) and ER stress. Reciprocally, UPR has been suggested to reduce insulin sensitivity through different axes, first by activation of IRE1, resulting in blunted insulin signaling due to increased JNK activity [24] and second by induction of JNK3, which directly inhibits the insulin receptor [25,26].

2.2. Lipotoxicity

Importantly, excessive uptake of saturated free fatty acids can result in hepatocellular stress and lipotoxicity. This injury is at least in part caused by induction of mitochondrial beta-oxidation of fatty acids, which results in the increased formation of reactive oxygen species (ROS) whose levels exceed the intracellular buffering mechanisms. Elevated ROS entails the activation of multiple stress signaling axes, such as TRAIL, JNK, and MLK3, DNA damage, lipid peroxidation, and the increased secretion of proinflammatory and profibrotic factors [27]. Furthermore, inflammation is associated with upregulation of CCR2/5 in the liver, promoting further infiltration of immune cells and exacerbating inflammation [28,29].

Hepatocellular stress in NASH has recently also been linked to an increase in the secretion of extracellular vesicles; while their detailed functions remain to be determined, they have been
suggested to participate in the activation of monocytes and hepatic stellate cells (HSCs) [30]. The importance of free fatty acids for the pathogenesis of NAFLD is emphasized by functional experiments in mice showing that knock-out of key lipogenic enzymes, such as Dgat2 or Scd, worsen hepatic inflammation, ROS-induced liver injury and fibrosis [31,32]. In addition to mitochondrial beta-oxidation of fatty acids, gamma-oxidation in the ER, mediated by CYP2E1 and CYP4A8, is also a major source of hepatocellular ROS in NAFLD [33]. In NASH patients, hepatic CYP2E1 activity is increased compared to individuals with benign steatosis and expression specifically localized to steatotic areas of the liver [34,35].

2.3. Hepatic fibrosis

The chronic injury and inflammatory milieu in NASH favor hepatic scarring, which manifests as increased deposition of extracellular matrix (ECM) due to ongoing wound-healing processes. Upon activation of HSCs via paracrine mechanisms, mostly pro-inflammatory cytokines and chemokines
secreted by liver macrophages, hepatocytes, liver sinusoidal endothelial cells and platelets, they assume a myofibroblastic phenotype and increase collagen deposition and ECM remodeling. TGFβ constitutes arguably the most important molecular mediator of HSC activation and has been suggested as a central factor linking hyperinsulinemia, hyperglycemia, and hepatic fibrogenesis [36–38]. Notably, recent single-cell sequencing analyses revealed the many subtypes of HSC, including the heterogeneity of activated myofibroblast along the hepatic zones [39]. As a detailed discussion of the underlying mechanisms is beyond the scope of this article, we refer the interested reader to recent excellent reviews on this topic [40,41].

3. Patient-specific factors underlying NAFLD risk

3.1. Metabolic conditions and demographic factors

NAFLD is strongly associated with a cluster of metabolic conditions including obesity, hypertension, hypertriglyceridemia, and hyperglycemia [42], which in turn are strongly linked to dietary, environmental and genetic factors. Furthermore, these factors favor the development of T2D and cardiovascular pathologies, which result in these diseases being considerably more prevalent in NAFLD patients compared to the general population. While NAFLD prevalence in lean individuals is around 4–10% [43,44], frequencies are around 50% in type 2 diabetics [45] and 60–95% in obese individuals [46]. Of note, the wide range of reported NAFLD prevalence in obesity is at least to a considerable extent due to a mixing of ‘overweight’ and ‘obesity’ terms and differences in BMI cutoffs that can range between 23 in Asia [47] and 30 in the US [48]. Furthermore, as lean NAFLD has been suggested as a separate clinical entity [49], whether these conditions are present or not in a given patient has to be considered in order to individualize treatment of NAFLD.

Age has been suggested as a further risk factor of NAFLD and NASH. While age is clearly correlated with NAFLD prevalence, it appears that effects are primarily due to increased frequencies of hypertension, T2D, and dyslipidemia, whereas slight inverse trends have been reported after correction for metabolic features [50]. In addition, there are considerable differences in NAFLD frequency and clinical outcomes between the sexes. Males are at higher risk of NAFLD than females at younger ages; however, risk increases in females after menopause ameliorating these disparities attributed to hormonal differences when corrected for lifestyle [51,52]. These differences have been attributed to sexual dimorphisms of liver metabolism, as well as hormonal disparities that are largely controlled by different patterns of pituitary growth hormone secretion [53–55]. Additionally, estrogen affects hepatic gene expression and has been shown to repress excessive hepatic lipid synthesis and steatosis through inhibition of genes involved in FA metabolism [56]. However, while clinical associations with sex are unambiguous, mechanistic studies are almost exclusively based on rodents and results should thus be interpreted carefully.

3.2. Genetic variability and epigenetic modifications

In addition to environmental factors, NAFLD is influenced by genetic predispositions, as elegantly shown by twin and familial aggregation studies that demonstrate that 22–50% of NAFLD risk is heritable [57]. Genetic variability has been found to account for 15–30% of liver steatosis risk factors [58] attributed to single nucleotide polymorphisms (SNPs) in or near genes involved in liver lipid droplet homeostasis and metabolism, VLDL secretion and carbohydrate metabolism (Table 1).

Among the genetic associations, the correlation with p. I148M (rs738409) in PNPLA3 was the first to be identified and shows the most robust association with both NAFLD onset, as well as its progression to NASH and fibrosis [59,60]. In contrast, the truncating reduced function variant rs72613567 in HSD17B13 is protective against hepatocellular injury, inflammation and fibrosis [61,62]. While the exact molecular function of TM6SF2 is not clear, its association with hepatic triglyceride content, NAFLD risk and liver injury has been confirmed in at least 11 genetic epidemiological studies [63]. More recently, MBOAT7 variations have been linked to a predisposition for NAFLD, NASH and fibrosis [64]. The MBOAT7 variant rs641738 was associated with fibrosis but not with histological inflammation, results which were confirmed by functional deletion studies in mice [65]. Jointly, these results suggest that reduced MBOAT7 function might cause fibrosis by an inflammation-independent pathway that alters hepatic lipid signaling, which might constitute an attractive target for pharmacological intervention. In addition to these associations, listed above, a multitude of additional associations of NAFLD risk or severity with variants in GCKR, NCAN, LYPLAY1, PPP1R3B, IL17RA, FABP1, TRIB1 and APOC3 have been identified, which however require further replication [58,66–68].

Besides genetic variants, there is increasing data on the epigenomic alterations from NAFLD-patient liver biopsies [69–73]. Jointly, these studies show that hypomethylated loci were enriched in tissue repair function, whereas folate and methionine metabolism genes were hypermethylated. Increased methylation was also observed in the promoter of PPARα in subjects with liver fibrosis. Furthermore, pathways

| Table 1. SNPs with the most robust association to NAFLD/NASH onset and progression. |
|-----------------|-----------------|-----------------|-----------------|
|Gene | Global MAF | Function | Phenotype | Population |
|rs738409 C > G | rs58542926 C > T | rs58542926 C > T |
|PNPLA3 | 0.37 (G) | Lipid remodeling | Increased risk for NAFLD, NASH, fibrosis |
| | TM6SF2 | VLDL secretion | Hepatic fibrosis progression, susceptibility to NAFLD |
| | MBOAT7 | Lipid remodeling | NASH progression, liver inflammation and fibrosis in chronic hepatitis C |
| | | | Caucasian |

Abbreviations: NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis (a progressive subtype of NAFLD); MAF: minor allele frequency; VLDL: very low density lipoprotein
related to nitric oxide and reactive oxygen species, LXR and FXR activation were differentially methylated. Notably, altered epigenetic profiles could be linked to in utero lipid and nutrient fluxes, which have been shown to have significant impacts on the predisposition to liver steatosis and NASH. Specifically, high birth weight has been associated with higher risk for steatosis and NASH in children whereas low birth weight was associated with elevated odds of liver fibrosis [74]. While these combined results suggest a role of DNA methylation in NAFLD, the data do not allow the drawing of conclusions regarding cause-consequence relationships [75] and, thus, suggestions of therapeutic targeting of epigenomic process seems in our opinion premature.

3.3. Contributions of the microbiome

Disturbances in the gut microbiome or dysbiosis has emerged as an important risk factor for various liver diseases including NAFLD [76,77]. Particularly, NAFLD patients have lower microbial diversity with an overrepresentation of *proteobacteria*, *fusobacteria* and *verucomicrobia* whereas *bacteriodetes* were decreased [78-80]. Importantly, recent studies in both mice and humans have suggested that these microbiomal alterations play a causal role in NAFLD etiology and progression. For instance, fecal microbiota transplants from steatotic or NASH patients increased hepatic steatosis and inflammation in mice [81,82]. Mechanically, these effects are thought to be mediated by microbial metabolites, including short-chain fatty acids (SCFAs), bile acids and ethanol, which can directly affect hepatic metabolism [83]. Furthermore, the gut microbiome impacts intestinal permeability and the presence of microbial products at the portal circulation resulting from disrupted tight junctions can trigger inflammatory responses and activation of Kupffer cells and HSCs [84].

Combined, the current data suggest that analysis of microbiome composition and complexity might provide powerful noninvasive biomarkers for NAFLD diagnosis, staging and prognosis. Furthermore, manipulation of the intestinal microbiome using probiotic products has yielded promising results as evident from a meta-analysis of 15 randomized, controlled trials involving 782 patients with NAFLD [85]. Thus, dietary supplementation with probiotics or synbiotics in NAFLD patients with dysbiosis might constitute an interesting avenue for personalized therapeutic interventions.

4. Treatments for NAFLD, NASH, and fibrosis

4.1. Currently available treatments and therapies

When successful, lifestyle interventions that include a Mediterranean diet and increased physical activity are highly effective in reducing body weight and ameliorating hepatic steatosis and inflammation [86]. For instance, histological analysis of liver biopsies from 293 patients with NASH showed that 138 (47%) had reductions in NAFLD activity score, 72 (25%) achieved resolution of NASH and 56 (19%) had regression of fibrosis after 52 weeks and weight loss was independently associated with improved NASH histology [87]. Subjects with carbohydrate-restricted diets (<20 g/d) showed significantly higher liver fat reduction than subjects on low-calorie diets (1,200–1,500 kcal/day) despite similar weight loss [88]. Furthermore, a recent 5-year longitudinal cohort study showed that glycemic control constituted an independent predictor for the progression of NASH-associated fibrosis [89]. Notably, carriers of the p.I148M (rs738409) risk variant in PNPLA3 experienced significantly higher loss in liver fat content in response to dietary interventions compared to non-carriers, suggesting that lifestyle modifications can counteract the effects of genetic predisposition [90,91].

Besides lifestyle interventions, bariatric surgery has been shown to be highly effective in reversing NASH and fibrosis in severely obese patients with difficulty to lose weight. Specifically, a meta-analysis including 2,374 patients showed an improvement of steatosis, NASH and fibrosis in 88%, 59% and 30% of patients, respectively [92].

Combined, these results indicate that weight loss due to lifestyle interventions or, if need be, surgery constitutes the most important factor for improving NAFLD and NASH. Furthermore, carbohydrate management and glycemic control are critical for the treatment of NAFLD and NASH.

4.2. Regulatory targets in NASH drug development

Regulatory agencies (FDA) have published guidance documents pertaining to NASH trial designs to show efficacy and safety of potential drugs for non-cirrhotic NASH with liver fibrosis [93]. Since it can take decades to assess NASH outcomes in the long-term, FDA has made recommendations on biopsy-based endpoints that could predict the clinical benefit of candidate drugs. Specifically, demonstration of reduced fibrosis at least by one stage with no worsening of the NAFLD activity score (NAS), overall resolution of steatohepatitis without worsening of fibrosis, or improvement in both NAS and fibrosis are required, with the latter being the primary endpoint to support an accelerated approval. The main challenges include the high sampling error related to biopsies as a primary endpoint and the presence of other comorbidities which may require the use of concomitant medications during the trials that may confound the clinical outcomes.

For the development of NASH drugs in the presence of compensated-cirrhosis, FDA has issued a separate guidance document [94]. The main challenge for this subtype of NASH is that there is insufficient evidence to suggest that histological improvement correlates with improvement of cirrhosis and reversal of cirrhosis may not be feasible. Though justified histological endpoints may still be proposed, composite endpoints in any of the following outcome events should be evaluated: complication of ascites, variceal hemorrhage, hepatic encephalopathy, worsening in the Model for End-Stage Liver Disease (MELD) score ≥15, liver transplantation, and all-cause mortality. Because of the stringency of criteria to prove efficacy and safety of new drugs for NASH in the presence of compensated cirrhosis, sponsors should expect FDA to consider their application through the traditional approval pathway.
4.3. Drugs and drug candidates in development

As detailed above, lifestyle interventions and surgery primarily focus on the various metabolic conditions associated with NAFLD, but not directly on NAFLD itself. Similarly, various pharmacological treatments have been used in NAFLD patients to treat insulin resistance, T2D and obesity. For instance, clinical trials with the antidiabetic GLP-1 analogues liraglutide and semaglutide showed improved resolution of NASH and reduced likelihood of fibrosis progression [95,96]. Similarly, significantly higher rate of improvement in NASH were detected with vitamin E [97]. However, for none of those therapies resulted in significant improvement of fibrosis. The antidiabetic thiazolidinedione pioglitazone achieved NASH resolution in 51% of 101 patients with prediabetes or T2D and biopsy-proven NASH in a randomized, double-blind, placebo-controlled trial [98,99]. In contrast to GLP-1 analogues and vitamin E, pioglitazone also showed slight but significant improvements of fibrosis.

A multitude of small molecules and biologics are currently being developed for NASH with the main goal of reducing inflammation and halting or reversing hepatic fibrosis (Figure 2; Tables 2 and 3). The FXR agonist obeticholic acid (OCA) [100] and the pan-PPAR agonist lanifibranor [101] have shown significant improvements in histological fibrosis compared to placebo in randomized controlled trials. However, for OCA, a significant increase in low-density lipoprotein cholesterol (LDL-C) was observed. On these grounds, FDA determined that the clinical benefit of OCA remained uncertain and did not outweigh the risks caused by elevated low-density lipoprotein cholesterol and total cholesterol. Thus, in response to its first application, the agency denied approval and suggested another submission with additional data to support OCA’s efficacy and safety [102]. Development of other non-steroidal FXR agonists for NASH is ongoing, including of GS-9674 (Gilead), AKN-083 (Allergan), and LBM763 (Novartis). Lanifibranor, on the other hand, has not been associated with severe adverse effects; however, larger phase 3 trials are currently pending.

As NAFLD and NASH constitute complex diseases, it is not surprising that the landscape of targets for drugs under development is highly diverse. Besides activation of FXR and PPAR mentioned above, inhibition of ACC (fisocostat) and CCR2/5 (cencriviroc) have shown significant improvements in steatosis, inflammation, and fibrosis [103,104]. Similarly, the FGFR9/21 mimetics, pegbelfermin and aldafermin, resulted in significantly reduced steatosis and a trend toward improvement of fibrosis and NASH [105–107]. Furthermore, activation of thyroid hormone signaling using the THRβ agonist resimetori showed significant improvement in hepatic steatosis [108]. While not nominally significant, trends toward a reduction in steatosis were also observed with the SCD1 inhibitor aramchol [109]. For an overview of the current drug development pipeline for NASH as well as recent project closures, we refer the interested reader to excellent recent reviews [110–113].

Table 2. Drugs in clinical trials showing efficacy in either NASH or fibrosis resolution with histological endpoints.

| Treatments | Mode of action | Length of study | Study outcomes | Indication for fibrosis improvement | Progress to the next phase? | Sponsors | Refs |
|------------|----------------|----------------|----------------|-----------------------------------|-----------------------------|----------|-----|
| Obeticholic acid (10 or 25 mg/d oral vs. placebo) FXR agonist | ≥18 months (interim) | Fibrosis resolution (≥1-stage reduction) without worsening of NASH | Yes for both doses | Yes | Rejected at registration | Intercept Pharmaceuticals | (82,84) |
| Lanifibranor/IVA337 (800 or 1200 mg/d oral vs. placebo) PPARα/γδ | 24 weeks | NASH resolution (≥2-point reduction in SAF-A scoring) | Yes for high dose | Yes | Yes, recruiting (NCT04849728) | Inventiva Pharma | (83) |
| Semaglutide (0.1/0.2/0.4 mg/d, s.c. injection) vs. placebo GLP1 receptor agonist | 72 weeks | NASH resolution (score 0–1 for inflammation and 0 for hepatic ballooning) without worsening of fibrosis | Yes for highest dose | No | Unknown, current phase still ongoing | Novo Nordisk | (80) |

Abbreviations: FXR: farnesoid X-activator receptor; PPAR: Peroxisome proliferation-activator receptor; GLP1: glucagon-like peptide 1
Table 3. Drugs in clinical trials showing efficacy in reducing hepatic steatosis with indication of fibrosis reduction by serum markers.

| Treatments                  | Mode of action | Length of study | Study outcomes | Indication for fibrosis improvement | Progress to the next phase? | Sponsors          | Refs |
|-----------------------------|----------------|-----------------|----------------|-------------------------------------|-----------------------------|-------------------|------|
| Aramchol (400 or 600 mg/d oral vs. placebo) | Partial SCD1 inhibitor | 52 weeks        | Steatosis reduction | MRS | Yes for high dose | Indirect, significant reduction in serum fibrosis markers, FIB4 and NFS. | Yes, recruiting (NCT0104321) | Galmed Pharmaceuticals ([88]) |
| Aldafermin (1 mg/d s.c. injection vs. placebo) | FGF19 analogue | 24 weeks        | Steatosis reduction | MRI-PDFF | Yes for high dose | Indirect, significant reduction in serum pro-collagen III level | Unknown, current phase still ongoing | NGM Biopharmaceuticals ([90]) |
| Pegbelfermin/ BMS-986036 (10 mg/d or 20 mg/wk s. c. injection vs. placebo) | PEGylated FGF21 analogue | 16 weeks        | Steatosis reduction | MRI-PDFF | Yes for high dose | Indirect, significant reduction in serum pro-collagen III level | Unknown, current phase still ongoing | Bristol-Myer Squibb ([91]) |
| Resmetirom/ MGL-3196 (80 mg/d oral vs. placebo) | THR agonist | 12 and 36 weeks | Steatosis reduction | MRI-PDFF | Yes for high dose | Indirect, reduction in pro-collagen III and advanced fibrosis scoring. | Yes, recruiting (NCT03900429, NCT04951219, NCT04197479) | Madrigal Pharmaceuticals ([86]) |

Abbreviations: SCD1: stearoyl-coA desaturase1; FGF21: fibroblast growth factor 21; THR: thyroid hormone receptor; MRS: magnetic resonance spectroscopy; MRI-PDFF: magnetic resonance imaging-derived proton density fat fraction

5. Conclusions

The clinical and economic burden of NAFLD is extensive. As of now, NAFLD has already surpassed both alcohol-induced and viral hepatitis as the main cause for liver transplantation and its prevalence is predicted to further increase over the next decade. Although our understanding of how genetic, epigenetic, demographic, and lifestyle factors shape NAFLD risk at the molecular and cellular level, these advances have not yet translated into successful pharmacological treatments. While moderate success was achieved in reducing liver fat levels, none of the drug candidates succeeded in reducing liver fibrosis with an acceptable risk-benefit profile. Thus, there remains a high unmet need to develop different approaches to tackle this disease.

6. Expert opinion

Over the recent years it has become increasingly clear that NAFLD constitutes a complex disease whose molecular underpinnings and driving forces differ both quantitatively and qualitatively between individuals. Incomplete understanding and consideration of this variability constitutes, in the opinion of the authors, one major reason for the large number of failures in NAFLD and NASH drug development. However, with further appreciation of the intricate interplay of genetic and environmental factors, the same complexity might also provide an important opportunity for patient stratification and the development of tailored treatments that take into account the specific risk profile of the individual.

Currently, the landscape of putative treatment strategies is highly diverse, ranging from metabolic manipulations to the targeting of inflammatory signals, reduction of oxidative stress, as well as direct anti-fibrotic and anti-apoptotic mechanisms of action. While multiple drugs have succeeded in reducing hepatic steatosis, the anticipated beneficial effects on downstream pathogenesis, such as liver inflammation and fibrosis, were, with few exceptions, not observed. As such, it appears that direct targeting of molecular pathways involved in liver fibrosis, such as stellate cell activation or ECM remodeling might be more promising to yield effects on disease resolution whereas improvement of insulin sensitivity, steatosis, ROS and inflammation might help in the prevention of disease progression. Furthermore, the authors would like to highlight that weight loss due lifestyle intervention and surgery results in considerably larger effects compared to any pharmacological treatment presented to date, indicating that i) non-pharmacological means should receive additional attention in the medical literature and ii) that support of weight loss using probiotic or pharmacological means can be expected to also have significant effects on NASH and fibrosis in overweight/obese patients.

It is furthermore important to revisit the diagnostic methods for staging liver inflammation and fibrosis. As of now, liver biopsies constitute the gold standard. However, biopsies are invasive, associated with considerable morbidity and limit the temporal resolution in longitudinal studies. More recent clinical trials with promising outcomes have employed noninvasive diagnostic tools, such as magnetic resonance imaging-derived proton density fat fraction (MRI-PDFF), to estimate whole-liver fat content and ultrasound-based transient elastography to gauge liver stiffness. Furthermore, biochemical analyses of blood draws for fibrosis surrogate markers, such as pro-collagen and TIMP1 [114–116], or more comprehensive molecular signatures of NASH and fibrosis [117] emerge as promising methods to complement clinical diagnostics. Furthermore, liver staging using automatic artificial intelligence-assisted histological scoring might further improve the objective identification of anti-fibrotic effects [118,119].
Major breakthroughs in comprehensive molecular profiling methods, combinatorial chemistry and machine learning promise to facilitate drug development. However, poor translatability of preclinical models and unreliable target identification continue to hamper drug development, particularly for complex diseases such as NAFLD. The development of preclinical model systems with increased pathophysiological relevance, such as specific genetic or dietary animal models \cite{120,121} or organotypic \textit{in vitro} tissue cultures using primary human liver cells \cite{122,123}, is thus imperative to open new possibilities for the investigation of disease mechanisms, target discovery and drug screening. Three-dimensional cell culture systems are especially appealing as they support the culture of primary human liver cells for multiple weeks, suitable for the emulation of disease with an indolent course such as NAFLD. Furthermore, the use of experimental models based on patient-derived cells will allow to mimic inter-individual differences in genetic predisposition, as well as in molecular and cellular phenotypes, which can provide useful tools to identify liver-specific biomarkers for treatment response \cite{124}.

Importantly, we expect such pathophysiologically relevant models to synergize with large-scale chemogenomic projects, such as Target2035 \cite{125}. The ambitious goal of this major global public-private partnership effort is to develop cell-active, potent, well-characterized and target-specific chemical probes or functional antibodies against all proteins in the human proteome. These tools promise to allow the systematic interrogation of genetic association at the functional level, including those that are currently deemed non-druggable, which in turn opens up exciting opportunities for catalyzing drug discovery and accelerating development programs for NAFLD, NASH, fibrosis and beyond.

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