NAFLD AND NASH

TIMELINE

Past, present and future perspectives in nonalcoholic fatty liver disease

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Abstract | Nonalcoholic fatty liver disease (NAFLD) was first described as a distinct clinical entity four decades ago. However, the condition has become the centre of attention within hepatology owing to its high prevalence and growing contribution to the burden of end-stage liver disease in the general population. This Perspective provides an overview on the development of knowledge related to NAFLD with a focus on landmark findings that have influenced current paradigms and key knowledge gaps that need to be filled to make progress. Specifically, a timeline of scientific discovery of both basic disease mechanisms (with a focus on human data) and the evolution of knowledge about the clinical course of the disease is provided and related to current approaches to treat and eventually prevent NAFLD.

Nonalcoholic fatty liver disease (NAFLD) has emerged as the most common form of chronic liver disease in most regions of the world[1]. It is a growing cause of end-stage liver disease globally and is recognized as an aetiology of hepatocellular cancer (HCC), even in the absence of underlying cirrhosis[2]. The prevalence of NAFLD is almost one-third of the general population in Western nations and is linked to excess body weight and type 2 diabetes mellitus (T2DM)[3]. The prevalence of the disease is also particularly high in the Middle East[1] and is growing in countries of the Asian subcontinent and the Far East[4]. It is estimated that the burden of end-stage liver disease will increase 2–3-fold in both Western nations as well as in several Asian countries by 2030 (REFS[2,5]). Over the past two decades, substantial progress has been made in understanding the spectrum of NAFLD, its clinical course and the biological factors (for example, lipotoxic stress) underlying its development and progression to cirrhosis, which has led to several therapeutic agents that are now in pivotal clinical trials. In this Perspective, I discuss the past accomplishments, present perspectives and future trends in NAFLD research from a translational perspective (FIG. 1).

NAFLD in the 20th century

The association between fat accumulation in the liver and development of hepatic injury and scarring was identified >50 years ago[6,7]; however, it was recognized as a distinct entity by Jurgen Ludwig and colleagues in 1980, who described the presence of macrovesicular steatosis, hepatocellular ballooning, lobular inflammation and pericellular fibrosis in individuals who either did not consume alcohol or consumed it only in quantities not considered to be harmful to the liver. They named this condition nonalcoholic steatohepatitis (NASH)[8]. NASH is now recognized to be part of a histological spectrum of disease that was later named NAFLD, ranging from a fatty liver alone to steatohepatitis[9].

Elizabeth Powell and colleagues further described the clinical features associated with NASH and identified obesity and T2DM as principal risk factors for the condition[10–12]. Importantly, these early descriptions noted that steatosis and other features of steatohepatitis diminished as the disease progressed to cirrhosis[10]. These findings have now been validated by many other groups, and it is recognized that many patients previously diagnosed with cryptogenic cirrhosis actually had NASH as the aetiology of their cirrhosis[13]. To date, these studies remain landmark descriptions of the clinical–histological course of NASH.

The early descriptions of NASH led to efforts to identify the biological basis of NAFLD. It was quickly noted that steatosis could be induced by a high-fat diet or by leptin receptor deficiency in the *ob/ob* mouse[14]. Although these models did not produce steatohepatitis, it was observed that injections of bacterial lipopolysaccharide could induce inflammation along with steatosis and that steatotic hepatocytes developed heightened susceptibility to injury from TNF[15]. This work led Day and James to postulate the 'two-hit' hypothesis of NASH in 1998, in which steatosis was the first hit and exposure to inflammatory cytokines was the second hit, causing cell death and inflammation in NASH[16]. They considered fat accumulation to be fairly benign, although studies have since found fibrosis to develop even in those with steatosis alone[17]. These early findings led to the first National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored workshop on NASH in 1999, at which the need for research investment was recognized. This research need fuelled a funding opportunity announcement that led to the creation of the NASH Clinical Research Network (CRN) that has since had a major effect, driving many concepts in the field such as the histological assessment of the condition, trial design elements and end points in clinical trials for NASH[9,18].

NAFLD in the 21st century

Translational science refers to the application of basic scientific discovery through preclinical models to clinical trials in humans, followed by delivery of care to affected populations. Over the course of the past 19 years, remarkable progress has been made along the entire spectrum of translational science in NAFLD and has led to an explosion of information related to this disease.

Basic scientific discoveries

Insulin resistance and oxidative stress. Two studies were key in reporting on the relationship of NAFLD with insulin resistance between 1999 and 2001 (REFS[19,20]) (BOX 1). First, it was reported that there was correlation between insulin resistance as measured by the homeostatic model assessment (HOMA) and NAFLD identified by an echogenic liver using ultrasonography[20]. Using euglycaemic–hyperinsulinaemic clamps in humans,

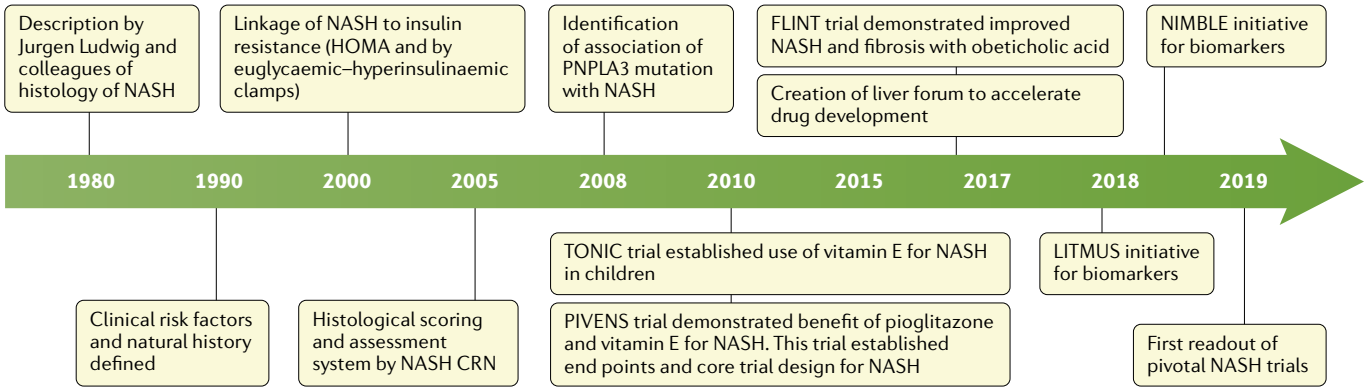

Fig. 1 | **A timeline of key developments in nonalcoholic steatohepatitis.** CRN, Clinical Research Network; HOMA, homeostatic model assessment; NASH, nonalcoholic steatohepatitis.

it was then demonstrated that, even in the absence of T2DM, glucose disposal was progressively impaired from healthy controls to those with histologically confirmed nonalcoholic fatty liver (NAFL) and then NASH with both low-dose and high-dose insulin infusions[19]. Simultaneous administration of labelled glycerol and glucose enabled quantification of peripheral lipolysis and hepatic glucose output, respectively, and demonstrated the presence of step-wise increased resistance to insulin-mediated suppression of peripheral lipolysis and hepatic glucose output from healthy controls to NAFL to NASH[19]. These studies have remained the anchor for the current understanding of the close relationship between insulin resistance and NAFLD (FIG. 2), while subsequent studies confirmed the frequent concordance of NAFLD with features of the metabolic syndrome such as T2DM and hypertension[21]. Together, these studies have formed the basis of the current concept that NAFLD represents the hepatic expression of the metabolic syndrome in the majority of patients.

Early studies were accompanied by a description of hepatic oxidative stress associated with NAFLD in humans[19], which occurred with the development of NAFL and increased further in NASH. Mitochondrial injury and both morphological and functional changes in mitochondria in the liver in humans with NASH were described in 1999 and confirmed in 2001, raising the possibility that these changes were involved in driving the oxidative stress in the liver[19,22]. Increased cycling of the cytochrome P450 system, especially CYP2E1, and changes in peroxisomal function were also described as potential drivers of oxidative stress in mouse models of NAFLD[23]. Although initial studies provided mixed data on the potential relevance of iron overload, it is now generally believed that iron overload is not present in most cases of NAFLD but, if present, might contribute to oxidative stress and disease progression[24].

Cell stress, apoptosis and lipotoxicity. A major paper from Gregory Gore's group described apoptotic cell death as the predominant form of cell death in human NASH and introduced the concept of lipotoxicity in NASH, in which lipids lead to activation of cell death pathways[25]. These studies demonstrated that saturated fatty acids could induce apoptosis and were increased in patients with NASH, who already had increased apoptosis owing to liver injury. This work led to a surge of findings describing multiple pathways by which excess lipids produced cell injury and death in NASH. A key finding in 2008 was the identification of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) in patients with NASH[26]. The development of the UPR was found to be dysregulated in those with NASH, with a failure to fully mount a spliced XBP1-mediated increase in EDEM1, which normally promotes proteosomal degradation of ubiquitinylated proteins[26]. This process was accompanied by an increase in alarm pathway activation with the phosphorylation of JNK1, which is well known to further impair insulin signalling and promote inflammation and apoptosis[26]. The UPR is now known to be a critical link between cell stress and inflammation, apoptosis and disease progression in NASH[27]. The identification of UPR-driven release of extracellular vesicles that promote inflammation and fibrosis by affecting inflammatory macrophages and hepatic stellate cells provides a major new insight into how metabolic stress drives the inflammatory fibrotic response in NASH[28].

Lipidomic studies in humans with NAFLD further revealed widespread perturbations in multiple lipid classes such as triglycerides, cholesterol and eicosanoids[29,30]. Additional studies demonstrated that hepatic cholesterol synthesis is inappropriately increased in NASH despite an accumulation of free cholesterol, suggesting a defect in intracellular lipid sensing[31]. The balance between liver X receptor (LXR) and farnesoid X receptor (FXR) signalling — two important lipid metabolic regulatory pathways — seems to be tilted in favour of LXR in NASH, providing a rational basis for the use of FXR agonists for the treatment of NASH[32]. The bile acid composition also changes with the onset of insulin resistance and NAFL D, with an increased proportion of circulating trihydroxylic bile acids that are poor agonists of FXR[33].

Inflammation. Another landmark in the evolution of knowledge on NASH pathogenesis was the observation of an activated innate immune system in this condition. With the discovery of Toll-like receptors (TLRs) in the 1990s and the recognition of increased inflammation in response to their activation by bacterial lipopolysaccharide or intracellular products associated with cellular injury, increased TLR signalling was noted in murine models of NAFLD[34,35]. It was further shown that palmitic acid could activate TLRs such as TLR2 and activate the inflammasome in NASH[36].

NASH has also been associated with increased systemic bacterial lipopolysaccharide levels, which are known to activate TLR4 (REF.[37]). Elegant studies have now further established a potential role for intracellular TLRs, such as TLR9, in NASH that are activated by denatured oligodeoxynucleotide fragments containing unmethylated CpG islands[38,39].

Studies have also shown the importance of macrophages as key cellular drivers of

disease progression in NASH. In 2006, David Brenner's group demonstrated that bone marrow ablation prevented the development of an inflammatory response in the liver following a high-fat diet and that repletion of the bone marrow cellular content restored this inflammatory response[40]. It is now recognized that inflammatory and pro-fibrogenic macrophages probably play a key part in disease progression[41,42], but gaps remain in our knowledge of what drives macrophage infiltration, whether liver-resident macrophages serve similar roles as bone-marrow-derived macrophages and what leads to a switch from a pro-inflammatory to a pro-fibrogenic macrophage profile. Similarly, there is still a paucity of data on the role of the adaptive immune response in the perpetuation and progression of the disease.

Fibrosis and disease progression. Fibrosis is widely recognized as the hallmark of disease progression in NASH, and the mechanisms underlying fibrogenic progression are currently an area of intense research. Although hepatic stellate cells are widely considered to be the primary cellular source of collagenous matrix in NASH, observations that portal inflammation is associated with fibrosis progression suggest a role for portal myofibroblasts as well[43].

TGFβ-mediated signalling is a driver of fibrogenesis in NASH[44,45]. Activation of Hedgehog signalling has also been implicated in fibrosis progression and as a link between hepatocellular ballooning and fibrogenic activation[46]. Although the cellular and signalling basis for hepatic stellate cell activation and fibrogenesis are well established[47], many gaps remain in our knowledge of how fibrolysis is regulated.

Concurrent with these developments, technological advances using unbiased omics approaches have enabled a description of the genetics, lipidomics, transcriptomics, microbiome and non-coding RNA profiles of progressive NAFLD[48–51]. The identification of the Ile148Met mutation in PNPLA3 in 2008 as a strong predictor of the risk of developing steatohepatitis and progressive disease, including cirrhosis, represents a landmark finding in the field[52]. Importantly, the presence of this risk variant decouples NASH from obesity. Loss of function of the triglyceride lipase activity of PNPLA3 provides an explanation of how this mutation drives steatosis but does not explain how it promotes steatohepatitis[53]. Increased expression of the mutant PNPLA3 leads to impaired proteosomal dysfunction and reduced turnover of the protein, which further impedes lipolysis and promotes steatosis[54]. A clearer understanding of how the PNPLA3 mutation leads to NASH represents another key knowledge gap. Other genetic markers such as variants in the gene encoding TM6SF2, which modulates triglyceride transport out of the liver, and in the lipid droplet protein 17-β-hydroxysteroid dehydrogenase 13 (encoded by *HSD17B13*), in which a splice variant (rs72613567:TA) has a protective effect from the development of NASH, suggest a critical role for lipid trafficking in driving lipotoxicity and thus disease progression[55–57]. This latter variant further mitigated the risk from the PNPLA3 $I^{148}M$ mutation, providing evidence of the complexity of genetic influences in NASH.

The microbiome. The past two decades have seen major advances in the methodologies for the analysis of the microbiome and for cataloguing its diversity in different regions of the body[58]. A faecal microbiome signature with increased Proteobacteria and Bacteriodetes along with a decrease in Firmicutes has been reported in patients with obesity and NASH[48]. Furthermore, a specific gut microbiota signature associated with increasing liver fibrosis has been found[59]. Although several mechanisms by which the gut microbiota might affect NASH have been proposed[60], such as an altered gut barrier, bile acid biology and activation of the innate immune system, more work is needed to fully understand these mechanistic relationships. A major challenge in elucidating such mechanisms is the redundancy in function across various microbial species and the resultant methodological difficulties in assessing causality relationships by gain or loss of function of individual groups of bacteria. The role of the intestinal viral and fungal microbiomes also remains virtually unexplored.

Preclinical models

A key factor that has limited progress in understanding how NASH develops and progresses in humans is the lack of highly characterized tissue samples from individuals who have undergone multiple biopsies over time. A potential solution is to use animal models that closely reflect human disease. Unfortunately, although it is relatively easy to induce steatosis and even inflammation in a variety of mouse models, translation of these findings to understanding human NASH and its progression to cirrhosis remains challenging because most models do not recapitulate many key elements of human disease, such as progressive fibrosis.

If animal models are to be used to better understand human NASH, it is imperative that the models recapitulate as many characteristics of human disease as possible. Thus, the model should be inducible by a diet in which the macronutrient composition resembles that of most humans with NASH. The model should also be associated with increased adiposity, weight gain, insulin resistance, dyslipidaemia and expression of the full spectrum of NAFLD histology with development of hepatocellular ballooning and fibrosis progression. Pathways known to be associated with human disease progression should also be activated, and the overall transcriptomic signature should be broadly concordant with human NAFLD of corresponding histological stage.

Box 1 | **Past, present and future perspectives on NASH pathogenesis**

Past perspectives

- Association with insulin resistance
- Two-hit hypothesis with steatosis as first hit and tissue injury as second hit

Present perspectives

- Current disease models based on excess metabolic substrate delivery to liver, resulting in cell stress, apoptosis, inflammation and fibrosis
- Oncogenesis believed to be linked to inflammation and increased cell turnover
- Changes in microbiome associated with presence of disease
- Disease development and progression models are linear

Future perspectives

- Clarity on the biology underlying the bidirectional evolution of the disease
- Clarification on the use and limitations of preclinical models of disease
- Integrated models of disease development based on genetics, clinical history, histology and changes in transcriptome, metabolome, proteome and microbiome
- Individual patient-level models of factors driving disease progression versus regression

NASH, nonalcoholic steatohepatitis.

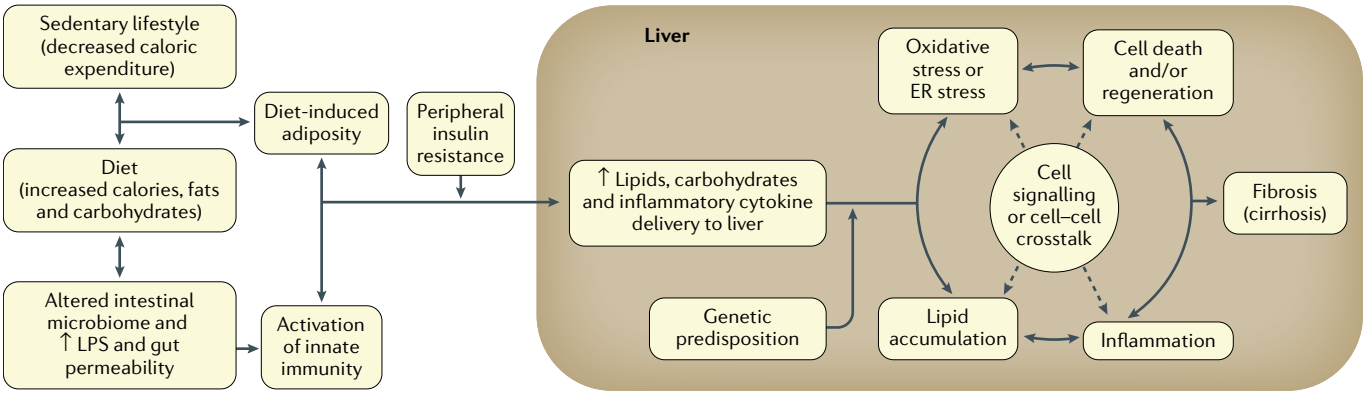

Fig. 2 | **A model of disease development for nonalcoholic steatohepatitis.** Several genetic variants, such as in *PNPLA3* or *HSD17B13*, predispose or protect from nonalcoholic steatohepatitis (NASH), respectively. On a genetic background that determines relative susceptibility to nonalcoholic fatty liver disease (NAFLD) or NASH, changes in diet and sedentary lifestyle-associated behaviour, as well as related gut microbiome changes, lead to increased metabolic substrate (mainly lipids and carbohydrates) delivery to the liver and activation of systemic inflammatory changes, producing insulin resistance. These changes drive increased circulating inflammatory cytokines such as TNF that induce cell stress (such as oxidative stress or endoplasmic reticulum (ER) stress) and modify cell–cell crosstalk, resulting in activation of cell injury or cell death, inflammation and fibrosis. Variable activation of these processes and the consequent metabolic wound healing response determine the development of disease phenotype and its progression to fibrosis and cirrhosis. LPS, lipopolysaccharide.

Unfortunately, many models currently in use do not meet these metrics. Some use specific gene knockouts such as *PTEN* whereas others use nonphysiological diets, such as those containing 2% cholesterol[61,62]. The methionine–choline-deficient diet, which is also commonly used to model NASH, is not associated with obesity and thus does not mimic typical human disease[63]. Use of streptozotocin to ablate the pancreatic islets, used in the Stelic mouse model[64], does not reproduce the systemic insulin-resistant state seen in human NASH. The C57BL/6J mouse does not develop a consistent phenotype or advanced fibrosis, but male mice with the J variant do develop HCC upon high-fat feeding[65]. Although carbon tetrachloride administration has been used to boost the fibrogenic response in mice, it is not yet clear whether this approach fully recapitulates the fibrogenic response in humans with NASH[66].

In 2016, a diet-induced animal model of NAFLD was introduced that does meet the requirements noted here[67]. The model is an inbred isogenic cross between C57BL/6J mice and S129 mice that sequentially develops NAFL, NASH, progressive fibrosis and HCC following initiation of a high-fat diet with a macronutrient composition similar to that in humans and ad libitum consumption of glucose and fructose. However, NASH takes 16 weeks to develop and advanced fibrosis takes ~36 weeks to develop in these mice. In addition, the frequency of HCC development is higher than that seen in humans. Thus, room exists for further refinement of animal models for NAFLD.

Human studies and diagnostics

The relevance of any disease is related to the consequences on the affected individual, the population incidence and the prevalence of the disease. The effect of the disease is assessed by the rates of development of clinically meaningful outcomes in various strata of patients with the disease. Numerous studies have reported a very high prevalence of NAFLD in the general population, and it is estimated that ~25% of adults and 10% of children have NAFLD in the USA[68,69]. Other studies have reported similar estimates of prevalence around the world, but given the larger size of the population at risk in Asia due to the growing prevalence of T2DM[70], there is a substantial burden of disease in this continent[1]. A high prevalence of NAFLD has also been reported in those of Hispanic origin, in elderly individuals and in those with diabetes[71,72].

Disease characteristics. A landmark observation in the study of NAFLD was in 2005 when Schwimmer and colleagues demonstrated that the phenotype of NASH varied from adults to children and that up to 51% of paediatric patients had periportal NASH and portal fibrosis[73], whereas adults mainly had zone 3 perisinusoidal fibrosis[73,74]. However, the relationship of these phenotypes to clinical outcomes is not well established. As clinical outcomes take many decades to develop, the literature has largely focused on disease subtypes and disease progression defined by histological scoring. In this regard, the most influential study was probably the validation of a system of classification and categorization of NAFLD phenotypes by the NIDDK NASH CRN in 2005 (REF.[9]) (BOX 2). Here, disease activity was defined by scoring the severity of three histological features (steatosis, inflammation and hepatocellular ballooning), which are combined to give the NAFLD activity score (NAS). Disease stage was defined by the fibrosis stage, which was based on the original description by Elizabeth Brunt in 1999 (REF.[75]). Importantly, these descriptions recognized the zone 3 perisinusoidal dominance of fibrosis in adults with NASH, which is distinct from the portal fibrosis seen with viral hepatitis captured by the Ishak or METAVIR scoring systems[76,77]. The NAS remains the most extensively validated and tested reference method for the assessment of NAFLD. Another scoring system has been introduced in which steatosis is considered separately from disease activity and includes only scores for hepatocyte ballooning and inflammation[78]. The utility of this approach in the assessment of long-term outcomes or as a predictor of clinically meaningful benefit from therapeutic interventions remains to be established.

Disease progression and outcomes. Whether disease activity is a relevant measure of outcome risk in NAFLD is controversial. These arguments are fuelled by several predominantly retrospective analyses that indicated that disease activity scores were not related to fibrosis progression and that failed to demonstrate a link between the NAS and clinical outcomes of fibrosis progression[79–81]. One highly cited paper reported clinical outcomes in 2015 from

a retrospective analysis of 522 patients with NAFLD who were followed in multiple centres across multiple continents[79]. In this study, in which only 17 patients had cirrhosis, a total of 193 deaths were reported after a median follow-up duration of 12 years, and fibrosis stage was the only driver of mortality. If true, this finding would translate to over a million deaths attributable to NAFLD over the past decade in the USA, which does not seem to be the case. Furthermore, the 14% cardiovascular mortality reported within this time frame[79] is not in line with the clinical experience of most large centres or large rigorously monitored cohorts such as the NIDDK NASH CRN (A.J.S., unpublished data). Thus, an urgent need exists for high-quality, rigorously generated, reproducible and reliable data to model the burden of disease and project the rates of development of cirrhosis and clinical outcomes in NAFLD.

Nonalcoholic fatty liver disease was first described four decades ago, but it is increasingly important owing to its high prevalence in the general population. This Perspective provides an overview on the development of knowledge related to NAFLD, focusing on landmark findings.Although several short-term studies have failed to identify a relationship between steatosis grade and fibrosis progression, which has fuelled efforts by the Fatty Liver Inhibition of Progression (FLIP) consortium to develop activity scores that separate steatosis from inflammation and hepatocyte ballooning and report it with the fibrosis stage[78], one study using Mendelian randomization on the basis of steatosis drivers linked fat accumulation to fibrosis[82]. Emerging data indicate that these scores are reproducible[78]. However, these findings need to be validated across large cohorts and in major clinical trials. A potential problem is that the scoring scales used by the NASH CRN and the FLIP consortium are not similar, which makes it difficult to make numerical comparisons. A risk of de-emphasizing steatosis is to ignore the metabolic underpinning of NAFLD. Furthermore, although changes in steatosis alone do not relate well with resolution of steatohepatitis, NASH rarely resolves without improvement in steatosis[83]. This concept is widely leveraged in phase IIa proof-of-concept studies of treatments of NASH for 'go or no-go' decisions to move to more advanced phase trials[84].

Conversely, some studies have demonstrated a link between disease activity, especially inflammation and hepatocyte ballooning, and fibrosis progression. One systematic review[85] of ten studies by Argo and colleagues identified a close relationship between lobular inflammation severity and disease progression[85]. Disease activity defined by the NAS has also been linked to disease stage in studies reported in abstract form, and this controversy has been reviewed in depth elsewhere[86]. Some studies have also related portal inflammation to fibrosis progression[43]. It is also recognized that with progression to cirrhosis, disease activity decreases and, in those with advanced fibrosis (bridging fibrosis or compensated cirrhosis), it is the fibrosis stage that is most directly related to clinical outcomes[87]. Thus, disease activity is linked to disease stage, which in turn reflects the progression towards cirrhosis that is linked to development of liver-related outcomes. Therefore, the interpretation of changes in disease activity versus disease stage over time must be context-specific.

Unfortunately, there is still a lack of high-quality longitudinal data in patients with various phenotypes, degrees of disease activity and stages of NAFLD to document either the rates of progression to cirrhosis or the actual clinical outcomes. The situation is further compounded by the potential effects of various comorbidities such as hypertension and T2DM and the use of concomitant medications such as statins and anti-diabetic medications. An early retrospective study in 1999 demonstrated that ~20% (4 out of 19) of patients with NASH progressed to cirrhosis over two decades[88]. Another study with a follow-up of >20 years in a cohort of patients with NAFLD corroborated these data[89]; however, only 79 of 130 patients had a follow-up biopsy, and the indications for the biopsy were not clarified. Moreover, after subgrouping the cohort on the basis of gender, race, age, comorbidities and histological subset, the low number of patients in various subgroups remains insufficient to make definitive statements about a complex heterogeneous disease that affects several million individuals.

The lack of large, prospectively collected data sets on disease progression has led to efforts to model fibrosis progression, with one meta-analysis reporting a one-stage progression every 7 years[90]. However, whether the core assumption of linear fibrosis progression is correct remains unresolved[91]. One less controversial aspect of NAFLD progression is the linkage between the disease and HCC. HCC can develop in the absence of cirrhosis, although the absolute risk is low (0.44 per 1,000 person-years of exposure)[1,92,93]. Overall, NASH is increasing as an aetiology for HCC and is the second most common cause for HCC requiring transplantation evaluation in the USA[94]. Of note, whereas the incidences of several obesity-associated cancers such as breast cancer and colon cancer have remained stable or declined over the past 10 years, the incidence of HCC has increased by 3% annually for the past 10 years[95]. The growing prevalence of NASH is probably related to this phenomenon[95].

In those who have developed cirrhosis, the rates of clinical decompensation have been reported to be ~3–4% annually[96]. In 2017, two rigorously performed controlled

Box 2 | Past, present and future clinical perspectives on NASH

Past perspectives

- Definition of the disease as a dichotomous condition (fatty liver versus steatohepatitis)
- Disease evolution model considered to be progressive and unidirectional

Present perspectives

- Disease represented by a continuum of phenotypes with fatty liver and steatohepatitis at its extreme ends
- Genetic variants (*PNPLA3* and *TM6SF2*) linked to more advanced disease
- Detailed and validated histological grading and staging system
- Disease progression often considered to be linear (although controversial)
- Disease stage linked to clinical outcomes

Future perspectives

- Greater emphasis on behavioural factors contributing to the disease
- Extending the dynamic range of disease, particularly fibrosis stage
- Clarifying the bidirectional changes in disease evolution
- Defining disease evolution with transition through adolescence to adulthood
- Establishing colinearity in end-organ status (liver, heart and pancreas) with disease progression
- Validation of noninvasive methods for disease assessment

NASH, nonalcoholic steatohepatitis.

Box 3 | **Past, present and future perspectives on treatment of NASH**

Past perspectives

- Treatments focused mainly on improving steatohepatitis
- Principal targets for treatment were insulin resistance and oxidative stress

Present perspectives

- Weight loss by lifestyle intervention shown to improve disease activity and stage
- Expansion of therapeutic approaches targeting metabolic targets
- Early proof of concept that a purely anti-fibrotic approach can decrease fibrosis progression
- Regulatory path to drug approval for pre-cirrhotic and cirrhotic stages of NASH established

Future perspectives

- Initial pivotal trials will report between 2020 and 2021
- Innovations in seamless phase II–IV trial designs
- Virtual placebo arm cohort analysis to eventually replace need for placebo-controlled trials
- Master protocols that enable multiple agents to be tested sequentially in the context of a single longitudinal study or multiple disease subtypes to be evaluated in a single study to accelerate assessment of combination therapies
- Precision medicine approaches
- Increased use of effectiveness trials and efficacy-to-effectiveness trials to demonstrate the value of therapies in real-world settings and routine clinical practice
- Increasing use of patient-centred outcomes assessment

NASH, nonalcoholic steatohepatitis.

trials of simtuzumab involving 219 and 258 individuals provided prospective data on the rates of progression from bridging fibrosis to cirrhosis, and from cirrhosis to decompensation in patients with NASH, respectively[97]. Over a duration of 24 months, 21% of patients with NASH with bridging fibrosis developed cirrhosis and 19% of those with compensated cirrhosis due to NASH developed clinical decompensation. Approximately two-thirds of the patients included in the trial in those with cirrhosis had clinically significant portal hypertension defined by a hepatic venous pressure gradient >10 mmHg (REF.[98]). This finding might explain the higher rate of portal hypertensive complications than those reported in prior studies[96].

Diagnostic strategies. Diagnostic strategies have continued to evolve over the past decade. Although steatosis can be diagnosed with accuracy using noninvasive tools[99,100], the diagnosis of steatohepatitis remains challenging. Practice guidelines from major professional organizations recommend pharmacological treatment in those with biopsy-proven NASH[101,102]. These guidelines proposed an initial diagnostic strategy that was heavily anchored by the need for a liver biopsy to diagnose NASH and determine the need for therapy. The evolution of knowledge regarding the natural history of the disease coupled with the rapid acceleration in drug development over the past 5 years have led to additional refinements and pragmatic recommendations focused not only on identifying whether NASH is present but also on identifying those individuals with high-risk NASH — that is, those patients with NASH at greatest risk of progression to cirrhosis and liver-related clinical outcomes[103]. Demand is also growing for the development of noninvasive methods to diagnose high-risk NASH owing to the invasive nature of liver biopsies, the sampling variability and the intraobserver and interobserver variability in assessment of liver histology[9]. Liver biopsies are also occasionally associated with severe morbidity and even mortality, making them unacceptable for large-scale deployment in routine clinical practice[104].

Those with cirrhosis have the greatest likelihood of having a liver-related outcome such as variceal haemorrhage, ascites and encephalopathy — when decompensation sets in, it is clinically evident[105]. However, cirrhosis can remain in a clinically silent, compensated state for a long period of time[96]. The presence of cirrhosis in those with NASH might be suspected from an aspartate aminotransferase (AST):alanine aminotransferase (ALT) ratio >1 and high scores for fibrosis from clinical aids such as FIB4, the AST:platelet ratio index (APRI) and the NAFLD fibrosis ratio[106,107]. In such cases, demonstration of high liver stiffness (for instance, >12.5 kPa for vibration-controlled transient elastography (VCTE)) can be used to make a working diagnosis of advanced fibrosis. Although a liver biopsy remains the reference standard, the combination of a high fibrosis score using a clinical aid along with elevated liver stiffness is often used to make follow-up and management decisions as though they had cirrhosis diagnosed in routine practice[103].

In those with obesity without other features of the metabolic syndrome, and with a FIB4 score <1.1 or APRI <0.5 and liver stiffness <6 kPa (VCTE), the likelihood of having clinically significant fibrosis is very low and their projected liver-related mortality risk is low within a 10 year time frame[108,109]. In such cases, there is growing consensus that lifestyle management is the best approach, combined with annual liver stiffness measurements[102].

In those with FIB4 or APRI and liver stiffness values that are intermediate between the low-risk and cirrhosis groups, a liver biopsy remains the reference standard when diagnostic certainty is essential and a therapy with potential toxicity is being considered. If only lifestyle management is to be offered, one can also monitor such individuals with serial measurements of liver stiffness. Data indicate that VCTE can be used at the point of care with relative accuracy and a failure rate of <5% when both the XL and M probes are available, where the XL probe is used for a skin-to-liver capsule distance between 2.5 and 5 cm (REF.[110]). Although MRI-based methods for assessment of hepatic steatosis and fibrosis are more accurate than VCTE, they are also more expensive and are currently used largely as research tools[111].

The development of therapeutics

The first drugs to be tested for NASH targeted insulin resistance and oxidative stress, which were also the first identified pathophysiological drivers of the disease[19] (BOX 3). Early pilot studies in 2000 demonstrated the ability of the antioxidant vitamin E to normalize liver enzymes in children with NAFLD, and steatosis, inflammation and hepatocyte ballooning improved in adults with biopsy-proven NASH[112,113]. Of note, a combination of the insulin sensitizer peroxisome proliferator-activated receptor γ (PPARγ) agonist pioglitazone and vitamin E was superior to vitamin E alone[112]. Interestingly, despite initial promising data, rosiglitazone, another PPARγ agonist, was not found to substantially improve hepatocyte ballooning, the hallmark lesion of NASH[114], and concerns regarding cardiovascular toxicity led to withdrawal of this drug from the market. Two studies performed by the

NASH CRN tested the utility of vitamin E or pioglitazone versus placebo in 247 adults with NASH (PIVENS trial)[18] and vitamin E or metformin versus placebo in 173 children with NAFLD (TONIC trial)[115]. The PIVENS trial established the utility of vitamin E for decreasing the NAS by ≥2 points (number needed to treat was 4.4 and resolution of NASH was 36%) and the TONIC trial also demonstrated increased resolution of NASH with vitamin E, although the improvement in ALT in the placebo arm rendered the decrease in ALT in the active arm nonsignificant. Pioglitazone also improved all aspects of steatohepatitis but did not meet the prespecified *P* value of 0.025 for the primary end point (a decrease of NAS by ≥2 points with contribution of at least 1 point from ballooning and no worsening of fibrosis), which was used instead of the usual value of 0.05 owing to the multiple comparisons being made. Metformin, a liver-specific insulin sensitizer, was not effective in improving steatohepatitis[115]. These studies form the basis for the current recommendations for the use of vitamin E and pioglitazone for the treatment of NASH by the American Association for Study of Liver Disease[101]. The utility of pioglitazone was also corroborated in an independent single-centre study[116].

Similarly, a phase IIb study of an n-3 polyunsaturated fatty acid also did not demonstrate improvement in histology; this finding was largely driven by a higher placebo response rate than expected[117]. It is also likely that the doses selected (1,800 and 2,700 mg per day of ethyl-eicosapentanoic acid) were too low because, even at the high dose, only a modest decrease in circulating triglycerides was found. Several other small studies of polyunsaturated fatty acids have also yielded disappointing results[118,119].

A major breakthrough was the realization that FXR, the nuclear receptor that is the cognate bile acid receptor, is a major modulator of lipid metabolism and insulin sensitivity in animal models[120]. These findings were translated in a proof-of-concept study using euglycaemic–hyperinsulinaemic clamps to demonstrate that the preclinical data could be reproduced in humans using the FXR agonist obeticholic acid[121]. This study was followed by the FLINT trial from the NASH CRN in which an overwhelming level of evidence for the efficacy of obeticholic acid for the improvement of NASH and improvement in fibrosis was obtained[122]. This trial met prespecified criteria for efficacy of obeticholic acid that led to early termination of the trial and demonstrated the ability to reverse fibrosis stage using pharmacological interventions. The success of the FLINT trial has spawned the pivotal REGENERATE trial[123], in which 1,300 patients will be treated for 72 weeks to confirm the histological improvement seen in the FLINT trial. In another major trial, the dual PPARα–PPARδ agonist elafibranor was tested in 276 patients with NASH[124]; the rationale for this compound was that PPARα would decrease steatosis by increasing lipid oxidation and PPARδ would target inflammatory macrophages and reduce inflammation. The a priori end points of this trial were not met; however, in a post hoc analysis, those with NASH and an NAS >4 had an improvement in both disease activity and fibrosis. This compound is now being tested in a pivotal trial (RESOLVE-IT)[125]. The success of these strategies targeting the metabolic underpinning of NASH (FIG. 3) are further supported by the benefits of bariatric surgery on liver histology in those with NASH[126]. However, the morbidity and occasional mortality associated with such surgery, especially in those with cirrhosis, precludes this option for all patients with NASH.

In the past 5 years, the potential to halt disease progression by the use of specific anti-inflammatory and anti-fibrotic agents has been tested. The 1-year findings from a 2-year trial of the CC-chemokine receptor 2 (CCR2)–CCR5 antagonist cenicriviroc, which was designed to reduce NASH fibrosis, has demonstrated a significant ($P<0.01$) improvement in one-stage or greater fibrosis reduction in patients with NASH without changing upstream aspects of the disease, such as steatosis and hepatocyte ballooning injury[127]. This trial further demonstrated that improvement in inflammation as defined by biochemical and molecular analyses is not recapitulated by traditional histological methods of inflammation assessment, raising questions about the validity of these conventional methods. Unfortunately, trials to inhibit fibrosis with the use of direct anti-fibrotics such as simtuzumab have been disappointing, suggesting that either more potent anti-fibrotic therapies are needed or that anti-fibrotic strategies should be combined with more metabolically targeted therapeutics. The current evidence, to date, supports the need for a metabolic anchor for the treatment of NASH.

A key advance in the development of therapeutics is the establishment of a clear development pathway and the evidence base needed for drug approval. The key evidence needed is demonstration of

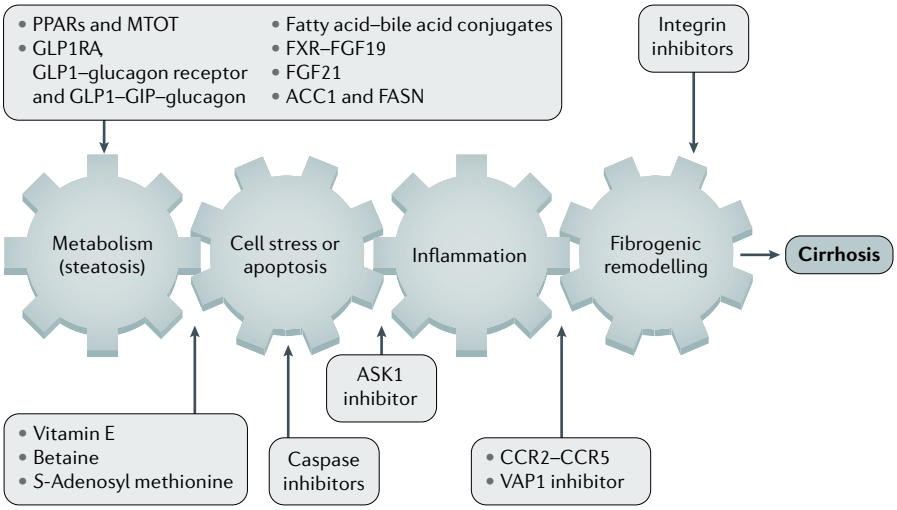

Fig. 3 | **Current therapeutic targets for nonalcoholic steatohepatitis.** The development of progressive nonalcoholic steatohepatitis (NASH) is linked to delivery of excess metabolic substrate and inflammatory cytokines to the liver that, in turn, induce cell stress, which can induce apoptotic and inflammatory signalling. Inflammation over time induces a fibrogenic response that can ultimately lead to cirrhosis. This simplified paradigm enables the evaluation of specific mechanisms underlying each of these elements and targeting them for treatment of NASH. A partial list of agents with a primary mechanism of action targeting specific nodes in the development of progressive NASH are shown. ACC1, acetyl-CoA carboxylase 1; ASK1, apoptosis signal-regulating kinase 1; CCR, CC-chemokine receptor; FASN, fatty acid synthase; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GIP, gastric inhibitory peptide; GLP1, glucagon-like peptide 1; GLP1RA, glucagon-like peptide 1 receptor agonist; MTOT, mitochondrial target of thiazolidinedione; PPAR, peroxisome proliferator-activated receptor; VAP1, vascular adhesion protein 1.

clinically meaningful benefit, which is defined by improvement in how an affected patient 'feels, functions or survives'[84]. Given the long duration for clinical end points to develop in patients with NASH, a conditional accelerated approval pathway, known as subpart H for the FDA, has been established on the basis of demonstration of histological improvement in the short term and either clinically meaningful benefit or decreased progression to cirrhosis[84]. The subpart H pathway is specifically used by the FDA, but an analogous accelerated approval path is used by the European Medicines Agency (EMA)[84].

Future perspectives

With the ageing of the current NAFLD population, it is expected that the burden of disease due to cirrhosis from NASH will increase over the next two decades unless effective preventive and therapeutic measures are implemented as part of a comprehensive public health strategy for metabolic syndrome and associated end-organ diseases. The good news is that the knowledge base related to NAFLD continues to evolve at a rapid pace, and it is likely that many of the current paradigms about the disease will be modified over the course of the next decade. It is becoming increasingly apparent that there is substantial heterogeneity in terms of the molecular and cellular processes driving the disease from one patient to the next. This understanding raises the possibility of matching specific therapeutic strategies to the specific disease drivers in a given patient. The development of such personalized approaches and the identification of subpopulations with unique disease drivers will require integration of phenotypic, molecular and genetic data to create a 'Liver Atlas', which should have a transformational effect on the field similar to The Cancer Genome Atlas project[128].

Human cohorts will provide longitudinal data both on the clinical course of patients and outcomes and on the molecular pathways involved in disease progression. These cohorts are also expected to provide critically needed information on the molecular heterogeneity of the disease and how it relates to clinical outcomes, as well as providing samples to enable qualification of biomarkers for the treatment of NASH.

A major gap in our knowledge is related to the evolution of NAFLD in children and through adolescence. Early data from the NASH CRN cohort indicate that the paediatric pattern of NASH is progressively lost through the teenage years[129]. However, the drivers of these changes and the implications for prognosis, therapeutic targeting and the confounding effects of alcohol use and other lifestyle changes with transition to adulthood all remain unclear. It is anticipated that substantial new information will emerge around these issues as the current paediatric cohort of the NASH CRN is prospectively monitored to adulthood.

Development of the regulatory science related to both diagnostics and therapeutics is expected from the work done by the Liver Forum, which was created to bring regulatory agencies such as the FDA and EMA together with other stakeholders in NASH, including academia, patients and the commercial sector, to identify gaps in knowledge and to develop methods to fill these gaps. Common case definitions and harmonization of assessments could enable development of a virtual placebo cohort, which might permit robust modelling of the natural course of the disease and might ultimately mitigate the need for placebo-controlled trials[130,131]. The anticipated participation of additional regulatory agencies in the Liver Forum such as the Chinese FDA is expected not only to harmonize drug development efforts globally but also to extend a global safety net by establishing standards for drug safety in the context of NASH. Specifically, a key area that will be highly effective is the development of global guidelines for the assessment of drug-induced liver injury in the context of chronic liver disease, in which the usual markers of liver injury are abnormal even before drugs are initiated. Several consortia are now collaborating to address drug safety in the context of chronic liver disease such as the IQ Consortium Drug-Induced Liver Injury Initiative (IQ-DILI). It is anticipated that these initiatives will generate new standards for drug safety for NASH, and their outputs are eagerly awaited.

Additional innovations such as the use of Master protocols are expected to further accelerate therapeutic development[132]. Such protocols enable multiple treatments to be tested in a seamless manner, or the efficacy of a treatment to be tested in different patient populations. The key element of these is a single placebo arm and faster assessment of drug efficacy and safety. Simultaneously, two major initiatives in Europe and the USA (LITMUS and NIMBLE consortia) have been initiated and are expected to qualify noninvasive assessments for NASH and permit simple point-of-care methods for the diagnostic evaluation of those with suspected NAFLD. Further evidence that will validate noninvasively assessed end points for NASH is expected, which will accelerate drug development and the testing of combination drug strategies.

A key finding in the past few years is that NASH, atherosclerosis and T2DM share many common pathogenic features such as ectopic fat deposits, inflammation, cell stress and death and fibrosis in affected organs, which are expected to drive common diagnostic tools that will inform the assessment of all of these diseases. Therapies that will beneficially affect all of these end-organ diseases will emerge as the first-line treatments of the condition.

Finally, it is becoming clear that weight loss, specifically reduced adiposity, is an important driver of histological improvement[133]. The benefits of weight loss will extend beyond those expected from drug treatment of high-risk NASH to include decreased cardiovascular and metabolic outcomes and potentially even cancer. Public health strategies will need to recognize the common elements driving the growing health consequences of T2DM, NAFLD, heart failure and several obesity-related cancers to reduce the burden of disease related to all of these conditions in the future.

Conclusions

NAFLD currently occupies centre stage in terms of research and therapeutic development in the area of liver diseases. Although the field is relatively young, it is an area of intense research given its public health implications. Early studies provided seminal information on its linkage to insulin resistance and the metabolic syndrome, and, over the past few years, the core elements of pathogenesis have been worked out along with the identification of several therapeutic targets, some of which have already been translated to treatment trials. Several agents are in pivotal trials and a regulatory pathway for drug approval has been established. Future studies are expected to identify specific disease drivers in individuals and subpopulations on the basis of molecular drivers of disease. The current efforts to develop and qualify specific biomarkers for NASH are expected to enable all health-care providers to rapidly assess the presence and severity of the underlying disease. Together with ongoing drug development efforts, these efforts will permit both identification of those at greatest risk of outcomes and potentially the reversal of the disease. Ultimately, from a societal perspective, it will be essential to attack the root cause

of NAFLD, T2DM and cardiovascular disease to reduce the burden of diseases related to caloric excess and disordered metabolism. This goal will require a broad effort of all stakeholders to address the social, economic, cultural and medical underpinning of obesity and its related conditions, including NAFLD.

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Competing interests
A.J.S. is President of Sanyal Biotechnology and has stock options in Akarna, Durect, Exhalenz, Genfit, Haemoshear, Indalo and Tiziana. He has served as a consultant to AbbVie, Amarin, Ardelyx, Astra Zeneca, Boehringer, Conatus, Fibrogen, Genfit, Gilead, Jannsen, Lilly, Nimbus, Nitto Denko, Novartis, Pfizer, Salix, Takeda, Tobira and Zafgen. He has been an unpaid consultant to Affimune, Bristol Myers Squibb, Chemomab, Echosens, Fractyl, Galectin, Immuron, Intercept, Nordic Bioscience, Novartis, Novo Nordisk and Syntlogic. His institution has received grant support from Astra Zeneca, Bristol Myers Squibb, Cumberland, Gilead, Intercept, Malinckrodt, Merck, Salix, Shire and Tobira. He receives royalties from Elsevier and UptoDate. Virginia Commonwealth University also has ownership interests in Sanyal Biotechnology.