Management of non-alcoholic fatty liver disease

State of the art

Advances in the management of nonalcoholic fatty liver disease

Maria Letizia Petroni,* M.D., Lucia Brodosi,* M.D., Prof. Elisabetta Bugianesi,^° M.D., Prof. Giulio Marchesini, M.D.*°

*Department of Medical and Surgical Sciences, "Alma Mater" University, Bologna, Italy

^Division of Gastro-Hepatology, University of Turin, Italy

Word count:	Text	7,997
	Summary	269
	Tables	6
	Figures	2
	Boxes	2
	References	193

Supplementary Tables 1

° Shared last name

Address for correspondence:

Prof. Giulio Marchesini, M.D.
Department of Medical & Surgical Sciences
"Alma Mater" University
Via Massarenti, 9
I-40135 Bologna, Italy

Phone: +39 051 2144889
e-mail: giulio.marchesini@unibo.it

Box1

MOST COMPELLING RESEARCH QUESTIONS IN NAFLD MANAGEMENT

- Which biomarkers or imaging tools are suitable to screen subjects at risk and and/or track meaningful changes in NAFLD progression/regression as part of the natural history of disease or in response to treatment strategies?
- How to identify distinct phenotypes on the basis of integrated models of history, histology and omics (genomic, metabolome, proteome and microbiome) (system medicine), also taking into account collinearity in organ status (liver, heart and pancreas), and the relation between phenotypes and liver disease progression?
- Should novel regulatory endpoints be established for drug development and biomarker approval (FDA/EMA guidance documents) to overcome the risks connected to liver biopsy and to be replicable in clinical practice?
- How to build a comprehensive network including primary care physicians, liver, diabetes, obesity specialists for the long-term management of disease, also sensitive to patient-reported outcomes, as well as to increase NAFLD awareness among healthcare professionals and the community?
- How to interact with public health authorities to implement the societal changes needed to address the obesogenic environment, the social determinants of health and food advertising, to facilitate nudging to healthy behavioral changes, thus reducing NAFLD burden?

Box 2

HOW PATIENTS WERE INVOLVED IN THE CREATION OF THIS ARTICLE

After e-mail communication, the manuscript was sent for review to the **Liver Pool** (Federazione Nazionale delle Associazioni di Volontariato per le Malattie Epatiche ed il Trapianto di Fegato) and to **FEDER** (Federazione Diabete Emilia-Romagna). Their comments addressed the issues of screening criteria for advanced disease and patients' reported outcomes. The former issue is discussed in a specific chapter; the latter is dealt with in the conclusion. The same associations will be contacted for the dissemination of the review.

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a very common medical condition, driven by a combination of genetic and lifestyle factors, ultimately producing a severe chronic liver disease and increased cardiovascular risk. The vast majority of cases are long free living and totally asymptomatic, hence the difficulty in identifying cases progressing to nonalcoholic steatohepatitis (NASH), to NASH-cirrhosis and eventually hepatocellular carcinoma for timely diagnosis and treatment. Despite advances in the understanding of pathogenic mechanism(s) and the identification of liver fibrosis as the most predicting risk factor for disease progression, no specific compounds have so far been approved by regulatory agencies. Outside controlled trials, treatment is generally limited to lifestyle intervention aimed at weight loss; pioglitazone remains the drug of choice to reduce fibrosis progression in subjects with diabetes – frequently used off-label also in the absence of diabetes –, whereas vitamin E is largely used in the paediatric population and may be considered in adults without diabetes. Several drugs are under investigation according to the agreed targets of reduced NASH activity without worsening of fibrosis or fibrosis improvement without worsening of NASH. Anti-inflammatory, anti-fibrotic agents or metabolism modulators have been tested either in phase 3 or in phase 2b randomized controlled trials; a few failed, others have produced marginally positive results, only a few are currently being tested in extension studies. The development of non-invasive, easy-to-repeat surrogate biomarkers and/or imaging tools remains the most critical issue to facilitate clinical studies and limit liver biopsy. Political commitment and concerted actions of the multiple stakeholders involved in prevention and treatment of NAFLD are mandatory to reduce the burden of disease in the population.

Introduction

As originally described by Ludwig et al,[1] nonalcoholic fatty liver disease (NAFLD) represents a condition of excessive liver fat accumulation in subjects consuming alcohol at doses below risk levels. The condition may be limited to excessive liver fat (NAFL) or progress to necroinflammation and fibrosis (nonalcoholic steatohepatitis – NASH),[1] to NASH-cirrhosis[2] and eventually to hepatocellular carcinoma (NASH-HCC).[3]

This definition carries two important biases: i) the necessary amount of liver fat remains undefined; ii) there is no pathogenic insight and it excludes the diagnosis of NAFLD for individuals consuming alcohol above an uncertain and debated threshold. The safe limits of alcohol use, as set by European and American guidelines,[4 5] are limited to 20 g/day in females and 30 g/day in males. Importantly, the definition excludes even modest alcohol intake as cofactor in liver fat accumulation driven by the metabolic dysfunction. Several studies identified insulin resistance, with/without obesity, as the underlying soil associated with NAFLD,[6 7] and identified NAFLD as the hepatic expression of metabolic syndrome (MetS).[8]

To overcome the negative definition originally attributed to NAFLD, a proposal was put forward to change the term NAFLD into MAFLD (Metabolic Associated Fatty Liver Disease),[9] assigning the disease a name linked with its pathogenesis. The new nomenclature is not yet accepted by regulatory agencies and dissenting comments have been raised.

The present review will particularly focus on screening methods to select patients for treatment, and on randomized clinical trials and real-world data to define treatment effects. These issues are covered by several clinical practice guidelines; the most recent documents, frequently used as reference in National guidelines, are compared to detect differences, strengths and weaknesses (Table 1).[4 5 10-12]

Search methods

Between January 1980 and May 2020, 15,087 articles were retrieved in PubMed, using the search term "non-alcoholic", "fatty liver" OR "steatosis" either [All Fields] OR [MeSH terms] AND "humans"[MeSH Terms]. After prioritizing articles in English and excluding duplicate reports, the search included 778 randomized trials and 4,099 review articles. Further manual searching for additional articles was done on relevant databases (*Clinicaltrials.gov*) and by scrutinizing review articles for missing references. A few additional data published up to September 30, 2020 were included.

Epidemiology of NAFLD

The prevalence of NAFLD in the general population is very high (~25%), peaking over 30% in the Middle East and South America and as low as 13% in Africa.[13] Although associated with MetS and obesity rates,[14] a recent meta-analysis of 84 studies (over 10 million cases) concluded that, within the NAFLD population, 40.8% of cases (95% confidence interval [CI], 36.6-45.1) were non-obese and 19.2% (95% CI, 15.9-23.0) were definitely lean.[15] These rates were calculated with body mass index (BMI) adjusted for ethnicity, i.e., <23kg/m^2 for normalweight and 23.0-27.5 for overweight in Asians.

The prevalence depends on the method of ascertainment, specific clinical conditions (e.g., obesity), and stage of disease. Ultrasonography (US) is the reference technique for epidemiological studies[16] and in clinical settings but remains operator-dependent and scarcely sensitive (only positive for liver fat ≥20-30% of the hepatic parenchyma).[17] More sensitive and quantitative methods have been developed for clinical trials, whereas surrogate biomarkers are used for epidemiological studies. Using proton magnetic resonance spectroscopy (MRS),[18] the physiologic amount of liver triglycerides was set at 5.0%.[19] Surrogate non-invasive markers include unexplained elevated liver enzymes in subjects with metabolic disturbances (namely, alanine aminotransferases-ALT) or specific algorithms (e.g., fatty liver index-FLI).[20] According to the different techniques, the prevalence varies from a mere 3.2% (elevated aminotransferases, NHANES population),[21] to 19% (ultrasonography, same population),[22] to 34% (Dallas Heart study population, proton magnetic resonance spectroscopy-MRS),[23] with age, gender and ethnicity differences.[13]

The prevalence of NASH in the general population varies between 1.5% and 6.5%,[13] i.e., one in 4-5 NAFLD patients, but these estimates are derived from biopsy studies, with a high risk of selection bias. From a clinical point of view, the prevalence of advanced fibrosis , the key feature of progressive liver disease and liver-related outcomes,[24] is measurable by non-invasive bio-markers[25] (preferably, NAFLD fibrosis score [NFS],[26] Fibrosis-4 index [Fib-4][27] and Enhanced Liver Fibrosis test [ELF™][28]). The prevalence of advanced fibrosis (fibrosis, ≥ F3)[29] in the general adult population is estimated around 1.5%, and similar data have been obtained by non-invasive imaging methods (transient elastography [TE, Fibroscan™]).[30]

In obesity and type 2 diabetes (T2DM), prevalence rates are two to four-fold increased,[31] depending on age and comorbidities. The prevalence of NAFLD in T2DM is estimated above 60%,[32] with two thirds of biopsied patients with NASH and 10% with advanced fibrosis.[33-35] In obesity (BMI ≥ 30kg/m^2), the prevalence of NAFLD exceeds 60%,[36] but exceeds 90% in

morbid obesity.[37] Of particular concern is the prevalence of NAFLD among children (approximately 7.6% in the general population),[38] rising in parallel with obesity,[38] and the finding that overweight/obesity in childhood/young adulthood increases the risk of liver-related morbidity and mortality in later life.[39]

Natural history of NAFLD

Liver disease progression may be extremely variable; pure fatty liver (NAFL) does not reduce life expectancy, whereas patients with NASH have increased all-cause and liver related mortality.[40] Liver biopsy remains the sole method for a correct disease classification, but guidelines suggest limiting its use to very specific settings. The NAFLD activity score (NAS), computed as sum of steatosis (0-3), lobular inflammation (0-3) and hepatocellular ballooning (0-2),[29] is largely used, but the European SAF (Steatosis-Activity-Fibrosis) score more precisely identifies the components of disease progression (Figure 1).[41 42] Fibrosis is indeed the most ominous predicting factor; it increases on average by one stage over 14.3 years in patients with NAFL and 7.1 years in patients with NASH.[43] In a recent meta-analysis on 4428 subjects with biopsy-proven NAFLD, the relative risks for events increased systematically from stage F2 onwards, to 3.42 (95% CI, 2.63-4.46) for all-cause mortality, 11.13 (4.15-29.84) for liver-related mortality, 5.42 (1.05-27.89) for liver transplant and 12.78 (6.85-23.85) for liver-related events in stage F4 (cirrhosis) vs. stage F0, irrespective of the presence of NASH.[24] In patients with F4, liver decompensation occurs at rates of 3.3-15.6 per 100 person-years, depending on Child-Pugh class.[44 45]

The whole cardiovascular (CV) system is frequently involved, driven by the atherogenic profile and features of MetS.[46 47] CV disease remains the most common cause of death;[44] diffuse atherogenic lesions, such as coronary artery disease [48] and increased carotid intima-media thickness,[49] are more common in NAFLD, independent of traditional risk factors. Left ventricular failure and altered cardiac energy metabolism have also been described.[50]

NAFLD doubles the risk of incident T2DM in a meta-analysis incorporating data from 20 observational studies (nearly 117,000 nondiabetic individuals), over a median 5-year follow-up.[51] The risk is diminished by NAFLD resolution,[52 53] pointing to liver fat accumulation as cofactor in T2DM pathogenesis.[54] Finally, the risk of incident chronic kidney disease is increased by 40% in association with T2DM.[55]

Lean NAFLD, although characterized by an apparently lower severity (lower ALT levels, lower insulin resistance and lower prevalence of features of MetS)[56 57] shares a similar or even higher risk of disease progression.[56 58 59]

Hepatocellular carcinoma (HCC) and extrahepatic cancers

NAFLD-associated hepatocellular carcinoma (HCC) is the third-most common cause of HCC in the United States (14%),[60] with a cumulative incidence of 2.4-12.8% over a median follow-up of 3.2-7.2 years.[61] NAFLD patients with advanced fibrosis (F3-F4) have an almost 7-fold increased risk of HCC compared to controls [60] and the risk can be even higher in T2DM and obesity.[62] At diagnosis, patients with NAFLD-related HCC are older and have a higher prevalence of extrahepatic comorbidities compared with viral- or alcohol-related HCC individuals, but a lower prevalence of cirrhosis (only two-third of cases),[61] leading to less systematic surveillance and late diagnosis.[63] Accordingly, NAFLD-related HCC may receive less treatment and more patients are likely to die of their HCC,[64] despite a lower prevalence of cirrhosis leading to higher resection rates (19% vs. 11% in HCV-related HCC).[65]

All cancer-related mortality is also increased, occurring in 1-2% of cases, possibly driven by metabolic alterations.[65] A large community cohort study showed that NAFLD was associated with a nearly double risk of extrahepatic cancers (particularly uterus, stomach, pancreas and colon) during a median follow-up of 8 years.[66] The association with incident cancer risk is stronger in NAFLD than in obesity,[66] suggesting that NAFLD might be the link between obesity and cancer.[67]

Approach to treatment - Screening

The natural history of NAFLD underlines the importance of timely diagnosis to reduce the burden of disease and the direct and indirect costs, potentially amenable to prevention and early diagnosis. The issue of effective screening in the community and in selected cohorts becomes mandatory to define treatment strategies, but not all screening criteria are fulfilled for NAFLD.[68] In particular, we still lack an easy-to-repeat, cheap and community-acceptable test to assess disease severity, and treatment is limited to lifestyle intervention. EASL guidelines suggested universal screening for NAFLD in patients with metabolic diseases,[5] according to resource availability. The position was criticized,[69 70] although limited to patients at higher risk of disease progression, and, as of 2019, the U.S. guidelines do not support screening.[4] Universal screening is not cost-effective,[71] but the cost-utility of

screening procedures to select patients for biopsy, follow-up and treatment is high, particularly in younger patients (below 45),[72 73] and programs for referral of patients with advanced disease to diagnostic procedures are needed. Two strategies are supported by all guidelines, with differences in relation to setting: i) community screening, ideally by primary care physicians, using cheap, non-invasive surrogate markers of steatosis and fibrosis – listed in Supplementary Table –, in particular FLI, FIB-4, NAFLD Fibrosis score (NFS) and ELF test,[20 26-28] ii) screening by non-invasive markers, also including transient elastography,[74 75] by specialists (i.e., diabetes specialists) in subjects at higher risk of disease progression. In both cases, patients identified with advanced disease should by referred to hepatologists for definite diagnosis (including liver biopsy), appropriate follow-up and treatment. Biopsy is mandatory for patients entering clinical trials, as well as in case of conflicting results or competing diagnoses (Table 1).

Primary care physicians are at the forefront in the community for early selection of at-risk cases. A two-step screening procedure by FIB-4 index and ELF test (tools having a high negative predictive value) reduced unnecessary referrals to liver specialists by 81%, and 5-fold increased the referral of cases with advanced fibrosis *versus* standard care.[76] This strategy also increased the detection of cases with cirrhosis in the community. Transient elastography as second step or as sole diagnostic procedure was similarly cost-effective.[77] Effectiveness is likely to further increase in selected cohorts at higher risk of progression to HCC, as diabetes cohorts. However, NAFLD awareness among primary care physicians and non-liver specialists remains scarce,[78 79] and this unconsciousness is also shared by patients.

Pathophysiologic approach to treatment

While simple steatosis is a reflection of non-progressive dysfunctional metabolism, NASH is a chronic liver disease that may progress undiagnosed for years, eventually emerging with liver failure and HCC. The burning question is why in some individuals a metabolic disease will translate into a progressive liver disease. Although NASH stems from the combination between environmental and genetic factors (Figure 2), reducing its aetiology to obesity comorbidity does not do justice to a far more complex disease. Unravelling the network of interacting factors that drive NASH development is essential for risk stratification and provides a roadmap of potential therapeutic targets.

Lipotoxicity

The earliest events initiating NAFLD reside in an absolute or relative calorie excess, as confirmed by the link between NAFLD and obesity. Limited physical activity, sedentary behaviors,[80-82] TV and computer watching [83 84] are complementary aspects of calorie imbalance, irrespective of BMI. Increased substrate flux will overload adipose tissue compartments, leading to dysfunctional adipose tissue, spill-over of free fatty acids into non-adipose tissues, *de novo* lipogenesis and disposal of lipids inside the liver. This process has been described by Unger as "lipotoxicity",[85] and occurs primarily in the liver (NAFLD), in the pancreas (nonalcoholic fatty pancreas, favouring T2DM), in the heart and diffusely in the arterial circulation (atherosclerotic CV disease). Under such circumstances, the liver, adipose tissue, muscle and gut interact via cytokine, growth factor and adipokine secretion, with the liver taking centre stage in metabolic regulation. These multiple insults would synergistically drive the development and progression of NAFLD, particularly in genetically predisposed individuals.[86] NASH is much less prevalent than simple steatosis in the general population and does not correlate with steatosis severity.[87] This suggests that most people with fatty liver are able to compensate for stressors that drive the progression to NASH in other individuals. Triglycerides are not *per se* hepatotoxic, and hepatocyte injury is likely generated by toxic precursors or products of triglyceride metabolism. Besides free fatty acids, candidate lipotoxic lipids include mono and diglycerides, ceramides, dihydroceramides and lysophosphatidyl choline species, as well as hepatic cholesterol accumulation, which may be responsible for necroinflammation, [88 89] while other lipids (mono- and poly-unsaturated fatty acids) may exert a protective role.[90] Increased *de novo* lipogenesis from carbohydrates, specifically fructose, [91 92] are expected to produce similar lipotoxic effects; consumption of sugar-sweetened beverages containing either fructose or sucrose (converted to fructose and glucose in the gut) may be even more toxic than lipids in promoting NASH.[93] Uncontrolled and incomplete lipid oxidation, oxidative stress and activation of the unfolded protein response are two well-characterized pathways that promote cell death in NASH.

Gut microbiota

An altered microbiome (i.e., 'dysbiosis') may contribute to liver damage. Human studies document a faecal microbiome signature characterized by increased Proteobacteria and Bacteriodetes along with a decrease in Firmicutes in patients with obesity and NASH.[94] Mechanistic links between altered microbiome and NASH include increased intestinal permeability as well as bacteria modulation of the gut-liver axis through intestinal farnesoid-X receptor (FXR) signalling which regulates the transcription of genes involved in bile acid

synthesis and transport, lipogenesis and glucose homeostasis, either directly or indirectly, via release of fibroblast growth factor-19 (FGF19).

Gene polymorphisms

Ethnic differences in hepatic fat accumulation have long been described,[95] leading to higher disease prevalence in subjects of Hispanic and Asian origin, and lower prevalence in Africans and African Americans. Genetic differences are in keeping with twin and family studies showing that steatosis and NAFLD progression to fibrosis and eventually to cirrhosis may be strong heritable traits.[96-98] Since the original finding of a close relationship of liver fat with a polymorphism in the patatin-like phospholipase domain-containing 3 gene *(PNPLA3)*,[99] other genes accounting for an increased susceptibility to NAFLD have been identified by genome-wide association studies (Table 1).[100] They act through totally different mechanisms,[101] interacting with dietary factors,[102] physical activity[103] and comorbidities,[104] sometimes producing epigenetic effect.[105] Of note, they are also differently associated with CV disease, potentially driving outcome. A novel gene variant reducing the risk of liver disease has also been described (a loss-of-function variant of hydroxysteroid 17-beta dehydrogenase 13 gene - *HSD17B13*),[106] as well as other polymorphisms linked with specific proteins in selected cohorts, offering a rationale for treatments.[101]

Fibrogenic response

Progression to liver fibrosis reflects the convergent impact of environment, metabolism, microbiome, genetic risk factors and comorbidities on cell death. In turn, dying hepatocytes trigger regenerative responses, enriching the liver with regenerative cell (myofibroblasts, immune cells, and liver-cell progenitors).[107] Liver fibrosis is the result of repeated and protracted wound healing, ultimately driven by hepatic stellate cells, and reflects the net balance between fibrogenesis and fibrosis degradation. In NASH, ongoing fibrogenesis does not proceed linearly from simple fatty liver to NASH to cirrhosis. Rather, progression appears to result from repetitive necro-inflammatory bouts interrupted by anti-inflammatory, reparative immune responses. Over time, futile regenerative responses also perpetuate the stimulus for neoplasia, increasing the risk of liver cancer.

According to the above mechanisms, treatment targets include attempts to repristinate calorie balance, lipid and glucose homeostasis, to reduce oxidative stress and systemic and local (hepatic) inflammatory signals, or to modulate stellate cell activation and fibrogenesis. Pleiotropic drugs such as FXR-agonists and glucagon-like peptide-1 receptor agonists, hit more than one target within the injury milieu. As both the mechanisms leading to NASH and

their phenotypic expression are highly heterogeneous, treatment should theoretically be tailored to individual patients and potentially consider combination therapy.

Accepted NAFLD Treatment

Lifestyle

Lifestyle intervention is the backbone and, at present, the sole treatment of NAFLD, as long as no drugs are approved by regulatory Agencies. The favourable effects of weight loss on surrogate biomarkers and imaging tests have been extensively demonstrated in real-world observational studies, but only a few RCTs are available and very few are based on histologic outcomes. An exhaustive analysis of this issue is outside the scope of this article, and several comprehensive reviews are available.[108-111] The targets of calorie restriction and physical activity are consistent among guidelines (Table 1). Both aerobic and resistance exercise and no specific diets are generally suggested, with a general indication to reduce simple sugars, industrial fructose and saturated fats, and with a preference for the Mediterranean diet in the European recommendations.[5] We shall discuss the most relevant observational studies and a few recent RCTs, offering clues to NAFLD management (Table 2).[112-119]

The first solid evidence for the beneficial effects of intensive lifestyle intervention (ILI) programs on NAFLD came from studies conducted using the strategy of the Diabetes Prevention Program,[120] based on cognitive-behavioural treatment carried out by a dedicated team. In individuals with/without T2DM,[112 113] ILI significantly reduced body weight and intra-hepatic fat, assessed by MRS,[126] and improved liver histology.[113] Of note, beneficial effects were also observed in control individuals achieving pre-defined weight loss targets (weight loss ≥7% of initial body weight).[113] The results were confirmed in a much larger sample of individuals with ultrasonographic-detected NAFLD, where ILI was also associated with improved metabolic and CV risk factors.[114] In a community-based study, ILI-treated subjects had a higher probability of NAFLD remission and reduced fibrosis (MRS and transient elastography) *vs.* standard care.[115] In the same population, a 7-10% weight loss was later confirmed to achieve clearance of liver fat in NAFLD with obesity, whereas a 3-5% was similarly effective in lean NAFLD (BMI <25 kg/m^2),[121] underlining the universal importance of diet and exercise to reduce NAFLD prevalence and disease progression, also improving health-related quality of life.[122]

Despite its observational nature, in 2015 the large Cuban experience signed a landmark step in support of the effectiveness of ILI in NAFLD, considering the large sample size and the

histologic assessment (293 cases, 261 follow-up biopsies).[116] The study confirmed a dose-response between weight loss at 12 months and NASH remission and set 10% weight loss as the target for fibrosis regression. Unfortunately, no data have been published on long-term follow-up, as well as on weight loss maintenance, the critical issue in behavioural treatment.

ILI requires a dedicated team, rarely present in liver units, and continuing patient/therapist interaction, limiting participation and adherence and increasing costs. These limits may be partly overcome by e-technology; in 278 motivated, young NAFLD patients, weight loss targets, dietary adherence and physical activity could be similarly achieved and maintained at 2-year follow-up by a web-based program, compared with a group-based educational approach, after adjustment for baseline differences.[117] The opportunities offered by new technologies for continuing motivation, support and education towards lifestyle changes need to be exploited. They will allow to reach larger groups of at-risk patients.

Finally, very few studies directly compared ILI and pharmacotherapy in NAFLD patients, using drugs approved for obesity or T2DM. A 26-week RCT did not demonstrate any difference between liraglutide (3mg/day) and ILI on weight loss, biochemistry and measures of fibrosis.[118] However, ILI was associated with sustained weight loss maintenance and reduced liver fat at follow-up, whereas weight regain and hepatic fat re-accumulation occurred after liraglutide stop.[119]

Bariatric surgery

Bariatric surgery very effectively promotes weight loss and weight loss maintenance; the effects on body weight largely exceed the 10% weight loss target associated with liver fat clearance, NASH resolution and fibrosis reversal. Accordingly, surgery candidates as a possible treatment to reduce NASH burden in patients fitting the agreed criteria for the management of obesity (BMI $\geq$40 kg/m^2 or BMI $\geq$35 with comorbidities). Roux-en-Y-gastric bypass and sleeve gastrectomy are the procedures of choice,[37 123] and surgical treatment becomes cost-effective in subjects at high risk of progression (F3 fibrosis).[124]

The evidence supporting bariatric surgery is exclusively derived from observational studies, where liver histology was measured at surgery and follow-up.[125] In 1236 cases, NAFLD improvement, including fibrosis regression, was associated with 5-year post-surgery weight loss.[123] Notably, NASH persistence one year after surgery was associated with less weight change (BMI, -9.1$\pm$1.5 kg/m^2) *vs.* NASH resolution (-12.3$\pm$0.6). In a retrospective analysis of a large insurance database, NAFLD patients with obesity who underwent bariatric surgery

were less likely to progress to cirrhosis *vs.* matched cases not receiving surgery (hazard ratio, 0.31; 95% confidence interval, 0.19-0.52).[126] In a bariatric French cohort prospectively-submitted to repeated biopsies, at 5 years NASH resolved, without fibrosis worsening, in 54/64 patients (84.4%; 95% CI, 73.1-92.2), while fibrosis decreased progressively along the years in 70.2% and completely disappeared in 56% of all cases (95% CI, 42.4-69.3), including 45.5% of patients with bridging fibrosis at baseline.[127] Cirrhosis *per se* does not contraindicate bariatric surgery, but requires a precise evaluation of hepatic functional reserve, portal hypertension and CV risk factors.[128]

Very recently, also bariatric/metabolic endoscopy has been proposed to facilitate rapid and large weight loss, particularly in type 2 diabetes. These procedures include endoscopic sleeve gastroplasty, endoscopic small-bowel by-pass and duodenal mucosal resurfacing. Although apparently safe and effective in the short-term,[129 130] many more data on histological outcomes and adverse events are needed for their extensive clinical application.

Drug treatment suggested by current clinical practice guidelines

Based on evidence from longitudinal studies, patients with intermediate and advanced fibrosis (F2-F4 fibrosis) are at greatest risk of overall and disease-specific mortality and have been identified as the target population for investigational drugs in phase 2-3 trials. As patients who are in pre-cirrhotic stages are not at short-term risk for liver-related outcomes, regulatory authorities accepted histological features as surrogates of liver-related events for accelerated or conditional approval with the requirement that additional studies are undertaken to demonstrate if short-term changes translate into reduced progression to cirrhosis and its complications.[131] The reversal of NASH (with no worsening of fibrosis) or the improvement of fibrosis (without NASH deterioration) are the endpoints for pre-cirrhotic patients, while in the cirrhotic population the main goals are to avoid decompensated cirrhosis, hepatocellular carcinoma, liver transplant and mortality. Thus, phase 2b and phase 3 trials require pre- and post-treatment liver biopsies to establish efficacy, a limitation that could change significantly in the future as newer non-invasive diagnostic methods are validated against biopsy.

No specific agents have so far been approved; nonetheless pioglitazone and vitamin E are frequently prescribed off label, following the results of large randomized studies with histologic end-points. Many more drugs have received or are undergoing evaluation in registered trials.

Pioglitazone

Pioglitazone is an antidiabetic agonist for peroxisome proliferator-activated receptor-γ (PPAR-γ), a member of a nuclear receptor family of proteins that modulate several responses, including insulin sensitivity. Its use in NAFLD has been proposed to counteract insulin resistance. Several RCTs and a meta-analysis [132] have consistently demonstrated an improvement in biochemistry and histology following pioglitazone administration at doses of 30-45 mg/day vs. placebo. In the PIVENS trial, also testing the effects of vitamin E, pioglitazone did not significantly improve NASH (34% vs. 19% in placebo), but aminotransferase levels were reduced, as were steatosis and lobular inflammation.[133] In 101 subjects with prediabetes or T2DM, pioglitazone (45 mg/day) was particularly effective, achieving the primary outcome (≥2 point improvement in NAS score without fibrosis worsening) in 58% of cases (*vs.* 17% in controls) and producing NASH resolution in 51% and change in fibrosis stage (-0.5 points; 95% CI, 0.0-0.9).[134] A more recent meta-analysis in 197 NASH patients and 195 controls confirmed that pioglitazone was associated with improvement of advanced fibrosis (OR, 4.53; 95% CI, 1.52-13.52) and in NASH resolution (OR, 3.51; 95% CI, 1.76-7.01).[135] Pioglitazone discontinuation is accompanied by an abrupt increase in ALT, possibly heralding NASH recurrence.[136] This makes pioglitazone the long-term pharmacologic treatment of choice, irrespective of T2DM. Notably, pioglitazone produces beneficial effects also on the CV system;[137 138] adverse events include increased body weight and an increased risk of non-osteoporotic fractures.

Vitamin E

Vitamin E has been proposed for the treatment of NAFLD, considering its anti-apoptotic and anti-oxidant properties, with conflicting results.[132] Following a series of negative data, in the PIVENS trial at the dose of 800IU/day, vitamin E was significantly better than placebo on NASH improvement (49% *vs.* 19%, respectively), as well as in reducing steatosis and lobular inflammation, without significant effects on fibrosis (41% *vs.* 31%; average change in score, -0.3 *vs.* -0.1). Accordingly, the U.S. guidelines consider the use of vitamin E in patients with biopsy-assessed NASH without diabetes or cirrhosis,[4] a recommendation not shared by the European guidelines.[5] A very recent trial in biopsy-proven NASH with T2DM, comparing vitamin E (800 IU/day) *vs.* vitamin E and pioglitazone (45mg/day) or placebo on the primary outcome (NAS reduction ≥2 points without worsening of fibrosis), found that only the combination therapy achieved the target (combination, 54%; vitamin E alone, 31%; placebo, 19%), although both treatments increased the rate of NASH resolution (43%, 33%, 12%,

respectively).[139] Fibrosis did not improve. As to safety, the evidence for increased all-cause mortality associated with a dose of 800IU/day, derived from an old meta-analysis, is no longer supported by data.[140] Vitamin E is the treatment of choice for paediatric NAFLD.[4]

Phase 3 drugs and hints at phase 2 (Tables 4-6)

Farnesoid X receptor (FXR) agonists

The farnesoid X receptor (FXR) belongs to the nuclear receptor superfamily mainly expressed in the liver, intestine, kidney and, to a lower extent, in adipose tissues. It regulates a wide variety of target genes critically involved in the control of bile acids, lipids and glucose (*via* augmented insulin sensitivity).[141] One of the many consequences of FXR activation is a decreased expression of enzymes involved in *de novo* lipogenesis; the release of fibroblast growth factor-19 (FGF19) from the intestine upon bile acid binding to FXR, major downstream mediators of FXR, potentiates FXR activity [141] and produces additional metabolic effects (PPAR-α activation and suppressed gluconeogenesis), decreased appetite and increased energy expenditure. Several FXR-activating drugs with differing structural characteristics and pharmacodynamic effects are thus under investigation in NAFLD.

Obeticholic acid (OCA), a 6α-ethyl derivative of chenodeoxycholic acid (CDCA), is a first-in-class selective FXR agonist, originally described for its anticholestatic and potentially broader hepatoprotective properties. The addition of the ethyl group to CDCA – the natural FXR agonist in human – approximately 100-fold multiplies its FXR agonistic activity.[141]

A phase 2B clinical trial of OCA (25 mg/day of oral OCA *vs.* placebo for 72 weeks) was terminated early following an interim pre-planned analysis at 24 weeks because of overt histological efficacy (≥2 points decrease in NAS, without worsening of fibrosis). 46/102 patients in the OCA group (45%) improved liver histology compared to 21/99 in placebo (relative risk 1.9, 95% CI 1.3-2.8).[142]

Obeticholic acid is currently being evaluated in phase 3 trial (REGENERATE, Intercept Pharmaceuticals) at doses of 10 and 25 mg/day *vs.* placebo in NASH with fibrosis; liver biopsies were scheduled at screening, at 18 and 48 months, and at the end of study. The results of the interim 18-month analysis in 931 patients with F2-F3 fibrosis have been recently published.[143] Improvement in fibrosis was achieved in 12% placebo-treated patients, 18% in the 10-mg OCA, and 23% in the 25-mg OCA group. The NASH resolution endpoint was not met in the whole intention-to-treat population (8%, 11% and 12%, respectively). However, a post-hoc analysis showed that approximately twice as many patients in 25 mg

OCA achieved NASH resolution *vs.* placebo, both by intention to treat (23% *vs.* 12%; relative risk, 1.9; 95% CI, 1.4-2.8) and per-protocol (29% *vs.* 16%, relative risk, 2.2; 95% CI, 1.4-3.2).[143] The evaluation is ongoing, to be completed by October 2022. Based on more than 1,700 patients treated with OCA, a dossier was submitted to the U.S. Food and Drug Administration (FDA) for regulatory approval, but the agency required additional efficacy and safety data to support accelerated approval, while continuing the long-term phase.[144]

Consistent with other OCA studies, dose-dependent pruritus, mild-to-moderate in severity and increased LDL cholesterol,[141] responsive to statin treatment, were the most commonly reported adverse events,[142 143] frequently leading to discontinuation. Combination studies of OCA with lipid-lowering agents are ongoing.

Other FXR-ligands are in earlier stages of clinical development. Tropifexor, a non-bile acid-derivative FXR agonist with potent activity on fibrosis in experimental NASH models,[145] is being evaluated in a phase 2, adaptive design NASH study (FLIGHT-FXR, Novartis). Treatment has been reported to cause a transient increase in serum ALT that decline with time, whereas the expected advantages *vs.* OCA on pruritus do not appear to be not fulfilled.

Another double-blind, multi-centre, phase 2b RCT is evaluating the safety and efficacy of a combination of tropifexor and cenicriviroc (see below) in patients with biopsy-proven NASH and advanced fibrosis (stages F2/F3).[146] Cilofexor, another non-steroidal FXR-ligand, is being evaluated alone or in combination with the acetyl-CoA carboxylase (ACC) inhibitor firsocostat and results are pending. In a phase 2 RCT, cilofexor alone was reported to decrease steatosis by over 30% at MRI-PDFF in 39% of cases at a daily dose of 100 mg for 24 weeks, in 14% at 30mg and in 13% on placebo, without any significant effect on fibrosis, measured by biomarkers and MRS-elastography.[147]

Elafibranor and Lanifibranor

Elafibranor is an oral, once-daily, first-in-class drug acting via dual agonism of PPAR-α/δ receptors, with proven efficacy in animal models of NASH and fibrosis. The pivotal phase 2 study (GOLDEN-505, GENFIT) tested elafibranor (80 and 120 mg *vs.* placebo) over 52 weeks in 276 patients with diagnosis of NASH and fibrosis (F0-F3); the primary outcome was set as defined by regulatory agencies, with several secondary outcomes.[148] The response rate was higher than placebo only in the 120-mg arm (19% vs 12%; OR, 2.31; 95% CI: 1.02-5.24), and was more pronounced with increasing baseline severity. In *post hoc* analysis, the exclusion of patients with mild activity revealed a significant effect of elafibranor 120 mg *vs.*

placebo (OR, 3.52; 95% CI: 1.32-9.40) in most severe cases (234 patients with NAS ≥4), doubling the proportion of responders. Both doses improved liver function tests and lipid parameters, and fasting serum glucose (-0.98 mmol/L at 120 mg) and HbA1c (-0.46%), in patients with T2DM (40% of total). Finally, elafibranor was safe and well tolerated.

Elafibranor was thus moved into a larger, confirmative phase 3 trial (RESOLVE-IT, GENFIT), to measure 4-year efficacy. At interim analysis, released on May 11, 2020, the trial did not achieve the expected results. The response rate on primary endpoint was 19.2% for elafibranor *vs.* 14.7% for placebo and the improvement of ≥1 fibrosis stage (key secondary endpoint) was 24.5% *vs.* 22.4%, respectively.[149] The trial was terminated early.

Another pan-PPAR agonist (lanifibranor, Inventiva) recently completed a phase 2b, biopsy-controlled study in 247 NASH patients receiving either 800 or 1200mg/day of active drug vs. placebo for 6 months. The primary endpoint was a 2-point reduction in the activity part of the SAF score [combining inflammation and ballooning] without worsening of fibrosis; the key secondary endpoints were NASH resolution without worsening of fibrosis and improvement of fibrosis without NASH worsening). The results, released on 15 June 2020, show that lanifibranor met both the primary (41% and 49% at the two doses *vs.* 27% on placebo) and the two secondary endpoints on intention to treat (33% and 45% *vs.* 19%; 34% and 44% *vs.* 9%).[150] The drug received the FDA designation as breakthrough therapy on 12 October 2020, intended to expedite the development of drugs candidate for serious or life-threatening conditions.[151]

Thyroid hormone receptor β agonists

Thyroid hormone receptor β (THR-β) is responsible for regulating specific metabolic pathways in the liver, frequently impaired in NAFLD, making NAFLD a condition of "hepatic hypothyroidism".[152] Resmetirom (MGL-3196- Madrigal Pharmaceutical) is a once daily, oral, highly selective agonist of THR-β specifically acting in the liver, without systemic effects (mediated through THR-α in the heart and bone).[152] The mechanism by which resmetirom reduces hepatic fat in NASH is probably dependent on the restoration of normal mitochondrial function and increased β oxidation.

Resmetirom was initially tested in a phase 2 quadruple-blind (participant, care provider, investigator, outcome assessors) RCT on 125 participants with ≥10% liver fat content at MRI-PDFF and biopsy-proven NASH (fibrosis F1-F3 and disease activity).[153] The primary outcome was the relative change from baseline in MRI-PDFF. Compared with placebo,

resmetirom significantly reduced MRI-PDFF from baseline, both after 12 weeks (least squares mean difference, -22.5; 95% CI, -32.9 to -12.2) and after 36 weeks (-28.8; -42.0 to -15.7), reduced the markers of liver injury and fibrosis, and finally reduced disease activity and prompted NASH resolution at liver biopsy in the drug-respondent cohort. Resmetirom was generally well tolerated. The most common adverse events were diarrhoea and nausea.

Two phase 3 resmetirom trials, MAESTRO-NASH and MAESTRO-NAFLD1, are ongoing. MAESTRO-NASH is estimated to be completed in 2024. It will include 2000 adults with biopsy-proven non-cirrhotic NASH and fibrosis. MAESTRO NAFLD1 study has recently started and will include 700 adults with MRI-PDFF liver fat fraction ≥8% and suspected NASH, randomized into four arms: open label, placebo (double-blind), resmetirom 80 mg (double-blind), resmetirom 100 mg (double-blind). The primary outcome is the incidence of adverse events after 52 weeks of treatment.

A second selective THR-β agonist (VK-2809, Viking Therapeutics) is currently being tested in a phase 2b trial in subjects with biopsy-proven NASH for 52 weeks. The results of a daily dose of 5 mg, 10 mg, or 10 mg on alternate days or placebo were extremely interesting, with an overall responder rate on >30% relative reduction in MRI-PDFF at 12 weeks of 88% *vs.* 17% in placebo.[154] Notably, alternate-day administration produced results comparable to the 5 mg/day dose, and lower doses are being tested in phase 2b (1-2.5 mg). The drug was safe and well tolerated, with no serious adverse events reported in the course of the study.

Cenicriviroc

Cenicriviroc is a once-daily oral drug that blocks two chemokine receptors, CCR2 and CCR5, involved in inflammatory and fibrogenic pathways. CCRs normally link C-C motif chemokine ligand, overexpressed in liver injury by activated Kuppfer cells or damaged hepatocyte.[155] Cenicriviroc inhibits monocyte recruitment, thereby modulating the hepatic macrophage pool toward less inflammatory and less fibrogenic macrophages.

Cenicriviroc has an established anti-inflammatory and antifibrotic activity in animal models of liver disease; in humans it has been used in HIV infection and, more recently, in NASH. In the phase 2 CENTAUR study (Tobira Therapeutics),[156] cenicriviroc has been tested in 289 participants with biopsy proven NASH (NAS≥4), and liver fibrosis (stages F1-F3). The primary endpoint was reached in a similar proportion of subjects on CVC (n=145, 16%) and placebo (n=144, 19%; OR, 0.82; 95% CI, 0.44-1.52), and NASH resolution was similarly not different (8% vs. 6%; OR, 1.40; 95% CI, 0.54-3.63). However, twice as many subjects on

cenicriviroc achieved improvement in fibrosis by ≥1 stage and no worsening of NASH *vs.* placebo (20% *vs.* 10%; OR, 2.20; 95% CI, 1.11-4.35). There were no differences in body weight and noninvasive biomarkers; safety and tolerability were comparable to placebo.

The 2-year results have recently been published, with a group of placebo-treated patients moved to cenicriviroc: group A (cenicriviroc for 2 years), group C (placebo for 2 years) and group B (crossover group). The primary endpoint (≥2-point improvement in NAS with ≥1-point improvement in either lobular inflammation or hepatocellular ballooning, no worsening of fibrosis) was again not met.[157]

A phase 3 cenicriviroc study (AURORA) in currently ongoing. It will involve up to 2000 adults, aged 18-75 years with NASH and fibrosis F2-F3, that will be followed-up for 5 years. Primary efficacy endpoints will also include time to occurrence of first adjudicated event: death, histopathologic progression to cirrhosis, liver transplant, model of end-stage liver disease (MELD) score ≥15, ascites, hospitalization due to liver failure.

The TANDEM trial is a phase 2b 48-week study in 200 adult patients with NASH and biopsy proven fibrosis (F2-F3) that will evaluate the safety and efficacy of a combination of cenicriviroc and tropifexor (LJN452, Novartis) in patients with NASH and fibrosis.[146]

Aramchol

Aramchol is a synthetic lipid molecule obtained by conjugating cholic acid and arachidic acid. Aramchol inhibits the liver enzyme stearoyl coenzyme A desaturase (SCD), reducing fatty acid synthesis while increasing fatty acid oxidation, with a lipid lowering effect, mainly via upregulation of the ABCA1 cholesterol transporter. Aramchol was shown to reduce liver fat in animal models with diet-induced fatty liver.[158]

In a phase 2 randomized, double-blind, placebo-controlled trial, aramchol (100-300 mg/day) or placebo were administered to 60 patients with biopsy-confirmed NAFLD (six with NASH) (NCT01094158). The primary aim was to test whether aramchol would safely and effectively reduce liver fat concentration (MRS-assessment). Over 3 months, liver fat content decreased by 12.6-22.1% in patients given 300 mg/day aramchol, by 2.9-28.2% with 100-mg aramchol, and increased in the placebo group. No serious adverse events were observed.[159]

A second multicentre, randomized, double blind, placebo-controlled phase 2b study evaluated the efficacy and safety of higher aramchol doses (400 and 600 mg) in NASH with overweight or obesity and diabetes or pre-diabetes (247 subjects, 52 weeks, and 13-week follow-up). The primary outcome was percent change in intra-hepatic triglyceride concentration measured by

MRS; histology was a secondary outcome. The study, only reported in abstract form,[160] confirmed that a larger number of patients in the aramchol 600 mg arm achieved NASH resolution without worsening of fibrosis (16.7% *vs.* 5% in placebo; OR, 4.74; 95% CI, 0.99-22.66), also improving biochemistry. A phase 3 RCT (ARMOR) is recruiting 2000 patients at high risk of progression. Subjects are randomized to receive aramchol 300 mg bid or matching placebo. Primary outcomes are the effects on liver histology at 52 weeks and the effects on composite long-term outcomes (all-cause mortality, transplant, hospitalization due to hepatic decompensation) at 5 years.

Glucagon-like peptide-1 receptor agonists

Glucagon-like peptide-1 (GLP-1) is an intestinal hormone released from L-cells in the small intestine in response to meals with multiple metabolic effects: it stimulates insulin secretion and inhibits glucagon secretion, increases energy disposal, delays gastric emptying and improves satiety.[161] GLP-1 analogues are commonly used to treat diabetes, and several studies incidentally reported a significant reduction of liver fat in response to treatment.[162]

Liraglutide is a long-acting human GLP-1 analogue licensed for glycaemic control in patients with type 2 diabetes. A meta-analysis based on individual patient data of registration trials with liraglutide (LEAD program, 2241 patients with elevated aminotransferase levels) confirmed a significant reduction of liver enzymes in response to treatment, and a trend towards reduced steatosis in the LEAD-2 study). Daily injection of liraglutide for 48 weeks improved NASH histology in a small phase 2 study (Liraglutide Efficacy and Action in NASH – LEAN study).[163] 9/23 patients who received liraglutide (39%) had resolution of NASH compared with 2/22 (9%) on placebo (relative risk, 4.3; 95% CI, 1.0–17.7). Notably, treatment with liraglutide was associated with significant weight loss (mean difference *vs.* placebo, -4.4 kg; 95% CI, -7.2 to -1.6). Adverse events included gastrointestinal disorders in 81% of liraglutide-treated patients and 65% in the placebo group.

A phase 2 study of semaglutide, a longer-acting, weekly dosing GLP-1 analogue, has recently been completed. A preliminary release after 72 weeks of therapy announced that 33/56 patients (59%) with fibrosis F2-F3 met the usual primary end-point with the highest dosage tested (0.4 mg) *vs.* 10/58 patients (17%) in the control arm.[164] Among patients taking the 0.1-0.2 mg doses, 40% and 36% achieved the end-point, respectively. Semaglutide is very effective on weight loss; a phase 3-4 trial in obesity reported a mean weight loss of 14.9% with semaglutide 2.4 mg/week for 68 weeks vs. 2.4% in placebo, and additional weight loss at follow up (to 17.4%), contrary to placebo-treated individuals who regained weight.[165]

Synergistic effects may be achieved by combining GLP-1RAs with lifestyle intervention,[166] with gastric inhibitory polypeptide (GIP) or glucagon receptor agonists. Treatment with a GIP/GLP-1 combined agonist, tirzepatide, improved several NASH biomarkers *vs.* placebo and, in part, *vs.* dulaglutide, another weekly-dosing GLP-1 receptor agonist.[167] Differences were partly explained by the larger weight loss achieved by tirzepatide treatment.

Drugs for selected cohorts

Individuals with T2DM constitute a relevant cohort of NASH patients, at higher risk of disease progression, and requiring pharmacologic control of their metabolic defects. A few classes of antidiabetic agents have demonstrated significant effects on liver enzymes and surrogate biomarkers of steatosis and fibrosis, potentially reducing the risk of end-stage liver disease. Trials with GLP-1RAs have previously been discussed; several cohort studies are also available supporting a beneficial effect of long-acting GLP-1RAs,[168] potentially making these drugs the treatment of choice in the presence of NASH, also improving CV outcomes.

MSDC-0602 (Cirius Therapeutics), an insulin sensitizer of the tiazolidinedione class acting through modulation of mitochondrial-pyruvate carrier with minimum PPAR-γ activity, although failing primary and secondary histologic outcomes in the general NASH population, fulfilled some end-points in the T2DM subset;[169] accordingly, a specific trial is still ongoing in NASH with fibrosis and diabetes.

Gliflozins, the sodium-glucose cotransporter-2 inhibitors (SGLT-2Is), by blocking glucose resorption from the proximal tubule, promote glycosuria, calorie waste and weight loss. This possibly translates into reduced lipid burden to the liver. Most approved gliflozins have been tested for their effects on biomarkers of steatosis and fibrosis,[170-172] and other compounds are under scrutiny, but very few histologic data are available. A network meta-analysis of 29 RCTs confirmed that gliflozin treatment was significantly associated with weight loss ≥5% *vs.* placebo (dapagliflozin 10 mg: OR, 8.57; 95% credible interval, 2.71-27.44; empagliflozin 25 mg: 10.20; 4.59-28.93).[173]

Unfortunately, very few comparative analyses exist on the impact of different antidiabetic treatments on liver disease progression in NAFLD with diabetes.[174]

Other compounds

Several other drugs, not discussed above and acting on different biochemical processes, are under investigation in phase 2 trials. Among them, nor-ursodeoxycholic acid (1500 mg/day), also under testing in primary biliary cholangitis, showed a reduction of serum ALT *vs.*

placebo in a 12-week RCT (mean difference, -27.8; 95% CI, -34.7 to -14.4) without relevant side-effects, but too few data on MRS-PDFF and liver stiffness were available to derive firm conclusions.[175] Much interest has also been given to an engineered version of fibroblast growth factor (FGF)-19 and to pegylated FGF-21, able to stimulate adiponectin secretion, thus reducing insulin resistance and inflammation, as well as to reduce body weight.

Placebo & risk stratification in clinical trials

Stratification is essential to define treatment effectiveness. T2DM highly impacts on the response rate of drugs; as an example, in the CENTAUR study,[156] the primary end point was achieved in 20% of cases in the experimental arm *vs.* 10.4% on placebo (OR 2.20); however, the drug was much more effective in subjects without diabetes (OR, 3.84; 95% CI, 1.26-11.7) *vs.* diabetes patients (1.40; 0.59-3.35).

Active changes in lifestyle may contribute to the heterogeneous and often high rate of "placebo response", driven by possible modifications in lifestyle during trial (Hawthorne effect). In a recent systematic review and meta-analysis of placebo groups from 39 histology-based RCTs of adults with NASH,[176] 25% of patients in the placebo groups (95% CI, 20%-30%) improved activity by ≥2 points, and 21% improved fibrosis, liver fat and liver enzymes.

A very recent document by the Liver Forum highlighted that only 26% of pharmacologic RCTs had nutritional counselling and/or exercise recommendations, 22% had undefined recommendation and 52% did not report interventions.[177] A similar bias is present in studies involving nutritional counselling and/or physical activity, where the placebo response was variable.[177] Clinical trials in diabetes and obesity confirm the importance of stable lifestyle prior to screening, as well as the need for improved delivery and reporting of lifestyle recommendations. The Liver Forum recommends that patients enrolled should: 1) be evaluated at screening for current diet and exercise habits; 2) have lifestyle stability prior to baseline screening; 3) be individually counselled on improving diet and physical activity, and decreasing sedentary behaviour; 4) all these practices should be appropriately documented throughout the trial.[177] Finally, changes in body weight and physical activity should be recorded and included in final analysis to avoid potential biases. Quantification of alcohol intake is also a challenging matter, with consistent variability across drinking patterns within NAFLD threshold, likely to influence the results.[178] Finally, gene polymorphisms associated with NASH (*PNPLA3 I148M* and *TM6SF2 E167K*), are likely to impact on trial response.

Follow-up and Surveillance

The presence of NASH and significant fibrosis prompt to systematic follow-up and surveillance, but four intertwined questions are still unanswered, both in community patients and in selected cohorts following a liver biopsy: a) who should be monitored? b) who should be responsible for surveillance? b) by which instruments? c) how frequently?

European guidelines suggest that patients at low risk of progression might be reconsidered at 2-year interval by surrogate biomarkers and eventually by ultrasonography or transient elastography.[5] This time interval is expanded to three years in NICE guideline.[10] Metabolic improvement is associated with reduced steatosis, measurable by FLI, largely heralded by weight loss.[179] Imaging modalities for a precise quantification of steatosis (e.g., MRI-PDFF) should be limited to research settings.[180]

Surrogate serum markers of hepatic inflammation, including ALT, show an overall correlation with the risk of fibrosis progression in large cohorts but are scarcely predictive of progression/regression on an individual basis. Nevertheless, sustained reduction or normalization of elevated ALT can be considered clinically meaningful end-points.[181]

Considering the obvious limitations to an extensive use of liver biopsy, changes in non-invasive biomarkers of fibrosis and transient elastography are at present the best tools to monitor disease progression,[74] although very few data are available on day-to-day variability and their correspondence with histological changes. A better performance is expected by new biomarkers reflecting fibrogenic activity,[182] or by MRE-elastography (15% worsening of liver stiffness on MRE is associated with fibrosis progression at histology).[183]

Monitoring and surveillance of patients with NAFLD need to be tailored on disease severity and resource availability,[5] in a complex network including primary care physicians as well as specialists of different branches. This will help detect early hepatic decompensation, prompting treatment and eventually inclusion in the waiting list for transplantation,[184] with limits due to CV comorbidities.[185-187]

There are no specific strategies for NASH-induced HCC screening, excluding the evidence-based procedures for cirrhosis (6-month ultrasonography),[188] but more than half of HCC arise in non-cirrhotic patients. Although the incidence is insufficiently high to deserve universal surveillance in non-advanced patients, the lack of systematic surveillance in pre-cirrhotic stages may be the reason for late HCC diagnosis.[63] We need to prospectively acquire

information on cohorts of patients with NASH, in order to define high-risk patients who should undergo surveillance at earlier stages.

Conclusions

Forty years after the original description of NAFLD, we have learnt a lot regarding its epidemiology and natural history, its pathogenesis, the underlying genetic background and the risks associated with disease progression, as well as the costs associated with disease. The condition produces a relevant impact on patients' quality of life, as it is expected to become the principal liver disease in future decades. However, we still lack a satisfactory treatment, and weight loss remains the treatment of choice. A matter of concern is the demonstration that epigenetic drives and/or obesity in childhood or young adulthood might be linked with the risks of cancer and liver failure in later life by a *fil rouge*,[39 189 190] having liver fat accumulation as common mechanism.[66]

The very high number of patients cannot be managed by specialists, and only selected cohorts at high risk of progression should be referred to their care. Initial experiences of network healthcare have provided interesting results,[76] and need to be exploited to larger samples. Meanwhile, accurate profiling of NAFLD individuals will help dissect different phenotypes to refine drug treatments, as well as plan sequential treatments based on disease stage.

Preventive healthcare strategies based on food-related policies to counteract the epidemics of obesity remain a priority to reduce the burden of NAFLD in the general population. Political commitment and concerted actions of the multiple stakeholders involved in prevention and treatment should be mandatory, but very few European countries have so far defined policies to tackle NAFLD in the community.[191] The proactive involvement of patients' associations is highly recommended to include patient-reported outcomes among relevant targets of future large-scale randomized and observational studies.[192 193]

COMPETING INTERESTS:

We have read and understood the BMJ policy on declaration of interests and declare the following interests: MLP participated in advisory board of NOVO NORDISK; LB declare none; EB received a grant from GILEAD and participated in advisory boards of BMS, GENFIT, GILEAD, INTERCEPT, INVENTIVA, NOVO-NORDISK, PFIZER; GM received honoraria from ELI LILLY and participated in Advisory boards of GILEAD, NOVARTIS, ASTRA-ZENECA, PFIZER, MUNDIPHARMA.

CONTRIBUTORSHIP STATEMENT AND GUARANTOR:

MLP and LB searched literature and drafted parts of manuscript; EB and GM planned the study, drafted parts of manuscript and critically revised the manuscript; all authors approved the final version. GM acts as guarantor

LICENCE TO PUBLISH:

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, a worldwide licence to the Publishers and its licensees in perpetuity, in all forms, formats and media (whether known now or created in the future), to i) publish, reproduce, distribute, display and store the Contribution, ii) translate the Contribution into other languages, create adaptations, reprints, include within collections and create summaries, extracts and/or, abstracts of the Contribution, iii) create any other derivative work(s) based on the Contribution, iv) to exploit all subsidiary rights in the Contribution, v) the inclusion of electronic links from the Contribution to third party material where-ever it may be located; and, vi) licence any third party to do any or all of the above.

References

1. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experience with an hitherto unnamed disease. *Mayo Clin Proc* 1980;55(7):434-8. [published Online First: 1980/07/01]
2. Hui JM, Kench JG, Chitturi S, et al. Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. *Hepatology* 2003;38(2):420-7. doi: 10.1053/jhep.2003.50320 [published Online First: 2003/07/29]
3. Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: From cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002;123(1):134-40. doi: 10.1053/gast.2002.34168 [published Online First: 2002/07/10]
4. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;67(1):328-57. doi: 10.1002/hep.29367 [published Online First: 2017/07/18]
5. European Association for the Study of the Liver, European Association for the Study of Diabetes, European Association for the Study of Obesity. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64(6):1388-402. doi: 10.1016/j.jhep.2015.11.004 [published Online First: 2016/04/12]
6. Marchesini G, Brizi M, Morselli-Labate AM, et al. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999;107(5):450-5. doi: 10.1016/s0002-9343(99)00271-5 [published Online First: 1999/11/24]
7. Marchesini G, Brizi M, Bianchi G, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001;50(8):1844-50. doi: 10.2337/diabetes.50.8.1844 [published Online First: 2001/07/27]
8. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003;37(4):917-23. doi: 10.1053/jhep.2003.50161 [published Online First: 2003/04/02]
9. Eslam M, Sanyal AJ, George J, International Consensus Panel. MAFLD: A consensus-driven proposed nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020;158(7):1999-2014 e1. doi: 10.1053/j.gastro.2019.11.312 [published Online First: 2020/02/12]
10. NICE. Non-alcoholic fatty liver disease (NAFLD): assessment and management: National Institute for Health and Care Excellence 2016.
11. Wong VW, Chan WK, Chitturi S, et al. Asia-Pacific working party on non-alcoholic fatty liver disease guidelines 2017-Part 1: Definition, risk factors and assessment. *J Gastroenterol Hepatol* 2018;33(1):70-85. doi: 10.1111/jgh.13857 [published Online First: 2017/07/04]
12. Chitturi S, Wong VW, Chan WK, et al. The Asia-Pacific Working Party on Non-alcoholic Fatty Liver Disease guidelines 2017-Part 2: Management and special groups. *J Gastroenterol Hepatol* 2018;33(1):86-98. doi: 10.1111/jgh.13856 [published Online First: 2017/07/12]
13. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;64(1):73-84. doi: 10.1002/hep.28431 [published Online First: 2015/12/29]
14. Yki-Jarvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol* 2014;2(11):901-10. doi: 10.1016/S2213-8587(14)70032-4 [published Online First: 2014/04/16]

15. Ye Q, Zou B, Yeo YH, et al. Global prevalence, incidence, and outcomes of non-obese or lean non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020;5(8):739-52. doi: 10.1016/S2468-1253(20)30077-7 [published Online First: 2020/05/16]
16. Bellentani S, Saccoccio G, Masutti F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000;132(2):112-7. doi: 10.7326/0003-4819-132-2-200001180-00004 [published Online First: 2000/01/22]
17. Saadeh S, Younossi ZM, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002;123(3):745-50. doi: 10.1053/gast.2002.35354 [published Online First: 2002/08/29]
18. Thomsen C, Becker U, Winkler K, et al. Quantification of liver fat using magnetic resonance spectroscopy. *Magn Reson Imaging* 1994;12(3):487-95. doi: 10.1016/0730-725x(94)92543-7 [published Online First: 1994/01/01]
19. Kotronen A, Westerbacka J, Bergholm R, Pietilainen KH, Yki-Jarvinen H. Liver fat in the metabolic syndrome. *J Clin Endocrinol Metab* 2007;92(9):3490-7. doi: 10.1210/jc.2007-0482 [published Online First: 2007/06/28]
20. Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006;6:33. doi: 10.1186/1471-230X-6-33 [published Online First: 2006/11/04]
21. Ioannou GN, Weiss NS, Boyko EJ, Mozaffarian D, Lee SP. Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. *Hepatology* 2006;43(5):1145-51. doi: 10.1002/hep.21171 [published Online First: 2006/04/22]
22. Lazo M, Hernaez R, Eberhardt MS, et al. Prevalence of nonalcoholic fatty liver disease in the United States: the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* 2013;178(1):38-45. doi: 10.1093/aje/kws448 [published Online First: 2013/05/25]
23. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004;40(6):1387-95. doi: 10.1002/hep.20466 [published Online First: 2004/11/27]
24. Taylor RS, Taylor RJ, Bayliss S, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Gastroenterology* 2020;158(6):1611-25 e12. doi: 10.1053/j.gastro.2020.01.043 [published Online First: 2020/02/07]
25. Hagstrom H, Talback M, Andreasson A, Walldius G, Hammar N. Ability of noninvasive scoring systems to identify individuals in the population at risk for severe liver disease. *Gastroenterology* 2020;158(1):200-14. doi: 10.1053/j.gastro.2019.09.008 [published Online First: 2019/09/30]
26. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45(4):846-54. doi: 10.1002/hep.21496 [published Online First: 2007/03/30]
27. Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007;46(1):32-6. doi: 10.1002/hep.21669 [published Online First: 2007/06/15]
28. Guha IN, Parkes J, Roderick P, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008;47(2):455-60. doi: 10.1002/hep.21984 [published Online First: 2007/11/27]
29. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological

lesions. Am J Gastroenterol 1999;94(9):2467-74. doi: 10.1111/j.1572-0241.1999.01377.x [published Online First: 1999/09/14]
30. Petta S, Di Marco V, Pipitone RM, et al. Prevalence and severity of nonalcoholic fatty liver disease by transient elastography: Genetic and metabolic risk factors in a general population. Liver Int 2018;38(11):2060-68. doi: 10.1111/liv.13743 [published Online First: 2018/03/27]
31. Rinella M, Charlton M. The globalization of nonalcoholic fatty liver disease: Prevalence and impact on world health. Hepatology 2016;64(1):19-22. doi: 10.1002/hep.28524 [published Online First: 2016/03/02]
32. Portillo-Sanchez P, Bril F, Maximos M, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. J Clin Endocrinol Metab 2015;100(6):2231-8. doi: 10.1210/jc.2015-1966 [published Online First: 2015/04/18]
33. Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. Metabolism 2016;65(8):1096-108. doi: 10.1016/j.metabol.2016.01.001 [published Online First: 2016/02/10]
34. Hossain N, Afendy A, Stepanova M, et al. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7(11):1224-9, 29 e1-2. doi: 10.1016/j.cgh.2009.06.007 [published Online First: 2009/06/30]
35. Jarvis H, Craig D, Barker R, et al. Metabolic risk factors and incident advanced liver disease in non-alcoholic fatty liver disease (NAFLD): A systematic review and meta-analysis of population-based observational studies. PLoS Med 2020;17(4):e1003100. doi: 10.1371/journal.pmed.1003100 [published Online First: 2020/05/01]
36. Wei JL, Leung JC, Loong TC, et al. Prevalence and severity of nonalcoholic fatty liver disease in non-obese patients: A population study using proton-magnetic resonance spectroscopy. Am J Gastroenterol 2015;110(9):1306-14; quiz 15. doi: 10.1038/ajg.2015.235 [published Online First: 2015/07/29]
37. Lassailly G, Caiazzo R, Buob D, et al. Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. Gastroenterology 2015;149(2):379-88; quiz e15-6. doi: 10.1053/j.gastro.2015.04.014 [published Online First: 2015/04/29]
38. Anderson EL, Howe LD, Jones HE, et al. The prevalence of non-alcoholic fatty liver disease in children and adolescents: A systematic review and meta-analysis. PLoS One 2015;10(10):e0140908. doi: 10.1371/journal.pone.0140908 [published Online First: 2015/10/30]
39. Hagstrom H, Stal P, Hultcrantz R, Hemmingsson T, Andreasson A. Overweight in late adolescence predicts development of severe liver disease later in life: A 39years follow-up study. J Hepatol 2016;65(2):363-8. doi: 10.1016/j.jhep.2016.03.019 [published Online First: 2016/06/21]
40. Ekstedt M, Franzen LE, Mathiesen UL, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44(4):865-73. doi: 10.1002/hep.21327 [published Online First: 2006/09/29]
41. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41(6):1313-21. doi: 10.1002/hep.20701 [published Online First: 2005/05/26]
42. Bedossa P, FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. Hepatology 2014;60(2):565-75. doi: 10.1002/hep.27173 [published Online First: 2014/04/23]
43. Singh S, Allen AM, Wang Z, et al. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy

studies. Clin Gastroenterol Hepatol 2015;13(4):643-54 e1-9; quiz e39-40. doi: 10.1016/j.cgh.2014.04.014 [published Online First: 2014/04/29]
44. Sanyal AJ, Harrison SA, Ratziu V, et al. The natural history of advanced fibrosis due to nonalcoholic steatohepatitis: Data from the simtuzumab trials. Hepatology 2019;70(6):1913-27. doi: 10.1002/hep.30664 [published Online First: 2019/04/18]
45. Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, et al. Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease: A multi-national cohort study. Gastroenterology 2018;155(2):443-57 e17. doi: 10.1053/j.gastro.2018.04.034 [published Online First: 2018/05/08]
46. Stepanova M, Younossi ZM. Independent association between nonalcoholic fatty liver disease and cardiovascular disease in the US population. Clin Gastroenterol Hepatol 2012;10(6):646-50. doi: 10.1016/j.cgh.2011.12.039 [published Online First: 2012/01/17]
47. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. J Hepatol 2016;65(3):589-600. doi: 10.1016/j.jhep.2016.05.013 [published Online First: 2016/05/24]
48. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med 2010;363(14):1341-50. doi: 10.1056/NEJMra0912063 [published Online First: 2010/10/01]
49. Fracanzani AL, Burdick L, Raselli S, et al. Carotid artery intima-media thickness in nonalcoholic fatty liver disease. Am J Med 2008;121(1):72-8. doi: 10.1016/j.amjmed.2007.08.041 [published Online First: 2008/01/12]
50. Lonardo A, Nascimbeni F, Mantovani A, Targher G. Hypertension, diabetes, atherosclerosis and NASH: Cause or consequence? J Hepatol 2018;68(2):335-52. doi: 10.1016/j.jhep.2017.09.021 [published Online First: 2017/11/11]
51. Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol 2016;31(5):936-44. doi: 10.1111/jgh.13264 [published Online First: 2015/12/17]
52. Sung KC, Wild SH, Byrne CD. Resolution of fatty liver and risk of incident diabetes. J Clin Endocrinol Metab 2013;98(9):3637-43. doi: 10.1210/jc.2013-1519 [published Online First: 2013/07/23]
53. Yamazaki H, Tsuboya T, Tsuji K, Dohke M, Maguchi H. Independent association between improvement of nonalcoholic fatty liver disease and reduced incidence of type 2 diabetes. Diabetes Care 2015;38(9):1673-9. doi: 10.2337/dc15-0140 [published Online First: 2015/07/15]
54. Taylor R, Al-Mrabeh A, Zhyzhneuskaya S, et al. Remission of human type 2 diabetes requires decrease in liver and pancreas fat content but is dependent upon capacity for beta cell recovery. Cell Metab 2018;28(4):547-56 e3. doi: 10.1016/j.cmet.2018.07.003 [published Online First: 2018/08/07]
55. Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: A meta-analysis. Diabetes Care 2018;41(2):372-82. doi: 10.2337/dc17-1902 [published Online First: 2018/01/24]
56. Hagstrom H, Nasr P, Ekstedt M, et al. Risk for development of severe liver disease in lean patients with nonalcoholic fatty liver disease: A long-term follow-up study. Hepatol Commun 2018;2(1):48-57. doi: 10.1002/hep4.1124 [published Online First: 2018/02/07]
57. Younes R, Bugianesi E. NASH in lean individuals. Semin Liver Dis 2019;39(1):86-95. doi: 10.1055/s-0038-1677517 [published Online First: 2019/01/18]

58. Dela Cruz AC, Bugianesi E, George J, et al. Characteristics and long-term prognosis of lean patients with nonalcoholic fatty liver disease. *Gastroenterology* 2014;146(5):S-909.
59. Chen F, Esmaili S, Rogers GB, et al. Lean NAFLD: A distinct entity shaped by differential metabolic adaptation. *Hepatology* 2020;71(4):1213-27. doi: 10.1002/hep.30908 [published Online First: 2019/08/24]
60. Younossi ZM, Otgonsuren M, Henry L, et al. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015;62(6):1723-30. doi: 10.1002/hep.28123 [published Online First: 2015/08/15]
61. Younes R, Bugianesi E. Should we undertake surveillance for HCC in patients with NAFLD? *J Hepatol* 2018;68(2):326-34. doi: 10.1016/j.jhep.2017.10.006 [published Online First: 2017/11/11]
62. Dyson J, Jaques B, Chattopadyhay D, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. *J Hepatol* 2014;60(1):110-7. doi: 10.1016/j.jhep.2013.08.011 [published Online First: 2013/08/28]
63. Marrero JA, Fontana RJ, Su GL, et al. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology* 2002;36(6):1349-54. doi: 10.1053/jhep.2002.36939 [published Online First: 2002/11/26]
64. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005;129(1):113-21. doi: 10.1053/j.gastro.2005.04.014 [published Online First: 2005/07/14]
65. Piscaglia F, Svegliati-Baroni G, Barchetti A, et al. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: A multicenter prospective study. *Hepatology* 2016;63(3):827-38. doi: 10.1002/hep.28368 [published Online First: 2015/11/26]
66. Allen AM, Hicks SB, Mara KC, Larson JJ, Therneau TM. The risk of incident extrahepatic cancers is higher in non-alcoholic fatty liver disease than obesity - A longitudinal cohort study. *J Hepatol* 2019;71(6):1229-36. doi: 10.1016/j.jhep.2019.08.018 [published Online First: 2019/08/31]
67. Marchesini G, Petroni ML, Cortez-Pinto H. Adipose-tissue-associated cancer risk: Is it the fat around the liver, or the fat inside the liver? *J Hepatol* 2019;71(6):1073-75. doi: 10.1016/j.jhep.2019.09.020 [published Online First: 2019/10/07]
68. Wilson JMG, Jungner G. Principles and Practice of Screening for Disease. Geneva: World Health Organization, 1968.
69. Toplak H, Stauber R, Sourij H. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease: guidelines, clinical reality and health economic aspects. *Diabetologia* 2016;59(6):1148-9. doi: 10.1007/s00125-016-3941-4 [published Online First: 2016/04/08]
70. Byrne CD, Targher G. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease: is universal screening appropriate? *Diabetologia* 2016;59(6):1141-4. doi: 10.1007/s00125-016-3910-y [published Online First: 2016/04/08]
71. Crossan C, Tsochatzis EA, Longworth L, et al. Cost-effectiveness of non-invasive methods for assessment and monitoring of liver fibrosis and cirrhosis in patients with chronic liver disease: systematic review and economic evaluation. *Health Technol Assess* 2015;19(9):1-409, v-vi. doi: 10.3310/hta19090 [published Online First: 2015/01/31]

72. Phisalprapa P, Supakankunti S, Charatcharoenwitthaya P, et al. Cost-effectiveness analysis of ultrasonography screening for nonalcoholic fatty liver disease in metabolic syndrome patients. *Medicine (Baltimore)* 2017;96(17):e6585. doi: 10.1097/MD.0000000000006585 [published Online First: 2017/04/27]
73. Tanajewski L, Harris R, Harman DJ, et al. Economic evaluation of a community-based diagnostic pathway to stratify adults for non-alcoholic fatty liver disease: a Markov model informed by a feasibility study. *BMJ Open* 2017;7(6):e015659. doi: 10.1136/bmjopen-2016-015659 [published Online First: 2017/07/07]
74. Boursier J, Vergniol J, Guillet A, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. *J Hepatol* 2016;65(3):570-8. doi: 10.1016/j.jhep.2016.04.023 [published Online First: 2016/05/07]
75. Eddowes PJ, Sasso M, Allison M, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2019;156(6):1717-30. doi: 10.1053/j.gastro.2019.01.042 [published Online First: 2019/01/29]
76. Srivastava A, Gailer R, Tanwar S, et al. Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease. *J Hepatol* 2019;71(2):371-78. doi: 10.1016/j.jhep.2019.03.033 [published Online First: 2019/04/10]
77. Srivastava A, Jong S, Gola A, et al. Cost-comparison analysis of FIB-4, ELF and fibroscan in community pathways for non-alcoholic fatty liver disease. *BMC Gastroenterol* 2019;19(1):122. doi: 10.1186/s12876-019-1039-4 [published Online First: 2019/07/13]
78. Marjot T, Sbardella E, Moolla A, et al. Prevalence and severity of non-alcoholic fatty liver disease are underestimated in clinical practice: impact of a dedicated screening approach at a large university teaching hospital. *Diabet Med* 2018;35(1):89-98. doi: 10.1111/dme.13540 [published Online First: 2017/11/03]
79. Patel PJ, Banh X, Horsfall LU, et al. Underappreciation of non-alcoholic fatty liver disease by primary care clinicians: limited awareness of surrogate markers of fibrosis. *Intern Med J* 2018;48(2):144-51. doi: 10.1111/imj.13667 [published Online First: 2017/10/31]
80. Croci I, Coombes JS, Bucher Sandbakk S, et al. Non-alcoholic fatty liver disease: Prevalence and all-cause mortality according to sedentary behaviour and cardiorespiratory fitness. The HUNT Study. *Prog Cardiovasc Dis* 2019;62(2):127-34. doi: 10.1016/j.pcad.2019.01.005 [published Online First: 2019/02/24]
81. Gerber L, Otgonsuren M, Mishra A, et al. Non-alcoholic fatty liver disease (NAFLD) is associated with low level of physical activity: a population-based study. *Aliment Pharmacol Ther* 2012;36(8):772-81. doi: 10.1111/apt.12038 [published Online First: 2012/09/11]
82. Keating SE, Parker HM, Pavey TG, et al. Objectively quantified physical activity and sedentary behavior in predicting visceral adiposity and liver fat. *J Obes* 2016;2016:2719014. doi: 10.1155/2016/2719014 [published Online First: 2016/10/26]
83. Meng G, Liu F, Fang L, et al. The overall computer/mobile devices usage time is related to newly diagnosed non-alcoholic fatty liver disease: a population-based study. *Ann Med* 2016;48(7):568-76. doi: 10.1080/07853890.2016.1219454 [published Online First: 2016/09/21]
84. Helajarvi H, Pahkala K, Heinonen OJ, et al. Television viewing and fatty liver in early midlife. The Cardiovascular Risk in Young Finns Study. *Ann Med* 2015;47(6):519-26. doi: 10.3109/07853890.2015.1077989 [published Online First: 2015/09/13]

85. Unger RH. Lipotoxic diseases. Annu Rev Med 2002;53:319-36. doi: 10.1146/annurev.med.53.082901.104057 [published Online First: 2002/01/31]
86. Ioannou GN. The role of cholesterol in the pathogenesis of NASH. Trends Endocrinol Metab 2016;27(2):84-95. doi: 10.1016/j.tem.2015.11.008 [published Online First: 2015/12/26]
87. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 2015;149(2):389-97 e10. doi: 10.1053/j.gastro.2015.04.043 [published Online First: 2015/05/04]
88. Yki-Jarvinen H. Nutritional modulation of non-alcoholic fatty liver disease and insulin resistance. Nutrients 2015;7(11):9127-38. doi: 10.3390/nu7115454 [published Online First: 2015/11/12]
89. Marra F, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. J Hepatol 2018;68(2):280-95. doi: 10.1016/j.jhep.2017.11.014 [published Online First: 2017/11/21]
90. Rosqvist F, Iggman D, Kullberg J, et al. Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. Diabetes 2014;63(7):2356-68. doi: 10.2337/db13-1622 [published Online First: 2014/02/20]
91. Chung M, Ma J, Patel K, et al. Fructose, high-fructose corn syrup, sucrose, and nonalcoholic fatty liver disease or indexes of liver health: a systematic review and meta-analysis. Am J Clin Nutr 2014;100(3):833-49. doi: 10.3945/ajcn.114.086314 [published Online First: 2014/08/08]
92. Vos MB, Lavine JE. Dietary fructose in nonalcoholic fatty liver disease. Hepatology 2013;57(6):2525-31. doi: 10.1002/hep.26299 [published Online First: 2013/02/08]
93. Abdelmalek MF, Suzuki A, Guy C, et al. Increased fructose consumption is associated with fibrosis severity in patients with nonalcoholic fatty liver disease. Hepatology 2010;51(6):1961-71. doi: 10.1002/hep.23535 [published Online First: 2010/03/20]
94. Brandl K, Schnabl B. Intestinal microbiota and nonalcoholic steatohepatitis. Curr Opin Gastroenterol 2017;33(3):128-33. doi: 10.1097/MOG.0000000000000349 [published Online First: 2017/03/04]
95. Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic steatosis: an insulin resistance paradox? Hepatology 2009;49(3):791-801. doi: 10.1002/hep.22726 [published Online First: 2008/12/24]
96. Loomba R, Schork N, Chen CH, et al. Heritability of hepatic fibrosis and steatosis based on a prospective twin study. Gastroenterology 2015;149(7):1784-93. doi: 10.1053/j.gastro.2015.08.011 [published Online First: 2015/08/25]
97. Caussy C, Soni M, Cui J, et al. Nonalcoholic fatty liver disease with cirrhosis increases familial risk for advanced fibrosis. J Clin Invest 2017;127(7):2697-704. doi: 10.1172/JCI93465 [published Online First: 2017/06/20]
98. Makkonen J, Pietilainen KH, Rissanen A, Kaprio J, Yki-Jarvinen H. Genetic factors contribute to variation in serum alanine aminotransferase activity independent of obesity and alcohol: a study in monozygotic and dizygotic twins. J Hepatol 2009;50(5):1035-42. doi: 10.1016/j.jhep.2008.12.025 [published Online First: 2009/03/24]
99. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40(12):1461-5. doi: ng.257 [pii]10.1038/ng.257 [published Online First: 2008/09/30]
100. Valenti LVC, Baselli GA. Genetics of nonalcoholic fatty liver disease: A 2018 update. Curr Pharm Des 2018;24(38):4566-73. doi: 10.2174/1381612825666190119113836 [published Online First: 2019/01/20]

101. Sookoian S, Pirola CJ. Genetics of nonalcoholic fatty liver disease: From pathogenesis to therapeutics. *Semin Liver Dis* 2019;39(2):124-40. doi: 10.1055/s-0039-1679920 [published Online First: 2019/03/27]
102. Wang S, Song J, Shang X, et al. Physical activity and sedentary behavior can modulate the effect of the PNPLA3 variant on childhood NAFLD: a case-control study in a Chinese population. *BMC Med Genet* 2016;17(1):90. doi: 10.1186/s12881-016-0352-9 [published Online First: 2016/12/03]
103. Nobili V, Liccardo D, Bedogni G, et al. Influence of dietary pattern, physical activity, and I148M PNPLA3 on steatosis severity in at-risk adolescents. *Genes Nutr* 2014;9(3):392. doi: 10.1007/s12263-014-0392-8 [published Online First: 2014/03/15]
104. Stender S, Kozlitina J, Nordestgaard BG, et al. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. *Nat Genet* 2017;49(6):842-47. doi: 10.1038/ng.3855 [published Online First: 2017/04/25]
105. Eslam M, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: Clinical impact. *J Hepatol* 2018;68(2):268-79. doi: 10.1016/j.jhep.2017.09.003 [published Online First: 2017/11/11]
106. Abul-Husn NS, Cheng X, Li AH, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. *N Engl J Med* 2018;378(12):1096-106. doi: 10.1056/NEJMoa1712191 [published Online First: 2018/03/22]
107. Schuppan D, Surabattula R, Wang XY. Determinants of fibrosis progression and regression in NASH. *J Hepatol* 2018;68(2):238-50. doi: 10.1016/j.jhep.2017.11.012 [published Online First: 2017/11/21]
108. Petroni ML, Brodosi L, Barbanti FA, et al. Lifestyle changes for the treatment of nonalcoholic fatty liver disease - A 2015-19 update. *Curr Pharm Des* 2020;26(10):1110-18. doi: 10.2174/1381612826666200204095401 [published Online First: 2020/02/06]
109. Romero-Gomez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. *J Hepatol* 2017;67(4):829-46. doi: 10.1016/j.jhep.2017.05.016 [published Online First: 2017/05/27]
110. Marchesini G, Petta S, Dalle Grave R. Diet, weight loss, and liver health in nonalcoholic fatty liver disease: Pathophysiology, evidence, and practice. *Hepatology* 2016;63(6):2032-43. doi: 10.1002/hep.28392 [published Online First: 2015/12/15]
111. Thoma C, Day CP, Trenell MI. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. *J Hepatol* 2012;56(1):255-66. doi: 10.1016/j.jhep.2011.06.010 [published Online First: 2011/07/05]
112. Lazo M, Solga SF, Horska A, et al. Effect of a 12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. *Diabetes Care* 2010;33(10):2156-63. doi: 10.2337/dc10-0856 [published Online First: 2010/07/29]
113. Promrat K, Kleiner DE, Niemeier HM, et al. Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis. *Hepatology* 2010;51(1):121-9. doi: 10.1002/hep.23276 [published Online First: 2009/10/15]
114. Sun WH, Song MQ, Jiang CQ, et al. Lifestyle intervention in non-alcoholic fatty liver disease in Chengyang District, Qingdao, China. *World J Hepatol* 2012;4(7):224-30. doi: 10.4254/wjh.v4.i7.224 [published Online First: 2012/08/03]
115. Wong VW, Chan RS, Wong GL, et al. Community-based lifestyle modification programme for non-alcoholic fatty liver disease: a randomized controlled trial. *J Hepatol* 2013;59(3):536-42. doi: 10.1016/j.jhep.2013.04.013 [published Online First: 2013/04/30]
116. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis.

Gastroenterology 2015;149(2):367-78 e5; quiz e14-5. doi: 10.1053/j.gastro.2015.04.005 [published Online First: 2015/04/14]
117. Mazzotti A, Caletti MT, Brodosi L, et al. An internet-based approach for lifestyle changes in patients with NAFLD: Two-year effects on weight loss and surrogate markers. J Hepatol 2018;69(5):1155-63. doi: 10.1016/j.jhep.2018.07.013 [published Online First: 2018/10/07]
118. Khoo J, Hsiang J, Taneja R, Law NM, Ang TL. Comparative effects of liraglutide 3 mg vs structured lifestyle modification on body weight, liver fat and liver function in obese patients with non-alcoholic fatty liver disease: A pilot randomized trial. Diabetes Obes Metab 2017;19(12):1814-17. doi: 10.1111/dom.13007 [published Online First: 2017/05/16]
119. Khoo J, Hsiang JC, Taneja R, et al. Randomized trial comparing effects of weight loss by liraglutide with lifestyle modification in non-alcoholic fatty liver disease. Liver Int 2019;39(5):941-49. doi: 10.1111/liv.14065 [published Online First: 2019/02/06]
120. The Diabetes Prevention Program Research Group. The Diabetes Prevention Program (DPP): description of lifestyle intervention. Diabetes Care 2002;25(12):2165-71. doi: 10.2337/diacare.25.12.2165 [published Online First: 2002/11/28]
121. Wong VW, Wong GL, Chan RS, et al. Beneficial effects of lifestyle intervention in non-obese patients with non-alcoholic fatty liver disease. J Hepatol 2018;69(6):1349-56. doi: 10.1016/j.jhep.2018.08.011 [published Online First: 2018/08/25]
122. Tapper EB, Lai M. Weight loss results in significant improvements in quality of life for patients with nonalcoholic fatty liver disease: A prospective cohort study. Hepatology 2016;63(4):1184-9. doi: 10.1002/hep.28416 [published Online First: 2015/12/25]
123. Caiazzo R, Lassailly G, Leteurtre E, et al. Roux-en-Y gastric bypass versus adjustable gastric banding to reduce nonalcoholic fatty liver disease: a 5-year controlled longitudinal study. Ann Surg 2014;260(5):893-8; discussion 98-9. doi: 10.1097/SLA.0000000000000945 [published Online First: 2014/11/08]
124. Klebanoff MJ, Corey KE, Chhatwal J, et al. Bariatric surgery for nonalcoholic steatohepatitis: A clinical and cost-effectiveness analysis. Hepatology 2017;65(4):1156-64. doi: 10.1002/hep.28958 [published Online First: 2016/11/24]
125. Laursen TL, Hagemann CA, Wei C, et al. Bariatric surgery in patients with non-alcoholic fatty liver disease - from pathophysiology to clinical effects. World J Hepatol 2019;11(2):138-49. doi: 10.4254/wjh.v11.i2.138 [published Online First: 2019/03/02]
126. Wirth KM, Sheka AC, Kizy S, et al. Bariatric surgery is associated with decreased progression of nonalcoholic fatty liver disease to cirrhosis: A retrospective cohort analysis. Ann Surg 2020;272(1):32-39. doi: 10.1097/SLA.0000000000003871 [published Online First: 2020/04/01]
127. Lassailly G, Caiazzo R, Ntandja-Wandji LC, et al. Bariatric surgery provides long-term resolution of nonalcoholic steatohepatitis and regression of fibrosis. Gastroenterology 2020;159(4):1290-301. doi: 10.1053/j.gastro.2020.06.006 [published Online First: 2020/06/20]
128. Goh GB, Schauer PR, McCullough AJ. Considerations for bariatric surgery in patients with cirrhosis. World J Gastroenterol 2018;24(28):3112-19. doi: 10.3748/wjg.v24.i28.3112 [published Online First: 2018/08/02]
129. Salomone F, Sharaiha RZ, Boskoski I. Endoscopic bariatric and metabolic therapies for non-alcoholic fatty liver disease: Evidence and perspectives. Liver Int 2020;40(6):1262-68. doi: 10.1111/liv.14441 [published Online First: 2020/03/18]
130. Abu Dayyeh BK, Bazerbachi F, Graupera I, Cardenas A Md MP. Endoscopic bariatric and metabolic therapies for non-alcoholic fatty liver disease. J Hepato

2019;71(6):1246-48. doi: 10.1016/j.jhep.2019.07.026 [published Online First: 2019/10/02]

131. Siddiqui MS, Harrison SA, Abdelmalek MF, et al. Case definitions for inclusion and analysis of endpoints in clinical trials for nonalcoholic steatohepatitis through the lens of regulatory science. *Hepatology* 2018;67(5):2001-12. doi: 10.1002/hep.29607 [published Online First: 2017/10/24]
132. Musso G, Gambino R, Cassader M, Pagano G. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. *Hepatology* 2010;52(1):79-104. doi: 10.1002/hep.23623 [published Online First: 2010/06/26]
133. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010;362(18):1675-85. doi: NEJMoa0907929 [pii]10.1056/NEJMoa0907929 [published Online First: 2010/04/30]
134. Cusi K, Orsak B, Bril F, et al. Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: A randomized trial. *Ann Intern Med* 2016;165(5):305-15. doi: 10.7326/M15-1774 [published Online First: 2016/06/21]
135. Musso G, Cassader M, Paschetta E, Gambino R. Thiazolidinediones and advanced liver fibrosis in nonalcoholic steatohepatitis: A meta-analysis. *JAMA Intern Med* 2017;177(5):633-40. doi: 10.1001/jamainternmed.2016.9607 [published Online First: 2017/02/28]
136. Bril F, Lomonaco R, Kalavalapalli S, Lai J, Cusi K. Pioglitazone discontinuation in patients with nonalcoholic steatohepatitis (NASH) is associated with disease recurrence. *Diabetes* 2019;68 (supplement 1)(6):223-OR. doi: 10.2337/db19-223-OR
137. Dormandy JA, Charbonnel B, Eckland DJ, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005;366(9493):1279-89. doi: 10.1016/S0140-6736(05)67528-9 [published Online First: 2005/10/11]
138. Kernan WN, Viscoli CM, Furie KL, et al. Pioglitazone after ischemic stroke or transient ischemic attack. *N Engl J Med* 2016;374(14):1321-31. doi: 10.1056/NEJMoa1506930 [published Online First: 2016/02/18]
139. Bril F, Biernacki DM, Kalavalapalli S, et al. Role of vitamin E for nonalcoholic steatohepatitis in patients with type 2 diabetes: A randomized controlled trial. *Diabetes Care* 2019;42(8):1481-88. doi: 10.2337/dc19-0167 [published Online First: 2019/07/25]
140. Abner EL, Schmitt FA, Mendiondo MS, Marcum JL, Kryscio RJ. Vitamin E and all-cause mortality: a meta-analysis. *Curr Aging Sci* 2011;4(2):158-70. doi: 10.2174/1874609811104020158 [published Online First: 2011/01/18]
141. Adorini L, Pruzanski M, Shapiro D. Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. *Drug Discov Today* 2012;17(17-18):988-97. doi: S1359-6446(12)00189-4 [pii]10.1016/j.drudis.2012.05.012 [published Online First: 2012/06/02]
142. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015;385(9972):956-65. doi: 10.1016/S0140-6736(14)61933-4 [published Online First: November 6]
143. Younossi ZM, Ratziu V, Loomba R, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 2019;394(10215):2184-96. doi: 10.1016/S0140-6736(19)33041-7 [published Online First: 2019/12/10]

144. Intercept release. Complete Response Letter (CRL) from the FDA regarding our new drug application for obeticholic acid (OCA) for the treatment of liver fibrosis due to NASH. https://ir.interceptpharma.com/news-releases/news-release-details/intercept-receives-complete-response-letter-fda-obeticholic-acid2020.[released 29 June 2020]
145. Hernandez ED, Zheng L, Kim Y, et al. Tropifexor-mediated abrogation of steatohepatitis and fibrosis is associated with the antioxidative gene expression profile in rodents. *Hepatol Commun* 2019;3(8):1085-97. doi: 10.1002/hep4.1368 [published Online First: 2019/08/08]
146. Pedrosa M, Seyedkazemi S, Francque S, et al. A randomized, double-blind, multicenter, phase 2b study to evaluate the safety and efficacy of a combination of tropifexor and cenicriviroc in patients with nonalcoholic steatohepatitis and liver fibrosis: Study design of the TANDEM trial. *Contemp Clin Trials* 2020;88:105889. doi: 10.1016/j.cct.2019.105889 [published Online First: 2019/11/16]
147. Patel K, Harrison SA, Elkashab M, et al. Cilofexor, a nonsteroidal FXR agonist, in non-Cirrhotic patients with nonalcoholic steatohepatitis: A phase 2 randomized controlled trial. *Hepatology* 2020;72(1):58-71. doi: 10.1002/hep.31205 [published Online First: 2020/03/03]
148. Ratziu V, Harrison SA, Francque S, et al. Elafibranor, an agonist of the peroxisome proliferator-activated receptor-alpha and -delta, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. *Gastroenterology* 2016;150(5):1147-59 e5. doi: 10.1053/j.gastro.2016.01.038 [published Online First: 2016/02/14]
149. GENFIT. GENFIT: Announces results from interim analysis of RESOLVE-IT phase 3 trial of elafibranor in adults with NASH and fibrosis. https://wwwglobenewswirecom/news-release/2020/05/11/2031418/0/en/GENFIT-Announces-Results-from-Interim-Analysis-of-RESOLVE-IT-Phase-3-Trial-of-Elafibranor-in-Adults-with-NASH-and-Fibrosishtml. GlobeNewswire 2020 [released 11 May 2020]
150. INVENTIVA. Inventiva's lanifibranor meets the primary and key secondary endpoints in the Phase IIb NATIVE clinical trial in non-alcoholic steatohepatitis (NASH). https://wwwglobenewswirecom/news-release/2020/06/15/2048284/0/en/Inventiva-s-lanifibranor-meets-the-primary-and-key-secondary-endpoints-in-the-Phase-IIb-NATIVE-clinical-trial-in-non-alcoholic-steatohepatitis-NASHhtml: GlobeNewswire 2020 [released 15 June 2020]
151. INVENTIVA. Inventiva receives FDA Breakthrough Therapy designation for lead drug candidate lanifibranor in NASH. https://wwwglobenewswirecom/news-release/2020/10/12/2107044/0/en/Inventiva-receives-FDA-Breakthrough-Therapy-designation-for-lead-drug-candidate-lanifibranor-in-NASHhtml: GlobeNewswire 2020 [released 12 October 2020]
152. Sinha RA, Bruinstroop E, Singh BK, Yen PM. Nonalcoholic fatty liver disease and hypercholesterolemia: Roles of thyroid hormones, metabolites, and agonists. *Thyroid* 2019;29(9):1173-91. doi: 10.1089/thy.2018.0664 [published Online First: 2019/08/08]
153. Harrison SA, Bashir MR, Guy CD, et al. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2019;394(10213):2012-24. doi: 10.1016/S0140-6736(19)32517-6 [published Online First: 2019/11/16]
154. Loomba R, Adams LA. Advances in non-invasive assessment of hepatic fibrosis. *Gut* 2020;69(7):1343-52. doi: 10.1136/gutjnl-2018-317593 [published Online First: 2020/02/19]

155. Krenkel O, Puengel T, Govaere O, et al. Therapeutic inhibition of inflammatory monocyte recruitment reduces steatohepatitis and liver fibrosis. Hepatology 2018;67(4):1270-83. doi: 10.1002/hep.29544 [published Online First: 2017/09/25]
156. Friedman SL, Ratziu V, Harrison SA, et al. A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. Hepatology 2018;67(5):1754-67. doi: 10.1002/hep.29477 [published Online First: 2017/08/24]
157. *Ratziu V, Sanyal A, Harrison SA, et al. Cenicriviroc teatment for adults with nonalcoholic steatohepatitis and fibrosis: Final analysis of the phase 2b CENTAUR study. Hepatology 2020 doi: 10.1002/hep.31108 [published Online First: 2020/01/17]
158. Konikoff FM, Gilat T. Effects of fatty acid bile acid conjugates (FABACs) on biliary lithogenesis: potential consequences for non-surgical treatment of gallstones. Curr Drug Targets Immune Endocr Metabol Disord 2005;5(2):171-5. doi: 10.2174/1568008054064904 [published Online First: 2005/08/11]
159. Safadi R, Konikoff FM, Mahamid M, et al. The fatty acid-bile acid conjugate Aramchol reduces liver fat content in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2014;12(12):2085-91 e1. doi: 10.1016/j.cgh.2014.04.038 [published Online First: 2014/05/13]
160. Ratziu V, Safadi R, Safadi R, et al. One-year results of the global phase 2b randomized placebo-controlled ARREST trial of aramchol, a stearoyl CoA desaturase inhibitor, in patients with NASH. Hepatology 2018;68(6):1447A.
161. Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell metabolism 2013;17(6):819-37. doi: 10.1016/j.cmet.2013.04.008 [published Online First: 2013/05/21]
162. Cusi K. Incretin-based therapies for the management of nonalcoholic fatty liver disease in patients with type 2 diabetes. Hepatology 2019;69(6):2318-22. doi: 10.1002/hep.30670 [published Online First: 2019/04/22]
163. Armstrong MJ, Gaunt P, Aithal GP, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet 2016;387(10019):679-90. doi: 10.1016/S0140-6736(15)00803-X [published Online First: 19 Nov 2015]
164. NOVO Nordisk. Semaglutide in NASH phase 2 trial successfully completed. Financial report for the period 1 January 2020 to 31 March 2020. https://www.novonordisk.com/content/dam/Denmark/HQ/investors/irmaterial/quarterly_financial_reports/2020/Financial%20report%20for%20Q1%202020.pdf2020 [released 6 May 2020]
165. NOVO Nordisk. Semaglutide 2.4 mg demonstrates superior and sustained weight loss versus placebo and in addition a 17.4% weight loss after 68 weeks in STEP 4 trial. https://ml-euglobenewswirecom/Resource/Download/4951d1a2-3bd1-47ea-840a-a1234109c018. GlobeNewswire 2020 [released 13 May 2020]
166. Petroni ML, Montesi L, Colosimo S, et al. Combination of GLP-1 receptor agonists and behavioural treatment in type 2 diabetes elicits synergistic effects on body weight: A retrospective cohort study. Endocrinol Diab Metab 2019;2(4):e00082. doi: 10.1002/edm2.82 [published Online First: 2019/10/09]
167. Hartman ML, Sanyal AJ, Loomba R, et al. Effects of novel dual GIP and GLP-1 receptor agonist tirzepatide on biomarkers of nonalcoholic steatohepatitis in patients with type 2 diabetes. Diabetes Care 2020;43(6):1352-55. doi: 10.2337/dc19-1892 [published Online First: 2020/04/16]
168. Seko Y, Sumida Y, Tanaka S, et al. Effect of 12-week dulaglutide therapy in Japanese patients with biopsy-proven non-alcoholic fatty liver disease and type 2 diabetes

mellitus. Hepatol Res 2017;47(11):1206-11. doi: 10.1111/hepr.12837 [published Online First: 2016/12/06]
169. Harrison SA, Alkhouri N, Davison BA, et al. Insulin sensitizer MSDC-0602K in non-alcoholic steatohepatitis: A randomized, double-blind, placebo-controlled phase IIb study. J Hepatol 2020;72(4):613-26. doi: 10.1016/j.jhep.2019.10.023 [published Online First: 2019/11/08]
170. Shimizu M, Suzuki K, Kato K, et al. Evaluation of the effects of dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on hepatic steatosis and fibrosis using transient elastography in patients with type 2 diabetes and non-alcoholic fatty liver disease. Diabetes Obes Metab 2019;21(2):285-92. doi: 10.1111/dom.13520 [published Online First: 2018/09/05]
171. Kuchay MS, Krishan S, Mishra SK, et al. Effect of empagliflozin on liver fat in patients with type 2 diabetes and nonalcoholic fatty liver disease: A randomized controlled trial (E-LIFT trial). Diabetes Care 2018;41(8):1801-08. doi: 10.2337/dc18-0165 [published Online First: 2018/06/14]
172. Cusi K, Bril F, Barb D, et al. Effect of canagliflozin treatment on hepatic triglyceride content and glucose metabolism in patients with type 2 diabetes. Diabetes Obes Metab 2019;21(4):812-21. doi: 10.1111/dom.13584 [published Online First: 2018/11/18]
173. Wang H, Yang J, Chen X, Qiu F, Li J. Effects of sodium-glucose cotransporter 2 inhibitor monotherapy on weight changes in patients with type 2 diabetes mellitus: a Bayesian network meta-analysis. Clin Ther 2019;41(2):322-34 e11. doi: 10.1016/j.clinthera.2019.01.001 [published Online First: 2019/02/04]
174. Yan J, Yao B, Kuang H, et al. Liraglutide, sitagliptin, and insulin glargine added to metformin: The effect on body weight and intrahepatic lipid in patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease. Hepatology 2019;69(6):2414-26. doi: 10.1002/hep.30320 [published Online First: 2018/10/21]
175. Traussnigg S, Schattenberg JM, Demir M, et al. Norursodeoxycholic acid versus placebo in the treatment of non-alcoholic fatty liver disease: a double-blind, randomised, placebo-controlled, phase 2 dose-finding trial. Lancet Gastroenterol Hepatol 2019;4(10):781-93. doi: 10.1016/S2468-1253(19)30184-0 [published Online First: 2019/07/28]
176. Han MAT, Altayar O, Hamdeh S, et al. Rates of and factors associated with placebo response in trials of pharmacotherapies for nonalcoholic steatohepatitis: Systematic review and meta-analysis. Clin Gastroenterol Hepatol 2019;17(4):616-29 e26. doi: 10.1016/j.cgh.2018.06.011 [published Online First: 2018/06/19]
177. Glass O, Filozof C, Noureddin M, et al. Standardization of diet and exercise in clinical trials of NAFLD-NASH: Recommendations from the Liver Forum. J Hepatol 2020;73(3):680-93. doi: 10.1016/j.jhep.2020.04.030 [published Online First: 2020/05/01]
178. Petroni ML, Brodosi L, Marchignoli F, Musio A, Marchesini G. Moderate alcohol intake in non-alcoholic fatty liver disease: To drink or not to drink? Nutrients 2019;11(12) doi: 10.3390/nu11123048 [published Online First: 2019/12/19]
179. Giorda C, Forlani G, Manti R, et al. Occurrence over time and regression of nonalcoholic fatty liver disease in type 2 diabetes. Diabetes Metab Res Rev 2017;33(4) doi: 10.1002/dmrr.2878 [published Online First: 2016/12/30]
180. Patel J, Bettencourt R, Cui J, et al. Association of noninvasive quantitative decline in liver fat content on MRI with histologic response in nonalcoholic steatohepatitis. Therap Adv Gastroenterol 2016;9(5):692-701. doi: 10.1177/1756283X16656735 [published Online First: 2016/09/02]

181. Ratziu V. A critical review of endpoints for non-cirrhotic NASH therapeutic trials. *J Hepatol* 2018;68(2):353-61. doi: 10.1016/j.jhep.2017.12.001 [published Online First: 2017/12/11]
182. Daniels SJ, Leeming DJ, Eslam M, et al. ADAPT: An algorithm incorporating PRO-C3 accurately identifies patients with NAFLD and advanced fbrosis. *Hepatology* 2019;69(3):1075-86. doi: 10.1002/hep.30163 [published Online First: 2018/07/18]
183. Ajmera VH, Liu A, Singh S, et al. Clinical utility of an increase in magnetic resonance elastography in predicting fibrosis progression in nonalcoholic fatty liver disease. *Hepatology* 2020;71(3):849-60. doi: 10.1002/hep.30974 [published Online First: 2019/09/27]
184. Haldar D, Kern B, Hodson J, et al. Outcomes of liver transplantation for non-alcoholic steatohepatitis: A European Liver Transplant Registry study. *J Hepatol* 2019;71(2):313-22. doi: 10.1016/j.jhep.2019.04.011 [published Online First: 2019/05/10]
185. Konerman MA, Fritze D, Weinberg RL, Sonnenday CJ, Sharma P. Incidence of and risk assessment for adverse cardiovascular outcomes after liver transplantation: A systematic review. *Transplantation* 2017;101(7):1645-57. doi: 10.1097/TP.0000000000001710 [published Online First: 2017/03/16]
186. Wang X, Li J, Riaz DR, et al. Outcomes of liver transplantation for nonalcoholic steatohepatitis: a systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2014;12(3):394-402 e1. doi: 10.1016/j.cgh.2013.09.023 [published Online First: 2013/10/01]
187. Tsochatzis E, Coilly A, Nadalin S, et al. International Liver Transplantation consensus statement on end-stage liver disease due to nonalcoholic steatohepatitis and liver transplantation. *Transplantation* 2019;103(1):45-56. doi: 10.1097/TP.0000000000002433 [published Online First: 2018/08/29]
188. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018;69(1):182-236. doi: 10.1016/j.jhep.2018.03.019 [published Online First: 2018/04/10]
189. Nobili V, Marcellini M, Marchesini G, et al. Intrauterine growth retardation, insulin resistance, and nonalcoholic fatty liver disease in children. *Diabetes Care* 2007;30(10):2638-40. doi: 10.2337/dc07-0281 [published Online First: 2007/05/31]
190. Berentzen TL, Gamborg M, Holst C, Sorensen TI, Baker JL. Body mass index in childhood and adult risk of primary liver cancer. *J Hepatol* 2014;60(2):325-30. doi: 10.1016/j.jhep.2013.09.015 [published Online First: 2013/10/01]
191. Lazarus JV, Ekstedt M, Marchesini G, et al. A cross-sectional study of the public health response to non-alcoholic fatty liver disease in Europe. *J Hepatol* 2020;72(1):14-24. doi: 10.1016/j.jhep.2019.08.027 [published Online First: 2019/09/14]
192. Balakrishnan M, Loomba R. PROs of patient-reported outcomes for nonalcoholic fatty liver disease and effects on treatment for nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2019;17(10):1950-53. doi: 10.1016/j.cgh.2019.04.018 [published Online First: 2019/04/14]
193. Younossi ZM, Stepanova M, Anstee QM, et al. Reduced patient-reported outcome scores associate with level of fibrosis in patients with nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2019;17(12):2552-60 e10. doi: 10.1016/j.cgh.2019.02.024 [published Online First: 2019/02/20]

Table 1. Comparative analysis of different guidelines on NAFLD/NASH

Recommendation	EASL-EASD-EASO[5]	AASLD[4]	NICE[10]	Asian-Pacific[11 12]
Diagnosis (after excluding alcohol and secondary causes)	Steatosis by imaging or histology or unexpectedly high liver enzymes	Steatosis by imaging or histology	Any evidence of excessive liver fat, regardless of liver enzymes. Use the fatty liver index (FLI) if testing adults for NAFLD	Steatosis by ultrasonography or transient elastography as first step (where available)
Community screening	Non cost-effective	Not considered	Non effective	Cost-effectiveness unknown
Screening in high-risk patients	All subjects with one or more features of metabolic syndrome	Not mentioned.	Not mentioned. Consider that NAFLD is common in type 2 diabetes and metabolic syndrome	Considered in subjects with type 2 diabetes and obesity
Screening by non-invasive tests	NFS or Fib-4, followed by elastography	NFS, Fib-4 and elastography	ELF test	Biomarkers and imaging effective (no specific test)
Genetic screening	Not cost-effective	Not mentioned	Not mentioned	Cost-effectiveness unknown
Screening for complications	Define cardiovascular and diabetes risk	Define cardiovascular and diabetes risk	Define cardiovascular and diabetes risk	Define the presence of all features of metabolic syndrome
Follow-up	Not at risk of progression, every 2 years; at risk, every 6 months	Not defined	Every 3 years in subjects not at risk of progression; if at risk, use NICE guidelines for cirrhosis	Not mentioned
Liver biopsy	Mandatory in drug trials	Consider in subjects at risk for NASH or advanced fibrosis and/or to exclude other coexisting liver disease	Gold standard, but not feasible also in patients at risk	When the diagnosis is unclear, or when fibrosis assessment by noninvasive tests is inconclusive.
Treatment: Diet & weight loss	Dietary restriction (deficit 500-1,000 kcal/day) Prefer Mediterranean diet	Dietary restriction (deficit 500-1,000 kcal/day) No specific diet	Consider NICE guidelines for obesity and weight gain prevention. No specific diet	Consider a multidisciplinary approach. Dietary restriction (deficit 500-1,000 kcal/day).
Treatment: Physical activity	Aerobic or exercise training (150-300 min/week), 3-5 sessions	Aerobic or exercise training (>150 min/week)	Consider NICE guidelines for obesity and weight gain prevention	Aerobic or resistance exercise (moderate-intensity ≥150 min/week or vigorous-intensity ≥75)
Treatment: Drugs	Pioglitazone (off label in the absence of diabetes) Vitamin E not indicated Other drugs not indicated	Pioglitazone and Vitamin E in patients with/without diabetes, respectively Other drugs not indicated	Consider pioglitazone in diabetic and vitamin E in non-diabetic cases with advanced fibrosis (only in secondary or tertiary care settings)	Consider pioglitazone for short-term use in diabetes or prediabetes. Consider vitamin E in non-cirrhotic, non-diabetic NASH. Other drugs not indicated

Table 2

Genes involved in NAFLD and in NAFLD progression

Gene	Metabolic effects	Prevalence in NAFLD and clinical significance
Patatin-like phospholipase domain-containing 3 (PNPLA3 I148M variant- Adiponutrin*)*	The mutated protein accumulates on the surface of lipid droplets preventing export from hepatocytes and favouring inflammation in hepatic stellate cells by interaction with retinol	• 10% vs. 5% in Caucasian populations (10-15% in Asian populations); 16% in NASH, 35% in NASH-cirrhosis and 45% in NASH-HCC. • To be considered as possible marker of disease progression
Transmembrane 6 superfamily member 2 (TM6SF2 E167K variant)	Decreased lipid secretion in VLDL, leading to reduced circulating lipids (both cholesterol and triglycerides)	• 13% vs. 7.2% in subjects of European ancestry, in 3.4% in African- and 4.7% in Hispanic-Americans • Increased risk of NASH and advanced fibrosis • Reduced risk of cardiovascular disease (Hazard Ratio, 0.67), totally explained by low cholesterol levels
Membrane bound O-acyltransferase domain-containing 7 (MBOAT7)	The variant promotes changes in hepatic phosphatidylinositol acyl-chain remodelling.	• Increased risk of NAFLD along the whole disease spectrum • Predisposes to cirrhosis in alcohol abusers
Glucokinase regulator (GCKR P446L variant)	The variant impairs glucokinase inhibition in response to fructose-6-phosphate, thus blocking fatty acid oxidation	• Associated with steatosis in children and adults, and with the presence of obesity, irrespective of ethnicity • In NAFLD, it predicts the risk of fibrosis (F1 or more)
Hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13)	The truncated protein has a reduced enzymatic activity	• Loss-of-function variant of the gene protects against chronic liver disease (both alcoholic and non-alcoholic) and reduces the risk of progressive NASH • It reduces the negative effects of PNPLA3 variant

At present, genome-wide screening for genes at risk of NAFLD and NAFLD progression is not advised by international and national guidelines

Table 3

Principal lifestyle intervention studies for NAFLD treatment

Author, year	Type of study, No. of pts	Treatment and duration	Study target & outcome measures	Results
Lazo et al, 2010 [112]	RCT 96 T2DM	Intensive LS intervention (ILI, n=46) *vs.* diabetes support & education (DSE, n=50); 12 months	7-10% WL. Biochemistry; intra-abdominal fat (steatosis ≤5.5% IHTG at MRS)	Data collected as part of the LookAhead study. At one year, ILI participants lost more weight (WL -8.0% vs. -0.5%) and had a larger decline in IHTG content (-50.8% vs. -22.8%) vs. participants in DSE
Promrat et al, 2010 [113]	RCT 31 biopsy proven NASH	Intensive LS intervention (ILI, n=21) *vs.* standard care (SC, n=10); 48 weeks	WL ≥ 7%, improved biochemistry; reduced NAS (≥3 points) or post-treatment NAS ≤ 2; NASH remission at histology	WL, 9.3±SD 7.5% in ILI vs. 0.2±6.1 in SC; NAS target reached in 72% *vs.* 30% (SC). In subjects who achieved ≥7% WL, liver fat, ballooning and lobular inflammation were improved, irrespective of treatment arm. Percent WL correlated with reduced ALT, steatosis and activity
Sun et al, 2012 [114]	RCT 1087 NAFLD (Ultrasounds)	LS-treated (LS, n=724) *vs.* basic education (SC, n= 363); 12 months	WL and liver enzymes; energy intake ≤ 25-30 kcal/kg BW; PA ≥23 METs/h/wk + 4 METs of exercise. Visceral fat area by CT	WL larger in LS (-11.6% vs. 0.4% in SC); liver enzymes, IR and parameters of MetS showed a larger improvement in LS vs. SC at 6- and 12-monts. VFA was reduced in LS at 12-mo.
Wong et al, 2013 [115]	RCT 154 NAFLD (IHTG ≥5% and high ALT)	Intensive LS intervention (ILI, n=77), standard care (SC, n=77); 12 months	NAFLD remission (IHTG content < 5%), WL, changes in ALT, improvement in fibrosis (transient elastography)	ILI was associated with NAFLD remission (64% vs. 20% SC; difference 44%, 95% CI 30–58%), normal ALT (53%) and reduced fibrosis. 39% of ILI patients and no patient in SC had WL ≥ 10% (difference 39%; 95% CI 28–50%). 97% of cases who achieved 10% WL target had NAFLD remission.
Vilar-Gomez et al, 2015 [116]	Cohort study 293 biopsy-proven NASH	All treated by intensive LS intervention (ILI), 261 cases had follow-up biopsies; 52 weeks	NASH resolution without fibrosis worsening; NAS improvement (≥2 points); improved histological lesions (≥1 point)	WL was ≥5% in 30% of cases. NASH remission was observed in 25%; NAS reduction in 47%, fibrosis regression in 19%. The amount of WL was independently associated with improvement in all histological parameters (ORs 1.1-2.0). WL ≥10% was associated with NASH remission (90% of cases) and fibrosis regression in 45%.
Khoo et al, 2017 [118 119]	Pilot RCT 24 obese MRI-diagnosed NAFLD	Liraglutide (3 mg/day, n=12) *vs.* LS (diet and exercise, n=12); 26 weeks + 26 weeks of weight loss maintenance	WL, biochemistry, MR elastography	Similar reduction in BW (-3.5 kg in both arms), liver enzymes and liver stiffness (LS, -0.21 kPa; liraglutide, -0.26); liraglutide as effective as structured LS modification. at 52 weeks, the LI group

regained weight (+1.8 ± 2.1 kg), and IHTG content (4.0 ± 5.3%), that were unchanged in the LS group

Mazzotti, 2018 [117]	Observational, cohort study 716 ultrasonography-assessed NAFLD	Web-based LS program (WEB, n=278) *vs.* group-based intervention (GROUP, n=438); Follow-up, 2 years	WL ≥10%, changes in liver enzymes, surrogate markers of steatosis and fibrosis (FLI, NFS, Fib-4)	Attrition rate was higher in WEB (OR, 1.87; 95% CI 1.20–2.90 at 6 months and OR 2.95; 95% CI 2.04–4.26, at 2 years). The 10% WL target was reached in 20% (WEB) *vs.* 15% (GROUP). 10% WL after two years was only associated with baseline BMI (OR 1.43; 95% CI 1.13-1.81 per BMI/5). After adjustment for confounders and attrition, the probability of reaching long-term 10% WL was not reduced in WEB (OR 0.70; 95% CI 0.38–1.27) *vs.* GROUP care.

Abbreviations: BW, body weight; CT, computed tomography; Fib-4, Fibrosis-4 index; FLI, Fatty Liver Index; IHTG, intra-hepatic triglyceride; IR, insulin resistance; LS, lifestyle; MetS, metabolic syndrome; MRS, magnetic resonance spectroscopy; MRI, magnetic resonance imaging; NAS, NAFLD activity score; NFS, NAFLD fibrosis score; NS, not significant; PA, physical activity; RCT, randomized controlled trial; SC, standard care; VFA, visceral fat area; WL, weight loss.

Table 4. Therapies for NASH in phase 3 development

Drug	Trial code Name (Pharma)	No. of Patients	Study population	Route of delivery	Time to surrogate end-point (biopsy)	Primary Endpoint	Long-term clinical outcome*
ANTI-INFLAMMATORY, ANTI-FIBROTIC							
Obeticholic acid [143] **(FXR agonist)**	NCT02548351 REGENERATE (Intercept)	2480	NASH with fibrosis F2/F3, NAS score ≥4 Fibrosis F1 and diabetes, obesity, or inflammation	oral	72 weeks	≥1 stage improvement of fibrosis w/o worsening of NASH OR NASH resolution w/o worsening of fibrosis	Time to first event
Cenicriviroc [157] **(dual CCR2/CCR5 antagonist)**	NCT03028740 AURORA (Allergan)	2000	NASH with fibrosis F2/F3, NAS score ≥4	oral	52 weeks	≥1 stage improvement of fibrosis w/o worsening of NASH	Time to first event (up to EOS, about 5 years)
METABOLISM MODULATORS							
Elafibranor [149] **(dual PPAR-α/δ agonist)°**	NCT02704403 RESOLVE-IT (Genfit)	2000	NAS score ≥4 Fibrosis F1/F2/F3 (F1, limited number) BMI ≤45kg/m^2	oral	72 weeks	NASH resolution (no ballooning, inflammation 0-1, no progression of fibrosis w/o worsening of steatohepatitis	Time to first event (up to EOS, about 4 years)
Resmetirom (THRβ agonist)	NCT03900429 MAESTRO-NASH (Madrigal)	2000	NASH with fibrosis F2/F3 High risk F1	oral	52 weeks	NASH resolution, no worsening of fibrosis Composite clinical outcome	% subjects experiencing >1 event (up to 54 months)
Aramchol (SCD-1 modulator)	NCT04104321 ARMOR (Galmed)	2000	NASH with fibrosis F2/F3, NAS score ≥4 overweight/obese, prediabetes/T2DM	oral	52 weeks	NASH resolution, no worsening of fibrosis OR ≥1 stage improvement of fibrosis, no worsening of NASH	% subjects experiencing >1 event (up to 5 years)

*Long term outcomes include all-cause mortality, transplant, hospitalization due to hepatic decompensation

°Recent early termination after interim analysis

Abbreviations: CCR2-CCR5, chemokine receptor 2-5; EOS, end-of-study; FXR, farnesoid-X receptor; NAS, NAFLD activity score (sum of steatosis (0-3), lobular inflammation (0-3), hepatocellular ballooning (0-2); PPAR, peroxisome proliferator-activated receptor; SDC-1, stearoyl-CoA desaturase modulator; THR-β (thyroid hormone

receptor β.

Table 5.
Therapies for NASH in late phase 2 development

Drug	Trial code Name (Pharma)	No of Patients	Study population	Route of delivery	Surrogate end-point Time to end-point	Primary Endpoint
METABOLISM MODULATORS						
Aldafermin (NGM282) (FGF19)	NCT03912532 ALPINE 2/3 (NGM)	152	NASH, fibrosis F2/F3	subcutaneous	Biopsy 24 weeks	% patients achieving histological treatment safety and tolerability
BFKB8488A (bi-specific FGF21/KLB ab)	NCT04171765 BANFF (Genentech)	260	NASH, fibrosis F2/F3 liver fat ≥8%	subcutaneous	Biopsy 52 weeks	NASH resolution without worsening of fibrosis
Icosabutate (structurally enhanced w-3 FA)	NCT04052516 ICONA (NorthSea)	264	NASH, fibrosis F1-F3 NAS score ≥4 liver fat ≥10%	oral	Biopsy 52 weeks	NASH resolution without worsening of fibrosis
Lanifibranor [150] **(Pan-PPAR agonist)**	NCT03008070 NATIVE (Inventiva)	247	NASH	oral	Biopsy 24 weeks	≥2 points reduction of SAF score without fibrosis progression
Licogliflozin (SGLT-1/2)	NCT03205150 (Novartis)	110	NASH, fibrosis F1-F3, elevated ALT or BMI ≥27kg/m^2 (≥23kg/m^2, Asian) A1c 6.5-10%	oral	MRI 12 weeks	change in ALT
MSDC-0602K [169] **(mTOT modulator, Insulin sensitizer)**	NCT03970031 MMONARCh (Cirius)	402	NASH, fibrosis + T2D	oral	Biopsy 52 weeks	change in HbA1c NASH resolution without worsening of fibrosis
Norursodeoxycholic acid [175] **(homolog of ursodeoxycholic)**	EudraCT2018-003443-31 (Dr. Falk)	363	NASH, fibrosis	oral	Biopsy 72 weeks	NASH resolution without worsening of fibrosis
Pegbelfermin (PEG-FGF21)	NCT03486899 FALCON 1 (BMS)	160	NASH, fibrosis F3 score≥1 for each NAS component	subcutaneous	Biopsy 24 weeks	≥1 stage improvement of fibrosis, no worsening of NASH or NASH resolution, no worsening of liver fibrosis

Semaglutide [164] **(GLP-1 receptor agonist)**	NCT02970942 (Novo Nordisk)	320	NASH, fibrosis F2/F3 NAS score ≥4	subcutaneous	Biopsy 72 weeks	NASH resolution without worsening of fibrosis
Tirzepatide [167] **(dual GLP-1/GIP agonist)**	NCT04166773 SYNERGY-NASH (Eli Lilly)	196	NASH, fibrosis F2/F3 BMI ≥27kg/m^2	subcutaneous	Biopsy 52 weeks	NASH resolution without worsening of fibrosis
VK2809 [154] **(THRβ agonist)**	NCT04173065 VOYAGE (Viking)	337	NASH, fibrosis F1/F2/F3 NAS score ≥4 liver fat ≥8%	oral	Biopsy 52 weeks	change in liver fat
ANTI-INFLAMMATORY, ANTI-FIBROTIC						
CC-90001 JNK-1 inhibitor	NCT04048876 (Celgene)	300	NASH, fibrosis <F4 NAS score ≥4 BMI 35-45kg/m^2	oral	Biopsy 52 weeks	≥1 stage improvement of fibrosis
Tropifexor (FXR agonist)	NCT02855164 FLIGHT-FXR (Novartis)	351	NASH, elevated ALT liver fat ≥10%	oral	MRI 12 weeks	safety and change in ALT and AST

Abbreviations: FA, fatty acid; FGF, fibroblast growth factor; FXR, farnesoid-X receptor; HbA1c, glycosylated haemoglobin; JNK, c-Jun N-terminal kinases; KLB, βKlotho; MRI, magnetic resonance imaging; mTOT, mitochondrial target of thiazolidinediones; PEG, pegylated; PPAR, peroxisome proliferator-activated receptor; SDC-1, stearoyl-CoA desaturase modulator; SGLT, sodium-glucose cotransporter; THR-β (thyroid hormone receptor β.

Table 6.

Trials for NASH-cirrhosis in late stage development

Drug	Trial code Name (Pharma)	N. of Patients	Study population	Route of administration	Surrogate end-point Time to end-point	Primary outcome
Aldafermin (NGM282) **(FGF19)**	NCT04210245 ALPINE 4 (NGM)	150	NASH, fibrosis F4 (compensated cirrhosis) liver fat ≥8% (MRI)	subcutaneous	Biopsy 48 weeks	≥1 stage improvement in fibrosis, no worsening of NASH Adverse events
Belapectin **(Galectin-3)**	NCT04365868 NASH-CX (Galectin)	162	NASH, fibrosis F4 HVPG ≥6mmHg	intravenous	HVPG 52 weeks	Change in HVPG
Obeticholic acid **(FXR agonist)**	NCT03439254 REVERSE (Intercept)	919	NASH, fibrosis F4	oral	Biopsy 78 weeks	≥1 stage improvement of fibrosis, no worsening of NASH or NASH resolution, no worsening of fibrosis
Pegbelfermin **(PEG-FGF21)**	NCT03486912 FALCON 2 (BMS)	152	NASH, fibrosis F4	subcutaneous	Biopsy 48 weeks	≥1 stage improvement of fibrosis, no worsening of NASH
Semaglutide SC **(GLP-1 receptor agonist)**	NCT03987451 (Novo Nordisk)	69	NASH, fibrosis F4 NAS score ≥3 BMI ≥27kg/m^2 stiffness >14kPa (MRE)	subcutaneous	Biopsy 48 weeks	≥1 stage improvement of fibrosis, no worsening of NASH

Abbreviations: FGF, fibroblast growth factor; FXR, farnesoid-X receptor; HVPG, hepatic vein pressure gradient; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; NAS, NAFLD activity score (sum of steatosis (0-3), lobular inflammation (0-3), hepatocellular ballooning (0-2); PEG, pegylated.

50

Figure 1

Histologic classification of NAFLD, according to the European Steatosis-Activity-Fibrosis (SAF) score.[42]

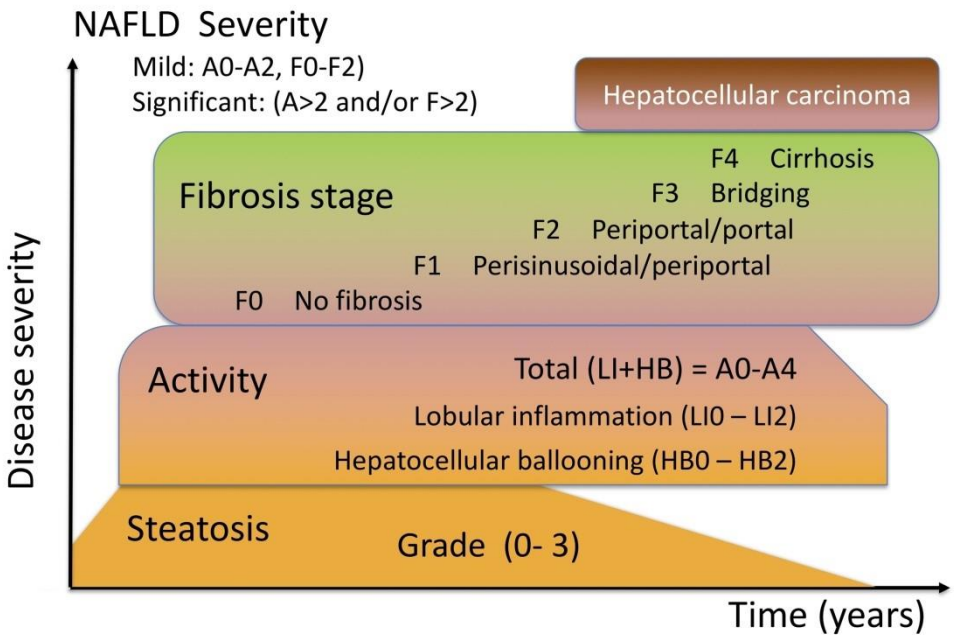
Legend

NASH is diagnosed by hepatocellular ballooning (HB) ≥ 1, independent of steatosis and lobular inflammation. Steatosis grade does not enter in the definition of disease severity Note that steatosis may disappear in subjects with advanced fibrosis; necro-inflammation too tends to decrease, but less sharply than steatosis. Both steatosis and necroinflammation may fluctuate during the years in response to intercurrent events.

Figure 2
Pathogenesis and progression of NAFLD.

Legend

Note that the disease may proceed totally asymptomatic to cirrhosis or liver failure, sometimes heralded by events associated with cardiovascular risk.



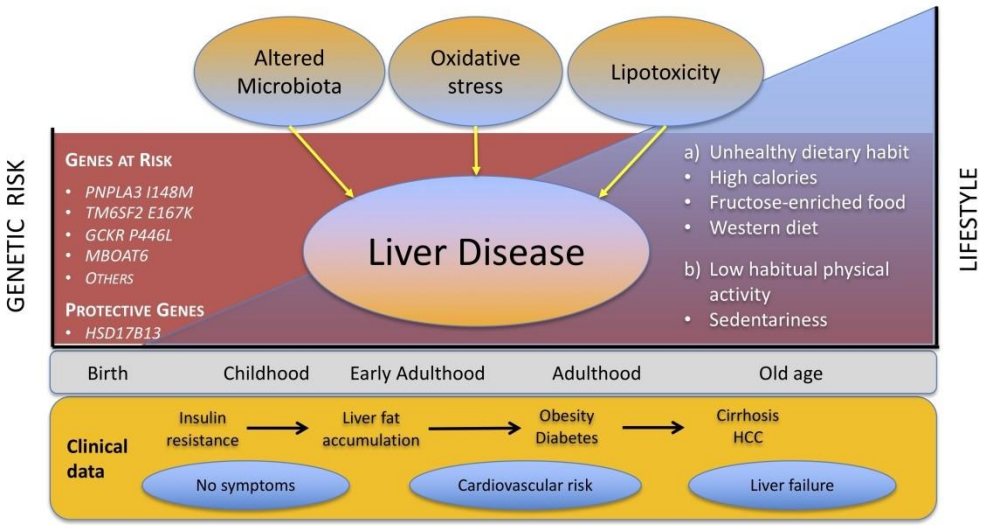
Histologic classification of NAFLD, according to the European Steatosis-Activity-Fibrosis (SAF) score.[42]

Legend

NASH is diagnosed by hepatocellular ballooning (HB) ≥ 1, independent of steatosis and lobular inflammation. Steatosis grade does not enter in the definition of disease severity

Note that steatosis may disappear in subjects with advanced fibrosis; necro-inflammation too tends to decrease, but less sharply than steatosis. Both steatosis and necroinflammation may fluctuate during the years in response to intercurrent events.

213x137mm (300 x 300 DPI)



Pathogenesis and progression of NAFLD.

Legend

Note that the disease may proceed totally asymptomatic to cirrhosis or liver failure, sometimes heralded by events associated with cardiovascular risk.

237x129mm (400 x 400 DPI)

Supplementary Table 1.
Most commonly used non-invasive markers for the diagnosis of NAFLD and for the assessment of disease severity

	Validation study	Strengths/Limitations
Biomarkers*		
Steatosis		
Fatty liver index (FLI) [1]	vs. US in the general population, AUROC 0.85	Two values (<30 and >60) to exclude or confirm the presence of steatosis
Hepatic steatosis index (HSI) [2]	In a US-assessed NAFLD cohort, AUROC 0.81	Only validated in a Korean population
Steatohepatitis		
NASH Test ™ [3]	In a biopsy-assessed NAFLD cohort (training and validation group), PPV 0.66 and NPV 0.72 for the diagnosis of NASH	NASH defined as NAS ≥5, non-NASH as NAS ≤2. Patented by Biopredictive, Paris, Fr, accessible on payment.
Fibrosis		
NAFLD fibrosis score (NFS) [4]	In a biopsy-assessed NAFLD training (n=480) and validation cohort (n=253). AUROC 0.84 for the global cohort. Two values (<-1.455 and ≥0.676) to exclude or confirm advanced fibrosis.	25% of cases classified as indeterminate. By applying the NAFLD fibrosis score, liver biopsy could have been avoided in 75% (549 of 733) of patients in the total cohort.
Enhanced liver fibrosis (ELF) ™ [5]	In a biopsy-assessed NAFLD cohort, AUROC 0.90 for severe fibrosis, 0.82 for moderate, 0.76 for no fibrosis. Improved diagnostic performance by inclusion of additional markers	82% and 88% of liver biopsies could be potentially avoided for the diagnosis of severe fibrosis using ELF and the combined panel, respectively. Accessible on payment.
FibroTest ™ [6]	In a biopsy-assessed NAFLD cohort, AUROC 0.81	Combined with Acti-Test. Patented by Biopredictive, Paris, Fr, accessible on payment.
Fibrometer ™ [7]	In a biopsy-assessed NAFLD cohort. Based on several variables, modified along the years. AUROC 0.94	Developed in patients with hepatitis C and in alcoholic hepatitis. Produced by Echosens, Paris, France
Fibrosis-4 index (FIB-4) [8]	In a biopsy-assessed NAFLD cohort, AUROC 0.80. Two values (< 1.3 and >2.67) to rule out or rule in advanced fibrosis (28% undetermined)	Based on simple, easily accessible variables. The test outperforms in comparison to six different markers of fibrosis in 541 adults with NAFLD
AST/Platelet Ratio Index (APRI) [9]	In biopsy-assessed NAFLD cohort, AUROC 0.87. Best cut-offs to rule out and rule in advanced fibrosis are 0.454 and 0.918, respectively	Developed in patients with hepatitis C. Based on very simple and accessible variables

Hepamet Fibrosis Score (HFS) [10]	In biopsy-assessed NAFLD training Spanish (n=758) and validation multiethnic cohort (n=1,694). AUROC 0.85 for the global cohort. Two values (< 0.12 and ≥0.47) to rule out or rule in advanced fibrosis.	Limited by need of non-routine tests (e.g., insulin). Not affected by BMI, high liver enzymes, diabetes. In liver unit samples, it outperforms compared with Fib-4 and NFS, limiting the "grey" intermediate zone.
Imaging		
Steatosis		
Ultrasounds [11]	Scoring system (0-6) validated in 94 biopsy-assessed NAFLD and in general population. AUROC 0.980	No possibility to distinguish NASH on the basis of ultrasonography
Controlled attenuation parameter (CAP) [12]	450 biopsy-assessed NAFLD patients. AUROC 0.87 (95% CI, 0.82-0.92)	Cutt-offs for steatosis mild, moderate, severe set at 302 dB/m, 331 dB/m, and 337 dB/m, respectively.
Magnetic resonance imaging (MRI) [13]	Meta-analysis of 10 studies with patients of different disease severity. Mean sensitivity, 82.0–97.4% and specificity, 76.1–95.3%.	MRI outperforms ultrasonography and CT scanning for all groups of steatosis severity. Usefulness limited by costs and availability of instruments for these analyses
Fibrosis		
Transient elastography (TE) (Fibroscan™) [14]	452 biopsy-assessed liver patients. Failure rates 14%. AUROC for advanced fibrosis 0.831 Accuracy of fibrosis stage, 80.8%.	In a retrospective analysis (mean follow-up 6.4 yrs), TE was the best tool predicting liver-related mortality, outperforming several blood fibrosis tests
Magnetic resonance elastography (MRE) [15]	104 biopsy-assessed NAFLD AUROC 0.82 (95% CI 0.74-0.91)	Outperforms TE, also for stage assessment. Exclusively available for research

*Components of biomarkers:

FLI: BMI, waist circumference, triglycerides, □-glutamyl-transferase;
HSI: BMI, aspartate and alanine aminotransferases;
NASH test: age, sex, □-glutamyl-transferase, bilirubin, haptoglobin, apoprotein A1, α2 macroglobulin, aspartate and alanine aminotransferases, cholesterol, triglycerides;
NFS: age, blood glucose, BMI, platelets;
ELF: hyaluronic acid, tissue inhibitor of metalloproteinase-1, amino-terminal pro-peptide of type III collagen;
Fibrometer: age, aspartate aminotransferases, platelet count, prothrombin index, α2 macroglobulin, hyaluronic acid, urea (with modifications);
FibroTest: γ-glutamyl-transferase, bilirubin, haptoglobin, apoprotein A1, α2 macroglobulin;
FIB-4: age, aspartate and alanine aminotransferases, platelets;
APRI, aspartate aminotransferase, platelet:
Hepamet: Age, sex, aspartate aminotransferase, albumin, HOMA (fasting glucose and insulin levels), diabetes, platelets.

https://mc.manuscriptcentral.com/bmj

Note that no imaging tools are available for NASH

Abbreviations not in the table: AUROC, area under the receiver operator characteristic; BMI, body mass index; CT, computed tomography; NAS, NAFLD activity score; NPV, negative predictive value; PPV, positive predictive value; US, ultrasonography

References

1. Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 2006;6:33. doi: 10.1186/1471-230X-6-33 [published Online First: 2006/11/04]
2. Lee JH, Kim D, Kim HJ, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis 2010;42(7):503-8. doi: 10.1016/j.dld.2009.08.002 [published Online First: 2009/09/22]
3. Poynard T, Ratziu V, Charlotte F, et al. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholic steatohepatitis in patients with non-alcoholic fatty liver disease. BMC Gastroenterol 2006;6:34. doi: 10.1186/1471-230X-6-34 [published Online First: 2006/11/14]
4. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45(4):846-54. doi: 10.1002/hep.21496 [published Online First: 2007/03/30]
5. Guha IN, Parkes J, Roderick P, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. Hepatology 2008;47(2):455-60. doi: 10.1002/hep.21984 [published Online First: 2007/11/27]
6. Poynard T, Lassailly G, Diaz E, et al. Performance of biomarkers FibroTest, ActiTest, SteatoTest, and NashTest in patients with severe obesity: meta analysis of individual patient data. PLoS One 2012;7(3):e30325. doi: 10.1371/journal.pone.0030325 [published Online First: 2012/03/21]
7. Cales P, Oberti F, Michalak S, et al. A novel panel of blood markers to assess the degree of liver fibrosis. Hepatology 2005;42(6):1373-81. doi: 10.1002/hep.20935 [published Online First: 2005/12/01]
8. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7(10):1104-12. doi: 10.1016/j.cgh.2009.05.033 [published Online First: 2009/06/16]
9. Cales P, Laine F, Boursier J, et al. Comparison of blood tests for liver fibrosis specific or not to NAFLD. Hepatol 2009;50(1):165-73. doi: 10.1016/j.jhep.2008.07.035 [published Online First: 2008/11/04]
10. Ampuero J, Pais R, Aller R, et al. Development and validation of Hepamet Fibrosis Scoring system - A simple, noninvasive test to identify patients with nonalcoholic fatty liver disease with advanced fibrosis. Clin Gastroenterol Hepatol 2020;18(1):216-25 e5. doi: 10.1016/j.cgh.2019.05.051
11. Hamaguchi M, Kojima T, Itoh Y, et al. The severity of ultras[published Online First: 2019/06/14] onographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. Am J Gastroenterol 2007;102(12):2708-15. doi: 10.1111/j.1572-0241.2007.01526.x [published Online First: 2007/09/27]
12. Eddowes PJ, Sasso M, Allison M, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 2019;156(6):1717-30. doi: 10.1053/j.gastro.2019.01.042 [published Online First: 2019/01/29]
13. Bohte AE, van Werven JR, Bipat S, Stoker J. The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. Eur Radiol 2011;21(1):87-97. doi: 10.1007/s00330-010-1905-5 [published Online First: 2010/08/04]
14. Boursier J, Vergniol J, Guillet A, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. J Hepatol 2016;65(3):570-8. doi: 10.1016/j.jhep.2016.04.023 [published Online First: 2016/05/07]
15. Park CC, Nguyen P, Hernandez C, et al. Magnetic resonance elastography vs transient elastography in detection of fibrosis and noninvasive measurement of steatosis in patients with biopsy-proven nonalcoholic fatty liver disease. Gastroenterology 2017;152(3):598-607 e2. doi: 10.1053/j.gastro.2016.10.026 [published Online First: 2016/12/03]