# Therapeutic pipeline in nonalcoholic steatohepatitis

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Abstract | Our understanding of nonalcoholic fatty liver disease pathophysiology continues to advance rapidly. Accordingly, the field has moved from describing the clinical phenotype through the presence of nonalcoholic steatohepatitis (NASH) and degree of fibrosis to deep phenotyping with a description of associated comorbidities, genetic polymorphisms and environmental influences that could be associated with disease progression. These insights have fuelled a robust therapeutic pipeline across a variety of new targets to resolve steatohepatitis or reverse fibrosis, or both. Additionally, some of these therapies have beneficial effects that extend beyond the liver, such as effects on glycaemic control, lipid profile and weight loss. In addition, emerging therapies for NASH cirrhosis would have to demonstrate either reversal of fibrosis with associated reduction in portal hypertension or at least delay the progression with eventual decrease in liver-related outcomes. For non-cirrhotic NASH, it is the expectation that reversal of fibrosis by one stage or resolution of NASH with no worsening in fibrosis will need to be accompanied by overall survival benefits. In this Review, we summarize NASH therapies that have progressed to phase II and beyond. We also discuss some of the potential clinical challenges with the use of these new therapies when approved.

Nonalcoholic fatty liver disease (NAFLD) is characterized by fatty infiltration of the liver in individuals with features of metabolic syndrome[1]. Although NAFLD can be non-invasively diagnosed by radiological assessment, a liver biopsy is currently necessary to diagnose nonalcoholic steatohepatitis (NASH) from nonalcoholic fatty liver (NAFL). The NASH phenotype is characterized by the presence of hepatocyte ballooning, lobular inflammation, macrovesicular steatosis and very often perisinusoidal fibrosis[1]. As the diagnosis of NASH is defined by the presence and pattern of specific histological abnormalities on liver biopsy, a scoring tool for the histological features of NAFLD (the NAFLD activity score (NAS)) was developed to measure changes during therapeutic trials[2]. Generally, a NAS of ≥5 correlates highly with a diagnosis of NASH, and most clinical trials, therefore, have an inclusion criterion of NAS ≥4. Currently, there are no approved therapies for the treatment of NASH[1]. Unsurprisingly, NASH is now one of the major indications for liver transplantation worldwide[3–10]. Unabated, it is likely to rise to become the leading indication[3,5,9]. In this Review, we navigate through the clinical trial results of several emerging therapies that have progressed to phase II and beyond, discussing both safety and efficacy. We believe that the safety, efficacy and differentiating features with benefits that extend beyond the liver will ultimately enable clinicians to personalize the treatment options for their patients with NASH.

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## NASH pathophysiology

The pathophysiology and associated severity of steatohepatitis seems to be a complex interaction between a patient's genetic determinants (such as polymorphisms in *PNPLA3*, *TM6SF2*, *MBOAT1* or *HSD17B13*), associated comorbidities (such as obesity, type 2 diabetes mellitus (T2DM), obstructive sleep apnoea, polycystic ovarian syndrome (PCOS) and gut dysbiosis) and a combination of environmental (socioeconomic) factors and behavioural factors (diet and exercise)[1,11–17]. Individuals with NASH are at risk of showing progression to cirrhosis with a natural history that seems to be highly variable and dynamic[18]. The change in disease activity, as evidenced by the change in NAS over time, is a strong predictor of fibrosis regression or progression[19]. In a study from the NASH Clinical Research Network (NASH CRN) published in 2019, in 446 patients with NAFLD over a mean interval of ~5 years, NAFL resolved in 13% and progressed to steatohepatitis in 42%[19]. Fibrosis progression or regression by at least one stage occurred in 30% and 34% of participants, respectively[19]. Metabolic syndrome, baseline NAS and a smaller reduction in NAS were associated with progression to advanced fibrosis (stage F3 or F4)[19]. Alternatively, fibrosis regression

**Key points**

- There are several novel treatments currently under development for treatment of non-cirrhotic and cirrhotic nonalcoholic steatohepatitis.
- Resolution of steatohepatitis and reversal of fibrosis are two important histological end points in the ongoing clinical trials.
- Emerging therapies are beginning to differentiate through extra-hepatic benefits such as improvement in glycaemic control, lipid profile and weight loss.
- Safety and tolerability of the emerging therapies will determine compliance that would result in sustained benefits and, ultimately, improvement in long-term survival.

was associated with a lower baseline insulin level and a decrease in all NAS components[19]. Changes in the aspartate aminotransferase (AST) level, the alanine aminotransferase (ALT) level and NAS were also associated with fibrosis regression and progression[19]. Although most patients could be slow progressors, it is estimated that up to 20% could be rapid progressors from underlying disease activity[18]. In two large completed clinical trials in patients with NASH, ~16–22% of those with bridging fibrosis showed progression to cirrhosis in under 2 years[20]. Notably, patients enrolled in the simtuzumab trial had an inclusion criterion of NAS ≥4, and more of those with Ishak fibrosis stage F4 showed progression to cirrhosis than those with Ishak fibrosis stage F3, suggesting that those with advanced disease are closer to a final outcome[20]. These observations are in line with the findings of previous studies showing that the degree of fibrosis is the single most important predictor of liver-related mortality[21–24]. Similarly, liver-related clinical events such as ascites and variceal bleeding occurred in 2–19% in patients with cirrhosis within 2 years[20]. The wide range in the outcomes reported in these two studies underscores the spectrum of disease severity in patients with compensated cirrhosis that extends beyond variables such as fibrosis stage, model for end-stage liver disease (MELD) score or Child–Turcotte–Pugh class[25,26]. Furthermore, these studies had selection bias based on the inclusion and exclusion criteria and might have led to overestimation of the progression rate[25,26]. Thus, it is now apparent that the clinical course is variable and, to a degree, unpredictable. In summary, disease activity as indicated by the NAS or the presence of NASH indicates the severity of underlying biology injuring the liver, whereas the stage of fibrosis reflects the proximity to cirrhosis and a liver-related event. These two variables are intimately connected and the ideal treatment for NASH has to address both.

Liver enzymes might or might not be elevated in patients with NASH; furthermore, they can fluctuate over time[1,27–29]. Monitoring of disease activity or disease progression with serial measurements of liver enzymes is, therefore, not a viable option. Monitoring with liver stiffness measurement (LSM) either with ultrasound-based or magnetic resonance-based technologies is fast emerging as a viable option, but has not yet been validated as a reasonably accepted surrogate end point for conditional approval by regulatory agencies in pre-cirrhotic NASH[30–34]. Because of the time required to accumulate enough clinical end points in patients with non-cirrhotic NASH in a phase III trial, the regulatory authorities currently provide conditional approval with either resolution of NASH by histology without worsening of fibrosis, or regression in fibrosis without worsening of NASH (TABLE 1) — two histological end points that are deemed indicative of improvement in the disease state[33–35]. Histology continues to be critically needed for late phase II trials for estimation of treatment effect size and sample size calculation for subsequent phase III trials[35]. However, for early phase II trials, improvement in hepatic steatosis as measured in terms of the MRI-derived proton density fat fraction (MRI-PDFF) and fibrosis as measured in terms of blood-based biomarkers or imaging-based modalities seems to be acceptable[35] (TABLE 1).

Guidance from FDA in 2019 recommended that the duration of late phase II trials should be at least 12–18 months for optimal characterization of histological changes[35]. By contrast, and understandably so, the regulatory pathway for cirrhosis is currently geared towards a decrease in clinical outcomes as the relationship between histological changes and clinical outcomes has not been well characterized[36]. Currently, a phase III trial design for compensated cirrhosis (with or without portal hypertension) is geared towards composite end points of clinical outcomes such as new-onset ascites, variceal bleeding, hepatic encephalopathy, or elevated bilirubin level or international normalized ratio[36]. Thus, patients with these clinical manifestations at baseline and those listed for liver transplantation, with a MELD score of >12 or hepatocellular carcinoma (HCC) are typically excluded from enrolment[36] (TABLE 1). This guidance is currently open for comments. We anticipate a few revisions to this guidance as patients with compensated cirrhosis would benefit from conditional approval owing to the risk of morbidity and mortality associated with a liver decompensation event. One end point to consider would be the prevention of new varices in a patient with compensated cirrhosis with no varices at baseline. By contrast, there is no clear guidance yet with regard to the phase II clinical trial for NASH cirrhosis. Most studies have used hepatic venous pressure gradient (HVPG) and a blood-based biomarker such as the enhanced liver fibrosis (ELF) test (Siemens) and NIS4 (REFS[37–40]). The ELF score is a single score from a proprietary algorithm that uses three serum biomarkers, hyaluronic acid, procollagen III amino-terminal peptide, and tissue inhibitor of metalloproteinase 1 (REF.[37]). The ELF test has been granted a Breakthrough Device designation by the FDA and is currently only available in the USA for clinical trials testing. We anticipate that both the ELF test and NIS4 will be cleared or approved as disease monitoring or treatment response tools[37].

In 2020, several experts reached consensus that the term NAFLD does not capture and convey the metabolic dysfunction driving the pathophysiology, and the term metabolic (dysfunction)-associated fatty liver disease (MAFLD) is a more appropriate and overarching term[41]. However, we do not anticipate the regulatory authorities to consider treatment of MAFLD as an approvable indication in the foreseeable future due to heterogeneity of the phenotype. However, further understanding and differentiation of these phenotypes into those with aggressive natural history due to steatohepatitis or progressive

fibrosis ultimately leading to liver-related or overall mortality could open up a regulatory pathway.

## Drugs beyond phase II

NASH has a substantial health-care burden: the at-risk population includes an estimated 13% of the world's adult population with obesity and 39% of the population with overweight[42,43]. Unfortunately, there are no FDA-approved NASH medications currently[1]. Two agents, vitamin E as an antioxidant and pioglitazone as an insulin sensitizer, have shown modest efficacy against NASH in randomized controlled trials (RCTs)[44,45]. For vitamin E, the initial concerns from observational trials on its association with stroke risk have precluded its widespread use[46,47]. However, these concerns seem to be unsubstantiated[48]. A meta-analysis published in 2018 showed a statistically significant inverse relationship between vitamin E and stroke, suggesting that a higher dietary vitamin E intake is associated with a lower stroke risk[48]. Another concern with the use of vitamin E came from observations made from the continued follow-up of participants in the SELECT trial: men who took 400 IU of vitamin E daily were more likely to have prostate cancer than men who took a placebo[49]. For every 1,000 men, 76 of those who took vitamin E supplements had prostate cancer over a 7-year period versus 65 of those taking placebo; that is, vitamin E supplementation was associated with 11 more cases of prostate cancer per 1,000 men. Although numerically small, this result represented a 17% increase in the rate of prostate cancer[49]. However, a meta-analysis published in 2015 that examined the association between vitamin E intake amount and the risk of prostate cancer among ever or never smokers failed to show any such increase[50]. Lastly, fluid retention, and possibly bladder cancer in patients treated with pioglitazone have limited its utilization by providers[1,51–54].

The increased understanding of mechanistic pathways that lead to NASH development and its progression has led to the development of numerous medical therapies for NASH that can be divided into four major categories (FIG. 1): metabolic targets that improve insulin sensitivity, inhibit different enzymes involved in de novo lipogenesis, or improve mitochondrial utilization of fatty acids; inflammation or cell injury targets that inhibit recruitment of inflammatory cells or block inflammatory signalling, reduce oxidative and/or endoplasmic reticulum stress, or inhibit hepatocyte apoptosis; liver–gut axis targets that modulate bile acid enterohepatic circulation and signalling, or alter the gut microbiota; and anti-fibrotic targets that directly target hepatic stellate cells, decrease collagen deposition in the liver, or enhance fibrolysis. A summary of medications in phase III clinical development is provided in TABLE 2.

### *Targeting bile acid receptors*

The most advanced drug in development is the farnesoid X receptor (FXR) agonist, obeticholic acid (OCA). FXR is a nuclear receptor that is highly expressed in the liver and small intestine[55]. Bile acids are the natural ligand of FXR[55] and, together, they regulate lipid and/or glucose homeostasis, promote insulin sensitivity and potentially modulate liver fibrosis[56]. A new drug application for OCA, which showed consistent efficacy on fibrosis regression in phase II and III trials in patients with NASH and significant fibrosis, has been submitted for potential approval[57–59]. In the phase III REGENERATE trial (NCT02548351), 1,968 patients were randomly assigned to receive OCA at 10 mg or 25 mg daily or placebo and an interim analysis was done at 18 months to assess for a histological response[59] (FIG. 2). The trial demonstrated significant improvement in fibrosis by one stage in patients receiving OCA 25 mg

Table 1 | **Summary of drug development strategies for NASH therapies**

| Phase | Blood-based | | | Radiology including elastography | Liver histology | Clinical outcomes | Biomarker strategy | Duration | Comments |
|---|---|---|---|---|---|---|---|---|---|
| | Hepatocyte inflammation | Fibrosis | Target engagement | | | | | | |
| *Non-cirrhotic with fibrosis* | | | | | | | | | |
| Early phase II | ✓ | ✓ | ✓ | ✓ | NA | NA | Optional | Variable depending on mechanism of action and anticipated effect | NA |
| Late phase II | ✓ | ✓ | ✓ | ✓ | ✓ | Optional | ✓ | 12–18 months | NA |
| Phase III | Optional | Optional | Optional | Optional | ✓ | ✓ | ✓ | 12–18 months for accelerated approval | Additional 4–5 years for clinical end points |
| *Compensated cirrhosisa* | | | | | | | | | |
| Early phase II | ✓ | ✓ | ✓ | ✓ | NA | NA | ✓ | Unclear | No guidance from FDA |
| Late phase II | ✓ | ✓ | ✓ | ✓ | NA | ✓ | ✓ | ≥2 years | |
| Phase III | ✓ | ✓ | ✓ | ✓ | NA | ✓ | ✓ | | Likely to be a traditional drug approval pathway |

NA, not applicable. [a]FDA guidance is currently in a draft stage and represents the current thinking of the FDA as of 30 July 2020.

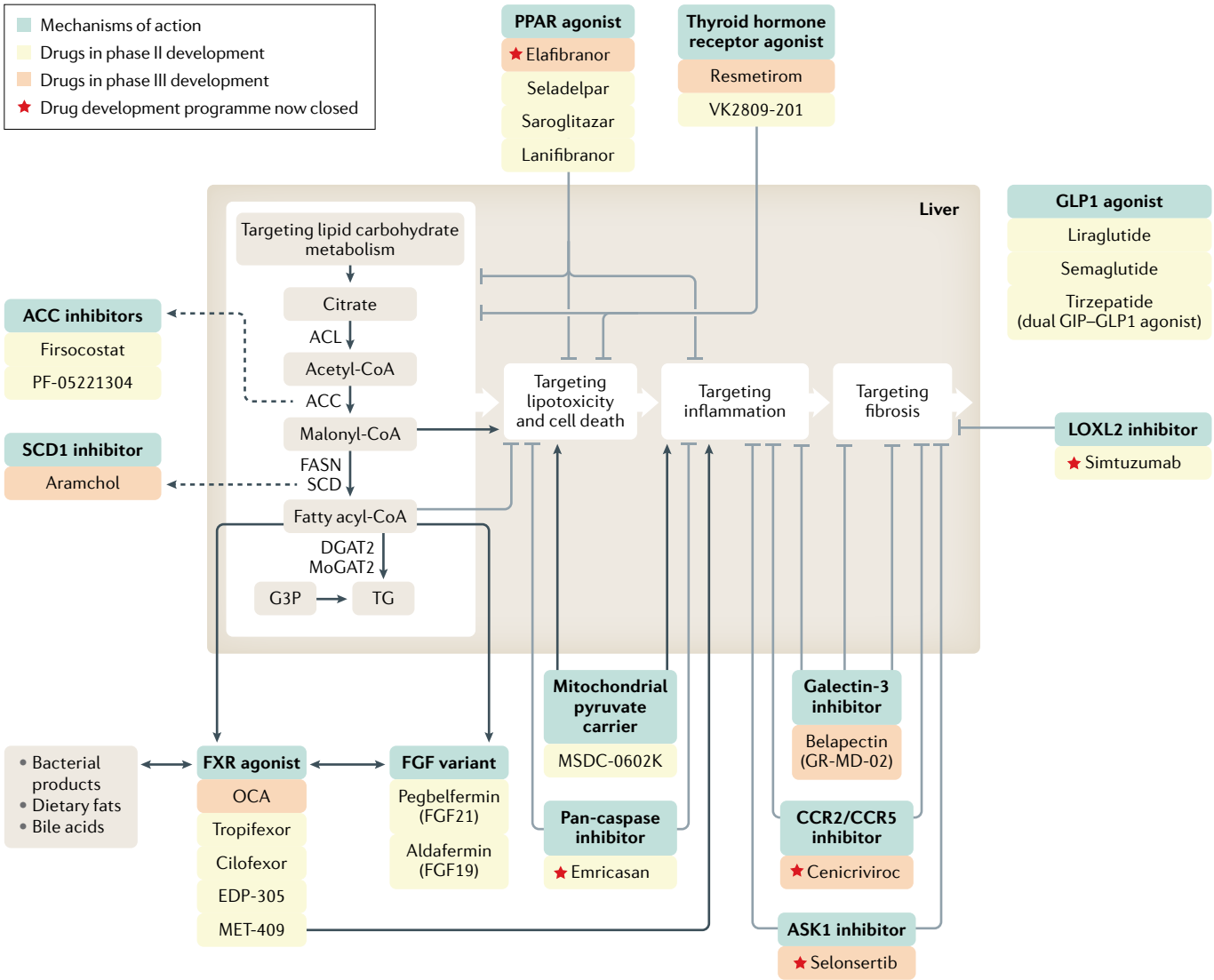


Fig. 1 | **Mechanism of action of NASH drugs currently in phase II and phase III development.** Several nonalcoholic steatohepatitis (NASH) drugs are now in late-stage development. ACC, acetyl CoA carboxylase; ACL, ATP-citrate lyase; CCR, CC-chemokine receptor; DGAT2, diacylglycerol *O*-acyltransferase 2; FASN, fatty acid synthase; FGF, fibroblast growth factor; FXR, farnesoid X receptor; G3P, glycerol-3-phosphate; GLP1, glucagon-like peptide 1; MoGAT2, monoacylglycerol *O*-acyltransferase 2; OCA, obeticholic acid; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase; TG, triglyceride. Figure is adapted from REF.[209], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

daily compared with those receiving placebo (23% versus 12%; $P = 0.0002$)[59]. Of patients who received at least 15 months of therapy with OCA 25 mg daily, 38% achieved improvement in fibrosis compared with 23% of those who received placebo[59]. Patients receiving OCA 25 mg also had a lower worsening of fibrosis; that is, 13% compared with 21% receiving placebo[59]. Taken together, it seems that up to five patients need to be treated for one patient to achieve either improvement or no worsening of fibrosis. Although NASH resolution was not seen in the intention-to-treat population with fibrosis stage F2 or F3 (11.7% versus 8%; $P = 0.13$), in both the post hoc analysis and the full efficacy analysis population with fibrosis stage F1–F3, the rate of NASH resolution without worsening of fibrosis was significantly higher in the OCA 25 mg arm than in the placebo arm (14.9% versus 7.9%; $P = 0.0013$)[59].

In terms of adverse events (AEs), OCA at 25 mg daily was associated with pruritus in half of patients, with severe intensity in 28%[59]. Although some data were presented showing that the quality of life was not altered in those who reported itching, it is unclear how many individuals will discontinue therapy due to pruritus in the real world. Furthermore, elevation in LDL cholesterol level was noted with a peak increase of 0.59 mmol/l (22.6 mg/dl) at 4 weeks that subsequently reversed and approached the baseline value at 18 months (0.1 mmol/l (4.0 mg/dl) increase from baseline) with medical management mainly consisting of adding a statin medication[59]. Studies examining the effect of OCA on the lipoprotein profile in patients with NASH participating in the phase II trials revealed increased levels of small VLDL particles, large and small LDL particles, and a reduction in HDL particles after 12 weeks of

therapy[60]. These lipoprotein concentrations had reverted to baseline values 24 weeks after drug discontinuation[60]. Although the incidence of cardiovascular AEs was similar across treatment groups in the REGENERATE trial, it is important to note that statin therapy was initiated in twice as many patients on OCA than on placebo and with reversal of increased levels occurring at 6 months after initiation. Thus, more monitoring is required when OCA is used in the real world.

With respect to hepatobiliary events, more patients receiving OCA 25 mg daily experienced hepatobiliary events in the form of gallstones or cholecystitis (3%) than those receiving placebo (<1%) and those receiving OCA 10 mg daily (1%)[59]. Whether these events occurred in patients who had gallstones at baseline is unclear. There is some suggestion that OCA increases human gallbladder cholesterol saturation and bile acid hydrophobicity, both of which decrease cholesterol solubility in bile[61]. Together with increased hepatobiliary fibroblast growth factor 19 (FGF19) expression, pharmacological FXR activation could increase the risk of gallstone formation[61]. It is possible that the hepatobiliary events could increase over time due to increased gallstone formation. These safety issues, therefore, raise concerns about long-term tolerability, cardiovascular morbidity and gallstone-related events. In the current study, long-term effects, including overall mortality, progression to cirrhosis, and liver-related morbidity (defined by the development of any of the following: a MELD score of ≥15, the need for liver transplantation, ascites requiring medical intervention, hospitalization for onset of variceal bleeding, hepatic encephalopathy, or spontaneous bacterial peritonitis), will also be evaluated through a 7-year period[62]. In our view, OCA demonstrates both improvement in histological markers of disease activity and fibrosis, and is likely to be an important addition to the therapeutic armamentarium, but will need monitoring and management of cholesterol, gallstones and pruritus. More recently, the FDA issued a complete response letter requesting additional post-interim efficacy and safety data to better determine the predicted benefit of OCA based on a surrogate histopathological end point[63].

The reversal of fibrosis by OCA in the phase II FLINT trial spurred substantial interest in FXR as a therapeutic target[57]. At the same time, the use of OCA during the study also brought to attention tolerability issues such as pruritus and AEs such as hyperlipidaemia[57,60]. These AEs were attributed to the steroidal bile acid-like chemical structure of OCA[64]. The steroidal structure with associated G protein-coupled bile acid receptor 1 (GPBAR; also known as TGR5) agonistic properties, which could have synergistic therapeutic potential but also enhance some TGR5-related adverse effects such as pruritus[64]. For these reasons, synthetic non-steroidal FXR agonists are in development, which theoretically could preserve the full therapeutic potential of FXR while avoiding its AEs (TABLE 3). However, contrary to expectations, phase II trials testing the synthetic non-steroidal FXR agonists continue to find a dose-dependent association with pruritus (TABLE 3).

### *Targeting PPARs*

Elafibranor is a dual agonist for peroxisome proliferator-activated receptor-α (PPARα) and PPARδ, which belong to a family of ligand-activated transcription factors that regulate several metabolic processes[65,66]. PPARα is expressed in metabolically active tissues, including the liver; its activity lowers lipid levels and drives the expression of genes that regulate fatty acid β-oxidation, lipid transport and the hormone FGF21 (REFS[67,68]). PPARδ is highly expressed in hepatocytes and is involved in fatty acid oxidation, decreases hepatic glucose production and improves insulin sensitivity[69,70]. More importantly, it exerts anti-inflammatory activities in macrophages and Kupffer cells[71]. Overall, elafibranor acts as an insulin sensitizer, leading to potential improvements in hepatic steatosis, inflammation and fibrosis, as suggested by preclinical models and early human investigations[66,72]. In the phase II GOLDEN-505 trial in 276 adults (18–75 years of age) with non-cirrhotic NASH, no statistically significant difference between the elafibranor and placebo groups was observed in the protocol-defined primary outcome[73]. However, in the post hoc analysis, a dosage of 120 mg daily for 52 weeks in patients with fibrosis

Table 2 | **Therapies currently beyond phase II for the treatment of non-cirrhotic NASH with fibrosis**

| Medication | Mechanism of action | Effective dose | Phase II efficacy data | | Phase III RCT | Planned interim analysis duration (weeks) | Comments |
|---|---|---|---|---|---|---|---|
| | | | Resolution of NASH | Decrease in fibrosis | | | |
| Obeticholic acid | FXR agonist | 25 mg per day | No | Yes | REGENERATE (NCT02548351) | 72 | Interim analysis data submitted to FDA and received a CRL for additional post-interim data |
| Elafibranor | PPARα/δ agonist | 120 mg per day | Yes | No | RESOLVE-IT (NCT02704403) | 72 | Interim analysis failed to show any treatment effect; the programme has been terminated |
| Resmetirom | THRβ agonist | 80–100 mg per day | Yes | No | MAESTRO (NCT03900429) | 52 | Recruiting |
| Aramchol | SCD1 inhibitor | 300 mg twice daily | Yes | No | ARMOR (NCT04104321) | 52 | Recruiting |
| Cenicriviroc | CCR2–CCR5 antagonist | 150 mg per day | No | Yes | AURORA (NCT03028740) | 52 | Terminated due to lack of efficacy |

CCR, CC-chemokine receptor; CRL, complete response letter; FXR, farnesoid X receptor; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; RCT, randomized controlled trial; SCD1, stearoyl-CoA desaturase 1; THRβ, thyroid hormone receptor-β.

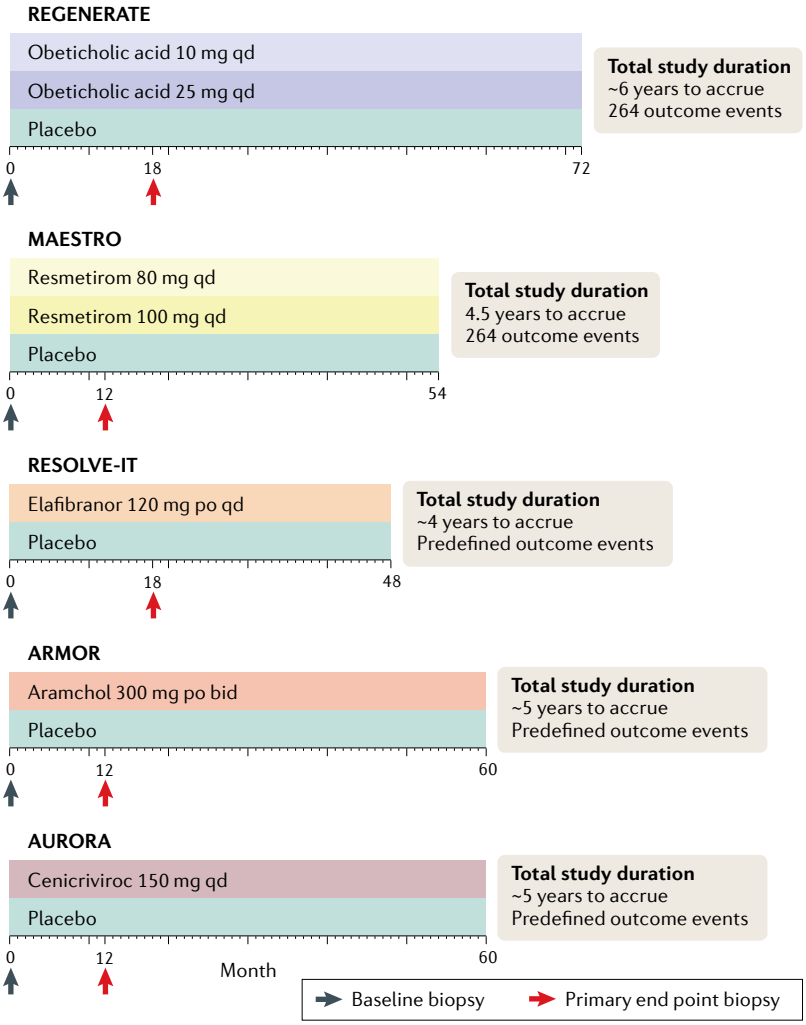


Fig. 2 | **The design of phase III RCTs currently testing medications to treat NASH.** All randomized controlled trials (RCTs) of drugs for the treatment of nonalcoholic steatohepatitis (NASH) have a planned interim analysis that will assess histological response based on liver biopsy at 12–18 months and extension arms to evaluate for long-term outcomes. The scale bars are time lines in months. bid, twice daily; po, oral; qd, once daily.

stage F2 or F3 led to significantly higher rates of NASH resolution than seen in the placebo group (19% versus 12%; $P = 0.045$)[73]. Also, after receiving elafibranor 120 mg daily, patients with NASH resolution had reductions in fibrosis score (mean reduction of $0.65 \pm 0.61$ in responders versus an increase of $0.10 \pm 0.98$ in non-responders; $P < 0.001$) and NAS due to improvement in hepatocyte ballooning, lobular inflammation and steatosis[73]. Additionally, metabolic benefits (such as improvement in lipids, glucose profiles and markers of systemic inflammation) were markedly reduced in the elafibranor 120 mg group versus the placebo group[74]. Subsequently, a phase III trial (RESOLVE IT, NCT02704403) has enrolled ~2,000 participants with histologically proven NASH with fibrosis stage F2 or F3 (based on post hoc analysis from a phase II trial) to assess the effects of elafibranor (120 mg per day or placebo) for 72 weeks on liver histology with long-term follow-up to evaluate the development of liver-related complications (FIG. 2).

The results of part 1 of this trial were announced in May 2020 and presented at a conference in November 2020 (REFS[75,76]). The trial did not meet the predefined primary end point of NASH resolution without worsening of fibrosis in the intention-to-treat population with a response rate of 19.2% in the elafibranor arm compared with 14.7% in the placebo arm[76]. With regard to fibrosis, 24.5% of patients who received elafibranor 120 mg daily showed improvement in fibrosis by at least one stage compared with 22.4% in the placebo arm[75,76]. The other key secondary end point related to metabolic parameters did not achieve statistical significance[75,76]. Subsequently, the sponsors closed the trial as it was determined that it was unlikely to yield the data to support regulatory approval[77].

### *Targeting liver-specific THRs*

Thyroid hormones regulate many processes involved in hepatic triglyceride and cholesterol metabolism to decrease serum cholesterol and intrahepatic lipid content[78–80]. The thyroid hormone functions as a ligand to its two receptors, thyroid hormone receptor-α (THRα) and THRβ[78–80]. Although both isoforms are expressed in most tissues, THRβ is the major form expressed in the liver, whereas THRα is highly expressed in the heart and bone[78–80]. The role of THRβ in hepatic lipid metabolism is well established from evidence from in vivo models[78–80], as discussed in-depth by Sinha et al., including the major mechanisms employed by thyroid hormone to regulate hepatic triglyceride and cholesterol metabolism and thyromimetics as potential therapies for NAFLD[78]. The incidence of clinical and subclinical hypothyroidism is higher in patients with NAFLD or NASH than in the general population, and evidence suggests that NASH could be associated with diminished liver thyroid hormone levels[28,81–83]. Thus, treatment of NAFLD or NASH with liver-specific thyromimetics is an attractive option due to additional metabolic benefits. Resmetirom and VK2809 are two orally active agonists of THR that are liver-directed with a severalfold higher selectivity than triiodothyronine (T3) for THRβ than THRα[84–87].

A phase IIb study enrolled 125 patients and randomly assigned 84 to resmetirom and 41 to placebo[87]. Based on MRI-PDFF measurements, resmetirom-treated patients had a relative reduction in liver fat compared with those receiving placebo both at week 12 (−36.3% resmetirom, −9.6% placebo; $P < 0.0001$) and week 36 (−37.3% resmetirom, −8.9% placebo; $P < 0.0001$)[87]. Based on liver biopsy, resmetirom treatment was associated with higher rates of NASH resolution (27.4% resmetirom versus 6.5% placebo; $P = 0.02$) with rates increasing to 39% in those who had 30% or more relative reduction in MRI-PDFF scores. However, the proportion of patients achieving at least a one-point reduction in fibrosis without worsening of NASH did not differ between the treatment groups[87]. Moreover, resmetirom had a favourable effect on lipid profiles compared with placebo with reductions in levels of LDL cholesterol by 22.3%, triglycerides by 30.8% and lipoprotein(a) by 37.9% ($P < 0.0001$ for all lipids)[87]. AEs were mostly mild with a few moderate AEs that were balanced between the groups; an increased incidence of

mild transient diarrhoea was noted with resmetirom[87]. Two phase III trials with different primary end points are currently active and recruiting. The phase III MAESTRO-NASH trial (NCT03900429) plans to enrol 2,000 participants with non-cirrhotic NASH and fibrosis stage F2 or F3 to evaluate the effect of resmetirom at 80 mg or 100 mg daily compared with placebo on achieving NASH resolution on liver histology obtained at 52 weeks (FIG. 2). In addition, the trial will evaluate the effect of resmetirom on a composite long-term outcome measured by the number of patients with the onset of cirrhosis, liver-related clinical outcomes and all-cause mortality up to 54 months. A second phase III trial with resmetirom, the MAESTRO-NAFLD-1 (NCT04197479), is a non-biopsy study in patients with NASH based on historical biopsy and presumed NASH using non-invasive techniques to collect additional safety data and support a broad potential therapeutic benefit of resmetirom on liver and cardiovascular end points in patients with NAFLD suspected to have NASH but with no histological confirmation[88].

### *Targeting liver lipid metabolism*

Aramchol is a conjugate of a bile acid (cholic acid) and a fatty acid (arachidic acid) that downregulates liver steatosis by inhibiting the stearoyl-CoA desaturase 1 (SCD1) enzyme that controls the rate-limiting step in mono-unsaturated fatty acid synthesis. A proof-of-concept trial using aramchol (100 mg or 300 mg per day) in 60 patients with NAFLD for 3 months showed a dose-dependent decrease in hepatic fat[89]. The phase IIb ARREST study (NCT02279524) enrolled 247 patients with biopsy-proven NASH with NAS ≥4 who had overweight or obesity and had confirmed prediabetes or T2DM with the primary end point being absolute percentage change in liver fat content measured by magnetic resonance spectroscopy[90]. Aramchol at 600 mg daily was associated with a trend towards higher rates of NASH resolution without worsening of fibrosis (16.7% compared with 5% in the placebo arm; $P=0.051$). In addition, aramchol was associated with liver fat reduction and biochemical improvement in liver enzymes. The phase III/IV ARMOR trial (NCT04104321) is a double-blind, placebo-controlled study that will enrol 2,000 individuals with NASH and fibrosis stage F2 or F3 who have overweight or obesity and have prediabetes or T2DM and will randomly assign participants to aramchol 300 mg twice daily or placebo (FIG. 2). The primary end points of the study are to evaluate the effect of aramchol on NASH resolution and/or fibrosis improvement at 52 weeks and then clinical outcomes related to the progression of liver disease over ~5 years.

### *Targeting liver chemokine receptors*

Cenicriviroc is a dual antagonist for CC-chemokine receptor 2 (CCR2) and CCR5 (REF.[91]). The activation of these receptors causes monocyte and macrophage recruitment to the inflamed tissue and activation of hepatic stellate cells, leading to fibrogenesis[92]. CCR2/CCR5 are upregulated in the liver of patients with obesity and NASH[93,94]. The CENTAUR phase IIb trial evaluated cenicriviroc in patients with non-cirrhotic NASH who had a NAS of ≥4, and NASH CRN fibrosis stage F1–F3 (REFS[95,96]). At the interim analysis, the primary end point at year 1 (an improvement of two or more points in NAS with an improvement of one or more points in either lobular inflammation or hepatocellular ballooning, with no worsening of fibrosis) was not met[95]. However, an improvement in fibrosis by at least one stage was achieved in significantly more patients on cenicriviroc than on placebo (20% versus 10%; $P=0.02$)[95]. Due to an unexpected improvement in fibrosis, the study was continued for the remainder of the duration and final data from year 2 exploratory analyses were published in 2020 (REF.[96]). Most individuals who achieved an improvement in fibrosis of one or more stages at year 1 maintained the improvement at year 2, with a greater effect observed in those with more advanced fibrosis[96].

Table 3 | **Summary of FXR agonists with clinical trial results in non-cirrhotic NASH**

| FXR agonist | Phase | Sample size | Daily dose | Duration (weeks) | Main efficacy end point | Main safety end point |
|---|---|---|---|---|---|---|
| ***Semi-synthetic steroidal FXR agonist*** | | | | | | |
| OCA (bile acid analogue)[59] | III | 933 | 10 mg or 25 mg | 72 | Fibrosis regression by one stage: placebo 12%, 10 mg 18%, 25 mg 23% | Pruritus (any grade): 28% at 10 mg, 51% at 25 mg |
| ***Synthetic non-steroidal FXR agonist*** | | | | | | |
| Cilofexor[128] | II | 140 | 30 mg or 100 mg | 24 | Relative HFF reduction: placebo +1.9%, 30 mg −1.8%, 100 mg −22.7% | Grade 2–4 pruritus: 4% at 30 mg, 14% at 100 mg |
| Tropifexor[131–133] | II | 152 | 140 µg or 200 µg | 12 | Relative HFF reduction: placebo −10%, 140 µg −17%, 200 µg −34% | Pruritus leading to discontinuation: 2% at 140 µg, 6% at 200 µg |
| EDP-305 (REF.[135]) | II | 134 | 1 mg or 2.5 mg | 12 | ALT reduction: placebo −15 U/l, 2.5 mg −28 U/l | Pruritus (any grade): 10% at 1 mg, 51% at 2.5 mg |
| MET-409 (REF.[136]) | II | 58 | 50 mg or 80 mg | 12 | Relative HFF reduction: placebo −6%, 50 mg −38%, 80 mg −55% | Pruritus (any grade): 10% at 50 mg, 35% at 80 mg |

Only phase II and III results listed. ALT, alanine amino transferase; FXR, farnesoid X receptor; HFF, hepatic fat fraction; NASH, nonalcoholic steatohepatitis; OCA, obeticholic acid.

The final analysis at year 2 showed that 24% of patients who switched to cenicriviroc and 17% who remained on placebo achieved an improvement in fibrosis by one or more stages and no worsening of NASH ($P = 0.37$)[96]. Over 2 years, a similar proportion on cenicriviroc or placebo achieved an improvement in fibrosis by one or more stages and no worsening of NASH (15% versus 17%)[96]. Overall, cenicriviroc has shown a favourable safety and tolerability profile in >1,000 individuals with up to 2 years of treatment[96]. AURORA (NCT03028740) is a phase III, multicentre, randomized, double-blind, placebo-controlled trial, which will be conducted in two parts. In part 1, ~1,200 study participants were randomly assigned 2:1 to cenicriviroc 150 mg orally daily or placebo to evaluate a surrogate histology end point at year 1 (FIG. 2). There will be ~2,000 study participants in part 2, which is expected to last >5 years overall to assess clinical outcomes, including liver-related events and overall mortality. Recently, this study was discontinued early due to lack of efficacy based on the results of part 1. The study results were not available in the public domain at the time of writing this manuscript; it is likely that the cenicriviroc drug development programme for NASH will be terminated.

## Drugs in phase II

Phase II studies can be divided into those that have used liver biopsy (phase IIb) to determine the primary outcome and those that have used MRI-PDFF and/or ALT (phase IIa) (Supplementary Table 1). Most drugs that have completed phase IIb and demonstrated efficacy with an acceptable safety profile are currently the subject of advanced discussion with the regulatory agencies to finalize the phase III trial design and end points.

### *Liver biopsy as the primary outcome*

***FGF analogues.*** NGM282 (aldafermin) is an engineered FGF19 analogue that regulates bile acid synthesis and glucose homeostasis. In a study of 82 patients with NASH (NCT02443116)[97] with fibrosis stage F1–F3 and at least 8% fat on MRI-PDFF, NGM282 safely and effectively reduced liver fat content. At 12 weeks, 74% of patients receiving 3 mg daily and 79% of those receiving 6 mg daily achieved at least a 5% reduction in absolute liver fat content from baseline. The study met the primary end point of a statistically significant reduction in liver fat content that was 5% higher than in those receiving the placebo. However, in 78 of these patients who underwent end of treatment biopsies at 24 weeks, NASH resolution with no worsening of fibrosis was observed in 24% of patients receiving aldafermin versus 9% of patients receiving placebo ($P = 0.20$). Improvement in fibrosis (by one or more stages) with no worsening of NASH was achieved in 38% of patients receiving aldafermin versus 18% of patients receiving placebo ($P = 0.10$)[98]. In a post hoc analysis, significantly more patients in the aldafermin group achieved the combined histological end point of both improvement in fibrosis and resolution of NASH compared with the placebo group (22% versus 0%)[98]. In another open-label study, of 43 patients who underwent liver biopsy before and after 12 weeks of treatment with aldafermin at 1 mg and 3 mg once daily, 50% and 63% showed improvement in NAS by two or more points, respectively, without worsening of steatohepatitis[99]. Improvement in fibrosis without worsening of steatohepatitis by one stage was seen in 25% and 42% of patients, respectively[99]. AEs associated with aldafermin treatment were few and either mild or moderate and gastrointestinal in nature[98,99].

***Insulin sensitizer.*** MSDC-0602K is an insulin sensitizer that targets the mitochondrial pyruvate carrier and minimizes binding to PPARγ[100]. The EMMINENCE phase IIb trial (NCT02784444) was a 52-week RCT to evaluate three doses of MSDC-0602K in patients with NASH and fibrosis stage F1–F3 (REF.[100]). Patients were randomly assigned to daily placebo ($n = 94$) or MSDC-0602K 62.5 mg ($n = 99$), 125 mg ($n = 98$) or 250 mg ($n = 101$). The primary end point, which was a decrease by two points in the NAS with at least one point in ballooning without worsening fibrosis, was not met, despite improvement in metabolic parameters (such as a reduction in levels of fasting glucose, fasting insulin and haemoglobin A1c)[100]. In our opinion, further advancement of this compound as a single agent for the treatment of NASH is unlikely but it is possible that it could be developed in combination with a potent anti-fibrotic agent due to these positive metabolic effects.

***GLP-1 receptor agonists.*** Liraglutide is a glucagon-like peptide 1 (GLP1) receptor agonist, also known as incretin mimetic. The FDA has approved the use of GLP1 receptor agonists for the treatment of T2DM, and their safety and efficacy are being tested in clinical trials for the treatment of NASH[101,102]. Administration of liraglutide improved liver histology in patients with NASH (LEAN, NCT01237119); the primary outcome of resolution of NASH at 48 weeks was achieved in 39% of patients who received liraglutide compared with 9% who received placebo. However, this study had some inherent limitations due to the small sample size (26 patients assigned to each treatment arm) and further stratification based on T2DM and geographical site[103]. Furthermore, the liraglutide group had a significantly lower mean BMI than the placebo group at baseline[103]. Additionally, the histological response was not statistically significantly different from that in the placebo group when adjusted for weight loss, suggesting that the benefits of liraglutide were perhaps related to the weight loss and independent of liraglutide treatment[103]. Nevertheless, improvement in liver fat content in patients with T2DM was also observed in the Lira-NAFLD study (NCT02048189)[104]. Importantly, multiple GLP1-related agents have shown benefit for both major adverse cardiovascular events and all-cause mortality in patients with T2DM who have cardiac risk factors[105–108]. Increasing evidence indicates that these agents also show stabilization of chronic kidney disease[106–110]. These extra-hepatic benefits will be of increasing importance particularly to show all-cause improvement in mortality (FIG. 3).

Results from a phase II trial using another GLP1 agonist, semaglutide, given subcutaneously in 320 patients with NASH for 72 weeks (NCT02970942) have now been published[111,112]. In this multicentre, randomized,

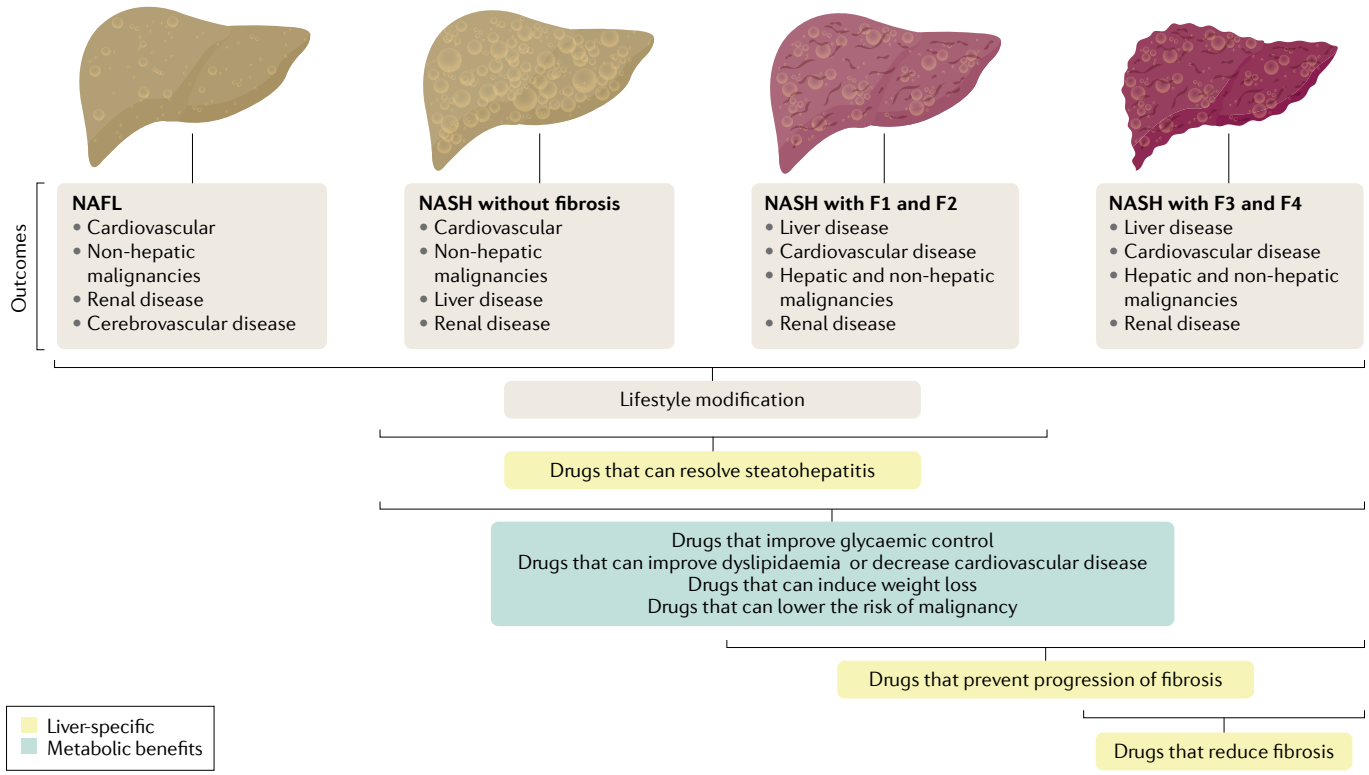


Fig. 3 | **Major causes of morbidity and mortality in various NAFLD subphenotypes.** The ideal drug for nonalcoholic steatohepatitis (NASH) should reduce liver-related effects such as resolution of steatohepatitis and reversal of fibrosis, and treat metabolic syndrome that addresses several associated comorbidities together. F1–F4, fibrosis stages 1–4; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease.

double-blind, placebo-controlled study, patients were randomly assigned to receive one of three doses of subcutaneous semaglutide once daily (0.1 mg, 0.2 mg or 0.4 mg) or placebo[111,112]. The primary analysis included patients with fibrosis stage F2 or F3 ($n = 230$) and the primary end point of resolution of NASH and no worsening in liver fibrosis was met for all doses of semaglutide compared with placebo[111]. Semaglutide exhibited a dose–response relationship with 59% of the group receiving 0.4 mg showing NASH resolution compared with 17% in the placebo group ($P < 0.001$, semaglutide 0.4 mg versus placebo). At this dosage, 43% of the patients showed an improvement in fibrosis stage, but this was not statistically significantly different from 33% in the placebo group ($P = 0.48$)[111]. Furthermore, in another study of 67 patients with NAFLD (MRI-PDFF ≥10% and increased LSM by magnetic resonance elastography (MRE)) who received semaglutide at 0.4 mg per day or placebo for 72 weeks (presented as a preliminary report in a conference abstract), no difference was found in LSM between the two groups at 48 or 72 weeks despite 70% of the semaglutide group achieving a reduction in liver fat of ≥30%[113].

Taken together, these two trials with semaglutide raise some perplexing issues of unusually high responses to placebo with regard to fibrosis improvement and lack of improvement in LSM, a biomarker of fibrosis, despite a reduction of ≥30% in hepatic steatosis, a threshold associated with high rates of NASH resolution and improvement in fibrosis. The safety profile of subcutaneous semaglutide was consistent with the observed profile in other trials and disease areas[114,115]. Semaglutide given once weekly is currently undergoing evaluation as a treatment for patients with NASH and compensated cirrhosis with no evidence of portal hypertension such as varices or ascites (NCT03987451). This study has now enrolled the target sample size of 65 patients. Interestingly, while the study is ongoing, the primary outcome was changed from improvement in LSM by MRE to a histological outcome of improvement in liver fibrosis by at least one stage with no worsening of NASH after 48 weeks of semaglutide therapy (NCT03987451) to align with a phase IIb study design.

In addition, a study investigating tirzepatide, a dual gastric inhibitory polypeptide (GIP) receptor and GLP1 agonist as a treatment for patients with non-cirrhotic NASH (SYNERGY-NASH, NCT04166773) has been initiated based on significant weight loss and improvement in features of metabolic syndrome observed in the T2DM trials[116–118]. In a post hoc analysis, levels of ALT, K-18, and Pro-C3, biomarkers associated with histological improvement, were significantly increased at 26 weeks in patients receiving tirzepatide[119]. This finding was also associated with a statistically significant increase in adiponectin levels compared with the levels in patients receiving placebo[119]. Cotadutide, a subcutaneous dual-receptor agonist with balanced GLP1 activity and glucagon activity, is also in trials in patients with biopsy-proven NAFLD–NASH (NCT04019561), but with a phase IIa study design.

### *HVPG, biopsy or clinical events as outcomes*

***Galectin 3 inhibitor.*** Belapectin, an inhibitor of galectin 3 that reduces liver fibrosis and portal hypertension, was evaluated in patients with non-cirrhotic NASH and NASH–cirrhosis with portal hypertension[120,121]. In the phase IIb study of 162 patients with NASH–cirrhosis and portal hypertension, 1 year of biweekly infusion of belapectin at 2 mg/kg and 8 mg/kg was safe but not associated with a statistically significant reduction in HVPG or fibrosis as compared with the placebo[120]. However, in the subgroup of 81 patients without oesophageal varices at baseline, 2 mg/kg of belapectin was associated with a reduction in HVPG at 52 weeks compared with baseline ($P = 0.02$) and reduced development of new varices ($P = 0.03$)[120]. The drug is currently advanced through an adaptive, two-stage, phase IIb/III trial in patients with NASH–cirrhosis without oesophageal varices (NCT04365868) with the proportion of patients in the belapectin treatment groups who develop new oesophageal varices at 18 months of treatment compared with the proportion in the placebo group as the primary outcome measure. The secondary outcome measures include cumulative incidence rates of patients who develop varices requiring treatment, variceal bleeding requiring hospitalization, clinically significant ascites requiring hospitalization, spontaneous bacterial peritonitis, hepatic encephalopathy requiring hospitalization, and the rates of liver transplantation and mortality.

***Caspase inhibitors.*** Caspase inhibitors have therapeutic potential for the treatment and prevention of apoptosis-mediated liver injury in patients with chronic liver diseases, including NASH. Emricasan is a first-in-class, orally active pan-caspase inhibitor and has been studied in several trials and across the whole spectrum of NASH[122–124]. Based on encouraging earlier studies and a positive signal from open-label studies, phase IIb studies were conducted in patients with NASH, including ENCORE-NF (for NASH fibrosis) for those with non-cirrhotic NASH, ENCORE-PH (for portal hypertension) for those with compensated or early decompensated cirrhosis and severe portal hypertension confirmed by an HVPG of ≥12 mmHg at baseline and ENCORE-LF (for liver function) for those with decompensated NASH–cirrhosis[39,122,125,126].

Unfortunately, all these studies failed to meet their primary efficacy end points, and the programme is now terminated[123,124]. Briefly, in the ENCORE-NF trial, the primary end point of improvement in fibrosis by one stage or more with no worsening of NASH at week 72 was achieved by 11.2% and 12.3% of patients receiving 5 mg and 50 mg emricasan twice daily, respectively, and by 19% of those receiving placebo[124]. In the ENCORE-LF trial, the trial's primary end point of event-free survival defined as a composite of all-cause mortality, new decompensation events or progression with an increase in MELD score of four or more points showed no statistically significant differences between the treatment and placebo arms and there was no clear trend indicating a potential treatment effect. In the ENCORE-PH study, the primary end point of change in mean HVPG from baseline to week 24 and week 48 was not different from the placebo arm when adjusted for baseline HVPG, compensation status and non-selective beta-blocker usage[123]. However, in a post hoc analysis of participants with compensated cirrhosis and an HVPG of ≥16 mmHg, emricasan resulted in statistically significant, clinically meaningful reductions in HVPG compared with placebo. The mean changes from baseline at week 24 in patients receiving emricasan twice daily were −1.6 mmHg (5 mg), −1.7 mmHg (25 mg), −1.5 mmHg (50 mg) compared with an increase of 0.5 mmHg in those receiving placebo ($P < 0.05$ versus placebo for all comparisons)[123].

### *MRI-PDFF and/or ALT as primary outcomes*

***FXR agonists.*** Cilofexor (GS-9674) is a selective non-steroidal FXR agonist that has improved markers of cholestasis and liver injury in patients with primary sclerosing cholangitis (PSC) and is currently in a phase III trial for the treatment PSC[127]. Patel et al. have tested GS-9674 in a phase II randomized, placebo-controlled trial in patients with NASH without cirrhosis (NCT02854605)[128]. After treatment for 24 weeks at a dose of 100 mg daily, there was a decrease in hepatic fat of ≥30% in 39% of patients compared with a decrease of 13% in those receiving placebo ($P = 0.011$), as revealed by the MRI-PDFF[128]. Moderate to severe pruritus was more common in patients receiving cilofexor 100 mg daily than in those receiving cilofexor 30 mg daily or placebo[128].

Tropifexor (TXR; also known as LJN452) is an FXR agonist[129,130] that is being assessed in a two-part phase IIb study (FLIGHT-FXR, NCT02855164). In the initial screening (part A), dose safety was examined in 77 patients with MRI-PDFF ≥10% and histological evidence of NASH or phenotypic diagnosis of NASH randomly assigned to 10, 30, 60 or 90 μg of TXR or placebo[131]. Part B examined doses of 60 μg and 90 μg daily in a 12-week treatment; a reduction in hepatic fat and ALT were recorded with both doses[131]. The Part C 12-week interim analysis in 152 patients with histological evidence of NASH and fibrosis F2 or F3 was presented in abstract form[132]. Patients were given placebo, or TXR 140 μg or 200 μg daily, and achieved a relative change in hepatic fat fraction (MRI-PDFF) of −10.26%, −16.99% and −31.37% ($P < 0.001$ versus placebo), respectively. Itching and weight loss were seen across all groups and were dose-dependent. The final 48-week efficacy and safety results from Part C, recently presented in abstract form, showed a progressive decrease in the hepatic fat fraction to −31.25% and −39.54% compared with −3.58% in the placebo arm[133]. The response rates in patients with a reduction in hepatic fat fraction of ≥30% were 55% and 68% in those receiving 140 μg and 200 μg daily, respectively, compared with 28% in the placebo arm. There was a dose-dependent decrease in NAS, but it was not statistically significant. A few patients showed NASH resolution and the number of patients acheiving the histological end point was not different between the placebo and treatment arms. There was no significant difference between the TXR and placebo groups regarding fibrosis improvement[133].

EDP-305, another FXR agonist[74], has been granted fast-track status by the FDA for a phase IIa randomized,

double-blind clinical trial (ARGON-1, NCT03421431) at two doses daily for 12 weeks in patients with non-cirrhotic NASH. In a press release, from the analysis of the 134 study participants, it was reported that the study's primary end point was achieved with a statistically significant reduction in ALT of 28 U/l in the EDP-305 2.5 mg per day arm versus 15 U/l in the placebo arm at week 12 ($P = 0.049$)[134]. The improvement in ALT was accompanied by a statistically significant reduction in liver fat content with EDP-305 at the 2.5 mg daily dose as measured by MRI-PDFF (7.1% versus 2.4%, $P < 0.001$), and[135] 45% of participants were MRI-PDFF responders (that is ≥30% fat reduction) as compared with 25% of participants in the placebo group. The most common (≥5%) treatment-emergent AEs included pruritus, gastrointestinal symptoms (nausea, vomiting, diarrhoea), headache and dizziness. Surprisingly, pruritus was present in ~51% of individuals receiving 2.5 mg daily compared with <10% of those receiving 1 mg daily, with mild or moderate severity in the majority. Treatment with EDP-305 was associated with a very modest effect on lipids[135].

MET409 is an FXR agonist that is designed to have a non-bile acid chemical scaffold and sustained FXR activation[136]. The results of a trial of MET409 dosed orally once daily for 12 weeks at 50 mg and 80 mg alongside placebo in 58 patients with biopsy-confirmed NASH or LSM ≥8.5 kPa and ≥10% liver fat content by MRI-PDFF were recently presented in abstract form[137]. The study found statistically significant reductions in relative mean liver fat content in those receiving MET409 relative to those receiving placebo: 55% for 80 mg, 38% for 50 mg and 6% for placebo[137]. Approximately 93% and 75% of patients receiving 80 mg and 50 mg MET409, respectively, had a reduction in liver fat from baseline of ≥30%. A significant reduction in ALT was observed in those receiving 80 mg but a subset of patients at both dose levels developed transient increases in ALT. The compound was associated with pruritus with an incidence (10–35%) that was similar to or better than those reported with other FXR drugs.

***ACC inhibitors.*** GS-0976 (firsocostat) is a liver acetyl-CoA carboxylase (ACC) direct inhibitor that reduced de novo lipogenesis and liver fat in a randomized, placebo-controlled study in patients without cirrhosis but with hepatic steatosis of at least 8% based on MRI-PDFF and a liver stiffness of at least 2.5 kPa based on MRE (NCT02856555) or had a liver biopsy with evidence of NASH and fibrosis stage F2 or F3. Of patients receiving 20 mg daily, 48% had a decrease of at least 30% from baseline in MRI-PDFF by 12 weeks compared with 15% of those receiving placebo ($P = 0.004$)[138]. Hypertriglyceridaemia was the main AE. PF-05221304 is another selective and reversible dual inhibitor of ACC1/2 with asymmetric distribution in the liver[139,140]. It was reported to be well tolerated at all single and multiple oral doses[139]. In an RCT in 305 patients given placebo or PF-05221304 at 2 mg, 10 mg, 25 mg or 50 mg daily over 16 weeks, reductions in liver fat fraction of ≥30% at week 16 were achieved in 6%, 22%, 74%, 87% and 90% of patients, respectively[141]. ALT and AST levels decreased, and, as expected, triglyceride values increased[141]. Interestingly, the proportion of patients with γ-glutamyl transpeptidase levels, a traditional marker of liver dysfunction, more than the upper limit of normal (ULN) was higher in a PF-05221304 dose-dependent fashion[141]. The relevance of this finding is yet to be ascertained.

***PPAR agonists.*** Saroglitazar is a dual PPARα/γ agonist that has undergone testing in rodent models, and it has shown promise for the treatment of NASH and diabetes[142,143]. The EVIDENCES IV phase II RCT (NCT03061721) has been presented in abstract form. In this study, 106 patients with NAFLD/NASH and elevated ALT levels were randomly assigned to placebo or saroglitazar at 1 mg, 2 mg and 4 mg daily doses[144]. The study results met the primary outcome of ALT level reduction at week 16. Patients on saroglitazar 4 mg daily had an absolute reduction in MRI-PDFF of −4.21% compared with −0.31% in those receiving placebo ($P = 0.01$)[144]. Improvement in haemoglobin A1c levels in the treatment arms was recorded, with no statistically significant weight change or lower extremity oedema. Lanifibranor (IVA337) is a moderately potent, well-balanced pan-PPAR agonist[145] and has a fast-track designation for the treatment of NASH. Lanifibranor was evaluated in the phase IIb NATIVE (NASH trial to validate IVA337 efficacy) trial (NCT03008070), and patients with T2DM and NAFLD are being recruited for a second trial (NCT03459079). The results of the NATIVE trial were recently reported through a press release in which lanifibranor given for 24 weeks met the primary end point at the dose of 1,200 mg/day with a significant (49% versus 27%; $P = 0.004$) decrease of at least two points in the SAF activity score (steatosis, activity and fibrosis), compared with the score at baseline, with no worsening of fibrosis[146] Additional results reported at The Liver Meeting 2020 (late breaking abstract 12) include a statistically significant reduction in both resolution of NASH and improvement in fibrosis (31% in the 1200 mg arm compared with 7% in the placebo arm, $P \leq 0.01$)[147].

***FGF21 analogues.*** BMS-986036, also known as pegbelfermin, is a pegylated FGF21 analogue[148,149]. It was tested in a phase IIa, 16-week trial (NCT02413372) in patients with NASH[150]. A significant decrease in absolute hepatic fat fraction was found in patients given 10 mg pegbelfermin daily (−6.8% versus −1.3% in those receiving placebo; $P = 0.0004$) or 20 mg weekly (−5.2% versus −1.3%, respectively; $P = 0.008$)[150]. The drug was well tolerated, and there were no discontinuations or deaths[150]. Phase IIb trials of pegbelfermin are underway in adults with NASH and liver fibrosis stage F3 or F4 (FALCON1 and FALCON2; NCT03486899 and NCT03486912, respectively).

Efruxifermin, previously known as AKR-001, an FGF21 analogue engineered to mimic the biological activity profile of native FGF21, has been evaluated in a phase IIa study with once weekly dosing. In the BALANCED study, 80 patients with biopsy-proven NASH (NCT03976401) were given efruxifermin at three daily dose levels (28 mg, 50 mg and 70 mg) or

placebo subcutaneously for 16 weeks[151]. Absolute liver fat decreases at 12 weeks, the primary efficacy end point, were significantly higher at 12%, 13% and 14% in those receiving the efruxifermin doses as compared with 0.3% in those receiving placebo[151]. All three groups also met the study's secondary end point of a statistically significant relative reduction in liver fat. The preliminary results of the 16-week analysis of secondary and exploratory end points in the 40 treatment responders (relative reduction in MRI-PDFF of ≥30%) who had end of treatment biopsies have been presented in abstract form[151]. Histological parameters reported included: 48% improvement in fibrosis by at least one stage without worsening of NAS across all dose groups, with a 62% response rate in the 50 mg daily dose group; 28% improvement in fibrosis by at least two stages across all dose groups, with a 38% response rate in the 50 mg daily dose group; and 48% NASH resolution without worsening of fibrosis across all dose groups, with a 54% response rate in the 50 mg daily dose group[146,151]. These results are promising considering that both NASH resolution and fibrosis regression occurred in such a short time. We look forward to the replication of these results in a larger cohort with safety and tolerability profiles that are favourable when the drug is used for longer.

***THR agonists.*** VK2809-201 is a liver-directed THRβ agonist was evaluated in a phase IIa randomized, placebo-controlled trial (NCT02927184) in patients with NAFLD, LDL-C >110 mg/dl and a liver fat content >8% according to MRI-PDFF[85,86]. Preliminary results showed a significant reduction in LDL-C, which was the primary outcome, and liver fat content at 12 weeks; the median change was −56.5% in patients receiving 10 mg VK2809-201 every other day and −59.7% in those receiving 10 mg VK2809-201 daily versus −8.9% in those receiving placebo (all *P* values <0.05)[86]. Patients receiving a lower daily dose of 5 mg also showed a median reduction of 54%, with 88% of patients showing a reduction of ≥30%[86]. VK2809-201 was safe and well tolerated at all doses, and no serious AEs were reported[86]. VK2809 is currently advanced to a phase IIb study with an anticipated enrolment of 339 patients with non-cirrhotic NASH with a NAS of ≥4 with a score of at least 1 for each of the following NAS components: ballooning degeneration (score 0–2), lobular inflammation (score 0–3) and steatosis (score 0–3). Subjects with fibrosis stage F1 must also have at least one of these risk factors: T2DM, BMI ≥30 mg/m$^2$ and/or ALT >1.5 × ULN. VK2809 will be administered for 52 weeks at daily doses of 1 mg, 2.5 mg, 5 mg and 10 mg followed by a 4-week off-drug phase (VOYAGE trial, NCT04173065).

## Combination drug trials

The pathogenesis of NASH is complex, so a one-drug therapeutic approach probably might not be successful. The disease's pathophysiological pathways can vary, leading to subtypes of NASH that might require personalized medicine approaches[12,152]. The delivery of excess metabolic substrate (agents that modulate PPARs, GLP1 receptor, FXR, FGF19, FGF21, ACC1 and FASN) and the development of inflammatory cytokines (vitamin E, S-adenosyl methionine) in the liver that can induce cell stress, which in turn can induce apoptotic and inflammatory signalling (apoptosis signal-regulating kinase 1 (ASK1) inhibitor and caspase inhibitors) and inflammation (CCR2/5 antagonist, VAP1 inhibitor), over time induce a fibrogenic response (integrin inhibitors) that can ultimately lead to cirrhosis[153]. This simplified paradigm enables the evaluation of specific mechanisms underlying each of these elements and targeting them for the treatment of NASH. Until these approaches are available, combined drug therapy to target several pathways simultaneously is being tested (Supplementary Table 2).

A study with the combination of the non-steroidal FXR agonist cilofexor (GS-9674) and the ACC inhibitor firsocostat (GS-0976) has been conducted in 20 patients with NASH; hepatic fat declined by at least 30% from baseline in 74% of patients at 12 weeks, as measured with MRI-PDFF (presented in Abstract form[92]). Subsequently, the sponsor tested this combination with the inclusion of selonsertib (GS-4997), an ASK1 inhibitor, in the double-blind, 48-week ATLAS RCT (NCT03449446) in patients with NASH and fibrosis stage F3 or F4 (REF.[154]). More patients treated with combination therapy achieved an improvement in fibrosis by one or more stages without worsening of NASH compared but none of the combinations was associated with a statistically significant improvement compared with placebo. Nevertheless, the study met multiple secondary end points, the most important being significant improvement by two points in the NAS score following treatment with the cilofexor–firsocostat combination[155]. Similarly, cilofexor (GS-9674) and firsocostat (GS-0976) were tested with semaglutide (GLP1 receptor agonist) in a proof-of-concept, open-label study comparing monotherapy and combination regimens in patients with NASH (NCT03987074). The results were presented at The Liver Meeting 2020 meeting[156] and the combination regimens were well tolerated with similar rates of AE across groups. All groups had a decrease in hepatic fat fraction ranging from −8.6% to −12.6%), and two combinations (semaglutide plus firsocostat and semaglutide plus firsocostat plus cilfexor) had significantly higher reductions than semaglutide alone[156]. The TANDEM phase IIa clinical trial is investigating the combination of TXR (LJN452) and cenicriviroc in patients with NASH with liver fibrosis (NCT03517540), and a phase IIa trial is testing dual therapy with PF-05221304 and PF06865571 (NCT03776175)[157]. The results of these studies are awaited.

Although trials of combination therapies are currently underway, the regulatory pathway regarding optimal design and choice of combination therapies is currently not established by the FDA. The combination strategy can include two or more investigational drugs (such as in the ATLAS trial), an investigational drug with a previously approved drug for a different indication, or two (or more) previously approved drugs for a different indication as a novel combination therapy. We anticipate that as the numbers of combination therapies and co-developed new investigational drugs increase, the study designs will need to accommodate more trial arms, and clinical trials will require larger numbers of

patients. There will be a need to balance the level of evidence needed for approval with the challenges of patient recruitment and costs associated with the conduct of large clinical trials with multiple arms.

## Insights from trials to date

### *Drugs that failed*

Several drugs have failed to show beneficial effects in clinical trials in patients with NASH, even though they target relevant mechanistic pathways. Simtuzumab, a monoclonal antibody against lysyl oxidase-2, did not benefit patients with either bridging fibrosis or compensated cirrhosis[26]. The ASK1 inhibitor selonsertib was not superior to placebo in improving fibrosis in patients with both bridging fibrosis and cirrhosis[25]. The safety and anti-fibrotic effects of 48 weeks of treatment with selonsertib in patients with advanced fibrosis due to NASH was evaluated in two phase III trials[25]. Neither trial met the primary efficacy end point and the programmes have been discontinued[25]. Furthermore, elafibranor failed the phase III study in patients with NASH. After 72 weeks of treatment, at the interim analysis, the study missed its primary end point, with 19% of patients in the treatment arm achieving NASH resolution without worsening of fibrosis compared with 15% of patients in the placebo group[75]. Unfortunately, the drug did no better on the study's secondary end points and the programme is currently shelved[78]. Treatment with the pan-caspase inhibitor emricasan did not meet the primary end points of improvement in portal hypertension or fibrosis in patients with NASH with and without cirrhosis[38,124]. Finally, seladelpar (MBX-8025), a selective PPARδ agonist, was tested in 175 patients with NASH (NCT03551522). In November 2019, the sponsor terminated all studies with seladelpar when biopsies in the NASH clinical study after 52 weeks of treatment revealed atypical histological findings such as interface hepatitis despite on-study improvement or stabilization of liver tests and no liver-related AEs[158]. An expert panel assembled by the sponsor subsequently found no clinical, biochemical or histological evidence of seladelpar-related liver injury and unanimously supported re-initiating the clinical development pending approval by the FDA. Based on the results of the investigation and the expert panel conclusions, the FDA concluded that clinical trials with seladelpar could resume[159]. The sponsor has reinitiated trials in patients with primary biliary cholangitis, but not in patients with NASH at the time of writing this Review.

### *Lessons learned*

It is dismaying to note the number of drugs that have failed in phase IIb and now in phase III[25,26,75]. The success of drug development in NASH is likely to be contingent on clarity of objectives, identification of an appropriately defined study population, careful selection of drug target, matching outcome measures to what can be realistically expected based on mechanism of action, and rigorous trial design. It is also prudent to proceed logically with thoughtful 'go–no go' decision gates to proceed to progressively advanced phases and larger resource-intense trials.

A key lesson learned is that it is increasingly difficult to alter the disease course the further downstream in the pathophysiological cascade the drug target is. So far, treatments targeting inflammation and fibrosis development, such as TLR4, ASK1 and lysyl oxidase have failed[25,26]. This aspect could be due to a redundancy of signalling pathways with progression from metabolic substrate overload to cell injury, inflammation and fibrosis. If so, unless a nodal target that is critical for regulation of the signalling network is targeted, it could be insufficient to inhibit one pathway while the other pathways working in parallel remain active and the upstream drivers of disease remain in place.

Also, some drugs that seemed promising in early phase trials ultimately failed in phase III trials (such as selonsertib, elafibranor, and now cenicriviroc)[25,160]. Careful review of the early phase trials could provide some insights into this lack of translation. For example, when there is improvement in both liver fat content and ALT levels in a small phase IIa trial, it is more likely to be a real finding if they occur in the same study participant[160]. Furthermore, the changes in fat and ALT levels during the trial are not normally distributed, with improvement in some individuals and worsening in others[160]. In such circumstances, measuring changes in mean fat content or ALT level might not be appropriate as they do not truly reflect the effects of the drugs.

Another important lesson is learned from the trials attempting to inhibit the caspase pathway to reduce cell death. Despite target engagement, not only did this approach not work but there was a suggestion of greater injury and hepatocellular death[124]. This suggests that once a cell is injured enough to activate death signalling, simply blocking it might be insufficient. In the phase II trial in patients with non-cirrhotic NASH who received emricasan, there was worsened fibrosis and hepatocyte ballooning despite robust target engagement[124]. It was suggested that the caspase inhibition lowered serum ALT in the short term (for example, 12 weeks) but in the long term might have directed cells to alternative mechanisms of cell death, resulting in more liver fibrosis. Liver histology showed increased hepatocyte ballooning Mallory hyaline[124]. Fewer placebo-treated individuals had worsening of fibrosis (20.4%) compared with the emricasan-treated individuals (41.1% of those receiving 5 mg emricasan daily; 38.1% of those receiving 50 mg emricasan daily)[124]. Together with the previously noted failure of downstream targets, these observations provide growing evidence that the treatment of NASH should be anchored in its root cause (that is, lipotoxic injury to the liver) or have pleiotropic effects at different points in the disease cascade. The success so far of pioglitazone, bariatric surgery and GLP1 support this concept[45,103,111,161,162].

Finally, there is now considerable consternation about the degree of variability in histological assessment of NASH[163]. The best practice for assessment of liver histology continues to evolve and be debated. Machine learning approaches are rapidly being developed to increase the precision of histological reading and to serve as an aid to pathologists[164,165]; however, their role in drug development is not yet fully established but is

currently being actively explored[154]. The failure of drugs at a late stage has understandably dampened the expectations of the clinicians and patients involved in trials, and highlights the perils of advancing drug programmes based on small and short-duration studies. It is, therefore, important to demonstrate target engagement and histological benefits in well-conducted phase IIb trials before engaging in a large phase III trial. Both selonsertib and elafibranor programmes lacked robust phase IIb data to support the decision to advance the programmes to phase III. Another aspect that is increasingly being appreciated is the placebo response[73,160]. Historical data generally support a NASH resolution rate of 9% in the placebo arm as seen in both OCA and selonsertib phase III trials[25,59]. However, in the elafibranor phase III study the placebo response rate was higher at 14.7%[75]. Furthermore, the NASH resolution rate in the placebo arm in the most recently reported phase IIb trial with semaglutide was 17%[111]. A comparable phase IIb study with resmetirom showed a placebo response rate of 6.5%, which is consistent with the placebo response rates seen in the phase III trials[87]. The failure of these advanced studies also highlights the shortcomings of current end points that are dependent on histology that is subject to inter-reader and intra-reader variability[163]. One other possibility is the increased awareness of lifestyle interventions for NASH through social media and targeted online advertising that might continue to increase the placebo response rates over time.

## Considerations for emerging therapies

### *Differentiating features*

In patients with NAFLD or NASH, the risk of liver-related mortality increases exponentially with increases in fibrosis stage[21,22,24,166]. However, many studies have also observed cardiovascular risk factors to be the major drivers of morbidity and mortality among patients with NAFLD[167,168]. In a 2019 analysis of NHANES (1988–1994) data, cardiovascular health metrics (particularly glycaemic control and hypertension) were associated with all-cause and cardiovascular mortality in patients with NAFLD[169]. Studies examining the top underlying causes of NAFLD-related deaths include cirrhosis, hepatic and non-hepatic cancers, T2DM, lung disorders (FIG. 3) and chronic kidney disease[170–172]. NAFLD with all metabolic syndrome components was associated with overall, cardiovascular and liver-related mortality, with increased number of metabolic syndrome components associated with lower survival[168,169]. In one study, compared with patients with NAFLD without any metabolic syndrome features, the risk of death in patients with NAFLD increased with each additional metabolic syndrome condition: HR 1.63 (95% CI 0.96–2.79), 3.57 (2.32–5.49), 5.87 (3.53–9.75) and 13.09 (7.49–22.87) for the presence of one, two, three and all four conditions, respectively[168].

Drugs that can induce weight loss and improve control of hypertension, dyslipidaemia and glycaemic control could provide added extrahepatic benefits and improve overall clinical outcomes. These differentiating and extrahepatic benefits could be particularly important in therapies approved for non-cirrhotic NASH that were approved through an accelerated pathway for which the long-term clinical outcomes are still awaited (FIG. 3). Such an approach is already being implemented in the MAESTRO-NAFLD-1 (NCT04197479) phase III trial of resmetirom. Key secondary end points in this trial include lowering of atherogenic lipids such as LDL cholesterol, apolipoprotein B and triglycerides, in addition to cardiovascular-related clinical end points[88]. Another much desired feature is weight loss associated with these emerging therapies. Agents such as GLP1 receptor agonists and dual GLP1–GIP agonists have been reported to be associated with significant weight loss approaching 10% or more[118]. Phase III studies with these agents have not been initiated yet.

Finally, some drugs are currently undergoing evaluation for treatment of NAFLD in populations at a higher risk of developing NASH with non-diabetic endocrine disorders such as PCOS in women and hypogonadism in men with low testosterone levels[173–181]. Women with PCOS have androgen excess as a defining feature but are also at increased risk of NAFLD due to an associated increase in features of metabolic syndrome such as insulin resistance and obesity[173,182,183]. In one meta-analysis of seven studies, NAFLD prevalence was significantly higher in patients with PCOS than in healthy controls, with an overall odds ratio of 3.93 (95% CI 2.17–7.11)[183]. Notable drugs in this space include saroglitazar for the treatment of NAFLD in patients with PCOS (EVIDENCES VII, NCT03617263). A research correspondence examined 159 randomly selected men in the NASH clinical research network cohort and found low free testosterone in 26% of men with NAFLD, including 24% of men less than 40 years old[181]. Men with low free testosterone were more likely to have NASH than simple steatosis (88% versus 67%), and advanced fibrosis (27% versus 14%). The prevalence of NASH was higher among men in the lower quartiles of free testosterone (in 88% of men in the lowest quartile versus 68% in the highest quartile)[181]. Furthermore, testosterone replacement in individuals with severe obesity and hypogonadism has been shown to improve insulin sensitivity and lipid handling. Therefore, a potentially protective role for testosterone on the progression of NAFLD is currently being explored by oral testosterone supplementation (LPCN 1144) in men with biopsy-confirmed NASH (NCT04134091).

### *Safety and tolerability considerations*

In general, patients with NASH can be particularly vulnerable to AEs due to associated comorbidities such as T2DM. Furthermore, patients with NASH are often on multiple drugs for treatment of their comorbidities, and unanticipated drug–drug interactions not identified in the clinical trials could occur in the real world. The class-related adverse effect of FXR agonists is pruritus that seems to be the most frequent AE in all the clinical trials evaluating its safety and efficacy. In the REGENERATE trial (for OCA), for instance, pruritus was the most frequent AE with up to 50% of patients experiencing it. Peak severity of pruritus at the 25 mg daily dose was observed early in the treatment course without subsequent worsening[59]. Although safety is

not much of a concern with this AE, we anticipate that pruritis could be a barrier to tolerability, particularly for a condition that was asymptomatic to begin with, and duration of treatment lasting several years. It is encouraging to know that patient-reported outcomes do not seem to be affected by this AE[184]. This class of drugs is also seemingly associated with gallstone-related AEs that occur at a rate of up to 3%[59]. Pancreatitis, a more serious and potentially gallstone-related AE, was rare and evenly distributed across treatment groups[59]. In general, liver-related AEs with OCA were rare and seemed to occur at higher dosages, but associated confounding factors for acute liver injury made the causality assessment often difficult[59].

### *Hepatocellular carcinoma risk*

Several factors, including genetic and epigenetic factors such as diet and lifestyle, can contribute to the development of HCC in patients with NAFLD or NASH[185]. The incidence of NASH-related HCC is increasing and is evident from the trends in liver transplantation[5,186,187]. In one study published in 2019 analysing 158,347 adult liver transplant candidates from a large registry in the USA, the proportion of patients with NASH who developed HCC increased 7.7-fold (from 2.1% to 16.2%) from 2002 to 2016 (REF.[188]). Furthermore, since 2002, the prevalence of HCC in liver transplant candidates with NASH in the USA increased 11.8-fold, which was a steeper increase than that for any other aetiology such a viral hepatitis and alcoholic liver disease[188].

The pathogenesis of NAFLD-associated HCC is complex, and proposed mechanisms include immune and inflammatory responses, DNA damage, oxidative stress and autophagy[185]. Any of the emerging therapies for NASH with a mechanism of action that interferes with progression to HCC could theoretically also demonstrate a reduction in the incidence of HCC, a benefit that extends beyond the immediate benefits of NASH resolution or regression of fibrosis. This expectation is not unrealistic and is supported by bariatric surgery outcome data that showed a statistically significant reduction in NASH (60% versus 10%) and NASH-related HCC (0.05% versus 0.34%) over a median follow up of 7.1 years[189].

In a number of studies in animal models, *FXR* knockout, including selective intestinal knockout, was associated with increased HCC, and FXR inhibits the tumour suppressor NDRG2 (REFS[190,191]). FXR activation also modulates IL-6–STAT3 to reduce oncogenesis and reduces HCC in xenograft mouse models[192]. Furthermore, OCA reduced HCC in preclinical studies and FXR agonists reduced tumour burden in xenograft models[191]. HCC tumours have reduced FXR expression, which has been linked to increased YAP expression in mice[193,194]. Furthermore, emerging data from patient samples and HCC cell lines implicate FGF19 as a potential HCC driver[195,196]. It is, therefore, a concern that therapeutic agents such as FXR agonists that raise the levels of FGF19 levels could increase the risk of HCC as the main FXR-regulated gene in the gut is *FGF19* (REFS[197,198]). The translatability of these in vitro and in vivo data remains debatable. Until now, despite exposure in a large number of patients with primary biliary cholangitis and also in the FLINT and REGENERATE trials, there is no signal for an increase in HCC[57,59]. Although these data are reassuring, the development of HCC will be a clinically important end point in the long-term phase IV component of all trials of FXR and FGF19 agonists.

### *Practical aspects after approval*

It is anticipated that the first few drugs approved for the treatment of non-cirrhotic NASH will be under the accelerated approval pathway while awaiting long-term outcomes. The accelerated approval pathway for NASH enables earlier approval of a drug based on interim analysis of a surrogate end point (such as liver histology) that is thought to predict clinical benefit[35,36]. The accelerated pathway enables earlier approval of a drug for serious medical conditions that do not have any approved drugs[199]. However, physicians and patients could be left wondering about the duration of treatment — should the therapy be continued beyond the period of the interim analysis? Furthermore, would insurers require a liver biopsy to assess the response to treatment, and if so, would it require reading by a liver pathologist to get an expert opinion? Finally, how is the treatment response defined? Is the responder status restricted to those with improvement in fibrosis? In the REGENERATE interim analysis, for example, the proportion of patients with worsening fibrosis on OCA treatment was lower than in the placebo group (13% compared to 21%)[59]. Thus, one could argue that the rate of fibrosis progression could have been slower in those with worsening fibrosis. One could then make an argument to continue treatment irrespective of biopsy results after 18 months of treatment. In that event, a liver biopsy might not even be necessary.

All newer therapies approved through the accelerated pathways are going to be available with limited safety data. OCA is anticipated to be the first drug to receive regulatory approval. As already discussed, one well-known AE with OCA therapy is pruritus. In the REGENERATE trial, up to one in four patients reported pruritus that was intense in severity[59]. These patients might require a drug holiday and subsequent treatment resumption on a less frequent dosing regimen if anti-pruritus management therapy is ineffective. Continued compliance of the patient with OCA therapy with a discontinuation rate of 13% in the trial could be much more in the real world, particularly among asymptomatic patients. Another aspect is the worsening hyperlipidaemia associated with OCA; in the REGENERATE trial, almost all patients who were not already on statins at baseline required statins for the management of hyperlipidaemia. Although generally well tolerated, a small subset of patients could experience statin-related myopathies that preclude their use[200–203].

### *Companion diagnostics*

A diagnostic test could be used as a companion to a therapeutic drug to determine its applicability to a specific patient either for treatment eligibility or treatment response. We do not anticipate that the FDA would impose the requirement that a liver biopsy is performed to document the degree of fibrosis to determine

treatment eligibility or to assess response to treatment. However, companion diagnostics would help to guide the therapy, particularly to determine eligibility if the label is restricted to a certain fibrosis stage or optimize therapy through early identification of responder status. All the ongoing clinical trials have rich datasets that include readily available tests such as liver biochemistry tests, combination tests such as NAFLD fibrosis score and FIB-4 and several other novel non-invasive tests such as the ELF test. In addition, LSM measured by transient elastography was captured at frequent intervals in most, if not all, of the ongoing clinical trials. For example, the REGENERATE trial is exploring the use of FIB-4 and LSM as companion diagnostics. We also anticipate that the ELF test will be examined for its use as a companion diagnostic in non-cirrhotic NASH trials[40]. However, we expect additional non-invasive tests that might be developed in-house from each of the trial sponsors at the time of approval. For example, the elafibrinor drug development programme has reported a non-invasive test known as NIS4 that considers four blood-based biomarkers to calculate a score from 0 to 1 (REF.[40]). A threshold score of 0.62 means that the patient has an 80% or higher chance of having disease severe enough to require treatment. This threshold score indicates that a patient has a NASH activity score higher than 4 and scarring classified as stage 2 or worse[204]. A NIS4 score of more than 0.63 classified patients as having at-risk NASH (ruled in) with 87% specificity and a positive predictive value of 79% (73–84%)[40].

Understandably, the clinician will have to navigate the results obtained from various modalities to evaluate the severity of NAFLD or NASH and assess treatment response using all the available non-invasive tests. A meta-analysis published in 2018 highlighted the marked heterogeneity in imaging devices, protocols, LSM methods and cut-off values used in various trials, suggesting that standardization was required[205]. Thus, there has been an initiative to standardize, compare, validate and advance the regulatory qualification of imaging and circulating biomarkers. Two biomarker consortiums, one in the USA, the Non-Invasive Biomarkers of Metabolic Liver Disease (NIMBLE) project and the other in the European Union, the Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS) project have studies that are ongoing to address the issues of validating and qualifying biomarkers for the testing of NAFLD.

### *Cost considerations*

Currently, non-cirrhotic NASH clinical trials include patients with fibrosis stage F2 or F3 and a small proportion of at-risk patients with fibrosis stage F1, that is, those with T2DM or extreme obesity. Depending on the label (restrictive versus broad) the number of patients eligible for treatment can vary. In our opinion, the product label that the sponsor seeks from the FDA (restricted to fibrosis stage F3 versus F1–F3) will influence the cost of therapy, which in turn will determine the payer's treatment eligibility criteria. The third-party payer perspective is the incremental value proposition of the newly approved therapies. The Institute for Clinical and Economic Review and other cost-effective analysis initiatives can help to provide guidance. Furthermore, pharmacoeconomic studies are needed to establish the value of specific treatments in specific populations based on the probability of current histological data translating into a certain degree of reduction in progression to cirrhosis and outcomes. Although these factors still play out after approval, the physician will need to determine the ideal patient who is likely to benefit the most from the initiation of treatment.

### *Era of personalized NASH therapeutics*

In the current era of personalized medicine and the availability of large-scale datasets through electronic health records, it might be possible that patients with NAFLD will be optimally matched with a combination of lifestyle modification and targeted therapies for improvement in both liver-related and overall survival. Obviously, this goal needs to be achieved through affordable and tolerable medications that will also improve the quality of life for patients with NAFLD or NASH.

The first drug approved for the treatment of NASH is forthcoming but perhaps not as soon as anticipated due to the FDA's complete response letter to the New Drug Application for OCA recommending that additional data be submitted to better understand the totality of the benefits[63]. It is also apparent that these therapies will need to be distinguished as those that are primarily anti-fibrotic and those that resolve steatohepatitis, or both. Furthermore, with limited efficacy of the first generation of these drugs, there will be the need to identify treatment responders early either through pretreatment patient factors such as *PNPLA3* genotype or through the use of companion diagnostics. Finally, clinicians prescribing these new therapies need to be aware of class-related or drug-specific AEs and should be adept at early recognition and optimal management.

## Conclusions

This Review familiarizes the reader about the regulatory pathways for approval of drugs to treat non-cirrhotic and cirrhotic NASH. The trial results, efficacy and safety profiles of drugs currently advanced through phase II and III are reviewed. A detailed list of drugs that are currently in phase II and III with sample sizes and study completion dates are presented in Supplementary Fig. 1. The enthusiasm for the approval of NASH-specific drugs is warranted but it should not diminish the value of the readily accessible and cheaper alternatives that are currently available. These alternatives include optimal management of comorbidities and lifestyle measures that include weight loss in the range of 10% in one year (FIG. 3). Furthermore, there is a suggestion that vitamin E and metformin could also benefit patients with NASH with fibrosis stage F3 and cirrhosis and its use might be extended beyond patients without cirrhosis and without diabetes that were included in the original PIVENS trial[45,206,207]. Other candidates, such as pioglitazone and GLP1 agonists (liraglutide or semaglutide), could be considered for management of diabetes mellitus with added beneficial effects on liver histology[45,208].

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### Author contributions
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