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Review article: new treatments in non-alcoholic fatty liver disease (NAFLD)

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Summary

Background

Non-alcoholic fatty liver disease is the fastest growing cause of liver disease in the Western world, yet there is no approved pharmacotherapy. While lifestyle modifications remain the mainstay of treatment, only a proportion of individuals are able to make or sustain them, and so more treatment options are required.

Aim

To review the potential benefit of drugs used in clinical practice, those entering phase II trials, and compounds being investigated in preclinical studies.

Methods

A literature search was performed using PubMed to identify relevant studies; linked references were also reviewed.

Results

Vitamin E and pioglitazone have shown efficacy in NASH, but long-term safety concerns, specifically bladder cancer and osteoporosis with pioglitazone, have limited their use. GLP-1 analogues and SGLT-2 inhibitors are currently approved for use in diabetes, have shown early efficacy in NASH and also have beneficial cardiovascular effects. PPARα/δ and FXR agonists have potent effects on lipogenesis, inflammation and fibrosis respectively, with their efficacy and safety being currently tested in phase 3. As inflammation and apoptosis are key features of NASH agents modulating these pathways are of interest; CCR2/5 antagonists downregulate inflammatory pathways and reduce fibrosis with caspase and apoptosis signal-regulating kinase 1 (ASK1) inhibitors reducing apoptosis and fibrosis.

Conclusions

Rising demand and an improved understanding of NASH pathophysiology has led to a surge in development of new therapies. Tailoring pharmacotherapy to the dominant pathogenic pathway in a given patient along with use of combination therapy is likely to represent the future direction in treatment of patients with NASH.

Introduction

Non-alcoholic fatty liver disease, defined as accumulation of excess fat in the liver, is the commonest cause of liver disease in Western countries. In the United States it is estimated to affect between 3 to 40% of individuals (1, 2) and is predicted to become the leading cause of liver transplantation over the next 10 years (3). Non-alcoholic steatohepatitis is mediated by inflammatory cytokines, mitochondrial dysfunction secondary to nutrient excess and oxidative stress (4, 5), resulting in hepatocyte inflammation, ballooning, apoptosis, and activation of hepatic stellate cells (HSC). These differentiate into a proliferative, contractile and matrix-secreting phenotype, depositing collagen I, and promoting liver fibrosis (6), which affects approximately one third of patients with NAFLD (7).

Weight loss, as part of lifestyle change, is the only recommended intervention, with a loss of >7% total body weight associated with clearance of histological NASH and a >10% loss associated with an improvement in fibrosis. However, even within trial settings where motivation is usually high, less than 20% achieve >7% weight loss, and so alternative treatments need to be found (8).

There remains uncertainty as to which patients with NASH need to be treated; cardiovascular and liver-related mortality and morbidity is directly related to fibrosis stage, and so patients with evidence of progressive fibrosis should be identified and prioritised (9, 10). Indeed, cardiovascular disease is the leading cause of death in NAFLD (10) and so emerging pharmacotherapy should ideally aim to reduce both liver-related and cardiovascular mortality.

Medications currently taken by patients with NASH target concomitant features of the metabolic syndrome such as hypertension, dyslipidaemia and diabetes (11). Agents such as Vitamin E and pioglitazone are recommended (11, 12), with caveats, for selected patients with NASH although there remain concerns about their side-effect profile. This review will consider medications currently used routinely in NAFLD clinics; those currently in phase II trials, and finally those compounds with promising preclinical data. Table 1 summarises the drugs that have evidence of efficacy in NAFLD, their proven histological and non-histological benefits.

Medications currently used in patients with NASH to treat co-morbidities

1. Dyslipidaemia and hypertension

Dyslipidaemia and hypertension should be managed according to evidence-based guidelines and should reduce cardiovascular morbidity and mortality in patients with NASH. Statins are not only safe in NASH (13) but are also associated with a reduced mortality (14). Fibrates are synthetic agonists of PPARα, but have not shown benefit in NAFLD outside the management of hypertriglyceridaemia (15).

There are no particularly favoured agents for control of hypertension, although some studies have suggested that angiotensinogen receptor blockers may have additional anti-fibrotic effects, albeit with small participant numbers (15, 16).

2. Weight management

Orlistat, a gut lipase inhibitor, reduces absorption of dietary fats, and is approved in obesity as an aid to weight loss in conjunction with a hypocaloric diet (17). Several studies have investigated orlistat in NAFLD, with conflicting results; one study comparing 9 months of hypocaloric diet plus vitamin E with diet plus thrice daily 120 mg orlistat showed improved insulin sensitivity ($P < 0.001$), adiponectin ($P = 0.03$), steatosis ($P = 0.005$), ballooning ($P = 0.04$), inflammation ($P = 0.045$), and NAS histology score ($P = 0.009$) in those who lost ≤9% body weight, but showed no correlation with the use of orlistat (18). Conversely, a smaller study comparing the same dose of orlistat with placebo for 6 months in subjects with biopsy confirmed NAFLD showed improved ALT and steatosis by USS in the orlistat group despite similar changes in weight in the two groups (19); similar results were seen in a more recent study following 4 months of treatment (20). Thus, Orlistat remains a part of weight-loss strategies with insufficient data for its use in NAFLD alone.

3. Management of diabetes

a) Metformin

Metformin is the first line agent for type 2 diabetes mellitus (T2DM), and reduces the risk of all diabetes-related end-points including microvascular disease, myocardial infarction, large vessel disease, and cardiovascular mortality, in addition to aiding weight loss (21). Although studies have not demonstrated any improvement in liver enzymes or liver histology (22), there is epidemiological evidence to suggest it is associated with a reduced incidence of both liver and non-liver malignancies including hepatocellular carcinoma (HCC) in those with NASH cirrhosis by as much as 7% (23, 24).

b) GLP-1 and DDPIV inhibitors

Glucagon-like peptide-1 (GLP-1) is a gut-derived hormone analogue that induces insulin secretion, reduces glucagon secretion, suppresses appetite and delays gastric emptying (25). This class of drugs is licensed for the treatment of diabetes and obesity, improvement of both of which is desirable in patients with NASH. GLP-1 analogues have been shown to improve liver histology in murine models of non-alcoholic steatohepatitis (26, 27), reduce liver enzymes in patients with T2DM (28, 29) and in clamp studies lead to a reduction in *de novo* lipogenesis and an increase in fatty acid oxidation in the liver (29-31)

Liraglutide

Liraglutide is a long-acting GLP-1 analogue which has been shown to induce improvements in peripheral, hepatic and adipose insulin resistance, and reductions in *de novo* lipogenesis in subjects with NASH (31). In a phase II 48-week randomised controlled trial liraglutide met its primary end-point and induced resolution of NASH in both diabetic and non-diabetic patients as well as improving steatosis and ballooning. It was not possible to establish whether these histological effects were solely determined by beneficial effects on weight, although there was a suggestion that the benefit remained after controlling for weight loss (32). Both liraglutide and the more biologically active

semaglutide reduced event rates in large cardiovascular outcome studies (33, 34). Semaglutide reduced rates of nonfatal myocardial infarction (2.9% vs 3.9%), and nonfatal stroke (1.6% vs 2.7%) compared with placebo in 1648 patients with type 2 diabetes who took 0.5 or 1.0 mg semaglutide weekly for 104 weeks, although death from cardiovascular cause was similar in the two groups (2.7% vs 2.8%). Notably, there was an increased risk of retinopathy (3.8% vs 1.8%) in the treated groups, which requires further monitoring and, like liraglutide, gastrointestinal side effects were common, which was the primary reason for discontinuation of treatment (33). Semaglutide is currently being examined in a phase II trial of patients with NASH and fibrosis (NCT02970942).

Dipeptidyl peptidase-4 (DDP-4) rapidly degrades endogenous GLP-1, and a pilot study of sitagliptin, a selective DDP-4 inhibitor, demonstrated an improvement in liver histology in diabetic patients after 1 year of treatment but included only 15 participants and no control arm (35). Reduction in liver fat content using magnetic resonance spectroscopy (MRS) has also been demonstrated following 24 weeks of sitagliptin or vildagliptin therapy (36). However, three subsequent studies have failed to show an effect of sitagliptin treatment on liver fat content (36), liver enzymes (37) or liver stiffness (38), and currently its use is reserved for the management of diabetes.

c) Sodium glucose co-transporter 2 (SGLT2) inhibitors

The sodium glucose co-transporter 2 is primarily expressed in the renal proximal tubules, and reabsorbs 90% of glucose filtered at the renal glomeruli; inhibition therefore facilitates urinary glucose excretion, and SGLT2 inhibitors are used in diabetes to improve plasma glucose levels and promote weight loss (39). A meta-analysis of placebo controlled randomised control trials for SGLT2 inhibitors showed improved all-cause (OR 0.72; 95% CI 0.59–0.86; $P < 0.001$), and cardiovascular

mortality (OR 0.67; 95% CI 0.51–0.87; P = 0.003), for empagliflozin but not all agents, suggesting it may not be a class effect (40, 41).

Several SGLT2 inhibitors have shown benefit in murine models of NAFLD, improving steatosis, inflammation, and fibrosis (42-45), and studies in humans with T2DM have demonstrated improved ALT and weight loss in patients with type 2 diabetes with ipragliflozin and canagliflozin (46, 47), as well as reducing fatty liver index score (from 70.1 ± 19.4 to 60.3 ± 25.5, P = 0.0009) with ipragliflozin (48). There are no reported human studies assessing changes in liver histology in NAFLD with SGLT2 inhibitors, although one study did demonstrate weight-independent improvements in serum ALT with ipragliflozin (44).

Dapagliflozin, canagliflozin and empagliflozin are currently approved for use in patients with T2DM, although some safety concerns have emerged from phase III trials and post marketing surveillance regarding urinary tract infections (UTI). The presence of urinary glucose is thought to increase the risk of mild genital mycotic infections and urinary tract infections (49, 50). Pooled data from randomized controlled trials showed that UTIs occurred in 3.8% of patients receiving placebo versus 5.9% and 4.4% of those receiving canagliflozin 100 mg and 300 mg once daily, respectively (50). Similar rates of UTIs were reported for dapagliflozin, (3.7% for placebo vs 5.7% and 4.3% for dapagliflozin 5 mg and 10 mg once daily, respectively) (51), and empagliflozin 10 mg once daily (9.3% vs 7.6% on placebo), although UTI rates for 25mg empagliflozin were the same as for placebo. (52). Post-marketing reports of cases of potentially fatal urosepsis, pyelonephritis and DKA in patients receiving SGLT2 inhibitors led to a warning from the FDA 2015 about the possibility of severe urinary tract infection and pyelonephritis with these agents (53).

In post-marketing studies, dual-energy X-ray absorptiometry revealed small but statistically significant reductions in bone mineral density (BMD) at the hip (placebo subtracted changes of –0.9% and –1.2% for the 100- and 300-mg dose groups, respectively) but no other sites (54) . The same risks do not appear to be associated with dapagliflozin or empagliflozin use (49, 55). Pooled

data for canagliflozin from nine clinical trials with a mean treatment time of 85 weeks reported incidence rates for bone fractures of 1.4 and 1.5 per 100 patient-years for those on 100mg and 300 mg of canagliflozin, respectively, vs 1.1 per 100 patient-years for those on placebo (31).

d) TZD

Peroxisome proliferator-activator receptors (PPARs) are nuclear receptors that play key roles in the regulation of lipid metabolism and insulin sensitivity, and recently have been implicated in pathways of inflammation and atherosclerosis (56). Drugs targeting PPAR receptors include pioglitazone, sometimes used in patients with NASH, as well as elafibranor, fibrates and saroglitazar, which were primarily developed to improve dylipidaemia, but may have additional benefit in NAFLD.

Whilst this class of drugs can be used in the treatment of diabetes, there has been use specifically to treat NASH. Pioglitazone is a PPARγ agonist that has been shown to improve hepatic steatosis, inflammation and fibrosis in a meta-analysis including both diabetic and non-diabetic patients with NASH (57). Other meta-analyses demonstrated cardiovascular benefit, with a reduction in death, myocardial infarction and stroke from 5.7% to 4.4% in patients with T2DM, the optimum benefit being seen after one year of therapy (58). Notably, in the PIVENS trial of 247 non-diabetic adults with NASH randomised to receive pioglitazone, vitamin E or placebo, for 96 weeks, pioglitazone did not meet its primary end-point although it demonstrated a reduction in hepatic steatosis ($p<0.001$), lobular inflammation ($p=0.004$) and serum alanine and aspartate aminotransferase levels ($p<0.001$). However, subjects receiving pioglitazone gained more weight than those who received vitamin E or placebo (59), a side effect seen in several other studies. There has been other concerns regarding the long-term safety of pioglitazone; two meta-analyses found an increased risk of congestive cardiac failure despite reductions in other cardiovascular mortality, and in the study by Lincoff et al,

heart failure was reported in 200 (2.3%) of the pioglitazone-treated patients compared with 139 (1.8%) of the control patients (HR, 1.41; 95% CI, 1.14-1.76; P = 0.002)(58, 60). A study based on adverse event reporting to the United States Food and Drug Administration (FDA) demonstrated an increased risk of bladder cancer with a relative odds ratio of 4.30 (95% CI 2.82-6.52) for pioglitazone compared with other antidiabetic medications (61). A more recent UK study with 689,616 person-year follow-up reports the risk of bladder cancer for pioglitazone vs other medications as 121.0 v 88.9 per 100 000 person years (HR 1.63, CI 1.22 to 2.19) (62), and the FDA have reiterated their warning about the risks. In addition, PPAR-γ activation increases bone resorption while decreasing bone formation, increasing the risk of osteoporosis, which is already a concern in those with diabetes (63). EASL and AASLD guidelines currently recommend pioglitazone for NASH, albeit with careful consideration of the potential long-term risks (11, 12).

Saroglitazar is a dual PPARα/γ agonist in clinical use, approved for the treatment of dylipidaemia in diabetic patients in India. In mouse models of NASH it decreased liver fat content and induced histological improvement (64), and retrospective analysis of a phase III trial conducted in diabetic patients with triglyceridaemia demonstrated a reduction in ALT without significant weight gain after 24 weeks of 2 or 4 mg saroglitazar (65). A smaller, open label phase II study in 32 patients with NAFLD and diabetes showed improvements in ALT, HBA1c, serum triglycerides and steatosis on USS following 24 weeks of treatment (66), and a further phase II trial comparing 3 doses of saroglitazar with placebo in NAFLD is planned (NCT03061721).

Elafibranor (GFT505) is an unlicensed dual agonist of the PPARα and δ receptors, and has been shown to improve lipid and glucose metabolism in type 2 diabetes mellitus (T2DM), and steatosis, inflammation and fibrosis in mouse models of NAFLD (67). A small study in 22 obese men has shown that GFT505 improved peripheral and hepatic insulin sensitivity, and significantly reduced plasma

free fatty acid concentrations, fasting plasma triglycerides and LDL cholesterol (68). *Post hoc* analysis of the GOLDEN-505, phase IIb randomised placebo controlled study, showed patients clearing NASH (as defined by disappearance of ballooning together with either disappearance of lobular inflammation or the persistence of mild lobular inflammation (score of 0 or 1) without worsening of fibrosis) with 120 mg oral elafibranor in those with NAS≥4 (19% vs 9%; p=0.013). Treatment was not effective in those with NAS ≤4 (19% vs 12%; p=0.045) (69, 70). A phase III trial is now evaluating the ability of elafibranor (dose 120mg od) to achieve resolution of NASH with no worsening of fibrosis after 72 weeks of treatment. (NCT02704403).

MSDC-0602 is a next-generation TZD with a diminished peroxisome proliferator–activated receptor γ (PPARγ) activity, stimulation of which is believed to cause the weight gain, oedema, and increased risk of fractures associated with usual TZD use. It is hoped that a PPARγ sparing TZD, may have some of the same benefits on insulin sensitivity and hepatic steatosis via the mitochondrial target of thiazolidinediones (mTOT), while sparing some of the associated side effects (71). In diabetes, a proof of concept study showed similar reductions in fasting glucose and HBA1c compared with pioglitazone, with reduced fluid retention and a lesser increase in adiponectin (suggestive of weight gain) following 12 weeks of treatment (72). Animal models of NASH have demonstrated reduced transaminases, histological NASH, and stellate cell activation following MSDC-0602 treatment (73), and a phase IIb study evaluating three doses of MSDC-0602K with a primary outcome of NAS improvement with no worsening of fibrosis after 12 months of treatment, is currently recruiting (NCT02784444).

Medications currently used to treat patients with NASH

Vitamin E is the most widely investigated antioxidant, with the potential to reduce oxidative stress in NASH. It has been shown to improve steatosis and inflammation in several RCTs in both diabetic and non-diabetic children and adults, although trials have been heterogeneous, comparing different doses of vitamin E against various agents as well as placebo (74, 75). In two studies weight loss may have accounted for some of the histological changes seen, making it difficult to draw robust conclusions. In the PIVENS trial, the arm taking 800mg/day of vitamin E met the primary end-point which was reduction in NAFLD Activity Score (43% vs. 19% in placebo; p =0 .001) but notably did not result in resolution of NASH. It reduced liver steatosis (p = 0.005) and ALT levels (P <0 .001), but had no effect on liver fibrosis (p = 0.240) (59); similar results were seen in children and adolescents in the TONIC RCT (75). However, concerns regarding the risk of prostate cancer and haemorrhagic stroke (50, 51) have limited its use. The SELECT study compared selenium vs vitamin E vs placebo for a primary outcome of Gleason grade ≥7 prostate cancer, and showed an increased relative risk of 17% with vitamin E (76). However, it is possible that single nucleotide polymorphisms (SNPs) affecting vitamin E metabolism may be responsible for the increased risk, as absolute risk of prostate cancer was lower at 1.6 per 1000 person-years (76). Furthermore, a meta-analysis investigating the effect of vitamin E on the incidence of stroke reported an increase in the relative risk of haemorrhagic stroke by 22%, while the risk of ischaemic stroke was reduced by 10% (77). Despite the potential benefits in NASH, the risks and benefits of therapy must be carefully discussed with patients in clinical practice.

Drugs in Phase II/III development

The therapeutic landscape for NASH is evolving rapidly with many compounds currently being assessed in phase II/III clinical trials. As detailed in this section these agents are targeting multiple aspects of the pathogenesis of NASH. Table 2 indicates the histological benefits demonstrated in patients with NAFLD and table 3 shows non-liver related benefits related to drugs used in NAFLD.

a) Farnesoid X receptor (FXR) bile acid axis

The nuclear hormone farnesoid X receptor (FXR) is primarily expressed in the liver, intestines, and kidneys, and has a key role in bile acid synthesis, and also influences carbohydrate and lipid metabolism, and insulin sensitivity (78). Both conjugated and unconjugated bile acids are the natural ligand of FXRs and can active the receptor at any point in the enterohepatic circulation (78, 79). In animal models, FXR activation has been demonstrated to reduce hepatic gluconeogenesis, lipogenesis and steatosis (79-81), and both synthetic bile acid variants (namely obeticholic acid, OCA) and non-bile acid FXR agonists have been developed and are currently being tested in clinical trials.

Obeticholic acid (OCA) is a synthetic variant of the natural bile acid chenodeoxycholic acid, a potent activator of the farnesoid X nuclear receptor, which negatively regulate bile acid synthesis and down-regulates lipogenesis (81). The FLINT study, a multicentre, phase IIb clinical trial conducted in subjects with subjects with NASH (NAS>4), compared placebo (n=142) with 25mg obeticholic acid (n=141) for 72 weeks. OCA met the pre-defined stopping rule for efficacy, namely reduction in the NAFLD activity score (NAS) by 2 points with no worsening in fibrosis, although it did not impact on resolution of NASH (22% in OCA arm vs 13% in placebo arm; p=0.08). Notably, OCA improved fibrosis stage in 35% of patients vs 19% (p=0.004) in the placebo arm (82). Of note, although insulin resistance, as assessed by euglycaemic hyperinsulinaemic clamps, improved in an earlier study (83), the opposite finding was observed in the FLINT trial. Whilst this may relate to the imbalance in insulin values at baseline in the FLINT trial or the different methods used to assess insulin resistance in the two studies it will be an important consideration in the ongoing phase III trial (NCT02548351). Moreover, pruritus was reported in 23% of the treated group, which may be an important consideration for a condition with minimal symptoms (82). Given the increased risk of cardiovascular morbidity and mortality in patients with NASH, the increased levels of low-density lipoprotein (LDL)

and reduced levels of high-density lipoprotein (HDL) on OCA therapy are a concern and will require careful attention/management in future studies (82).

Ursodeoxycholic acid (UDCA), a naturally occurring secondary bile acid, has not been shown to improve histological features of NASH (84, 85), and there is as yet no human histological data for the bile acid derivative tauroursodeoxycholic acid, or the UDCA derivative 24 norursodeoxycholic acid.

Selective non-bile acid synthetic farnesoid X receptor agonists have been developed, which have the potential to provide the metabolic effects of FXR agonism without the side effects of pruritus and elevated LDL. GS-9674 has been shown to significantly reduce serum transaminases, hepatic steatosis and fibrosis in murine models of NASH (86) and is being assessed at two dosages for 12 weeks in patients with clinical, radiological or histological NAFLD (NCT02856555). A further study compares GS-9674 with the Acetyl Co–A carboxylase inhibitor, GS-9676 (see lipid lowering drugs), and selonsertib, (apoptosis signal-regulating kinase 1 inhibitor), as well as selonsertib and GS-9674 in combination, for 12 weeks (NCT02781584).

Two other FXR agonists, LMB763 and LJN452 have been developed and are in phase II trials. A phase IIa study in patients with phenotypical or histological NASH for LMB763 is recruiting (NCT02913105), and LJN452 is being tested across a range of doses in patients with phenotypical or histological NASH and hepatic fat content >10% by MRI (NCT02855164).

b) Bile acid sequestrants/transporter inhibitors

Bile acid sequestrants bind bile acids in the gut and can be used to treat dyslipidaemia and hyperphosphatemia, although more recently these agents have been reported to lower blood glucose and increase insulin sensitivity through altered bile acid signalling pathways, possibly mediated by the presence of increased bile acids in the distal colon stimulating GLP-1 and peptide YY

(87). Whilst the bile acid sequestrant colesevelam was ineffective for the treatment of NASH, Sevelamer, a phosphate binding drug used to treat hyperphosphataemia in patients with chronic kidney disease, appeared more promising as it reduced steatosis and lobular inflammation in murine studies with a potential additional indirect impact on FXR signalling (79).

In another approach, inhibitors of the ileal apical sodium-dependent bile acid transporter (ASBT) have been developed which target bile acid reabsorption in the terminal ileum, and have been demonstrated to improve glycaemic control in diabetic rats (87), and restore glucose tolerance, reduce hepatic triglyceride and total cholesterol concentrations, as well as improve NAS in HFD-fed mice (88). An oral inhibitor of ASBT, volixibat (SHP-626), was well tolerated in phase I studies (89) and a phase II trial in patients with NASH is currently enrolling (NCT02787304).

c) Hormone signalling

Fibroblast growth factor 21 is a hormone secreted predominantly from the liver, which acts primarily in the fasting state to coordinate carbohydrate and lipid metabolism, enhance insulin sensitivity, decrease triglyceride levels, and cause weight loss, thus ameliorating obesity-associated hyperglycaemia and hyperlipidaemia (90). In animal models, an FGF-21 analogue (BMS-986036) improved insulin sensitivity, hepatic fat content, and *de novo* lipogenesis (91), and a subsequent phase II RCT is evaluating the effectiveness of 16 weeks of BMS-986036 treatment on hepatic fat content measured by magnetic resonance spectroscopy (MRS) in patients with NASH (NCT02413372). FGF-19 is another peptide hormone secreted in response to reabsorption of bile acids and FXR activation in the terminal ileum, reducing bile acid synthesis and gluconeogenesis independently of insulin (92). NGM-282 is a recombinant variant of FGF-19 that has shown

reduction of hepatic fat, ALT and improved NAS scores in mouse models (93) and is currently in a phase II study (NCT02443116) in patients with biopsy-proven NASH.

d) Anti-inflammatory and anti-apoptotic agents

Cenicriviroc, a C-C chemokine receptor types 2 and 5 antagonist, has been shown to reduce CD14 which is involved in inflammatory cell activation, and improve the aspartate aminotransferase-to-platelet count ratio index (APRI) and fibrosis-4 scores in patients with HIV (94). The C-C chemokine receptor types 2 and 5 (CCR2 and CCR5) and their respective ligands, C-C chemokine ligand types 2 (CCL2/monocyte chemo attractant protein-1 [MCP-1]) and 5 (CCL5/RANTES) play a role in inflammatory cell recruitment to the liver and also the activation of hepatic stellate cells. In a phase II study in obese adults with NASH (NCT02330549) cenicriviroc showed a reduction in fibrosis scores, but did not meet its pre-defined primary end-point of a two-point reduction in NAFLD activity scores; primary analysis of a phase IIb study in NASH shows similar results (95, 96).

Apoptosis signal-regulating kinase 1 (ASK1) is activated by extracellular TNFα, intracellular oxidative or endoplasmic reticulum stress and initiates the p38/JNK pathway, resulting in hepatocyte apoptosis and fibrosis (97). In animal models ASK1 inhibition reduced hepatic fibrosis, steatosis, and insulin resistance (98). Two doses of an ASK1 inhibitor, selonsertib, in combination with simtuzumab, or alone, were studied in a phase II trial in patients with NASH and stage 2-3 fibrosis (NCT02466516). Abstract data suggested that high dose selonsertib was effective at reducing fibrosis but did not improve the histological NAS score. Side effects such as headache, nausea, and abdominal pain were more prominent, however, on higher dose combination therapy and are likely to remain a concern in longer term trials (99).

The adhesion molecule vascular adhesion protein-1 (VAP-1), is a membrane-bound amine oxidase that promotes leukocyte recruitment to the liver, and its soluble form (sVAP-1) which accounts for

most circulating monoamine oxidase activity, has insulin-like effects and can initiate oxidative stress. An absence or blockade of functional VAP-1 in murine hepatic injury models has been shown to reduce inflammatory cell recruitment to the liver and attenuate fibrosis (100). Furthermore, serum sVAP-1 levels are elevated in patients with NAFLD compared with those in control individuals (100), and targeting VAP-1 is believed to have therapeutic potential for NAFLD and other chronic fibrotic liver diseases. A phase II clinical trial is currently underway for patients with primary sclerosing cholangitis (NCT02239211), and further trials in NAFLD are anticipated.

Emricasan is an irreversible caspase inhibitor, which has been investigated in pathways of inflammation and apoptosis in liver disease. It was demonstrated to reduce the histological NAS and fibrosis scores in murine models of NAFLD (101) and liver enzymes and markers of apoptosis in a phase II, placebo control trial in patients with NAFLD and elevated transaminases (102). A phase IIb study in subjects with biopsy-proven NASH and fibrosis is evaluating the efficacy of 72 weeks of 10mg or 100mg/day of emricasan, the primary endpoint being improvement in fibrosis, and secondary endpoint being resolution of NASH (NCT02686762). Emricasan has also shown efficacy in reducing the hepatic venous pressure gradient in cirrhotics with portal hypertension(103) and a phase II trial is assessing efficacy of 3 doses of emricasan on portal hypertension in individuals with NASH cirrhosis and a hepatic venous pressure gradient ≥12mmHg (NCT02960204).

e) Inhibition of *de novo* lipogenesis ("Lipid altering")

Aramchol is an arachidic and cholic acid conjugate that was shown to inhibit stearoyl CoA desaturase (SCD) *in vitro* and *de novo* lipogenesis in animal models on high fat diet (104, 105). A randomized, double-blind, placebo-controlled trial of 60 patients with biopsy-confirmed NAFLD evaluating 100mg

and 300mg Aramchol daily for 3 months showed a 12.57% ± 22.14% reduction in liver fat content in the 300mg dose group, compared with an insignificant reduction in the 100mg group and an increase in those on placebo. However, there were no other significant improvements in metabolic parameters and changes in ALT were minimal (106). Higher doses of aramchol, 400mg and 600mg, are currently being tested on individuals with NASH in a 52 week phase IIb study investigating their effect on hepatic triglycerides using nuclear magnetic resonance spectroscopy, and NAS score as a secondary endpoint (NCT02279524).

Malonyl-CoA is a key regulator of fatty acid metabolism, controlling the balance between *de novo* lipogenesis and fatty acid oxidation (107), and Acetyl Co–A carboxylase (ACC) is an enzyme that regulates the conversion of malonyl-CoA to Acetyl Co–A. Inhibition of ACC in a murine model of NAFLD increased fatty acid oxidation, reduced lipogenesis and hepatic fat content, and improved insulin sensitivity (107). A phase I trial using an allosteric inhibitor in obese adults demonstrated a reduction in *de novo* lipogenesis (108) and the same compound (GS-9676) is being assessed at two dosages for 12 weeks in patients with clinical, radiological or histological NAFLD (NCT02856555). The compound is also being compared with 3 others in another study, as mentioned previously.

The thyroid hormone receptor beta (THR-β) is the predominant liver thyroxine (T4) receptor, through which increased cholesterol metabolism and excretion through bile is mediated (109). The highly selective (THR-β) agonist MGL-3196 has been developed to target dyslipidaemia but has also been shown to reduce liver steatosis in animal models (110); this is now being tested in phase II trials in patients with biopsy proven NASH and ≥10% liver steatosis using change from baseline hepatic fat fraction assessed by MRI-PDFF as a primary outcome (NCT02912260).

f) Targeting the gut microbiome

IMM-124e is an IgG-rich extract of bovine colostrum from cows immunised against lipopolysaccharide (LPS), and is believed to reduce exposure of the liver to gut-derived bacterial products and LPS (111). In phase I studies and pre-clinical data, IMM-124 was found to improve liver enzymes, insulin resistance (OGTT and HgbA1c), and dyslipidaemia (LDL)(111, 112). A phase II RDBPCT is currently evaluating the effects of 24 weeks of IMM-124e in patients with biopsy-proven NASH (NCT02316717).

g) Anti-fibrotic agents

Lysyl oxidase like-2 (LOXL2) is one of a family of enzymes involved in modifying the extracellular matrix, promoting cross-linking of cellular collagen, and enhancing fibrosis (113). Serum LOXL2 levels have been shown to correlate with fibrosis in NAFLD (114), and an antibody has been developed and studied in a phase IIb clinical trial in patients with NAFLD-related fibrosis; the results are yet to be published (NCT01672866). The anti-fibrotic potential of the antibody simtuzumab is also being investigated in combination with drugs that target the metabolic or inflammatory component of NASH (see combination therapies, NCT02466516), and more phase II trials are anticipated. In addition, a LOXL2 enzyme inhibitor has also been developed, and shows promise in pre-clinical models of fibrosis (115)

GR-MD-02 is a carbohydrate-based galectin inhibitor that has been shown to reduce hyaluronic acid (a marker of fibrosis) in animal models of NASH with fibrosis (116). Phase I clinical studies confirmed safety, tolerability, and pharmacokinetics for single and multiple doses of GR-MD-02, and a reduction in the non-invasive FibroTest®(116). Phase II studies in patients with advanced fibrosis

(NCT02421094) and portal hypertension (NCT02462967) assessed changes in transient elastography by MR and Fibroscan, but have not yet been reported.

Combination therapy

Combination therapy, targeting both inflammation and fibrosis, may be the most effective strategy to improve both cardiovascular and liver outcomes, but few of those carried out so far have shown improvement in fibrosis. A prospective, double-blind, randomized, placebo-controlled trial in 45 patients with biopsy proven NASH investigated vitamin E plus vitamin C vs placebo for 6 months, showed a significant improvement in fibrosis but no improvement in necro-inflammation or ALT (117). Another combination study involving vitamin E with UDCA for two years showed improved serum aminotransferases in the combination group compared to UDCA alone, but no changes in fibrosis (118). NASH but not fibrosis improved following 48 weeks of rosiglitazone with no additional benefit of metformin or losartan in another combination study (119). In a 12-month prospective study in patients with type 2 diabetes, both pioglitazone and the combination of pioglitazone and exenatide (synthetic exendin-4) led to significant reductions in liver fat and ALT, and the combination therapy was superior (−60 %) compared to pioglitazone alone (−40 %)(120), but no combination studies have been carried out in NASH. Targeting fibrosis more directly, Gilead have completed a phase II study in selonsertib (a potent ASK1 inhibitor) and simtuzumab, a LOXL2 antibody, which showed some histological improvement in fibrosis but not NAS score with high dose selonsertib, but little suggestion that the addition of simtuzumab was effective (99) (NCT02466516).

Treatment for NASH – what will the model of care be?

This is an exciting time for pharmacotherapy in NAFLD. Improved understanding of the pathophysiology of NASH has facilitated development of new agents, and evidence is emerging that

some already established treatments may have additional benefit. In trials, primary endpoint have focused on improvement in liver-related outcomes, namely NASH resolution and improvement in fibrosis; these goals have been difficult to achieve, likely due to poor efficacy of treatments tested within limited time frames. Whilst the rationale of treatment for NAFLD is to prevent progression to end-stage liver disease, cardiovascular end-points will be critical in enabling licensing of new drugs.

As NAFLD is an asymptomatic condition with a long natural history, it is difficult to predict how patients will perceive the benefits of therapies with few tangible short-term outcomes. It is likely that drugs will need to be well tolerated and relatively side effect-free if patients are to adhere to medical therapy. Other challenges relate to the duration of treatment in NAFLD and the role of non-invasive markers in determining dose and duration of treatment.

Combination therapy is likely to be the utilised in the future, but with results of phase III studies pending, it is difficult to predict which combinations therapies are likely to be most effective. It seems likely that drugs will target different aspects of pathogenesis ie. an anti-fibrotic in combination with one that targets *de novo* lipogenesis, or an anti-inflammatory agent paired with one that improves lipid metabolism. There is also the potential for drug therapy to be tailored according to patient needs, ie. using drugs that improve glucose metabolism in those with diabetes, using therapies that promote weight loss in the obese, or anti-inflammatories or anti-fibrotics in those for which these are the predominant histological features.

Notwithstanding the exciting developments of new pharmacological agents, it is important not to lose sight of the importance of modification to not only treat but also to prevent the onset of metabolic syndrome and NAFLD.

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Class	Drug	Latest phase of development in NAFLD	Preclinical data	Clinical data
Incretin based therapy	Liraglutide (GLP-1 analogue)	Proof of concept phase IIb trial completed (approved for use in type 2 diabetes)	Improved steatosis, ALT, and insulin sensitivity; hypertension and cardiac hypertrophy also reduced (27)	Histology: NASH resolution*(28, 32) Non-histological: weight loss, improved diabetic control
	Exenatide (Synthetic exendin-4)	Proof of concept phase IIb trial completed in patients with NASH and diabetes (approved for use in type 2 diabetes)	Improved steatosis (26, 121)	Results from phase IIb trial not published Non histological: Improved insulin resistance and weight loss (diabetics) (29, 30)
	Sitagliptin (DDP-4 inhibitor)	Phase IIa trial in NAFLD completed Approved for use in type 2 diabetes	Improved steatosis and insulin sensitivity (122)	Histology: improved hepatocyte ballooning and reduction in NAS scores in diabetic subjects with NASH (35). (Non-histological studies have shown mixed results for steatosis (36,38) and no improvement in serum markers (37,38))
SGLT-2 inhibitor	Canagliflozin Ipragliflozin Luseogliflozin	Ipragliflozin: Rodent studies completed. Luseogliflozin: Rodent studies completed (all approved for use in type 2 diabetes)	Ipragliflozin: improved steatosis, apoptosis and fibrosis (43-45) Luseogliflozin: improved steatosis and fibrosis (42)	Non-histological: weight loss (39) and improved ALT/FLI demonstrated in trials for T2DM (Canagliflozin, Ipragliflozin) (47, 48, 50)
PPAR agonists	Pioglitazone (PPARγ agonist)	Phase IIb trial completed Approved for use in type 2 diabetes	Improved inflammation and fibrosis (123)	Histology: improved hepatic steatosis, inflammation, ballooning and fibrosis (57, 59) Non-histological: reduction in death, myocardial infarction and stroke in patients with T2DM
	Elafibranor (Dual PPARα/δ agonist)	Phase IIb trial in NASH completed. Global phase III trial recruiting	Improved steatosis, inflammation and fibrosis (67)	Histology: NASH resolution in those with NAS≥4[§] (69, 70)
	Saroglitazar (Dual PPARα/γ agonist)	Phase IIa trial in NASH and diabetes completed. Approved for treatment of dyslipidaemia in diabetics	Reduced steatosis, NASH and fibrosis (64)	Non-histological: improved ALT , HBA1c and serum triglycerides, improved steatosis on USS (65, 66)
	MSDC -0602 (PPARγ sparing TZD)	Phase IIb	Reduced transaminases, histological NASH, and stellate cell activation (73)	Non-histological: Improved fasting glucose and HBA1c in patients with diabetes (72)
FXR-bile acid axis	Obeticholic acid (Synthetic bile acid)	Phase IIb trial in NAFLD completed in US, Global phase III trial recruiting Approved for use in PBC in the US	Improved steatosis (124)	Histology: improved NAS scores and fibrosis (82)
	GS-9674 (Selective Farnesoid X receptor agonist)	Phase IIa recruiting	Reduce serum transaminases, hepatic steatosis and fibrosis in NASH (86)	-
	INT-767 (Dual FXR/TGFR agonist)	Phase I trial anticipated	Improved histological NASH in mice (125)	-
	Volixibat ASBT inhibitor	Phase IIa trial in NASH recruiting	Improved glycaemic control in rats (78); improved glucose tolerance, reduced hepatic triglyceride and total cholesterol concentrations, and improved NAFLD activity scores in mice (84)	-
	Sevelamer (Bile acid sequestrant)	Animal studies only	Reduced steatosis and lobular inflammation in mice (79)	-
Hormone signalling	BMS-986036 (Fibroblast growth factor 21 analogue)	Phase IIa trial evaluating effect on hepatic fat content measured by MRS in NASH registered but not yet recruiting	Improved insulin sensitivity, reduced hepatic fat content, and de novo lipogenesis in rats (91)	-
	NGM-282 (Recombinant FGF-19 variant)	Phase IIa trial in NASH recruiting	Reduction of hepatic fat, ALT, and improved NAS scores (93)	-
De novo lipogenesis/lipid	Aramchol (arachidic and cholic acid conjugate)	Phase IIa complete. Phase IIb trial recruiting	Reduced *de novo* lipogenesis (104, 105)	Non-histological: reduction in liver fat content by MRS at higher dose (106)
	NDI-010796 (Acetyl Co–A carboxylase inhibitor)	Phase I trial completed. Phase II recruiting	Increased fatty acid oxidation, reduced lipogenesis and hepatic fat content, and improved insulin sensitivity in rats (107)	Non-histological: reduced de novo lipogenesis in obese adults (108)
	MGL-3196 (thyroid hormone receptor beta (THR-β) agonist)	Phase 1 complete. Phase IIa trial recruiting	Reduced hepatic steatosis as well as reduced plasma FFA and triglycerides (110)	Reductions in LDL cholesterol of up to 30%, and trend to reduce triglycerides (109)
Antioxidant	Vitamin E	Phase IIb trials completed	Reduced steatohepatitis (126) and fibrosis (127)	Histology: reduced steatosis and improved NAS scores** Non-histological: reduced ALT (59, 75)
	Cysteamine (Aminothiol)	Proof of concept phase IIb trial in children with NAFLD completed.	-	Results of proof of concept phase IIb trial not published Non-histological: reduced AST, ALT and CK-18 fragment levels (children) (128)
Targeting apoptosis	Emricasan (Caspase inhibitor)	Phase IIb in NASH and NASH cirrhosis recruiting	Improved NAS score and fibrosis (101)	Non-histological: reduced ALT and markers of apoptosis (102)
	Selonsertib (ASK-1 inhibitor)	Phase IIb trial in NASH and fibrosis and cirrhosis recruiting	Reduced hepatic fibrosis, steatosis, and insulin resistance in mice (98)	Histology: improved fibrosis demonstrated in phase IIb trial using selonsertib in combination with simtuzumab (with no additional benefit of simtuzumab) (99)
Anti-inflammatory	Cenicriviroc (C-C chemokine receptor types 2/5 antagonist)	Phase IIb trial completed. Phase III trial opening soon.	-	Histology: Did not meet primary end-point of 2 point reduction in NAS, but reduced fibrosis (secondary end-point) (96)

	BTT1023 (Anti-VAP antibody)	Phase IIa trial in NAFLD open	Animal data: reduced inflammatory cell recruitment and fibrosis (mice) (100)	-
Gut microbiome	IMM-124e (IgG-rich extract of bovine colostrum)	Phase IIa trial in NASH ongoing	Improved ALT and glucose tolerance (111)	-
Anti-fibrotic	Simtuzumab (LOXL2 antibody)	Phase IIb trial terminated	Reduced collagen cross linking in fibrosis models only (129)	Results of phase IIb trial in NASH and fibrosis not published Histology: no benefit from the addition of simtuzimab to selomsertib in phase IIb combination trial (99).
	GR-MD-02 (Galectin inhibitor)	Phase IIa trial completed, further proof of concept phase IIb in NASH related cirrhosis and portal hypertension ongoing	Reduced hyaluronic acid in mice (116)	Results of phase IIa trial not published
Dual therapies	Vitamin E + Vitamin C	Proof of concept phase IIb trial completed	-	Histology: improved fibrosis (117)
	Vitamin E + UDCA	Proof of concept phase IIb trial completed	-	Histology: no improvement in fibrosis Serum markers: improved liver enzymes (118)
	Selonsertib + Simtuzumab	Proof of concept phase IIb trial completed	-	Histology: improved fibrosis (99)
	Selonsertib + GS-9674	Phase IIa recruiting	-	-

Table 1. Drugs with proven benefit in NAFLD.

*Defined as the resolution of steatohepatitis (disappearance of hepatocyte ballooning) without worsening of fibrosis by the Kleiner fibrosis classification. [§]Post-hoc analysis demonstrated NAS resolution in those with NAS≥4 resolution. **Defined as an improvement by 1 or more points in the hepatocellular ballooning score and no increase in fibrosis.

Trial phases are defined as follows: phase I, safety study; phase IIa – non-histology randomised control trial (RCT); proof of concept phase IIb – small histology RCT; phase IIb – large histology RCT.

Drug	Lipid profile	Weight	Insulin resistance	Cardiovascular outcomes
Pioglitazone	No effect	Results in weight gain [59]	Improves [59]	Reduced incidence of MI and stroke demonstrated in patients with T2MD [58]
Obeticholic acid	Increase in LDL and reduced HDL seen in the phase IIb FLINT trial [76]	Weight loss observed in FLINT trial [76]	Improved insulin sensitivity in clamp studies during phase IIa trial, but higher fasting serum insulin and greater hepatic insulin resistance by HOMA demonstrated in FLINT[76,77]	No known effect
Elafibranor	Reduced plasma free fatty acid, fasting plasma triglycerides, and LDL cholesterol levels [68]	No effect	Improved peripheral and hepatic insulin sensitivity in obese men [68]	No known effect
GLP-1 inhibition	No known effect	Weight loss [32]	Improved peripheral, hepatic and adipose insulin resistance in subjects with NASH [31]	Reduced cardiovascular mortality (liraglutide) and non-fatal MI and stroke (semaglutide) in patients with T2DM [34]
Saroglitazar	Improved serum triglycerides in patients with NAFLD and diabetes [66]	No effect [65]	Improved HBA1c in patients with NAFLD and diabetes [66]	No known effect

Table 2. Non-liver related benefits for potential NAFLD therapies. MI, myocardial infarct; T2DM, type 2 diabetes mellitus; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HOMA, homoeostasis model of assessment.

Table 3. Histological benefits demonstrated during clinical trials for potential therapies in NAFLD

Drug	Steatosis	Reduction in NAS	NASH resolution	Fibrosis
Pioglitazone [57]	✓	✓	✓*	✓**†
Vitamin E [59,72]	✓	✓	✗	✗
Obeticholic acid[76]	✓	✓	✗	✓**
Elafibranor [69]	✓	✗	✓*‡	✗
Liraglutide [32]	✓	✗	✓	✗§
Cenicriviroc [96]	✗	✗	✗	✓**
Vitamin E + Vitamin C[120]	✗	✗	✗	✓

*NASH resolution with no stipulation of any worsening of fibrosis. †Improved fibrosis demonstrated at meta-analysis.**Fibrosis measured as secondary end-point in clinical trials. ‡Observed in post-hoc analysis in those with NAS≥4. §Reduced progression of fibrosis in those on liraglutide.