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REVIEWS

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The therapeutic landscape of non-alcoholic steatohepatitis

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Abstract

Non-alcoholic steatohepatitis (NASH) is characterized by lobular inflammation and hepatocellular ballooning, and may be associated with liver fibrosis leading to cirrhosis and its complications. A pharmacological approach is necessary to treat NASH because of failure to change dietary habits and lifestyle in most patients. Insulin resistance with an increased release of free fatty acids, oxidative stress and activation of inflammatory cytokines seem to be key features for disease progression. Thiazolidinediones, such as pioglitazone and antioxidant agents, such as vitamin E, were the first pharmacological options to be evaluated for NASH. In recent years, several new molecules that target different pathways related to NASH pathogenesis, such as liver metabolic homeostasis, inflammation, oxidative stress and fibrosis, have been developed. Obeticholic acid (INT-747) and elafibranor (GFT-505) have provided promising results in phase IIb, randomized, placebo-controlled clinical trials and they are being evaluated in ongoing phase III studies. Most of the potential treatments for NASH are under investigation in phase II studies, with some at phase I. This diversity in possible treatments calls for a better understanding of NASH in order to enrich trial populations with patients more susceptible to progress and to respond. This manuscript aims to review the pharmacological NASH treatment landscape.

KEYWORDS
fatty liver, fibrosis, pharmacological treatment, steatohepatitis

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease worldwide, especially owing to its close relationship with metabolic features, such as type 2 diabetes mellitus (T2DM), dyslipidaemia and obesity.[1] NAFLD presents a clinical spectrum, ranging from simple steatosis or non-alcoholic fatty liver (NAFL) to its progressive form, known as non-alcoholic steatohepatitis (NASH), which is characterized by lobular inflammation and hepatocellular ballooning.[2] In some patients, NASH is associated with progression of fibrosis leading to cirrhosis and its complications, such as portal hypertension and hepatocellular carcinoma (HCC).[3] Several studies have reported a strong relationship between NAFLD and cardiovascular disease.[4] NAFLD has been significantly associated with an increased risk of fatal and non-fatal cardiovascular events.[5] An optimal treatment of NASH might reduce liver-related complications [6] and the risk of cardiovascular events.[7]

Changes in dietary habits and lifestyle have been recommended as standard of care for NAFLD.[2] However, this behavioural strategy fails more often than it succeeds, and therefore, a pharmacological

Abbreviations: AEs, adverse events; ALT, alanine aminotransferase; APRI, aspartate to platelet ratio index; ASK1, apoptosis signal-regulating kinase 1; AST, aspartate aminotransferase; CCL2/MCP-1, chemokine (C-C motif) ligand 2/monocyte chemoattractant protein 1; CCL5/RANTES, chemokine (C-C motif) ligand 5/regulated on activation normal T cell expressed and secreted; CCR, C-C chemokine receptor; CI, confidence interval; CVC, cenicriviroc; DPP, dipeptidyl peptidase-4; ELF, Enhanced Liver Fibrosis; FC, liver fat content; Fex, fexaramine; FGF, fibroblast growth factor; FIB-4, fibrosis-4 score; FXR, farnesoid X receptor; GGT, gamma-glutamyl transpeptidase; GIP, glucose-dependent insulinotropic polypeptide; GIR, glucose infusion rate; GLP-1, glucagon-like peptide-1; HCC, hepatocellular carcinoma; HDL, high-density cholesterol; HOMA, homoeostasis model of assessment; HVPG, hepatic venous pressure gradient; IgG4, immunoglobulin G4; LDL, low-density cholesterol; LOXL, lysyl oxidase and lysyl oxidase like; LS, liver stiffness; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NAS, NAFLD Activity Score; OCA, obeticholic acid; OR, odds ratio; PK, pharmacokinetics; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acids; SCD1, stearoyl coenzyme A desaturase 1; SIM, simtuzumab; T2DM, type 2 diabetes mellitus; TGF-β, transforming growth factor beta; TNF, tumour necrosis factor; UDCA, ursodeoxycholic acid; US, ultrasound; VAP-1, vascular adhesion protein-1; VLX-103venlafaxine-103.

approach is often necessary to treat NASH.[8] So far, few molecules, such as thiazolidinediones and vitamin E, have been evaluated as pharmacological options for NASH.[9] However, several new molecules targeting different pathways, such as liver metabolic homeostasis, inflammation, oxidative stress and fibrosis, have been developed for the treatment of NASH (Table 1 and Figure 1).

TABLE 1 Summary of drugs candidates for non-alcoholic steatohepatitis (NASH) treatment

Drug class	Drug name	Company
Metabolism homeostasis		
Insulin sensitizer	Rosiglitazone	GlaxoSmithKline
Insulin sensitizer	Pioglitazone	Takeda Pharmaceutical
FXR agonist	Obethicolic acid (INT-747)	Intercept Pharmaceuticals
FXR agonist	GS-9674	Gilead Sciences
FXR agonist	LJN-452	Novartis
FXR agonist	EDP-305	Enanta Pharmaceuticals
PPAR α/δ agonist	Elafibranor (GTF-505)	Genfit
PPAR α/γ agonist	Saroglitazar (ZYH1)	Zydus Cadila
PPAR α, δ and γ (Pan PPAR)	IVA-337	Inventiva Pharma
FGF19 analogue	NGM-282	NGM Biopharmaceuticals
FGF21 analogue	PF-05231023	Pfizer
SCD1 inhibitor	Aramchol	Galmed Pharmaceuticals
GLP-1 analogue	Liraglutide (NN2211)	Novo Nordisk
NorUDCA	NorUDCA	NA
Oxidative stress		
Anti-oxidant agent	Vitamin E	NA
ASK1 inhibitor	GS-4997	Gilead Sciences
VAP-1 inhibitor	PXS-4728A	Boehringer Ingelheim
Inflammation		
CCR2/CCR5 antagonist	Cenicriviroc	Tobira Therapeutics
Pentamidine	VLX-103	Verlix Pharma
Apoptosis		
Caspase inhibitor	Emricasan (IDN-6556)	Conatus Pharmaceuticals
Fibrosis		
LOXL2 inhibitor	Simtuzumab (GS-6624)	Gilead Sciences
Galectin-3 protein inhibitor	GR-MD-02	Galectin Therapeutics

ASK1, apoptosis signal-regulating kinase 1; CCR, C-C chemokine receptor; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; LOXL, lysyl oxidase and lysyl oxidase-like; NA, not applicable; PPAR, peroxisome proliferator-activated receptor; SCD1, stearoyl coenzyme A desaturase 1; UDCA, ursodeoxycholic acid; VAP-1, vascular adhesion protein-1.

Key points

- Non-alcoholic fatty liver disease (NAFLD), a leading cause of chronic liver disease worldwide, ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) that might be associated with cirrhosis and its complications.
- Changes in dietary habits and lifestyle have been recommended as standard of care for NAFLD. However, this behavioural strategy mostly fails.
- Thiazolidinediones and antioxidant agents, such as vitamin E, have been extensively evaluated in phase III clinical trials as options for NASH treatment
- New molecules targeting different pathways, such as liver metabolic homeostasis, inflammation, oxidative stress and fibrosis, are being tested for the treatment of NASH in ongoing clinical trials

Insulin resistance with an increased released of free fatty acids, oxidative stress and activation of inflammatory cytokines seem to be key features for transition from NAFL to NASH and progression of fibrosis.[10] Therefore, insulin sensitizers, such as thiazolidinediones, as well as antioxidant agents, such as vitamin E, have been extensively evaluated in phase III clinical trials as options for NASH treatment. Several molecules have also been evaluated in phase II and III randomized clinical trials (Table 2), along with several drugs in the pipeline that are being tested for the treatment of NASH in ongoing clinical trials (Table 3). Health authorities have been recognized that the resolution of NASH, defined as the disappearance of necroinflammatory features in histologic analysis without progression of fibrosis, must be the end point for phase IIb and phase III trials of patients with NASH and early stage fibrosis.[11] The decrease in NAFLD Activity Score (NAS) can be used as an end point in clinical trials. However, further studies are needed to determine whether patients with lower scores have a reduced risk for progression to advanced fibrosis. Thus, the great heterogeneity in criteria for enrolment and definition of primary outcomes have been a main limitation in interpreting the results of NASH treatment trials. This manuscript aims to review the therapeutic landscape for NASH treatment.

2 | WEIGHT LOSS

The major treatment offered for NAFLD remains lifestyle changes including weight reduction by a healthy diet and performing regular physical activity.[8] Studies have described that a weight loss of 7%-9% should be the goal to reduce necroinflammation and weight loss of more than 10% might lead to regression of fibrosis in patients with NASH.[12] Weight loss leads to a reduction in oxidative stress and improvement in lipid profile and insulin sensitivity.[13] Bariatric surgery

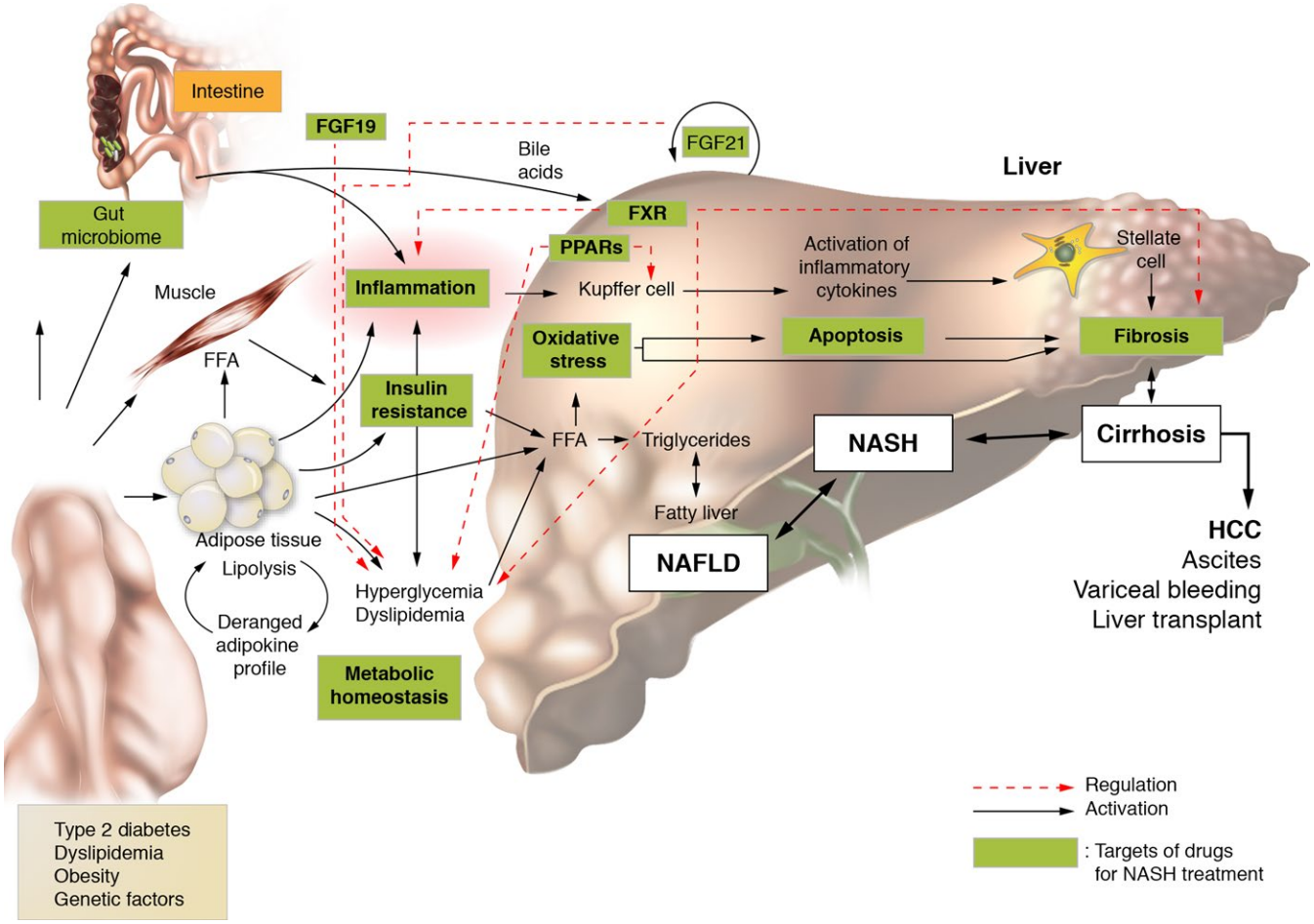

FIGURE 1 Pathogenesis of non-alcoholic fatty liver disease (NAFLD) and potential targets for treatment of non-alcoholic steatohepatitis (NASH)

can be an option for weight loss in morbid obese patients. Bariatric surgery induces long-term weight loss, resolution of NASH and reduction in all of the histologic components of NASH, including fibrosis.[14]

3 | METABOLIC HOMEOSTASIS

3.1 | Thiazolidinediones

Thiazolidinediones, known as glitazones (pioglitazone and rosiglitazone), are oral drugs that currently provide the best evidence-based data for NASH treatment, and the rationale for their use in NASH is based on the fact that insulin resistance is central to the pathogenesis of NASH.15 These drugs act by improving insulin resistance through different pathways: (i) promoting the differentiation of insulin-resistant large pre-adipocytes into small and insulin-sensitive adipocytes16; (ii) reducing inappropriate fat storage in muscle and adipocyte tissue with subsequent improvement in insulin sensitivity despite the expansion in fat mass; and (iii) upregulating production of adiponectin, an insulin-sensitizing and anti-steatogenic adipokine that increases fatty acid beta-oxidation in liver and muscle.17

The PIVENS was a large randomized phase III trial that compared once-daily pioglitazone (30 mg in 80 subjects) to once-daily vitamin E (800 IU in 84 subjects) and placebo (83 subjects) in non-diabetic patients with NASH over 96 weeks.9 In this trial, NASH was defined as definite or possible steatohepatitis with a NAFLD Activity Score (NAS) of 5 or more, or definite steatohepatitis (confirmed by two pathologists) with NAS of 4. The primary outcome was an improvement in histological findings, which required an improvement by one or more points in: hepatocellular ballooning score; no increase in the fibrosis score; and a two-point decrease in NAS. Steatosis (69% vs 31%, P<.001) and lobular inflammation (60% vs 35%, P=.004) were substantially improved by pioglitazone compared with placebo. In addition, the rate of clearance of steatohepatitis was higher in pioglitazone-treated patients compared with placebo (47% vs 21%, P<.001). However, the pre-planned 0.025 level of significance for the primary outcome for pioglitazone vs placebo (P=.04) was not reached.

A recent randomized, double-blind placebo-controlled trial. (NCT00994682) compared once-daily pioglitazone (45 mg in 50 subjects) to placebo (51 subjects) in prediabetes or type-2 diabetes mellitus patients with NASH over 72 weeks.18 Patients treated had higher rates of a two-point decrease in NAS without worsening fibrosis (65% vs 19%, P<.001) and resolution of NASH (58% vs 21%, P<.001) compared to placebo. In addition, pioglitazone treatment was associated

TABLE 2 Summary of phase II/III randomized clinical trials that have evaluated potential treatments for non-alcoholic steatohepatitis (NASH)

Reference	NCT number	Trial Acronym	Phase	Treatment arms	Duration, wk	Sample, n	Population	Primary endpoint	Main results	AEs
Pioglitazone and vitamin E										
Sanyal et al. 2010[9]	NCT00063622	PIVENS	III	Pioglitazone 30 mg/d vitamin E 800 IU/d placebo	96	247	Non-diabetic patients with NASH	Improvement in histological findings	NASH clearance (pioglitazone vs placebo): 47% vs 21%, *P*<.001; histological improvement (vit E vs placebo): 43% vs 19%, *P*<.001	Weight gain; similar rate cardiovascular events; no hepatotoxicity
Cusi et al. 2016[18]	NCT00994682	–	III	Pioglitazone 45 mg/d placebo	72	101	Prediabetres and T2DM patients with NASH	Improvement in histological findings	Pioglitazone vs placebo: 2-point decrease in NAS (65% vs 19%, *P*<.001); NASH clearance (58% vs 21%, *P*<.001)	Weight gain was greater with pioglitazone (2.5 kg vs placebo)
FXR agonist—OCA (INT-747)										
Mudaliar et al. 2013[37]	NCT00501592	–	II	OCA 25 mg/d OCA 50 mg/d placebo	6	64	T2DM and NAFLD	Changes in IR and glucose homeostasis by a two-step hyperinsulinaemic euglycemic clamp	Increased insulin sensitivity (OCA vs placebo):+24.5% vs −5.5%, *P*=.011. Reduction in fibrosis by biomarkers (OCA vs placebo)	OCA vs placebo (≥1 AE): 22% vs 26%
Neuschwander et al. 2015[38]	NCT01265498	FLINT	IIb	OCA 25 mg/d placebo	72	283	Liver-biopsy NASH; non-cirrhotic	NAS reduction≥2 points without worsening of fibrosis by liver biopsy	OCA vs placebo: histological improvement (45% vs 21%, *P*=.002), fibrosis (*P*=.004), ballooning (*P*=.03), steatosis (*P*=.001) and lobular inflammation (*P*=.006)	Pruritus (OCA vs placebo): 23% vs 6%, *P*<.0001
PPAR α/δ agonist—elafibranor (GFT-505)										
Ratziu et al. 2016[28]	NCT01694849	GOLDEN-505	IIb	Elafibranor 80 mg/d elafibranor 120 mg/d placebo	52	274	Liver-biopsy NASH; non-cirrhotic	NASH reversal without worsening of fibrosis by liver biopsy (protocol-defined and post-hoc definition)	Post-hoc analysis (elafibranor 120 mg vs placebo): NASH reversal (19% vs 12%; *P*=.045). Improvement of lipids, glucose homeostasis and insulin sensitivity	Elevation of creatinine reversible after elafibranor stopped. No deaths or CV events in all groups
SCD1inhibitor—aramchol										
Safadi et al. 2014[54]	NCT01094158	–	II	Aramchol 100 mg/d aramchol 300 mg/d placebo	12	60	Liver-biopsy NAFLD or NASH; non-cirrhotic	The difference between initial and final liver fat content by MRS. PK analysis	Aramchol 300 mg/d vs placebo: LFC (-12.6% vs +6.4%, *P*=.020. Serum levels of aramchol were stable	AEs were mild or moderate, not related to the treatment and similar among groups
GLP-1—liraglutide										
Armstrong et al. 2016[57]	NCT01237119	LEAN	II	Liraglutide 1.8 mg/d placebo	48	52	Liver-biopsy NASH; 12% with cirrhosis	NASH reversal (disappearance of hepatocyte ballooning) without worsening of fibrosis by liver biopsy	Reversal of NASH: 39% liraglutide vs 9% placebo; *P*=.019). Trend to improve steatosis and hepatocyte ballooning in liraglutide	Higher rates of gastrointestinal disorders (liraglutide vs placebo): 81% vs 65%

AEs, adverse events; MRS, magnetic resonance spectroscopy; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD Activity Score; NASH, non-alcoholic steatohepatitis; OCA, obeticholic acid; PK, pharmacokinetics; T2DM, type 2 diabetes mellitus.

TABLE 3 Summary of ongoing phase II/III randomized clinical trials that will evaluate potential treatments for non-alcoholic steatohepatitis (NASH)

Drug	NCT number	Phase	Treatment arms	Duration wk	Sample, n	Population	Primary endpoint	Estimated study completion	Countries	Last updated
Elafibranor	NCT02704403	III	Elafibranor120 mg vs placebo	72	2000	Liver-biopsy NASH; non-cirrhotic	NASH resolution without worsening of fibrosis	December 2021	EU	March 2016
Saroglitazar	NCT02265276	III	Saroglitazar 4 mg/d vs pioglitazone 30 mg/d	24	100	NAFLD by US, BMI>23 Kg/m² and elevated transaminases	Change in the NAFLD fibrosis score	September 2015	India	October 2014
OCA	NCT02548351	III	OCA 10 mg vs 25 mg vs placebo	72	2000	Liver-biopsy NASH; non-cirrhotic	NASH resolution without worsening of fibrosis	October 2021	USA, Australia, EU, Canada	June 2016
LNJ452	NCT02855164	II	LNJ452 dose TBD vs placebo	12	250	Liver-biopsy NASH with elevated transaminases	AE profile, change in transaminases	November 2017	USA, Australia, EU, Canada	August 2016
NGM282	NCT02443116	II	NGM282 doe TBD vs placebo	12	75	Liver-biopsy NASH	Change in absolute LFC by MRS	December 2016	USA, Australia	November 2015
Aramchol	NCT02279524	IIb	Aramchol 400 mg vs 600 mg vs placebo	52	240	Liver-biopsy NASH; LFC>5.5% by MRS; non-cirrhotic	% change in liver triglycerides concentration by MRS	June 2017	USA, EU. and America	October 2015
Aramchol	NCT02684591	II	Aramchol 600 mg vs placebo	12	50	HIV with LFC>5% by MRS; non-cirrhotic	Improvement of hepatic steatosis assessed by MRS	January 2020	USA	February 2016
GS-4997	NCT02466516	II	GS-4997 6 mg (±SIM 125 mg) vs 18 mg (±125 mg) vs SIM 125 mg	24	72	Liver-biopsy NASH; non-cirrhotic	Adverse event profile of GS-4997	October 2016	USA, Canada	April 2016
GS-4997	NCT02781584	II	GS-4997 vs placebo	12	10	MRS>10% steatosis and MRE with LS≥2.88 kPa	Incidence of AEs	October 2016	USA	May 2016

(continues)

TABLE 3 (continued)

Drug	NCT number	Phase	Treatment arms	Duration wk	Sample, n	Population	Primary endpoint	Estimated study completion	Countries	Last updated
CVC	NCT02217475	II	CVC 150 mg vs placebo	52	289	Liver-biopsy NASH; non-cirrhotic	Improvement of NAS without worsening of fibrosis by liver biopsy	October 2017	USA, EU, Australia, Hong Kong	September 2015
CVC	NCT02330549	II	CVC 150 mg vs placebo	24	45	Liver-biopsy NAFLD and pre-diabetes or T2DM; non-cirrhotic	Changes in insulin sensitivity	September 2016	USA; Puerto Rico	May 2016
Emricasan	NCT02686762	II	Emricasan 5 mg/BID vs 50 mg/BID vs placebo	72	330	Liver-biopsy NASH with NAS≥4; non-cirrhotic	Fibrosis improvement by at least one stage without worsening of NASH	October 2018	USA	June 2016
SIM	NCT01672866	IIb	SIM 75 mg vs 125 mg vs placebo	96	222	Liver-biopsy NASH with Ishak stages 3-4; non-cirrhotic	Change in morphometric quantitative collagen on liver biopsy	September 2016	USA, Canada, EU, Puerto Rico	August 2015
SIM	NCT01672879	IIb	SIM 200 mg vs 400 mg vs placebo	240	259	Liver-biopsy NASH with cirrhosis	Change in HVPG	September 2016	USA, Canada, EU, Puerto Rico	August 2015
GR-MD-02	NCT02421094	II	GR-MD-02 vs placebo	16	30	Liver-biopsy NASH	Change in liver fibrosis by LiverMultiScan	September 2016	USA	September 2015
GR-MD-02	NCT02462967	II	GR-MD-02 2 mg/kg vs 8 mg/kg every other week vs placebo	52	156	Liver-biopsy NASH and HVPG≥6 mm Hg	HVPG reduction	February 2018	USA	March 2016

AEs, adverse events; CVC, cenicriviroc; EU, European Union; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD Activity Score; NASH, non-alcoholic steatohepatitis; HVPG, hepatic venous pressure gradient; LFC, liver fat content; LS, liver stiffness; MRS, magnetic resonance spectroscopy; OCA, obeticholic acid; SIM, simtuzumab; T2DM, type 2 diabetes mellitus; USA, United States of America. Source: www.clinicaltrial.gov accessed on August 2016.

with improvement of steatosis, inflammation, ballooning (P<.001 for all) and fibrosis (P=.04).

However, the use of glitazones have been associated with adverse effects, such as weight gain, which tends to persist after drug discontinuation,19 bone fractures in women,20 and increased risk of bladder cancer for pioglitazone 21 and cardiovascular events with rosiglitazone.22 In addition, rosiglitazone is not available in European Union and many countries such as UK, New Zealand and South Africa, due to safety concerns and a raised risk of cardiovascular events.23

3.2 | Peroxisome proliferator-activated receptor (PPAR) agonists

Peroxisome proliferator-activated receptor alpha and delta (PPAR α/δ) regulate lipid metabolism in liver and glucose homeostasis.[24] PPARα activation leads to control of lipid flux and, in the liver, inhibition of inflammatory genes induced by nuclear factor k-B and improvement of necro-inflammatory activity.[25] In addition, active PPARδ improves glucose homeostasis and inhibits hepatic lipogenesis, and has anti-inflammatory activity in macrophages and Kupffer cells.[26] The activation of both PPAR α/δ leads to improvement of different pathways to regulate liver metabolism involved in NASH pathogenesis. In preclinical trials, elafibranor (GTF-505) was described as a PPAR α/δ modulator that decreases hepatic steatosis and inflammation, and has antifibrotic properties.[27]

The dual PPAR α/δ, elafibranor (GFT-505), was evaluated in a large, phase IIb, randomized, placebo-controlled trial that included 274 patients from 56 medical centres across Europe and the USA (GOLDEN-505 trial; NCT01694849).[28] Non-cirrhotic patients with NASH were randomized (1:1:1) into one of three treatment arms, elafibranor 80 or 120 mg/day, or placebo, for 52 weeks. NASH was defined as presence of steatosis, hepatocellular ballooning and lobular inflammation by a liver biopsy at least 9 months before enrolment. Patients with cirrhosis (stage 4 of NASH Clinical Research Network (NASH CRN) fibrosis staging system) were excluded. The protocol-defined primary outcome was NASH reversal—absence of at least one of the three components of NASH—without worsening of liver fibrosis. For a post-hoc analysis, NASH resolution was modified and defined as disappearance of ballooning (score=0) with absent or mild lobular inflammation (score=0 or 1) without any stage increase in fibrosis. There was no difference between the elafibranor arms and placebo according to the protocol-defined primary outcome. However, considering the post-hoc definition, the response rate was significantly higher for elafibranor 120 mg than placebo (19% vs 12%; odds ratio (OR)=2.31 [95% CI 1.02-5.24], *P*=.045). There was no significant difference between elafibranor and placebo regarding the histological secondary endpoints. Fibrosis was significant improved only in those patients who cleared NASH (responder patients). Patients treated with elafibranor had improvement in liver enzymes, lipid parameters [triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol], and serum biomarkers of inflammation, steatosis and fibrosis. In diabetic patients, elafibranor improved glucose homeostasis and markers of insulin resistance, such as homoeostasis model of assessment (HOMA). Elafibranor was safe and well tolerated, although some patients had a transient increase in serum creatinine, which was reversible after discontinuation of the drug. Clinical AEs were similar in placebo and elafibranor arms, and there were no deaths nor cardiovascular events as a result of treatment.

Efficacy and safety of elafibranor is being evaluated in an ongoing, phase III, multicentre, randomized, double-blind, placebo-controlled study in patients with NASH (NCT02704403). This study includes 2000 patients with liver biopsy-proven NASH compares elafibranor 120 mg with placebo for 72 weeks to evaluate NASH resolution without worsening of fibrosis, and long-term outcomes such as all-cause mortality, cirrhosis, and liver-related complications. The secondary outcomes to be evaluated include improvement of fibrosis and individual components of NAS, as well as improvement in cardiometabolic and liver markers.

Saroglitazar (ZYH1) is a PPAR agonist with predominant PPARα and moderate PPARγ activity. This drug was developed for the treatment of dyslipidaemia and has favourable effects on glycaemic parameters in T2DM.[29] A phase I clinical trial described the pharmacokinetics (PK) of saroglitazar and showed that it was safe and well tolerated in healthy volunteers.[30] The GLAZED, a phase III randomized clinical trial (NCT02265276) will evaluate the safety and efficacy of saroglitazar plus pioglitazone in NAFLD patients diagnosed by abdominal ultrasound (US) with elevated liver enzymes.

IVA337 is a chemical entity that activates the PPAR α, δ and γ (pan PPAR). Given the antifibrotic and anti-inflammatory effects of PPARs, the rationale behind the use of agonists for all three PPARs is that it is expected to lead to greater improvement in efficacy compared with targeting a single PPAR isoform.[31] This molecule has demonstrated safety and efficacy in suppression of inflammation and fibrosis in systemic sclerosis and skin fibrosis of animal models.[32] Indeed, both anti-inflammatory and antifibrotic properties of IVA337 make it an appealing candidate for further development in fibrotic diseases, such as NASH.

3.3 | Farnesoid x receptor agonists

It is thought that bile acids might modulate several metabolic pathways by targeting the farnesoid X receptor (FXR).[33] This receptor controls glucose and lipid metabolisms through regulation of insulin sensitivity in skeletal muscle and adipose tissue, and regulation of gluconeogenesis and glycogenolysis in the liver, while also decreasing circulating triglycerides.[34] Preclinical studies have described the anti-inflammatory and antifibrotic properties of FXR agonists.[35] There are FXR agonists that are bile acids, such as obethicolic acid (OCA), but also there are synthetic FXR agonists that have a different chemical structure compared to the bile acids.

A semi-synthetic variant of the bile acid analogue, which has the chemical structure 6α-ethyl-chenodeoxycholic acid, known as OCA (INT-747) selectively activates FXR.[36] A phase II, multicentre, double-blind, randomized, placebo-controlled, multiple-dose study evaluated the safety and efficacy of OCA treatment in patients with T2DM and (presumed) NAFLD (NCT00501592).[37] This trial enrolled

a limited sample (n=64) of T2DM patients who were treated for 6 weeks with OCA 50 or 25 mg, or placebo. NAFLD was defined by altered aminotransferases, or fatty liver in abdominal US, or a 5-year prior liver biopsy. The primary outcome was evaluated by a two-step hyperinsulinaemic-euglycaemic clamp procedure performed both before the first and after the last dose of study treatment. Enhanced Liver Fibrosis (ELF) score was used as a biomarker of fibrosis. Most patients had NAFLD defined by abdominal US. The mean percent change in glucose infusion rate (GIR) showed improvement of 28% and 20% with OCA 25 and 50 mg respectively, compared with a decrease of 5.5% in the placebo group (P=.019 and P=.060 respectively). Patients treated with OCA 25 mg had a significant decrease in alanine aminotransferase (ALT) levels (P=.003) and there was a significant decrease in mean ELF score compared with the placebo group (P=.004).

The FLINT trial (NCT01265498) was a large, phase IIb, multicentre, randomized, double-blind, placebo-controlled trial conducted to assess efficacy of OCA in non-cirrhotic and liver biopsy-proven NASH patients.[38] A total of 283 patients were randomized (1:1) to once-daily OCA 25 mg or placebo for 72 weeks. NASH was defined as NAS≥4 [39] in a liver biopsy obtained at least 90 days before randomization. The primary outcome was histological improvement of NASH assessed by NAS reduction in at least two points without worsening of fibrosis. A pre-planned interim analysis, showed a significant improvement on liver histology in the OCA group compared with the placebo group (45% vs 21%; P=.002), which led to early trial termination to avoid unnecessary biopsies. More patients from the OCA group compared with placebo had improvement in fibrosis (P=.004), hepatocellular ballooning (P=.03), steatosis (P=.001) and lobular inflammation (P=.006). On the other hand, NASH resolution was not statistical different between the groups. Significant reduction on ALT and aspartate aminotransferase (AST) levels occurred during treatment in OCA group. By contrast, these patients had a significant increase in alkaline phosphatase levels whereas gamma-glutamyl transferase (GGT) levels decreased. In addition, OCA-treated patients had significant weight loss (2.3 vs 0.0 kg, P=.008) and reduction in systolic blood pressure (P=.05) compared with placebo. There were similar rates of AEs in both groups, mostly (83%) judged to be unrelated to therapy. At the end of treatment, patients treated with OCA had higher fasting serum insulin concentrations and HOMA indicated greater hepatic insulin resistance compared with placebo at week 72. In addition, treatment with OCA was associated with higher concentrations of total serum cholesterol and LDL cholesterol, and a decrease in HDL cholesterol compared with placebo. Pruritus was significantly more frequent in the OCA group than the placebo group (23% vs 6%, P<.0001).

An ongoing, phase III, double-blind, randomized, placebo-controlled, international multicentre study is evaluating the efficacy of OCA in patients with NASH (NCT02548351). A total of 2000 non-cirrhotic patients with liver biopsy-proven NASH were be included in three arms: OCA 10 or 25 mg/day, or placebo for 72 weeks. Improvement of at least one stage of fibrosis without worsening of NASH, NASH resolution with no worsening of liver fibrosis, all-cause mortality and liver-related complications up to 6 years are being evaluated in this study.

GS-9674 is a synthetic FXR agonist that has been shown to significantly reduced serum transaminases, hepatic steatosis and fibrosis in a murine model of NASH.[40] The safety, tolerability and PK of this drug are being evaluated in an ongoing phase I study of 150 healthy volunteers (NCT02654002). This study is being conducted in subjects who are receiving GS-9674 300 mg 600 mg, or placebo for 96 hours. In addition, PK and safety of this molecule is being evaluated in a phase I, open-label, single-dose (10 mg) study in patients with mild-to-severe hepatic impairment (NCT02808312). The primary outcomes of both studies include single- and multiple-dose PK up to 96 hours post-dose, and incidence of AEs up to 30 days after end of treatment.

LJN452 and EDP-305 are examples of other synthetic FXR agonists that are potential candidates for NASH treatment.[41] The safety, tolerability and efficacy of LJN452 are been assessed in primary biliary cirrhosis (NCT02516605) and NASH in ongoing studies. LJN452 is being evaluated in phase II, double-blind, randomized, placebo-controlled trial that is enrolling 250 patients with liver biopsy-proven NASH with elevated transaminases (NCT02855164). Preclinical and phase I clinical studies to evaluate the safety and efficacy of EDP-305 are due to start in the second half of 2016. The gut-restricted FXR agonist, fexaramine (Fex) has been shown to induces enteric fibroblast growth factor 15 (FGF15), leading to alterations in bile acid composition.[42] A study reports that Fex reduced weight gain, systemic inflammation and hepatic glucose production, while enhancing 'browning' of adipose tissue in animal models.[43]

3.4 | Fibroblast growth factor 19 and 21 analogues

Fibroblast Growth Factor 19 (FGF19) is an intestinal hormone that regulates bile acid metabolism, glucose homeostasis and triglyceride regulation.[44] However, overexpression of FGF19 has been shown to promote liver tumour development in transgenic mice.[45] NGM282 is a molecule that has been developed to eliminate the elements of FGF19 signalling that are associated with its tumourigenic properties while retaining signalling activities of the native hormone that direct the regulation of metabolism and bile acid synthesis.[46] A phase II, randomized, double-blind, placebo-controlled, multicentre study is ongoing to evaluate the safety, tolerability and efficacy of NGM282 for 12 weeks in patients with histologically confirmed NASH (NCT02443116). A total of 75 patients were randomized to NGM282 (two doses) or placebo to evaluate change in absolute liver fat content (LFC) (as measured by MRS) from baseline to end of treatment as the primary endpoint.

Fibroblast Growth Factor 21 (FGF21) is a metabolic hormone mainly produced by the liver and expressed by adipocytes and the pancreas.[47] In preclinical studies, recombinant FGF21 demonstrated potent in vivo beneficial effects on glucose and lipid metabolism, insulin sensitivity and body weight.[48] A placebo-controlled, multiple ascending-dose study in overweight/obese subjects with T2DM reported that the long-acting FGF21 analogue, PF-05231023, resulted in a significant decrease in body weight, improved plasma lipoprotein profile and increased adiponectin levels.[49]

3.5 | Stearoyl coenzyme a desaturase 1 inhibitor

Inhibition of stearoyl coenzyme A desaturase 1 (SCD1) activity decreases the synthesis and increases β oxidation of fatty acids, which regulate fatty acid metabolism in the liver.[50] In addition, SCD1 inhibition activates cholesterol efflux by stimulating the adenosine triphosphate-binding cassette transporter A1.[51] Aramchol is a synthetic lipid molecule obtained by conjugating cholic acid (bile acid) and arachidic acid (saturated fatty acid) through a stable amide bond, to achieve up to 83% SCD1 inhibition.[51] This drug has significantly reduced fatty content in experimental animal models [52] and was well tolerated in short-term clinical trials of healthy volunteers.[53]

Safety, PK and efficacy of aramchol was evaluated in a multicentre, randomized, double-blind, placebo-controlled trial (NCT01094158) that included a limited sample (n=60) of patients with proven histology of NAFLD or NASH.[54] Patients with NAS of 0-2 defined NAFLD, and NAS≥3 defined NASH in a liver biopsy performed at least 18 months before randomization to aramchol 300 or 100 mg/day, or placebo for 12 weeks. The primary efficacy endpoint was the difference in LFC, measured by MRS between baseline and the end of treatment. Patients treated with once-daily aramchol 300 mg had a significant reduction in LFC compared with an increase in the placebo group (−12.6% vs +6.4%, *P*=.020). There was no difference between low-dose aramchol (100 mg/day) and placebo in the primary endpoint. In addition, there were no statistically significant differences among the three arms for any of the secondary endpoints, despite a trend for reduction in adiponectin levels in aramchol-treated patients (*P*=.088). In this trial, AEs were mild or moderate, not related to the treatment and similar among groups.

3.6 | Glucagon-like peptide-1 analogue

Glucagon-like peptide-1 (GLP-1) is a gut-derived incretin hormone that induces insulin secretion and reduces glucagon secretion leading to a potent control of serum glucose. In addition, this hormone induces weight loss, suppression of appetite and delayed gastric emptying.[55] Liraglutide was described as a long-acting human GLP-1 analogue that may be used for NASH therapy.[56]

The LEAN trial (NCT01237119) was a phase II, multicentre, double-blind, randomized trial that evaluated the efficacy of liraglutide in the treatment of NASH.[57] A total of 52 participants from UK medical centres were randomized to daily, subcutaneous liraglutide 1.8 mg or placebo for 48 weeks. NASH was defined as a liver biopsy obtained within 6 months of screening with steatosis (>5% hepatocytes), hepatocellular ballooning and lobular inflammation confirmed by two independent pathologists. The primary outcome was NASH resolution—disappearance of hepatocyte ballooning—without worsening of fibrosis (any increase in fibrosis stage using the NASH CRN system). A total of 40% of patients had stage 3 fibrosis and 12% had cirrhosis in liver biopsy. NASH reversal was observed more often in patients treated with liraglutide than placebo (39% vs 9%, *P*=.019). In addition, patients in the liraglutide group showed a trend towards improvement in steatosis and hepatocyte ballooning. However, no differences were seen in lobular inflammation and overall NAS. Liraglutide-treated patients had significantly lower GGT levels and reduction in fibrosis by ELF score compared with placebo. There were no differences in aminotransferases levels in both groups. Gastrointestinal disorders were the most frequent AE in both groups (liraglutide, 81% vs placebo, 65%). Most AEs were mild or moderate, and there were no deaths or cases of pancreatitis or liver failure during the trial.

3.7 | Nor-ursodeoxycholic acid (NORUDCA)

Ursodeoxycholic acid (UDCA) is a bile acid used as a therapeutic agent in primary biliary cholangitis, but has limited efficacy in NASH. NorUDCA is a side-chained shortened derivative of UDCA that has demonstrated improvement in liver injury in mouse models of cholestatic liver and bile duct injury.[58] NorUDCA has specific physicochemical and therapeutic properties distinct from UDCA. NorUDCA enables 'ductular targeting' and induces a bicarbonate-rich hypercholeresis, with cholangioprotective effects. This drug targets the liver and the bile duct system at multifactorial and multicellular levels.[58] A preclinical study in an experimental animal model reported that NorUDCA treatment improved NASH features such as inflammation, glucose tolerance and liver cell injury.[59] NorUDCA was tested in primary sclerosing cholangitis in a phase II randomized clinical trial (NCT01755507) and might be a new option of pharmacological treatment for NASH.

3.8 | Polyunsaturated fatty acids

The diet of NASH patients seems to be richer in saturated fat and cholesterol and poorer in polyunsaturated fatty acids (PUFA) compared with age, gender and BMI matched controls.[60] Therefore, PUFA could be an option for NASH treatment. Experimental studies have shown that diets enriched with omega-3 PUFA increased insulin sensitivity, reduced intrahepatic triglyceride content and ameliorated steatohepatitis.[61] However, randomized clinical trials that evaluated the effect of omega-3 PUFA supplementation on NAFLD in humans have shown conflicting results.[62,63] Thus, it remains premature to recommend omega-3 fatty acids for the specific treatment of NAFLD or NASH.[64]

4 | OXIDATIVE STRESS

4.1 | Vitamin E

Vitamin E is an antioxidant that prevents liver damage from oxygen free radicals.[65] In addition, this vitamin protects against mitochondrial toxicity and blocks intrinsic apoptotic pathways.[66] Vitamin E was also evaluated for NASH treatment in PIVENS trial.[9] The rate of achievement of the primary outcome of thisstudy, described above, was higher in vitamin E-treated patients compared with placebo (43% vs 19%, *P*<.001). In addition, vitamin E significantly improved steatosis (54% vs 31%, *P*=.005), lobular inflammation (54% vs 35%, *P*=.02) and ballooning (50% vs 29%, *P*=.01). The main concerns about long-term vitamin E use are related to its safety and potential adverse events

(AEs). An increased overall mortality,[67] an increased risk of prostate cancer [68] and higher incidence of haemorrhagic stroke [69] have been reported.

4.2 | Apoptosis signal-regulating kinase 1 inhibitor

Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen-activated protein kinase that is involved in transduction of apoptotic signals under oxidative stress conditions.[70] Studies have suggested that inhibition of ASK1 could possibly be used for treatment of cardiometabolic, neurodegenerative diseases and cancer.[71] GS-4997 is an ASK1 inhibitor that was developed for NASH treatment, with two, phase II randomized clinical trials ongoing to evaluate its safety and efficacy. Incidence of AEs has been evaluated in 10 patients with steatosis and liver stiffness (LS)>2.88 kPa (measured by MRS) treated with GS-4997 for 12 weeks (NCT02781584). The second study is assessing 24 weeks of GS-4997 (stratified by dose: 6 vs 18 mg) with or without simtuzumab (an antifibrotic) in 72 NASH-biopsied and non-cirrhotic patients (NCT02466516).

4.3 | Vascular adhesion protein-1 inhibitor

Vascular adhesion protein-1 (VAP-1) is an enzyme located on endothelial cells, adipocytes and smooth muscle cells that promotes leucocyte recruitment to the liver, leading to inflammation, fibrosis and cirrhosis driven by oxidative stress.[72] PXS-4728A is a very selective inhibitor of VAP-1 with nanomolar potency and good oral bioavailability. A phase I, double-blind, randomized trial reported the safety and efficacy (inhibition of VAP-1 activity) of PXS-4728A in healthy volunteers.[73] Therefore, PXS-4728A may act as an anti-inflammatory and antifibrotic drug in NASH by reducing the migration of leucocytes and lymphocytes to the site of inflammation and diminishing oxidative stress.

5 | INFLAMMATION

5.1 | C-C chemokine receptor types 2 and 5 antagonists

The C-C chemokine receptor types 2 (CCR2) and 5 (CCR5) and their respective ligands [chemokine (C-C motif) ligand 2/monocyte chemoattractant protein 1 (CCL2/MCP-1) and chemokine (C-C motif) ligand 5/regulated on activation, normal T cell expressed and secreted (CCL5/RANTES)] mediate interactions that lead to liver inflammation and fibrosis, and should be considered as potential targets for NASH treatment.[74] The activation of these receptors promotes recruitment and migration of monocytes to the liver, which maturate into pro-inflammatory macrophages leading to activation of Kupffer cells, hepatic stellates cells, collagenous production and fibrogenesis.[75] Cenicriviroc (CVC) is a potent CCR2/CCR5 antagonist that has demonstrated anti-inflammatory and antifibrotic activity in animal models.[76] Treatment with CVC led to an improvement of serum biomarkers of liver fibrosis, such asAspartate-to-Platelet Ratio Index (APRI) and Fibrosis-4 score (FIB-4),that correlated with a decrease in a surrogate marker of monocyte activation (sCD14).[77] A phase I, open-label, non-randomized, single-centre study has described the safety and PK of once-daily CVC 150 mg during 14 days in a small sample of patients with mild or moderate hepatic impairment and healthy controls.[76]

Two, phase II, randomized, placebo-controlled clinical trials have been running to evaluate the efficacy and safety of CVC (CCR2/CCR5 antagonist) in NAFLD and NASH patients. The ORION trial (NCT02330549) are including 45 patients with liver biopsy-proven NAFLD and pre-diabetes or T2DM randomized to CVC 150 mg/day or placebo for 24 weeks. Changes in insulin sensitivity from baseline to end of treatment is the primary outcome of this trial. In addition, the CENTAUR trial (NCT02217475), an international, phase IIb study, are enrolling 289 non-cirrhotic liver biopsy-proven NASH patients to assess hepatic histological improvement in NAS after 1 year of CVC treatment compared with placebo (primary endpoint).[78] Improvement in NAS was defined by a minimum two-point decrease in score, with at least a one-point improvement in more than one individual component of NAS. In addition, no fibrosis worsening must be present to characterize the improvement in NAS. Complete resolution of NASH without fibrosis worsening, change in collagen proportionate area in CVC vs placebo arms; and change from baseline to end of treatment of anthropometric measures, metabolic features and non-invasive imaging methods, as well as biomarkers of inflammation and fibrosis will be evaluated as secondary endpoints.

5.2 | Dipeptidyl peptidase-4 inhibitor

The selective dipeptidyl peptidase-4 (DPP-4; CD26 antigen) inhibitor controls glucose levels by preventing the breakdown of the incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and GLP-1. Sitagliptin, a DPP-4 inhibithor, was not significantly better than placebo in reducing liver fat measured by MRS in 50 NAFLD patients with prediabetes or early diabetes randomized to sitagliptin orally 100 mg/day or placebo for 24 weeks (NCT01963845).[79] Evogliptin Suganon (Dong-A ST; Seoul, Korea) is an orally bioavailable DPP-4that has recently been approved for the treatment of T2DM.[80] Pharmaceutical companies are developing a NASH treatment of evogliptin in combination with CVC. The main aim of this potential fixed-dose combination tablet will be to achieve potent DPP-4 inhibition complemented by the anti-inflammatory action of CVC.

5.3 | Venlafaxine -103

Venlafaxine-103 (VLX-103) is an oral form of pentamidine that exhibits hepatoprotective activity by reducing serum concentration of proinflammatory cytokines, such as tumour necrosis factor (TNF).[81] VLX-103 has markedly decreased hepatic steatosis, hepatocyte injury and cell death, and inflammation in experimental models for alcoholic liver disease.[82] The hepatic uptake, PK, safety and tolerability of VLX103 has been evaluated in a phase I clinical study. There is a randomized, double-blind, placebo-controlled, sequential-group study being conducted in patients with HCC to evaluate the liver

concentration of VLX103 over three days after oral administration at different doses, measured in liver biopsies obtained during thermal ablation or partial hepatectomy procedures (NCT02210182). Further phase II trials need to be implemented for evaluation of this molecule in NAFLD/NASH patients.

6 | APOPTOSIS

6.1 | Caspase inhibitor

Caspases are enzymes that play a central role in liver apoptosis and inflammation. Caspase-mediated apoptosis is driven by the enzymatic action of caspase 3 and 7 on a wide variety of cellular substrates.[83] Inhibition of caspases may therefore reduce the disease-driven loss of hepatocytes and production of apoptotic bodies and microparticles that promote disease progression in NASH. Emricasan (IDN-6556) is a potent irreversible pan-caspase inhibitor that may be used for NASH treatment.[84] A phase I study showed that a single dose (50 mg) of emricasan was safe and reduced biomarkers of inflammation in patients with severe hepatic impairment.[85] However, emricasan did not affect serum caspase enzymatic activity in healthy subjects (NCT02121860). A phase II, randomized, placebo-controlled study is ongoing to evaluate the safety and efficacy of twice-daily emricasan (5 and 50 mg vs placebo) for 72 weeks in 330 non-cirrhotic patients with liver biopsy-proven NASH and NAS≥4 (NCT02686762). The primary endpoint of this trial is fibrosis improvement by at least one stage without worsening of steatohepatitis.

6.2 | Pentoxifylline

Pentoxifylline is a xanthine derivative that inhibits TNF- alpha which is a proinflammatory cytokine that promotes necroinflammation, fibrogenesis, hepatic insulinresistance and apoptosis.[86] Randomized placebo-controlled trials with limited sample size of patients with biopsy proven NASH that evaluated the efficacy of pentoxifylline vs placebo described conflicting results.[87,88] Larger and well-designed, randomized placebo-controlled trials are still needed to confirm the benefit of NASH treatment by this drug.

7 | FIBROSIS

7.1 | Lysyl oxidase and lysyl oxidase like inhibitor

Lysyl oxidase and lysyl oxidase-like (LOXL) are enzymes secreted and expressed by fibrogenetic cells.[89] LOXL protein-2 (LOXL2) is upregulated in hepatocytes, which regulates fibroblast activation, transforming growth factor beta (TGF-β) signalling and latent TGF-β activation leading to liver collagen deposition and, consequently, progression of fibrosis.[90] Decreases in liver and lung fibrosis were observed in an experimental study that evaluated inhibition of LOXL2 with a monoclonal antibody, called simtuzumab.[91] Simtuzumab (GS-6624), a LOXL2 inhibitor, is a humanized monoclonal immunoglobulin G4 (IgG4) antibody that has a long half-life (10-20 days) and can be administered subcutaneously to address liver fibrosis in NASH.

Two, phase II, international, multicentre, randomized, double-blind, placebo-controlled trials are evaluating the safety and efficacy of simtuzumab in non-cirrhotic and cirrhotic patients with NASH. Firstly, a randomized clinical trial (NCT01672866) of 222 liver biopsy-proven NASH patients without cirrhosis (Ishak stages 3 or 4) is ongoing, and will assess change in collagen proportionate area with simtuzumab 45 and 125 mg vs placebo over 96 weeks. A second trial (NCT01672879) will assess change in hepatic venous pressure gradient (HVPG) after 240 weeks of simtuzumab 200 or 400 mg vs placebo in 259 liver biopsy-proven NASH and cirrhotic patients (Ishak stages≥5).

7.2 | Galectin-3 protein inhibitor

GR-MD-02 is a proprietary polysaccharide pharmaceutical preparation that inhibits galectin-3 protein, and acts as an antifibrotic drug in lung, kidney and liver diseases. In experimental models, GR-MD-02 treatment resulted in a marked improvement in liver histology with significant reduction in NASH activity and collagen deposition, suggesting that galectin-targeting drugs may have potential in human NASH with fibrosis.[92] Efficacy (HVPG reduction) of GR-MD-02 (2 vs 8 mg/kg every other week vs placebo for 52 weeks) is being evaluated in 156 cirrhotic NASH patients who are participating in a phase II randomized, placebo-controlled clinical trial (NCT02462967).

8 | CONCLUSIONS

NAFLD remains the most prevalent chronic liver disease worldwide. Changes in dietary habits and lifestyle have been recommended as standard of care for NAFLD, however, this behavioural strategy tends to fail in most patients. Several new molecules have been developed and evaluated in randomized clinical trials for the treatment of NASH. These drugs target a variety of different pathways, such as metabolic homeostasis, inflammation, oxidative stress and fibrosis. Despite a great heterogeneity among primary endpoints,most of the drugs being investigated can be administered orally and, so far, appear well tolerated, with satisfactory efficacy for improvement of histological findings and metabolic features. Currently, several phase IIb and III trials with new molecules are ongoing. Preliminary results of randomized clinical trials pave the way for address NASH treatment in the next years.

CONFLICT OF INTEREST

HP: this author has no conflicts of interest. JFD: Advisory committees: Abbvie, Bayer, BMS, Genfit, Gilead Science, Intercept, Merck, Novartis, Sillagen. Speaking and teaching: Abbvie, Bayer, Gilead Science. Unrestricted research grant: Bayer.

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