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# New Drugs on the Block—Emerging Treatments for Nonalcoholic Steatohepatitis

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## Abstract

Patients with nonalcoholic steatohepatitis (NASH) are at higher risk of progression to advanced stages of fibrosis, cirrhosis, hepatocellular carcinoma and other end-stage liver disease complications. When addressing treatment of NASH, we have limited approved options, and the mainstay of therapy is lifestyle intervention. Extensive research and revelation in the field of pathogenesis of NASH has offered new possibilities of treatment and emerging new drugs that are being tested currently in numerous preclinical and clinical trials. These drugs target almost all steps in the pathogenesis of NASH to improve insulin sensitivity, glucose and lipid metabolism, to inhibit *de novo* lipogenesis and delivery of lipids to the liver, and to influence apoptosis, inflammation and fibrogenesis. Although NASH is a multifactorial disease, in the future we could identify the predominating pathological mechanism and, by choosing the most appropriate specific medication, tailor the treatment for every patient individually.

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## Introduction

Nonalcoholic steatohepatitis (NASH) and nonalcoholic fatty liver are histologically two distinguishable subtypes of nonalcoholic fatty liver disease (NAFLD). Nonalcoholic fatty liver requires more than 5% fat infiltration of the liver and NASH, alongside fat infiltration, is characterized by inflammation

and hepatocyte injury.<sup>1</sup> Although hepatic steatosis within NAFLD is a widespread disease, with prevalence in some parts of the world up to 40%,<sup>2</sup> NASH is present in only 10% to 20% of individuals with NAFLD, but when accompanied by significant fibrosis is associated with increased overall mortality, primarily from cardiovascular diseases.<sup>1</sup> Population projection models estimate that 3% to 6% of adults have NASH, and according to current trends, the prevalence of NASH is expected to rise by 15% to 56% until 2030.<sup>3,4</sup>

Patients with NASH have a higher risk of progression to advanced stages of fibrosis, cirrhosis, hepatocellular carcinoma and other end-stage liver disease complications.<sup>5</sup> At the time of NASH diagnosis about 25% of patients have a moderate to severe stage of fibrosis (F>2), and in around 40% of NASH patients fibrosis will progress at a rate of 1 stage per 10 years.<sup>6</sup> Although often clinically silent, more than 20% of patients with NASH will develop end-stage liver disease over their lifetime.<sup>7,8</sup> A meta-analysis of 86 studies and more than 8 million patients from 22 countries has shown that in comparison with NAFLD, NASH has greater overall mortality (11.77 to 0.77 per 1,000 person-years) and liver related mortality (25.56 to 15.44 per 1,000 person-years).<sup>6</sup>

When addressing treatment of NASH, we have limited approved options, and the mainstay of therapy is lifestyle intervention, including changes in diet and exercise regimens, with an emphasis on weight reduction of more than 7%.<sup>1,9,10</sup>

Several drugs have been proposed for treating NASH, but according to the guidelines of European and American societies, metformin (an insulin sensitizer) is not recommended because it showed no effect on liver histology although it has a beneficial effect on insulin resistance and alanine aminotransferase (ALT) levels.<sup>1,11</sup> Pioglitazone, a thiazolidinedione and intra-nuclear peroxisome proliferator-activated receptor (PPAR)  $\gamma$  agonist that is in use for diabetes mellitus treatment, was evaluated in several trials for the treatment of NASH. In the PIVENS randomized controlled trial, patients with biopsy-proven NASH without diabetes mellitus received vitamin E (800 IU/day) and pioglitazone (30 mg/day) for 96 weeks and were compared with patients who received placebo.<sup>12</sup> The therapy with vitamin E was associated with amelioration of NASH (43% vs. 19%); however, when compared with the placebo, the pioglitazone therapy was not that successful (34% and 19%).<sup>12</sup> Both therapies were associated with reduction of hepatic steatosis, inflammation and liver laboratory tests but without an improvement in fibrosis scores.<sup>12</sup> Pioglitazone is, however, associated with substantial side-effects, such as weight gain, fluid retention, heart failure and bone loss, so the American Association for the Study of Liver Diseases 2018 Guidelines state that pioglitazone may be used in biopsy-proven NASH

**Keywords:** Fatty liver; Steatohepatitis; Nonalcoholic fatty liver disease; Treatment.

**Abbreviations:** ALT, alanine aminotransferase; ASK-1, apoptosis signal-regulating kinase 1; BMI, body mass index; CCR, C-C chemokine receptor type; DPP4, dipeptidyl peptidase 4; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; MAFLD, metabolic-associated fatty liver disease; MR, magnetic resonance; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; NF $\kappa$ B, nuclear factor kappa B; PPAR, peroxisome proliferator-activated receptor; SGLT, sodium-glucose co-transporter.

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**Table 1. New drugs in phase 2 and phase 3 clinical trials**

Agent	Mechanism of action	Treatment/intervention	Patients	Phase	ClinicalTrials.gov identifier
Elafibranor	Dual PPAR $\alpha$ and PPAR $\delta$ agonist	Elafibranor (120 mg) vs. placebo	NASH (NAS>4) and F2/F3	3	NCT02704403
Saroglitazar	Dual PPAR $\alpha$ and PPAR $\gamma$ agonist	Saroglitazar (1,2 or 4 mg) vs. placebo	NASH and/or NAFLD (biopsy or noninvasive)	2	NCT03061721
Lanifibranor	pan-PPAR agonist	Lanifibranor (800 mg and 1,200 mg) vs. placebo	NASH (biopsy)	2	NCT03008070
Liraglutide	GLP-1 receptor agonist	Liraglutide (0,6–3 mg) vs. exercise + diet vs. bariatric surgery	NASH (biopsy or noninvasive)	3	NCT02654665
Semaglutide	GLP-1 receptor agonist	Semaglutide (0.1, 0.2, 0.4 mg) vs. placebo	NASH (biopsy)	2	NCT02970942
Dapagliflozin	SGLT inhibitor	Dapagliflozin (10 mg) vs. placebo	NASH and DM (biopsy)	3	NCT03723252
Pegbelfermin	FGF21 analog	Pegbelfermin (3 doses) vs. placebo	NASH and F3 (biopsy)	2	NCT03486899
Cenicriviroc	CCR2/CCR5 antagonist	Cenicriviroc (150 mg) vs. placebo	NASH and F2/F3 (biopsy)	3	NCT03028740
Tropifexor	FXR agonist	Tropifexor monotherapy vs. combination with cenicriviroc	NASH with F2/F3 (biopsy)	2	NCT03517540
Resmetirom	Thyroid hormone receptor $\beta$ agonist	Resmetirom (80 mg, 100 mg) vs. placebo	NASH (NAS>4)	3	NCT03900429
Firsocostat	Acetyl-CoA carboxylase inhibitor	Firsocostat vs. fenofibrate, cilofexor, selonsertib	NASH (F2/F3 and F4/cirrhosis)	2	NCT02781584, NCT03449446

Abbreviations: CCR2/CCR5, C-C chemokine ligand types 2 and 5; DM, diabetes mellitus; FGF21, fibroblast growth factor-21; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; SGLT, sodium-glucose co-transporter.

patients after discussing the risks and benefits.<sup>1</sup> Vitamin E has been associated with increased incidence of intracranial bleeding and prostate cancer; however, NASH patients who could have benefited from vitamin E were not included in the study nor did the study take into account other confounding factors (smoking, supplements).<sup>13</sup> Orlistat, as well as lipid lowering agents used in treating hyperlipidemia, have been used in patients with NASH, and although their use is safe, the outcomes of their effect on treating NASH are inconclusive.<sup>1,11</sup>

As a result of better understanding the underlying processes in the development of NAFLD and NASH, as well as a long withstanding opinion that the nomenclature for NAFLD and NASH doesn't suffice, an international working group represented by Eslam *et al.*<sup>14</sup> proposed a change in nomenclature to metabolic-associated fatty liver disease or "MAFLD".<sup>14</sup> This change would, in their opinion, better depict the character of this disease and patients that suffer from it, and alienate the labeling alcoholic burden. This could also propose a change in the study design of pre-clinical and clinical trials that would take into consideration the specific heterogeneity of the pathological pathways and specific characteristics of the patients and thus give more reliable results for the studied drugs.<sup>14</sup>

Extensive research and revelation in the field of pathogenesis of NASH has offered new possibilities of treatment and emerging new drugs that are being tested now in numerous preclinical and clinical trials (Table 1). These drugs target almost all steps in the pathogenesis of NAFLD and NASH, aiming to improve insulin resistance, glucose and lipid metabolism, to inhibit lipogenesis and delivery of lipids to the liver, and to influence apoptosis, inflammation and fibrogenesis.<sup>15</sup> Figure 1 depicts the proposed mechanisms of action of these agents in the pathogenesis of NAFLD and NASH.

Intestinal microbiome and associated dysbiosis has

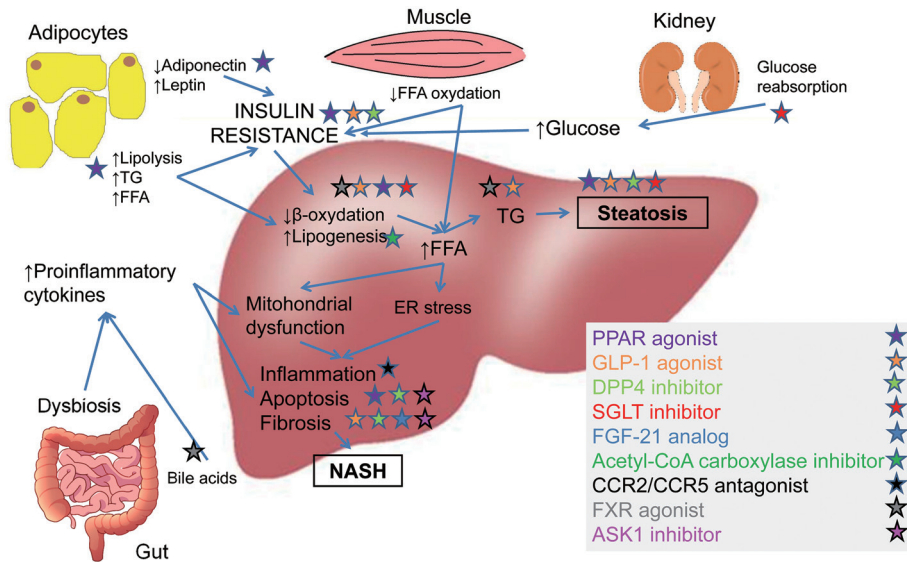
emerged as an important factor that indirectly, through intestinal permeability and bacterial metabolites, influences several mechanisms responsible for NASH progression. The therapeutic options that could influence these steps are depicted in Figure 2.

### Drugs improving insulin sensitivity and modulating glucose and lipid metabolism

#### PPARs

PPARs are nuclear receptors that regulate metabolic homeostasis, cell differentiation and immune-inflammation.<sup>16</sup> We distinguish several PPAR intracellular receptor subtypes, as they exert different distribution and actions in different tissues.<sup>16</sup> The first extensively studied PPAR $\gamma$  agonist, pioglitazone, already mentioned in the introduction, modulates glucose uptake, insulin signaling, fatty acid uptake, triglyceride synthesis and hydrolysis, as well as inflammation and maturation of macrophages.<sup>16</sup>

PPAR $\alpha$  is most extensively pronounced in the liver and exerts its regulatory roles through fatty acid transport and  $\beta$ -oxidation to deliver lipids in the liver, and has been shown to decrease triglycerides and increase high-density lipoprotein cholesterol in serum.<sup>17</sup> It also influences inflammation through nuclear factor-kappa B (NF $\kappa$ B) action modulation and reduces the expression of acute-phase genes.<sup>17</sup> PPAR $\gamma$  is mostly expressed in the adipose tissue and controls lipogenesis, adipocyte differentiation, and glucose metabolism.<sup>18</sup> By promoting the storage of fatty acids, such as triglycerides, PPAR $\gamma$  acts as an insulin sensitizer and prevents ectopic fat accumulation.<sup>18,19</sup> Maeda *et al.*<sup>20</sup> showed that PPAR $\gamma$  agonists caused a significant rise in plasma adiponectin concentrations. Adiponectin is a protein derived



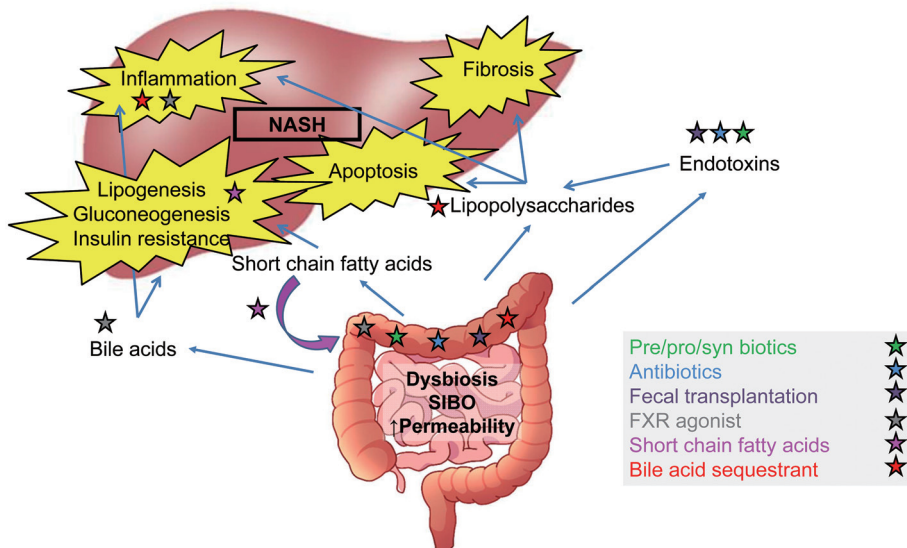
**Fig. 1. Proposed mechanisms of action of new agents for treatment of NASH.** Abbreviations: ASK-1, apoptosis signal-regulating kinase 1; CCR2/CCR5, C-C chemokine ligand types 2 and 5; DPP4, dipeptidyl peptidase 4; ER, endoplasmic reticulum; FFA, free fatty acids; FGF21, fibroblast growth factor-21; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator-activated receptor; SGLT, sodium-glucose co-transporter; TG, triglycerides.

from fat tissue that possesses anti-atherogenic properties and suppresses cytokine production from macrophages and expression of adhesion molecules in vascular endothelial cells. PPAR $\delta$  agonists produce similar effects as PPAR $\alpha$  on liver lipid metabolism, exhibit a positive influence on insulin sensitivity, and promote an alternative activation effect on macrophages and Kupffer cells that leads to attenuation of tissue inflammation.<sup>21,22</sup>

Elafibranor is a dual PPAR $\alpha$  and PPAR $\delta$  agonist, that was investigated in a phase II multicenter, randomized placebo-controlled study at two dose regimes (80 mg and 120 mg once a day versus placebo) over 52 weeks in biopsy-proven NASH patients.<sup>23</sup> Treatment with 120 mg of elafibranor reduced liver enzymes, lipid values, glucose profiles,

and markers of systemic inflammation in comparison with placebo, and there was a statistically significant amelioration of NASH activity without aggravation of fibrosis.<sup>23</sup> Furthermore, the patients that experienced NASH regression had a significant decline in fibrosis stage compared to those without NASH regression.<sup>23</sup> Elafibranor, although, was well-tolerated and caused an increase in serum creatinine level that was reversible.<sup>23</sup> A phase III multicenter study (RESOLVE-IT) on elafibranor (120 mg per day versus placebo) is ongoing and planned to enroll 2,000 patients with liver biopsy-proven NASH (NAFLD activity score (NAS) of >4) and F2-3 fibrosis (NCT02704403).<sup>24</sup>

Saroglitazar is a dual PPAR $\alpha$  and PPAR $\gamma$  agonist that exhibits a predominant PPAR $\alpha$  effect with a moderate PPAR $\gamma$



**Fig. 2. Proposed mechanism of action of treatments for NASH targeting microbiome changes.** Abbreviations: NASH, nonalcoholic steatohepatitis; FXR, farnesoid X receptor; SIBO, small intestinal bacterial overgrowth.

effect; as such, it provides a positive effect on lipid metabolism and insulin sensitivity without the side effects caused by PPAR $\gamma$  activation.<sup>25</sup> Since it has a nonrenal route of elimination, it has been shown to be safe in patients with deteriorated renal function.<sup>25</sup> A review of 18 selected studies on patients with diabetic dyslipidemia that were treated with saroglitazar 4 mg once daily for at least 12 weeks showed a consistent mean regression in lipid levels and glycosylated hemoglobin levels with an increase in mean high-density lipoprotein cholesterol levels from baseline as well as an incline in ALT levels and fatty liver (evaluated by FibroScan™) in NAFLD patients with diabetic dyslipidemia.<sup>26</sup> There is an ongoing phase II study on saroglitazar on NASH/NAFLD patients that will investigate the safety of treatment and impact on serum ALT levels (NCT03061721).<sup>27</sup>

Lanifibranor is a next-generation pan-PPAR agonist that in a NAFLD mouse model improved insulin resistance and steatohepatitis (biopsy-assessed hepatic steatosis, inflammation, ballooning and fibrosis), that combines and exceeds specific effects of the single PPAR agonists.<sup>18</sup> Currently, efficacy and the safety of two doses (800 mg and 1,200 mg) of lanifibranor per day for 24 weeks is being evaluated in a phase II study versus placebo in adult NASH patients with moderate to severe necroinflammation without cirrhosis (NCT03008070).

### **Glucagon-like peptide receptor agonists and dipeptidyl peptidase 4 inhibitors**

Glucagon-like peptide-1 (GLP-1), recognized as physiologic incretin, is a hormone secreted from the distal ileum and colon that increases insulin synthesis and secretion, decreases glucagon secretion, decreases hepatic gluconeogenesis, suppresses appetite, and delays gastric emptying.<sup>28</sup> It has been shown that hepatocytes express GLP-1 receptors and that GLP-1 agonists reduce steatosis and influence lipid metabolism by decreasing lipogenesis and increasing oxidation of fatty acids.<sup>29</sup>

Liraglutide, a first class GLP-1 receptor agonist, was studied on a hepatic stellate cell (rat and human) model, and it was found that liraglutide markedly improved the stellate cell phenotype and diminished cell proliferation.<sup>30</sup> Rats with cirrhosis treated with liraglutide had lower portal pressure, lower intrahepatic vascular resistance, and significant improvement in fibrosis and endothelial function.<sup>30</sup> These antifibrotic effects of liraglutide therapy were also recorded in human liver and the proposed mechanism is an GLP1-R-independent and NF $\kappa$ B-Sox9-dependent one.<sup>30</sup> Furthermore, metabolic and hepatic beneficiary effects of liraglutide were studied in an obese NASH mouse model, and found reduced body weight, reduced hepatic steatosis, and reduced collagen 1a1 and galectin-3 content.<sup>31</sup> In a randomized, phase 2 study, liraglutide was compared to placebo in obese, biopsy-confirmed NASH patients.<sup>31</sup> After the treatment with liraglutide, 40% of patients had NASH resolution compared with 9% in the placebo group. Only 9% of patients on liraglutide versus 36% of patients on placebo had progression of fibrosis.<sup>32</sup> Results also showed improvement in the metabolic risk factors such as weight, glucose and high-density lipoprotein cholesterol levels; the main reported adverse events were mild to moderate, and included diarrhea, constipation and loss of appetite.<sup>32</sup> Currently ongoing is a phase 3 study that is comparing effects of liraglutide and bariatric surgery on anthropometric measures, liver function, insulin resistance, endothelial function and biomarkers of NASH (CGH-LiNASH, NCT02654665). A recently published study on 30 obese NAFLD patients showed that liraglutide administered at 3 mg daily for 26 weeks, followed by 26 weeks of only weight gain prevention, compared with diet and exercise

modifications only, had significant reductions in weight, liver fat fraction (measured by magnetic resonance imaging), serum ALT and caspase-cleaved cytokeratin-18 at 26 weeks.<sup>33</sup> However, those benefits were not sustained after discontinuation of treatment, in contrast with effects of lifestyle modification.<sup>33</sup>

Exenatide, also a GLP-1 receptor agonist, in a NASH mouse model showed an improvement of mitochondrial tricarboxylic acid cycle flux after a 8-week treatment, a significant decrease in insulin resistance, steatosis, hepatocyte lipotoxicity and hepatic triglyceride content as well as lower expression of hepatic lipogenic genes (Srebp1C, Cd36) and genes involved in inflammation and fibrosis.<sup>34</sup> Exenatide treatment for 12 weeks (5 mg twice daily for 4 weeks then 10 mg twice daily for 8 weeks) combined with insulin glargine in diabetic, obese, NAFLD patients was associated with a greater reduction of body weight, waist circumference, liver fat (appraised by ultrasound) and liver enzymes than with intensive insulin therapy (93% vs. 67%); at the end of treatment, up to 43% of patients had no liver steatosis.<sup>35</sup> The most common side effects were similar to liraglutide, and were found in up to 40% of patients.<sup>35</sup> A relatively small open-label study on eight patients with diabetes mellitus and biopsy-proven NASH found that 28 weeks of exenatide treatment made no significant difference in liver histology, and only three of the eight subjects did meet the primary end point of improved histopathology, with 1 to 2 point fibrosis improvement seen in four subjects and fibrosis worsening by 1 point in one subject and staying the same in three subjects.<sup>36</sup> More studies on exenatide are needed to draw firmer conclusions on its role in treating NASH patients.

Semaglutide, a novel GLP-1 receptor agonist, was investigated in a recently completed phase 2 placebo-controlled trial comparing the efficacy and safety of different doses in 320 NASH patients and the results are awaited (NCT02970942). Since NASH patients have a greater risk of cardiovascular mortality, semaglutide could have potential benefit compared to other GLP-1 receptor agonists, since it was shown to be able to prevent cardiovascular events as well as reduce body mass and ALT level.<sup>37,38</sup>

Dipeptidyl peptidase 4 (DPP4) inhibitors exert their effect by blocking the enzyme DPP4, which is involved in the degradation of GLP-1 and other incretins.<sup>28</sup> Serum DPP4 levels are elevated in NASH patients and correlate well with the histopathological severity of NASH. DPP4 levels are also positively associated with liver fibrosis and hepatocyte apoptosis.<sup>28</sup> Sitagliptine, an DPP4 inhibitor, prevented infiltration of adipose tissue by CD8(+) T-cells and M1 macrophages, decreased PAI-1 expression, and had a positive effect on liver lipid metabolism and liver fat infiltration.<sup>39</sup> It was also shown in a mouse model that sitagliptine could prevent the progression of hepatocellular carcinoma related to NASH.<sup>40</sup> However, a relatively small study on biopsy-proven NASH patients after 24 weeks of sitagliptine (100 mg) showed no improvement of fibrosis or NAS versus placebo.<sup>41</sup> Conflicting results were found in larger studies on biopsy-confirmed NASH patients, where the same dose of sitagliptine (100 mg) given for 1 year improved NAS by ameliorating steatosis and ballooning, regardless of diabetic state,<sup>42</sup> and in another 24-week administration trial showed no superiority compared to placebo in reducing liver fat infiltration in prediabetic patients with NAFLD or those with diabetes mellitus and NAFLD.<sup>43</sup> Linagliptine, another DPP4 inhibitor, was shown to have both anti-inflammatory and antisteatotic activity in NASH.<sup>44</sup> It was evaluated in a NASH mouse model as well, in combination with empagliflozine, a sodium-glucose co-transporter (SGLT)-2 inhibitor. A combination of linagliptine and empagliflozine ameliorated NASH with a stronger anti-fibrotic effect.<sup>45</sup> There are still no human studies being conducted

to verify the safety and utility of linagliptine in the treatment of NASH.

### **SGLT inhibitors**

SGLT inhibitors act by reducing glucose reabsorption in the proximal tubule in the kidney, leading to glucosuria and plasma glucose reduction.<sup>46</sup> They have been used in treating diabetes mellitus, but as several mouse model studies showed beneficial effect on liver function and prevention of fibrosis associated with NASH, it has become an interesting option for treating NASH in humans.<sup>47,48</sup> Ipragliflozin, a SGLT2 inhibitor was investigated in a NASH mouse model, where ipragliflozin had a positive effect on free fatty acid serum concentration, liver lipid metabolism, reduced apoptosis and fibrosis.<sup>46</sup> The same results were confirmed in a similar study using a mouse NASH model and 4-week therapy, in which ipragliflozin improved glucose metabolism, reduced insulin resistance, and improved liver steatosis and fibrosis by reducing inflammation and oxidative stress in the liver.<sup>48</sup> In humans, in patients with diabetes mellitus type 2 and NAFLD, ipragliflozin reduced liver fat (as estimated indirectly by calculating liver fat index).<sup>49</sup> A Japanese study retrospectively included 130 diabetes mellitus patients with proven NASH, and selected patients with altered liver enzymes when adding ipragliflozin to their DPP-4 inhibitor or GLP-1 receptor antagonist therapy, and found after treatment significantly decreased ALT and the Fibrosis-4 score.<sup>50</sup>

In a randomized, active-controlled, open-label trial on 57 patients with diabetes mellitus type 2 and NAFLD who were treated with dapagliflozin (5 mg/d) for 24 weeks or placebo, hepatic steatosis and stiffness were assessed noninvasively (by transient elastography and controlled attenuation parameter).<sup>51</sup> Based on their findings, dapagliflozin improved liver steatosis in diabetes mellitus type 2 and NAFLD patients, and ameliorated liver fibrosis only in patients with significant liver fibrosis assessed non-invasively by transient elastography.<sup>51</sup> In a randomized placebo-controlled double-blind multicenter study on participants with diabetes mellitus type 2 and NAFLD, dapagliflozin monotherapy reduced liver serum markers, cytokeratin (CK) 18-M30 and CK 18-M65, and plasma fibroblast growth factor (FGF)-21.<sup>52</sup> A phase 3 study on the histological efficacy and safety of dapagliflozin in NASH patients is ongoing (NCT03723252).

The effect of empagliflozin on liver steatosis in type 2 diabetes mellitus patients and NAFLD was the focus of an investigator-initiated, prospective, open-label, randomized clinical study to examine the effect of 10 mg of empagliflozin per day when included in the treatment of type 2 diabetes mellitus versus standard treatment without empagliflozin.<sup>53</sup> Hepatic liver fat was measured by magnetic resonance (MR) imaging proton density fat fraction, and found that the empagliflozin group had a significant reduction of liver fat and ALT levels.<sup>53</sup> A multicenter study on patients with diabetes mellitus type 2 to evaluate the impact of empagliflozin (25 mg daily) or placebo for 24 weeks on lipid content, liver energy metabolism and body composition evaluated by 1H magnetic resonance (MR) spectroscopy was recently completed, and results are awaited (NCT02637973).

### **FGF21 analog (pegbelfermin)**

FGF21 is a regulator of energy metabolism and in a study on patients with NAFLD (defined by MR proton spectroscopy) and NASH (defined by biopsy), plasma FGF21 levels were higher in patients with NASH compared to those without NASH or NAFLD. Plasma FGF21 levels correlated positively

with the stage of necroinflammation ( $p=0.02$ ) and fibrosis ( $p<0.001$ ) but not with steatosis ( $p=0.60$ ).<sup>54</sup> Endogenous FGF21 has a short half-life of 1 to 2 hours, but various modification strategies have been used to create longer acting FGF21 analogues.<sup>55,56</sup> Pegbelfermin is a recombinant analog of human FGF21 that was evaluated in a multicenter randomized, double-blind, placebo-controlled study in biopsy-proven NASH in overweight adults (body mass index of  $>25$  kg/m<sup>2</sup>) in subcutaneous two dose group administration (10 mg daily and 20 mg weekly).<sup>56</sup> After 16 weeks of treatment, patients in both groups showed a significant amelioration of steatosis (assessed by MR spectroscopy), levels of noninvasive fibrosis biomarker (N-terminal type III propeptide), and amelioration of liver stiffness and transaminase levels.<sup>56</sup> A phase 2b randomized, double-blind, placebo-controlled study evaluating the safety and efficacy of pegbelfermin in patients with NASH and stage 3 fibrosis is active but not recruiting any new patients. The primary outcome was determined as achievement of  $\geq 1$  stage amelioration in fibrosis without progression of NASH or NASH improvement with no progression of fibrosis (as determined by liver biopsy) (NCT03486899). Results are awaited.

### **Statins**

Although statins have been extensively used in treating cardiovascular diseases, their use has been widely underestimated in treating NASH, probably due the common misinterpretation that statins damage the liver, as seen in elevation of liver enzymes during treatment. In a multicenter cohort of 1,201 European individuals who underwent liver biopsy for suspected NASH, statin use was recorded in 107 subjects and was associated with an improvement in liver steatosis, NASH and fibrosis stage F2–F4 development; however, this effect was limited in patients with the I148M PNPLA3 variant.<sup>57</sup>

### **Resmetirom (thyroid hormone receptor $\beta$ -agonist)**

Resmetirom, a thyroid hormone receptor  $\beta$ -agonist has been shown in a mouse model to reduce liver steatosis by targeting dyslipidemia.<sup>58</sup> In a 36-week randomized, double-blind, placebo-controlled multicenter study on adults with biopsy-confirmed NASH (fibrosis stages 1–3) and hepatic fat fraction of at least 10% (assessed by MR proton density fat fraction) resmetirom treatment resulted in improvement in steatosis in NASH patients.<sup>59</sup> Adverse events were mostly mild or moderate, with a higher incidence of transient diarrhea and nausea caused by the resmetirom.<sup>59</sup> A phase 3, multinational study (MAESTRO-NASH) on resmetirom at 80 mg or 100 mg compared to placebo to achieve NASH resolution on liver histology in non-cirrhotic NASH patients with stage 2 or 3 fibrosis is recruiting and expected to end in 2024 (NCT03900429).

### **Firsocostat (acetyl-CoA carboxylase inhibitor)**

Firsocostat is an acetyl-CoA carboxylase inhibitor that targets *de novo* lipogenesis through inhibition of acetyl-CoA to malonyl-CoA conversion. In a phase 2 trial that included 126 patients with NASH and fibrosis, 20 mg daily of firsocostat for 12 weeks showed significant reduction in liver fat by 29%; however, during treatment, an increase in plasma triglyceride levels was recorded, with 16 patients having levels of more than 500 mg/dL.<sup>60</sup> Two phase 2 clinical trials on firsocostat in monotherapy or in combination with other therapies for NASH regarding safety, efficacy and tolerabil-

ity, one on patients with stage 2 and 3 fibrosis and another in participants with bridging fibrosis or compensated cirrhosis are expected to finish soon, and results of these studies are awaited (NCT02781584, NCT03449446).

### Drugs modulating hepatocyte injury, inflammation, apoptosis and fibrosis

Inflammation is a crucial step in NASH pathogenesis, and it has been found that Kupffer cells secrete C-C chemokine ligand types 2 (CCL2) in response to hepatocyte injury which, downstream, leads to monocyte recruitment and influx to the liver, where they mature into proinflammatory macrophages.<sup>61-63</sup> These activated macrophages express proinflammatory cytokines, which in turn activate hepatic stellate cells, promote their survival, and stimulate fibrogenesis.<sup>63</sup> C-C chemokine receptor types 2 (CCR2) and 5 (CCR5), and their ligands, C-C chemokine ligand types 2 (CCL2) and type 5 (CCL5) were found to be up-regulated in NASH.<sup>64</sup>

Cenicriviroc, a dual CCR2/CCR5 antagonist with a long plasma half-life (30–40 h in humans), was studied in a phase 2b study (CENTAUR) in the treatment of NASH and liver fibrosis.<sup>63</sup> The study evaluated efficacy and safety of 150 mg per day of cenicriviroc over 2 years for obtaining improvement in NAS at the first year relative to screening biopsy, without progression of fibrosis.<sup>63</sup> After 1 year of cenicriviroc, two times more patients achieved regression of fibrosis without worsening of steatohepatitis compared with placebo (20% vs. 10%;  $p=0.02$ ).<sup>63</sup> A phase III study to evaluate the efficacy and safety of 150 mg per day of cenicriviroc versus placebo for the treatment of liver fibrosis in adult subjects with NASH and stage 2 or 3 liver fibrosis (AURORA trial) is still recruiting patients and results are awaited (NCT03028740).

### Farnesoid X receptor agonists

Farnesoid X receptor (FXR) is a nuclear receptor expressed in the liver, gallbladder, and intestines. It is a modulator of bile acid, glucose and lipid metabolism.<sup>65-67</sup> In the intestine, FXR modulates FGF15 and FGF19 synthesis and delivery to the liver through the portal circulation.<sup>65</sup> In the liver, FGF15 and FGF19 stimulate glycogen synthesis and suppress gluconeogenesis but also decrease triglyceride accumulation.<sup>66</sup> FXR activation reduces activity of SREBP-1c, a key transcription factor in regulation of triglyceride synthesis.<sup>67</sup> Obeticholic acid, which acts as an FXR agonist, has been evaluated in a phase 2 study on NASH patients without cirrhosis (FLINT trial), which randomized 283 patients to 25 mg of obeticholic acid or placebo for 72 weeks.<sup>68</sup> Although obeticholic acid was successful in the FLINT trial in reducing NAS by 2 points without worsening of fibrosis, a large number of patients developed significant pruritus and experienced a rise in total serum cholesterol and low-density lipoprotein.<sup>68</sup> Since these changes were attributed to suppression of *de novo* bile acid synthesis and an escalation in reverse cholesterol transport, future research was focused on development of FXR agonists that would have better efficacy, safety and tolerability profiles.<sup>69</sup> Tropifexor, a novel FXR agonist, that modulates gene expression in the liver and intestines in low doses with low systemic exposures was found to have a good tolerability profile in healthy volunteers and is now being evaluated in patients with NASH.<sup>69</sup> A combination therapy of tropifexor and cenicriviroc is also being evaluated in a phase 2 study (TANDEM trial) in patients with NASH and liver fibrosis (NCT03517540).

### Selonsertib (apoptosis signal-regulating kinase 1 inhibitor)

Selonsertib is an apoptosis signal-regulating kinase 1 (known as ASK1) inhibitor that is intended to target the p38/JNK pathway, which is activated by TNF $\alpha$  and intracellular oxidative stress, and results in apoptosis and fibrosis.<sup>70</sup> A phase 2 multicenter study of selonsertib, given for 24 weeks once daily in doses of 6 mg and 18 mg, with or without simtuzumab, was undertaken in NASH patients with liver fibrosis (stage 2 or 3).<sup>71</sup> Paired pretreatment and post-treatment liver biopsies and noninvasive diagnostic methods were used to evaluate the efficacy of treatment. After the treatment, a significant number of patients in the 18 mg selonsertib group achieved one or more stage amelioration in fibrosis compared to the 6 mg selonsertib group and simtuzumab alone (43% vs. 30% vs. 20%, respectively).<sup>71</sup> Reduction in fibrosis was associated with reduction in liver stiffness and apoptosis markers measured by noninvasive methods.<sup>71</sup> However, further trials of selonsertib on NASH patients but with stage 3 fibrosis (STELLAR-3) and stage 4 fibrosis/compensated cirrhosis (STELLAR-4) found that 48 week treatment with selonsertib (6 or 18 mg daily dose) had no significant effect on liver serum tests or fibrosis progression evaluated noninvasively.<sup>72</sup>

### Drugs targeting gut microbiome changes

Although the composition of gut microbiome varies among individuals, the prevailing bacterial phyla are *Firmicutes* and *Bacteroidetes*, that make up around 90% of the microbiome, and *Actinobacteria* and *Proteobacteria*. The prevailing bacterial phyla is responsible for a specific metabolite profile that can influence liver and overall metabolism, specifically metabolites such as bile acids, lipopolysaccharides and short-chain fatty acids.<sup>73</sup> It has been shown that the changes in gut microbiome and concentrations of the aforementioned metabolites is important in NAFLD pathogenesis and progression, but a specific microbiome composition in NAFLD has not yet been identified.<sup>74,75</sup> A prospective study on fecal microbiome in adult NAFLD patients, carried out by Loomba et al.<sup>76</sup> on the association between microbiome composition and advanced stages of fibrosis in NAFLD, identified 37 bacterial species that vary depending on the disease stage. However, the authors state that these results could reflect changes within the microbiome that occur with age and that further studies are needed to see if specific microbial species are responsible for the gut-liver crosstalk and progression of NAFLD.<sup>76</sup> Prebiotics, probiotics, and antibiotics have been investigated in animal and human studies, in attempts to modify the microbiome and influence NAFLD. In animal studies, it has been shown that prebiotics and synbiotics can influence gene expression to modify  $\beta$ -oxidation and lipogenesis, thus effecting liver fat infiltration, inflammation, and insulin resistance.<sup>77,78</sup> Results in human studies regarding their use in NAFLD were modest; although, most of these studies were not accompanied by a histological confirmation and had a small population sample.<sup>79,80</sup> A recent meta-analysis on the use of prebiotics, probiotics and synbiotics for NAFLD concluded that prebiotic and probiotic use was associated with a reduction in body mass index (BMI) and modest influence on serum aminotransferase levels and lipid profile, without ameliorating inflammation.<sup>79</sup>

### Antibiotics

Antibiotics have been relatively successfully used in treating

NAFLD. An example is a trial with rifaximin, where a 28-day treatment induced a decrease in BMI, serum aminotransferases, and gamma-glutamyl transferase.<sup>80</sup> Since then, there have been many trials with opposing results, but the antibiotic treatment for NAFLD and obesity is still intriguing, based on the high number of ongoing trials.<sup>81</sup> However, although short-term antibiotic treatment could prove beneficial, long-term and frequent use of antibiotics could cause a much greater problem of antibiotic resistance.

### **Fecal transplantation**

Fecal transplantation, successful in treatment of *Clostridium difficile* infection, has been a promising treatment for microbial dysbiosis in NAFLD. However, recently published results from a randomized controlled trial on 21 patients with NAFLD that underwent autologous and allogenic fecal transplantation, and were followed up until 6 months from the procedure, found that although allogenic transplantation reduced small intestinal permeability it did not influence insulin resistance (measured by HOMA-IR) or hepatic steatosis measured by MR proton density fat fraction.<sup>82</sup>

### **Modification of bacterial metabolites**

A specific microbiome composition is also responsible for various points in bile acid metabolism and circulation in the body. Enterohepatic circulation of bile acids is regulated by the microbiome since it affects synthesis of amino acids necessary for bile acid liver conjugation as well as the expression of terminal ileum transporters that reabsorb around 95% of intestinal bile acids.<sup>83,84</sup> As mentioned in the previous section, the FXR receptor is a nuclear receptor expressed in the liver, gallbladder and intestines, and a modulator of bile acid metabolism.<sup>65-67</sup> Obeticholic acid is a synthetic derivative of chenodeoxycholic acid that as well as cholic acid is a primary bile acid synthesized in the liver. In study by Friedman *et al.*,<sup>85</sup> obeticholic acid induced suppression of bile acid synthesis (measured by reduced levels of 7 $\alpha$ -hydroxy-4-cholesten-3-one) and caused an increase in Gram-positive bacteria species (*S. thermophilus*, *L. casei*, and *L. lactis*). The FLINT trial on obeticholic acid in NASH patients, as elaborated earlier, emphasized the need for FXR agonists with better efficacy, safety and tolerability profiles.<sup>68</sup>

Specific short chain fatty acids have been found to indirectly influence NASH progression, as well as previously described bile acids, in interplay with microbiome composition.<sup>86</sup> Short chain fatty acids are products of complex carbohydrate fermentation that cannot be digested by the organism, and the most prevalent are acetate, butyrate, and propionate.<sup>87</sup> They exert their effects by binding to specific receptors in the colon to increase GLP-1 and other insulin sensitizing peptides.<sup>88</sup> Their overall effects on liver, skeletal muscle and adipose tissue *in vitro* promote *de novo* lipid synthesis, fat oxidation, anabolism, and insulin sensitivity.<sup>89</sup> Moreover, studies have shown that they strengthen the intestinal barrier and reduce gut permeability and, by that, exhibit anti-inflammatory effects that are an important component in NASH development.<sup>90,91</sup> In humans, the interplay between specific short fatty acids and the residing microbiome is more complex, and studies that addressed specific therapeutic procedures influencing short fatty acid intestine composition are inconsistent in their conclusions, so further research is necessary.<sup>89</sup>

Lipopolysaccharides are a structural component of Gram-negative bacteria and of endotoxins that in healthy microbiota and intact intestinal barrier enter the hepatic circulation in only small amount and are thereby eliminated by Kupffer

cells after recognition by Toll-like receptors.<sup>92</sup> In patients with NASH, there is an up-regulation of the pro-inflammatory response in liver that results from a major influx of lipopolysaccharides and other bacteria metabolites due to impaired intestinal barrier and altered microbiome.<sup>93</sup> Agents that could affect this pathway are extensively researched, and a recently published study on sevelamer (that acts as a hydrophilic bile acid sequestrant) showed great potential in affecting liver fibrosis in a diet-induced NASH animal model.<sup>94</sup> Sevelamer improved the composition of the gut microbiome, improved the intestinal barrier, promoted fecal excretion of lipopolysaccharides and, by that, reduced the concentration of lipopolysaccharides in liver and suppressed the proinflammatory Toll-like receptor pathway.<sup>94</sup> Studies on sevelamer in human NASH patients have not yet been conducted.

### **Conclusions**

NASH represents an important global health burden with significant morbidity and the available treatment options are still unsatisfactory. However, new treatments for NASH are emerging. There is a large number of new drugs that are being tested in the preclinical setting and understanding NASH pathogenesis has a crucial role in their development. The microbiome composition has been shown to be related to changes in gut permeability, leaking lipopolysaccharides, and metabolism of short-chain fatty acids, all indirectly influencing proinflammatory and profibrotic pathways in the liver. Fecal microbiota transplantation and influencing the microbiome composition through bile acids and agents affecting other microbial metabolites have, in that way, been recognized as possible mechanisms to influence the development of NASH, potentially to reverse the changes preceding NASH and even influence NASH stage regression.

After years of not being able to actively treat NASH other than with diet and exercise modification, with only limited pharmacological possibilities, we are now expecting drugs which target specific points in NASH pathogenesis. Although NASH is a multifactorial disease, in the future, we could identify the predominating pathological mechanism and, by choosing specific medications, tailor the treatment for every patient individually.

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### **Author contributions**

Study concept and design (MD, SSS), acquisition of data (SSS, LVJ, DH), analysis and interpretation of data (MD, SSS, LVJ, DH), drafting of the manuscript (SSS, LVJ), critical revision of the manuscript for important intellectual content (MD, LVJ, DH), administrative, technical, or material support, study supervision (LSD, MD).

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