



## Current and Future Treatment Options in Non-Alcoholic Steatohepatitis (NASH)

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**Current and Future Treatment Options in Non-Alcoholic Steatohepatitis (NASH)**

For Peer Review Only

**Abstract**

Introduction: Non-alcoholic steatohepatitis (NASH) is a chronic liver disease that can progress to cirrhosis and hepatocellular carcinoma. Diagnosis of NASH requires liver biopsy and is defined as presence of hepatic steatosis and inflammation with or without fibrosis. Although NASH is the most common cause of liver disease in the west world and among the top three indications for liver transplantation, there are no universally accepted pharmacological therapies and advances have been slow.

Areas covered: Current evidence about lifestyle interventions, bariatric surgery and pharmacotherapy is reviewed. Dietary recommendations and lifestyle interventions have shown promising results but are difficult to maintain. At the moment, there is no universally approved medical treatment for NASH. Pioglitazone and vitamin E are recommended by guidelines in selected patients. An increasing number of phase II and III trials in non-cirrhotic NASH are currently recruiting and their preliminary results discussed.

Expert commentary: As NASH is classified as a medical condition of an unmet therapeutic need, it has gained an accelerated access pathway for drug approval based on surrogate endpoints. It is therefore expected that within the next five years, there will be at least one approved agent for the pharmacological treatment of pre-cirrhotic NASH.

**Keywords:** fibrosis, obeticholic acid, elafibranor, fatty liver, cirrhosis, bariatric surgery

## 1.0 Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as the presence of hepatic steatosis in patients who do not consume excessive alcohol and who do not have other secondary causes of steatosis such as steatogenic medication [1, 2, 3]. It is considered the hepatic manifestation of the metabolic syndrome and is associated with type II diabetes, obesity and dyslipidaemia [4, 5, 6]. The prevalence of obesity and metabolic syndrome have increased exponentially over the recent years hence contributing to the rising prevalence of NAFLD [7]. NAFLD is projected to become the main indication for liver transplantation in the next 10 years [2, 3, 8]. Based on the histological features, NAFLD is differentiated in simple steatosis (NAFL) and non-alcoholic steatohepatitis (NASH) [2, 3], which is characterized by lobular inflammation, ballooning and higher risk of progression to cirrhosis [9]. Although there is rigorous scientific interest in approaching novel therapeutic pathways to treat NASH, the gold standard remains lifestyle modification with a combination of weight loss and exercise [10, 11]. During the last decades, a variety of different agents, which target specific pathophysiologic mechanisms, have been studied in controlled trials in the treatment of NASH. A better understanding of NAFLD pathogenesis will not only help clarify how the disease progresses but may also result in the discovery of new treatment strategies. Potential therapeutic targets and approaches include the use of insulin sensitizers, antioxidants/anti-inflammatory and anti-fibrotic agents. This review focuses on therapeutic approaches that have already been tested in patients with NASH, with a brief mention on promising future agents that are already at least in phase II trials.

## 2.0 Management principles

NAFL is considered a benign condition and is not associated with increased liver-related morbidity hence no liver-specific treatment is required. The presence of coexisting conditions like diabetes mellitus, dyslipidaemia and obesity should be investigated and managed [2, 3]. Conversely, patients with NASH might potentially progress to cirrhosis and treatment is necessary. Patients with NASH have increased risk of death from liver-related and cardiovascular causes [12].

### 2.1 Diet and lifestyle modification / exercise

NAFLD and NASH are strongly associated with the metabolic syndrome and the majority of patients are overweight or obese, with co-morbidities like diabetes mellitus or insulin resistance and hyperlipidemia. Hence the cornerstone of treatment is a combination of weight reduction and exercise through a combination of diet, physical activity and general lifestyle changes [13]. Behavioural change through diet and exercise may not suffice alone as other parameters are still under investigation, such as smoking, which is associated with increased insulin resistance and possibly with advanced liver fibrosis [14, 15].

From as early as 1970, studies indicated that weight loss is associated with improvement in liver histology as evidenced by follow up biopsies [16] and liver enzyme levels [17]. Small studies verified that weight reduction is correlated with lower levels of aminotransferases and showed that liver biochemistry was aggravated when patients regained weight [18]. The prevalence of metabolic syndrome in obese patients was also reduced when weight loss was achieved by diet and medical treatment [19]. Patients who accomplish a reduction of more than 5% of their body weight have improved insulin sensitivity and steatosis. Furthermore, when patients

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3 lose more than 9% of body weight, ballooning, inflammation and nonalcoholic fatty  
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5 liver disease score (NAS) are also improved [20].  
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8 A randomized controlled trial (RCT) with 31 patients tested the efficacy of  
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10 intense dietary and lifestyle interventions with exercise compared with general  
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12 education for 48 weeks. The lifestyle interventions arm achieved an average of 9.3%  
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14 reduction of body weight versus 0.2% in the other group, which was also associated  
15  
16 with a beneficial effect in liver histology since liver steatosis was improved. Overall a  
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18 weight loss of more than 7% of body weight was linked to significant improvement in  
19  
20 steatosis, lobular inflammation, ballooning and NAS, but not fibrosis. Whether liver  
21  
22 fibrosis may not be affected as much from weight loss or whether a longer treatment  
23  
24 period is needed, is unclear [11]. However a recent retrospective study of 45 patients  
25  
26 showed regression of fibrosis when patients lost more than 10% of body weight  
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28 regardless of the method used (bariatric therapy, diet, medical therapy for weight  
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30 loss). In addition when patients regained weight, fibrosis worsened [21].  
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36 The largest clinical study thus far, assessed 293 individuals with biopsy  
37  
38 proven NASH for one year. Patients received dietary advice, motivation to exercise  
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40 and were instructed to log their daily caloric consumption and activities. Assessment  
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42 of follow up biopsies recorded a 25% resolution of NASH and a 47% of NAS  
43  
44 improvement. Improvement in portal inflammation and fibrosis was greater in  
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46 patients with weight loss more than 10% of body weight. In total 19% of patients  
47  
48 achieved regression of fibrosis after a year. It is noteworthy that from those patients  
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50 who reached 10% of weight loss, 45% showed regression of fibrosis thus underlining  
51  
52 the importance of weight reduction as well as the dose-response relationship between  
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54 weight loss and hepatic histology improvement. On the other hand the majority of  
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56 patients with deteriorating liver histology had minimal or no weight loss. In total, less  
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3 than 50% of patients lost more than 7% of body weight and 25% achieved resolution  
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5 of NASH questioning the efficacy of these interventions in real life where usually  
6  
7 success rates are lower [22].  
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11 Published studies highlight the association between diet and exercise with  
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13 improvement in the biochemical levels and histological findings of NAFLD patients  
14  
15 [23]. Further supporting the role of dietary intervention, small studies displayed  
16  
17 histological improvement though without statistical significance [24]. The focus of  
18  
19 current research is to characterize unhealthy dietary habits for NAFLD patients. In  
20  
21 this setting, a prospective study showed that NAFLD patients tend to consume more  
22  
23 soft drinks and meat and less omega-3 fatty acids [25]. Similarly fructose  
24  
25 consumption is increased in NAFLD patients and is associated with increased fibrosis  
26  
27 [26]. Interestingly, diets with either restriction of fat or carbohydrate result in weight  
28  
29 reduction [27] while the Mediterranean diet seems to improve liver steatosis and  
30  
31 insulin sensitivity even without weight loss, albeit in a small group of patients [28].  
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37 Exercise, along with diet, is a key part of behavioral changes with both  
38  
39 beneficial effects in cardiovascular risk factors and leading to body weight reduction  
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41 [29]. Despite the fact that physical activity plays an important role in NAFLD  
42  
43 management, the majority of patients do not exercise and fitness is inversely  
44  
45 associated with the prevalence of NAFLD [30]. Indeed even when weight loss is not  
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47 accomplished through exercise there are beneficial results from physical activity. A  
48  
49 placebo controlled RCT of 19 sedentary obese patients assessed hepatic function by  
50  
51 magnetic resonance imaging and blood testing after 4 weeks of aerobic training or  
52  
53 placebo (stretching), documenting a reduction in hepatic triglyceride concentration  
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55 [31]. In a similar study, resistance exercise decreased fat concentration in the liver, as  
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3 measured by magnetic resonance, and ameliorated insulin resistance, glucose control  
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5 and lipid oxidation, despite the fact that once again weight loss was not achieved [32].  
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8 Data from a recent meta-analysis suggests that exercise has a beneficial role in  
9  
10 reducing intrahepatic lipids but not in the improvement of liver biochemistry even  
11  
12 when weight reduction is minimal or none. The vast heterogeneity and small number  
13  
14 of studies included in the analyses indicates that more research regarding exercise  
15  
16 interventions is required in order to accurately assess its role in NAFLD treatment  
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18 [33]. Furthermore the same researchers concluded that changes in the intensity or  
19  
20 amount of aerobic exercise do not have a significant effect on liver fat reduction and  
21  
22 fail to improve weight loss [34]. Despite this finding, a significant difference between  
23  
24 all exercise groups and placebo was documented in regards to intrahepatic lipid and  
25  
26 visceral adipose tissue, as measured by magnetic resonance [34].  
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## 31 **2.2 Orlistat**

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34 Weight reduction through diet and lifestyle modification is advocated as the  
35  
36 first line of intervention in NASH but the majority of real life patients are unable to  
37  
38 comply with dietary modifications. Orlistat, an oral inhibitor of gastric and pancreatic  
39  
40 lipases, was introduced in obese patients with NASH in order to assist with weight  
41  
42 loss. Initially a case series of 3 patients showed marked improvement in histological  
43  
44 and biochemical markers after 6 or 12 months of orlistat treatment [35]. In a RCT of  
45  
46 52 patients, orlistat in conjunction with a weight loss program reduced ALT levels  
47  
48 and steatosis by ultrasound after 6 months. Interestingly, there was no significant  
49  
50 difference in weight reduction between orlistat and placebo group [36]. Another RCT  
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52 with fifty overweight patients demonstrated that the reduction of aminotransferases  
53  
54 and body weight were similar when comparing treatment with vitamin E plus diet or  
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3 vitamin E plus diet and orlistat. In addition, no improvement in liver histology was  
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5 documented after 9 months of treatment regarding hepatic steatosis, ballooning,  
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7 inflammation or fibrosis [20].  
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### 10 **2.3 Bariatric surgery**

11  
12 Bariatric surgery is indicated in severely or morbidly obese patients and a  
13  
14 variety of procedures have been performed. These procedures include bilio-intestinal  
15  
16 bypass, gastric band, laparoscopic adjustable gastric banding, Roux-en-Y gastric  
17  
18 bypass and sleeve gastrectomy. Jejunio-ileal bypass was abandoned because of liver  
19  
20 complications that developed after surgery ranging from increased fatty infiltration to  
21  
22 cirrhosis and liver failure [37]. The majority of patients undergoing bariatric  
23  
24 procedures also have NAFLD. A recent Cochrane review found insufficient data from  
25  
26 RCTs and quasi-randomised clinical studies in order to consider bariatric surgery as a  
27  
28 therapeutic resource in patients with NASH [38]. Although recommendations have  
29  
30 not yet established bariatric procedures as an eligible treatment for NAFLD and  
31  
32 NASH, weight loss is known to increase insulin sensitivity and also act beneficially to  
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34 visceral fat loss [39].  
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41 In a small study, laparoscopic adjustable gastric band placement was  
42  
43 associated with sustained weight loss and improved liver biochemistry and insulin  
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45 sensitivity. Follow up biopsies indicated that steatosis, inflammation, ballooning and  
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47 fibrosis also reduced, although a lot of patients had to undergo revision surgery [40].  
48  
49 A prospective study from France evaluated the presence of fibrosis and non-alcoholic  
50  
51 steatohepatitis in 381 obese patients after bariatric surgery. They concluded that the  
52  
53 majority of patients had decreased steatosis and ballooning after one and five years,  
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55 albeit inflammation remained unchanged compared with baseline measurements. The  
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3 most significant improvement was observed the first year after the procedure and was  
4 sustained. Interestingly, there was a statistically significant increase in fibrosis but  
5 with low fibrosis score of F1 or less at 5 years [41]. Conversely a recent study  
6 presented evidence of improvement of fibrosis along with the histological features of  
7 NAFLD in liver biopsies after bariatric surgery [42]. Intriguingly, steatosis and  
8 inflammation resolved in 75% and fibrosis of any grade improved in half of the 160  
9 patients, although the second liver biopsy was done in different time intervals and was  
10 not always accompanied with biochemical liver markers. The team from Lille  
11 published a new study that included 109 obese patients, over the last twenty years,  
12 who had a follow up liver biopsy one year after bariatric surgery. Body mass index,  
13 insulin resistance index and other markers were significant improved and NASH  
14 disappeared from 85% of the patients [43]. Bariatric surgery induced histological  
15 improvement of NASH and fibrosis amelioration. The persistence of NASH was  
16 correlated with less weight loss and refractory insulin resistance.  
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35 In conclusion, foregut bariatric surgery is not yet an established treatment of  
36 NASH and more RCTs are awaited. However, it is frequently practiced in overweight  
37 non-cirrhotic NAFLD patients with encouraging results.  
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#### 42 **2.4.0 Insulin sensitizing agents**

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45 There is a well-documented association between non-alcoholic steatohepatitis  
46 and the metabolic syndrome. This association has led to a rigorous investigation of the  
47 majority of type 2 diabetes regimens for the treatment of NAFLD and NASH.  
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##### 52 **2.4.1 Metformin**

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3 One of the first anti-diabetic agents assessed in NASH was metformin. A comparison  
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5 between metformin, vitamin E and diet alone exhibited the benefit of metformin in an  
6  
7 open label, randomized trial with 110 patients, by improving aminotransferases levels  
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9 and decreasing liver fat and necroinflammation in a limited number of 17 patients  
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11 [44]. Besides liver function tests improvement, metformin facilitated weight loss and  
12  
13 had a beneficial role in necroinflammation and fibrosis in a small number of patients  
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15 as shown in the follow up biopsy. However more recent trials have not confirmed  
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17 these results, as metformin did not to have a significant effect neither on liver  
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19 histology nor on liver biochemical results [45]. In a double blind, placebo-controlled  
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21 RCT with 173 patients, metformin did not significantly improve liver biochemistry or  
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23 histology in children and adolescents with NAFLD and NASH [46]. Furthermore,  
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25 metformin provided no additional benefit when combined with another anti-diabetic  
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27 agent such as rosiglitazone [47]. Although metformin does not improve NASH, there  
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29 is evidence that it might reduce the incidence of hepatocellular carcinoma. In a recent  
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31 nationwide case control study from Taiwan with 97,430 HCC patients and 194,960  
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33 matched controls, the use of metformin was associated with a decreased risk of HCC  
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35 in a dose dependent manner, as HCC risk was reduced by 7% for each incremental  
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37 year of metformin use [48].  
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#### 43 **2.4.2 Thiazolidinediones**

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46 Pioglitazone and rosiglitazone are agonists of the nuclear peroxisome  
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48 proliferator activated receptor-gamma (PPAR $\gamma$ ), thus acting by improving insulin  
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50 sensitivity. The effect of rosiglitazone and pioglitazone on liver biochemistry and  
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52 histology (steatosis, ballooning, inflammation and fibrosis) has been vigorously  
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54 investigated in NASH both in diabetic and non-diabetic patients.  
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3 In 2008, the FLIRT RCT tested the effect of rosiglitazone in 63 patients with  
4 biopsy-proven NASH for one year. Steatosis and transaminase levels improved after  
5 treatment, although fibrosis and other histology scores remained unchanged. There  
6 was a documented increase in weight in the rosiglitazone group due to peripheral  
7 oedema [49]. The study showed a rapid reduction in liver biochemistry in the first  
8 months of treatment and a gradual return to baseline levels after the end of the  
9 treatment period. An extension of this trial was designed aiming to assess further  
10 histological improvement with longer treatment. Unfortunately the goal of fibrosis  
11 regression was not achieved and no additional benefit was documented after 2 years  
12 of treatment [50]. Researchers attempted to combine rosiglitazone with other agents  
13 like metformin and losartan however the results were not as promising [47].  
14 Moreover, rosiglitazone has been associated with an increased risk of myocardial  
15 infarction and heart failure and as a result is no more accessible in Europe [51, 52].  
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32 In a pilot study of 18 non-diabetic patients with NASH treated with  
33 pioglitazone for 48 weeks, liver biochemistry and histology significantly improved  
34 [53]. This improvement may be modulated by an adiponectin-mediated effect on  
35 insulin sensitivity and hepatic fatty acid metabolism rather than by changes in pro-  
36 inflammatory cytokines [54]. In a RCT of pioglitazone and dietary restriction versus  
37 diet restriction alone in 55 patients with NASH, pioglitazone significantly improved  
38 all histological measures apart from fibrosis [55]. Interestingly discontinuation of  
39 pioglitazone treatment reverses the beneficial effects on aminotransferase levels and  
40 inflammation markers [54]. In a UK RCT of 74 patients, pioglitazone treatment for 12  
41 months resulted in significant weight gain and improvements in metabolic and  
42 histological parameter, including liver fibrosis [56].  
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3 The PIVENS trial was the largest RCT to assess the use of pioglitazone in  
4 biopsy proven NASH compared with vitamin E or placebo therapy. 247 non-diabetic  
5 patients were randomized in three groups and received vitamin E, pioglitazone or  
6 placebo for 96 weeks with additional lifestyle and dietary recommendations.  
7 Pioglitazone was associated with significant reductions in hepatic steatosis, lobular  
8 inflammation and resolution of steatohepatitis as compared with placebo although no  
9 improvement in fibrosis was seen. The study concluded that there was no benefit of  
10 pioglitazone over placebo since the drug failed to meet the pre-specified level of  
11 significance for the improvement in histologic findings [57].  
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24 There are adverse effects of pioglitazone that need to be acknowledged.  
25 Congestive heart failure increases with pioglitazone, nevertheless without an  
26 associated increase in cardiovascular mortality [58, 59]. Furthermore, pioglitazone  
27 presents the same side effect profile with the other thiazolidinediones regarding  
28 weight gain, bone loss and pedal edema [60].  
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36 Pioglitazone is included in the AASLD and EASL management  
37 recommendations for patients with biopsy proven NASH although the optimal  
38 duration of treatment is yet to be determined [2, 3]. However the long-term efficacy is  
39 debatable and the experience with rosiglitazone suggests no additional benefit with  
40 longer therapy schemes [50]. Moreover both biochemical and histological  
41 improvement return to baseline after treatment discontinuation while weight gain is  
42 not easily reversible [54].  
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### 51 **2.4.3 Liraglutide**

52 Liraglutide is a glucagon-like peptide-1 (GLP-1) analogue with longer  
53 duration and was introduced for the treatment of diabetes mellitus in obese patients in  
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3 2009 [61]. Liraglutide improves glycaemic control and promotes weight loss [62],  
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5 with only minor adverse events initially documented. Liraglutide and other GLP-1  
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7 analogues have two advantages. Firstly, they have been used for a number of years  
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9 already in other diseases and thus the safety profile is known. Secondly, they improve  
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11 to a large extent the metabolic syndrome parameters that are implicated in NASH,  
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13 hence making them attractive therapeutic options. Liraglutide was recently  
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15 investigated in 52 patients in a phase II double blind RCT in patients with NASH,  
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17 with the primary outcome being resolution of NASH and no deterioration of fibrosis  
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19 [63]. The results were promising and showed that 39% (9/23) of patients in the  
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21 liraglutide group had resolution of the histological features of NASH compared to 9%  
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23 (2/22) of patients in the placebo arm. In addition, patients in the liraglutide arm had  
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25 significantly reduced weight and BMI at the end of treatment compared with those  
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27 that received placebo.  
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#### 32 **2.4.4. Sitagliptin**

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35 Sitagliptin, a dipeptidyl peptidase 4 (DDP-4 inhibitor) is another anti  
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37 hyperglycemic agent studied in animal models with hopeful results [64]. An open  
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39 label observational pilot study with 15 NASH patients with diabetes assessed the  
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41 effect of sitagliptin for one year. Paired liver biopsies revealed a significant decrease  
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43 in ballooning and histological activity scores [65]. However, when further trials  
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45 evaluated sitagliptin on patients with NASH, one study was terminated early  
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47 (NCT01260246) and another was withdrawn prior to enrollment (NCT02263677). In  
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49 a recent RCT of sitagliptin for 24 weeks in 50 NAFLD patients, there was no  
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51 significant reduction in liver fat as measured by MRI in comparison with the placebo  
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53 group [66].  
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## 2.5 Statins and ezetimibe

One of the main therapies for dyslipidaemia are statins, hence the efficacy of these agents has also been assessed in the treatment of NASH but without robust results. The use of statins is indicated in patients with NASH and dyslipidaemia in order to reduce cardiovascular risk, however there is no strong evidence yet to prove that they can improve or delay liver disease progression. A prospective randomised open label study from Greece with 1600 patients, demonstrated a significant improvement of liver enzymes in patients receiving atorvastatin albeit no liver histology was assessed with biopsy after treatment [67]. Patients underwent liver biopsy after a year of statin therapy in small studies from USA and Japan, though with conflicting results. Simvastatin appeared to have no effect on the histopathological signs of NASH [68], however pravastatin leads to improvement of the biochemical markers and even histology in some patients [69]. Nevertheless, progression of fibrosis was evident in some patients. Recently a prospective open label uncontrolled study with 20 NASH patients reported resolution of NASH after 12 months of monotherapy with rosuvastatin (10 mg/d). All patients underwent liver biopsy before and after treatment and although body weight and waist circumference remained unchanged, 19 patients had histological resolution of NASH [70]. Furthermore statin use was associated with a reduction in incident of hepatocellular carcinoma among patients with diabetes and HCV in large cohorts of patients [71, 72].

A recent double blind, placebo-controlled RCT with 50 biopsy proven NASH patients examined the efficacy of ezetimibe (10mg/d) for 24 weeks mainly by assessing liver fat change as measured by magnetic resonance imaging and was negative [73].

## 2.6 Polyunsaturated fatty acids (PUFAs) – Omega-3 fatty acids

In both animal and human intervention trials it was suggested that polyunsaturated fatty acids (PUFA) had an impact on both lipid metabolism and insulin sensitivity through multiple pathways. Thus it has been hypothesized that PUFA supplementation, which cannot be synthesized by mammals, can have beneficial effect on NAFLD [74]. Administration of eicosapentaenoic acid and docosahexaenoic acid for 48 weeks showed no positive effect over placebo on both histological markers and insulin resistance or lipid control [75]. In a phase 2 RCT in 243 patients with biopsy proven NASH, two dosages of ethyl-eicosapentaenoic acid were tested and no histological improvement was demonstrated after one year of treatment, whilst the effect on serum triglycerides appeared moderate [76]. A possible explanation for those negative results is the lack of an optimal dosage of polyunsaturated fatty acids hence higher dosages should be used and standardised according to their lipid lowering effect.

## 2.7 Ursodeoxycholic acid (UDCA)

UDCA, a bile acid, was thought to be a promising treatment for NASH based on small pilot studies with the potential to improve liver biochemistry and histology. In the first large RCT including 166 patients, no significant difference between the UDCA (13-15 mg/Kgr/day) and placebo was identified in biochemistry or histology [77]. The result was partially attributed to low dosage and a higher dosage of UDCA (23-28 mg/Kgr/day) was tried in a further RCT that included 185 patients for 18 months [78]. Nonetheless, the result remained the same and no liver histology improvement was documented regardless of the histological score used. Hence the



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3 EASL and AASLD guidelines do not recommend the use of UDCA in NAFLD or  
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5 NASH patients [2, 3].  
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## 8 **2.8 Vitamin E**

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11 The hypothesis that an antioxidant agent such as vitamin E (a-tocopherol) can  
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13 ameliorate liver histology has been frequently tested in NASH patients. Vitamin E  
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15 improved histological findings in a small double-blind RCT of 49 patients and a  
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17 prospective open label study of 23 patients after 6 and 12 months of treatment  
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19 respectively [79, 80]. In those studies the number of patients was small and the dosage  
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21 and formulation of vitamin E was variable. A combination with pioglitazone was  
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23 initially tested in a small randomized prospective trial with 20 NASH patients with  
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25 positive results [81]. Lately the PIVENS and TONIC studies assessed the efficacy of  
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27 vitamin E in large numbers of patients with biopsy confirmed NASH in adult and  
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29 paediatric populations respectively. The PIVENS study compared pioglitazone with  
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31 vitamin E and placebo for 96 weeks and demonstrated the superiority of vitamin E  
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33 over placebo in the improvement of liver histology [57]. When compared to placebo,  
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35 vitamin E treatment demonstrated a higher percentage of NAS score improvement  
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37 (43% vs. 19%) and liver biochemistry improvement [57]. In the children and  
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39 adolescents cohort, although vitamin E was not superior to placebo in ALT reduction,  
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41 it resulted in significantly higher resolution of NASH [46]. Both PIVENS and TONIC  
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43 used 800IU/day of vitamin E for duration of 96 weeks but neither presented sufficient  
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45 evidence of regression of fibrosis. When vitamin E was used as the control treatment  
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47 in a study that combined metformin and bicyclol, the histological features of steatosis,  
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49 inflammation and ballooning were decreased in both groups [82]. On account of those  
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51 results, EASL and AASLD guidelines consider vitamin E as a potential short term  
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53 treatment for non-diabetic NASH patients [2, 3] despite a potential increase in all-  
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3 cause mortality, haemorrhagic stroke and prostate cancer that has been documented  
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5 [83, 84, 85].  
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## 8 **2.9 Obeticholic acid (6 $\alpha$ -ethyl-chenodeoxycholic acid)**

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11 A very promising agent that has emerged from animal and human studies is  
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13 obeticholic acid, a bile acid derivative that acts as an agonist of the farnesoid X  
14  
15 receptor (FXR). Bile acids might interfere in the metabolic pathways of glucose and  
16  
17 lipids through the FXR [86]. In a small RCT of 64 diabetic patients with NASH,  
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19 obeticholic acid administered for 6 weeks increased insulin sensitivity and reduced  
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21 liver biochemical markers compared to placebo in the expense of a rise in LDL levels  
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23 [87]. A large, multicentre RCT of obeticholic acid versus placebo for 72 weeks was  
24  
25 subsequently performed in 283 patients with biopsy proven NASH [88]. The  
26  
27 obeticholic acid group showed improved liver histology compared with placebo,  
28  
29 including fibrosis, ballooning, steatosis and inflammation. Furthermore obeticholic  
30  
31 acid was associated with weight loss. The most common adverse event were the  
32  
33 presence of pruritus, elevated LDL and reduced HDL cholesterol levels in the  
34  
35 obeticholic acid group that reversed after treatment [88]. Albeit the presence of  
36  
37 pruritus and the rise of total and LDL cholesterol, obeticholic acid appears to be a  
38  
39 promising agent for the treatment of NASH, once long term safety is assured and is  
40  
41 currently tested in a phase III trial (NCT02548351).  
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## 47 **2.9 Elafibranor**

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49  
50 Elafibranor (formerly known as GFT505) is a dual peroxisome proliferator-  
51  
52 activated receptor alpha/delta agonist (PPAR $\alpha$ / $\delta$  agonist), which provided some  
53  
54 hopeful results in patients with metabolic syndrome and obesity [89, 90]. Human liver  
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56 PPAR $\alpha$  gene expression negatively correlates with NASH severity, while histological  
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3 improvement is associated with an increase in expression of PPAR $\alpha$  and its target  
4 genes [91]. In animal models of NAFLD/NASH, elafibranor improved liver histology  
5 by both PPAR- $\alpha$  dependent and -independent mechanisms [92]. Subsequently  
6 elafibranor was introduced in a double blind placebo controlled RCT with 276 non-  
7 cirrhotic NASH patients in two doses of 80mg and 120mg daily for 52 weeks in order  
8 to define histological response. The primary outcome of reversal of NASH without  
9 worsening of fibrosis was not achieved in the elafibranor arms compared with  
10 placebo. Nevertheless, when a post hoc analysis assessed the response using a  
11 modified definition of NASH resolution, the 120mg arm exhibited a significant  
12 improvement. Furthermore liver biochemistry, glucose and lipids profiles were  
13 reduced as well, without weight gain [93]. An ongoing phase III trial will further  
14 evaluate the efficacy and long term safety of elafibranor versus placebo in NASH  
15 patients (NCT02704403).  
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### 3.0 The future

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36 Future research for effective pharmacotherapy treatment in NAFLD and  
37 NASH is challenging. A lot of agents are being investigated and some emerging data  
38 from rodent trials are promising. As NASH pathogenesis and progression are  
39 illuminated, more potential therapeutic targets emerge [94]. Novel drugs aim at  
40 multiple targets and pathways, such as inflammation and oxidative stress, the gut liver  
41 axis, the metabolic factor and progression of fibrosis [95].  
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50 Aramchol, a fatty acid-bile conjugate is a potential therapeutic agent which  
51 seems to reduce liver fat as assessed by magnetic resonance spectroscopy [96]. An  
52 ongoing trial is recruiting patients to evaluate the efficacy and safety of two aramchol  
53 doses versus placebo in NASH patients (NCT02279524). Volixibat (SHP-626), is an  
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3 apical sodium dependent bile acid transporter inhibitor (ASBTi) and is being  
4  
5 evaluated in an ongoing phase 2 RTC for 48 weeks. The primary outcome measures  
6  
7 the effect of volixibat on liver histology (NCT02787304).  
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10 Saroglitazar is a dual PPAR agonist that has demonstrated significant  
11  
12 improvement in glycemic and lipid control in diabetic patients and appears to  
13  
14 ameliorate aminotransferases in 31 NAFLD patients after 24 weeks [97]. A series of  
15  
16 trials from India are evaluating potential benefits from this agent [98]. A RCT  
17  
18 evaluated a pradigastat, which is a diacylglycerol acyltransferase-1 inhibitor, in 52  
19  
20 NAFLD patients for 24 weeks with two different doses. Pradigastat improved liver fat  
21  
22 after 12 and 24 weeks when administered at the high dose (10/20 mg/day) [99].  
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27 An inhibitor of phosphodiesterase-4 (ASP9831) with promising results from a  
28  
29 phase 1 trial was evaluated in 93 patients with NASH compared with placebo but no  
30  
31 significant change was observed in liver biochemistry after 12 weeks of  
32  
33 administration at either of the two investigated doses [100]. In murine models of  
34  
35 NASH, a pan-caspase inhibitor, emricasan (IDN-6556) reduced hepatocyte apoptosis  
36  
37 and consequently attenuated liver injury and fibrosis [101]. Emricasan significantly  
38  
39 reduced transaminases after 28 days in 38 patients with NAFLD [102]. A larger RCT  
40  
41 is currently recruiting participants (NCT02686762).  
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45 Cenicriviroc, a dual CCR2/CCR5 antagonist is evaluated in a phase 2b  
46  
47 randomized, double blind, placebo controlled study with 289 NASH patients for a  
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49 period of 2 years [103].  
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52 GS-6624 (simtuzumab) is a monoclonal antibody against lysyl oxidase-like 2  
53  
54 (LOXL2) and GS-4497 is a molecule that inhibits apoptosis signal-regulating kinase 1  
55  
56 (ASK1) which acts as a mediator of oxidative stress. Both these agents are being  
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3 tested in at least three ongoing clinical trials to assess the efficacy of simtuzumab  
4 alone or in combination with GS-4997 in patients with NASH with and without  
5 cirrhosis. (NCT02466516, NCT01672866, NCT01672879). Finally anti-CD3  
6  
7 antibodies that reduce insulin resistance in animals were included in small trial  
8  
9 designs for NASH patients and led to positive results demanding further research  
10  
11 in the immediate future [104].  
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#### 15 16 17 **4.0 Conclusions** 18

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20 There is a documented global increase of NAFLD and NASH related to the  
21 epidemic of the metabolic syndrome and obesity leading to increasing morbidity,  
22 mortality and associated health costs. The research for better understanding the  
23 pathogenesis of NASH and determining new biomarkers of prognosis, disease  
24 progression and regression is of cardinal importance. This needs to take into account  
25 influence of genetics and the molecular mechanisms that are associated with the  
26 progression from NAFLD to NASH and which are not completely understood. Of the  
27 available therapeutic options, weight loss of at least 10% results in resolution of  
28 NASH and fibrosis in the majority of patients within one year. No pharmacological  
29 treatment to date has produced such results. Pioglitazone and vitamin E are the only  
30 treatments that are recommended in the EASL and AASLD guidelines in selected  
31 cases, however both are associated with side effects and their long-term safety and  
32 efficacy are questionable. There is an increasing interest from the pharmaceutical  
33 industry in conducting studies in NASH, which will hopefully result in effective  
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#### 5.0 Expert commentary

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3 NAFLD is a slowly progressing disease and expected liver-related outcome take more  
4 than 25 years to occur. It is therefore unrealistic to expect the conduction of trials of  
5 such duration. The Federal Drug Agency (FDA) in the US has agreed to the use of a  
6 co-primary endpoint for trials in pre-cirrhotic NASH that consists of resolution of  
7 steatohepatitis without worsening of fibrosis or improvement in the fibrosis score  
8 without worsening of the steatohepatitis. This will grant approval through the  
9 accelerated access pathway, with the sponsor obligation to conduct a post-market trial  
10 to demonstrate that the improvement in these surrogate end-points translates into a  
11 clinical meaningful benefit to patients [105]. The presence or severity of NASH has  
12 not been associated with clinical outcomes in long-term cohort studies [106], whereas  
13 only the presence of advanced fibrosis was associated with liver-related events [107].  
14 Moreover, there is significant intra- and inter-observer variability in the assessment of  
15 the NAS score, which has so far jeopardized the outcome of well-conducted RCTs  
16 [57, 93]. Therefore, the resolution of NASH endpoint (or any decrease of the NAS  
17 score) is of questionable utility. We propose the exploration of quantitative fibrosis  
18 assessment using the collagen proportionate area as a surrogate endpoint, that would  
19 allow for the detection of finer changes in fibrosis and could potentially shorten the  
20 duration of clinical trials [108, 109, 110]. CPA assessment would probably require a  
21 modified method to capture and measure the finer pericellular fibrosis which is  
22 present in NASH.

23  
24 Moreover, the identification of clinically relevant biomarkers of disease regression  
25 that could potentially alleviate the need for serial biopsies and would increase study  
26 recruitment is an unmet need. A current innovative medicines initiative call, which  
27 represents a collaboration between the European Commission and the European  
28 pharmaceutical industry, will hopefully address this issue.

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3 Since NASH has multiple potential pathways of development, combination treatment  
4 will also be explored at some point in the future. Ultimately, if there are no large scale  
5 interventions from a public health policy perspective to actively implement changes in  
6 lifestyle, the toll of morbidity and mortality will continue to rise irrespective of any  
7 small scale success in pharmaceutical therapy.  
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### 10 11 12 13 14 **6.0 Five-year view**

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17 At the moment, there are promising agents in large scale phase III trials (obeticholic  
18 acid, elafibranor) and others in phase II trials (cenicriviroc, simtuzumbab, volixibat)  
19 that target different pathways of NASH development and progression. As NASH is  
20 classified as a medical condition of an unmet therapeutic need, it has gained an  
21 accelerated access pathway for drug approval based on surrogate endpoints. It is  
22 therefore expected that within the next five years, there will be at least one approved  
23 agent for the pharmacological treatment of pre-cirrhotic NASH. It is also expected  
24 that biomarker research will have identified candidate markers that will allow better  
25 characterization of patients and reduce the need for a liver biopsy.  
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### 37 38 **7.0 Key issues**

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41 \* NAFLD has become the most common cause for chronic liver disease in the  
42 developed world.  
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45 \* Dietary and lifestyle interventions provide beneficial results but are difficult to  
46 maintain in the long term.  
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50 \* Although there is no universally approved medical treatment, both the EASL and  
51 AASLD guidelines suggest pioglitazone and vitamin E use in selected cases.  
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54  
55 \* As NASH is classified as a medical condition of an unmet therapeutic need, it has  
56 gained an accelerated access pathway for drug approval based on surrogate endpoints.  
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3 \* New agents as obeticholic acid and elafibranor have shown promising results in  
4  
5 phase II and are currently in phase III trials.  
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7  
8 \* Novel drugs aim at multiple targets and pathways, such as inflammation and  
9  
10 oxidative stress, the gut liver axis, the metabolic factor and progression of fibrosis.  
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12  
13 \* The exploration of surrogate outcomes with clinical relevance, such as quantitative  
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15 fibrosis assessment using the collagen proportionate area, and biomarkers of disease  
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17 progression and regression is important.  
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For Peer Review Only



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**Table 1. Randomized controlled trials in patients with non-alcoholic steatohepatitis that included more than 50 patients and used liver histology as the primary outcome.**

A/a	Author	Number of patients	Intervention	Duration	Primary Outcome	Result
1	Ratziu V, 2016 [93]	276 patients	Elafibranor 2:1	52 weeks	Resolution of steatohepatitis without worsening of fibrosis	Predefined end point not met
2	Armstrong M.J, 2016 [63]	52 patients	Liraglutide (1.8mg) 1:1	48 weeks	Resolution of steatohepatitis without worsening of fibrosis	Significant effect
3	Neuschwander-Tetri B, 2015 (FLINT) [88]	283 patients	Obeticholic acid (25mg/d) 1:1	72 weeks	Improvement of NAS by at least 2 points, without worsening of fibrosis	Significant effect
4	Sanyal A, 2014 [76]	243 patients	Ethyl-Eicosapentanoic Acid	12 months	Improvement of NAS, without	No significant effect

			(EPA-E) 2:1		worsening of fibrosis	
5	Wong VW, 2013 [111]	60 patients	Phyllanthus urinaria (herb) 2:1	24 weeks	Change in NAFLD activity score	No significant effect
6	Torres D, 2011 [47]	137 patients	Rosiglitazone vs Rosiglitazone & metformin vs rosiglitazone & losartan 1:1:1	12 months	Improvement of steatosis, inflammation and fibrosis	No significant effect
7	Lavine J, 2011 (TONIC) in children [46]	173 patients	Vitamin E vs. metformin vs. Placebo 1:1:1	96 weeks	Sustained reduction in ALT	No significant effect
8	Malaguara M, 2010 [112]	74 patients	L-Carnitine supplementation to diet 1:1	24 weeks	Liver biochemistry	Significant improvement
9	Sanyal A, 2010 (PIVENS) [57]	247 patients	Pioglitazone vs Vitamin E vs placebo 1:1:1	96 weeks	Improvement in NAS score	Vitamin E was superior to placebo, no benefit of pioglitazone over placebo

10	Leuschner U, 2010 [78]	185 patients	Ursodeoxycholic acid 1:1	18 months	An overall improvement of liver histology	No significant effect
11	Vilar Gomez E, 2009 [113]	60 patients	Nutritional supplement Viusid 1:1	6 months	Improvement in NAS and fibrosis score	Improvement in the NAS score but not in fibrosis
12	Aithal G.P, 2008 [56]	74 patients	Pioglitazone 1:1	12 months	Reduction in hepatocyte injury and fibrosis	Significant effect
13	Ratziu V, 2008 (FLIRT) [49]	63 patients	Rosiglitazone	1 year	Improvement of steatosis, necroinflammation & fibrosis Normalization of transaminases	Improved steatosis Normalized transaminase levels No improvement in fibrosis and NAS
14	Lindor K, 2004 [77]	166 patients	Ursodeoxycholic acid 1:1	2 years	Histological and biochemical improvement	No significant effect

**Table 2. Ongoing clinical trials with new pharmacologic agents for patients with non-alcoholic steatohepatitis (clinical trials.gov accessed 20 Oct 2016).**

A/a	ClinicalTrials.gov Identifier	Phase	Stage	Agent	Action
1	NCT02704403	Phase III	Recruiting	Elafibranor (GFT505)	dual <u>PPAR<math>\alpha</math>/<math>\delta</math></u> agonist
2	NCT02654665	Phase III	Recruiting	Liraglutide & bariatric surgery	GLP-1 analogue
3	NCT02548351	Phase III	Recruiting	Obeticholic acid	FXR agonist
4	NCT02541045	Phase III	Recruiting	Metadoxine	Pyridoxine-pyrrolidone carboxylate
5	NCT02605616	Phase II	Recruiting	A novel AZ agent	
6	NCT02913105	Phase II	Not yet recruiting	LMB763	
7	NCT02443116	Phase II	Recruiting	NGM282	Recombinant variant of FGF-19 (FGF-19 is a peptide hormone)
8	NCT02279524	Phase IIb	Recruiting	Aramchol	Conjugate of cholic and arachidic acid
9	NCT02912260	Phase II	Recruiting	MGL-3196	small-molecule liver- directed $\beta$ -selective THR agonist
10	NCT02855164	Phase II	Recruiting	LJN452	FXR agonist
11	NCT02854605	Phase II	Not yet recruiting	GS-9674	A synthetic non-steroidal FXR agonist

12	NCT02927314	Phase II	Not yet recruiting	CF102	A3 adenosine receptor agonist
13	NCT02856555	Phase II	Recruiting	GS-0976	ACC inhibitor
14	NCT02574325	Phase II	Ongoing but not recruiting	Niacin/ARI-3037MO	Vitamin B3 analog
15	NCT02413372	Phase II	Ongoing but not recruiting	BMS-986036	Pegylated FGF-21 analogue (FGF-21 is a peptide hormone)
16	NCT02217475	Phase II	Ongoing but not recruiting	Cenicriviroc	dual CCR2/CCR5 antagonist
17	NCT02686762	Phase II	Recruiting	Emricasan	pan caspase inhibitor
18	NCT02466516	Phase II	Ongoing but not recruiting	GS-4997 alone or with simtuzumab	inhibitor of ASK1
19	NCT02421094	Phase II	Ongoing but not recruiting	GR-MD-02	A complex carbohydrate drug that targets galectin-3
20	NCT02316717	Phase II	Recruiting	IMM-124E	is composed of anti-LPS antibodies and adjuvants, many of which are glycosphingolipids, targeting the gut microbiome and the innate immune system of the gut
21	NCT02784444	Phase II	Recruiting	MSDC 0602K	Insulin sensitizer
22	NCT02442687	Phase II	Recruiting	JKB-121	Non-selective opioid antagonist



23	NCT01679197	Phase II	Ongoing but not recruiting	Metreleptin	Recombinant human leptin
24	NCT02681055	Phase II	Recruiting	MN-001/Tipelukast	orally bio-available small molecule compound which demonstrates anti-inflammatory activity
25	NCT02510599	Phase II	Recruiting	CEM-101/Solithromycin	a new generation macrolide antibiotic
26	NCT01919294	Phase II	Recruiting	Testosterone replacement	
27	NCT01672879	Phase II	Ongoing but not recruiting	GS-6624/Simtuzumab	Humanized anti-LOXL2 monoclonal IgG4 antibody
28	NCT01672866	Phase II	Ongoing but not recruiting	GS-6624/Simtuzumab	
29	NCT02923154	Phase II	Recruiting	MT-3995	a selective aldosterone receptor antagonist
30	NCT02612662	Phase I	Recruiting	AZD4076/RG-125 (tetracosasodium)	an anti-Mir directed against miR-103/107
31	NCT02469272	Phase I	Recruiting	Fecal microbiota transplantation	
32	NCT02721264		Recruiting	Fecal microbiota therapy	
33	NCT02196831		Recruiting	Tesamorelin	GHRH analogue
<p>PPAR, peroxisome proliferator-activated receptor; GLP, glucagon-like peptide; FXR, farnesoid X receptor; FGF, Fibroblast Growth Factor; THR, thyroid hormone receptor; ACC, Acetyl-CoA carboxylase; CCR, CC-</p>					

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chemokine receptor; ASK, apoptosis signal-regulating kinase; LPS, lipopolysaccharide; LOXL, lysyl oxidase-like protein; GHRH, growth hormone-releasing hormone

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