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

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Current and Future Treatment Options in Non-Alcoholic Steatohepatitis (NASH)
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
lose more than 9% of body weight, ballooning, inflammation and nonalcoholic fatty liver disease score (NAS) are also improved [20].

A randomized controlled trial (RCT) with 31 patients tested the efficacy of intense dietary and lifestyle interventions with exercise compared with general education for 48 weeks. The lifestyle interventions arm achieved an average of 9.3% reduction of body weight versus 0.2% in the other group, which was also associated with a beneficial effect in liver histology since liver steatosis was improved. Overall a weight loss of more than 7% of body weight was linked to significant improvement in steatosis, lobular inflammation, ballooning and NAS, but not fibrosis. Whether liver fibrosis may not be affected as much from weight loss or whether a longer treatment period is needed, is unclear [11]. However a recent retrospective study of 45 patients showed regression of fibrosis when patients lost more than 10% of body weight regardless of the method used (bariatric therapy, diet, medical therapy for weight loss). In addition when patients regained weight, fibrosis worsened [21].

The largest clinical study thus far, assessed 293 individuals with biopsy proven NASH for one year. Patients received dietary advice, motivation to exercise and were instructed to log their daily caloric consumption and activities. Assessment of follow up biopsies recorded a 25% resolution of NASH and a 47% of NAS improvement. Improvement in portal inflammation and fibrosis was greater in patients with weight loss more than 10% of body weight. In total 19% of patients achieved regression of fibrosis after a year. It is noteworthy that from those patients who reached 10% of weight loss, 45% showed regression of fibrosis thus underlining the importance of weight reduction as well as the dose-response relationship between weight loss and hepatic histology improvement. On the other hand the majority of patients with deteriorating liver histology had minimal or no weight loss. In total, less
than 50% of patients lost more than 7% of body weight and 25% achieved resolution of NASH questioning the efficacy of these interventions in real life where usually success rates are lower [22].

Published studies highlight the association between diet and exercise with improvement in the biochemical levels and histological findings of NAFLD patients [23]. Further supporting the role of dietary intervention, small studies displayed histological improvement though without statistical significance [24]. The focus of current research is to characterize unhealthy dietary habits for NAFLD patients. In this setting, a prospective study showed that NAFLD patients tend to consume more soft drinks and meat and less omega-3 fatty acids [25]. Similarly fructose consumption is increased in NAFLD patients and is associated with increased fibrosis [26]. Interestingly, diets with either restriction of fat or carbohydrate result in weight reduction [27] while the Mediterranean diet seems to improve liver steatosis and insulin sensitivity even without weight loss, albeit in a small group of patients [28].

Exercise, along with diet, is a key part of behavioral changes with both beneficial effects in cardiovascular risk factors and leading to body weight reduction [29]. Despite the fact that physical activity plays an important role in NAFLD management, the majority of patients do not exercise and fitness is inversely associated with the prevalence of NAFLD [30]. Indeed even when weight loss is not accomplished through exercise there are beneficial results from physical activity. A placebo controlled RCT of 19 sedentary obese patients assessed hepatic function by magnetic resonance imaging and blood testing after 4 weeks of aerobic training or placebo (stretching), documenting a reduction in hepatic triglyceride concentration [31]. In a similar study, resistance exercise decreased fat concentration in the liver, as
measured by magnetic resonance, and ameliorated insulin resistance, glucose control and lipid oxidation, despite the fact that once again weight loss was not achieved [32].

Data from a recent meta-analysis suggests that exercise has a beneficial role in reducing intrahepatic lipids but not in the improvement of liver biochemistry even when weight reduction is minimal or none. The vast heterogeneity and small number of studies included in the analyses indicates that more research regarding exercise interventions is required in order to accurately assess its role in NAFLD treatment [33]. Furthermore the same researchers concluded that changes in the intensity or amount of aerobic exercise do not have a significant effect on liver fat reduction and fail to improve weight loss [34]. Despite this finding, a significant difference between all exercise groups and placebo was documented in regards to intrahepatic lipid and visceral adipose tissue, as measured by magnetic resonance [34].

2.2 Orlistat

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

2.3 Bariatric surgery

Bariatric surgery is indicated in severely or morbidly obese patients and a variety of procedures have been performed. These procedures include bili-intestinal bypass, gastric band, laparoscopic adjustable gastric banding, Roux-en-Y gastric bypass and sleeve gastrectomy. Jejuno-ileal bypass was abandoned because of liver complications that developed after surgery ranging from increased fatty infiltration to cirrhosis and liver failure [37]. The majority of patients undergoing bariatric procedures also have NAFLD. A recent Cochrane review found insufficient data from RCTs and quasi-randomised clinical studies in order to consider bariatric surgery as a therapeutic resource in patients with NASH [38]. Although recommendations have not yet established bariatric procedures as an eligible treatment for NAFLD and NASH, weight loss is known to increase insulin sensitivity and also act beneficially to visceral fat loss [39].

In a small study, laparoscopic adjustable gastric band placement was associated with sustained weigh loss and improved liver biochemistry and insulin sensitivity. Follow up biopsies indicated that steatosis, inflammation, ballooning and fibrosis also reduced, although a lot of patients had to undergo revision surgery [40]. A prospective study from France evaluated the presence of fibrosis and non-alcoholic steatohepatitis in 381 obese patients after bariatric surgery. They concluded that the majority of patients had decreased steatosis and ballooning after one and five years, albeit inflammation remained unchanged compared with baseline measurements. The
most significant improvement was observed the first year after the procedure and was sustained. Interestingly, there was a statistically significant increase in fibrosis but with low fibrosis score of F1 or less at 5 years [41]. Conversely a recent study presented evidence of improvement of fibrosis along with the histological features of NAFLD in liver biopsies after bariatric surgery [42]. Intriguingly, steatosis and inflammation resolved in 75% and fibrosis of any grade improved in half of the 160 patients, although the second liver biopsy was done in different time intervals and was not always accompanied with biochemical liver markers. The team from Lille published a new study that included 109 obese patients, over the last twenty years, who had a follow up liver biopsy one year after bariatric surgery. Body mass index, insulin resistance index and other markers were significant improved and NASH disappeared from 85% of the patients [43]. Bariatric surgery induced histological improvement of NASH and fibrosis amelioration. The persistence of NASH was correlated with less weight loss and refractory insulin resistance.

In conclusion, foregut bariatric surgery is not yet an established treatment of NASH and more RCTs are awaited. However, it is frequently practiced in overweight non-cirrhotic NAFLD patients with encouraging results.

2.4.0 Insulin sensitizing agents

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

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

2.4.2 Thiazolidinediones

Pioglitazone and rosiglitazone are agonists of the nuclear peroxisome proliferator activated receptor-gamma (PPARγ), thus acting by improving insulin sensitivity. The effect of rosiglitazone and pioglitazone on liver biochemistry and histology (steatosis, ballooning, inflammation and fibrosis) has been vigorously investigated in NASH both in diabetic and non-diabetic patients.
In 2008, the FLIRT RCT tested the effect of rosiglitazone in 63 patients with biopsy-proven NASH for one year. Steatosis and transaminase levels improved after treatment, although fibrosis and other histology scores remained unchanged. There was a documented increase in weight in the rosiglitazone group due to peripheral oedema [49]. The study showed a rapid reduction in liver biochemistry in the first months of treatment and a gradual return to baseline levels after the end of the treatment period. An extension of this trial was designed aiming to assess further histological improvement with longer treatment. Unfortunately the goal of fibrosis regression was not achieved and no additional benefit was documented after 2 years of treatment [50]. Researchers attempted to combine rosiglitazone with other agents like metformin and losartan however the results were not as promising [47]. Moreover, rosiglitazone has been associated with an increased risk of myocardial infarction and heart failure and as a result is no more accessible in Europe [51, 52].

In a pilot study of 18 non-diabetic patients with NASH treated with pioglitazone for 48 weeks, liver biochemistry and histology significantly improved [53]. This improvement may be modulated by an adiponectin-mediated effect on insulin sensitivity and hepatic fatty acid metabolism rather than by changes in pro-inflammatory cytokines [54]. In a RCT of pioglitazone and dietary restriction versus diet restriction alone in 55 patients with NASH, pioglitazone significantly improved all histological measures apart from fibrosis [55]. Interestingly discontinuation of pioglitazone treatment reverses the beneficial effects on aminotransferase levels and inflammation markers [54]. In a UK RCT of 74 patients, pioglitazone treatment for 12 months resulted in significant weight gain and improvements in metabolic and histological parameter, including liver fibrosis [56].
The PIVENS trial was the largest RCT to assess the use of pioglitazone in biopsy proven NASH compared with vitamin E or placebo therapy. 247 non-diabetic patients were randomized in three groups and received vitamin E, pioglitazone or placebo for 96 weeks with additional lifestyle and dietary recommendations. Pioglitazone was associated with significant reductions in hepatic steatosis, lobular inflammation and resolution of steatohepatitis as compared with placebo although no improvement in fibrosis was seen. The study concluded that there was no benefit of pioglitazone over placebo since the drug failed to meet the pre-specified level of significance for the improvement in histologic findings [57].

There are adverse effects of pioglitazone that need to be acknowledged. Congestive heart failure increases with pioglitazone, nevertheless without an associated increase in cardiovascular mortality [58, 59]. Furthermore, pioglitazone presents the same side effect profile with the other thiazolidinediones regarding weight gain, bone loss and pedal edema [60].

Pioglitazone is included in the AASLD and EASL management recommendations for patients with biopsy proven NASH although the optimal duration of treatment is yet to be determined [2, 3]. However the long-term efficacy is debatable and the experience with rosiglitazone suggests no additional benefit with longer therapy schemes [50]. Moreover both biochemical and histological improvement return to baseline after treatment discontinuation while weight gain is not easily reversible [54].

2.4.3 Liraglutide

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

2.4.4. Sitagliptin

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

One of the main therapies for dyslipidaemia are statins, hence the efficacy of this 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 rosvastatin (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
EASL and AASLD guidelines do not recommend the use of UDCA in NAFLD or NASH patients [2, 3].

2.8 Vitamin E

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

2.9 Obeticholic acid (6α-ethyl-chenodeoxycholic acid)

A very promising agent that has emerged from animal and human studies is obeticholic acid, a bile acid derivative that acts as an agonist of the farnesoid X receptor (FXR). Bile acids might interfere in the metabolic pathways of glucose and lipids through the FXR [86]. In a small RCT of 64 diabetic patients with NASH, obeticholic acid administered for 6 weeks increased insulin sensitivity and reduced liver biochemical markers compared to placebo in the expense of a rise in LDL levels [87]. A large, multicentre RCT of obeticholic acid versus placebo for 72 weeks was subsequently performed in 283 patients with biopsy proven NASH [88]. The obeticholic acid group showed improved liver histology compared with placebo, including fibrosis, ballooning, steatosis and inflammation. Furthermore obeticholic acid was associated with weight loss. The most common adverse event were the presence of pruritus, elevated LDL and reduced HDL cholesterol levels in the obeticholic acid group that reversed after treatment [88]. Albeit the presence of pruritus and the rise of total and LDL cholesterol, obeticholic acid appears to be a promising agent for the treatment of NASH, once long term safety is assured and is currently tested in a phase III trial (NCT02548351).

2.9 Elafibranor

Elafibranor (formerly known as GFT505) is a dual peroxisome proliferator-activated receptor alpha/delta agonist (PPARα/δ agonist), which provided some hopeful results in patients with metabolic syndrome and obesity [89, 90]. Human liver PPARα gene expression negatively correlates with NASH severity, while histological

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improvement is associated with an increase in expression of PPARα and its target genes [91]. In animal models of NAFLD/NASH, elafibranor improved liver histology by both PPAR-α dependent and -independent mechanisms [92]. Subsequently, elafibranor was introduced in a double blind placebo controlled RCT with 276 non-cirrhotic NASH patients in two doses of 80mg and 120mg daily for 52 weeks in order to define histological response. The primary outcome of reversal of NASH without worsening of fibrosis was not achieved in the elafibranor arms compared with placebo. Nevertheless, when a post hoc analysis assessed the response using a modified definition of NASH resolution, the 120mg arm exhibited a significant improvement. Furthermore liver biochemistry, glucose and lipids profiles were reduced as well, without weight gain [93]. An ongoing phase III trial will further evaluate the efficacy and long term safety of elafibranor versus placebo in NASH patients (NCT02704403).

3.0 The future

Future research for effective pharmacotherapy treatment in NAFLD and NASH is challenging. A lot of agents are being investigated and some emerging data from rodent trials are promising. As NASH pathogenesis and progression are illuminated, more potential therapeutic targets emerge [94]. Novel drugs aim at multiple targets and pathways, such as inflammation and oxidative stress, the gut liver axis, the metabolic factor and progression of fibrosis [95].

Aramchol, a fatty acid-bile conjugate is a potential therapeutic agent which seems to reduce liver fat as assessed by magnetic resonance spectroscopy [96]. An ongoing trial is recruiting patients to evaluate the efficacy and safety of two aramchol doses versus placebo in NASH patients (NCT02279524). Volixibat (SHP-626), is an
apical sodium dependent bile acid transporter inhibitor (ASBTi) and is being evaluated in an ongoing phase 2 RTC for 48 weeks. The primary outcome measures the effect of volixibat on liver histology (NCT02787304).

Saroglitazar is a dual PPAR agonist that has demonstrated significant improvement in glycemic and lipid control in diabetic patients and appears to ameliorate aminotransferases in 31 NAFLD patients after 24 weeks [97]. A series of trials from India are evaluating potential benefits from this agent [98]. A RCT evaluated a pradigastat, which is a diacylglycerol acyltransferase-1 inhibitor, in 52 NAFLD patients for 24 weeks with two different doses. Pradigastat improved liver fat after 12 and 24 weeks when administered at the high dose (10/20 mg/day) [99].

An inhibitor of phosphodiesterase-4 (ASP9831) with promising results from a phase 1 trial was evaluated in 93 patients with NASH compared with placebo but no significant change was observed in liver biochemistry after 12 weeks of administration at either of the two investigated doses [100]. In murine models of NASH, a pan-caspase inhibitor, emricasan (IDN-6556) reduced hepatocyte apoptosis and consequently attenuated liver injury and fibrosis [101]. Emricasan significantly reduced transaminases after 28 days in 38 patients with NAFLD [102]. A larger RCT is currently recruiting participants (NCT02686762).

Cenicriviroc, a dual CCR2/CCR5 antagonist is evaluated in a phase 2b randomized, double blind, placebo controlled study with 289 NASH patients for a period of 2 years [103].

GS-6624 (simtuzumab) is a monoclonal antibody against lysyl oxidase-like 2 (LOXL2) and GS-4497 is a molecule that inhibits apoptosis signal-regulating kinase 1 (ASK1) which acts as a mediator of oxidative stress. Both these agents are being
tested in at least three ongoing clinical trials to assess the efficacy of simtuzumab alone or in combination with GS-4997 in patients with NASH with and without cirrhosis. (NCT02466516, NCT01672866, NCT01672879). Finally anti-CD3 antibodies that reduce insulin resistance in animals were included in small trial designs for NASH patients and leaded to positive results demanding further research in the immediate future [104].

4.0 Conclusions

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

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

Moreover, the identification of clinically relevant biomarkers of disease regression that could potentially alleviate the need for serial biopsies and would increase study recruitment is an unmet need. A current innovative medicines initiative call, which represents a collaboration between the European Commission and the European pharmaceutical industry, will hopefully address this issue.
Since NASH has multiple potential pathways of development, combination treatment will also be explored at some point in the future. Ultimately, if there are no large scale interventions from a public health policy perspective to actively implement changes in lifestyle, the toll of morbidity and mortality will continue to rise irrespective of any small scale success in pharmaceutical therapy.

6.0 Five-year view

At the moment, there are promising agents in large scale phase III trials (obeticholic acid, elafibranor) and others in phase II trials (cenicriviroc, simtuzumbab, volixibat) that target different pathways of NASH development and progression. 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. It is also expected that biomarker research will have identified candidate markers that will allow better characterization of patients and reduce the need for a liver biopsy.

7.0 Key issues

* NAFLD has become the most common cause for chronic liver disease in the developed world.

* Dietary and lifestyle interventions provide beneficial results but are difficult to maintain in the long term.

* Although there is no universally approved medical treatment, both the EASL and AASLD guidelines suggest pioglitazone and vitamin E use in selected cases.

* 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.
* New agents as obeticholic acid and elafibranor have shown promising results in phase II and are currently in phase III trials.

* Novel drugs aim at multiple targets and pathways, such as inflammation and oxidative stress, the gut liver axis, the metabolic factor and progression of fibrosis.

* The exploration of surrogate outcomes with clinical relevance, such as quantitative fibrosis assessment using the collagen proportionate area, and biomarkers of disease progression and regression is important.
REFERENCES

Reference annotations

* Of interest

** Of considerable interest


** The European Association for the Study of the Liver (EASL) latest guidelines for an updated management of non-alcoholic fatty liver disease.


URL: https://mc.manuscriptcentral.com/erh Email: Anna.Woolman@informa.com


* Metformin did not significantly improved liver histology in children and adolescents with NAFLD and NASH.


* An important study investigating the role of pioglitazone and vitamin E in NASH.


* Liraglutide can ameliorate liver histology in NASH patients compared with placebo and reduce body weight at the same time.


71. Simon TG, Bonilla H, Yan P, Chung RT, Butt AA. Atorvastatin and fluvastatin are associated with dose-dependent reductions in cirrhosis and HCC, among patients with HCV Results from ERCHIVES. Hepatology. 2016.


82. Han Y, Shi JP, Ma AL, Xu Y, Ding XD, Fan JG. Randomized, vitamin E-controlled trial of bicyclol plus metformin in non-alcoholic fatty liver disease patients with impaired fasting glucose. Clinical drug investigation. 2014;34:1-7.


** The first large RCT assessing the beneficial role of obeticholic acid in NASH patients. Obeticholic acid group showed improved liver histology compared with placebo, including fibrosis, ballooning, steatosis and inflammation.


* The first RCT study with elafibranor in NASH patients failed to achieve the primary outcome of reversal of NASH.

A recent review about new compounds and molecules for NASH treatment based on the different pathogenic pathways.


*Recent developments in the principals of drug approval for NASH through surrogate end points.

* Patients with NASH exhibit increased mortality compared with the general population in Sweden.


111. Wong VW, Wong GL, Chan AW, Chu WC, Choi PC, Chim AM, Yiu KK, Yu J, Chan FK, Chan HL. Treatment of non-alcoholic steatohepatitis with Phyllanthus

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.

<table>
<thead>
<tr>
<th>A/a</th>
<th>Author</th>
<th>Number of patients</th>
<th>Intervention</th>
<th>Duration</th>
<th>Primary Outcome</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ratziu V, 2016 [93]</td>
<td>276 patients</td>
<td>Elafibranor 2:1</td>
<td>52 weeks</td>
<td>Resolution of steatohepatitis without worsening of fibrosis</td>
<td>Predefined end point not met</td>
</tr>
<tr>
<td>2</td>
<td>Armstrong M.J, 2016 [63]</td>
<td>52 patients</td>
<td>Liraglutide (1.8mg) 1:1</td>
<td>48 weeks</td>
<td>Resolution of steatohepatitis without worsening of fibrosis</td>
<td>Significant effect</td>
</tr>
<tr>
<td>3</td>
<td>Neuschwander-Tetri B, 2015 (FLINT) [88]</td>
<td>283 patients</td>
<td>Obeticholic acid (25mg/d) 1:1</td>
<td>72 weeks</td>
<td>Improvement of NAS by at least 2 points, without worsening of fibrosis</td>
<td>Significant effect</td>
</tr>
<tr>
<td>4</td>
<td>Sanyal A, 2014 [76]</td>
<td>243 patients</td>
<td>Ethyl-Eicosapentanoic Acid</td>
<td>12 months</td>
<td>Improvement of NAS, without</td>
<td>No significant effect</td>
</tr>
<tr>
<td>No.</td>
<td>Author(s)</td>
<td>Year</td>
<td>Patients</td>
<td>Intervention</td>
<td>Duration</td>
<td>Outcome</td>
</tr>
<tr>
<td>-----</td>
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</tr>
<tr>
<td>5</td>
<td>Wong VW, 2013 [111]</td>
<td>60</td>
<td>Phyllanthus urinaria (herb) 2:1</td>
<td>24 weeks</td>
<td>Change in NAFLD activity score</td>
<td>No significant effect</td>
</tr>
<tr>
<td>6</td>
<td>Torres D, 2011 [47]</td>
<td>137</td>
<td>Rosiglitazone vs Rosiglitazone &amp; metformin vs rosiglitazone &amp; losartan 1:1:1</td>
<td>12 months</td>
<td>Improvement of steatosis, inflammation and fibrosis</td>
<td>No significant effect</td>
</tr>
<tr>
<td>7</td>
<td>Lavine J, 2011 (TONIC) in children [46]</td>
<td>173</td>
<td>Vitamin E vs. metformin vs. Placebo 1:1:1</td>
<td>96 weeks</td>
<td>Sustained reduction in ALT</td>
<td>No significant effect</td>
</tr>
<tr>
<td>8</td>
<td>Malaguarnera M, 2010 [112]</td>
<td>74</td>
<td>L-Carnitine supplementation to diet 1:1</td>
<td>24 weeks</td>
<td>Liver biochemistry</td>
<td>Significant improvement</td>
</tr>
<tr>
<td>9</td>
<td>Sanyal A, 2010 (PIVENS) [57]</td>
<td>247</td>
<td>Pioglitazone vs Vitamin E vs placebo 1:1:1</td>
<td>96 weeks</td>
<td>Improvement in NAS score</td>
<td>Vitamin E was superior to placebo, no benefit of pioglitazone over placebo</td>
</tr>
<tr>
<td></td>
<td>Name</td>
<td>Number of Patients</td>
<td>Treatment</td>
<td>Duration</td>
<td>Effect</td>
<td>Comment</td>
</tr>
<tr>
<td>---</td>
<td>------</td>
<td>--------------------</td>
<td>-----------</td>
<td>----------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>10</td>
<td>Leuschner U, 2010 [78]</td>
<td>185 patients</td>
<td>Ursodeoxycholic acid 1:1</td>
<td>18 months</td>
<td>An overall improvement of liver histology</td>
<td>No significant effect</td>
</tr>
<tr>
<td>11</td>
<td>Vilar Gomez E, 2009 [113]</td>
<td>60 patients</td>
<td>Nutritional supplement Viusid 1:1</td>
<td>6 months</td>
<td>Improvement in NAS and fibrosis score</td>
<td>Improvement in the NAS score but not in fibrosis</td>
</tr>
<tr>
<td>12</td>
<td>Aithal G.P, 2008 [56]</td>
<td>74 patients</td>
<td>Pioglitazone 1:1</td>
<td>12 months</td>
<td>Reduction in hepatocyte injury and fibrosis</td>
<td>Significant effect</td>
</tr>
<tr>
<td>13</td>
<td>Ratziu V, 2008 (FLIRT) [49]</td>
<td>63 patients</td>
<td>Rosiglitazone</td>
<td>1 year</td>
<td>Improvement of steatosis, necroinflammation &amp; fibrosis</td>
<td>Improved steatosis</td>
</tr>
<tr>
<td>14</td>
<td>Lindor K, 2004 [77]</td>
<td>166 patients</td>
<td>Ursodeoxycholic acid 1:1</td>
<td>2 years</td>
<td>Histological and biochemical improvement</td>
<td>No significant effect</td>
</tr>
</tbody>
</table>
Table 2. Ongoing clinical trials with new pharmacologic agents for patients with non-alcoholic steatohepatitis (clinical trials.gov accessed 20 Oct 2016).

<table>
<thead>
<tr>
<th>A/a</th>
<th>ClinicalTrials.gov Identifier</th>
<th>Phase Stage</th>
<th>Agent</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NCT02704403</td>
<td>Phase III Recruiting</td>
<td>Elafibranor (GFT505)</td>
<td>dual PPARα/δ agonist</td>
</tr>
<tr>
<td>2</td>
<td>NCT02654665</td>
<td>Phase III Recruiting</td>
<td>Liraglutide &amp; bariatric surgery</td>
<td>GLP-1 analogue</td>
</tr>
<tr>
<td>3</td>
<td>NCT02548351</td>
<td>Phase III Recruiting</td>
<td>Obeticholic acid</td>
<td>FXR agonist</td>
</tr>
<tr>
<td>4</td>
<td>NCT02541045</td>
<td>Phase III Recruiting</td>
<td>Metadoxine</td>
<td>Pyridoxine-pyrrolidone carboxylate</td>
</tr>
<tr>
<td>5</td>
<td>NCT02605616</td>
<td>Phase II Recruiting</td>
<td>A novel AZ agent</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>NCT02913105</td>
<td>Phase II Not yet recruiting</td>
<td>LMB763</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>NCT02443116</td>
<td>Phase II Recruiting</td>
<td>NGM282</td>
<td>Recombinant variant of FGF-19 (FGF-19 is a peptide hormone)</td>
</tr>
<tr>
<td>8</td>
<td>NCT02279524</td>
<td>Phase II Recruiting</td>
<td>Aramchol</td>
<td>Conjugate of cholic and arachidic acid</td>
</tr>
<tr>
<td>9</td>
<td>NCT02912260</td>
<td>Phase II Recruiting</td>
<td>MGL-3196</td>
<td>small-molecule liver-directed β-selective THR agonist</td>
</tr>
<tr>
<td>10</td>
<td>NCT02855164</td>
<td>Phase II Recruiting</td>
<td>LJN452</td>
<td>FXR agonist</td>
</tr>
<tr>
<td>11</td>
<td>NCT02854605</td>
<td>Phase II Not yet recruiting</td>
<td>GS-9674</td>
<td>A synthetic non-steroidal FXR agonist</td>
</tr>
<tr>
<td></td>
<td>NCT Number</td>
<td>Phase</td>
<td>Status</td>
<td>Investigational Drug</td>
</tr>
<tr>
<td>---</td>
<td>-----------------</td>
<td>--------</td>
<td>-------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>12</td>
<td>NCT02927314</td>
<td>Phase II</td>
<td>Not yet recruiting</td>
<td>CF102</td>
</tr>
<tr>
<td>13</td>
<td>NCT02856555</td>
<td>Phase II</td>
<td>Recruiting</td>
<td>GS-0976</td>
</tr>
<tr>
<td>14</td>
<td>NCT02574325</td>
<td>Phase II</td>
<td>Ongoing but not recruiting</td>
<td>Niacin/ARI-3037MO</td>
</tr>
<tr>
<td>15</td>
<td>NCT02413372</td>
<td>Phase II</td>
<td>Ongoing but not recruiting</td>
<td>BMS-986036</td>
</tr>
<tr>
<td>16</td>
<td>NCT02217475</td>
<td>Phase II</td>
<td>Ongoing but not recruiting</td>
<td>Cenicriviroc</td>
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<tr>
<td>17</td>
<td>NCT02686762</td>
<td>Phase II</td>
<td>Recruiting</td>
<td>Emricasan</td>
</tr>
<tr>
<td>18</td>
<td>NCT02466516</td>
<td>Phase II</td>
<td>Ongoing but not recruiting</td>
<td>GS-4997 alone or with simtuzumab</td>
</tr>
<tr>
<td>19</td>
<td>NCT02421094</td>
<td>Phase II</td>
<td>Ongoing but not recruiting</td>
<td>GR-MD-02</td>
</tr>
<tr>
<td>20</td>
<td>NCT02316717</td>
<td>Phase II</td>
<td>Recruiting</td>
<td>IMM-124E</td>
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<tr>
<td>21</td>
<td>NCT02784444</td>
<td>Phase II</td>
<td>Recruiting</td>
<td>MSDC 0602K</td>
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<tr>
<td>22</td>
<td>NCT02442687</td>
<td>Phase II</td>
<td>Recruiting</td>
<td>JKB-121</td>
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<tr>
<td></td>
<td>NCT</td>
<td>Phase</td>
<td>Status</td>
<td>Study Arm</td>
</tr>
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<td>-------</td>
<td>---------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>23</td>
<td>NCT01679197</td>
<td>Phase II</td>
<td>Ongoing but not recruiting</td>
<td>Metreleptin</td>
</tr>
<tr>
<td>24</td>
<td>NCT02681055</td>
<td>Phase II</td>
<td>Recruiting</td>
<td>MN-001/Tipelukast</td>
</tr>
<tr>
<td>25</td>
<td>NCT02510599</td>
<td>Phase II</td>
<td>Recruiting</td>
<td>CEM-101/Solithromycin</td>
</tr>
<tr>
<td>26</td>
<td>NCT01919294</td>
<td>Phase II</td>
<td>Recruiting</td>
<td>Testosterone replacement</td>
</tr>
<tr>
<td>27</td>
<td>NCT01672879</td>
<td>Phase II</td>
<td>Ongoing but not recruiting</td>
<td>GS-6624/Simtuzumab</td>
</tr>
<tr>
<td>28</td>
<td>NCT01672866</td>
<td>Phase II</td>
<td>Ongoing but not recruiting</td>
<td>GS-6624/Simtuzumab</td>
</tr>
<tr>
<td>29</td>
<td>NCT02923154</td>
<td>Phase II</td>
<td>Recruiting</td>
<td>MT-3995</td>
</tr>
<tr>
<td>30</td>
<td>NCT02612662</td>
<td>Phase I</td>
<td>Recruiting</td>
<td>AZD4076/RG-125 (tetracosasodium)</td>
</tr>
<tr>
<td>31</td>
<td>NCT02469272</td>
<td>Phase I</td>
<td>Recruiting</td>
<td>Fecal microbiota transplantation</td>
</tr>
<tr>
<td>32</td>
<td>NCT02721264</td>
<td>Recruiting</td>
<td>Fecal microbiota therapy</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>NCT02196831</td>
<td>Recruiting</td>
<td>Tesamorelin</td>
<td>GHRH analogue</td>
</tr>
</tbody>
</table>

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