Review Article

Nonalcoholic fatty liver disease: current therapies and future perspectives in drug delivery

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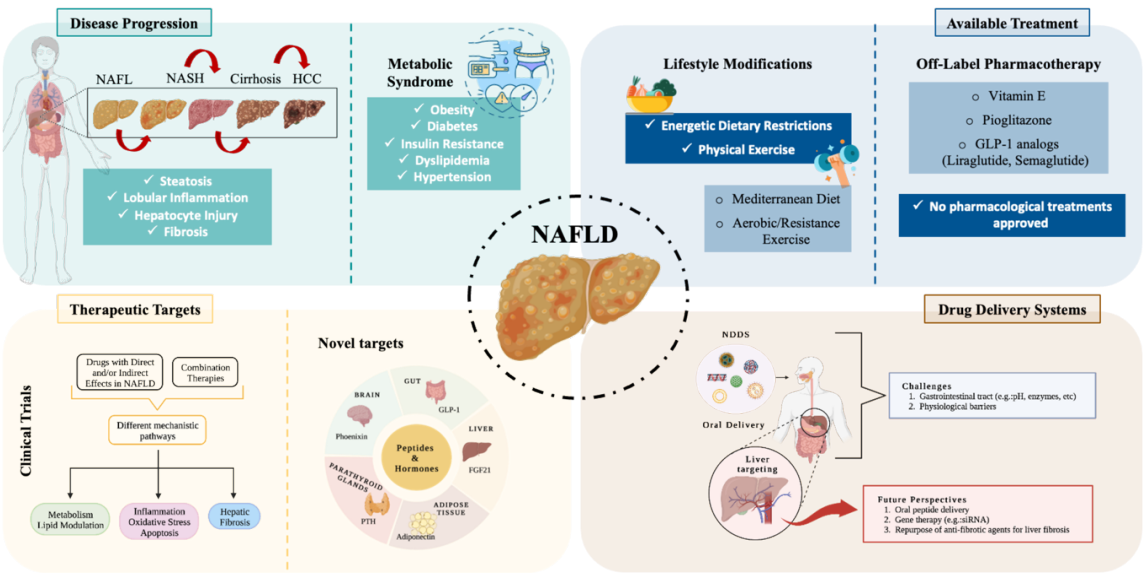
ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) affects approximately 25% of the adult population worldwide. This pathology can progress into end-stage liver disease with life-threatening complications, and yet no pharmacologic therapy has been approved. NAFLD is commonly characterized by excessive fat accumulation in the liver and is in closely associated with insulin resistance and metabolic disorders, which suggests that NAFLD is the hepatic manifestation of metabolic syndrome. Regarding treatment options, the current validated strategy relies on lifestyle modifications (exercise and diet restrictions). Although there are no approved drug-based treatments, several clinical trials are ongoing. Novel targets are being discovered, and the repurposing of drugs that show promising effects in NAFLD is starting to gain more interest. The field of nanotechnology has been growing at an increasing rate, with new and more efficient drug delivery strategies being developed for NAFLD treatment. Nanocarriers can easily encapsulate drugs that need to be better protected from the organism to exert their effect or that need help at reaching their target, thereby helping achieve a better bioavailability. Drug delivery systems can also be designed to target the site of the disease, in this case, the liver. In this review, we focus on the current knowledge of NAFLD pathology, the targets being considered for clinical trials, and the current guidelines and ongoing clinical trials, with a specific focus on potential oral treatments for NAFLD using promising drug delivery strategies.

Keywords: NAFLD, NASH, Peptides, Oral drug delivery

GRAPHICAL ABSTRACT



1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is commonly characterized by excessive fat accumulation in the liver not due to secondary causes of hepatic steatosis (e.g., steatogenic medication, monogenic hereditary diseases, autoimmune liver diseases, hepatitis B and C, excessive alcohol consumption, among others). NAFLD is closely associated with insulin resistance and metabolic abnormalities. [1-3] The spectrum of NAFLD mainly comprises two conditions defined by the presence of several distinct histological findings. Nonalcoholic fatty liver (NAFL) is considered the first stage and is mostly characterized by more than 5% of hepatocytes containing lipid droplets, with or without inflammation. Nonalcoholic steatohepatitis (NASH) requires the presence of hepatic steatosis, lobular inflammation, and hepatocyte injury with or without fibrosis. The evolution of NAFL into NASH increases the risk of disease progression into even more advanced stages, including the development of cirrhosis, hepatocellular carcinoma (HCC), and end-stage liver disease. NAFLD affects both adults and children, although the histological features may differ between these two populations. Imaging techniques, such as ultrasounds or magnetic resonance imaging-derived proton density fat fraction (MRI-PDFF), are helpful for identifying steatosis. Similarly, noninvasive techniques (FibroScan® or MRI elastography) faithfully detect significant fibrosis associated with severe disease. Liver biopsy is needed to diagnose NASH. Liver biopsy is still regarded as the gold standard to differentiate between simple steatosis and steatohepatitis [1-4], although it is an invasive procedure involving errors concerning sampling and interpretation.

The prevalence of NAFLD varies according to the diagnostic method, age, sex, and ethnicity. Based on an imaging diagnosis, it affects approximately 25% of the adult global population, and the overall prevalence of NASH in biopsied NAFL patients is approximately 59%. [1, 5] NAFLD is becoming one of the most common indications for liver transplantation and is one of its fastest-growing causes. [6] Despite NAFLD's slow development, as the disease evolves, the liver condition deteriorates faster, especially in the fibrotic stage, with 20% of patients having an accelerated progression. [3]

NAFLD is tightly associated with metabolic disorders and metabolic syndrome (MetS), which can be defined when presenting three or more of the following five features: increased waist circumference, high blood pressure, impaired fasting glycemia, hypertriglyceridemia, and decreased high-density lipoprotein (HDL) levels. MetS is one of the risk factors for developing NAFLD and vice versa. Due to their close association, NAFLD is considered as a potential hepatic manifestation of MetS. [1-3, 7] This fact can be observed by the higher

prevalence of this chronic liver disease in patients with comorbidities related to MetS, such as obesity and type 2 diabetes mellitus (T2DM). In a meta-analysis conducted using PubMed and MEDLINE databases (1989-2015), the general prevalence of obesity, diabetes, dyslipidemia, hypertriglyceridemia, hypertension and MetS in patients with NAFL was 51%, 23%, 69%, 41%, 39%, and 43%, respectively. In NASH patients, the overall prevalence for the same metabolic disorders was found to be almost double in some cases, with 81%, 44%, 72%, 83%, 68%, and 71%, respectively. [5] Despite this close relationship with MetS, NAFLD can also affect people with an average body mass index (BMI), defining this pathogenesis as "lean" NAFLD. Despite the presence of normal weight, patients still have associated metabolic abnormalities and complications and can display insulin resistance and altered body fat distribution, affecting approximately 4.1% of the general population and 9.7% of the overall lean population. [1, 8]

There is an unmet therapeutic need for NAFLD treatment, which is currently solely relies on lifestyle modifications (exercise and diet restrictions) as the only validated strategy approved. This approach has been shown to significantly improve all features of NAFLD; however, a 'healthy' lifestyle is not easily maintained in the long run, and not all patients comply with the recommended guidelines, which is necessary for the overall improvement of NAFLD. Although no approved pharmacological treatments are available, several compounds are being evaluated in ongoing phase II and phase III clinical trials. When needed, off-label medications (e.g., pioglitazone, vitamin E, liraglutide, etc.) can be used in specific situations and in defined subgroups of patients suffering from NAFLD (e.g., obese-NAFLD, T2DM-NAFLD, among others). [1-3, 9]

A new nomenclature has been recently proposed, with metabolic dysfunction-associated steatotic liver (MASLD) replacing NAFLD and metabolic dysfunction-associated steatohepatitis (MASH) replacing NASH. This change was implemented due to problems with accurately capturing the etiology of the disease and in stigmatizing language ("nonalcoholic" and "fatty"). With this new definition, there is also the inclusion of at least one of five cardiometabolic risk factors. [10] For the sake of this review, the old terminology will still be used due to its recent alteration and to avoid confusion.

This review will focus on the current knowledge of NAFLD pathology, the identified therapeutic targets, current guidelines for therapy and ongoing clinical trials with a specific focus on promising oral drug delivery strategies.

2. PATHOGENESIS OF NAFLD

Over two decades ago, a “two hit” theory for the pathogenesis of NAFLD was proposed. The “first hit” was considered the increase in fat accumulation in the liver. Over time, the liver would be sensitized to further injuries leading to the induction of stress, inflammation and its progression into NASH (“second hit”). [11] Currently, that hypothesis is considered too simplistic due to the growing knowledge of NAFLD pathogenesis and due to the large heterogeneity of NAFLD and NAFLD patients. A “multiple hits” theory was proposed in which a myriad of factors are considered to contribute to the disease's initial state and progression although the relative importance and hierarchy of such factors has not yet been established yet (Figure 1). [12] Lifestyle, environmental and genetic factors are included because they promote insulin resistance, MetS, the presence of T2DM, obesity, hypertension, and gut dysbiosis. Specific genetic hits, such as polymorphisms in the patatin-like phospholipase domain containing 3 (PNPLA3) gene, have been associated with the incidence of steatosis and disease severity. [13] Other polymorphisms have also been identified (e.g., TM6SF2), and carriers of these genes have a higher predisposition to NAFLD development. [13]

Lipid deposition in the liver is a normal metabolic process. However, it can reach undesired concentrations, leading to hepatic steatosis. There are three sources of free fatty acids (FFAs) First, in the adipose tissue, from which FFAs and glycerol are broken down from stored triglycerides (TGs), are released into the bloodstream and taken up by the liver. Second, *de novo* lipogenesis is a pathway that generates lipids from carbohydrates within hepatocytes. This pathway has been shown to be increased in NAFLD patients compared to normal patients. [14-16] Third, FFA is provided to the liver via the gut-liver axis from the diet. [7, 17-19] This increase in fat storage favors lipotoxicity due to a higher concentration of toxic lipids, such as saturated fatty acids, free cholesterol, and ceramides, and can promote endoplasmic reticulum stress, oxidative stress due to mitochondrial dysfunction, defective autophagy, lipoapoptosis, and inflammation. These processes are, in part, mediated by the activation of the c-Jun NH_2-terminal kinase pathway and by inflammasomes, which are multiprotein receptors belonging to the innate immune system and capable of inducing inflammatory processes. [20-24]

Consequent cell injury leads to the release of stress signals that drive the secretion of inflammatory mediators and the recruitment of immune cells to the liver. For instance, both in mice and humans, the expression of vascular cell adhesion molecule 1 (VCAM-1) is overexpressed in the liver sinusoidal endothelial cells, which is induced by the presence of toxic lipids, increasing the pool of monocyte-derived macrophages that infiltrate the liver. [24-

[27] Due to this increased expression, the molecular imaging of VCAM-1 could possibly be utilized as a noninvasive method to detect the presence of inflammation preceding the development of fibrosis. [28]

Insulin resistance is tightly related to the development and progression of NAFLD. Insulin favors glucose uptake by peripheral tissues, inhibits hepatic glucose production, favors lipid storage in adipose tissue, and favors lipogenesis, TG synthesis and packaging in the liver and exportation as very-low-density lipoproteins (VLDL).[7] In obesity and conditions of systemic low-grade inflammation, the liver, adipose tissue and muscle may become resistant to insulin. In the presence of inflamed adipose tissue and insulin resistance, inflammatory mediators and FFAs are released, which reach the liver and drive the inflammation and fat accumulation process. This has been established and observed in humans, where metabolically healthy obese patients with normal insulin sensitivity in the adipose tissue do not normally develop hepatic steatosis. However, a slight increase in adipose tissue insulin resistance leads to a rapid increase in liver aminotransferases, circulating lipids, liver and muscle insulin resistance, hepatic steatosis, and NASH secondary to lipotoxicity. [29, 30] Furthermore, an imbalance in adipokine secretion has been demonstrated to occur with lower levels of adiponectin and higher levels of leptin present in NAFLD patients. [31] The idea of an adipose tissue-liver axis, where the degree of inflammation shown in the adipose tissue positively correlates with the inflammation in the liver, has been proposed. [32, 33]

The gut-liver axis plays an important role in NAFLD, and the gut microbiota has been shown to significantly impact the trajectory of NAFLD development, representing an important factor in disease progression. It has been shown that NASH patients have different gut microbiota signatures when compared to those of healthy subjects and that overnutrition changes the intestinal barrier, leading to an increase in endotoxins able to reach the liver via the portal system. This increase in endotoxins further fuels inflammation mostly through the interactions with Toll-like receptors. [34-36]

Oxidative stress, inflammatory mediators and damaged hepatocytes in turn activate the once quiescent hepatic stellate cells (HSCs) to drive fibrogenesis in the liver. For instance, the most potent cytokine with profibrogenic properties is transforming growth factor β (TGFβ), which is produced by several hepatic cell populations and interacts with the integrin αV receptors expressed in HSCs. [37] Other mediators are involved in fibrosis development, such as platelet-derived growth factor. [38-40]

Therapeutic interventions that target impaired lipid metabolism, lipotoxicity or its downstream signals, inflammation and/or fibrosis, directly or indirectly, would eventually lead to the development and progression of NAFLD.

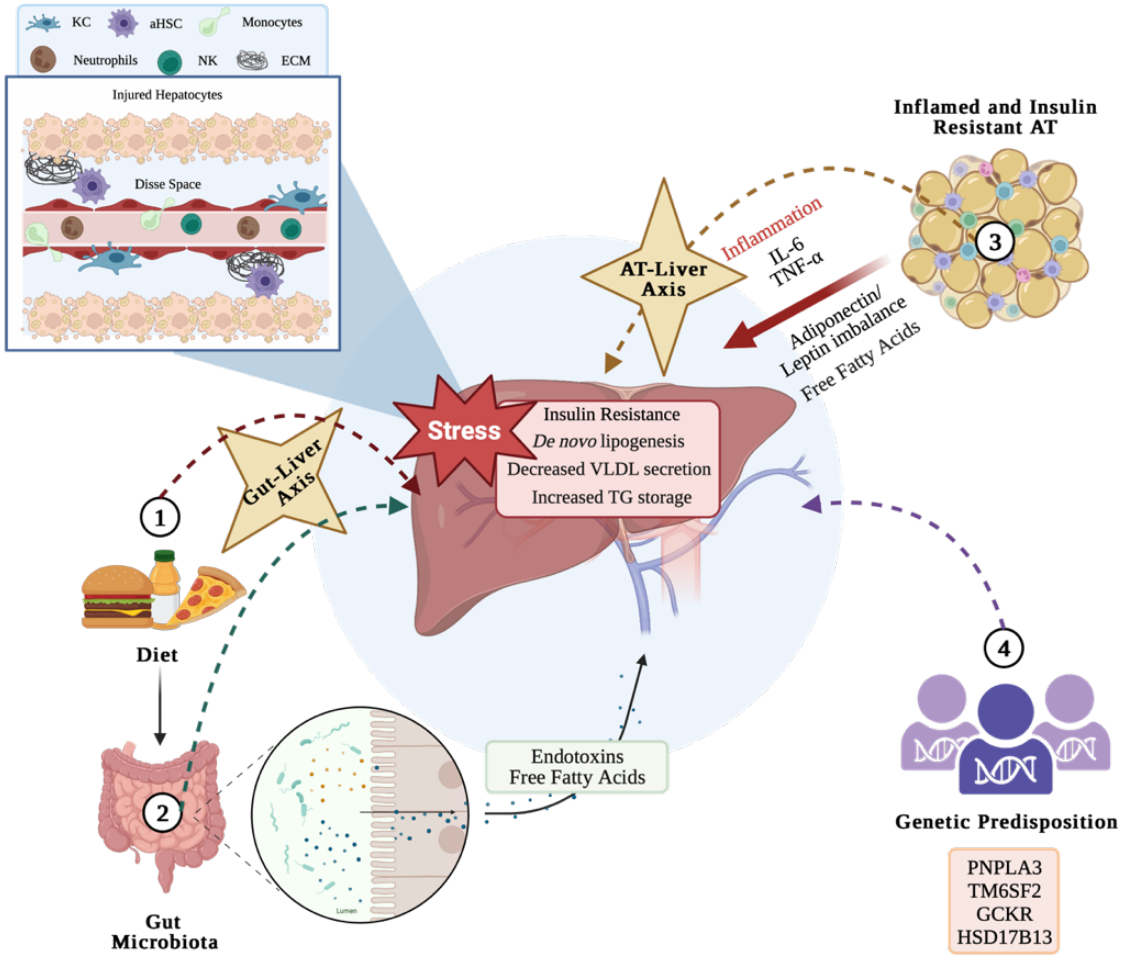


Figure 1: A simplified representation of the multiple hit theory in NAFLD. (1) The source of fatty acids to the liver is provided by the diet, which can also interfere with the intestinal epithelium and gut microbiota; (2) gut dysbiosis, increased absorption of endotoxins, and increased inflammation in the liver; (3) inflamed and insulin-resistant adipose tissue further increases the uptake of fatty acids in the liver and the inflammatory state; and (4) genetic predispositions of individuals with genetic polymorphisms further increase the probability of developing NAFLD. Abbreviations: NAFLD, non-alcoholic fatty liver disease; KC, Kupffer cells; αHSC, activated hepatic stellate cells; NK, natural killer cells; ECM, extracellular matrix; AT, adipose tissue; VLDL, very-low-density lipoproteins; TG, triglycerides; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; PNPLA3, patatin-like phospholipase domain-containing 3; TM6SF2, transmembrane 6 superfamily 2; GCKR, glucokinase regulatory protein-encoding gene; HSD17B13, hydroxysteroid 17-beta dehydrogenase 13. Created by Biorender.com

3. CURRENT APPROACHES IN NAFLD TREATMENT

3.1. Lifestyle modifications

To date, the only validated NAFLD treatment to date that has shown results at improving the outcome of NAFLD is the modification of the lifestyle with the establishment of healthy eating habits and the promotion of physical activity. Together, these two strategies lead to

weight loss, which is important for NAFLD resolution, and has been shown to reverse the disease condition when the weight loss is between 7 and 10%. [1, 2, 9]

Current guidelines do not recommend a particular type of diet; however, energy restriction and the avoidance of processed food (rich in fat, sugar, and fructose) and sweetened beverages high in fructose are highly recommended. [1, 2, 9] Several studies highlight the impact of Mediterranean diet (MED), low carbohydrate (carb) diets, at helping to reduce hepatic steatosis, [41, 42] likely contributed to by reduced *de novo* lipogenesis pathway, increased β-oxidation and modification of the gut microbiota. [43] More recently, a clinical study was conducted in which MED was compared to a green-MED enriched in plant-based products, phytocompounds and walnuts and poor in red meat. A higher weight loss was reached with the green-MED when compared to that with MED, showing the potential positive impact, in NAFLD patients, of plant-based products in the reduction of the hepatic fat content in NAFLD patients. [44]

Regarding physical activity, different types of exercise, duration time, and intensity, with or without dietary changes in both mice and humans, have been studied. Both resistance and aerobic/high intensity training ameliorated NAFLD, independent of weight loss. [45-48] According to the most recent clinical guidelines, approximately 150 min/week and/or aerobic/resistance training is recommended. [1, 2, 9] However, the choice regarding which type of physical activity, duration and intensity should be tailored to each individual patient to increase adherence in the long term. [49]

Overall, an equilibrated diet, MED with low carb and potentially plant-based, along with 150 min/week of exercise would be favorable for improving in NAFLD symptoms.

3.2. Off-label pharmacotherapy

Based on ongoing and completed clinical trials, certain drugs are prescribed off-label to certain subpopulations of NAFLD patients, such as “obese-NAFLD”, “T2DM-NAFLD” and “lean-NAFLD” patients. Recommendations in clinical guidelines from Europe, Asia, and America are not always concordant. According to the European guidelines, pharmacotherapy should be reserved for patients with NASH, particularly those with significant fibrosis or patients with less severe disease but at a high risk of disease progression (e.g., associated comorbidities). [1] Considering the pathogenesis of NAFLD (Section 2), there are different existing therapeutics that can be considered potential treatments for NAFLD. Such options can be used off-label according to the clinical guidelines for the management of NAFLD consisting mostly of drugs approved for other pathologies closely related to NAFLD. Through the

amelioration of those comorbidities, we reduce the cardiovascular risk, having a positive effect on the liver and NAFLD progression.

According to two guidelines, statins can be safely used in patients with NASH to reduce low-density lipoprotein-cholesterol (LDL-cholesterol) and prevent the risk of developing cardiovascular complications, but only in NASH patients with dyslipidemia.[2] The effects of statins on NAFLD are due to their action on cholesterol synthesis through the inhibition of HMG-CoA.[50] Pioglitazone, an insulin sensitizer used for the treatment of T2DM, can be used in diabetic or nondiabetic NALFD patients with biopsy-proven NASH. [1, 2, 9, 51] PPARα is a master regulator of lipid metabolism; therefore, its activation increases fat combustion. This is true in rodents, where β-oxidation is quasi exclusively under the control of PPARα which is not the case in humans. Studies have revealed that it might be helpful to screen for PPARα expression to select subjects who might be more likely to respond to these agonist drugs.[52] PPARδ is mainly expressed in the liver, muscle, and brown adipose tissue, and it increases mitochondrial biogenesis and thus the capacity to burn fat for metabolic demand.[52] Dual-PPAR or pan-PPAR agonists seem to be more effective, as clinical trials are focusing more on these types of agonists.[52] Vitamin E can be used in the short term to treat nondiabetic, noncirrhotic NAFLD patients. [1, 2, 9] Although some of the results in clinical trials are controversial, vitamin E has been shown to have histological benefits in patients with NAFLD. [2] Although not mentioned in all clinical guidelines, glucagon-like peptide-1 receptor agonists (GLP1-RAs), such as liraglutide and semaglutide, can be a possible off-label recommendation and could potentially be used in diabetic/obese NAFLD patients due to their protective cardiovascular effects. [9, 51] Although the use of certain drugs is only recommended in particular cases, there is still missing information related to their overall efficacy and safety in NAFLD patients. Before starting any treatment, it is always necessary to fully weigh the advantages that a given treatment might provide as well as its possible side effects in short- and/or long-term use.

3.3. Pharmacological treatments in phase II/III

Due to the lack of therapeutic options approved for NAFLD, multiple drugs have been and/or are being tested in clinical trials. Whether they are already approved drugs for a different pathological application, new drugs, or new drug delivery systems, the use of these drugs in NAFLD is a “hot topic” due to its prevalence and risk factors, as discussed previously. Based on NAFLD pathogenesis, there are a myriad of targets to treat the disease. In this section, an

exhaustive overview of the current and future clinical trials being carried out in the context of NAFLD (Phase II and III) will be discussed alongside its mechanistic target (Tables 1 and 2).

3.3.1. Targeting metabolism/lipid modulation

Peroxisome proliferator-activated receptor (PPAR) agonists are composed of three different isoforms (α, β/δ and γ) and help, via different pathways, regulate the metabolism of lipids and carbohydrates. In the context of NAFLD, PPARα can improve lipid metabolism through regulation of fatty acid transport and β-oxidation. [53] PPARα has an impact on inflammation by acting on hepatocytes and reducing intestinal permeability and is involved in decreasing portal pressure (cirrhosis) and reducing insulin resistance in skeletal muscle. [54, 55] The second isoform, PPARβ/δ, acts by inhibiting inflammatory macrophages and favoring the anti-inflammatory phenotype; it also stimulates fat burning within brown adipose tissue. [56] PPARγ is involved in regulating insulin sensitivity within adipose tissue and is involved in the prevention of HSC activation. [57] [54] Lanifibranor, a pan-PPAR agonist (an agonist of all 3 isoforms), has been tested in clinical trials, and the steatosis-activity-fibrosis (SAF) score was evaluated. The SAF score consists of separately assessing steatosis by the activity of the disease (lobular inflammation and ballooning), enabling the differentiation from simple steatosis to steatohepatitis and ranging from 0 to 4. A decrease of at least 2 points was one of the endpoints of this trial, and it was obtained in 55% of the patients receiving a high dose compared to 33% in the placebo arm, supporting the entry of lanifibranor in phase III clinical trials [58] (currently in the recruiting phase) with the primary end point of NASH resolution and fibrosis improvement (ClinicalTrials.gov, number NCT04849728). Other PPAR agonists currently in the recruiting phase for phase II clinical trials are saroglitazar (ClinicalTrials.gov, number NCT05011305) and pioglitazone (ClinicalTrials.gov, number NCT04501406), both dual-PPAR agonists (PPARα/γ).

Farnesoid X receptor (FXR) is expressed in the liver and small intestine with actions in bile acid and glucose homeostasis, lipid metabolism, and gut microbiota, among others. [53] The actions of FXR in NAFLD reflect its effects on lipogenesis and β-oxidation, reducing FFA uptake and VDLD secretion in the liver. [53, 59, 60] In a multicenter, randomized, placebo-controlled trial, obeticholic acid (OCA), an FXR agonist, improved the histological features of NAFLD, but its long-term benefits and safety need to be further assessed. Owing to these observations, a phase III clinical trial is in its recruiting phase where the aim is to evaluate the effect of OCA on mortality, liver-related clinical outcomes, and long-term safety (ClinicalTrials.gov, number NCT02548351). [61, 62] EDP-305 is another FXR agonist in

development. It reduced alanine transaminase (ALT) levels and liver fat content, prompting a longer-term trial with histological endpoints in patients with NASH [63] (recruitment phase for Phase II clinical trials - ClinicalTrials.gov, number NCT04378010). Bile acid derivatives, such as norursodeoxycholic acid (NorUDCA) are also being exploited in the treatment of NAFLD. NorUDCA is orally administered and is a side chain-shortened homolog of ursodeoxycholic acid (UDCA) that undergoes hepatic enrichment with hepatoprotective, anti-inflammatory, and antifibrotic activity. The efficacy study of two doses of NorUDCA versus placebo for the treatment of NAFLD was concluded, and NorUDCA at 1,500 mg resulted in a significant reduction in serum ALT within 12 weeks of treatment when compared with that from the placebo. This dose was considered safe and well tolerated, which encouraged further studies (ClinicalTrials.gov, number NCT05083390). [64] HTD1801 is an ionic salt of berberine and UDCA, and it has shown effects on hepatic lipid reduction and an improvement in glycemia control alongside weight loss and reduced enzymatic levels.[65] A new phase II clinical trial is recruiting patients with the primary endpoint being the decrease of at least 2 points in the NAFLD activity score (NAS) or resolution of NASH without worsening of fibrosis (ClinicalTrials.gov, number NCT05623189). The NAS score sums up the degrees of steatosis, lobular inflammation, and ballooning ranging from 0-8. Fibrosis degree is measured separately and can be added to the score (0-4), and its purpose is to evaluate histological changes during clinical trials.

Fibroblast growth factor 21 (FGF21), is a hepatokine that elicits pleiotropic metabolic actions**.** [66] Several FGF receptors exist; however, FGF21 exerts its effects by binding to FGF receptor 1c in the presence of its coreceptor β-Klotho. Its activity is thus restricted to the tissues where both receptors and coreceptors are co-expressed (adipose tissue, liver, pancreas, and CNS). FGF21 is produced mainly in the liver but also in adipose tissue and the pancreas, and its levels are increased in stress-inducing states such as metabolic abnormalities. Increased circulatory values of this hormone have been reported in metabolic-related pathologies such as T2DM, obesity and NAFLD. These increased concentrations do not seem to have any beneficial impact, suggesting the potential presence of resistance to FGF21. Under normal conditions, FGF21 promotes ketogenesis, inhibits growth hormone signaling, and stimulates glucose uptake in adipose tissue and β-cell proliferation, restoring insulin synthesis. [53, 67-69]. Due to its mechanism of action, FGF21 analogs have been and/or are being developed and studied in NAFLD. In ongoing phase II clinical trials, efruxifermin, a fusion protein of the human immunoglobulin G1 fragment crystallizable domain (IgG1 Fc) with a modified human FGF21 having affinity for all three coreceptor complexes, (ClinicalTrials.gov, number

NCT05039450 and NCT04767529), has met the primary end point of reduction in the hepatic fat fraction by week 12 of treatment with good tolerance. [70] Other FGF21 analogs currently studied are BIO89-100, a glycoPEGylated FGF21 [71] (ClinicalTrials.gov, number NCT04929483), and analogs targeting only 1 coreceptor complex (FGFR1c/KLB), such as MK-3655 (ClinicalTrials.gov, number NCT04583423) and BFKB8488A (ClinicalTrials.gov, number NCT04171765).

Thyroid hormone receptors (THRs) are activated by thyroid hormones and play a role in increasing energy expenditure by exerting catabolic activity. [72] Thyroid hormones activate the β-receptor (THR-β) in hepatocytes, with key effects on mitochondria. [60] In NAFLD, the inflammatory process can interfere with the levels of these hormones, with low concentrations in NAFLD patients being observed. [60, 72, 73] THRβ agonists, such as resmetirom, have shown a positive impact on the reduction of liver fat as well as the reduction in liver markers related to inflammation and fibrosis, [74] granting the opportunity for a phase III clinical study (ClinicalTrials.gov, number NCT03900429). Other THRβ agonists are also being investigated in phase II clinical trials, such as ASC41 (ClinicalTrials.gov, number NCT05462353), TERN-501 (ClinicalTrials.gov, number, NCT05415722) and VK2809, a prodrug that exhibited promising effects on liver fat content and LDL cholesterol [72] (ClinicalTrials.gov, number NCT04173065). More recently, a new highly potent THR agonist that resulted from the cyclization of resmetirom was discovered, and its further optimization allowed us to obtain a higher selectivity for the THR-β receptor with reduced systemic exposure and a higher ratio of liver to blood. This compound, which is similar to resmetirom, showed beneficial effects and the improvement of steatotic, inflammatory and fibrotic states. [75]

Metabolic inhibitors of enzymes such as acetyl-CoA carboxylase (ACC), diacylglycerol acyltransferase 2 (DGAT2) and fatty acid synthase (FAS) act on liver lipid metabolism. More specifically, the steps related to the *de novo* lipogenesis pathway that convert dietary carbs into triglycerides that can be stored in lipid droplets in hepatocytes. [76] More specifically, FAS has been shown to be increased in NAFLD patients and is involved in the production of palmitate, a saturated fatty acid involved in the development of steatosis. Palmitate, a substrate used to synthetize proinflammatory and fibrogenic mediators, is also involved in the activation of HSCs, helping the progression of the disease and the development of lipotoxicity with the activation of the inflammasome. [77] Studies in preclinical and clinical trials evaluating TVB-2640, an FAS inhibitor, demonstrated reduced hepatic steatosis, along with reduced serum markers of liver injury, inflammation, and fibrosis. [77, 78] TVB-2640 is in ongoing phase II

clinical trials with the primary endpoint of NASH resolution without worsening of fibrosis. (ClinicalTrials.gov, number NCT04906421).

Sodium-glucose cotransport 2 (SGLT2) inhibitors are a class of drugs already used for the treatment of T2DM and are involved in suppressing the reabsorption of glucose on the proximal tubule of the nephrons in the kidneys, promoting urinary glucose excretion. Preclinical studies have shown that SGLT2 inhibition decreases liver lipid accumulation and reduces body weight and fat mass by promoting the use of fatty acids instead of glucose as an energy source. [79] Dapagliflozin is currently being tested in NAFLD in a phase III clinical trial (ClinicalTrials.gov, number NCT03723252). Previous clinical studies showed an effect on reducing hepatic fat but with discordant results regarding the effect of dapagliflozin on adipocytokine concentrations (e.g., adiponectin, leptin, TNF-α). [79]

Incretin mimetics, such as glucagon-like peptide 1 receptor agonists (GLP-1RAs) reduce liver lipids through indirect and direct mechanisms involving multiple organs in the body. [80] The activation of GLP-1 receptors (GLP-1R) in neurons reduced food intake and caused weight loss, contributing to the reduction in liver lipids. GLP-1RA also potentiates insulin secretion from pancreatic β cells. Although there is no GLP-1R expression in enterocytes and hepatocytes, GLP-1RAs indirectly reduce hepatic glucose production, *de novo* lipogenesis, TG secretion, gut chylomicron synthesis, and gut motility while increasing liver glucose uptake due to their direct action on insulin and glucagon signaling, helping maintain liver homeostasis. [81, 82] GLP-1RA administered as a subcutaneous injection is a promising therapeutic approach. For example, liraglutide (ClinicalTrials.gov, number NCT01237119) [83] and semaglutide (ClinicalTrials.gov, number NCT02970942) [84] have both completed phase II clinical trials and have met their primary endpoint of NASH resolution without worsening fibrosis. A phase III clinical trial for semaglutide has already been launched (ClinicalTrials.gov, number NCT04822181). Furthermore, orally administered semaglutide will be used in a phase IV clinical trial, that is currently recruiting to assess its impact in liver transplant patients with poorly controlled diabetes. Although it is not focused on NAFLD, having NASH is not an exclusion criterion, and liver parameters will be assessed as well (ClinicalTrials.gov, number NCT05195944). Another clinical trial using Rybelsus® is evaluating its effect in patients with polycystic ovary syndrome, with the primary endpoint being related to the reduction in the hepatic fat fraction over the 12 weeks of treatment (ClinicalTrials.gov, number NCT03919929). More recently, incretin combination therapies with dual or triple agonists have been developed to target not only GLP-1R but also glucagon G-protein coupled receptor

(GCGR) and gastric inhibitory polypeptide receptor (GIPR). Currently, there are two dual agonists for GCGR and GLP-1R in clinical trials, namely, cotadutide (ClinicalTrials.gov, number NCT05364931 and NCT05364931) [85] and BI456906 (ClinicalTrials.gov, number NCT04771273), and one dual agonist for GIPR and GLP-1R, namely tirzepatide (ClinicalTrials.gov, number NCT04166773). [86, 87]

Another example of a promising treatment for NAFLD acting on lipid metabolism is bempedoic acid, which is currently approved for the treatment of heterozygous familial hypercholesterolemia and atherosclerotic cardiovascular disease.[88] By activating adenosine monophosphate-activated protein kinase (AMPK) and inhibiting ATP citrate lyase (ACLY), bempedoic acid regulates of lipid, carbohydrate, and energy metabolism.[89] Due to its action in reducing hepatic steatosis, bempedoic acid is a promising candidate for NAFLD treatment. Several animal studies showed reduced lipogenesis, gluconeogenesis, dyslipidemia, and HSC activation/proliferation, consequently reducing NASH and fibrosis in mice treated with bempedoic acid.[88, 90] There are no ongoing or available trials in the context of NAFLD; however, there are several recruiting trials in the context of dyslipidemia (ClinicalTrials.gov, number NCT05488431, NCT05694260, NCT05687071, NCT05683340).

3.3.2. Targeting Inflammation/Oxidative Stress/Apoptosis

Icosabutate is an engineered omega-3 fatty acid that has demonstrated its efficacy in preclinical studies in mice in the context of NAFLD. [91] The treatment of NAFLD with omega-3 fatty acids has shown contradictory results in human trials that could be due to their peroxidation susceptibility (Section 6). Icosabutate can escape incorporation into complex lipids and resist β-oxidation, which does not happen with eicosapentaenoic acid (EPA). Icosabutate fully activates the G protein coupled receptor 120 (GPR-120), which is highly expressed in Kupffer cells/macrophages, and mediates potent anti-inflammatory and insulin sensitizing effects. Icosabutate is currently under investigation in a phase II clinical trial (ClinicalTrials.gov, number NCT04052516). [92-94]

Aspirin is a well-known drug with antipyretic and analgesic effects due to the inhibition of cyclooxygenase 2 (COX2), which is responsible for the conversion of arachidonic acid into prostaglandins, inflammatory mediators, resulting in the inhibition of the inflammatory process. Recent data suggest that aspirin is a promising antifibrotic agent for NAFLD because it limits HSC activation through the suppression of the proinflammatory COX2 enzyme and the platelet-derived growth factor (PDGF) signaling pathway. [95, 96] Aspirin is being tested in

phase II clinical trials to evaluate changes in intrahepatic TG (ClinicalTrials.gov, number NCT04031729).

TNF-α is a well-known proinflammatory cytokine and is involved in the development of NAFLD by promoting inflammation, apoptosis, fibrogenesis, and interfering with the insulin signaling pathway, leading to insulin resistance. [53, 97] Pentoxifylline, a TNF-α inhibitor, has been shown to ameliorate NASH in human and animal studies, exhibiting improved body weight, liver enzymes, cytokines, and oxidative stress levels through the stimulation of necrosis and apoptosis pathways and a decrease in genes related to lipid metabolism. [97, 98] Currently, pentoxifylline is being evaluated in a phase III clinical trial (ClinicalTrials.gov, number NCT05284448).

Adenosine receptors are involved in intracellular signaling pathways and physiological functions and are comprised of several subtypes. A3 adenosine receptor (A3AR) is one of them, and is overexpressed in inflammatory cells. The anti-inflammatory effect of A3AR is due to the decrease in the nuclear factor kappa B (NF-kB) signaling pathway, leading to the apoptosis of inflammatory cells. [53] Namodenoson is a synthetic highly selective A3AR agonist currently in the recruiting phase of a phase II clinical trial (ClinicalTrials.gov, number NCT04697810) that has been shown previously to improve liver enzymes and adiponectin levels with a decrease in different parameters analyzing steatosis and fibrosis (CAP, Fib4 scores). [99]

3.3.3. Targeting Hepatic Fibrosis

Chemokines are cytokines with known functions in the liver that drive NAFLD progression. [53] The chemokines exert an effect on inflammation by promoting the M1 phenotype of macrophages (proinflammatory), recruiting immune cells and directly interacting with hepatocytes and HSCs leading to increased lipid accumulation and collagen production, respectively. [53, 100] Hence, chemokine inhibitors are also potential targets for NAFLD treatment under investigation, and several clinical trials have been conducted targeting different chemokines and chemokine receptors. One example is leronlimab, a monoclonal antibody targeting C-C chemokine receptor type 5 (CCR5), first used in HIV, and now being tested in NAFLD (ClinicalTrials.gov, number NCT04521114). [101]

The nanomedicine field has been growing, and an increasing number of nanoparticle-based therapies are reaching clinical trials. Nanoparticles can help the drug attain a certain effect, notwithstanding the fact that the carrier itself can also exert a positive effect. In NAFLD, there is one clinical trial in the recruiting stage for phase II, where lipid nanoparticles (LNPs) are used to target HSCs to improve hepatic fibrosis. BMS-986263 has been formulated with the

active targeting of HSCs in mind. LNPs were conjugated with a retinol-specific target (di-retinamide-PEG-di-retinamide) on their surface, and small interfering ribonucleic acids (siRNA) on the inside were targeted to silence the translation of the HSP47 protein, which is necessary for the optimal deposition of fibrotic collagen. The conjugate on the outside interacts directly to target HSCs as they express the retinol-binding protein on their membranes, facilitating its uptake by the cells, the disruption of collagen and the promotion of HSC apoptosis. [102-104] This formulation has been evaluated in phase I clinical trials in patients with advanced hepatic fibrosis secondary to NAFLD or to other hepatic diseases, such as hepatitis C. Currently, BMS-986263 is in the recruiting phase for phase II trials with the primary end point of at least one stage improvement in the fibrosis score in patients with compensated cirrhosis due to NAFLD (ClinicalTrials.gov, number NCT04267393).

3.3.4. Targeting multiple pathways

Cyclophilins (Cyp) are a family of enzymes that mainly regulate protein folding and trafficking, controlling the structure and function of many proteins. [105] One mechanism of action responsible for improvements in NAFLD is the prevention of collagen synthesis via Cyp B inhibition. [106] Other mechanisms, such as anti-inflammatory and mitochondrial permeability pore mechanisms, may also be involved via Cyp A and D inhibition, respectively. Rencofilstat is a cyclophilin inhibitor proposed to directly target the hepatotoxic effects of steatosis via CypB, CypA, and CypD inhibition. In a previous clinical trial, that evaluated the safety of rencofilstat, the administration of the drug for only 28 days was enough to downregulate the expression of relevant collagen genes, but its efficacy should be evaluated more in depth (ClinicalTrials.gov, number NCT05402371). [105, 106]

Endogenous metabolic modulators (EMMs) represent a potential strategy focused on targeting complex mechanisms that affect multiple systems. EMMs involve physiologically intrinsic components such as amino acids, fatty acids and other lipids, bile acids, etc. The formation of a new EMM can be achieved to target multiple metabolic pathways of interest in a certain disease. [107] Amino acid supplementation has been shown to have a positive impact on liver conditions such as NAFLD. AXA1125 is a novel, orally administered EMM that simultaneously targets pathways related to liver metabolism, inflammation, and fibrosis. AXA1125 is composed of 5 amino acids (leucine, isoleucine, valine, arginine, and glutamine) and an amino acid precursor (N-acetylcysteine [NAC]). Regarding the amino acid composition of AXA1125, it has been suggested that glutamine and arginine might contribute to the effects on inflammatory pathways through the enhancement of mucosal integrity and the tight

junctions of gut epithelial cells, leading to a reduction in endotoxemia. NAC has also been suggested to exert therapeutic effects by reducing the proinflammatory and profibrotic pathways and by increasing anti-inflammatory pathways. [108] AXA1125 is currently being tested in phase II clinical trials (ClinicalTrials.gov, number NCT04880187).

3.3.5. Targeting the gut microbiota

The use of probiotics as a way to improve liver health has been a focus with preclinical and clinical studies showing its benefits in improving liver function. To date, several studies have revealed the efficacy of probiotic use in improving liver enzymes, TG, and cholesterol concentrations; the longer the exposure to the treatment, the better the results.[109] However, information regarding bacterial gut composition is still controversial and not consistent due to the high complexity of the disease and the multiple factors involved that can alter the gut environment (lifestyle, age, body weight, etc.).[109, 110] Currently, there is one phase II recruiting clinical trial involving the use of capsules containing probiotics and prebiotics as a way of performing a microbiota transplantation in patients with biopsy-proven NASH (ClinicalTrials.gov, number NCT05821010).

Table 1: Ongoing and recruiting clinical trials for NAFLD – Phase II

Mechanistic Pathway	Agent (Trial Name)	Primary Mechanism	Major Inclusion Criteria	Primary Outcomes	Estimated Completion	Trial Number
Active, not recruiting						
Metabolism/Lipid Modulation	TVB-2640	FAS inhibitor	NASH on biopsy; NAS≥4; F2-F3	Histological improvement in NAS score without worsening of fibrosis	September 2023	NCT04906421
	PF-06865571 PF-05221304 (MIRNA)	DGAT2 inhibitor ACC inhibitor	NASH on biopsy; F2-F3	Resolution of NASH without worsening of fibrosis or improvement in fibrosis by ≥1 point or both	February 2024	NCT04321031
	ION224	DGAT2 inhibitor	NASH on biopsy; HS≥10% MRI-PDFF	At least 2-point reduction in NAS score with at least 1-point improvement in ballooning or lobular inflammation, without worsening in fibrosis stage	December 2023	NCT04932512
	MK-3655	FGFR1c/KLB agonist	Histological confirmation of NASH	NASH resolution without worsening of fibrosis	August 2024	NCT04583423
	BFKB8488A (BANF)	FGFR1c/KLB agonist	NASH on biopsy; F2-F3	NASH resolution on overall histopathological reading without worsening of fibrosis	January 2023	NCT04171765
	BIO89-100 (ENLIVEN)	FGF21 analog	NASH on biopsy; NAS≥4; F2-F3	Histological resolution of NASH without worsening of fibrosis; ≥1 stage reduction in fibrosis with no worsening of NASH	September 2023	NCT04929483
	Efruxifermin	FGF21 analog	NASH on biopsy; Compensated cirrhosis due to NASH	Change from baseline in fibrosis with no worsening steatohepatitis	April 2024	NCT05039450
	Efruxifermin (HARMONY)	FGF21 analog	NASH on biopsy; NAS≥4; F2-F3	Change from baseline in fibrosis with no worsening steatohepatitis	May 2024	NCT04767529
	BI 456906	GCGR and GLP-1R agonist	NASH on biopsy; NAS≥4; F2-F3	Histological improvement of NASH (NAS reduction of 2 points)	December 2023	NCT04771273
	Tirzepatide (SYNERGY-NASH)	GIP and GLP-1R agonists	NASH with F2-F3	Resolution of NASH without worsening of fibrosis	December 2023	NCT04166773
Inflammation/Oxidative Stress/Apoptosis	Icosabutate (ICONA)	GPR-120 agonist	NASH on biopsy; NAS≥4; F2-F3	Resolution of NASH defined as the disappearance of ballooning with lobular inflammation score 0 or 1, with no worsening of fibrosis	January 2023	NCT04052516
	Vitamin E	Free radical scavenger	Histological diagnosis of NASH	Reduction in hepatic fat mass by MRI-PDFF	December 2025	NCT03669133
Hepatic Fibrosis	Leronlimab	CCR5 inhibitor	NASH on biopsy, FibroScan or equivalent	Change from baseline in hepatic fraction assessed by MRI-PDFF	August 2022	NCT04521114
Recruiting						
	FM101	A3AR agonist	Histological NASH; F1-F3; At least 3 components of the MetS	Improvement in ALT concentrations	August 2023	NCT04710524
	Cholecalciferol	Vitamin D	Fatty liver on imaging	Improvement in ALT concentrations	February 2026	NCT05578404
	Tesamorelin	GHRHH analog	HS on biopsy or ^{1}H-MRS	Fat liver content improvement	September 2024	NCT03375788
	TERN-501	THRβ agonist	NASH on biopsy or imaging	Change in HS	June 2023	NCT05415722
	ASC41	THRβ agonist	HS≥7.5% (MRI-PDFF)	Histological changes in NASH	June 2024	NCT05462353

Metabolism/Lipid Modulation	VK2809 (VOYAGE)	THRβ agonist	NASH on biopsy; NAS≥4; F1-F3	Improvement liver fat	December 2023	NCT04173065
	Lanifibranor	Pan-PPAR agonist	HS≥10% (^{1}H-MRS)	Changes in IHTG	April 2023	NCT03459079
	Pioglitazone	PPARγ1/2 and PPARα agonist	AST/ALT no more than 8 times the ULN	Improvement of at least 2 points in NAS score without an increase in fibrosis stage	February 2024	NCT04501406
	Saroglitazar Magnesium (NASH)	Dual PPAR agonist	NASH on biopsy; NAS≥5; F2-F3	Resolution of NASH without worsening of fibrosis	December 2023	NCT05011305
	EDP-305	FXR agonist	NASH on biopsy; NAS≥4; F2-F3	At least 1 stage improvement in fibrosis without worsening NASH or resolution of NASH without worsening of fibrosis	December 2022	NCT04378010
	HTD1801 (CENTRICITY)	FXR agonist	NASH on biopsy; F2-F3	Decrease of at least 2 points in NAS score or resolution of NASH without worsening of fibrosis	December 2024	NCT05623189
	Norursodeoxycholic Acid	FXR agonist	Histological evidence of NASH and fibrosis	Resolution of NASH without worsening of fibrosis and/or fibrosis improvement without worsening of NAS	April 2022	NCT05083390
	Semaglutide (NAFLD HEROES)	GLP-1R agonist	NASH on biopsy or imaging	Histological improvement with at least 1 point decrease in NAS score	August 2024	NCT03884075
	Semaglutide (Rybelsus®)	GLP-1R agonist	Sedentary, BMI equal or greater than the 90Th percentile for age and gender	Change in hepatic fat fraction	July 2024	NCT03919929
	Cotadutide (PROXYMO-ADV)	GCGR and GLP-1R agonist	NASH on biopsy; NAS≥4; F2-F3	Resolution of NASH and no worsening of fibrosis; Improvement of fibrosis by at least one stage without worsening of NASH	March 2026	NCT05364931
Inflammation/Oxidative Stress/Apoptosis	Vitamin E (VEDS)	Free radical scavenger	FibroScan CAP≥280 dB/m; ALT≥60 U/L	Improvement of ALT concentrations	December 2024	NCT04801849
	Aspirin	COX inhibitor	NASH on biopsy or imaging; FI-F2	Changes in IHTG	September 2024	NCT04031729
	Namodenoson	A3AR agonist	NASH on biopsy; NAS≥4; F1-F3	Improvement in NAS score	April 2023	NCT04697810
	AZD4831 (COSMOS)	MPO7 inhibitor	NASH on biopsy; NAS≥4; F2-F3	Improvement in ALT concentrations	May 2024	NCT05638737
Inflammation/Oxidative Stress/Apoptosis/Hepatic Fibrosis	Rencofilstat (ASCEND)	Cyclophilin inhibitor	NASH on biopsy; NAS≥4	Improvement in fibrosis by at least 1 stage or NASH resolution without worsening fibrosis	September 2025	NCT05402371
Hepatic Fibrosis	*BMS-986263	HSP47 mRNA silencing	NASH on biopsy; F4	At least one stage improvement in fibrosis	December 2026	NCT04267393
All	AXA1125	Endogenous metabolic modulator	NASH on biopsy with fibrosis	Improvement in steatohepatitis	October 2023	NCT04880187
Gut microbiota	LFMT-Capsules	Microbiota transplantation	NASH on biopsy; SAF steatosis≥1, Activity≥2, Fibrosis□4	Improvement in liver histology with 1 or more point improvement in the SAF	August 2025	NCT05821010

	(probiotics and prebiotics)	score or a change in the stage of fibrosis

Criteria used: (1) include a liver-related primary end point; (2) enroll more than 50 patients; (3) ongoing or recruiting clinical trials (4) Phase II (search conducted in clinicaltrials.gov on the 17th of February 2023) Abbreviations: FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; FGFR1c/KLB, fibroblast growth factor receptor-1c/βKlotho; FGF21, fibroblast growth factor 21; CCR5, C-C chemokine receptor type 5; GCGR, glucagon G-protein coupled receptor; GLP-1R, glucagon-like peptide 1 receptor; GPR-120, G-protein coupled receptor; DGAT2, diacylglycerol acyltransferase 2; A3AR, adenosine A3 receptor; PPAR, peroxisome proliferator-activated receptor; THRβ, thyroid hormone receptor beta; COX, cyclo-oxygenase; GHRH, growth hormone-releasing hormone; FXR, farnesoid X receptor; GIP, gastric inhibitory polypeptide; HSP47, heat shock protein 47; MPO7, myeloperoxidase; HS, hepatic steatosis; ^{1}H-MRS, proton magnetic resonance spectroscopy; MRI-PDFF, magnetic resonance imaging-derived proton density fat fraction; IHTG, intrahepatic triglycerides; ALT, alanine transaminase; NAS, NAFLD activity score; CAP, controlled attenuation parameter, SAF, steatosis-activity-fibrosis. *Clinical trial involving nanomedicine

Table 2: Ongoing and recruiting clinical trials for NAFLD – Phase III

Mechanistic Pathway	Agent (Trial Name)	Primary Mechanism	Major Inclusion Criteria	Primary Outcomes	Estimated Completion	Trial Number
Active, not recruiting						
Metabolism/Lipid Modulation	Obeticholic Acid (REGENERATE)	FXR agonist	NASH on biopsy	At least one stage of liver fibrosis improvement with no worsening of NASH, or NASH resolution with no worsening of liver fibrosis	September 2025	NCT02548351
Inflammation/Oxidative Stress/Apoptosis	Pentoxifylline	TNF-α inhibitor	NASH on clinical examination, radiological criteria, or laboratory investigation	Improvement in liver aminotransferases	October 2022	NCT05284448
Recruiting						
Metabolism/Lipid Modulation	MGL-3196 (Resmetirom) (MAESTRO-NASH)	THRβ agonist	NASH on biopsy; NAS≥4; F1-F3	Resolution of NASH: 2-point reduction in NAS without worsening of fibrosis or at least one point improvement in fibrosis with no worsening of NAS	May 2026	NCT03900429
	Dapagliflozin (DEAN)	SGLT2 inhibitor	NASH on biopsy	Improvement in scored liver histology	November 2023	NCT03723252
	Semaglutide (ESSENCE)	GLP-1R agonist	NASH on biopsy; NAS≥4; F2-F3	Resolution of NASH and no worsening of fibrosis; Improvement in liver fibrosis and no worsening of NASH;	May 2028	NCT04822181
	Cotadutide (PROXYMO-ADV)	GCGR and GLP-1R agonist	NASH on biopsy; NAS≥4; F2-F3	Resolution of NASH and no worsening of fibrosis; Improvement of fibrosis by at least one stage without worsening of NASH	March 2026	NCT05364931
	Lanifibranor (IVA337) (NATiV3)	Pan-PPAR agonist	NASH on biopsy; SAF score	Resolution of NASH and improvement of fibrosis	September 2028	NCT04849728

Criteria used: (1) include a liver-related primary end point; (2) enroll more than 50 patients; (3) ongoing or recruiting clinical trials; (4) Phase III (search conducted in clinicaltrials.gov on the 17th of February 2023) Abbreviations: FXR, farnesoid X receptor; TNF-α, tumor necrosis factor alpha; THRβ, thyroid hormone receptor beta; SGLT2, sodium-glucose cotransporter-2; GLP-1R, glucagon-like peptide 1 receptor; GCGR, glucagon G-protein coupled receptor; PPAR, peroxisome proliferator-activated receptor; NAS, NAFLD activity score; SAF, steatosis-activity-fibrosis.

4. COMBINATION THERAPIES FOR NAFLD TREATMENT

Due to the complexity and heterogeneity of NAFLD and the failure of clinical trials in monotherapy studies to meet primary endpoints, the use of drug combinations has been proposed. These combination therapies could potentially be advantageous over monotherapies by simultaneously targeting different relevant pathways related to metabolism, lipid modulation, inflammation, and/or fibrosis. The ongoing and anticipated phase II clinical trials consisting of combination therapies are summarized in Table 3.

The FXR agonist cilofexor and the acetyl-CoA-carboxylase (ACC) inhibitor firsocostat are well tolerated in monotherapy and efficient at reducing hepatic fat accumulation in patients with NAFLD. In patients with a more advanced pathology, the combination therapy of cilofexor and firsocostat has been shown to be even more beneficial than each drug alone and is still well tolerated. The GLP-1RA semaglutide has shown promising results in NASH resolution. However, significant differences were not encountered regarding fibrosis improvement were not observed by histological analysis. [84] These three drugs together show different but complementary mechanisms of action targeting metabolic and anti-inflammatory pathways, as well as the liver directly. Adding semaglutide to cilofexor and firsocostat can help mitigate hypertriglyceridemia caused by ACC and FXR inhibitors. A phase II clinical trial is underway to evaluate the efficacy and safety of this triple therapy with clear histological endpoints (ClinicalTrials.gov, number NCT04971785). [111]

NNC0194, an FGF21 analog, is also being tested in combination with semaglutide with the primary endpoint set at improving liver fibrosis without worsening steatohepatitis. Considering that semaglutide improved NASH resolution with no differences regarding fibrosis, combining semaglutide with other drugs that exert a complementary effect represents a plausible alternative for NAFLD treatment (ClinicalTrials.gov, number NCT05016882).

Licogliflozin inhibits both SGLT1 and SGLT2 receptors leading to the reduction of glucose absorption in the intestine (SGLT1) and reabsorption in the kidneys (SGLT2). The use of licogliflozin leads to weight loss with improved insulin sensitivity and increased secretion of incretin hormones. The potential for a higher effect with dual inhibition of SGLT1/2 compared to the targeting of SGLT2 alone may be of interest in the context of NAFLD. However, side effects need to be taken into consideration due to the potential development of diabetic ketoacidosis and hypoglycemia. In a previous clinical study licogliflozin met the primary endpoint with reduced ALT levels, and further studies either in combination (e.g., FXR agonists) or monotherapy should be conducted to assess the potential of this drug in NAFLD.

[112] Tropifexor is an FXR agonist that effectively targets the liver and intestines with very low doses and minimal systemic exposure, and it was found to be well tolerated at the administered doses. In NAFLD patients with fibrosis, tropifexor favorably decreased all the parameters analyzed (e.g., ALT, gamma glutamyl transferase, hepatic fat fraction and body weight) with a good safety profile. These results provided us with a rationale to conduct further studies both in mono- and combination therapies (e.g., licogliflozin) (ClinicalTrials.gov, number NCT04065841). [113, 114]

Combination therapy with both an acetyl-CoA-carboxylase (ACC) inhibitor and a DGAT2 inhibitor is being used to target the initial and later steps of lipogenesis. One of the benefits of combining these two strategies is the fact that the increase in serum TG provided by ACC inhibitors is not observed when using the two strategies combined. Following conclusive effects in preclinical studies [115, 116], PF-06865571 or ervogastat (DAGT2 inhibitor) and PF-05221304 or clesacostat (ACC inhibitor) are being tested as combination therapies (ClinicalTrials.gov, number NCT04321031).

Table 3: Ongoing and recruiting clinical trials for combination therapies in NAFLD

Mechanistic Pathway Combo	Agent (Trial Name)	Primary Mechanism	Major Inclusion Criteria	Primary Outcomes	Estimated Completion	Trial Number
Active, not recruiting						
Inflammation/Oxidative Stress/Apoptosis	Tocotrienols + Vitamin E (T3-NAFL)	Free radical scavenger + Inhibitor of free radical production	NASH on ultrasound or FibroScan	Reduction of Liver Fat	June 2023	NCT04704063
Metabolism/Lipid Modulation	PF-06865571 + PF-05221304 (MIRNA)	DGAT2 + ACC inhibitor	NASH on biopsy; F2-F3	Resolution of NASH without worsening of fibrosis or improvement in fibrosis by ≥1 point or both	February 2024	NCT04321031
Recruiting						
Metabolism/Lipid Modulation	Semaglutide + Cilofexor + Firsocostat	GLP-1R agonist + FXR agonist + ACC inhibitor	NASH on biopsy; F4	Stage improvement in fibrosis without worsening of NASH; Resolution of NASH	March 2024	NCT04971785
	NNC0194 + Semaglutide	FGF21 analog + GLP-1R agonist	NASH on biopsy; NAS≥4; F2-F4	Improvement in liver fibrosis and no worsening of NASH	March 2025	NCT05016882
	Tropifexor + Licogliflozin	FXR agonist + SGLT1/2 inhibitor	NASH on biopsy; NAS≥4; F2-F3	At least one stage improvement in fibrosis without worsening of NASH; Resolution of NASH without worsening fibrosis	March 2024	NCT04065841

Criteria used: (1) include a liver-related primary end point; (2) enroll more than 50 patients; (3) ongoing or recruiting clinical trials; (4) combination therapies; (5) Phase II and III (search conducted in clinicaltrials.gov on the 17th of February 2023) Abbreviations: GLP1-R, glucagon-like peptide 1 receptor; FXR, farnesoid X receptor; DGAT2, diacylglycerol acyltransferase 2; ACC, acetyl-CoA carboxylase; FGF21, fibroblast growth factor 21; SGLT1/2, sodium-glucose cotransporter 1 and 2; NAS, NAFLD activity score.

5. PEPTIDES AND HORMONES IN NAFLD

Therapeutic peptides are intrinsic signaling molecules in several physiological pathways. The use of peptides and hormones as a treatment is a replacement therapy where their levels are replenished or supplemented to the organism when they are dysregulated due to pathological conditions. Despite being biologics, these therapeutic peptides show less immunogenicity and lower production costs than antibodies. Some major drawbacks are their low membrane permeability and stability. [117, 118]

In NAFLD several treatment options rely on the use of peptides and hormones that are diminished in the disease condition. By increasing the levels of these peptides and hormones, we could achieve beneficial effects. Some examples of therapeutic peptides are summarized in Table 4.

5.1. Gut-derived hormones

Some examples of gut-derived hormones that play an important role in metabolic-related disorders include GLP-1, GIP, cholecystokinin (CCK), peptide tyrosine-tyrosine (PYY), fibroblast growth factor 19 (FGF19), obestatin and ghrelin, all with potential therapeutic effects in NAFLD.

GLP-1 is an incretin hormone produced by the enteroendocrine L cells present in the small intestine that is released after food intake, and its direct effects involve mostly the stimulation of insulin secretion from the pancreatic β cells promoting glycemic control and its action in the brain leading to satiety. [80] As previously mentioned, the effects observed in the liver reflect indirect mechanisms and are secondary to weight loss. [80, 81] GLP-1 analogs are being extensively studied in preclinical and clinical trials for their proven therapeutic benefits in NAFLD treatment. GIP is another incretin hormone secreted from enteroendocrine K cells in the upper part of the small intestine, and when used in NAFLD patients, it has a mild effect on glycemia or body weight loss. [119] However, the dual agonist for both GIPR and GLP-1R (tirzepatide) did not. When studied in clinical trials, GIP exerted a better outcome when compared to that of a GLP-1 analog alone (dulaglutide), demonstrating a significant decrease in ALT levels with a 10 and 15 mg dose of tirzepatide when compared to dulaglutide, and a significant decrease in the tirzepatide group was observed when compared to placebo in a cell apoptosis and fibrosis biomarker, K-18, and procollagen 3, respectively. [87] This improved effect could possibly be related to the action of GLP-1 on glycemic control. It has been observed that improvements in glycemia could help re-establish the insulinotropic effects of

GIP, which are reduced in patients with T2DM. [120] Therefore, it is possible that by raising the levels of GLP-1 the resistance to the action of GIP is lowered, through the normalization of glucose levels. [121] Through the restoration of GIP sensitivity and the combined effect of GLP-1 and GIP, there is a higher glucose lowering and higher satiety effect when compared to those in the individual hormones. [122] Furthermore, GLP-1 and GIP act in different hypothalamic neurons to induce weight loss [123] further explaining the higher change in weight observed in clinical trials. [119] Another dual agonist developed alongside GLP-1 is glucagon. Glucagon is secreted from the pancreatic α-cells, and it has opposite effects when compared to insulin, which in fasting conditions, leads to the production of glucose by the liver and into the blood stream to meet the energy demands in the different tissues. Glucagon also impacts weight loss by interacting with both GCGR and GLP-1R receptors. The mechanism by which glucagon impacts weight loss involves actions in the central nervous system and in the liver with the upregulation of FGF21. [122, 124] Cotadutide was tested in obese and diabetic patients to evaluate its effect on metabolic and hepatic parameters. When compared to liraglutide, the highest dose of cotadutide (300 □g) reduced body weight with statistical significance. Cotadutide (300 □g) improved ALT and aspartate aminotransferase (AST) levels, as well as fibrosis scores exhibiting a significantly improved outcome when compared to the placebo but with no significant differences when compared to liraglutide. [85]

Cholecystokinin (CCK) is also a peptide hormone secreted from enteroendocrine I cells in the upper part of the small intestine and is responsible for stimulating the digestion of fat and protein, and regulating satiety and food intake. [125] NN9056 is a highly selective CCK-1 agonist and a CCK analog, and it has been tested in obese pigs to assess its effect on weight loss. NN9056 significantly reduced food intake with slight improvements in insulin sensitivity, possibly secondary to weight loss. CCK represents a potential therapy for metabolic-related diseases as a monotherapy or most likely in combination with other peptide hormones. [126] However, caution must be taken regarding the possible side effects of CCK that could contribute to the progression of pancreatic cancer. [127] Agonists targeting both the GLP-1R and cholecystokinin receptor (CCKR) have also been designed using exenatide, and in a preclinical model, they reduce body weight in mice with diet-induced obesity. [128]

Peptide tyrosine-tyrosine (PYY) is a peptide that reduces food intake, inducing weight loss, and is in close relationship with the gut-brain axis. Mice lacking PYY become hyperphagic and obese. In addition to its role in appetite regulation and body weight, PYY can also impact the pancreas through improved β-cell survival, proliferation, and function with

known benefits in insulin resistance, a major player in NAFLD and diabetes. This effect is observed through the activation of the neuropeptide Y1 receptor (NPYR1) present in the pancreatic islets. [125] Two novel PYY analogs were developed, ($(P^{3}L^{31}P^{34})$ PYY (1-36) and PYY (1-36) ($Lys^{12}PAL$), to target the NPYR1 receptor and evaluate its effect on β cells. An *in vitro* study showed enhanced growth and survival of pancreatic β cells. Since insulin resistance and T2DM are intrinsically related to NAFLD, having a strategy with effects on insulin resistance is a good approach. [129] Furthermore, the degradation product of PYY due to dipeptidyl peptidase-4 (DPP4), PYY(3-36), is highly selective for the NPYR2 receptor. An analog was developed by cyclization and antibody conjugation or PEGylation. The analog led to reduced food intake, weight loss and diminished gastric side effects in obese rhesus macaques. [130]

Fibroblast growth factor 19 (FGF19) is a hormone secreted after food intake, and its main functions are related to the repression of bile acid synthesis and the stimulation of gallbladder refilling. In addition to these functions, FGF19 is also capable of inhibiting gluconeogenesis without leading to lipogenesis and is tightly associated with features of NAFLD development, such as FA oxidation, *de novo* lipogenesis, VDLD secretion, inflammation, cell damage and endoplasmic reticulum stress. [131] NGM282 is an FGF19 analog, differing only at the amino terminus, and shows selectivity to activate the FGFR4 receptor but not the FGF19 signaling pathway related to the development of hepatocarcinogenesis, the signal transducer and activator of transcription 3 (STAT3) pathway. In clinical trials, the potential of this variant was studied in patients with biopsy-proven NASH. Following 12 weeks of treatment, the patients displayed improvements in the NAFLD activity and fibrosis scores. Therefore, NGM282 is a therapeutic option to consider. [132]

Obestatin and ghrelin are hormones secreted in the stomach with opposing effects. Ghrelin's effects in NAFLD can be contradictory depending on the disease stage because it induces fat accumulation in the liver, but it also exerts a protective effect toward liver inflammation and fibrosis. [133] Obestatin is derived from the pro-ghrelin gene and has opposing actions when to those of ghrelin, leading to a reduction in food intake and body weight, although its mechanism of action remains unclear. Several studies have shown the ability of obestatin to increase β-cell survival, enhancing insulin secretion and preventing the appearance of inflammation and insulin resistance in adipose tissue. Obestatin levels have been reported to be decreased in NAFLD patients, and high levels of this hormone reveal a low risk of disease development. In a preclinical study using a rat NAFLD model, obestatin administration

improved insulin resistance, increased adiponectin levels, and decreased the levels of active ghrelin (acetylated ghrelin). [134]

5.2. Liver-derived hormones

FGF21, as previously mentioned, is a hepatokine functioning as a hormone with insulin-sensitizing and hepatoprotective effects. In preclinical studies, FGF21 has been shown to improve insulin resistance through its effects on adipose tissue (reduction in adiposity, increased glucose uptake, modulation of lipolysis, increased activity of PPARγ, increased adiponectin release and protection of pancreatic β-cells against apoptosis). [135] Several strategies have been exploited to develop synthetic analogs of this hormone. Pegbelfermin, a PEGylated long-acting FGF21, reduced the hepatic fat fraction, improved dyslipidemia, raised adiponectin values and improved markers of cell injury and fibrosis in humans in a randomized, placebo-controlled phase II trial. However, there was no impact on weight loss. More than half of the subjects treated with pegbelfermin developed antibodies against the synthetic peptide and the endogenous hormone, which can pose a limitation for the use of this drug as a treatment due to concerns regarding immunogenicity. [136] In another clinical trial (FALCON 1), which was a randomized phase IIb trial in which the primary endpoint was at least one point improvement in the fibrosis score, the use of pegbelfermin did not meet the primary endpoint.[137]

5.3. Adipose tissue-derived hormones

Leptin is secreted by white adipose tissue and regulates food intake and energy homeostasis. In obesity, higher levels of leptin have been found, indicating a resistance state to leptin actions. The important roles of leptin are related to the reduction of TG storage in adipose tissue and to minimizing TG storage in other tissues, such as the liver, thus serving as hepatoprotective. Leptin action is mediated by the activation of 5' adenosine monophosphate-activated protein kinase (AMPK), leading to decreased activity of ACC in skeletal muscle with increased β-oxidation. Leptin action also inhibits the activity of hepatic stearoyl-CoA desaturase-1 (SCD-1), which is involved in lipoprotein metabolism and energy consumption. In the hypothalamus, leptin action leads to less food intake. Leptin exerts different effects in NAFLD depending on the stage of the disease. In preclinical studies, leptin seems to be protective against fat accumulation in the liver in the first stages, but it also promotes the inflammatory and fibrogenic development of the disease. [133] A study using a recombinant

methionyl-human leptin, metreleptin, was conducted in NAFLD patients with relative leptin deficiency and partial lipodystrophy that led to improvements in the NAS score of patients. In this subgroup of NAFLD patients, the re-establishment of leptin normal levels could be beneficial. [138] In another preclinical study, the administration of leptin to leptin-deficient mice (*ob/ob*) led to improvements in NAFLD due to the normalization of adiponectin levels, reducing inflammation and oxidative stress. [139]

Adiponectin is another chemokine secreted by white adipose tissue, and in NAFLD patients, it has been shown to be reduced. Adiponectin elicits effects on glucose lowering and insulin sensitization. In a preclinical study following chronic administration of adiponectin to a high-fat diet (HFD)-fed mouse model of NAFLD, two major mechanisms were proposed for that action. Adiponectin promotes the uptake of TG into the adipose tissue and promotes fatty acid oxidation in skeletal muscle, leading to the activation of different pathways and resulting in an amelioration of insulin signaling in tissues. [140] A dual agonist of adiponectin, JT003, was developed to bind to the adiponectin receptors AdipoR1 and AdipoR2. In two mouse models of NAFLD, JT003 halted the progression of NAFLD and the fibrotic state through the AMPK, PPARα and phosphatidylinositol-3-kinase-protein kinase B (PI3K-Akt) pathways, inducing a reduction in liver steatosis, inflammatory markers, and fibrosis, where JT003 reversed the activation of HSCs and improved endoplasmic reticulum and mitochondrial functions. [141] Another adiponectin agonist is AdipoRon, which has been studied in the context of alleviating myosteatosis in obese aged mice. The administration of this compound for almost a year reduced the severity of NAFLD, protecting against liver fat accumulation. [142] Analogs of AdipoRon have also been developed in an attempt to increase its anti-inflammatory effects. In a recent study, thirteen compounds were developed, and one was tested in a diet-induced mouse model of NAFLD with promising results in attenuating steatosis, inflammation, and fibrosis markers. [143]

5.4. Parathyroid gland-derived hormones

Parathyroid hormone (PTH) is secreted from the parathyroid glands and is important for regulating calcium-phosphate homeostasis and bone remodeling. [144] Increasing evidence has suggested its potential benefit in metabolic disorders. PTH exerts its effect on adipose tissue, inducing lipolysis and hepatic lipid metabolism. In a recent study using two mouse models of NAFLD, PTH upregulated genes related to β-oxidation and downregulated genes involved in *de novo* lipogenesis and lipid uptake. The mechanism of action was through the direct activation of the parathyroid hormone 1 receptor (PTH1R) receptor which is expressed in

hepatic cells leading to the downstream signaling pathway via the activation of the cyclic adenosine 3',5'-monophosphate (cAMP), the cAMP-dependent protein kinase A (PKA) and the cAMP response element-binding protein (CREB) pathway involved in hepatic lipid metabolism. The systemic administration of PTH could raise some concerns regarding the risk of side effects in the bones and kidneys and increase the risk of osteosarcoma. To circumvent these limitations, other targeted liver delivery systems could potentially help mitigate them. Additionally, the restriction to patients who have concomitant NAFLD and osteoporosis could potentially limit these constrictions. [144]

5.5. Brain-derived hormones

Phoenixin 14 is a neuropeptide (active short peptide) mainly expressed in the brain with a wide range of actions in glucose metabolism, pain, appetite, anxiety, and cardiovascular regulation and plays a role in the gut-brain axis. [145] Studies in fish have shown that this peptide targets the liver via growth hormone receptor 1 (GHR1), and it was also detected in the liver cells of zebrafish. [145] The molecular targets of phoenixin are still unclear; however, in a preclinical study using a mouse model of NAFLD, this peptide decreased body and liver weight, improved liver functions by decreasing ALT and AST levels and decreased serum TG and cholesterol. The amelioration observed in inflammation and liver injury could be secondary to weight loss. However, it was suggested that the mechanism responsible for the action of phoenixin is related to the activation and crosstalk of AMPK, which regulates energy expenditure and metabolism with sirtuin 1 (SIRT1) pathways. When activated, phoenixin elicits beneficial effects on lipid metabolism, oxidative stress, and inflammation. The nuclear factor E2-related factor 2 (NFR2)/heme-oxygenase-1 (HO-1) pathway is also activated and is involved in improving of the inflammation and oxidative stress present in the liver. [145]

Humanin (HN) is a polypeptide discovered in patients with Alzheimer's disease and has been proven to be protective against this disorder. [146, 147] Its effects on NAFLD hallmarks were assessed *in vitro* in human primary hepatocytes. HN, after palmitate treatment of the cells to induce steatosis, could prevent lipid accumulation and reduce the expression of lipogenic genes. [147] Treatment with HN led to decreased cell apoptosis markers such as caspase 3 and decreased insulin resistance. Insulin sensitivity was evaluated by examining the expression of signaling proteins of insulin transduction pathways, such as insulin receptor substrate-1 (IRS-1) and protein kinase 3 (Akt), after the treatment of hepatocytes with HN. The main suggested mechanism of action suggested is via the modulation of the AMPK/mammalian target of rapamycin (mTOR) pathway. Further investigation is needed to confirm these results in animal

models to determine whether HN treatment could represent a therapeutic option for NAFLD. [147] A synthetic version of this peptide exists (S14G), which has increased biological activity compared with that of humanin. [148] However, to the best of our knowledge, S14G has not been tested in NAFLD. [146]

Table 4: Therapeutic peptides and hormones as potential therapeutic options for NAFLD treatment

Source	Therapeutic Peptides	Secretion	Mechanism of action in NAFLD	Delivery approaches in NAFLD	References
Gut	GLP-1	Distal small intestine (L cells)	Stimulates insulin secretion and reduces food intake	SC/Oral	[122, 125]
	Cholecystokinin	Upper small intestine (I cells)	Binds to the CCK1 receptors	SC	[122, 125]
	PYY	Distal small intestine (L cells)	Binds to the NPYR receptors	SC	[122, 125]
	FGF19	Intestinal epithelia	Controls bile acid metabolism by acting on the CYP7α1	SC	[122, 131, 132]
	Obestatin and Ghrelin	Stomach	Unknown	Oral	[125, 134]
Liver	FGF21	Mainly in the Liver	PPARγ activation	SC	[135, 136]
Adipose Tissue	Leptin	White adipose tissue	AMPK, ACC, and SCD-1 pathways	SC/IP	[133]
	Adiponectin	White adipose tissue	AMPK, PPARα and PI3K-Akt pathways	IV/SC	[140, 141]
Parathyroid Glands	Parathyroid hormone	Parathyroid Glands (Chief cells)	cAMP/PKA/CREB pathways	SC	[144]
Brain	Phoenixin 14	Hypothalamus region	SIRT1/AMPK and Nrf2/HO-1 pathways	IG	[145]
Unknown	Humanin	Unknown	AMPK/mTOR pathway	NA	[147]

Abbreviations: FGF19, fibroblast growth factor 19; FGF21, fibroblast growth factor 21; GLP-1, glucagon-like peptide 1; PYY, peptide tyrosine-tyrosine; AMPK, 5'adenosine monophosphate-activated protein kinase; PPARα, peroxisome proliferator activated receptor alpha; PI3K-Akt, phosphatidylinositol-3-kinase-protein kinase B; CCK, cholecystokinin; CYP7α1, cholesterol 7 alpha-hydroxylase; cAMP, cyclic adenosine 3',5'-monophosphate; PKA, protein kinase A; mTOR, mammalian target of rapamycin; ACC, acetyl-CoA carboxylase; SCD-1, stearoyl-CoA desaturase 1; CREB, cAMP response element-binding protein; SIRT1, sirtuin 1; NRF2, nuclear factor erythroid 2-related factor 2; HO-1, heme-oxygenase-1; NPYR, peptide tyrosine-tyrosine receptor 2; IV, intravenous; SC, subcutaneous; IP, intraperitoneal; IG, intragastric; NA, nonapplicable

6. EFFECTS OF EXCIPIENTS IN NAFLD TREATMENT

Undoubtedly, the physicochemical characteristics of drug delivery systems will drive the fate of the system and/or the cargo in the body. By controlling different parameters of the formulation, we can, for instance, attain a higher accumulation in the liver when compared to that in other organs. In addition, the excipient can exert a therapeutic effect on its own. Additionally, there are certain excipients that do not elicit an effect *per se* but, in combination with other excipients, exert a therapeutic effect. In this section, we will overview the therapeutic effect observed for excipients and/or drug delivery systems that could be exploited in the context of NAFLD.

Our group was the first to describe that lipid nanocapsules (LNCs), when orally administered to mice, could induce the secretion of endogenous GLP-1. [149, 150] *In vitro*, it was demonstrated that the nanocarriers, and not the individual components of the formulation analyzed separately or as mixtures, led to this therapeutic effect. [149-151]. In an HFD-mouse model of T2DM, the stimulation of endogenous GLP-1 by the LNC plus the absorption of the LNC-encapsulated exogenous exenatide (GLP-1RA) was sufficient to normalize the glycemia of obese/diabetic mice after either acute or chronic treatment, while LNC or exenatide alone did not. [149] This approach was found to be as efficient as the current subcutaneous marketed drug as a comparison (at a different dose) and could even be more potent at improving oral glucose tolerance, insulin resistance and hepatic steatosis. This marked effect on liver weight and hepatic steatosis led to further evaluation of the potential of this approach in NAFLD treatment. The results in two different mouse models of early NAFLD, without the development of fibrosis, led to improvements in the metabolic syndrome associated with NAFLD (e.g., glucose homeostasis).[152] Both unloaded and exenatide (GLP-1RA)-loaded LNCs exhibited similar effects, which proved the therapeutic effect of the nanocarrier itself.[152] A hypothesis explaining the lack of differences between loaded and unloaded nanocarriers could be that exenatide is not strong enough to induce a more potent effect. Exenatide presents a 2.5 h half-life versus semaglutide presenting a 160 h half-life; thus, replacing exenatide with another GLP-1RA that has a longer half-life, as per the ones under evaluation in current clinical trials, might be more effective for NAFLD treatment. Additionally, the treatment duration of one month might be too short to show the effect of our synergistic approach in NAFLD treatment compared to T2DM treatment. In current clinical trials, GLP-1RAs evaluated in NAFLD (e.g., liraglutide and semaglutide) are administered during treatment periods ranging from 48 to 72 weeks. Furthermore, we observed an anti-inflammatory effect in the liver that

was not seen in the context of T2DM, probably due to the severity of the disease. However, further studies are guaranteed to evaluate the mechanism by which these nanocarriers exert this effect, considering that the effect of GLP-1RA in the liver is indirect, as previously explained.

One of the components of these LNCs is phosphatidylcholine, which is considered to be an essential phospholipid (EPL) and holds a great potential *per se* as a treatment for NAFLD. [153] Essential phospholipids (EPLs) are present in cell membranes and lipoproteins functioning as a barrier against apoptosis and inflammation. [154] At low concentrations in the body, VLDL secretion from the liver is reduced, increasing the storage of TG in lipid droplets. Low EPL levels may also trigger *de novo* lipogenesis.[153] Previous clinical trials suggested that the treatment with EPL was associated with improved ALT levels and reductions in TG and total cholesterol. [153] In another study, a bile acid-phospholipid conjugate of ursodeoxycholic acid and lysophosphatidylethanolamide showed promising results at reducing fibrogenic markers such as transforming growth factor beta (TGFβ), demonstrating its role in a late-stage diet-induced mouse model of NAFLD. [155]

Hydroxypropyl methylcellulose (HPMC) is a modified cellulose, and it has been shown to have the same physiological properties as soluble dietary fibers. [156] HPMC shows an inulin-like effect with changes in microbiota in mice fed a HFD with an increase in bacteria of the phylum Firmicutes. [157] In both animal and human studies, HPMC has been shown to have beneficial effects on hypocholesterolemia, postprandial hypoglycemia, adipocytokines and body weight. In a preclinical study with mice fed a HFD and supplemented with 6% HPMC, a reduction in body weight, improved insulin sensitivity, and glucose tolerance was observed. HPMC upregulated genes involved in cholesterol and bile acid synthesis and downregulated genes involved in steroid biosynthesis, reducing plasma LDL cholesterol. Due to the modulation of genes that are related to TG synthesis and fatty acid oxidation and to a reduced energy intake and an increase in fecal lipid excretion, HPMC could decrease hepatic steatosis. [156] In drug delivery, HPMC is commonly used as a thickening, as a tablet binder or as a tablet matrix for extended release, among other uses. [158]

Chitosan, a polysaccharide with the characteristics of a dietary fiber, exhibits promising effects on hypertension and hypercholesterolemia. [159] The evaluation of different types of chitosan (molecular weight and solubility) has shown that water-soluble, low molecular weight chitosan exerts a beneficial effect of preventing body weight and adipose tissue weight gain in mice. This is probably due to its effect on the suppression of dietary fat absorption in the small intestine, linked to the polymer's positive charge, which attracts the negatively charged fatty

acids and bile acids, preventing them from being absorbed. [159, 160] Chitosan is classified as generally recognized as safe (GRAS), and it has its own biological properties that could be applied to NAFLD as a therapeutic strategy, e.g., as supplementation or as part of a drug delivery system (e.g., nanoparticles). [160, 161]

Chitosan oligosaccharide (COS), a degradation product of chitin, has been shown to suppress inflammatory responses, possessing a strong radical scavenging and antioxidative capacity. [162] COS has been demonstrated to reduce body weight and serum, and hepatic lipid profiles in preclinical studies. [162] Its ability to decrease glycemia and insulin resistance and suppress inflammatory responses in the adipose tissue of diabetic mice suggests a role in ameliorating metabolic disorders. In NAFLD-induced mice, COS activated the AMPK signaling pathway to regulate lipid metabolism and inhibit the inflammatory response and oxidative stress, leading to the prevention of liver damage. Altogether, COS is an effective dietary supplement for ameliorating NAFLD and related metabolic diseases. [162]

Omega-3 fatty acids can be used as excipients, e.g., for the formation of lipid nanocarriers. Due to their effects on lipid metabolism, inflammation, and oxidative stress, as mentioned previously, omega-3 fatty acids are interesting excipients for use with lipophilic drugs in nanoformulations. As an example, nanostructured lipid carriers (NLCs) were formulated for the treatment of hyperlipidemia with encapsulated atorvastatin. These NLCs were formulated using stearic acid and omega-3 fatty acids as the lipidic phase. The aqueous phase contained 80% w/w Tween (emulsifier), poloxamer 188 (surfactant) and soya lecithin (cosurfactant). [163] The results showed higher hypolipidemic effects of atorvastatin in combination within the NLCs containing omega-3 fatty acids than with the respective components alone (blank NLC and atorvastatin alone). [163]

7. ORAL DELIVERY STRATEGIES IN NAFLD TREATMENT

Nanomedicine is a field of nanotechnology that consists of the use of particles in the nanometer range (1-1000 nm) for the prevention, diagnosis, and treatment of diseases. [164] Drug delivery systems, especially those involving the use of nanoparticles (e.g., lipidic, polymeric, etc.), have been studied and investigated as vehicles to improve the delivery of certain drugs to the human body and effectively exert their therapeutic effects. Not all active substances are easily delivered orally due to their lack of solubility, stability, degradation along the gastrointestinal tract, etc. [165] To overcome these difficulties, advanced drug delivery strategies are being tested to facilitate the oral delivery of those molecules. Nanoparticles can

be modified to achieve both passive and active drug targeting to the organs or regions of interest. [164, 166] The use of nanomedicine provides several benefits, such as an increase in drug stability, solubility, and bioavailability, by shielding the drug from the harsh environment encountered and increasing intracellular penetration. [165] By orally administering a treatment, in addition to the benefits of convenience and compliance for the patients, higher amounts of the drug reach the liver via the hepatic portal vein, reducing systemic exposure to the drug and its possible side effects. [154, 165, 167] The use of nanomedicine has been increasingly growing, and the same can be observed in NAFLD. [168]

Regarding NAFLD treatment, throughout the years, several NDDSs have been developed to treat the liver either by passive or active targeting and for the administration of different drugs. Examples of oral drug delivery systems for the treatment of NAFLD are summarized in Table 5. In these studies, the most common types of NPs used were lipidic or polymeric, and the drugs of choice were generally poorly soluble and within the phytocompound category with proven effects in several "hits" of NAFLD development (e.g., inflammation, oxidative stress, insulin resistance, lipid accumulation). [169] A recent review overviewed the use of lipid nanocarriers for NAFLD treatment. [154]

Berberine (BBR) is a therapeutic constituent of the Chinese plant *Coptis chinensis* known to have a potential effect in the treatment of T2DM and NAFLD through the improvement of lipid and glucose metabolism, possibly through a mechanism that stimulates the AMPK pathway, improving insulin sensitivity. However, poor absorption in the GIT, poor bioavailability upon oral administration, and low plasma levels limit oral delivery of berberine. To overcome these limitations, berberine was encapsulated into solid lipid nanoparticles (SLNs) composed of soybean phospholipid and glycerol tripalmitate, showing improved bioavailability when compared to that of berberine alone. *In vivo* experiments performed in *db/db* mice showed that BBR-SLN downregulated lipogenic gene expression, such as stearoyl-CoA desaturase, fatty acid synthase and SREBP1c, whereas the expression of lipolytic gene-like carnitine palmitoyltransferase-1 (CPT1A) was increased in the liver. [170, 171]

Curcumin is a polyphenol with pleiotropic effects, including antioxidant and anti-inflammatory activities. In a recent double-blind, randomized, placebo-controlled clinical trial, improvements in anthropometric measures, blood pressure, TG, total cholesterol, LDL-cholesterol, fasting glycemia and liver function (ALT, AST) were observed in moderate-to-high NAFLD patients supplemented with curcumin plus piperine (daily capsule). [172] As per BBR, curcumin presents a low oral bioavailability that limits its clinical efficacy. The use of nanocarriers is another effective approach to enhance the bioavailability of curcumin. One

example is the use of berberine in combination with curcumin encapsulated in bilosomes, which are liposomes that use bile acids to reinforce themselves against the GIT environment and enhance its absorption across the intestinal barrier. The nanocarriers were further coated with diethylaminoethyl dextran (DEAE-dextran), a cationic polymer, to help improve stability in the GIT fluids, inhibit uptake by phagocytic cells in the circulation and promote uptake by hepatocytes. In NAFLD treatment the use of these oral NDDSs induced a positive effect on inflammation and oxidative stress pathways due to the better biodistribution of the drugs, especially to the liver. [173]

Silymarin is a polyphenolic flavonoid derived from *Silybum marianum*. It has antioxidant and anti-inflammatory properties and is used in chronic liver diseases. [161] Due to the low water solubility of silymarin, its oral bioavailability is very low. Various nanoparticle delivery platforms were prepared, including hybrid lipid-polymeric nanoparticles coated with chitosan. These nanoparticles have been shown to significantly change the total cholesterol concentrations and induce reductions in the amount of lipid droplets in the liver compared to that with the drug alone, indicating an improved effect of the drug through its incorporation within NDDSs. [161]

Naringenin, a dihydroflavone, shows potent anti-inflammatory properties; however, it has inadequate pharmacodynamics due to its poor solubility and susceptibility to oxidation limiting its bioavailability. [174] Naringenin was successfully incorporated into different types of nanocarriers, such as liposomes and nanostructured lipid carriers (NLCs). When incorporated into liposomes, the bioavailability increased 6.7-fold when compared to that of the crude drug, and the administration of 25 mg of naringenin in the liposomes showed the same reduction impact on ALT, AST, and hepatic lipid accumulation as the administration of 100 mg of naringenin alone. However, only a significant reduction in triglyceride levels was reported upon the oral intake of naringenin-NLC in comparison to naringenin alone in a diet-induced mouse model of NAFLD. [175] The attenuation observed in NAFLD by naringenin was via the downregulation of the NLR family pyrin domain containing 3 (NLRP3)/nuclear factor kappa B (NF-kB) signaling pathway, as previously demonstrated in mice. [176]

Another strategy employed to treat NAFLD orally and via NDDSs, is the targeting of oxidative stress, which leads to the formation of reactive oxygen species (ROS), triggering liver damage and leading to NAFLD progression. One approach that has been evaluated is the use of redox nanoparticles (RNPs). Low molecular weight polymers were selected because they tend to be absorbed into the bloodstream when orally administered and avoid internalization in healthy cells, reducing side effects. These polymers consisted of

poly(ethylene glycol)-*b*-polyc [4-(2,2,6,6-tetramethylpiperidine-1-oxyl) aminomethylstyrene] (MeO-PEG-*b*-PMNT) (10 kDa) and formed polymeric micelles under physiological conditions. These RNPs disaggregated in acidic conditions, separated into individual polymers in the stomach and were absorbed in the intestinal epithelium. Once absorbed, these polymers reached the liver, and a significant reduction in ROS and inflammation (NLRP3 inflammasome, IL-1β) was observed, especially in the number of inflammatory cell infiltrates (macrophages and neutrophils). A moderate reversal of liver fibrosis was also observed after 4 weeks of treatment with RNP. [177]

Vitexin, an apigenin belonging to the bioflavonoid compounds, has been formulated into pegylated liposomes to facilitate its absorption and increase its bioavailability. [178] Vitexin has been reported to reduce inflammation in the liver in high-fat diet mice, ameliorating lipid metabolism and cytokines in NAFLD mouse models, and it is closely associated with the inhibition of lipid synthesis and the TLR4/NF-kB pathways. [178-180]

Luteolin is a flavonoid, commonly found in vegetables and fruits, that is able to inhibit the liver X receptor, thus influencing lipid metabolism. [181] The effect of luteolin on NAFLD has been reported to be mainly due to its interaction with the gut microbiota, thus influencing the gut-liver axis pathway and alleviating the dysbiosis observed in this pathology. To overcome its poor solubility and oral bioavailability, a colon-targeted nanocarrier was developed to facilitate the transportation of luteolin into the colon and more effectively interact with the gut microbiota. The purpose of this formulation was to help prevent the release of luteolin in the small intestine and to prevent its total release before reaching the colon by using pH-sensitive polymers such as methoxy poly(ethylene glycol)–poly(dl-lactide-co-glycolic acid) (mPEG-PLGA), which is biodegradable, biocompatible and provides prolonged circulation times. The nanoparticles were further coated with an anionic copolymer (Eudragit S100) to prevent degradation from the acidic environment in the stomach and in the small intestine, making it a colon-targeted approach.[181, 182]

Fenofibrate (FNB) is a drug that is already approved for dyslipidemia treatment and its action is mainly due to the direct activation of the PPARα receptor, which is involved in lipid metabolism. [183] The activation of the PPARα receptor leads to hydrolysis of TG and secretion of VLDL, helping to reduce hepatic fat accumulation. Oral absorption of FNB is low and variable among individuals. Addressing these limitations would make FNB as a candidate in NAFLD treatment. Liposomes encapsulating FNB into the phospholipid bilayer were developed. Liposomes are biodegradable and safe to use for drugs to be released in a sustained

way, staying longer in circulation. In a preventive preclinical study of NAFLD using FNB-liposomes, the reduction observed in lipid content was mainly due to increased oral absorption. Increased plasma levels of the drug provided by the nanocarrier led to prolonged and enhanced activation of the PPARα receptor, exhibiting greater effects on preventing NAFLD than the drug administered alone. [55, 183]

Inositol hexanicotinate (IHN) is a substitute for the normally used nicotinic acid (Vitamin B3), which is available with a prescription as a lipid-lowering drug and most commonly available over the counter as a diet supplement. [184] The effects of IHN on NAFLD have rarely been investigated due, in part, to its low availability. [184] IHN was incorporated into a solid dispersion with glycyrrhetinic acid and arabic gum. [184] Glycyrrhetinic acid (GA) is used mostly to reduce toxicity and improve the efficacy of drugs. Arabic gum (AG) is a biocompatible, nontoxic, biodegradable excipient with good water solubility. These two excipients contain hydroxyl and carboxyl groups that can interact with pyridine groups, changing the physical properties of drugs such as IHN. IHN/GA/AG was more effective at treating NAFLD than the commercialized version (280 mg/tablet, IHN content 64 mg/100 mg) and pure IHN, allowing lower doses to obtain a therapeutic effect. [184]

Vitamin D_3 levels in patients with NAFLD are reduced, and this reduction in vitamin D_3 levels is associated with liver steatosis, which makes vitamin D_3 supplementation a therapeutic option. [185] Vitamin D_3 is converted to 25-hydroxycholecalciferol ($25(OH)D_3$) and then to the active form, 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), with the help of different enzymes present mainly in the liver and kidneys. In NAFLD, active vitamin D_3 inhibits immune cell activation and the synthesis of collagen in HSCs and reinforces the intestinal epithelium by improving tight junctions and maintaining a balance in the gut microbiota. To deliver active vitamin D_3 to the intestines and liver without the risk of hypercalcemia, an NLC formulation was developed. These NLC were used to treat NAFLD in a mouse model of the disease. [185] Although both NLCs and vitamin alone ameliorated intestinal permeability by increasing the expression of genes related to the tight junctions in the intestinal wall, only NLC proved to exert a therapeutic effect in NAFLD, possibly through the suppression of the permeation of small pathogen-associated molecular patterns (PAMPs), which were not detected by the analysis conducted in this study due to their small size. NLC treatment ameliorated or halted the progression of NAFLD through the suppression of steatosis and inflammation. The halt of these liver injuries also delayed the progression to liver fibrosis. This is due to the higher concentrations of vitamin D_3 in both the intestines and liver. [185]

Table 5: Use of oral drug delivery systems in NAFLD treatment

Type of NP	Formulation excipients	Drug	Class of compound/Source	Actions in NAFLD	Year	References
Solid Lipid Nanoparticles	Glycerol tripalmitate; Soybean phospholipid	Berberine	Isoquinoline alkaloid *Coptis chinensis*	Improvement in glucose, lipid metabolism and insulin sensitivity	2015	[170, 171]
Redox Nanoparticles	MeO-PEG-*b*-PMNT	Redox polymers	Synthetic polymers	Oxidative stress suppression	2015	[177]
Liposomes	Soybean lecithin; Cholesterol	Fenofibrate	Antilipemic agent	PPARα agonist	2016	[183]
Liposomes	Soybean lecithin; Cholesterol	Naringenin	Dihydroflavone Citrus fruits	Anti-inflammation, anti-oxidation, insulin sensitivity and normalization of hepatic TG	2017	[174]
Chitosan-functionalized Lipid-polymer Hybrid Nanoparticles	Soybean lecithin; DSPE-PEG 2000; PLGA; Chitosan	Silymarin	Flavonoid compounds *(Silybum marianum)*	Hepatoprotective, antioxidant, anti- inflammatory, anti-tumor, and with hypolipidemic effects	2018	[161]
Nanostructured Lipid Carriers	Glycerol monostearate; L-*α* phosphatidylcholine; L-3-phosphatidyl-L-serine sodium; *α*-tocopherol; $1,25(OH)_2D_3$	Vitamin D_3	Supplement	Suppress immune cell activation, collagen synthesis and ameliorates the intestinal barrier	2020	[185]
Solid Dispersions	Glycyrrhizic acid; Arabic gum	Inositol hexanicotinate;	Niacin ester	Reduction in TG levels	2021	[184]
Dextran-coated Bilosomes	Diethylaminoethyl dextran; sodium deoxycholate; soybean lecithin; cholesterol; octadecylamine	Berberine Curcumin;	Isoquinoline alkaloid (*Coptis chinensis)* Polyphenol *(Curcuma longa)*	Insulin sensitivity, anti-oxidation, anti-inflammation, anti-fibrosis, and reduction of liver steatosis	2021	[173]
Nanostructured Lipid Carriers	Soybean lecithin; stearic acid; monostearin; oleic acid; Poloxamer 188	Naringenin	Dihydroflavone Citrus fruits	Anti-inflammation, anti-oxidation, insulin sensitivity and normalization of hepatic TG	2021	[175]
Eudragit S100-Coated mPEG-PLGA Nanoparticles	PEG; PLGA; Eudragit S100	Luteolin	Flavonoid Vegetables and fruit	Gut microbiota modulation	2022	[181, 182]
PEG-Liposomes	DPPC and cholesterol	Vitexin	Apigenin flavone glycoside Passionflower, bamboo leaves and pearl millet	Antioxidant, anti-inflammatory, antihypertensive	2022	[178]

Abbreviations: PEG, polyethylene glycol; PLGA, polylactic-co-glycolic acid; MeO-PEG-*b*-PMNT, poly(ethylene glycol)-*b*-poly[4-(2,2,6,6-tetramethylpiperidine-1-oxyl) aminomethylstyrene]; DSPE-PEG, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol); PPARα, peroxisome proliferator-activated receptor alpha; TG, triglycerides.

8. CHALLENGES AND FUTURE PERSPECTIVES IN DRUG DELIVERY

The field of drug delivery, more specifically the use of the oral route, has advanced in the treatment of NAFLD, as summarized in Section 7. Despite the difficulties encountered via this route of administration (e.g., the harsh environment throughout the gastrointestinal tract and the multiple physical barriers) that can be daunting and hard to overcome, when using peptides or oligonucleotides as a therapeutic approach, strategies can be applied to overcome these hurdles and/or target specific organs, especially in the field of nanomedicine. Targeting the liver via the oral route is not the most frequent strategy used in the clinic when we think of biologics as a therapeutic approach. However, utilizing the oral route can help reduce systemic exposure after administration and help drugs reach the liver at higher concentrations, as previously mentioned.

There are no approved drug-based treatment options for NAFLD, and the treatment relies on a stage-based approach. As a "hot topic", NAFLD is being extensively investigated, and increasing knowledge, regarding its pathogenesis, is being discovered, as well as new potential targets. NALFD's close association with metabolic abnormalities has led to the investigation of drugs already approved for other pathologies (e.g., T2DM, obesity, dyslipidemia, etc.). Some drugs exert their effect by directly impacting the liver, by specifically targeting it, or by having an indirect effect (e.g., ameliorating the overall MetS symptoms). The use of GLP-1 analogs in NAFLD represents a promising approach, with preclinical and clinical studies demonstrating the impact observed hepatically and extrahepatically. [81] In clinical trials, the route of administration is mainly subcutaneous. However, research has been performed to deliver these peptides via the oral route with promising results regarding their protection, absorption, and therapeutic effects, using nanoformulations such as polymeric nanoparticles. [186, 187] In Section 6, the use of LNC to orally deliver exenatide in T2DM and NAFLD was mentioned, demonstrating a new approach for oral peptide delivery as well as a novel nanocarrier that on its own can lead to the physiological secretion of GLP-1 after oral administration. [149] This result shows the positive impact of nanoformulations *per se* on exerting a therapeutic effect. In addition to peptide delivery, gene therapy has also been applied using GLP-1 plasmids inside a nanosystem made of protamine, a succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) linker, and a fragment of the human immunoglobulin G1 (IgG1) antibody. [186] This nanosystem could protect the plasmid from the GIT, helping in its transport across the intestinal barrier via the neonatal Fc receptor (FcRn), entering systemic circulation. [186]

The field of oral peptide delivery has evolved tremendously, and one example is the recent approval of an oral GLP-1 analog for T2DM (Semaglutide: Rybelsus®). [188] Despite its relatively low bioavailability (~1%), this GLP-1 analog has shown an impressive result in T2DM, offering a more convenient method of administration for patients. The approach is based on transcellular delivery through the intestinal epithelia using a permeation enhancer, salcaprozate sodium (SNAC), facilitating the absorption of macromolecules when orally delivered. [189] Similar strategies have been employed with the development of another GLP-1 analog, MEDI7219, which is structurally different from semaglutide. [190] MEDI7219 has twelve amino acid substitutions, five α-methyl amino acids and a bilipidation. On the other hand, semaglutide has one α-methyl amino acid and a mono-lipidation when compared to native GLP-1. [190] This peptide was developed with permeation enhancers such as sodium chenodeoxycholate and propyl gallate. It demonstrated a half-life period of 15 h and a 5-fold increase in bioavailability when compared to previous studies of semaglutide conducted in dogs. [190]

Another important therapeutic approach would be the use of small interfering RNA (siRNA) to silence key pathways of disease progression, potentially reverting the fibrotic state. Many studies have already demonstrated the ability of siRNA to impact NAFLD, especially when incorporated into lipid-based nanocarriers. [191] Different strategies have been applied using siRNA to silence different genes related to fibrosis development, such as high mobility group box 1 (HMGB1) [192] and heat shock protein 47 (HSP47), but with different targeting techniques. [103, 193, 194] For example, the use of liposomes with a vitamin A coupled to their surfaces to target the once inactive vitamin A-storing HSC with siRNA against HSP47 has been developed. [103] This formulation was able to reach clinical trials (Section 3.3). [102] A recent paper developed lipid nanoparticles (LNPs) with active targeting toward HSCs incorporated into the lipidoids used to form them. The intravenous administration of the LNP containing siRNA directed at silencing HSP47 led to a higher silencing and selectivity of both the gene of interest and the HSC, with an outperformance of the marketed version, Onpattro®, by 2-fold. [194] These positive results and the fact that Onpattro is already commercialized, pave the way for continuing the efforts in formulation development and improvement for future oral delivery of these nanosystems containing oligonucleotides. The use of silencing techniques using the oral route has been more focused on diseases affecting the GIT. [191] Evaluation of the fate of orally administered LNPs containing siRNA has been evaluated in preclinical studies, and although digestive enzymes could impact gene silencing *in vitro*, *in vivo* the LNPs

could enter enterocytes in mice after oral delivery. There was no significant gene silencing by using oral administration, and higher doses are necessary when switching from an IV to an oral route, with 50x higher doses orally. [195] Suggestions for the use of siRNA-based LNPs via the oral route are protection of the nanocarriers from inactivation in the stomach (e.g., by pepsin) [195], the use of pH-responsive excipients that could lead to the release of the drug only at the site of the disease in the GIT [195] or the use of multicompartmental systems [191], among others. Currently, there are two recruiting clinical trials using siRNA therapies for NAFLD but as a subcutaneous injection: ALN-HSD, a hydroxysteroid dehydrogenase expression inhibitor (ClinicalTrials.gov, number NCT05519475) and LY3849891, targeting the PNPLA3 domain (ClinicalTrials.gov, number NCT05395481).

Regarding hepatic fibrosis, there are drugs approved for fibrosis in other organs, such as the lungs, that could potentially be tested in the liver. However, the difficulty of accessing to the liver when there is an excess of ECM may complicate its delivery. Liver sinusoidal endothelial cells under normal conditions present fenestrae of approximately 50-200 nm. [154] However, in the disease state of hepatic fibrosis, those fenestrae close, making it difficult to access the space of Disse and consequently target HSCs or hepatocytes. A recent study showed the effects of a peptidomimetic agonist of the αV integrin family in reverting fibrosis in pancreatic cancer when subcutaneously administered. [196] This family of transmembrane receptors is overexpressed in activated HSCs posing a possible therapeutic option. [197] In clinical trials, regarding liver fibrosis and NAFLD, the repurposing of drugs is evident. For instance, ZED1227, a drug used in celiac disease, is recruiting for a phase II trial (ClinicalTrials.gov, number NCT05305599). Dasatinib is used in certain forms of leukemia as an antineoplastic drug and is also going to be repurposed in fibrotic NAFLD (ClinicalTrials.gov, number NCT05506488).

The field of drug delivery is constantly evolving, with new types of drug delivery systems being developed and engineered to possess a therapeutic effect and to carry, protect, and improve the effects of drugs. The treatment of NAFLD will likely require the use of combination therapies, and nanomedicine can be useful in merging different drugs, with distinct but complementary mechanisms of action, within the same formulation. Some examples of nanosystems can be found in several preclinical studies for use in cancer for combination therapy. [198] The transport of more than one drug in the nanocarrier can be a challenge, and it depends on the types of drug and carriers used, where the active principle can be either entrapped, dissolved/dispersed, or adsorbed to the surface. [198]

The possibilities that nanomedicine can provide to patients with NAFLD can reduce side effects by limiting off-target effects. Other advantages include the possibility of tailoring the nanosystem to our needs and to the disease of interest. However, improvements in patient screening and testing should also be made to detect patients in the early stages of NAFLD.

The drug delivery field could help overcome the need for treatment in NAFLD. Progress in the understanding of the disease along with novel drugs/targets will help increase the study of liver drug delivery for NAFLD treatment.

9. CONCLUSION

NALFD is caused by an interplay between all the cells present in the liver and surrounding organs, creating very complex pathways. This leads to the progression from simple steatosis into steatohepatitis with inflammation, cell injury and fibrosis development with possible progression into cirrhosis. This plethora of mechanisms involved in disease progression can vary from patient to patient, making NAFLD a very heterogeneous and difficult pathology, where its mechanisms are not fully understood. However, increasing knowledge has led to a multitude of drugs/targets being investigated to treat NAFLD. Nanomedicine can be one of the methods used to achieve a better therapeutic efficacy, with possible active targeting of the liver with direct effects in, e.g., hepatic fibrosis and/or indirect effects ameliorating the MetS associated with NAFLD. Here, the different ongoing and recruiting clinical trials were revised alongside possible novel therapeutic approaches. Oral drug delivery systems being tested in preclinical studies in NAFLD have been discussed as well as the challenges that the drug delivery field faces as the current trend to NAFLD treatment seems to pass by the use of combination therapies targeting one or more “hits” of disease development.

Declaration of Competing Interest

The authors declare no competing financial interests.

Acknowledgments

A. Beloqui is a research associate from the FRS-FNRS (Fonds de la Recherche Scientifique), Belgium and a WELBIO investigator (grant agreement WELBIO-CR-2022 S–01). This work was supported by the Belgian National Funds of Research (FRS-FNRS) (convention J.0009.22). All figures were created with BioRender.com and PowerPoint.

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