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Combination therapies in nonalcoholic fatty liver disease using antidiabetic and disease-specific drugs

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is one of the most common diseases in the world, affecting approximately one fourth of the worldwide population**.** Glucose metabolism dysregulation and type 2 diabetes mellitus (T2DM), as part of the metabolic syndrome, are important factors implicated in the pathogenesis and progression of NAFLD to nonalcoholic steatohepatitis (NASH) and cirrhosis. Although a great deal of research has already been conducted regarding possible therapeutic medications for NAFLD/NASH, no drugs have been approved until now. Combination therapies in NAFLD seem to represent an attractive approach concerning treatment of the disease, as multiple pathophysiologic pathways contribute to the development and advance of NAFLD. In this review we discuss the impact of combining antidiabetic drugs, focusing on pioglitazone, sodium glucose cotransporter 2 inhibitors and glucagon-like peptide-1 receptor agonists. We also include data from the literature concerning combinations of newer "NAFLD-specific" drugs.

Keywords Nonalcoholic fatty liver disease, pioglitazone, sodium glucose cotransporter 2 inhibitors, glucagon-like peptide-1 receptor agonists

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease encountered in hepatology departments, particularly in western countries [1,2]. Metabolic syndrome (MetS) and its individual components are associated with NAFLD pathogenesis and progression [3-5]. Abnormal fasting blood glucose levels (≥100 mg/dL) or diabetes mellitus type II (T2DM) comprise one of the components of MetS [6-8]. Several studies have shown that patients with T2DM and/or MetS are at increased risk of developing advanced stages of NAFLD [9-12], i.e., nonalcoholic steatohepatitis (NASH), advanced fibrosis/cirrhosis and hepatocellular carcinoma [13-16]. Although many trials have investigated the role of different agents in the treatment of NAFLD and NASH, none of these agents have been approved [17], and currently the only recommendation for these patients is lifestyle modification consisting of exercise and diet [18-20]. In view of the complex pathophysiology of NAFLD/NASH [21-24], combinations of treatments targeting different pathogenetic mechanisms have been studied [25-27], and several trials related to this topic are ongoing. To write this article, we reviewed the literature reporting combination treatments in NAFLD/NASH, focusing on antidiabetic medications, namely pioglitazone—a peroxisome proliferator-activated receptor (PPAR)-γ agonist—as well as the newer antidiabetic drugs, including sodium glucose cotransporter 2 (SGLT2) inhibitors and glucagon-like peptide-1 receptor (GLP-1) agonists, all of which have shown promising results in NAFLD/NASH. The recently investigated "NAFLD-specific" drugs in this field, such as selonsertib-targeting apoptosis, cilofexor-a farnesoid X receptor agonist, and the acetyl coenzyme A carboxylase inhibitor (ACCi) firsocostat, were also included. Clinical and experimental studies were reviewed.

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Conflict of Interest: None

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Materials and methods

A comprehensive literature search was conducted for relevant literature using the PubMed database, in which only studies written in the English language and published until September 2022 were included. The following search terms

were used: "non-alcoholic fatty liver disease" or "NAFLD" or "non-alcoholic steatohepatitis" or "NASH" or "fatty liver" AND "pioglitazone" or "sodium glucose co-transporter 2 inhibitor" or "SGLT2 inhibitors" or "glucagon-like peptide-1 receptor agonist" or "GLP-1 agonist" or "acetyl CoA carboxylase inhibitor" or "farnesoid X receptor agonist" or every drug included in the last 4 categories. In addition, we searched for the terms "combination treatment in NAFLD", "combination treatment in NASH", "combined in NAFLD" and "combined in NASH". Animal and human studies involving a combination of 2 or more of the above-mentioned categories of agents were included in the present review.

NAFLD combination therapies: animal studies (Table 1)

Ipragliflozin and pioglitazone

Tahara *et al* [28] conducted a study to examine the effects of ipragliflozin-a SGLT-2 inhibitor, alone or in combination with pioglitazone, in high-fat diet-fed KK/Ay T2DM mice with NASH. Diabetic mice received vehicle, or ipragliflozin, or pioglitazone, or ipragliflozin in combination with pioglitazone, for 4 weeks. At week 4, hepatic lipid contents and transaminases levels were significantly reduced after ipragliflozin and combination therapy, while the ipragliflozin and pioglitazone combination increased adiponectin levels ($P<0.05$ vs. vehicle group).

Liraglutide and ipragliflozin

Koike *et al* [29] evaluated the effects of liraglutide (a GLP-1 agonist) and ipragliflozin as monotherapy or in combination in mouse models with T2DM. Diet-induced obese (DIO) mice, representing an early-stage diabetes model, and leptin receptor deficient C57BL/6 +Lepr <db>/+Lepr <db> (db/db) mice, as an advanced stage diabetes model, were studied. Four groups of DIO mice were evaluated: liraglutide group, ipragliflozin group, combination group and controls (vehicles). Alanine aminotransferase (ALT) levels were significantly lower in the liraglutide ($P<0.01$) and combination group ($P<0.05$) and tended to be lower in the ipragliflozin group, compared to the control group. Ipragliflozin, liraglutide and their combination reduced the NAFLD activity score to similar degrees. All treatments also reduced liver lipid accumulation, ipragliflozin to a lesser degree than the other treatment arms. However, hepatic triglycerides were significantly lower in the liraglutide and combination groups compared to the ipragliflozin group ($P<0.01$).

Regarding the db/db mice model, plasma ALT levels were lower in the liraglutide ($P<0.01$) and combination treatment groups ($P<0.001$) compared with the control group. Ipragliflozin and combination therapy reduced the NAFLD activity score ($P<0.001$ and $P<0.05$ vs. control, respectively), but no significant differences between groups were observed in reductions of hepatic lipid accumulation.

"NAFLD-specific" drugs

Vijayakumar *et al* [30] performed 5 *in vivo* studies in 3 mouse models and evaluated whether enhancing hepatocyte fatty acid oxidation by combining ACCi with PPAR agonist or thyroid hormone receptor β (THRβ) agonist would result in greater liver triglyceride reduction and NASH/antifibrotic efficacy along with amelioration of ACCi-induced hypertriglyceridemia. The duration of the studies was 2-6 weeks. In high-fat diet-fed dyslipidemic rats, it was found that the addition of PPAR agonists (fenofibrate, elafibranor, lanifibranor, seladelpar or saroglitazar) or resmetirom (a THRβ agonist) to an analog of firsocostat (ACCi) prevented ACCi-induced hypertriglyceridemia, while only PPARα agonists (fenofibrate, elafibranor) and resmetirom provided additional liver triglyceride reduction. In the choline-deficient high-fat diet rat model of advanced liver fibrosis, neither PPARα (fenofibrate) nor THRβ agonist augmented the antifibrotic efficacy of ACCi.

Combination anti-diabetic therapies in NAFLD: clinical studies (Table 2A and 2B)

Pioglitazone

Exenatide and pioglitazone

Sathyanarayana *et al* [31] evaluated the effects of exenatide, a GLP-1 receptor agonist, in combination with pioglitazone, on hepatic fat content and levels of plasma adiponectin (the most common adipokine to be inversely linked with insulin resistance, inflammation, lipid accumulation and NAFLD) in patients with T2DM. Twenty-four diabetic patients on diet and/or metformin were enrolled, of whom 21 completed the study. Liver fat content was assessed by magnetic resonance spectroscopy (MRS). Patients were randomized to receive pioglitazone, either alone or combined with exenatide 5 μg, subcutaneously b.i.d. for 2 weeks, followed by exenatide 10 μg subcutaneously b.i.d. All patients in both arms started pioglitazone 30 mg/day for 2 weeks, followed by pioglitazone 45 mg/day for 48 weeks.

In the combination therapy, a significant reduction in hepatic fat content was observed after 12 months (12.1±1.7% at baseline vs. 4.7±1.3% at 12 months, $P<0.001$). This reduction was significantly greater than under pioglitazone alone (11.0±3.1% at baseline vs. 6.5±1.9% at 12 months, $P<0.05$). In addition, a greater improvement in ALT was observed in the combination group compared to pioglitazone alone. Interestingly, in both treatment arms adiponectin levels increased compared to baseline (pioglitazone arm: from 8.5±0.8 to 15.8±1.4 μg/mL, combination arm: from 7.9±0.9 to 23.2±2.7 μg/mL, $P<0.001$),

Table 1 Animal trials concerning combinations of antidiabetic (pioglitazone, GLP-1 agonists, SGLT-2 inhibitors) and "NAFLD-specific" drugs in NAFLD/NASH

Authors/ [ref.] /year	Treatment/ population/ duration	Effects on histologic findings	Effects on liver enzymes	Changes in body weight, liver or visceral fat weight	Changes in laboratory values concerning glucose metabolism	Changes in other laboratory values	Changes in metabolic parameters /Gene expressions	Effects on scores related to NAFLD	Conclusions
Tahara *et al* [28], 2019	Vehicle or IPRA (0.1-3 mg/kg) or PIO (3-30 mg/ kg) or IPRA (1 mg/kg)+ PIO (10 mg/kg)/ diabetic mice with NASH/4 wks	IPRA and combo treatment significantly improved liver injury. PIO improved liver injury but to a lesser degree compared to IPRA.	Transaminase levels significantly ↓with IPRA and combo therapy. PIO also showed a trend towards ↓ or significantly ↓ in these parameters.	↓ in body and visceral fat weight with IPRA compared to vehicle group. ↑ Body and visceral fat weight with PIO (P<0.05 vs. vehicle group). Combo treatment significantly ↓ body and visceral fat weight compared to PIO alone	Combo treatment significantly and additively ↓ blood glucose and insulin levels (P<0.05 vs. vehicle group and PIO group). Combo treatment significantly ↓non-fasting blood glucose, HbA1c, insulin and increased urinary glucose excretion compared to PIO alone	Combo treatment significantly ↓ lipid levels, body and visceral fat weight compared to PIO alone. Liver weight, hepatic lipid contents and transaminases levels significantly ↓ with IPRA and combo therapy.	Liver weight and hepatic lipid contents significantly ↓ with IPRA and combo therapy. Combo treatment significantly and additively ↓ levels of leptin and FGF-21 and ↑ adiponectin levels (P<0.05 vs. vehicle group). Combo treatment also ↓or showed a trend towards ↓ plasma and liver levels of IL-6, monocytechemotactic protein -1, TNF-α, CRP and oxidative stress biomarkers (P<0.05 vs. vehicle and PIO monotherapy)	NA	IPRA improves hyperglycemia as well as NASH in T2DM mice. Treatment with IPRA monotherapy or co-administered with PIO is expected to be a potential therapeutic optionv for the treatment of T2DM with NASH.
Koike *et al* [29], 2021	LIRA and IPRA as monotherapy or in combo/ mouse models of T2DM/4 wks	DIO mice: All treatments ↓liver lipid accumulation -IPRA to a lesser degree that the other treatments. ↓Hepatic TGs with LIRA and combo group compared to IPRA (P<0.01).	DIO mice: ALT levels were significantly ↓ in the LIRA (P<0.01) and combo group (P<0.05) and tended to ↓in the IPRA group compared to control group. AST levels were similar in all groups.	DIO mice: LIRA and combo treatment ↓body weight after 4 wks (P<0.001 vs. control and IPRA group); IPRA ↑ body weight.	DIO mice: All treatments ↓ glycemic excursions during iPGTT -IPRA was the least efficient in controlling glucose levels. All groups had similar glucagon and fasting plasma insulin levels.	DIO mice: IPRA slightly but significantly ↓ Chol levels compared to controls (P<0.05) Further ↓with LIRA and combo treatment (P<0.001 vs. control and vs. IPRA for both).	DIO mice: Fatty acid transporter isoforms, Slc27a2, 4 and 5 were ↓ in all groups (except for Slca4 in LIRA group). Acc1, Fas, Acox1, Cpt1a and Mttp genes were also ↓ vs. control group.	DIO mice: IPRA, LIRA and their combo ↓ NAFLD activity score to similar degrees (P<0.001 vs. control	LIRA is more efficient at an earlier stage of T2DM and IPRA can be effective in early and advanced stages. Their combined use is a potential option for treating advanced stage diabetes with NAFLD

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Table 1 *(Continued)*

Authors/ [ref.] /year	Treatment/ population/ duration	Effects on histologic findings	Effects on liver enzymes	Changes in body weight, liver or visceral fat weight	Changes in laboratory values concerning glucose metabolism	Changes in other laboratory values	Changes in metabolic parameters /Gene expressions	Effects on scores related to NAFLD	Conclusions
		Db/db mice: no significant differences in ↓ of hepatic lipid accumulation between groups	Db /db mice: ALT levels ↓in the LIRA (P<0.01) and combo group (P<0.001) vs. the controll. AST levels were similar across groups	Db/db mice those in the LIRA group weighted significantly ↓than the ones in the other groups (P<0.001). IPRA ↑ weight to a greater degree than the control group. Combo therapy had an intermediate effect on body weight	Db/db mice: LIRA showed no beneficial effect on blood glucose levels /IPRA and combo treatment ↑ plasma insulin levels.	Db/db mice: Plasma TGs levels were significantly ↓with LIRA and combo treatment (P<0.05 and P<0.01 vs. control respectively).	Db/db mice: Pancreatic insulin content was significantly ↑ on combo treatment group (P<0.001 vs. control and LIRA group) and tended to be ↑ in the IPRA group. Srebp1c was significantly ↓by combination therapy and tended to be ↓ by LIRA and IPRA monotherapy. Slc27a2,4 and 5 isoforms, Acox1, Cpt1a and Mttp genes were also ↓	Db/db mice: IPRA and combo therapy ↓ NAFLD activity score (P<0.001 and P<0.05 vs. control respectively)	
Vijayakumar *et al* [30], 2022	ACCi+Feno or elafibranor or lanifibranor or seladelpar or saroglitazar or resmetirom/FFD fed mice and dyslipidemic rat models/2-6 wks	ACCi+Feno combo caused further ↓ in liver TG vs. ACCi in the FFD mouse model of early NASH. ACCi+Feno further ↓ liver TG (40%–45% vs. vehicle; p≤0.01 vs. ACCi). Great ↓ in liver total chol content (58%–66%) with ACCi+Feno	Serum ALT and AST levels were significantly ↓by 40%–71% with all treatments ACCi+Feno were more effective than ACCi alone	FFD mouse model of early NASH: Feno (150 mg/kg) caused significant weight loss in the monotherapy and combo groups	ACCi monotherapy ↑ blood glucose levels and ACCi+Feno normalized blood glucose levels	ACCi+Feno combo normalized circulating TGs in the FFD mouse model of early NASH/ Serum total chol levels were significantly ↓ by 14%–20% with ACCi monotherapy and ACCi+Feno combo, vs. vehicle	ACCi+Feno synergistically ↑ FAO in the mouse FFD model ACCi+Feno synergistically ↑ plasma and liver β-OHB levels relative to ACCi or Feno alone. ACCi+Feno ↓and even normalized FGF-21 levels were significantly ↓with ACCi and dose-dependently and similarly ↑with Feno and ACCi+Feno combinations	NA	Combo therapies targeting hepatocyte lipid metabolism may have beneficial effects on liver TG reduction. However, they may not be sufficient to drive fibrosis regression.

GLP-1, glucagon-like peptide-1; SGLT-2, sodium glucose cotransporter 2; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ref, reference; IPRA, ipragliflozin; PIO, pioglitazone; wk, week; combo, combination; HbA1c, hemoglobin A1c; FGF-21, fibroblast growth factor-21; IL, interleukin; TNF-a, tumor necrosis factor-a; CRP, C-reactive protein; NA, not applicable; T2DM, type 2 diabetes mellitus; LIRA, liraglutide; DIO, diet-induced obese; TGs, triglycerides; db/db, leptin receptor deficient; ALT, alanine aminotransferase; AST, aspartate aminotransferase; iPGTT, intraperitoneal glucose tolerance test; chol, cholesterol; Mttp, microsomal triglyceride transfer protein; ACCi, analog of firsocostat; Feno, fenofibrate; FFD, fast food diet; FAO, fatty acid oxidation; β-HOB, β hydroxybutyrate

Table 2 (A) Clinical trials that evaluated combinations of antidiabetic (pioglitazone, GLP-1 agonists, SGLT-2 inhibitors) and "NAFLD-specific" drugs in NAFLD/NASH)

Authors/ [ref.] /year, type of study	Treatment/ population/ duration	Effects on liver enzymes	Changes in anthropometric parameters	Changes in laboratory values	Changes in other metabolic parameters
Sathyanarayana *et al* [31], 2011, open label RCT	PIO (n=10) vs. EXE+PIO (n=11) add on to metformin or diet / T2DM/50 wks	Compared to baseline greater↓ of ALT levels with combo (ALT 35 IU/L to 18 IU/L at 12 months), vs. monotherapy (at baseline ALT: 25 IU/L to 19 IU/L at 12 months)	↑Body weight with PIO (93.1 kg to 96.8 kg, P<0.05). No change with combo therapy	Compared to baseline: Combo treatment: ↓HbA1c (8.1% to 6.8%, P<0.01), FFA (603 to 369 μmol/L, P<0.01) and TGs (136 to 85 mg/dL, P<0.01) and ↑ HDL (48 to 54 mg/dL, P<0.05). PIO alone:↓ HbA1c (8.3 to 7.3%, P<0.01, FFA (487 to 331 μmol/L, P<0.05) and TGs (192 to 165 mg/dL, P<0.05)	Both treatments ↑ adiponectin levels PIO:8.5 to 5.8 μg/mL. PIO+EXE: 7.9 to 23.2 μg/mL, P<0.001. greater ↑with combo therapy (86% vs. 193%, P<0.001).
Yoneda *et al* [32], 2022, open label RCT	TOFO+PIO (n=32) vs. TOFO (n=21) or PIO (n=19)/ T2DM/ 48 wks	Compared to baseline: ↓ALT levels in all groups (-19.3 IU/L, P=0.0219 with TOFO, -34 IU/L, P<0.001 with PIO and -35.7 IU/L, P<0.001 with combo therapy). In patients who firstly received PIO and then TOFO was added, additional ↓ in ALT levels compared to monotherapy (P<0.01) ↓AST levels in all groups (-13.8 IU/L, P=0.0195 with TOFO, -31.2 IU/L, P=0.0102 with PIO and -25.2 IU/L, P<0.001 with combo therapy). ↓ γGT with TOFO (-15.3 IU/L, P=0.0189) and combo treatment (-37.4 IU/L P=0.0081)	↓Body weight with TOFO (-3.25 kg, P<0.001), but ↑ with PIO (2.46 kg, P=0.0341). No change with combo treatment	Compared to baseline: ↓HbA1c with combo therapy (-0.80%, P<0.001) and ↓HOMA-IR (-3.12, P<0.001). ↓HbA1c with TOFO and PIO monotherapy (-0.36%, P=0.0027 and -0.73%, P=0.0014, respectively) with no change in HOMA-IR. No change in TGs or chol levels with TOFO. PIO and combo therapy ↓ TGs (-48.3 mg/dL, P=0.0077 and -24.5 mg/dL, P= 0.0073 respectively) and ↑ HDL (8.83 mg/dL, P<0.001 and 8.28 mg/dL, P<0.001, respectively). ↓uric acid in TOFO and combo therapy group (-0.90 mg/dL, P<0.001 and -0.88 mg/dL, P<0.001, respectively)	Compared to baseline: ↓cytokeratin-18 fragment M30 antigen from baseline with combo therapy and PIO (-377.5 U/L, P<0.001 and -252.1 U/L, P=0.0156 respectively). ↓urinary 8-hydroxydeoxyguanosine in all groups (-8.93 ng/ mL, P<0.001 with TOFO; -6.608 mg/mL, P=0.0428 with PIO; -4.86ng/ mL, P=0.0309 with TOFO+PIO. ↑adiponectin from baseline in all groups (0.40 μg/mL, P=0.0107 vs. 7.21 μg/mL, P<0.001 vs. 5.45 μg/mL, P<0.001, respectively). ↑3-hydroxybutyrate, ketone bodies and acetate in pts under combo therapy (56.7 μmol/L, P=0.0109, 74.3 μmol/L, P=0.0120 and 17.63 μmol/L, P=0.0190, respectively)
Gastaldelli *et al* [33], 2019, *post hoc* analysis of RCT	EXE+DAPA (n=228) vs. EXE+ PLB (n=227) vs. DAPA+ PLB(n=230) / T2DM uncontrolled by metformin /52 wks	↓ ALT and AST levels at wks 28 and 52 with EXE +DAPA compared to EXE +PLB (P=0.0026 for ALT at both wks and 0.0052 for AST at wk 28 and 0.0551 at wk 52). ALT ↓with DAPA+PLB at wks 28 and 52 (P<0.001 and 0.0072 respectively) compared to baseline. EXE +DAPA ↓ γ-GT at wks 28, 52 and at wk 28 with DAPA+PLB	↓Body weight from baseline in all groups (P<0.05) at wks 28 and 52	EXE +DAPA ↓ TGs at wks 28 and 52 (P<0.001 and 0.0143 respectively), EXE+PLB ↓ TGs at wk 28 (P=0.0237) compared to baseline	Compared to baseline EXE +DAPA ↓HOMA-IR at wks 28 and 52 similarly with DAPA+PLB (but to a greater degree compared to EXE+PLB, P<0.001). Adipo-IR was reduced with EXE +DAPA (P=0.0148) and DAPA+PLB at wk 52 (P=0.0073)

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Table 2 (A) *(Continued)*

Authors/ [ref.] /year, type of study	Treatment/ population/ duration	Effects on liver enzymes	Changes in anthropometric parameters	Changes in laboratory values	Changes in other metabolic parameters
Harreiter *et al* [34], 2021, RCT	EXE+DAPA (n=16) vs. DAPA+PLB (n=14) /T2DM under metformin / 24 wks	EXE+DAPA: ALT ↓ compared to baseline (P<0.01). Trend to ↓ levels with DAPA +PLB (P=0.06). ↓ AST in both arms (P<0.05 for EXE+DAPA and P<0.01 for DAPA +PLB). Significant ↓ of γGT compared to baseline only with DAPA+PLB (P<0.01). No between- groups differences for liver enzymes at the end of study	↓BMI (P<0.001 with EXE +DAPA and P<0.01 with DAPA+PLB), ↓waist (P<0.01 and P<0.001 respectively) and hip circumference from baseline. No significant differences between groups	No difference in TGs, HDL and LDL cholesterol between groups at the end of study. EXE+PLB ↓fasting glucose and HbA1c to a greater degree than DAPA+PLB (P=0.03 and <0.01 respectively) at the end of study. 68% of pts on EXE+DAPA had HbA1c <6.5% (vs. 0% at baseline, P=0.001) and 35.7% on DAPA+PLB (vs. 15.4% at baseline, P=0.25)	HOMA-IR ↓in the DAPA+PLB group, No differences between groups at the end of study
Loomba *et al* [35], 2020, Phase 2b trial	PLB (n=39) or selonsertib (n=39) or cilofexor (n=40) or firsocostat (n=40) or firsocostat +selonsertib (n=79) or cilofexor+ selonsertib (n=77) or cilofexor+ firsocostat (n=78)/NASH bridging fibrosis or compensated cirrhosis/48 wks	Compared with PLB, cilofexor + firsocostat statistically significantly ↓ ALT (P=0.033), AST (P=0.05), ALP (P=0.017) and total bilirubin (P=0.010). No statistically significant changes compared to PLB with the other treatment regimens	Cilofexor + firsocostat ↓ body weight at wk 48 compared to PLB (P=0.060)	Cilofexor + firsocostat statistically significantly ↓fasting insulin (P=0.020) and estimated glomerular filtration rate (P=0.029) vs. PLB. Cilofexor +firsocostat ↑ total chol (P=0.005), VLDL and TGs (P<0.001 for both) and ↓ HDL (P=0.012). Firsocostat+ selonsertib: ↑total chol (P=0.035). Firsocostat monotherapy ↑ VLDL and TGs vs. PLB at wk 48 (P<0.001 and P=0.005 respectively)	Cilofexor + firsocostat statistically significantly ↓ total bile acids (P=0.005) and CK18 M30 (P=0.006) vs. PLB at wk 48
Alkhouri *et al* [36], 2022, RCT phase II	SEMA alone (n=21) vs. SEMA with firsocostat (n=22) or cilofexor 30 mg (n=22) or cilofexor 100 mg (n=22) or firsocostat + cilofexor (n=21)/ NASH/ 24 wks	Greater ↓of ALT levels in the combo treatment arms compared with SEMA alone (-32 to -40 U/L vs. -13 UL/L, P<0.05) Normalization of ALT after 24 wks in 50% of pts under SEMA monotherapy vs. 85.7 -100% under combo therapies.	Significant ↓ of body weight in SEMA+ cilofexor 30 mg group. Relative ↓in body weight from baseline to wk 24 similar across groups	Significant ↓of fasting glucose with SEMA +cilofexor 100 mg. Changes from baseline in HbA1c similar between groups. LDL ↑ at wk 24 in pts who received SEMA+cilofexor 100 mg (P<0.05 vs. SEMA alone); no changes with cilofexor 30 mg Fircosostat containing regimens ↑ TGs and VLDL but ↓ HDL (P<0.05 vs. SEMA monotherapy).	Greater ↓ of CK -18 M30 levels with SEMA+firsocostat compared to SEMA monotherapy (P=0.0102).

GLP-1, glucagon-like peptide-1; SGLT-2, sodium glucose cotransporter 2; NAFLD, nonalcoholic fatty disease; NASH, nonalcoholic steatohepatitis; ref, reference; RCT, randomized controlled trial; PIO, pioglitazone; EXE, exenatide; T2DM, type 2 diabetes mellitus; wk, week; ALT, alanine aminotransferase; combo, combination; HbA1c, hemoglobin A1c; FFA, free fatty acids; TGs, triglycerides; HDL, high-density lipoprotein; TOFO, tofogliflozin; AST, aspartate aminotransferase; γ-GT, γ-glutamyl transpeptidase; HOMA-IR, homeostatic model assessment for insulin resistance; chol, cholesterol; pts, patients; DAPA, dapagliflozin; PLB, placebo; Adipo-IR, adipose tissue insulin resistance index; BMI, body mass index; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; SEMA, semaglutide

Table 2 (B) Clinical trials that evaluated combinations of antidiabetic (pioglitazone, GLP-1 agonists, SGLT-2 inhibitors) and "NAFLD-specific" drugs in NAFLD/NASH

Authors/ [ref.] /year	Effects on histologic findings	Effects on imaging findings	Effects on scores related to NAFLD	Conclusions
Sathyanarayana *et al* [31], 2011	NA	Compared to baseline PIO +EXE: significantly ↓ hepatic fat content (measured by MRS (4.7% vs. 12.1%, P<0.001) greater than PIO alone (6.5% vs. 11.0%, P<0.05).	NA	PIO +EXE is associated with a greater ↓ in hepatic fat content compared to PIO alone in pts with T2DM under metformin or diet
Yoneda *et al* [32], 2022	NA	Compared to baseline improvement of MRI-PDFF after 24 wks (-3.38%, P=0.0061 with TOFO and -5.56%, P<0.001 with PIO), but further improvement with combo therapy (-2.60% and -0.42%, respectively). PIO and combo therapy significantly ↓ MRE-LSM (-0.43 kPa, P=0.00364 and -0.40 kPa, P<0.001, respectively). ↓ type IV collagen 7S with combo therapy (-0.41ng/mL, P=0.0193) but no improvement with monotherapies. All treatments ↓ WFA+-M2BP (-0.09 with TOFO, -0.20 with PIO and -0.19 with combo therapy).	NA	In addition to the additive effects of PIO and TOFO in pts with T2DM and NAFLD, combo therapy ↓weight gain and induce cardioprotective effect.
Gastaldelli *et al* [33], 2019	NA	NA	Greater changes in FLI with EXE+DAPA vs. DAPA+PLB at wk 28 (P=0.0162) and in FLI and NLFS with EXE +DAPA vs. EXE +PLB at wks 28 and 52 (P=0.008 and 0.0036 for FLI and P<0.001 and P<0.001 for NLFS, respectively). FIB-4 ↓only in pts under combo therapy. At wk 28, combo treatment ↓ the proportion of pts with scores suggestive of fibrosis and severe fibrosis (i.e. FIB-4 ≥1.3 and NFS >0.676) by 4.1% and 2.8 %, respectively	EXE+DAPA had stronger effects in improvement of markers of hepatic steatosis and fibrosis than EXE +PLB or DAPA+PLB in pts with T2DM
Harreiter *et al* [34], 2021	NA	In both groups, HCL, VAT and SAT ↓ similarly. HCL: positive correlation with changes in body weight (r=0.54, P=0.002), waist and hip circumference (r=0.40, P=0.03 for waist and hip), VAT (r=0.41, P=0.04) and SAT (r=0.62, P=0.001)	No differences between the 2 arms regarding FIB-4 score or FLI at the end of study. Both treatments ↓FLI (P< 0.002). FIB-4 score ↓ with DAPA +PLB (P=0.028)	After 24 weeks HCLs were significantly but comparably ↓ in EXE+DAPA and DAPA+PLB groups, despite better glycemic control in the EXE +DAPA group. Changes in HCLs were associated with ↓visceral adiposity

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Table 2 (B) *(Continued)*

Authors/ [ref.] /year	Effects on histologic findings	Effects on imaging findings	Effects on scores related to NAFLD	Conclusions
Loomba *et al* [35], 2020	No significant differences for the primary end point (≥1 stage improvement in fibrosis without worsening of NASH) between groups. Cilofexor+firsocostat more likely achieved a ≥2-point improvement in NAS compared to PLB (35% vs. 11%, P=0.002) and ≥1-grade improvements in steatosis (26% vs. 6%, P=0.009), ballooning (29% vs. 13%, P=0.04) and lobular inflammation (57% vs. 29%, P=0.004). Progression to cirrhosis less frequently with cilofexor + selonsertib than PLB (8% vs. 41%, P=0.018). Compared with PLB, cilofexor+firsocostat significantly ↓ ML NASH CRN fibrosis score (P=0.04)	With firsocostat, steatosis based on MRI-PDFF and liver histology was ↓ compared to baseline (P=0.033 and P=0.017 vs. PLB at wk 48 respectively). Steatosis according to MRI-PDFF was ↓ in all combo treatments vs. PLB at week 48 (P=0.003 for firsocostat+selonsertib, P=0.043 for cilofexor+selonsertib and P=0.002 for cilofexor/firsocostat)	ELF score was↓ with cilofexor +firsocostat compared to PLB at the end of study (P=0.024)	In pts with bridging fibrosis and cirrhosis, 48 wks of cilofexor+ firsocostat was well tolerated, improved NASH activity and may have an antifibrotic effect
Alkhouri *et al* [36], 2022	NA	Greater ↓in liver steatosis (MRI -PDFF) with combo therapies compared with SEMA alone -significant only for SEMA+firsocostat arm (-11% vs. -8% with SEMA alone, P=0.0353). Greater proportion of pts achieved relative ↓ in MRI -PDFF of ≥50% from baseline with combo therapy, compared to SEMA alone (58.8%-76.2% vs. 38.9%, respectively, P>0.05). 29.4% of pts who received SEMA alone achieved liver fat <5% in MRI-PDFF vs. 38.1%-41.2% under combo therapy, P>0.05).	↓FAST score in all combo regimens except for SEMA+cilofexor 100 mg compared to SEMA alone. In all arms ↓ liver stiffness from baseline (ELF score or transient elastography). No significant differences between groups. No change in liver stiffness from baseline measured by MRE -no differences between groups. No differences in ↓ of Fibrosure and Fibrotest between combo treatment and monotherapy	In pts with NASH and mild to moderate fibrosis SEMA with firsocostat and/or cilofexor was well tolerated. Combination treatments resulted in greater improvement in hepatic steatosis, liver biochemistry and several hepatic and metabolic parameters compared to SEMA monotherapy

GLP-1, glucagon-like peptide-1; SGLT-2, sodium glucose cotransporter 2; NAFLD, nonalcoholic fatty disease; NASH, nonalcoholic steatohepatitis; ref, reference; NA, not applicable; PIO, pioglitazone; EXE, exenatide; MRS, magnetic resonance spectroscopy; pt, patient; T2DM, type 2 diabetes mellitus; MRI-PDFF, magnetic resonance imaging proton density fat fraction; wk, week; combo, combination; TOFO, tofogliflozin; MRE, magnetic resonance elastography; LSM, liver stiffness measurements; WFA+-M2BP, wisteria floribunda agglutinin-positive Mac-2 binding protein; FLI, fatty liver index; NLFS, NAFLD liver fat score; DAPA, dapagliflozin; PLB, placebo; FIB-4, fibrosis-4 index; NFS, NAFLD fibrosis score; HCL, hepatocellular lipid; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; NAS, NAFLD activity score; ML, machine learning; CRN, Clinical Research Network; ELF, enhanced liver fibrosis; SEMA, semaglutide; FAST, fibroscan-AST

but the increase was greater in the latter arm (193% vs. 86%, P<0.001).

Tofogliflozin and pioglitazone

Yoneda *et al* [32] conducted an open-label, prospective randomized trial in which tofogliflozin, an SGLT2 inhibitor, and pioglitazone were combined to treat hepatic steatosis in patients with T2DM and NAFLD, defined as ≥10% liver fat content on magnetic resonance imaging proton density fat fraction (MRI-PDFF). This study was actually the second half of the ToPiND trial, which investigated the effectiveness of tofogliflozin and pioglitazone monotherapy on NAFLD. Forty patients were initially assigned to receive tofogliflozin 20 mg or pioglitazone 15-30 mg q.d. for 24 weeks. In 20 patients who received tofogliflozin and 12 who received pioglitazone and met the inclusion criteria, combination treatment was administered for an additional 24 weeks.

In patients who first received pioglitazone with the later addition of tofogliflozin, combination therapy showed an additional improvement in ALT levels compared to monotherapy (P<0.01). MRI-PDFF was improved after 24 weeks of monotherapy treatment (-3.38±4.90%, P=0.0061 with tofogliflozin, and -5.56±3.92%, P<0.001 with pioglitazone), but combination treatment further improved MRI-PDFF by -2.60% and -0.42%, respectively. Interestingly, pioglitazone and combination therapy significantly reduced magnetic resonance elastography liver stiffness measurements (MRE-LSM) (-0.43±0.61 kPa, P=0.00364 and -0.40±0.54 kPa, P<0.001, respectively). Adiponectin increased from baseline in all groups (0.40±0.63 μg/mL, P=0.0107 vs. 7.21±5.12 μg/mL, P<0.001, vs. 5.45±3.90 μg/mL, P<0.001 respectively).

Combination of newer antidiabetic agents

Exenatide and dapagliflozin

Two studies investigated the effects of the combination of exenatide and dapagliflozin, a SGLT2 inhibitor, in patients with T2DM and NAFLD/NASH. The first was a *post hoc* analysis of the DURATION-8 study, which enrolled patients with T2DM uncontrolled by metformin monotherapy. In this study, Gastaldelli *et al* [33] assessed the efficacy of exenatide once weekly subcutaneously combined with dapagliflozin once daily, versus each drug alone, in lowering noninvasive biomarkers of liver steatosis and fibrosis along with liver biochemistry and insulin resistance. In total, 695 participants were randomized to receive exenatide 2 mg once weekly plus dapagliflozin 10 mg/day orally, exenatide 2 mg once weekly plus placebo or dapagliflozin 10 mg/day plus placebo for 104 weeks. The biomarkers that were evaluated at weeks 28 and 52 were fatty liver index (FLI) (based on serum triglyceride levels, γ-glutamyltranspeptidase [γ-GT], body mass index [BMI], and waist circumference), NAFLD liver fat score [NLFS] (which includes the presence of T2DM and MetS, fasting serum insulin, AST and the AST: ALT ratio) for evaluation of steatosis, as well as the fibrosis-4 index [FIB-4] (which comprises age, platelet count [PLT], AST, and ALT) and the NAFLD fibrosis score [NFS] (which is based on the presence of impaired fasting glucose or T2DM and includes age, BMI, PLT, AST: ALT ratio and albumin) for the evaluation of fibrosis. Interestingly, greater changes were observed in: a) FLI with the combination of exenatide/dapagliflozin versus dapagliflozin/placebo at week 28 (P=0.0162); and b) FLI and NLFS in the combination treatment group compared with the exenatide/placebo group at weeks 28 and 52 (P=0.008 and 0.0036 for FLI and P<0.001 and P<0.001 for NLFS, respectively). At weeks 28 and 52, similar reductions in NFS were found in all groups, whereas FIB-4 decreased only in patients under combination therapy (P=0.0135 and 0.0308, respectively). At week 28, combination treatment reduced the proportion of patients with noninvasive scores suggestive of severe fibrosis (i.e., FIB-4 ≥1.3 and NFS >0.676) by 4.1% and 2.8%, respectively.

Harreiter *et al* [34] investigated the effects of combined exenatide and dapagliflozin versus dapagliflozin and placebo on hepatocellular lipid (HCL) concentrations in patients with T2DM under metformin therapy. Subjects were randomized and stratified by BMI to receive either exenatide 2 mg subcutaneously once a week and dapagliflozin 10 mg/day orally, or dapagliflozin 10 mg/day and placebo for 24 weeks. A hepatic triglyceride threshold of ≥5.56% was used to determine hepatic steatosis.

HCL, assessed by MRS, decreased similarly in both treatment groups compared to baseline. As regards liver enzymes, after 24 weeks of treatment, ALT levels were lower in the combination treatment group compared to baseline (P<0.01). The authors did not detect any differences between the 2 arms regarding FIB-4 score or FLI. Both therapeutic approaches reduced FLI (P=0.002 for both), whereas FIB-4 score was lower under dapagliflozin treatment (P=0.028) compared to baseline.

Combination of "NAFLD-specific" with or without antidiabetic agents

Selonsertib, cilofexor, and firsocostat

Loomba *et al* [35] evaluated the effects of selonsertib (an apoptosis signal-regulating kinase 1), cilofexor (a farnesoid X receptor agonist) and firsocostat (an ACCi), alone or in 2-drug combinations, in patients with biopsy-proven NASH-related bridging fibrosis or compensated cirrhosis. However, 20% of the controls were enrolled based on noninvasive markers consistent with advanced fibrosis: vibration-controlled transient elastography ≥14.4 kPa and enhanced liver fibrosis test ≥9.8. Patients were randomized to 7 groups: placebo, or selonsertib 18 mg, or cilofexor 30 mg, or firsocostat 20 mg, or combination treatment with either cilofexor/selonsertib or firsocostat/selonsertib or cilofexor/firsocostat. The regimens were administered orally once daily for 48 weeks. Liver biopsies were also performed at week 48 and were evaluated *post hoc* by a machine learning (ML) approach validated for the assessment

of NASH pathology. A weighted average of the proportionate areas of each fibrosis stage pattern was calculated (ML NASH Clinical Research Network [CRN] fibrosis score).

Differences in the primary endpoint (i.e., a ≥1-stage improvement in fibrosis without worsening of NASH) did not reach statistical significance between groups. However, combination treatment with cilofexor/firsocostat was more likely to achieve a ≥2-point improvement in NAFLD activity score compared to placebo (35% vs. 11%, P=0.002) and ≥1-grade improvements in steatosis (26% vs. 6%, P=0.009), ballooning (29% vs. 13%, P=0.04), and lobular inflammation (57% vs. 29%, P=0.004), while progression to cirrhosis was less frequent in patients treated with the combination of cilofexor/selonsertib than in those receiving placebo (8% vs. 41%, P=0.018).

With firsocostat monotherapy, steatosis based on MRI-PDFF and liver histology was decreased compared to baseline (P=0.033 and P=0.017 vs. placebo at week 48, respectively), while steatosis according to MRI-PDFF was also reduced in all combination treatments compared to placebo at week 48. Interestingly, compared with placebo, cilofexor/firsocostat significantly decreased ML NASH CRN fibrosis score (P=0.04) Finally, all combination groups reduced the proportionate area of steatosis compared to placebo (P-values always <0.05).

Semaglutide, cilofexor, and firsocostat

A phase II open-label, randomized proof-of-concept trial [36] evaluated the safety and tolerability of subcutaneous semaglutide (a GLP-1 agonist) alone or in combination with cilofexor and/or firsocostat in NASH patients with mild-to-moderate fibrosis (F2-F3) on biopsy or fat fraction ≥10% on MRI-PDFF and liver stiffness ≥7 kPa on transient elastography). Patients were randomized to receive semaglutide alone once a week (at a starting dose of 0.24 mg and increased monthly to 0.5 mg, 1.0 mg and 1.7 mg and to 2.4 mg after week 17). or combined with cilofexor 30 mg/day or cilofexor 100 mg/day or firsocostat 20 mg/day or cilofexor 30 mg and firsocostat 20 mg for 24 weeks.

All combination treatments achieved greater reduction in liver steatosis, evaluated by MRI-PDFF, compared with semaglutide alone, but the decrease was statistically significant only in the semaglutide plus firsocostat arm (-11% vs. -8% in semaglutide alone, P=0.0353). However, in a sensitivity analysis, excluding patients with imaging data at least 1 month after the last dose of the study, the difference between semaglutide compared to semaglutide plus cilofexor plus firsocostat was also significant (-8.6% vs. -12.6%, P=0.0078). The proportion of patients who achieved a relative reduction in MRI-PDFF of ≥50%, compared to baseline, was greater for the combination regimens than for semaglutide alone (58.8-76.2% vs. 38.9%, respectively, always P>0.05). Interestingly, 29.4% of the patients who received semaglutide alone achieved normalization of liver fat content by MRI-PDFF (i.e., liver fat <5%), compared to 38.1-41.2% of patients under combination regimens (always P>0.05). Treatment with semaglutide plus firsocostat and semaglutide plus cilofexor 30 mg significantly reduced liver steatosis assessed by the controlled attenuation parameter (CAP), compared to semaglutide monotherapy (P=0.0034 and 0.0379, respectively). Liver stiffness measured by magnetic resonance elastography (MRE) did not change from baseline to the end of study and no differences were observed between groups.

Discussion

The highly heterogenous pathogenesis of NAFLD/NASH implies that an individualized approach would be a reasonable option to treat and control the consequences of the disease [37,38]. Combining medications that have the same or, preferably, different targets would appear to be an interesting approach with many potential benefits. The concomitant use of drugs may have synergistic effects, enhancing the efficacy of the regimen. Additionally, this strategy allows the use of lower doses of each drug, increasing the tolerability and attenuating the possible side-effects [27]. Table 3 summarizes the combinations of drugs in these categories that have been studied so far. Several trials that investigated the efficacy of combination therapies in NAFLD/NASH are ongoing, and antidiabetic drugs, including pioglitazone or the newer classes of antidiabetic regimens, as well as "NAFLD-specific" drugs, are part of them (Table 4). Interestingly, newer antidiabetic drugs with more than one way of action—such as tirzepatide, a dual glucose-dependent insulinotropic polypeptide and GLP-1 receptor agonist, and cotadutide, a dual glucagon-like protein-1 receptor and glucagon receptor agonist—seem promising agents for the therapy of NAFLD/NASH [39,40].

So far, animal studies regarding this topic have shown encouraging results. In the study of Tahara *et al* [28], a combination of ipragliflozin and pioglitazone significantly and additively improved liver fibrosis in T2DM mice compared to monotherapy. However, in 5 *in vivo* studies using preclinical models of NASH and fibrosis [30], the combination of ACCi with hepatic lipid modulating agents did not augment antifibrotic efficacy. In a study by Koike *et al* [29], pancreatic insulin content and β cell area were further increased in db/db mice under combination therapy with liraglutide plus ipragliflozin, compared to ipragliflozin monotherapy, leading to better glycemic control. On the other hand, liraglutide and/or ipragliflozin reduced hepatic lipid accumulation similarly in DIO mice. However, no evaluation of fibrosis parameters was performed in this study, although fibrosis is considered to be an optimal target for these therapies.

Regarding the clinical studies published so far, 2 randomized controlled trials evaluated the combination of pioglitazone with either a GLP-1 receptor agonist or an SGLT-2 inhibitor. Sathyanarayana *et al* [31] found that, in patients with T2DM, combination treatment with pioglitazone and exenatide resulted in a greater reduction of ALT as well as hepatic fat content, compared to pioglitazone alone, although no significant change in body weight was observed. However, the effects of combined treatment on liver fibrosis were not evaluated in this study. In another study from Japan [32], the

Table 3 Summary of the combinations of antidiabetic (pioglitazone, GLP-1 agonists, SGLT-2 inhibitors) and "NAFLD-specific" drugs in NAFLD/NASH

Authors/ [ref.] /drugs	Drugs mechanism of action	Type of study
Tahara *et al* [28] / IPRA + PIO	SGLT-2 inhibitor + PPAR-γ agonist	Animal study
Koike *et al* [29] / IPRA + LIRA	SGLT-2 inhibitor + GLP-1 agonist	Animal study
Vijayakumar *et al* [30] / Analog of firsocostat+Feno or elafibranor or lanifibranor or seladelpar or saroglitazar or resmetirom	ACCi + PPAR agonist or ACCi +THRβ agonist	Animal study
Sathyanarayana *et al* [31] / EXE + PIO	GLP-1 agonist + PPAR-γ agonist	Clinical study
Yoneda *et al* [32] / TOFO+PIO	SGLT-2 inhibitor + PPAR-γ agonist	Clinical study
Gastaldelli *et al* [33] and Harreiter *et al* [34] / DAPA+EXE	SGLT-2 inhibitor + GLP-1 agonist	Clinical study
Loomba *et al* [35] / fircosostat +selonsertib or cilofexor+selonsertib or cilofexor+firsocostat	ACCi + apoptosis signal-regulating kinase 1 or FXR agonist + apoptosis signal-regulating kinase 1or FXR agonist + ACCi	Clinical study
Alkhouri *et al* [36] / SEMA+ firsocostat or SEMA + cilofexor or SEMA + firsocostat + cilofexor	GLP-1 agonist+ACCi or GLP-1 agonist + FXR agonist or GLP-1 agonist+ ACCi+ FXR agonist	Clinical study

GLP-1, glucagon-like peptide-1; SGLT-2, sodium glucose cotransporter 2; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; IPRA, ipragliflozin; PIO, pioglitazone; PPAR-γ, peroxisome proliferator-activated receptor-γ; LIRA, liraglutide; ACCi, acetyl-CoA carboxylase inhibitor (analog of firsocostat); Feno, fenofibrate; THRβ, thyroid hormone receptor β; EXE, exenatide; TOFO, tofogliflozin; DAPA, dapagliflozin; FXR, farnesoid X receptor; SEMA, semaglutide

Table 4 Ongoing trials evaluating combinations of antidiabetic (pioglitazone, GLP-1 agonists, SGLT-2 inhibitors) and "NAFLD specific" drugs in NAFLD/NASH

Number/ phase	Drugs	Arms	Population/ enrollment	Duration	Primary endpoints	Secondary endpoints
NCT05140694/4	Empagliflozin, Dulaglutide	1) Empagliflozin 10 mg pos once daily (available to control over ~25 mg) 2) Dulaglutide 0.75 mg sc once weekly (available to control over ~1.5 mg) 3) Empagliflozin 10 mg pos once daily + dulaglutide 0.75 mg sc once weekly	Metabolic-associated fatty liver disease and T2DM / 135	24 wks	HbA1c changes /CAP score changes	Changes of: LSM score, noninvasive liver fibrosis markers, body weight and body composition, lipid levels, ketone levels, liver parenchyma by ultrasonography, liver function parameters, liver fibrosis biomarkers, inflammation biomarkers
NCT04971785/2	SEMA, CILO, FIR, PTM SEMA, PTM CILO/ FIR	1) SEMA 0.24-2.4 mg once weekly and fixed-dose of CILO/FIR 30 mg/20 mg once daily for 72 weeks 2) SEMA 0.24-2.4 mg once weekly (dose escalation every 4 weeks) and PTM CILO/FIR administered once daily for 72 wks 3) PTM SEMA once weekly and CILO/FIR 30 mg/20 mg FDC administered once daily for 72 wks 4) PTM SEMA once weekly and PTM CILO/FIR once daily for 72 wks	Compensated cirrhosis due to NASH/ 440	72 wks	Percentage of participants: 1) who achieve ≥ 1-stage improvement in fibrosis According to the NASH CRN classification without worsening of NASH in participants treated With SEMA + CILO/ FIR vs PLB 2) with NASH resolution in participants treated with SEMA+CILO/ FIR vs PLB	1) Percentage of participants with NASH resolution in participants treated with SEMA+CILO/ FIR vs CILO/FIR 2) Percentage of participants who achieve ≥1-stage improvement in fibrosis (according to the NASH CRN Classification) without worsening of NASH in participants treated with SEMA+CILO/ FIR vs SEMA alone

(Contd...)

Table 4 *(Continued)*

Number/ phase	Drugs	Arms	Population/ enrollment	Duration	Primary endpoints	Secondary endpoints
NCT04639414/4	Empagliflozin SEMA, PLB	1) Empagliflozin 10 mg pos/ SEMA 1 mg inj 2) Empagliflozin 10 mg pos and PLB matching SEMA 3) PLB matching empagliflozin and PLB matching SEMA	T2DM with NASH/ 192	48wks	Histological resolution of NASH without worsening of fibrosis	1) Overall NAS 2) Stage of fibrosis according to the Kleiner Fibrosis Classification 3) Activity component of NASH according to the SAF score 4) Hepatic steatosis grade
NCT04976283/4	Empagliflozin, PIO	1) PIO up to 45 mg/ day with (or without) metformin and/or DPP4 inhibitor 2) Empagliflozin up to 25 mg/day with (or without) metformin and/or DPP4 inhibitor 3) PIO up to 45 mg/day with (or without) metformin and/or DPP4 inhibitor, plus empagliflozin up to 25 mg/day	T2DM and NAFLD/ 123	12 months	Change in radiologic liver parameters	Change in 1) liver enzymes, 2) FIB-4 Score and NAFLD Fibrosis Score, 3) body weight, 4) waist circumference, 5) liver fat mass with total body fat, 6) HbA1C levels, 7) fasting blood sugar, 8) lipid profile
NCT05232071/2	Lanifibranor (PPAR agonist), PLB, empagliflozin	1) lanifibranor 800 mg 2) PLB 3) lanifibranor 800 mg+empagliflozin 10 mg	T2DM and NASH / 63	24wks	Assessment of the effect of lanifibranor alone and in combo with empagliflozin compared to PLB on absolute change from baseline to wk 24	NA
NCT04065841/2	Tropifexor (FXR agonist) Licogliflozin (FXR agonist)	1) tropifexor +licogliflozin 2) tropifexor alone (+licogliflozin PLB) 3) licogliflozin alone(+tropifexor PLB) 4) PLB licogliflozin + PLB tropifexor	NASH and fibrosis(stages 2,3)/ 380	48 wks	To evaluate the efficacy of tropifexor and licogliflozin in combo and as monotherapy, as assessed by histologic improvement compared to PLB in NASH and stage 2 or 3 fibrosis 1) achievement of at least one stage of improvement in fibrosis without worsening of NASH	1) Achievement of NASH resolution and no worsening of fibrosis OR improvement in fibrosis by at least one stage without worsening of NASH 2) At least one stage improvement in fibrosis 3) At least two stage improvement in fibrosis without worsening of NASH 4) ≥5% reduction in body weight 5) Change in liver fat content based on MRI -PDFF

(Contd...)

Table 4 *(Continued)*

Number/ phase	Drugs	Arms	Population/ enrollment	Duration	Primary endpoints	Secondary endpoints
					2) NASH resolution without worsening of fibrosis	6) AST and ALT changes over time 7) γGT changes over time 8) Occurrence of adverse events, serious adverse events, adverse events resulting in discontinuation of treatment, changes in vital signs and laboratory parameters
NCT03646292/4	PIO, Empagliflozin	1) PIO 15 mg daily 2) Empagliflozin 10 mg daily 3) PIO 15 mg +Empagliflozin 10 mg	T2DM and NAFLD / 60	6 months	Liver fat change measured by MRI-PDFF in co-localized regions of interest within 9 liver segments	1) Liver fibrosis measured by MRE 2) Changes in lipid profile, liver enzymes, glucose metabolism, inflammation status

GLP-1, glucagon-like peptide-1; SGLT2, sodium glucose cotransporter 2; NAFLD, nonalcoholic fatty disease; NASH, nonalcoholic steatohepatitis; pos, per os; sc, subcutaneous; T2DM, type 2 diabetes mellitus; wk, week; HbA1c, hemoglobin A1c; CAP, controlled attenuation parameter; LSM, liver stiffness measurement; SEMA, semaglutide; CILO, cilofexor; FIR, firsocostat; PTM, placebo to match; CRN, clinical research network; PLB, placebo; inj, injection; NAS, NAFLD activity score; SAF, steatosis-activity-fibrosis; PIO, pioglitazone; DPP4, dipeptyl peptidase 4; FIB-4, Fibrosis-4; NA, not applicable; PPAR, peroxisome proliferator-activated receptor; combo, combination; FXR, farnesoid X receptor; MRI-PDFF, magnetic resonance imaging proton density fat fraction; AST, aminotransferase; ALT, alanine aminotransferase, γGT, γ glutamyl transpeptidase; MRE, magnetic resonance elastography

combination of pioglitazone and tofogliflozin improved ALT levels, liver steatosis and stiffness compared to tofogliflozin alone, in patients with T2DM and NAFLD. Interestingly, the combination treatment also resulted in an improvement of lipidemic profile and increased adiponectin levels.

Regarding the newer antidiabetic agents, the combination of exenatide and dapagliflozin has been studied in 2 trials, with contradictory results. In the first study [33], the combination treatment improved markers of liver steatosis and fibrosis in patients with T2DM, uncontrolled by metformin; however, in the second study [34], which was a small pilot study, combination therapy had no additive effects on the reduction of hepatocellular lipids in patients with T2DM, despite better glycemic control.

As for the use of "NAFLD-specific" drugs, in a phase 2b trial [35], which enrolled patients with bridging fibrosis or compensated cirrhosis attributable to NASH, steatosis was reduced in all studied combination treatments (cilofexor/firsocostat, cilofexor/selonsertib and firsocostat/selonsertib) versus placebo. However, only the combination of cilofexor/firsocostat was found to improve NASH activity, and there were indications that it may also exert an antifibrotic effect, so this combination regimen seems to be a better option for this category of patients. In another phase 2 trial [36], which studied the combinations of semaglutide/cilofexor, semaglutide/firsocostat and semaglutide/cilofexor/firsocostat in patients with mild to moderate fibrosis due to NASH, only semaglutide/firsocostat significantly reduced liver steatosis measured by MRI-PDFF or CAP, whereas semaglutide plus cilofexor 30 mg reduced only steatosis evaluated by CAP, compared to monotherapy with semaglutide. However, no differences in liver stiffness were observed between groups. Interestingly, compared to semaglutide monotherapy, the FAST score, which incorporates liver stiffness, liver steatosis and AST levels, was reduced in all combination regimens except for semaglutide plus cilofexor 100 mg.

Concluding remarks

Combining new antidiabetic medicines as well as new "NAFLD-specific" drugs is a promising approach to the treatment of NAFLD/NASH, and many trials are ongoing in this area (Table 4). As no treatment is currently approved for this entity, further research is needed to specify the categories of patients that could benefit more from this strategy, focusing on patients with or without T2DM/MetS and taking into account the complexity of NASH pathophysiology.

References

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016;**64**:73-84.
2. Vernon G, Baranova A, Younossi ZM. Systematic review: the

epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011;**34**:274-285.
3. Jinjuvadia R, Antaki F, Lohia P, Liangpunsakul S. The association between nonalcoholic fatty liver disease and metabolic abnormalities in the United States population. *J Clin Gastroenterol* 2017;**51**:160-166.
4. Saklayen MG. The global epidemic of the metabolic syndrome. *Curr Hypertens Rep* 2018;**20**:12.
5. Kim D, Chung GE, Kwak MS, et al. Body fat distribution and risk of incident and regressed nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2016;**14**:132-138.e4.
6. Eslam M, Sanyal AJ, George J; International Consensus Panel. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* 2020;**158**:1999-2014.
7. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* 2020;**73**:202-209.
8. Polyzos SA, Mantzoros CS. Making progress in nonalcoholic fatty liver disease (NAFLD) as we are transitioning from the era of NAFLD to dys-metabolism associated fatty liver disease (DAFLD). *Metabolism* 2020;**111S**:154318.
9. Cusi K. Time to include nonalcoholic steatohepatitis in the management of patients with type 2 diabetes. *Diabetes Care* 2020;**43**:275-279.
10. Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. *J Hepatol* 2019;**71**:793-801.
11. Dai W, Ye L, Liu A, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a meta-analysis. *Medicine (Baltimore)* 2017;**96**:e8179.
12. Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. *Metabolism* 2016;**65**:1096-1108.
13. Angulo P. Long-term mortality in nonalcoholic fatty liver disease: is liver histology of any prognostic significance? *Hepatology* 2010;**51**:373-375.
14. Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE. From NAFLD to NASH to cirrhosis-new insights into disease mechanisms. *Nat Rev Gastroenterol Hepatol* 2013;**10**:627-636.
15. Shetty K, Chen J, Shin JH, Jogunoori W, Mishra L. Pathogenesis of hepatocellular carcinoma development in non alcoholic fatty liver disease. *Curr Hepatol Rep* 2015;**14**:119-127.
16. Wainwright P, Scorletti E, Byrne CD. Type 2 diabetes and hepatocellular carcinoma: risk factors and pathogenesis. *Curr Diab Rep* 2017;**17**:20.
17. Kenneally S, Sier JH, Moore JB. Efficacy of dietary and physical activity intervention in non-alcoholic fatty liver disease: a systematic review. *BMJ Open Gastroenterol* 2017;**4**:e000139.
18. Hallsworth K, Adams LA. Lifestyle modification in NAFLD/NASH: Facts and figures. *JHEP Rep* 2019;**1**:468-479.
19. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology* 2015;**149**:367-378.e5.
20. Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. *J Hepatol* 2017;**67**:829-846.
21. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016;**65**:1038-1048.
22. Polyzos SA, Kountouras J, Mantzoros CS. Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. *Metabolism* 2019;**92**:82-97.
23. Bedossa P. Pathology of non-alcoholic fatty liver disease. *Liver Int* 2017;**37**(Suppl 1):85-89.
24. Makri E, Goulas A, Polyzos SA. Epidemiology, pathogenesis, diagnosis and emerging treatment of nonalcoholic fatty liver disease. *Arch Med Res* 2021;**52**:25-37.
25. Polyzos SA, Kang ES, Boutari C, Rhee EJ, Mantzoros CS. Current and emerging pharmacological options for the treatment of nonalcoholic steatohepatitis. *Metabolism* 2020;**111S**:154203.
26. Dufour JF, Caussy C, Loomba R. Combination therapy for non-alcoholic steatohepatitis: rationale, opportunities and challenges. *Gut* 2020;**69**:1877-1884.
27. Makri ES, Makri E, Polyzos SA. Combination therapies for nonalcoholic fatty liver disease. *J Pers Med* 2022;**12**:1166.
28. Tahara A, Takasu T. SGLT2 inhibitor ipragliflozin alone and combined with pioglitazone prevents progression of nonalcoholic steatohepatitis in a type 2 diabetes rodent model. *Physiol Rep* 2019;**7**:e14286.
29. Koike M, Saito H, Kohno G, Takubo M, Watanabe K, Ishihara H. Effects of GLP-1RA and SGLT2i, alone or in combination, on mouse models of type 2 diabetes representing different disease stages. *Int J Mol Sci* 2021;**22**:11463.
30. Vijayakumar A, Okesli-Armlovich A, Wang T, et al. Combinations of an acetyl CoA carboxylase inhibitor with hepatic lipid modulating agents do not augment antifibrotic efficacy in preclinical models of NASH and fibrosis. *Hepatol Commun* 2022;**6**:2298-2309.
31. Sathyanarayana P, Jogi M, Muthupillai R, Krishnamurthy R, Samson SL, Bajaj M. Effects of combined exenatide and pioglitazone therapy on hepatic fat content in type 2 diabetes. *Obesity (Silver Spring)* 2011;**19**:2310-2315.
32. Yoneda M, Kobayashi T, Honda Y, et al. Combination of tofogliflozin and pioglitazone for NAFLD: Extension to the ToPiND randomized controlled trial. *Hepatol Commun* 2022;**6**:2273-2285.
33. Gastaldelli A, Repetto E, Guja C, et al. Exenatide and dapagliflozin combination improves markers of liver steatosis and fibrosis in patients with type 2 diabetes. *Diabetes Obes Metab* 2020;**22**:393-403.
34. Harreiter J, Just I, Leutner M, et al. Combined exenatide and dapagliflozin has no additive effects on reduction of hepatocellular lipids despite better glycaemic control in patients with type 2 diabetes mellitus treated with metformin: EXENDA, a 24-week, prospective, randomized, placebo-controlled pilot trial. *Diabetes Obes Metab* 2021;**23**:1129-1139.
35. Loomba R, Noureddin M, Kowdley KV, et al; ATLAS Investigators. Combination therapies including cilofexor and firsocostat for bridging fibrosis and cirrhosis attributable to NASH. *Hepatology* 2021;**73**:625-643.
36. Alkhouri N, Herring R, Kabler H, et al. Safety and efficacy of combination therapy with semaglutide, cilofexor and firsocostat in patients with non-alcoholic steatohepatitis: A randomised, open-label phase II trial. *J Hepatol* 2022;**77**:607-618.
37. Polyzos SA, Kountouras J, Anastasiadis S, Doulberis M, Katsinelos P. Nonalcoholic fatty liver disease: is it time for combination treatment and a diabetes-like approach? *Hepatology* 2018;**68**:389.
38. Polyzos SA, Kountouras J, Zavos C, Deretzi G. Nonalcoholic fatty liver disease: multimodal treatment options for a pathogenetically multiple-hit disease. *J Clin Gastroenterol* 2012;**46**:272-284.
39. Muzurović EM, Volčanšek, Tomšić KZ, et al. Glucagon-like peptide-1 receptor agonists and dual glucose-dependent insulinotropic polypeptide/glucagon-like peptide-1 receptor agonists in the treatment of obesity/metabolic syndrome, prediabetes/diabetes and non-alcoholic fatty liver disease-current evidence. *J Cardiovasc Pharmacol Ther* 2022;**27**:10742484221146371.
40. Boland ML, Laker RC, Mather K, et al. Resolution of NASH and hepatic fibrosis by the GLP-1R/GcgR dual-agonist Cotadutide via modulating mitochondrial function and lipogenesis. *Nat Metab* 2020;**2**:413-431.