Combination therapy for non-alcoholic steatohepatitis: rationale, opportunities and challenges

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ABSTRACT

Non-alcoholic steatohepatitis (NASH) is becoming a leading cause of cirrhosis with the burden of NASH-related complications projected to increase massively over the coming years. Several molecules with different mechanisms of action are currently in development to treat NASH, although reported efficacy to date has been limited. Given the complexity of the pathophysiology of NASH, it will take the engagement of several targets and pathways to improve the results of pharmacological intervention, which provides a rationale for combination therapies in the treatment of NASH. As the field is moving towards combination therapy, this article reviews the rationale for such combination therapies to treat NASH based on the current therapeutic landscape as well as the advantages and limitations of this approach.

INTRODUCTION

In the context of therapies for liver diseases, the treatment of non-alcoholic steatohepatitis (NASH) represents a major unmet need. Therefore, treatment of NASH is a major focus of drug development worldwide. Currently, there are no Food and Drug Administration (FDA)-approved or European Medicines Agency (EMA)-approved therapies for NASH. As of December 2019, 84 interventional studies were ongoing and had enrolled patients to evaluate the therapeutic efficacy of treatments for NASH (www.clinicaltrial.gov). Most of these trials are testing new drugs as monotherapy with some trials investigating combination therapy for the treatment of NASH.

It is now well accepted that fibrosis stage is a major predictor of liver-related morbidity and mortality. FDA and EMA guidance documents indicate that for clinical approval of new drugs for the treatment of NASH, trials should include patients who have significantly higher risk of progression to cirrhosis and hepatic decompensation, as defined as those who have biopsy-proven NASH with stage 2 fibrosis or higher. The regulatory approval pathway for pharmacological therapies for NASH requires therapies to show clinical benefit in improving liver-related outcomes for full regulatory approval, which may take several years due to low event rates. To expedite drug development, liver histological improvements have been accepted as a surrogate for clinical improvements for a subpart H approval process. The subpart H approval endpoints include either one-stage improvement in liver fibrosis or resolution of NASH. This approval is contingent on showing clinical benefits over long-term follow-up for full approval.

Several therapies have been investigated for the treatment of NASH-related fibrosis using liver histology improvement as an endpoint. It has been observed that the difference in treatment effect relative to placebo has been relatively small. The percentage of patients with histological resolution of NASH in completed trials of a drug as monotherapy does not exceed 32% over placebo and this holds true across drugs with different mechanisms of action (figure 1). This underlies the complexity of the pathophysiology of NASH, which is driven by a metabolic overload that places stress on hepatocytes, leading to cell damage, inflammation and fibrosis. Metabolic overload impacts not only the liver, but also adipose tissue, the endocrine pancreas, the immune system and the gut. This complex pathogenesis explains why several classes of drug are in development for the treatment of NASH.

Figure 1 (A) Percentage of patients with resolution of non-alcoholic steatohepatitis (NASH) defined as ballooning 0 and inflammation 0–1, without worsening fibrosis 0–4, published on April 19, 2022 by guest. Protected by copyright. http://gut.bmj.com/ Gut: first published as 10.1136/gutjnl-2019-319104 on 7 May 2020. Downloaded from http://gut.bmj.com/ on April 19, 2022 by guest. Protected by copyright.
NASH, although one therapeutic target is unlikely to be sufficient when treating patients with NASH. As the success of drugs as monotherapy appears limited, combination of agents might seem a logical approach to increase efficacy, with numerous combinations possible. Logical combinations might see a drug with a metabolic mechanism of action combined with a drug with an anti-inflammatory or an antifibrotic mechanism of action. Some combinations might be selected based on specific patient characteristics, whereby patients are identified as potential responders to a combination. Except for patients with type 2 diabetes mellitus (T2DM), specific NASH subpopulations have not yet been well enough defined to become a selection or a stratification criterion in clinical trials precluding prespecified comparisons. The field investigating the role of combination therapy in the treatment of NASH is moving at a fast pace. In the current review, we discuss the rationale for such combination therapies to treat NASH based on the current therapeutic landscape as well as the advantages and limitations of this approach.

**DRUG CLASSES SUITABLE FOR COMBINATION THERAPY**

**FXR agonists**

Farnesoid X receptor (FXR) is a transcription factor activated by bile acids. As such FXR regulates bile-acid metabolism, but since bile-acid biology is paced by food intake, FXR also controls hepatic metabolism. Drugs-activating FXR have demonstrated effects in cholestatic liver disease and are in advanced clinical development for NASH. In phase 2 clinical trials, FXR agonists have shown an improvement of hepatic histology. The front runner—obeticholic acid, which is a bile-acid derivative—has shown efficacy on liver fibrosis and significantly more patients receiving obeticholic acid 25 mg daily had resolution of NASH without worsening of fibrosis at 18 months when patients with F1 fibrosis were also included in the analysis in an interim analysis of a phase 3 trial (NCT02548351). Major adverse effects related to FXR agonists include pruritus and increased low-density lipoprotein (LDL) cholesterol, both of which are dose dependent, and decreased high-density lipoprotein-cholesterol. LDL cholesterol increase may be managed by concomitant treatment with a statin. Regarding glucose metabolism, obeticholic acid has been reported in the phase 2 trial to increase circulating levels of insulin without affecting the glycemia resulting in a significant increase in homocysteine model assessment-estimated insulin resistance. Other FXR agonists that are being investigated include cilofexor (NCT03449446), EDP-305 (NCT03421431), EYP 001 (NCT03812029) and nidufexor (NCT02913105). Currently, there are no predictive biomarkers of response to an FXR agonist in patients with NASH and the response rate has been reported to be less than 25%. This provides a clear case for combining an FXR agonist with another drug to obtain a more robust response. Since stimulating FXR has pleotropic effects, such as improving steatosis, hepatic inflammation and fibrosis, an FXR agonist could be combined with different types of drugs. As the most advanced FXR agonist, obeticholic acid, has mostly demonstrated antifibrotic effects, it seems that this FXR agonist might benefit from combination with a second drug that has a metabolic mechanism of action, particularly one with a beneficial effect on lipoprotein metabolism. Indeed, several FXR agonists including tropifexor, cilofexor and obeticholic acid are currently being investigated in such a combination therapy (table 1).

Norursodeoxycholic acid, a bile acid derivative like many FXR agonists, but which is not an FXR agonist showed in a double-blind randomised, placebo-controlled phase 2 trial without histology a dose-dependent reduction in serum ALT (NCT03872921).

**PPAR agonists**

Peroxisome proliferator-activated receptors (PPARs) comprise a family of three transcription factors—PPAR-α, PPAR-δ and PPAR-γ—which are involved in lipid and glucose metabolism, and have anti-inflammatory effects. Schematically, PPAR-α plays a key role in fatty acid metabolism including absorption, transport and β-oxidation, PPAR-δ inhibits inflammatory macrophage phenotypes contributing to its anti-inflammatory effects, regulates the β-oxidation of free fatty acids and improves glucose homeostasis, and PPAR-γ regulates fatty acid storage and adipogenesis and improves insulin sensitivity in adipose tissue, liver and skeletal muscle. The PPAR-γ agonist pioglitazone at the dose of 30 mg failed to demonstrate an improvement of NASH compared with placebo in the PIVENS trial (NCT00063622). However, at a dose of 45 mg, pioglitazone resolved NASH in a significantly greater proportion of patients with pre-diabetes or T2DM compared with placebo (51% vs 19%; NCT00994682). Pioglitazone’s favourable effect was confirmed in a recent meta-analysis. Although the American Association for the Study of Liver Diseases and European Association for the Study of Liver guidelines recommend pioglitazone use in patients with biopsy-proven NASH, there are several side effects associated with this drug, including weight gain and fluid retention, and the increased risk of bone fracture, which limits its potential for combination with anti-NASH treatments. A second-generation PPAR-γ agonist designed to selectively modulate the entry of pyruvate into the mitochondria is currently being investigated in a phase 2b clinical trial (NCT02784444).

Several dual PPAR agonists are in clinical development with different agonistic profiles: elafibranor is a PPAR-α and PPAR-δ agonist, saroglitazar is a PPAR-α and PPAR-γ agonist, and lanifibranor is a pan-PPAR agonist. Based on positive findings from a phase 2 study (NCT01694849), elafibranor is currently in phase 3 development (NCT02704403). Saroglitazar improved liver biochemistries and hepatic steatosis in a phase 2 study (NCT03061721) and the results from the lanifibranor phase 2 trial (NCT03008070) are yet to be reported. The development of seladelpar, a PPAR-δ agonist, was halted due to unexpected findings in a NASH phase 2 trial (NCT03551522). Combination trials are being planned for PPAR agonists in NASH. As drugs with strong metabolic effects, it would be logical to combine PPARs with drugs with anti-inflammatory and antifibrotic properties.

**Metabolic enzyme inhibitors**

Steroyl-CoA desaturase-1 (SCD-1) converts saturated fatty acids to monounsaturated fatty acids. SCD-1 downregulation reduces hepatic lipogenesis, enhances insulin sensitivity and promotes lipid oxidation. Aramchol is a liver-targeted SCD-1 inhibitor that, in a 52-week, phase 2b, placebo-controlled, randomised trial, promoted NASH resolution and fibrosis-stage reduction with a favourable safety and tolerability profile (NCT02279524), and is currently being tested in the phase 3/4 ARMOR clinical trial (NCT04104321).

Acetyl-CoA carboxylase (ACC) converts acetyl-CoA to malonyl-CoA. Inhibition of ACC reduces hepatocellular malonyl-CoA levels, which in turn increases mitochondria beta-oxidation and

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**Table 1:** Combination therapies for NASH

| Drug Class | Agonist | Combination | NASH Phase 2/3 Trial |
|------------|---------|-------------|---------------------|
| FXR Agonist | Obeticholic Acid | Cilofexor | NCT02548351 |
| PPAR Agonist | Pioglitazone | Seladelpar | NCT03061721 |
| Metabolic Enzyme Inhibitor | Aramchol | | NCT02279524 |

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**References:**

1. Dufour J-F, et al. *Gut* 2020; 69:1877–1884. doi:10.1136/gutjnl-2019-319104
A DGAT2 inhibitor, PF-06865571, decreases polyunsaturated fatty-acid synthesis; the net effect is improvement in hepatic steatosis. In a phase 2 trial of firsocostat, an ACC inhibitor, 126 patients with NASH treated at a dose of 20 mg daily for 12 weeks had a 29% relative reduction of liver fat (NCT02856555).\textsuperscript{14} PF-05221304, a liver-directed ACC inhibitor, is being investigated in a phase 2 trial to assess its pharmacodynamics, safety and tolerability over 16 weeks in patients with biopsy-proven NASH (NCT03256526).\textsuperscript{16}

Diacylglycerol acyltransferase 2 (DGAT2) controls the final step in triglyceride synthesis. A DGAT2 inhibitor, PF-06865571, is in early clinical development and so there is currently limited published information on this drug.

Ketohexokinase (KHK) or hepatic fructokinase metabolises dietary fructose by phosphorylation to produce fructose-1-phosphate. PF-06835919 is a KHK inhibitor that has been shown to decrease steatosis in a phase 2 trial in patients with NAFLD (NCT03256526).\textsuperscript{16}

### Thyroid hormone receptor beta agonists

Selective thyroid hormone receptor beta (TRβ) agonist can modulate lipid metabolism without the side effects which are mediated by thyroid hormone receptor α. VK2809 and resmetirom are two TRβ agonists that are currently in clinical development. In phase 2 trials, both of these medications have been shown to effectively reduce liver fat content and LDL cholesterol (NCT02927184 and NCT02912260).\textsuperscript{17} 18 The efficacy and safety of VK2809 are currently being investigated in patients with biopsy-proven NASH in the phase 2B VOYAGE clinical trial (NCT04173065). In a phase 2b trial, Harrison et al have

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#### Table 1: Trials of combination therapies currently ongoing for the treatment of non-alcoholic steatohepatitis (NASH)

| Name/number | First drug                  | Second drug                  | Arms                              | Population | Duration, weeks | Endpoints                  | Secondary endpoints |
|-------------|-----------------------------|-----------------------------|----------------------------------|------------|-----------------|----------------------------|---------------------|
| CONTROL     | Obeticholic acid            | Atorvastatin                | Placebo, 5, 10, 25 mg            | NASH F1–F3 | 16              | LDL cholesterol            | Safety, tolerability |
| Phase 2     | Obeticholic acid with       | atorvastatin 10, 20 mg      |                                    |            |                 |                            |                     |
| NCT02633956 | (NCT02927184)              |                             | F4 No decompensation             |            |                 |                            |                     |
| TANDEM      | Tropifexor                  | Cenicriviroc                | Tropifexor dose 1                 | NASH F2/3  | 48              | AE                         | One-stage improvement |
| Phase 2     | Cenicriviroc                |                             | Cenicriviroc + cvc               |            |                 |                            |                     |
| NCT03517540 | Tropifexor dose 1 + cvc    |                             | Tropifexor dose 2 + cvc          |            |                 |                            |                     |
| ELIVATE     | Tropifexor                  | Licoglflozin                | Tropifexor + Licoglflozin        | NASH F2/F3 | 48              | Resolution of NASH and no worsening of fibrosis | Improvement of fibrosis by two stages, reduction in body weight, change in liver fat content on MRI-PDFF, improvement of liver tests |
| Phase 2     | Tropifexor                  |                             | Tropifexor dose 1 + Licoglflozin |            |                 |                            |                     |
| NCT04065841 |                             |                             | Tropifexor dose 2 + Licoglflozin  |            |                 |                            |                     |
| Proof-of-concept study | Cilofexor | Firsoconstat Selonsertib | Selonsertib Firsoconstat          | NASH F2/3 some F4 | 12 | TEAEs, TESAEs, TELAs |                      |
| NCT02781584 | Cilofexor + Sel             | Selofexor + Firso + Cilof + Cilof + Firso + Cilof cinnithic Cilof cinnithic Cilof + Sel + Firso Firso + Feno 48 | | | | | |
| ATLAS       | Cilofexor                   | Firsoconstat Selonsertib   | Sel + Firso + placebo             | NASH F3/4  | 48              | AE                         | One-stage improvement |
| Phase 2     | Sel + Firso + Cilof + placebo | Sel + Cilof + placebo Sel + placebo + placebo Sel + Cilof + placebo | | | | | |
| NCT03449446 |                             |                             | Cilof + placebo + placebo + Cilof + placebo | | | | |
| Phase 2     | Cilofexor                   | Semaglutide Firsoconstat    | Semaglutide Firso + Semagl + Cilof + Semagl + Cilof + Cilof + Semagl + Cilof + Cilof | NASH F2/3  | 24              | TEAEs, TESAEs, TELA        |                      |
| NCT03987074 |                             |                             |                                    |            |                 |                            |                     |
| Phase 2A    | PF-05221304, ACC inhibitor | PF-06865571, DGAT2 Inhibitor | PF-05221304 Placebo PF-06865571 + Placebo PF-05221304 + PF-06865571 | NAFLD 6    | Safety, tolerability |                            |                      |
| NCT03776175 | PF-05221304 ACC inhibitor  | PF-06865571 DGAT2 Inhibitor |                                    |            |                 |                            |                     |

ACC, acetyl-CoA carboxylase; AEs, adverse events; Cilof, cilofexor; CVC, cenicriviroc; DGAT2, diacylglycerol acyltransferase 2; Firso, firsocostat; LDL, low-density lipoprotein; MRI-PDFF, MRI proton density fat fraction; NAFLD, non-alcoholic steatohepatitis; Sel, selonsertib; Sema, semaglutide; TEAEs, treatment-emergent adverse events; TELAs, treatment-emergent serious adverse events.
Mitochondria pyruvate carrier inhibitors

Pyruvate fuels the tricarboxylic acid cycle to produce citrate and oxaloacetate, which supports lipogenesis and neogluconeogenesis, respectively. The mitochondrial pyruvate carrier (MPC) transports pyruvate across the mitochondria so that it can interact with the enzymes of the cycle. MSDC-0602K is an MPC inhibitor that has been evaluated in a 52-week, phase 2b dose-ranging clinical trial in subjects with biopsy-proven NASH. MSDC-0602K led to significant reductions in glucose, glycated haemoglobin (HbA1c), insulin, liver enzymes and NAFLD Activity Score (NAS) compared with placebo (NCT02784444). The efficacy of MSDC-0602K in both glycaemic control and NAS resolution will be assessed in a phase 3 clinical trial in patients with TD2M and NASH (NCT03970031).

FGF21 agonists

Fibroblast growth factor 21 (FGF21) is produced by the liver, adipose tissue and pancreas, and has pleiotropic metabolic effects including increasing energy expenditure, improving insulin sensitivity, reducing sugar intake and browning adipose tissue. The beta receptor of FGF21 is expressed in hepatocytes where it stimulates mitochondria beta-oxidation. In adipocytes, FGF21 stimulates the production of adiponectin. Pegbelfermin, a pegylated FGF21 analogue, administered for 16 weeks is associated with GI side effects, such as nausea and vomiting, which occur at the initiation of the treatment. Furthermore, it is administered, like pegbelfermin, by subcutaneous injections making it inconvenient for some patients to self-administer. The new generation of GLP-1 agonists (dulaglutide, semaglutide, extended-release exenatide and albiglutide) has a longer duration of action with the advantage of weekly subcutaneous injection. Semaglutide 0.1, 0.2 and 0.4 mg once daily, which are different doses than the dose of semaglutide approved for the treatment of TD2M (1 mg weekly), is currently being tested in a phase 2b clinical trial in patients with NASH (NCT02970942). This large, multicentre, randomised clinical trial will provide confirmation of the effect of GLP-1 agonist on NAS resolution. Oral GLP-1 therapies are currently being tested with promising results for improving glycaemic control and weight loss. Cardiovascular safety has been demonstrated for GLP-1 agonists as a class effect in patients with TD2M. These trials have demonstrated a significant reduction of major adverse cardiac events using the composite endpoint of cardiovascular death, non-fatal myocardial infarction or non-fatal stroke.

Tirzepatide is a dual glucose-dependent insulinotropic polypeptide and GLP-1 receptor agonist, which has been investigated in patients with TD2M. In a 26-week, double-blind, randomised, phase 2 study in patients with TD2M, tirzepatide demonstrated a significant dose-dependent reduction of HbA1c up to −1.94% at 15 mg compared with −0.06% for placebo, and a potent dose-dependent weight reduction up to −11.3 kg for tirzepatide compared with −0.4 kg for placebo and −2.7 kg for dulaglutide. The efficacy of tirzepatide in patients with NASH is currently being investigated in the phase 2b SYNERGY-NASH trial (NCT04166773). Cotadutide is a dual GLP-1 and glucagon receptor agonist that has been investigated in a 26-week, double-blind, phase 2b trial in overweight subjects with TD2M. It showed superior reduction in body weight and serum aminotransferases levels compared with liraglutide (NCT03235050).
on cardiovascular and kidney diseases, and glucose homeostasis, SGLT2 inhibitors may provide collateral benefits if these agents show improvement in resolution of NASH and fibrosis in future trials.

Chemokine inhibitors
C-C motif chemokine receptor (CCR) type 2 plays a role in the recruitment, migration and infiltration of proinflammatory monocytes and macrophages at the site of liver injury, and CCR5 in the activation and proliferation of collagen-producing activated hepatic stellate cells/myofibroblasts. Cenicriviroc is an oral, dual CCR2/CCR5 receptor inhibitor. In the phase 2b CENTAUR trial, cenicriviroc showed no effect on resolution of NASH, but an improvement in fibrosis stage after 1 year, although this effect was not significant after 2 years; however, patients with a decrease in fibrosis at year 1 maintained this benefit at year 2 (NCT02217475). The efficacy and safety of cenicriviroc are currently being tested in patients with NASH in a phase 3 AURORA clinical trial (NCT03028740).

TREATMENT COMBINATIONS FOR NASH
There are several reasons for treating patients with NASH with a combination of drugs, as shown in figure 2.

Increasing response rate with combination therapy
Trials of drugs as monotherapy for the treatment of NASH have reported response rates <32% in comparison with placebo. Combination of two or more therapies may increase these response rates, meaning that the proportion of patients improving is larger with the combination than with a monotherapy. This strategy aims to convert non-responders or partial responders to monotherapy into responders. Given the heterogeneity in the drivers of fibrosis and NASH among the spectrum of patients with NASH, it is likely that multiple mechanistic pathways may need to be targeted to achieve an optimal histological response. In order to enhance the response rates for either one-stage improvement in fibrosis or resolution of NASH, one can speculate that two or three distinct pathways would need to be targeted to optimise response, for example, by combining drugs with metabolic activity along with predominantly anti-inflammatory activity may further enhance the likelihood of a histological response.

Several drugs are currently being tested in combination with FXR agonists (table 1). The results of 20 patients with NASH who received cilofexor 30 mg plus firsocostat 20 mg once daily in combination for 12 weeks in a ‘proof-of-concept’ study have been reported (NCT02781584): 74% of patients had >30% decrease in liver fat, as determined by MRI-PDFF, and serum ALT and GGT were significantly improved. ATLAS, a phase 2, randomised, double-blind, placebo-controlled study (NCT03449446), evaluated the safety and efficacy of monotherapy and dual combination regimens of cilofexor 30 mg, firsocostat 20 mg and selonsertib 18 mg in patients with advanced fibrosis, including bridging fibrosis and cirrhosis due to NASH. The selonsertib monotherapy arm was discontinued in the ATLAS trial following the negative results of selonsertib monotherapy in the STELLAR trials. In 392 treated patients, of whom 56% had compensated cirrhosis, a ≥1-stage improvement in fibrosis without worsening of NASH after 48 weeks of treatment was numerically higher in the combination therapy group (cilofexor and firsocostat) compared with placebo (20.9% vs 10.5%, p=0.17), respectively. Although the trial did not meet its primary endpoint, probably due to a small sample size, the numerical results were higher in the combination therapy group compared with cilofexor or firsocostat monotherapy. Furthermore, the combination therapy group (cilofexor plus firsocostat) was statistically significant for decreases in ≥2-points improvement in NAS, serum ALT and serum based non-invasive fibrosis markers compared with placebo. These results are to be interpreted with caution given the small sample size.

The ATLAS trial provides an example of efforts to use a lower dose of an FXR agonist with an ACC inhibitor with the aim of reducing side effects related to FXR, namely pruritus and LDL cholesterol increase, but still retaining efficacy in showing
fibrosis improvements in patients with advanced fibrosis due to NASH (NCT03449446). Another study will test the combination of cificlofen and fircoscatost with semaglutide (NCT03987074). Other trials that are planned include a trial of the FXR agonist tropifexor combined with the SGLT1/2 inhibitor licogilofloz (NCT04063841), and a trial combining tropifexor with the leukotriene A4 hydrolase inhibitor, LYS006 (NCT04147195). PF-05221304 and PF-06865371 are also being investigated in combination in a randomised, double-blind, placebo-controlled, phase 2 study to assess their pharmacodynamics, safety and tolerability for 6 weeks in adults with NAFLD (NCT03776175).

Maximising response with combination therapy
Primary endpoints of phase 3 trials of patients with NASH are either improvement of fibrosis without worsening of NASH or resolution of NASH without worsening of fibrosis. Maximising the response with combination therapy means that for a given patient the response is bigger with the combination than with monotherapy. The optimal response would be to improve fibrosis and have resolution of NASH. Fibrosis is not part of the definition of NASH but it is a consequence of the chronic metabolic overload and inflammation. Fibrosis is a relevant therapeutic endpoint since it dictates the prognosis of the disease. It is logical from a clinical perspective to combine drugs to improve the fibrosis and to decrease the metabolic stress and inflammation that drives the fibrotic process. The phase 2 TANDEM trial assesses the combination of cenicriviroc with two doses of the FXR agonist tropifexor over 48 weeks (NCT03517540).

Combination with antidiabetic drugs
Patients with T2DM have a high prevalence of NAFLD, 60%–80% depending on diagnostic methods used46–52; 30%–40% are estimated to have NASH53,54 and 7%–20% to have advanced fibrosis.52,53,56 Several studies have demonstrated that the coexistence of T2DM and NAFLD worsen the course of either disease. Indeed, T2DM is an independent risk factor for the progression of NAFLD to NASH and advanced fibrosis, which increases the risk of progression to cirrhosis, liver-related mortality and hepatocellular carcinoma.57-60 Also, the presence of NAFLD in patients with T2DM hampers maintenance of optimal glycaemic control as it increases hepatic and peripheral insulin resistance.61 Moreover, an increased risk of both macrovascular and microvascular complications of T2DM has been reported when NAFLD is present.62-65 Combination of antidiabetic drugs with anti-NASH drugs may help improve both liver-related and diabetes-related outcomes while improving glucose homeostasis. The addition of vitamin E to pioglitazone was tested over 18 months in a randomised trial in patients with T2DM. Pioglitazone alone resulted in significantly more frequent resolution of NASH and improvement of fibrosis than placebo; the addition of vitamin E resulted in a numerically greater response, although this was not significantly different from pioglitazone alone.66 Of particular interest are antidiabetic drugs that lead to weight loss, such as GLP-1 agonist and SGLT2 inhibitors. The FXR agonist, tropifexor, is being investigated in combination with the SGLT1/2 inhibitor, licogilofloz, for a duration of 48 weeks as a treatment for adults with fibrotic NASH; this study will assess a histological endpoint (NCT04063841). The GLP-1 agonist, semaglutide, is being investigated as monotherapy and in combination with the ACC inhibitor, fircoscatost, in a phase 2 proof-of-concept trial (NCT03987074).

Decreasing side effects with combination therapy
Drug combination may decrease side effects in two ways. First, drugs may have dose-dependent side effects and their use in combination may allow using lower doses to increase tolerability without compromising efficacy. Second, addition of a drug may be prescribed to mitigate the side effect of the first drug. There are currently two examples of this strategy. FXR agonists, including obeticholic acid, increase LDL cholesterol; combination with a statin may decrease this side effect. This was tested in the randomised, placebo-controlled, double-blind CONTROL phase 2 study. After 4 weeks of obeticholic acid, LDL cholesterol increased; addition of atorvastatin subsequently decreased LDL cholesterol below baseline values (NCT02633956).67 In the second example, ACC inhibition may be associated with hypertriglyceridaemia; combination with fenofibrate may decrease this side effect. In a phase 2 randomised study, fenofibrate was prescribed 2 weeks before the addition of fircoscatost in patients with advanced fibrosis due to NASH. Not only did the combination prevent increase in triglycerides, but it also improved hepatic fat and liver biochemistry (NCT02781584).58

Addressing loss of effects
A drug with a narrow mechanism of action may lose its effects over time due to adaptive mechanisms. Combinations may reduce the rate of escape to a monotherapy. There is currently no combination trial designed with this rationale.

CHALLENGES OF COMBINATION THERAPIES FOR NASH
Selection of drugs for combination
Only considering drugs which have shown an effect in clinical trials as monotherapy, the number of combinations is so high that many of them will never be tested. Besides, focusing only on drugs with demonstrated effects is not correct: a drug without individual effects as monotherapy such as, for example, selonsertib should not necessarily be discarded as it may display synergistic effects in a combination. One way to select combination partners may be based on different and complementary mechanisms of action. Development strategy to test combinations in phase 3 clinical trials may include drugs which have not been tested in a phase 3, but in a phase 2 trial as monotherapy; whether such acceleration in drug development is acceptable is debatable.

Chronology
Possible sequences for when to introduce each component drug of a combination therapy for NASH can be outlined as follows:
1. Overlapping. The combination is given from the start to the end of the treatment.
2. Outlasting. The combination is given from the start and one drug is stopped as it reaches a specific endpoint whereas the second drug is given longer as a maintenance therapy.
3. Addition. One drug is prescribed, with a second drug introduced when the effect of the first drug declines or is insufficient.

All current trials follow an overlapping combination sequence, except for addition of a statin to obeticholic acid.

Safety and side effects
Side effects are a major concern in a population of patients with chronic liver disease. Combination approaches need to have no more side effects or safety signals than monotherapies. As combination trials are ongoing, particular attention has to be
paid to safety and side effects and these protocols should capture information on quality of life and patient-related outcomes.

Selecting a target population
Given the complexity of the pathophysiology of NASH and patient heterogeneity, it is essential to select adequate populations for a specific combination. This requires the development of predictive biomarkers of response. Determination of genetic polymorphisms could provide relevant information regarding response to treatment. Kawaguchi-Suzuki et al reported that a single nucleotide polymorphism rs903361 in the ADORA1 gene was associated with resolution of NASH in patients treated with pioglitazone. Recently, a genome-wide analysis study identified several loci associated with response to obeticholic acid in patients with NASH. More research in this field is needed before we see the introduction of predictive biomarkers in NASH.

Trial design
Variability of the reponse rate due to unaccountable changes in lifestyles may lead to uncontrolled improvement and jeopardise the outcome of a trial also in case of combination therapy. Another difficulty with combination trials is the number of arms necessary to demonstrate an advantage of the combination. Ideally, four arms with placebo should be considered, where each drug would be tested as monotherapy and in combination. With numerous combinations of interest, the number of patients to be included in trials will increase as well as the costs of clinical development.

CONCLUSION
A strategy of combining therapies to treat NASH seems like a natural progression and several combinations are already being tested in phase 2 trials. Further studies are needed to improve our understanding for better identifying patients who would have a higher likelihood of treatment response with a specific combination therapy. This would only be possible if credible non-invasive biomarkers can be developed to reliably predict histological and clinical responses to facilitate efficient screening of suitable individual therapies that provide synergistic effects when combined.

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Competing interests
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Although the study was a randomized controlled trial, the population was primarily female, and the results may not be generalizable to the male population. The study also included older individuals, and the results may not be applicable to younger individuals.

In conclusion, the study suggests that metformin may be an effective treatment for type 2 diabetes, particularly in individuals with hyperglycemia. However, further research is needed to confirm these findings and to identify the optimal dosage and duration of treatment.