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Rationale and design of Metabolic Interventions to Resolve NASH with fibrosis (MIRNA): a phase II randomised study to assess efficacy and safety of an orally administered DGAT2 inhibitor alone and when coadministered with a liver-targeted ACC inhibitor

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ABSTRACT (300/300 word limit)

Introduction: Nonalcoholic steatohepatitis (NASH) is a subtype of nonalcoholic fatty liver disease, defined by cellular injury and inflammation. Small molecule inhibitors of the terminal step in intrahepatic triglyceride synthesis (diacylglycerol acyltransferase 2 inhibitor [DGAT2i, PF-06865571]) and upstream blockade of *de novo* lipogenesis via acetyl-coenzyme A carboxylase inhibitor (ACCi, PF-05221304) have shown promise in reducing hepatic steatosis in early clinical trials. This study assesses efficacy and safety of these metabolic interventions to resolve NASH with fibrosis.

Methods and analysis: This phase II, randomised, dose-ranging, dose-finding study evaluates DGAT2i 25-300 mg twice-daily (BID) or 150-300 mg once-daily, DGAT2i 150-300 mg BID+ACCi 5-10 mg BID coadministration, or matching placebo in adults with biopsy-confirmed NASH and liver fibrosis stages 2-3. A triage approach including double-confirmation via noninvasive markers is included prior to screening/baseline liver biopsy. Upon confirmation of histological diagnosis, participants transition into a minimum 6-week run-in period to stabilise baseline parameters. A double-blind, double-dummy dosing period of 48 weeks is followed by a standard 4-week observation period. The primary endpoint of this study is the proportion of participants achieving histological resolution of NASH without worsening of fibrosis, improvement in fibrosis by ≥1 stage without worsening of NASH, or both, as assessed by central pathologists. Other endpoints include assessment of hepatic steatosis (imaging substudy), overall safety and tolerability across DGAT2i, DGAT2i+ACCi and placebo arms, and evaluation of blood-based biomarkers and quantitative ultrasound parameters over time.

Ethics and dissemination: MIRNA is conducted in accordance with the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, International Council on Harmonisation Good Clinical Practice guidelines, and applicable laws and regulations, including privacy laws. Results will be published in a peer-reviewed journal and publicly disclosed through ClinicalTrials.gov, EudraCT, and/or www.pfizer.com, and other public registries as per applicable local laws/regulations.

Trial registration: NCT04321031 (https://clinicaltrials.gov/ct2/show/NCT04321031)

Strengths and limitations of this study

- This is the first clinical study to evaluate histological endpoints after oral administration of DGAT2i and DGAT2i+ACCi in participants with biopsy-confirmed NASH and fibrosis stage F2 or F3.
- A triage approach (including double-confirmation via noninvasive blood and quantitative ultrasound-based markers prior to screening/baseline liver biopsy), coupled with central reading of all liver biopsies with consensus required to determine eligibility and assess drug(s) effects, is designed to improve efficiency in identifying participants likely to meet histological entry criteria and robust confidence in histological findings.
- The dosing period includes a longitudinal evaluation of noninvasive imaging and blood-based biomarkers, to identify correlations between histological parameters and noninvasive imaging and/or blood-based biomarkers, in order to assess drug effects.
- The Bayesian dose-response modelling methodologies employed enable an efficient and complete characterisation of dose response, to aid phase III dose selection.
- This study is limited in that the design relies on assumptions around the translation of effects observed in earlier nonbiopsy studies to a histological endpoint; the impact of the drug(s) on clinical outcomes will need confirmation in an adequately sample-sized phase III trial.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is characterised by excessive accumulation of intrahepatic lipids, especially triglycerides (steatosis) and estimated to affect >25% of the global population.[1 2] A progressive subtype of the disease, nonalcoholic steatohepatitis (NASH), is defined by cellular injury and inflammation[2] and affects 21–25% of people with NAFLD.[3 4] NASH drives fibrogenesis,[5] and fibrosis stage is linked to disease outcome and mortality.[6-9]

Targeting molecular pathways involved in the early pathogenesis and abnormal accumulation of hepatic steatosis could prevent inflammation, cellular injury, and fibrosis, thus offering potential treatments for patients with NASH and liver fibrosis. Acetyl-coenzyme A carboxylase (ACC) and diacylglycerol acyltransferase 2 (DGAT2) each play a role in hepatic steatosis (figure 1). *De novo* lipogenesis (DNL) is more active in patients with NAFLD than in healthy individuals and may contribute to excess hepatic triglycerides,[10] with ACC being the first committed enzyme in the hepatic DNL pathway.[11] DGAT2 is highly expressed in the liver and adipose tissue[12] and catalyses the terminal step of DNL, specifically the esterification of a fatty acid with diacylglycerol to form triglyceride.[13] Independent inhibition of each of these steps has been shown to reduce hepatic steatosis. Sterol regulatory element-binding protein 1c (SREBP1c) is a metabolic switch that governs hepatic lipogenesis,[14 15] and ACC inhibition is associated with upregulation of SREBP1c activity but reduced steatosis in hepatocytes.[11] Conversely, inhibition of DGAT2 was found to down-regulate SREBP1c activity, which in turn reduced hepatic lipogenesis[16]; in addition, a small molecule inhibitor of DGAT2, PF-06427878, reduced hepatic steatosis in a rodent model and clinically after 2 weeks of dosing.[17] In patients with NAFLD, oral administration of another small molecule inhibitor of DGAT2 (DGAT2i, PF-06865571) for 14 days resulted in dose-dependent reductions in both liver fat and serum triglycerides.[18] In addition to its effects on steatosis, ACC inhibition may have direct antifibrotic effects in hepatic stellate cells, the collagen-producing fibroblast population in the liver; in rodent models, ACC inhibition abrogated a metabolic switch necessary for induction of glycolysis

and oxidative phosphorylation during hepatic stellate cell activation *in vitro,* thereby reducing hepatic fibrosis.[19]

In clinical trials, liver-targeted ACC-inhibiting agents have been associated with potent reductions in hepatic steatosis, but with accompanying elevations in serum triglycerides.[11 20 21] Doses ≥40 mg/day of a liver-targeted ACC inhibitor (ACCi; PF-05221304) showed near-complete DNL inhibition for 0–10 hours after 14 days of dosing in healthy adults, but these doses were accompanied with increases in serum triglycerides.[22] However, doses <40 mg/day still inhibited DNL by up to 80% but without elevated serum triglycerides.[22] Based on this observation in healthy adults, doses ≤50 mg/day were evaluated in a phase IIa dose-ranging trial with the ACCi in participants with NAFLD and presumed NASH.[23] Reductions in liver steatosis, markers of liver inflammation (alanine aminotransferase [ALT], aspartate aminotransferase [AST]), and markers of hepatocyte cell death (cytokeratin [CK]18-M30 and CK18-M65) were observed following 16 weeks of dosing.[23] However, these potentially beneficial effects were accompanied by marked elevations in fasting serum triglycerides,[23] which is a known mechanistic consequence of hepatic ACC inhibition.[11 20] Notably, the magnitude of this serum triglyceride increase appears to be higher in adults with NAFLD/presumed NASH than in healthy adults.[22 23] It could be hypothesised that the increased SREBP1c tone in patients with NAFLD, potentially due to hyperinsulinaemia, make patients more sensitive to ACCi and resultant SREBP activation, compared with healthy adults.[24-26]

While independent inhibition of either upstream (ACC) or downstream (DGAT2) pathways may have limited efficacy for pharmacological treatment of NASH, based on the scientific evidence (figure 1), evaluation of DGAT2i and ACCi together is intriguing given the opposing effects on SREBP1c. Indeed, in rodent models, DGAT2i+ACCi reduced steatosis as well as inflammation and fibrosis markers without the expected ACCi-associated increases in serum triglycerides.[27] In a 6-week, phase IIa trial in patients with NAFLD, DGAT2i 300 mg twice-daily (BID)+ACCi 15 mg BID reduced hepatic steatosis to a similar degree as ACCi alone and to a greater degree than DGAT2i alone, as assessed

by magnetic resonance imaging-proton density fat fraction (MRI-PDFF).[23] The nadir for effect on liver fat was observed at 12–16 weeks with ACCi alone and was not determined for DGAT2i+ACCi[23]). Notably, ACCi-induced elevations in triglycerides (47% increase relative to placebo) were effectively mitigated by DGAT2i+ACCi.[23]

MIRNA is a phase II, randomised, placebo-controlled, dose-ranging, dose-finding study (clinicaltrials.gov: NCT04321031) that assesses the efficacy and safety of an investigational, orally administered DGAT2i and DGAT2i+ACCi in adults with biopsy-confirmed NASH and liver fibrosis stage 2 or 3, as defined using NASH-Clinical Research Network (NASH-CRN) criteria.[28] MIRNA is envisioned to add to the body of scientific evidence by assessing histological endpoints, such as NAFLD Activity Score (NAS), and liver fibrosis. MIRNA is supplemented by a concurrent, short-term (6-week dosing), phase IIa trial of DGAT2i+ACCi (NCT04399538) that aims to identify the lowest dose of DGAT2i that can mitigate ACCi-induced adverse effects on serum lipids, to further aid in the optimal selection of dose(s) of DGAT2i+ACCi for subsequent pivotal studies.

METHODS AND ANALYSIS

Study design

MIRNA is a randomised, double-blind, double-dummy, placebo-controlled, dose-ranging, dose-finding, 9-arm, parallel-group study conducted in North America, Europe, and Asia initiated in June 2020. This study includes a total of 22 on-site visits and telephone contacts, and each participant's time in the study ranges from 62–68 weeks (figure 2). Given the prior clinical experience of ≤6 weeks with DGAT2i and DGAT2i+ACCi,[23] frequent post-randomisation visits have been planned to permit close monitoring of safety. Procedures throughout MIRNA are summarised in supplementary table 1.

Eligibility is determined in a three-step process

Guidance from the European Association for the Study of the Liver and American Association for the Study of Liver Disease recognises the significant interest in noninvasive biomarkers for identifying NASH[30 31]; as such, MIRNA aims to reduce the burden of liver biopsies by using noninvasive techniques to identify eligible participants that are most likely to exhibit NASH, thus increasing selection efficiency. Medical history review, medication use, and blood-derived assessments are used to exclude other causes of NAFLD and other liver diseases. Stability of liver function measurements is confirmed during the pre-qualification and first screening visits. Double-confirmation of liver fat and stiffness using quantitative ultrasound (FibroScan®, EchoSens, Paris, France), along with AST to derive FAST™ scores,[32] are used to identify participants qualifying for a screening/baseline liver biopsy. In a prospective derivation and global validation study, FAST score cut-offs for sensitivity (≥0.90) of 0.35 and for specificity (≥0.90) of 0.67 were reported, leading to a positive predictive value of 0.83 (84/101) and a negative predictive value of 0.85 (93/110).[32] A slightly lower cut-off of ≥0.30 is used in MIRNA since this threshold needs to be met twice, with an expected missed case rate (participants with NASH and F2 or F3 who do not undergo biopsy based

on FAST score) of 9.7% and expected screen fail rate (participants undergoing biopsy based on FAST but fail on biopsy) of 49.5%, indicating that two participants would need to be biopsied to detect one eligible participant.[32]

Participants who qualify based on the noninvasive assessments at the pre-qualification and first screening visits undergo a standardised, ultrasound-guided biopsy of the right lobe of the liver using either a 16 or 18 gauge suction or cutting needle, to acquire tissue ≥1.5 cm in length to determine eligibility based on liver histology. Biopsies are graded and scored, using the NASH-CRN definition,[28 33] by central, NASH-CRN pathologists (figure 3). The eligible population is defined as participants with a NAS ≥4 and either F2 or F3 fibrosis. All eligibility criteria are listed in supplementary table 2.

To optimise the evaluable data and limit sampling variability that can confound biopsy results,[34] MIRNA employs careful standardisation of biopsy collection including ultrasound guidance, use of specific biopsy needle size, and assessment limited to the right lobe only. MIRNA also utilises prospective, central biopsy reading by two blinded pathologists for eligibility (and evaluating endpoints), using digitised images to shorten the time needed to judge eligibility. Each pathologist qualitatively assesses each domain of NAS and fibrosis.

For assessment of biopsy-related endpoints at Week 48 or discontinuation, review by central pathologists initially independently – and when needed, consensus review to reach agreement – will be performed via paired, blinded assessment of digitised images. In this process, each pathologist assesses the eligibility/screening (baseline biopsy) alongside the Week 48/discontinuation biopsy, for a given participant. The pathologists are blinded to the nominal timepoints and treatment arm, minimising bias when assessing drug(s) effects. Divergence in grading or staging between pathologists is handled in the same way as outlined in Figure 3 except that for endpoint assessment, agreement between the two pathologists is required for all four domains (steatosis, inflammation, ballooning, fibrosis), thus adding rigour to the determination of histological-based endpoints.

Participants are stabilised during Run-In and Baseline periods

After the liver biopsy is performed (ie. Screen 2), participants start a 6-week run-in period prior to randomisation to generate an in-study, stable state for all participants in terms of medical history and medication use (including compliance). In consideration of potential drug–drug interactions, participants taking gemfibrozil are switched to another permitted agent for lipid control, and those taking metformin >1 g/day have their dose adjusted down by one-third to one-half to 1 g/day, starting at the run-in visit (supplementary table 3). Standardisation of lifestyle guidelines across all sites and countries is advocated;[35 36] in MIRNA, accounting for operational considerations and local practices, lifestyle guidelines advocating healthy choices that do not result in overt weight loss during the course of the study are implemented to minimise placebo response. The Alcohol Use Disorders Identification Test (AUDIT) questionnaire is used before randomisation and at the end of study dosing to confirm that alcohol intake is in moderation during the study (supplementary table 1). Single-blind placebo is administered over a 2-week period before randomisation to confirm that participants can comply with dosing instructions for the study drug (i.e. three tablets/dose, BID with meals). Participants are provided with electronic devices auto-programmed with periodic reminders to enhance compliance (from baseline to end of dosing period). These steps are intended to account for the 'Hawthorne effect', wherein changes in participant behaviour occur because of increased knowledge or interest or due to the perception of being observed. They help limit placebo response, thus permitting ascertainment of drug effect.

Randomised participants are treated for up to 48 weeks and followed for an additional 4 weeks

On day 1, eligible participants are randomised to one of nine arms using a computer-generated randomisation code (random permuted blocks method) and stratified by fibrosis stage (F2 or F3), to ensure a balance of participants across regimens. Study drugs are self-administered in a double-

blind, double-dummy manner. A follow-up on-site visit occurs 2 weeks post-last dose with a follow-up phone call approximately 4 weeks after the last dose.

Half of the total sample size are participating in an imaging substudy to characterise effect on liver steatosis and liver volume over time

Some participants are enrolled in an imaging substudy to 1) characterise the dose-response for effect on liver steatosis using MRI-PDFF; 2) characterise the drug effect over time and define the time to maximum effect (i.e. nadir); and 3) assess the correlation of liver fat and volume assessed by MRI-PDFF (and other associated imaging and laboratory-based endpoints) with histology endpoints.

Concomitant medications are allowed with some adjustments

All concomitant medications taken during the study (supplementary table 3), including herbal supplements in countries where they are part of standard of care to lower liver function test measurements, are recorded along with indication of use. Additional information including daily dose and duration of administration are captured for medications used for glycaemic control, lipid control, and blood pressure control.

Patient and public involvement

Input from patients with biopsy-confirmed diagnoses of NASH was sought while designing MIRNA. Their feedback led to revisions in the protocol regarding the manner by which information is provided to prospective participants in the informed consent document. These included explaining why the intervention may work and the associated benefits and risks, information about efforts to minimise biopsy for diagnosis and about tracking drug effects, and the rationale for the blood volume collected and the intent to use blood samples to evaluate pre-identified and new biomarkers at a later date. Blister packs (rather than bottles) are being utilised to aid compliance and acknowledge pill burden, while balancing the requirements of the double-blind, double-dummy

design. Additionally, participant-friendly reminders and milestone communications were incorporated into the study.

Selection of DGAT2i and ACCi doses to maximise liver fat reduction

Dose selection was informed by exposure–response modelling of historical pharmacokinetic and reduction in liver fat data observed following 2 weeks of DGAT2i dosing;[18] 6 weeks of DGAT2i, ACCi and DGAT2i+ACCi; and 16 weeks of ACCi.[23] The half-maximal effective concentration (EC_{50}) for liver fat reduction was estimated as 41 ng/mL for DGAT2i, and represents a dose of approximately 30 mg BID. A 300-mg BID dose was projected to achieve a near maximal effect on liver fat reduction, and it was hypothesised that DGAT2i 300 mg BID coadministered with ACCi would further extend the effect of DGAT2i. Using both exposure–response analysis and quantitative systems pharmacology modelling, DGAT2i doses of 25, 75, 150, and 300 mg BID were chosen. Additionally, DGAT2i doses of 150 mg and 300 mg once-daily (QD) were selected to compare QD with BID regimens at the same daily dose (75 mg BID and 150 mg BID). This will help determine if similar efficacy is achievable with equivalent QD and BID dosing while testing for potential dissociation between plasma pharmacokinetic profiles and pharmacology.

Two dose levels of DGAT2i+ACCi are being evaluated – DGAT2i 300 mg BID+ACCi 10 mg BID and DGAT2i 150 mg BID+ACCi 5 mg BID – to assess whether ACCi coadministration extends DGAT2i efficacy. ACCi 10 mg BID yields 80% DNL inhibition;[22] on a mg-per-mg basis for both doses the ratio is maintained at 30:1 (DGAT2i:ACCi) to enable evaluation of both efficacy and safety of DGAT2i+ACCi relative to DGAT2i alone. Although ACCi has a pharmacokinetic half-life conducive to QD dosing,[22] a BID dosing regimen was selected to match the likely frequency of clinical dosing for DGAT2i.

Dose selection and dose range from this and the ongoing phase IIa trial (NCT04399538) investigating a wider dose range for DGAT2i+ACCi in patients with presumed NASH may aid in determining the optimal dose(s) of DGAT2i and DGAT2i+ACCi to evaluate in confirmatory, phase III trials.

Objectives, estimands, and endpoints

Clinical responders based on histological evidence

The primary endpoint of MIRNA is the proportion of participants achieving resolution of NASH (i.e. absence of ballooning with no or minimal inflammation by histology)[37] without worsening of fibrosis, or improvement in fibrosis by ≥1 stage without worsening of NASH, or both, at week 48, as assessed by central pathologists. This is based on histological assessment at screening/baseline liver biopsy, and at end of drug administration (i.e. week 48 or earlier in cases of premature withdrawal of study drug, provided the study drug was administered up to at least week 24), in all randomised and treated participants with evaluable baseline biopsy data. Using a composite estimand strategy, drug effect is estimated in terms of the proportion of 'clinical responders', defined as participants achieving the primary endpoint.[38] All cases of withdrawal from study drug(s) due to lack of efficacy or toleration are treated as nonresponders. Participants who withdraw from study drug(s) for other reasons but have evaluable biopsy data at withdrawal or Week 48 will have their biopsy data assessed to determine whether they are responders or not. Participants with no Week 48 biopsy data are considered to be nonresponders.

Secondary endpoints include the percent change in liver fat (assessed via MRI-PDFF in the substudy population), the proportion of participants achieving improvements in responder definitions (resolution of NASH without fibrosis worsening, ≥1 or ≥2-stage fibrosis improvement without NASH worsening, ≥2-point improvement in total NAS score), and safety up to week 48. The secondary efficacy endpoints employ a composite estimand strategy for the histological assessments, whereby the responder definitions are evaluated based on histological assessment at week 48 relative to baseline.

The tertiary endpoint is the proportion of the population with worsening disease at week 48, defined as progression of fibrosis by ≥1 stage and worsening of ≥2 points in total NAS.

Secondary and tertiary objectives include evaluation of safety/tolerability and clinical response on imaging and blood-based biomarkers

Safety and tolerability

These assessments include 12-lead electrocardiogram, blood pressure, pulse rate, body weight measurements, open-ended enquiries of adverse events, collection of blood and urine for assessment of haematology, chemistry, and urinalysis (supplementary table 4). Reasons for discontinuation of study drug include sustained fasting serum triglyceride levels ≥800 mg/dL (≥9 mmol/L), platelet count <75,000/mm^3, or other adverse events based on medical judgement.

Imaging assessments

Considering the primary pharmacology of DGAT2i and ACCi, liver fat and volume (via MRI-PDFF) are assessed as a secondary objective in the imaging substudy. In addition, FibroScan® is being used to measure liver fat via the controlled attenuation parameter (CAP™), and liver stiffness via vibration-controlled transient elastography (VCTE™), over time. Study-specific manuals emphasise the use of M and XL probes guided by SMART tools on the FibroScan® device, including evaluation of tissue change in TM-mode and ultrasound signal strength and propagation in A-mode.

Both MRI-PDFF and FibroScan® assessments are performed following a fast (except water) of ≥4 hours. The MRI-PDFF acquisition protocol is standardised *a priori* across all sites participating in the substudy, with images centrally analysed using validated, two-dimensional, six-echo, spoiled gradient-recalled-echo, breath-hold pulse sequences.[39] MRI-PDFF image analyses are performed by a central, blinded reader; a 2.5 cm-diameter region of interest is applied on each of nine anatomical liver segments, except for the caudate where a 1.5 cm-diameter region of interest is identified.

Blood-based biomarkers

Any potential pharmacological consequences of DGAT2i and DGAT2i+ACCi are being studied via blood-based biomarkers summarised in table 1.

Sparse blood sampling for pharmacokinetic analyses over the 48-week dosing period is included to estimate drug exposure and help describe the relationship between dose, concentration, and efficacy/safety of DGAT2i and DGAT2i+ACCi via population pharmacokinetics and pharmacokinetic/pharmacodynamic analyses. Additionally, blood samples are collected for exploration of other plasma- or serum- biomarkers and single nucleotide polymorphisms linked to NASH.

Table 1. Blood-based biomarkers assessed in MIRNA. Additional samples collected for exploratory biomarker analysis are listed in supplementary table 4.

Parameter	Biomarker
Liver function tests	• Alanine aminotransferase • Aspartate aminotransferase • Alkaline phosphatase • γ-glutamyl transferase • Total bilirubin
NASH-related	• 3-parameter derived enhanced liver fibrosis™ score (marker of liver fibrosis used to track disease progression) • Cytokeratin-18-M30 fragment (marker of apoptotic activity) • Cytokeratin-18-M65 fragment (marker of necrotic activity) • N-terminal propeptide of procollagen type III (marker of fibrinogenesis) • C-terminal fragment of α3 chain of procollagen type VI (marker of fibrinolysis)

Fasting lipid parameters/markers of target engagement	• Fasting serum lipid panel:
	  ○ Total cholesterol
	  ○ Triglycerides
	  ○ High density lipoprotein cholesterol
	  ○ Direct low density lipoprotein cholesterol
	  ○ Direct very low density lipoprotein cholesterol
	• Fasting serum apolipoproteins:
	  ○ A1
	  ○ B_{total}
	  ○ B_{100}
	  ○ B_{48}
	  ○ C3
	  ○ E
	• High-sensitivity C-reactive protein
	• Proprotein convertase subtilisin/kexin type 9

Glycaemic	• HbA1c • Fasting plasma glucose

- Fasting plasma insulin
- Homeostatic model assessment of insulin resistance
- Adiponectin

HbA1c, glycated haemoglobin; MIRNA, Metabolic Interventions to Resolve NASH with Fibrosis;

NASH, nonalcoholic steatohepatitis.

Statistical considerations

Sample size

Sample size estimation is driven by the characterisation of dose response and drug effect using a Bayesian maximum effect of drug (E_{max}) study design and modelling approach, which utilises weakly informative priors for model parameters. This approach increases the precision in drug/dose comparisons (supplementary table 5) and enables the required sample size to be reduced by almost half compared with conventional pairwise comparisons. Nonetheless, MIRNA is over-enrolling by approximately 20% (450 participants with 50 per arm) to minimise the risk of an underpowered study due to a lack of primary endpoint data. Anticipated reasons for insufficient primary endpoint data include nonevaluable biopsies, participant withdrawal, and inconsistencies in scoring/grading digitised slides when determining eligibility and pair-wise (second screening and end-of-dosing period visits) blinded review.[40] The decision to over-enrol in MIRNA was informed by learnings from a previous trial, which reported statistically significant improvements in some secondary endpoints (glucose, HbA1c, fasting plasma insulin, liver enzymes, and NAS), but not primary and secondary histological endpoints after treatment with an insulin sensitiser for NASH, due to issues with interpretation of liver biopsies.[41]

Priors for E_{max} model parameters were evaluated and for DGAT2i, ED_{50} was estimated to be approximately 30 mg BID (based on the projected EC_{50}) and the placebo responder rate (E_0) was estimated to be 16%. Based on the above assumptions, at the theoretical E_{max} of 0.6 (i.e. a 60% responder rate) and an estimated sample size of 450 participants, there is enough precision to show a >24% difference in the primary endpoint responder rate between placebo and the second-highest DGAT2i dose, 150 mg BID, with a probability of ≥89%. In addition, a sample size of 450 participants provides 75% power to demonstrate a 24% difference in the primary endpoint responder rates between QD doses and placebo, and adequate precision to assess whether DGAT2i+ACCi provides a higher responder rate than DGAT2i with a probability of 82% if the true effect size is at least 6%.

Inter and intra-pathologist variability

Variability is assessed quarterly on a randomly selected sample comprising 10% of screening biopsies and Week 48/discontinuation biopsies from randomized participants. Pathologists review the same biopsy images ≥3 months apart, to ascertain if the same levels of calibre are maintained over time (intra-pathologist variability). Reviews by the pathologists are compared to NASH-CRN peers using weighted kappa statistics (inter-pathologist variability).

Statistical models

In assessing the primary objective, a Bayesian dose-response model will characterise the dose response across all DGAT2i BID arms, to estimate the proportion of responders (and 95% confidence intervals [CI]) for each dose, and to estimate the placebo-adjusted proportion of responders for each dose (with 95% CI). The Bayesian estimation of the E_{max} dose-response model uses prior distributions on the placebo response (E_0), as well as the ED_{50} (30 mg BID) and E_{max} parameters. A normal prior distribution for the logit of the placebo response centred at logit (0.16) with a prior standard deviation of 2.0 (logistic scale) is planned to be used, and similarly, the prior for the E_{max} parameter will be centred at logit (0.6) with a prior standard deviation of 2.0. These are diffuse parameters on the logistic scale, which will ensure that the data collected in this study are not overly influenced by these prior distributions, while ensuring convergence of the Bayesian dose-response model. If an E_{max} dose-response model cannot be fitted to the data, other models that allow dose response to be estimated will be fitted (i.e. linear, log-linear, or exponential).

Similar Bayesian dose-response models will be utilised for the secondary objectives of achievement of different responder definitions based on histological outcomes. Other comparisons (DGAT2i QD doses vs placebo, and DGAT2i+ACCi BID doses vs placebo and vs corresponding DGAT2i BID doses) will be analysed using logistic regression models to estimate the proportion of responders in each arm and odds ratio (95% CI) for each comparison.

For the secondary objective of percent change from baseline in liver fat, all drug effect contrasts will be based on a hypothetical estimand strategy, which assumes that all participants remained in the trial for 48 weeks and received study drug(s) as planned without withdrawal. Any available MRI data for all participants is included, including those who withdrew from study drug(s) due to lack of efficacy or toleration. If the Week 48 response is missing, this is imputed using a model-based analysis based on the treatment arm assigned at randomisation. This will follow the average treatment effect as observed in the same assigned treatment arm. A Bayesian E_{max} dose-response model for the DGAT2i BID doses will also be utilised. Other dose group comparisons will use an analysis of covariance (ANCOVA) performed on log-transformed relative change from baseline, with dose group and baseline fibrosis stage (F2 or F3) as factors and log-transformed baseline liver fat value as a covariate. Estimates of the mean relative changes for each dose comparison and 95% CI will be obtained from the model and will be exponentiated to provide estimates of the percent change. For comparisons of DGAT2i+ACCi BID doses versus corresponding DGAT2i BID doses, 50% CI will also be calculated. No adjustment for multiple comparisons will be made. Safety analyses will be summarised descriptively.

Ethics and dissemination

MIRNA is conducted in accordance with ethical principles derived from the Declaration of Helsinki and CIOMS International Ethical Guidelines, applicable International Council for Harmonisation Good Clinical Practice guidelines, and applicable laws and regulations, including privacy laws. The protocol, protocol amendments (if any), informed consent and other forms were reviewed and approved by the independent review board/ethics committee before the study was initiated. Study participants provide informed consent at pre-qualification and first screening, with additional consent required for the imaging substudy. Given a screening population without biopsy-confirmed NASH, the triage approach necessitates screening many prospective participants. Only those participants with biopsy-confirmed NASH and fibrosis receive study-specific information to minimise anxiety in those who do

not have NASH with fibrosis. Information provided at pre-qualification incorporates education about NAFLD and NASH including risk factors. All parties are required to comply with all applicable laws, including laws regarding the implementation of organisational and technical measures to ensure protection of participant data. Participants may withdraw from the study at any time.

As per patient feedback that was incorporated into the design of MIRNA, aggregate-level summaries of study results in lay language are to be disseminated to randomised participants; study results will be publicly disclosed 24 months after completion, through posting on www.clinicaltrials.gov, the EudraCT, and/or www.pfizer.com, and other public registries in accordance with applicable local laws/regulations. Participant-level data will be anonymised in accordance with applicable privacy laws and regulations. The results of MIRNA may be published or presented at scientific meetings by the investigators after disclosure of the overall study results or 1 year after the end of the study (or study termination), whichever comes first.

DISCUSSION

MIRNA represents the first clinical study to assess an oral DGAT2i alone and coadministered with another investigational chemical entity, ACCi, in patients with biopsy-confirmed NASH with F2 or F3.

The rationale for MIRNA is supported by nonclinical and clinical data. Reduced liver steatosis (accompanied by increasing fibrosis) was observed with an antisense oligonucleotide DGAT2 inhibitor in a specific rodent model[42] but this increase in fibrosis has not been replicated with orally administered DGAT2i.[17] Dose-dependent reductions in liver fat of ≤41% (vs 11% with placebo) and fasting serum triglycerides of ≤24.5 mg/dL (vs 7.0 mg/dL with placebo) were reported following oral administration of the DGAT2i ≤300 mg BID for 14 days in patients with NAFLD.[18] Moreover, preliminary data suggest that DGAT2i+ACCi could extend the efficacy of DGAT2i and also mitigate ACCi-induced increases in serum triglycerides.[23] Further data from MIRNA will help elucidate the

benefit-to-risk profile of these new chemical entities, particularly when considering that hepatic fibrosis (defined by VCTE ≥8.2 kPa) is associated with several cardiometabolic disease risk factors.[43]

Assessing a wide range of DGAT2i doses via QD and BID regimens along with DGAT2i+ACCi allows for a thorough assessment of several objectives, using statistically efficient methodology to identify potentially well-tolerated and efficacious dose(s) and dosing regimen(s) for pivotal phase III/IV trials.[44] The ongoing phase IIa trial (NCT04399538) will provide additional information on optimal doses of DGAT2i+ACCi. Furthermore, evaluating drug effects on imaging and blood-based biomarkers alongside regulatory-mandated histological endpoints could help identify surrogate endpoints for NASH with F2 or F3 fibrosis.[37 45] This is particularly pertinent as European and US guidelines currently recommend striving for validated, noninvasive endpoints for NASH.[46 47] MIRNA is designed to satisfy this recommendation with adequate statistical power to assess the primary endpoint. Enrolment above the minimum requirement, double screening by two pathologists, and collection of plasma and serum samples for future noninvasive endpoints further supports the robustness of the study design.

MIRNA incorporates learnings from previous trials in patients with NASH and F2 or F3 fibrosis, and other metabolic diseases (e.g. type 2 diabetes).[48 49] This dose-ranging, dose-finding study in patients with NASH and F2 or F3 fibrosis aims to collect robust data for histological, imaging, and blood-based biomarkers to provide confidence in the efficacy of DGAT2i and ACCi, so that the pivotal phase III trials can focus on confirming efficacy and evaluate safety in a much larger sample size.

Funding statement

This work is supported by Pfizer Inc; the study sponsor is involved in the design, analysis, interpretation, and reporting of this study.

Competing interests

NBA, AD, DSL, MV and CY are employees of, and hold stock or stock options with, Pfizer Inc. QMA has received fees for consultancy on behalf of Newcastle University, grant funding via the EU IMI2 scheme and speaker fees from Pfizer Inc in relation to the submitted work. QMA has received grant funding from AbbVie, Allergan/Tobira, AstraZeneca, Genfit SA, GlaxoSmithKline, Glympse Bio, Intercept Pharma Europe Ltd (via the EU IMI2 scheme), Novartis Pharma AG, Pfizer Inc (via the EU IMI2 scheme); speaker fees from Bristol Myers Squibb, Gilead, Kenes, Novo Nordisk, Pfizer Inc; consultancy fees on behalf of Newcastle University from 89Bio, Allergan/Tobira, Altimmune, AstraZeneca, Axcella, BGMBio, Blade, BNN Cardio, Bristol Myers Squibb, Celgene, Cirius, CymaBay, E3Bio, EcoR1, Eli Lilly & Co., Galmed, Genentech, Genfit SA, Gilead, Grunthal, HistoIndex, Imperial Innovations, Indalo, Intercept Pharma Europe Ltd., Inventiva, IQVIA, Janssen, Madrigal, MedImmune, Metacrine, NewGene, NGMBio, North Sea Therapeutics, Novartis Pharma AG, Novo Nordisk, PathAI, Pfizer Inc, Poxel, Raptor Pharma, Servier, Terns, Viking Therapeutics, outside the submitted work. VWSW has received medical writing and article processing charges support from Pfizer Inc for the submitted work. VWSW's institution has received grant funding from Gilead and support for meetings attendance from AbbVie and Gilead; VWSW has received consulting fees and payments for participation on a Data Safety Monitoring Board or Advisory Board from 3V-Bio, AbbVie, Allergan, Boehringer Ingelheim, Center for Outcomes Research in Liver Diseases, Echosens, Gilead, Hanmi Pharmaceutical, Intercept, Inventiva, Merck, Novartis, Novo Nordisk, Perspectum Diagnostics, Pfizer Inc, ProSciento, Sagimet Biosciences, TARGET PharmaSolutions, Terns; VWSW has received payment or honoraria for lectures, presentations, speakers bureaux, manuscript writing or educational events

from Abbott, AbbVie, Bristol Myers Squibb, Echosens, Gilead; VWSV has stock or stock options in Illuminatio Medical Technology Ltd.

FT's institution has received grants or contracts from Allergan, Bristol Myers Squibb, Galapagos, Gilead and Inventiva; FT has received consulting fees from AbbVie, Allergan, Boehringer Ingelheim, Bristol Myers Squibb, Galapagos, Gilead, Ionis, Ipsen, Inventiva, Novartis, Pfizer Inc and Roche; FT has received payment or honoraria for lectures, presentations, speakers bureaux, manuscript writing or educational events from Falk, Intercept and Gilead; he has received payment for expert testimony from Alnylam.

NA has received grants or contracts from Akero, Allergan, Bristol Myers Squibb, DSM, Genentech, Genfit, Gilead, Intercept, Inventiva, Madrigal, NGMBio, North Sea Therapeutics, Novo Nordisk, Pfizer Inc, Poxel and Zydus; NA has received payment or honoraria for lectures, presentations, speakers bureaux, manuscript writing or educational events from Gilead and Intercept.

MC and AN declare no competing interests.

A patent rationalising the invention of administering DGAT2i+ACCi to mitigate effects of ACCi alone has been submitted.

Contributors

- Conception and study design: NBA, AD, DSL, MV, QMA, VWSW, FT, CY
- Oversight and leadership of the study planning and execution: NBA, AD, CY
- Study conduct: NBA, AD, QMA, VWSW, FT, MV, MC, NA, AN, CY
- Drafting, revising, and final approval of the manuscript: NBA, AD, QMA, VMSM, FT, MV, DSL, MC, NA, AN, CY

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FIGURE LEGENDS

Figure 1. Effects of ACC and DGAT2 inhibition on hepatic lipid metabolism.[11 13 16 27 29]

Footnotes for Figure 1:

[a]Adaptive effects.

[b]In nonclinical models.

ACC, acetyl-coenzyme A carboxylase; ACCi, ACC inhibitor; ACS, acyl-CoA synthetase; CoA, coenzyme A; CPT1, carnitine palmitoyl transferase 1; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; DGAT2i, DGAT2 inhibitor; DNL, *de novo* lipogenesis; FAS, fatty acid synthase; FFA, free fatty acid; G-3-P, glycerol-3-phosphate; MUFA, monounsaturated fatty acid; PPARα, peroxisome proliferator-activated receptor α; SCD1, stearoyl-CoA desaturase 1; SREBP, sterol regulatory element-binding protein; TAG, triacylglycerol (also known as triglyceride); TG, triglyceride; VLDL, very low density lipoprotein.

Figure 2. MIRNA study design.

The intervals depict the maximum time between the various periods in the study.

Footnotes for Figure 2:

[a]In addition, metformin dose reduced if dose is >1 g/day.

ACCi, acetyl-CoA carboxylase inhibitor; BID, twice-daily; DGAT2i, diacylglycerol acyltransferase 2 inhibitor; MIRNA, Metabolic Interventions to Resolve NASH with Fibrosis; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; n, target number of participants; QD, once-daily.

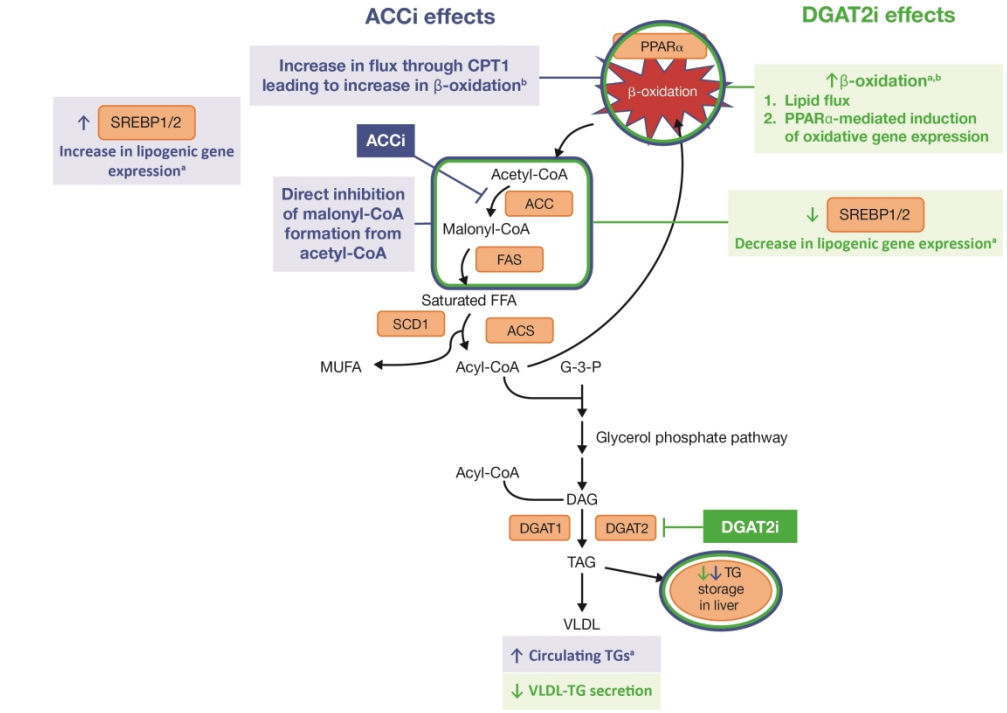
Figure 3. Prospective, centralised grading and scoring of liver biopsies at screening for eligibility using the NASH-CRN definition.

Footnotes for Figure 3:

NAFLD, non-alcoholic fatty liver disease; NASH-CRN, Nonalcoholic steatohepatitis Clinical Research Network.

[a]Consensus review to reach agreement is not required if both pathologists agree that either the NAFLD Activity Score or fibrosis grade renders the participant ineligible

Figure 1



258x189mm (300 x 300 DPI)

Figure 2

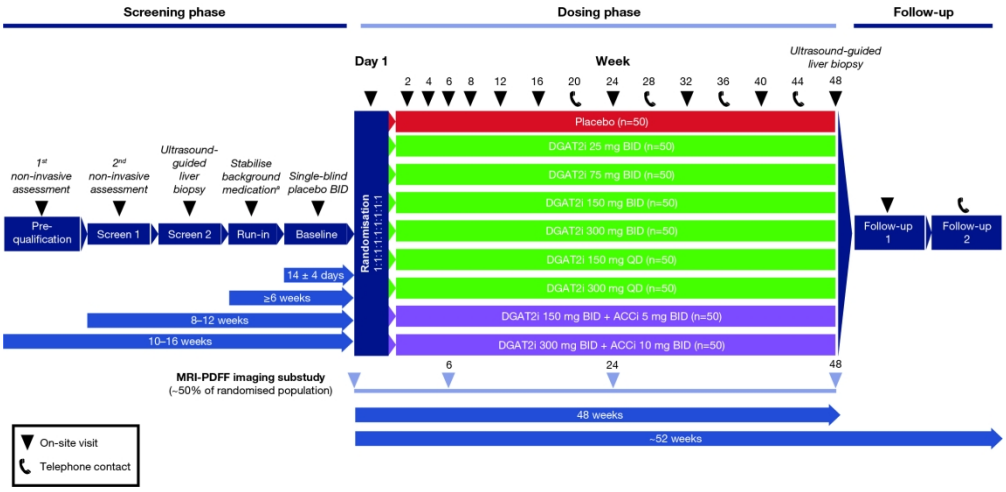


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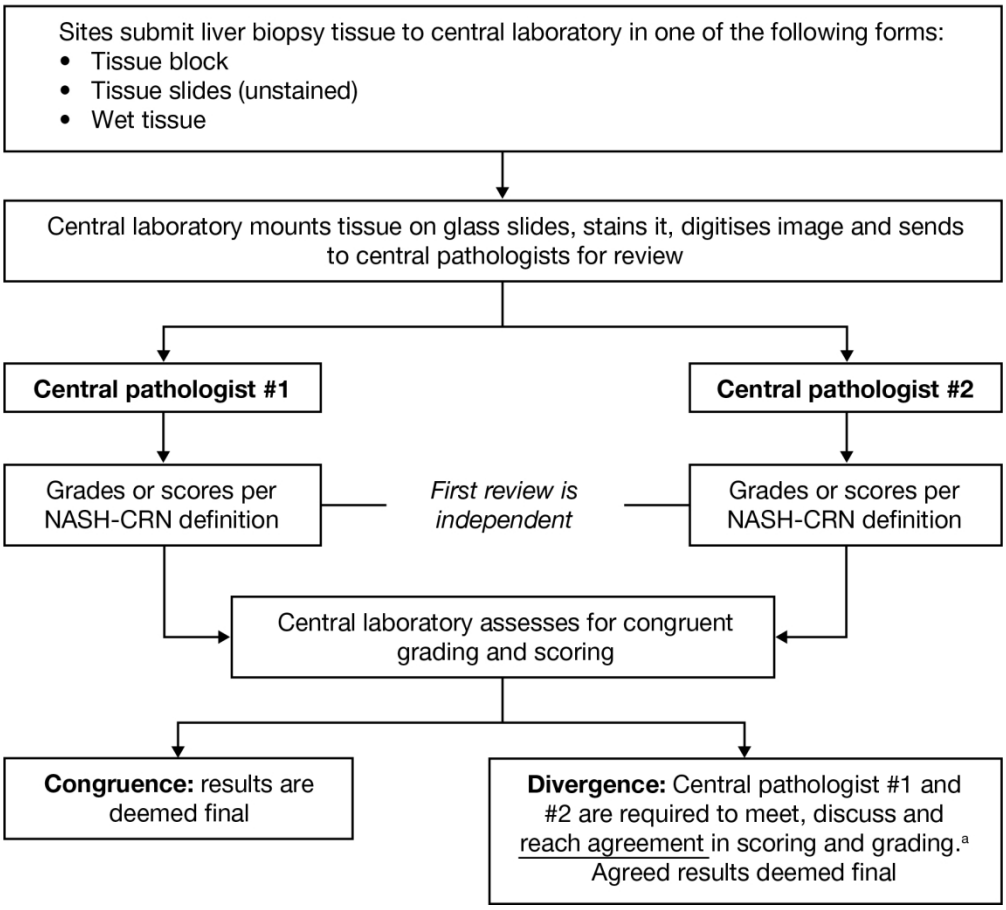
Figure 3



159x160mm (300 x 300 DPI)

Rationale and design of Metabolic Interventions to Resolve NASH with fibrosis (MIRNA): a phase II randomised study to assess efficacy and safety of an orally administered DGAT2 inhibitor alone and when coadministered with a liver-targeted ACC inhibitor

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SUPPLEMENTARY INFORMATION

Supplementary Table 1. Collection of data during MIRNA

	Pre-qualification	Screen 1	Screen 2	Run-in	Baseline	Dosing week																Follow-up	Discontinuation
Week	–	–	–	–6	–2	0	2	4	6	8	12	16	20	24	28	32	36	40	44	48	50	52	
Procedures																							
Informed consent, demography	✓	✓																					
Medical & medication history (update)	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ultrasound-guided liver biopsy			✓																	✓			✓
Liver fat and stiffness (FibroScan®)	✓	✓			✓				✓		✓			✓		✓		✓		✓			✓
Liver MRI-PDFF (Imaging substudy)					✓				✓					✓						✓			✓
Physical exam	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓		✓	✓		✓
Alcohol intake assessed (AUDIT)	✓	✓				✓														✓			✓
Counselling on diet/exercise guidelines				✓		✓																	
Adverse events (open-ended query)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Single supine 12-lead ECG	✓	✓			✓	✓								✓						✓	✓		✓

	Pre-qualification	Screen 1	Screen 2	Run-in	Baseline	Dosing week																Follow-up	Discontinuation
Week	–	–	–	–6	–2	0	2	4	6	8	12	16	20	24	28	32	36	40	44	48	50	52	
Singled seated vitals (blood pressure, pulse rate) and body weight	✓	✓			✓	✓	✓		✓		✓			✓		✓		✓		✓	✓		✓
Study intervention taken with morning meal					✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓			
Blood collection (after overnight fast of ≥8 hours)																							
FSH (females only), HBsAg, HCVAb, HIV, α1-antitrypsin, ceruloplasmin	✓	✓																					
% carbohydrate deficient transferrin	✓	✓			✓	✓														✓			✓
Haematology, chemistry, coagulation, triglycerides, direct LDL-C, HDL-C, total cholesterol, pregnancy (females only)	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓		✓	✓	✓	✓
HbA1c, plasma glucose	✓	✓			✓	✓		✓		✓		✓		✓		✓		✓		✓	✓		
Direct VLDL, ApoA1, $ApoB_{total}$, ApoB100, ApoB48, ApoC3, ApoE, PCSK9, plasma insulin, adiponectin, CK18-M30, CK18-M65, ProC3, ProC6, enhanced liver fibrosis test, hs-CRP					✓	✓		✓		✓		✓		✓		✓		✓		✓	✓		
Pre-dose PK – DGAT2i and ACCi						✓	✓			✓	✓	✓				✓				✓			
Post-dose PK – DGAT2i and ACCi							✓			✓	✓	✓											
Spot urine collection																							

	Pre-qualification	Screen 1	Screen 2	Run-in	Baseline	Dosing week																	Follow-up	Discontinuation
Week	–	–	–	–6	–2	0	2	4	6	8	12	16	20	24	28	32	36	40	44	48	50	52		
Urine drug test	✓	✓			✓																			
Urinalysis	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓		✓	✓	✓	✓	
Pregnancy test (women of child-bearing potential)					✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓		✓	✓	✓	✓	

ACCi, acetyl-coenzyme A carboxylase inhibitor; Apo, apolipoprotein; AUDIT, Alcohol Use Disorders Identification Test; CK18-M30, cytokeratin-18-M30 fragment; CK18-M65, cytokeratin-18-M65 fragment; DGAT2i, diacylglycerol acyltransferase 2 inhibitor; ECG, electrocardiogram; FSH, follicle-stimulating hormone; HbA1c, glycated haemoglobin; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; HDL-C, high density lipoprotein-cholesterol; HIV, human immunodeficiency virus; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low density lipoprotein-cholesterol; MIRNA, Metabolic Interventions to Resolve NASH with Fibrosis; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; NASH, nonalcoholic steatohepatitis; PCSK9, proprotein convertase subtilisin/kexin type 9; PK, pharmacokinetics; ProC3, N-terminal propeptide of type III procollagen; ProC6, C-terminal fragment of α3 chain of procollagen type VI; VLDL, very low density lipoprotein.

Supplementary Table 2. Inclusion and exclusion criteria in MIRNA

Inclusion Criteria

• At pre-qualification and the first screening, participants must meet ≥2 of the following:
  ○ Fasting plasma glucose ≥100 mg/dL (or taking agents to improve glycaemic control)
  ○ Fasting serum HDL-C <40 mg/dL for males and <50 mg/dL for females (or taking agents to increase HDL-C)
  ○ Fasting serum triglycerides ≥150 mg/dL (or taking agents to reduce triglycerides)
  ○ Seated blood pressure ≥130/85 mmHg (or taking agents for blood pressure control)
  ○ Waist circumference ≥40 inches for males and ≥35 inches for females

• At both the pre-qualification and the first screening, FAST™ ≥0.30

• At the second screening, ultrasound-guided liver biopsy meeting the NASH-CRN definition
  ○ Total NAS ≥4 with steatosis, inflammation, and ballooning grades all ≥1
  ○ Fibrosis scoring of F2 or F3

• Participants are willing and able to comply with all scheduled visits, dosing plan, laboratory tests, lifestyle considerations, and other study procedures including a second biopsy while in the study
• At pre-qualification and first screening, BMI ≥25 kg/m^2 or ≥22.5 kg/m^2 (Asia only) and ≤40 kg/m^2
• Demonstration of stable body weight (within 5%) for ≥12 weeks before the first screening
• Capable of giving signed informed consent
Exclusion Criteria

• At pre-qualification and first screening visit, current significant alcohol consumption defined by any of the following:
  ○ >14 or >7 drinks/week for males or females, respectively
  ○ % carbohydrate deficient transferrin ≥1.5 x ULN
  ○ Total score of ≥8 on the interview-based AUDIT questionnaire[1]

• At pre-qualification and first screening, evidence of other causes of liver disease, including:
  ○ Alcoholic steatohepatitis, compensated and decompensated cirrhosis, histological presence of cirrhosis on screening/baseline liver biopsy, HIV infection, hepatocellular carcinoma or other types of liver cancer
  ○ Active viral hepatitis B, defined by presence of HBsAg
  ○ Active viral hepatitis C, defined as presence of HCVAb
  ▪ Those cured are eligible so long as there is evidence of SVR for ≥3 years
  ○ Wilson's disease, defined as ceruloplasmin level <0.1 g/L
  ○ A1AT deficiency, defined as A1AT level <LLN
  ○ Upper gastrointestinal bleed due to oesophageal varices, liver transplant, or current MELD-Na score >12

• At pre-qualification, history of pancreatitis
• At pre-qualification, any condition possibly affecting absorption (eg. prior bariatric surgery, gastrectomy, ileal resection)

- Within 12 weeks prior to first screening, diagnosis of type 2 diabetes mellitus which requires management with >3 medications
- Within 12 weeks prior to first screening, dyslipidaemia which requires management with >3 lipid-modifying agents
- Severe hypertension (≥180 mmHg systolic and ≥105 mmHg diastolic) at pre-qualification and first screening, or management with >3 agents to control blood pressure within 12 weeks prior to first screening
- A cardiovascular event within 12 months prior to pre-qualification
- Recent (within 5 years of pre-qualification) systemically administered treatments for malignancy
- Known participation in a trial involving DGAT2i or ACCi, or previous administration with an investigational product, ≤30 days or 5 half-lives preceding the first dose of investigational product
- Any of the following diagnostic measurements, at both pre-qualification and first screening:
    - ALT <0.5x ULN or >5x ULN
    - AST >5x ULN
    - ALP >2x ULN
    - Total bilirubin >ULN and direct bilirubin >ULN
    - HbA1c >9%
    - Fasting plasma glucose >270 mg/dL
    - Fasting serum triglycerides >400 mg/dL
    - Platelet count <LLN
    - INR ≥1.3
    - Albumin <LLN
    - eGFR of <30 mL/min/1.73 m^2, using Cystatin-C and CKD-EPI equation
    - Positive urine test for illicit drugs
- Supine ECG QTc interval >480 msec or QRS interval >120 msec at pre-qualification and first screening
- Participants meeting criteria for contraindication to undergoing imaging assessments
- Investigator site staff or Pfizer employee directly involved in the conduct of the study, site staff otherwise supervised by the investigator, and their respective family members

A1AT, alpha-1-antitrypsin; ACCi, acetyl-coenzyme A carboxylase inhibitor; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUDIT, alcohol use disorders identification test; BMI, body mass index; CAP™, controlled attenuation parameter; CRN, clinical research network; DGAT2i; diacylglycerol acyltransferase 2 inhibitor; ECG, electrocardiogram; eGFR, enhanced glomerular filtration rate; FAST™, a derived score (using CAP™, VCTE™, and AST) to identify those with progressive NASH; HbA1C, glycated haemoglobin; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; HIV, human immunodeficiency virus; HDL-C, high density lipoprotein-cholesterol; INR, international normalised ratio; LLN, lower limit of normal; MELD-Na,

model of end-stage liver disease including serum sodium, serum creatinine, total bilirubin and INR; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD Activity Score; NASH, nonalcoholic steatohepatitis; SVR, sustained virology response; ULN, upper limit of normal; VCTE™, vibration-controlled transient elastography.

Supplementary Table 3. Concomitant medications in MIRNA

Medication for glycaemic control

• Participants are permitted to be on stable doses of ≤3 agents for glycaemic control, for ≥12 weeks prior to first screening and until first on-site follow-up, across the country-specific approved classes of agents for glycaemic control. For example:
  ○ Biguanides
  ○ Dipeptidyl peptidase-IV inhibitors
  ○ Sodium-glucose cotransporter 2 inhibitors
  ○ Sulphonylureas
  ○ α-glucosidase inhibitors
  ○ Meglitinide analogues
• Those on thiazolidinediones/peroxisome proliferator-activated receptor gamma (e.g. pioglitazone) must be on a stable dose for ≥24 weeks before first screening
• Those on metformin at doses >1 g/day must decrease the dose by one-third or one-half starting at the run-in visit.[a]
  ○ Upward adjustment is permitted post-randomisation based on fasting plasma glucose
• Those on insulin must be on stable doses for ≥12 weeks before first screening
  ○ Short-term use of sliding scale insulin to manage glycaemic control during a concomitant acute medical condition is acceptable
• Those on glucagon-like peptide-1 receptor agonists must be on stable doses for ≥12 weeks before first screening

Lipid-modifying medications

• Participants are permitted to be on stable doses of ≤3 lipid-modifying oral agents, for ≥12 weeks prior to first screening and until the first on-site follow-up/week 50, across the country-specific, approved classes of agents including the following:
  ○ Those on selected statins which are BCRP substrates will only be permitted if on:
  ▪ Rosuvastatin doses up to 10 mg/day
  ▪ Atorvastatin doses up to 40 mg/day
  ▪ Simvastatin or fluvastatin doses up to half-maximum in-country approved dose
  ○ Bile acid sequestrants such as cholestyramine, colestipol, as well as colesevalam
  ○ Fibric acid derivatives such as fenofibrate, bezafibrate, pemfibrate
  ○ Nicotinic acid/niacin
  ○ Ezetimibe
  ○ Participants on gemfibrozil at first screening are to be switched to another acceptable agent starting at the Run-In visit, with stable dose of the acceptable agent achieved for ≥6 weeks before day 1.

Medications for controlling blood pressure
• Participants are permitted to be on stable doses of ≤3 agents for blood pressure control, for ≥12 weeks prior to first screening and until the first on-site follow-up
Other acceptable concomitant medications
• Multi-vitamins are permitted, but vitamin E doses must be stable for ≥24 weeks before first screening

- Aspirin ≤325 mg/day
- Oral agents that alter gastric pH
- Inhaled and topical corticosteroids
    - Intercurrent treatment with systemic steroids may be permitted if treatment does not exceed 14 days
- Thyroid replacement therapy
- Postmenopausal hormone therapy
- Antipsychotic medications such as tricyclic agents, selective serotonin reuptake inhibitors, and serotonin/norepinephrine reuptake inhibitors
- Select supplements (herbal or approved agents) as a part of standard care to lower liver function markers: glutathione, glycyrrhizic acid, polyene phosphatidylcholine, silymarin, ursodeoxycholic acid
- Chronic and intermittent use of nonsteroidal anti-inflammatory drugs
- Intermittent use of acetaminophen/paracetamol at doses up to 2 g/day is acceptable.

Prohibited medications

- Use of drugs historically associated with fatty liver, taken within any interval lasting ≥4 weeks in the previous 12-months prior to first screening:
    - Amiodarone, methotrexate, systemic glucocorticoids (such as prednisone, dexamethasone, triamcinolone, budesonide, betamethasone), anabolic steroids, tetracyclines, tamoxifen, oestrogens at doses greater than those used for hormone replacement, valproic acid, other known hepatotoxins
- Use of the following medications ≤ 12 weeks prior to first screening, or likely to need these medications based on medical history at any time until first on-site follow-up:
    - Chronic use of immunosuppressants (e.g. cyclosporine and tacrolimus)
    - Agents with approved indication for weight loss (e.g. orlistat and sibutramin)
    - Over-the-counter appetite-stimulants or appetite-suppressants
- P-gp substrates with narrow therapeutic index (e.g. digoxin)
- Potent inducers and inhibitors CYP-3A
- CYP-2C9 substrates with narrow therapeutic index (e.g. warfarin or phenytoin)
- Blood thinners (e.g. apixaban, dabigatran, rivaroxaban, edoxaban, fondaparinux, heparin, and vitamin K antagonists [such as warfarin])
- Clinically significant OATP inhibitors (e.g. cyclosporine, gemfibrozil, rifampin)

[a]DGAT2i 300 mg BID was shown to increase metformin exposures approximately 2-fold (data on file).

BCRP, breast cancer resistant protein; BID, twice-daily; CYP, cytochrome P-450; DGAT2i, diacylglycerol acyltransferase 2 inhibitor; OATP, organic anion-transporting polypeptide; P-gp, P-glycoprotein.

Supplementary Table 4. Clinical laboratory tests performed in MIRNA

Haematology	Chemistry	Urinalysis	Other
• Haemoglobin • Haematocrit • Red blood cell count • Reticulocyte count (absolute) • Mean corpuscular volume • Mean corpuscular haemoglobin • Mean corpuscular haemoglobin concentration • Platelet count • White blood cell count • Total neutrophils (absolute) • Eosinophils (absolute) • Monocytes (absolute) • Basophils (absolute) • Lymphocytes (absolute)	• Blood urea nitrogen • Creatinine • Plasma glucose • Calcium • Sodium • Potassium • Chloride • Total carbon dioxide (bicarbonate) • Aspartate aminotransferase • Alanine aminotransferase • Alkaline phosphatase • γ-glutamyl transferase • Total bilirubin • Direct (conjugated) bilirubin • Indirect (unconjugated) bilirubin • Total bile acids • Creatine kinase • Uric acid • Albumin • Total protein	• pH • Glucose • Protein • Blood • Ketones • Nitrites • Leukocyte esterase • Urobilinogen • Urine bilirubin • Microscopy[a]	• Cystatin-C (and enhanced glomerular filtration rate using Chronic Kidney Disease-Epidemiology Collaboration equation-Cystatin-C) • Plasma activated partial thromboplastin time, prothrombin time, and international normalised ratio • Serum follicle-stimulating hormone[b] • Serum and urine pregnancy test • Urine drug test[c] • α1-antitrypsin[d] • Ceruloplasmin[d] • Serology:[d] hepatitis B surface antigen, hepatitis C virus antibody (and if positive, reflex hepatitis C virus ribonucleic acid), human immunodeficiency virus • % carbohydrate deficient transferrin relative to total transferrin[e] • Glycated haemoglobin • Fasting serum lipid panel[f] • Adiponectin
Additional exploratory biomarker assessments[g] include:			

- Serum apolipoprotein A1, B (total), B100, B48, C3, E and direct very low density lipoprotein
- Plasma insulin
- High-sensitivity C-reactive protein
- Cytokeratin-18-M30 fragment; cytokeratin-18-M65 fragment
- N-terminal propeptide of type III procollagen
- C-terminal fragment of α3 chain of procollagen type VI
- Plasma proprotein convertase subtilisin/kexin type 9
- Enhanced liver fibrosis test

[a]Only if urine dipstick is positive for blood, protein, nitrites, or leukocyte esterase

[b]In females, at pre-qualification, and first screening, only

[c]At pre-qualification, first screening, and baseline only; minimum requirement for urine drug test include cocaine, opiates/opioids, benzodiazepines, and amphetamines; this test not permitted to be repeated at scheduled visits.

[d]At pre-qualification and first screening only

[e]At pre-qualification, first screening, baseline, day 1, week 48, and when study intervention is prematurely stopped (with participant remaining in study or permanently withdrawn)

[f]Includes triglycerides, high density lipoprotein-cholesterol, direct low density lipoprotein-cholesterol, and total cholesterol

[g]At selected visits starting from baseline to first on-site follow-up

Supplementary Table 5. Summary of the probability of meeting decision criteria for drug/dose comparisons, in order to establish sample size

Arm	Dose group	Comparator	Δ	Analysis method	Criteria	Probability of meeting criteria	N evaluable per group
Placebo	Placebo	–	–	–	–		40
DGAT2i	25 mg BID	Placebo	24%	E_{max} DR modelling	≥95% certainty of ≥0% Δ vs placebo and ≥67% certainty of ≥24% Δ vs placebo	0.004[a]	40
	75 mg BID	Placebo	24%	E_{max} DR modelling	≥95% certainty of ≥0% Δ vs placebo and ≥67% certainty of ≥24% Δ vs placebo	0.626[a]	40
	150 mg BID	Placebo	24%	E_{max} DR modelling	≥95% certainty of ≥0% Δ vs placebo and ≥67% certainty of ≥24% Δ vs placebo	0.892[a]	40
	300 mg BID	Placebo	24%	E_{max} DR modelling	≥95% certainty of ≥0% Δ vs placebo and ≥67% certainty of ≥24% Δ vs placebo	0.945[a]	40
	150 mg QD	Placebo	24%	Pairwise/ER modelling	Power for 24% Δ vs placebo	0.75 (power)	40
	300 mg QD	Placebo	24%	Pairwise/ER modelling	Power for 24% Δ vs placebo	0.75 (power)	40
DGAT2i+ACCi	150 mg BID + 5 mg BID	150 mg BID	(3%)	Pairwise/linear DR modelling	≥75% certainty of ≥0% Δ vs 150 mg BID	0.67	40
	300 mg + 10 mg BID	300 mg BID	(6%)	Pairwise/linear DR modelling	≥75% certainty of ≥0% Δ vs 300 mg BID	0.82	40

[a]Assuming an E_{max} = 0.6.

DR, dose response; E_{max}, maximum effect of drug; ER, exposure–response.

References

1. Saunders JB, Aasland OG, Babor TF*, et al.* Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. *Addiction* 1993;88:791–804.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Manuscript: 1 Protocol: 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Manuscript: 4, 7 Protocol: 1
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	Protocol: 2
Funding	4	Sources and types of financial, material, and other support	Manuscript: 25
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	See Authors: manuscript: 1
	5b	Name and contact information for the trial sponsor	Manuscript: 23 (corresponding author: 1)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Manuscript: 23
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Protocol: 88
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Manuscript: 5-7 Protocol: 17-19
	6b	Explanation for choice of comparators	Manuscript: 6-7 Protocol: 19, 25, 28
Objectives	7	Specific objectives or hypotheses	Manuscript: 7, 13-14 Protocol: 25-26
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Manuscript: 8-12, Figure 2 Protocol: 26-27
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Manuscript: 8 Protocol: 45
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Manuscript: 9, Supplementary Table 2 Protocol: 36-42, 60
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Manuscript: 10-11 Protocol: 31, 35-36, 43
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Manuscript: 14 Protocol: 55-56
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Manuscript: 10, 11 Protocol: 49-50
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Manuscript: 11, Supplementary Table 3 Protocol: 50

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Manuscript: 13-14 Protocol: 25-26
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Manuscript: Figure 2, Supplementary Table 1 Protocol: 27
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Manuscript: 18 Protocol: 79
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Manuscript: 10 Protocol: 48-49
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Manuscript: 10 Protocol: 48-49
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Protocol: 49
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Manuscript: 9-10, 14 Protocol: 11, 49, 98

	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Protocol: 49
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Manuscript: 9, 14-15, Figure 3 Protocol: 58-60
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Manuscript: 9, 13 Protocol: 55
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Protocol: 92-93
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Manuscript: 18-20
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Manuscript: 11, 15 Protocol: 59
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Manuscript: 20
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Protocol: 87-88

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Protocol: 87
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Manuscript: 14, Suppl Table 1 Protocol: 63-72
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Protocol: 93
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Manuscript: 20-21 Protocol: 89
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Protocol: 90
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Manuscript: 20 Protocol: 90-91
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Protocol: 91
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	-
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Manuscript: 21 Protocol: 91-92
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Manuscript: 20-21
	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Protocol: 92
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Manuscript: 15, Table 1, Suppl Table 1, Suppl Table 4

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and safety of an orally administered DGAT2 inhibitor alone or coadministered with a liver-targeted ACC inhibitor in adults with nonalcoholic steatohepatitis (NASH): rationale and design of the phase II, dose-ranging, dose-finding, randomised, placebo-controlled MIRNA (Metabolic Interventions to Resolve NASH with fibrosis) study

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Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Pharmacology and therapeutics, Research methods
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Efficacy and safety of an orally administered DGAT2 inhibitor alone or coadministered with a liver-targeted ACC inhibitor in adults with nonalcoholic steatohepatitis (NASH): rationale and design of the phase II, dose-ranging, dose-finding, randomised, placebo-controlled MIRNA (Metabolic Interventions to Resolve NASH with fibrosis) study

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Short/running title: DGAT2 and ACC inhibitors in NASH

Keywords (3-6 required, from list provided by journal)

- Internal medicine - hepatology
- Therapeutics - clinical trials
- Radiology & imaging – magnetic resonance imaging

ABSTRACT (298/300 word limit)

Introduction: Small molecule inhibitors of the terminal step in intrahepatic triglyceride synthesis (diacylglycerol acyltransferase 2 inhibitor [DGAT2i, PF-06865571, ervogastat]) and upstream blockade of *de novo* lipogenesis via acetyl-coenzyme A carboxylase inhibitor (ACCi, PF-05221304, clesacostat) showed promise in reducing hepatic steatosis in early clinical trials. This study assesses efficacy and safety of these metabolic interventions to resolve NASH with fibrosis.

Methods and analysis: This phase II, randomised, dose-ranging, dose-finding study evaluates DGAT2i 25-300 mg twice-daily (BID) or 150-300 mg once-daily, DGAT2i 150-300 mg BID+ACCi 5-10 mg BID coadministration, or matching placebo in a planned 450 adults with biopsy-confirmed NASH and liver fibrosis stages 2-3 from approximately 220 sites in 11 countries across North America, Europe and Asia. A triage approach including double-confirmation via noninvasive markers is included prior to screening/baseline liver biopsy. Upon confirmation of histological diagnosis, participants enter a ≥6-week run-in period, then a 48-week double-blind, double-dummy dosing period. The primary endpoint is the proportion of participants achieving histological NASH resolution without worsening fibrosis, ≥1 stage improvement in fibrosis without worsening NASH, or both, assessed by central pathologists. Other endpoints include assessment of hepatic steatosis (imaging substudy), overall safety and tolerability, and evaluation of blood-based biomarkers and quantitative ultrasound parameters over time.

Ethics and dissemination: MIRNA is conducted in accordance with the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, International Council on Harmonisation Good Clinical Practice guidelines, applicable laws and regulations, including privacy laws. Local independent review board/ethics committees (IRB/ECs) review/approve the protocol, any amendments, informed consent and other forms. Participants provide written informed consent. Details of all IRB/ECs, as well as results, will be published in a

peer-reviewed journal and publicly disclosed through ClinicalTrials.gov, EudraCT, and/or www.pfizer.com, and other public registries as per applicable local laws/regulations.

Trial registration: NCT04321031 (https://clinicaltrials.gov/ct2/show/NCT04321031)

Strengths and limitations of this study

- This is the first clinical study to evaluate histological endpoints after oral administration of DGAT2i and DGAT2i+ACCi in participants with biopsy-confirmed NASH and fibrosis stage F2 or F3.
- A triage approach (including double-confirmation via noninvasive blood and quantitative ultrasound-based markers prior to screening/baseline liver biopsy), coupled with central reading of all liver biopsies with consensus required to determine eligibility and assess drug(s) effects, is designed to improve efficiency in identifying participants likely to meet histological entry criteria and robust confidence in histological findings.
- The dosing period includes a longitudinal evaluation of noninvasive imaging and blood-based biomarkers, to identify correlations between histological parameters and noninvasive imaging and/or blood-based biomarkers, in order to assess drug effects.
- The Bayesian dose–response modelling methodologies employed enable an efficient and complete characterisation of dose–response, to aid phase III dose selection.
- This study is limited in that the design relies on assumptions around the translation of effects observed in earlier nonbiopsy studies to a histological endpoint; the impact of the drug(s) on clinical outcomes will need confirmation in an adequately sample-sized phase III trial.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is characterised by excessive accumulation of intrahepatic lipids, especially triglycerides (steatosis) and estimated to affect >25% of the global population.[1, 2] A progressive subtype of the disease, nonalcoholic steatohepatitis (NASH), is defined by cellular injury and inflammation[2] and affects 21–25% of people with NAFLD.[3, 4] NASH drives fibrogenesis,[5] and fibrosis stage is linked to disease outcome and mortality.[6-9]

Targeting molecular pathways involved in the early pathogenesis and abnormal accumulation of hepatic steatosis could prevent inflammation, cellular injury, and fibrosis, thus offering potential treatments for patients with NASH and liver fibrosis. Acetyl-coenzyme A carboxylase (ACC) and diacylglycerol acyltransferase 2 (DGAT2) each play a role in hepatic steatosis (figure 1). *De novo* lipogenesis (DNL) is more active in patients with NAFLD than in healthy individuals and may contribute to excess hepatic triglycerides,[10] with ACC being the first committed enzyme in the hepatic DNL pathway.[11] DGAT2 is highly expressed in the liver and adipose tissue[12] and catalyses the terminal step of DNL, specifically the esterification of a fatty acid with diacylglycerol to form triglyceride.[13] Independent inhibition of each of these steps has been shown to reduce hepatic steatosis. Sterol regulatory element-binding protein 1c (SREBP1c) is a metabolic switch that governs hepatic lipogenesis,[14, 15] and ACC inhibition is associated with upregulation of SREBP1c activity but reduced steatosis in hepatocytes.[11] Conversely, inhibition of DGAT2 was found to down-regulate SREBP1c activity, which in turn reduced hepatic lipogenesis[16]; in addition, a small molecule inhibitor of DGAT2, PF-06427878, reduced hepatic steatosis in a rodent model and clinically after 2 weeks of dosing.[17] In patients with NAFLD, oral administration of another small molecule inhibitor of DGAT2 (DGAT2i, PF-06865571, ervogastat) for 14 days resulted in dose-dependent reductions in both liver fat and serum triglycerides.[18] In addition to its effects on steatosis, ACC inhibition may have direct antifibrotic effects in hepatic stellate cells, the collagen-producing fibroblast population in the liver; in rodent models, ACC inhibition abrogated a metabolic switch necessary for induction of glycolysis

and oxidative phosphorylation during hepatic stellate cell activation *in vitro,* thereby reducing hepatic fibrosis.[19]

In clinical trials, liver-targeted ACC-inhibiting agents have been associated with potent reductions in hepatic steatosis, but with accompanying elevations in serum triglycerides.[11, 20, 21] Doses ≥40 mg/day of a liver-targeted ACC inhibitor (ACCi; PF-05221304, clesacostat) showed near-complete DNL inhibition for 0–10 hours after 14 days of dosing in healthy adults, but these doses were accompanied with increases in serum triglycerides.[22] However, doses <40 mg/day still inhibited DNL by up to 80% but without elevated serum triglycerides.[22] Based on this observation in healthy adults, doses ≤50 mg/day were evaluated in a phase IIa dose-ranging trial with the ACCi in participants with NAFLD and presumed NASH.[23] Reductions in liver steatosis, markers of liver inflammation (alanine aminotransferase [ALT], aspartate aminotransferase [AST]), and markers of hepatocyte cell death (cytokeratin [CK]18-M30 and CK18-M65) were observed following 16 weeks of dosing.[23] However, these potentially beneficial effects were accompanied by marked elevations in fasting serum triglycerides,[23] which is a known mechanistic consequence of hepatic ACC inhibition.[11, 20] Notably, the magnitude of this serum triglyceride increase appears to be higher in adults with NAFLD/presumed NASH than in healthy adults.[22, 23] It could be hypothesised that the increased SREBP1c tone in patients with NAFLD, potentially due to hyperinsulinaemia, make patients more sensitive to ACCi and resultant SREBP activation, compared with healthy adults.[24-26]

While independent inhibition of either upstream (ACC) or downstream (DGAT2) pathways may have limited efficacy for pharmacological treatment of NASH, based on the scientific evidence (figure 1), evaluation of DGAT2i and ACCi together is intriguing given the opposing effects on SREBP1c. Indeed, in rodent models, DGAT2i+ACCi reduced steatosis as well as inflammation and fibrosis markers without the expected ACCi-associated increases in serum triglycerides.[27] In a 6-week, phase IIa trial in patients with NAFLD, DGAT2i 300 mg twice-daily (BID)+ACCi 15 mg BID reduced hepatic steatosis to a similar degree as ACCi alone and to a greater degree than DGAT2i alone, as assessed

by magnetic resonance imaging-proton density fat fraction (MRI-PDFF).[23] The nadir for effect on liver fat was observed at 12–16 weeks with ACCi alone and was not determined for DGAT2i+ACCi[23]. Notably, ACCi-induced elevations in triglycerides (47% increase relative to placebo) were effectively mitigated by DGAT2i+ACCi.[23]

MIRNA is a phase II, randomised, placebo-controlled, dose-ranging, dose-finding study (clinicaltrials.gov: NCT04321031) that assesses the efficacy and safety of an investigational, orally administered DGAT2i and DGAT2i+ACCi in adults with biopsy-confirmed NASH and liver fibrosis stage 2 or 3, as defined using NASH-Clinical Research Network (NASH-CRN) criteria.[28] MIRNA is envisioned to add to the body of scientific evidence by assessing histological endpoints, such as NAFLD Activity Score (NAS), and liver fibrosis. MIRNA is supplemented by a concurrent, short-term (6-week dosing), phase IIa trial of DGAT2i+ACCi (NCT04399538) that aims to identify the lowest dose of DGAT2i that can mitigate ACCi-induced adverse effects on serum lipids, to further aid in the optimal selection of dose(s) of DGAT2i+ACCi for subsequent pivotal studies.

METHODS AND ANALYSIS

Study design

MIRNA is a randomised, double-blind, double-dummy, placebo-controlled, dose-ranging, dose-finding, 9-arm, parallel-group study conducted across approximately 220 planned sites in Bulgaria, Canada, China, Hong Kong, India, Japan, South Korea, Poland, Slovakia, Taiwan and the USA (including Puerto Rico). Recruitment initiated in June 2020 in the USA and is ongoing. Randomisation is estimated to complete in December 2022. This study includes a total of 22 on-site visits and telephone contacts, and each participant's time in the study ranges from 62–68 weeks (figure 2). Given the prior clinical experience of ≤6 weeks with DGAT2i and DGAT2i+ACCi,[23] frequent post-randomisation visits have been planned to permit close monitoring of safety. Procedures throughout MIRNA are summarised in supplementary table 1. A blinded Steering Committee comprising both external and internal medical/clinical representatives from each country/region of study operations will oversee recruitment, retention and quality issues within the country/region.

Eligibility is determined in a three-step process

Guidance from the European Association for the Study of the Liver and American Association for the Study of Liver Disease recognises the significant interest in noninvasive biomarkers for identifying NASH[29, 30]; as such, MIRNA aims to reduce the burden of liver biopsies by using noninvasive techniques to identify eligible participants that are most likely to exhibit NASH, thus increasing selection efficiency. Medical history review, medication use, and blood-derived assessments are used to exclude other causes of NAFLD and other liver diseases. Stability of liver function measurements is confirmed during the pre-qualification and first screening visits. Double-confirmation of liver fat and stiffness using quantitative ultrasound (FibroScan®, EchoSens, Paris, France), along with AST to derive FAST™ scores,[31] are used to identify participants qualifying for a screening/baseline liver biopsy. In a prospective derivation and global validation study, FAST score

cut-offs for sensitivity (≥0.90) of 0.35 and for specificity (≥0.90) of 0.67 were reported, leading to a positive predictive value of 0.83 (84/101) and a negative predictive value of 0.85 (93/110).[31] A slightly lower cut-off of ≥0.30 is used in MIRNA since this threshold needs to be met twice, with an expected missed case rate (participants with NASH and F2 or F3 who do not undergo biopsy based on FAST score) of 9.7% and expected screen fail rate (participants undergoing biopsy based on FAST but fail on biopsy) of 49.5%, indicating that two participants would need to be biopsied to detect one eligible participant.[31]

Participants who qualify based on the noninvasive assessments at the pre-qualification and first screening visits undergo a standardised, ultrasound-guided biopsy of the right lobe of the liver using either a 16- or 18-gauge suction or cutting needle, to acquire tissue ≥1.5 cm in length to determine eligibility based on liver histology. Biopsies are graded and scored, using the NASH-CRN definition,[28, 32] by central, NASH-CRN pathologists (figure 3). The eligible population is defined as participants with a NAS ≥4 and either F2 or F3 fibrosis. All eligibility criteria are listed in supplementary table 2.

To optimise the evaluable data and limit sampling variability that can confound biopsy results,[33] MIRNA employs careful standardisation of biopsy collection including ultrasound guidance, use of specific biopsy needle size, and assessment limited to the right lobe only. MIRNA also utilises prospective, central biopsy reading by two blinded pathologists for eligibility (and evaluating endpoints), using digitised images to shorten the time needed to judge eligibility. Each pathologist qualitatively assesses each domain of NAS and fibrosis.

For assessment of biopsy-related endpoints at Week 48 or discontinuation, review by central pathologists initially independently – and when needed, consensus review to reach agreement – will be performed via paired, blinded assessment of digitised images. In this process, each pathologist assesses the eligibility/screening (baseline biopsy) alongside the Week 48/discontinuation biopsy, for a given participant. The pathologists are blinded to the nominal timepoints and treatment arm, minimising bias when assessing drug(s) effects. Divergence in grading or staging between

pathologists is handled in the same way as outlined in Figure 3 except that for endpoint assessment, agreement between the two pathologists is required for all four domains (steatosis, inflammation, ballooning, fibrosis), thus adding rigour to the determination of histological-based endpoints.

Participants are stabilised during Run-In and Baseline periods

After the liver biopsy is performed (i.e. Screen 2), participants start a 6-week run-in period prior to randomisation to generate an in-study, stable state for all participants in terms of medical history and medication use (including compliance). In consideration of potential drug–drug interactions, participants taking gemfibrozil are switched to another permitted agent for lipid control, and those taking metformin >1 g/day have their dose adjusted down by one-third to one-half to 1 g/day, starting at the run-in visit (supplementary table 3). Standardisation of lifestyle guidelines across all sites and countries is advocated[34, 35]; in MIRNA, accounting for operational considerations and local practices, lifestyle guidelines advocating healthy choices that do not result in overt weight loss during the course of the study are implemented to minimise placebo response. The Alcohol Use Disorders Identification Test questionnaire is used before randomisation and at the end of study dosing to confirm that alcohol intake is in moderation during the study (supplementary table 1). Single-blind placebo is administered over a 2-week period before randomisation to confirm that participants can comply with dosing instructions for the study drug (i.e. three tablets/dose, BID with meals). Participants are provided with electronic devices auto-programmed with periodic reminders to enhance compliance (from baseline to end-of-dosing period). These steps are intended to account for the 'Hawthorne effect', wherein changes in participant behaviour occur because of increased knowledge or interest or due to the perception of being observed. They help limit placebo response, thus permitting ascertainment of drug effect.

Randomised participants are treated for up to 48 weeks and followed for an additional 4 weeks

On Day 1, eligible participants are randomised to one of nine arms using a computer-generated randomisation code (random permuted blocks method) and stratified by fibrosis stage (F2 or F3), to ensure a balance of participants across regimens. Participants are randomly allocated to treatment groups by blinded investigators using an interactive response technology system (interactive web response) programmed with instructions for unblinding only in emergency situations for reasons of participant safety, as determined by the investigator. Study drugs are self-administered in a double-blind, double-dummy manner for 48 weeks, in line with regulatory guidance for agents in development for NASH with fibrosis.[36, 37] A follow-up on-site visit occurs 2 weeks post-last dose with a follow-up phone call approximately 4 weeks after the last dose. Participants and all persons involved in trial conduct, participant interactions and data analysis are blinded to treatment assignment.

Approximately half of the total sample size are participating in an imaging substudy to characterise the effect on liver steatosis and liver volume over time

Approximately 50% of participants are forecast to be enrolled in an imaging substudy to 1) characterise the dose–response for effect on liver steatosis using MRI-PDFF; 2) characterise the drug effect over time and define the time to maximum effect (i.e. nadir); and 3) assess the correlation of liver fat and volume assessed by MRI-PDFF (and other associated imaging and laboratory-based endpoints) with histology endpoints.

Concomitant medications are allowed with some adjustments

All concomitant medications taken during the study (supplementary table 3), including herbal supplements in countries where they are part of standard of care to lower liver function test measurements, are recorded along with indication of use. Additional information including daily

dose and duration of administration are captured for medications used for glycaemic control, lipid control, and blood pressure control.

Patient and public involvement

Input from patients with biopsy-confirmed diagnoses of NASH was sought while designing MIRNA. Their feedback led to revisions in the protocol (final version 22 January 2020) regarding the manner by which information is provided to prospective participants in the informed consent document. These included explaining why the intervention may work and the associated benefits and risks, information about efforts to minimise biopsy for diagnosis and about tracking drug effects, and the rationale for the blood volume collected and the intent to use blood samples to evaluate pre-identified and new biomarkers at a later date. Blister packs (rather than bottles) are being utilised to aid compliance and acknowledge pill burden, while balancing the requirements of the double-blind, double-dummy design. Additionally, participant-friendly reminders and milestone communications were incorporated into the study.

Selection of DGAT2i and ACCi doses to maximise liver fat reduction

Dose selection was informed by exposure–response modelling of historical pharmacokinetic and reduction in liver fat data observed following 2 weeks of DGAT2i dosing[18]; 6 weeks of DGAT2i, ACCi and DGAT2i+ACCi; and 16 weeks of ACCi.[23] The half-maximal effective concentration (EC_{50}) for liver fat reduction was estimated as 41 ng/mL for DGAT2i, and represents a dose of approximately 30 mg BID. A 300-mg BID dose was projected to achieve a near maximal effect on liver fat reduction, and it was hypothesised that DGAT2i 300 mg BID coadministered with ACCi would further extend the effect of DGAT2i. Using both exposure–response analysis and quantitative systems pharmacology modelling, DGAT2i doses of 25, 75, 150, and 300 mg BID were chosen. Additionally, DGAT2i doses of 150 mg and 300 mg once-daily (QD) were selected to compare QD with BID regimens at the same daily dose (75 mg BID and 150 mg BID). This will help determine if similar efficacy is achievable with

equivalent QD and BID dosing while testing for potential dissociation between plasma pharmacokinetic profiles and pharmacology.

Two dose levels of DGAT2i+ACCi are being evaluated – DGAT2i 300 mg BID+ACCi 10 mg BID and DGAT2i 150 mg BID+ACCi 5 mg BID – to assess whether ACCi coadministration extends DGAT2i efficacy. ACCi 10 mg BID yields 80% DNL inhibition[22]; on a mg-per-mg basis for both doses the ratio is maintained at 30:1 (DGAT2i:ACCi) to enable evaluation of both efficacy and safety of DGAT2i+ACCi relative to DGAT2i alone. Although ACCi has a pharmacokinetic half-life conducive to QD dosing,[22] a BID dosing regimen was selected to match the likely frequency of clinical dosing for DGAT2i.

Dose selection and dose range from this and the ongoing phase IIa trial (NCT04399538) investigating a wider dose range for DGAT2i+ACCi in patients with presumed NASH may aid in determining the optimal dose(s) of DGAT2i and DGAT2i+ACCi to evaluate in confirmatory, phase III trials.

Objectives, estimands and endpoints

Clinical responders based on histological evidence

The primary endpoint of MIRNA is the proportion of participants achieving resolution of NASH (i.e. absence of ballooning with no or minimal inflammation by histology)[36] without worsening of fibrosis, or improvement in fibrosis by ≥1 stage without worsening of NASH, or both, at Week 48, as assessed by central pathologists. This is based on histological assessment at screening/baseline liver biopsy, and at end of drug administration (i.e. Week 48 or earlier in cases of premature withdrawal of study drug, provided the study drug was administered up to at least Week 24), in all randomised and treated participants with evaluable baseline biopsy data. Using a composite estimand strategy, drug effect is estimated in terms of the proportion of 'clinical responders', defined as participants achieving the primary endpoint.[38] All cases of withdrawal from study drug(s) due to lack of efficacy or toleration are treated as nonresponders. Participants who withdraw from study drug(s) for other

reasons but have evaluable biopsy data at withdrawal or Week 48 will have their biopsy data assessed to determine whether they are responders or not. Participants with no Week 48 biopsy data are considered to be nonresponders.

Secondary endpoints include the percent change in liver fat (assessed via MRI-PDFF in the substudy population), the proportion of participants achieving improvements in responder definitions (resolution of NASH without fibrosis worsening, ≥1 or ≥2-stage fibrosis improvement without NASH worsening, ≥2-point improvement in total NAS score), assessment of adverse events (AEs) up to Week 52 and safety-related clinical laboratory tests (including full blood and platelet counts), vital signs and 12-lead ECGs to at least Week 50. The secondary efficacy endpoints employ a composite estimand strategy for the histological assessments, whereby the responder definitions are evaluated based on histological assessment at Week 48 relative to baseline.

The tertiary endpoint is the proportion of the population with worsening disease at Week 48, defined as progression of fibrosis by ≥1 stage and worsening of ≥2 points in total NAS.

Secondary and tertiary objectives include evaluation of safety/tolerability and clinical response on imaging and blood-based biomarkers

Analysis of all imaging and laboratory parameters is performed by external vendors who are blinded to treatment assignment to ensure the blind is preserved and to minimise any bias in assessment of the study endpoints.

Safety and tolerability

These assessments include 12-lead electrocardiogram, blood pressure, pulse rate, body weight measurements, open-ended enquiries of AEs, collection of blood and urine for assessment of haematology, chemistry, and urinalysis (supplementary table 4). Reasons for discontinuation of study drug include sustained fasting serum triglyceride levels ≥800 mg/dL (≥9 mmol/L), platelet count <75,000/mm^3, or other AEs based on medical judgement. An independent external data

monitoring committee consisting of medical experts and a statistician will be responsible for ongoing review of unblinded data to assess safety. Unblinded data analysis for this explicit purpose is undertaken by a dedicated independent external vendor (Statistical Data Analysis Center, University of Wisconsin, USA). In addition, an independent adjudication committee consisting of external experts will perform blinded review of all potential fatal events, hepatic events (including decompensation, histological progression to cirrhosis, hepatocellular carcinoma or drug-induced liver injury) or cardiovascular events (including major adverse cardiovascular events) to confirm that the data support the endpoint designation. Interim analyses will be performed to assess safety, at a minimum, after approximately 25%, 50% and 75% of planned total sample size have been randomised in the study. Interim analysis results may be used for future study planning, including adapting safety-related endpoints.

Imaging assessments

Considering the primary pharmacology of DGAT2i and ACCi, liver fat and volume (via MRI-PDFF) are assessed as a secondary objective in the imaging substudy. In addition, FibroScan® is being used to measure liver fat via the controlled attenuation parameter (CAP™), and liver stiffness via vibration-controlled transient elastography (VCTE™), over time. Study-specific manuals emphasise the use of M and XL probes guided by SMART tools on the FibroScan® device, including evaluation of tissue change in TM-mode and ultrasound signal strength and propagation in A-mode.

Both MRI-PDFF and FibroScan® assessments are performed following a fast (except water) of ≥4 hours. The MRI-PDFF acquisition protocol is standardised *a priori* across all sites participating in the substudy, with images centrally analysed using validated, two-dimensional, six-echo, spoiled gradient-recalled-echo, breath-hold pulse sequences.[39] MRI-PDFF image analyses are performed by a blinded external vendor; a 2.5 cm diameter region of interest is applied on each of nine anatomical liver segments, except for the caudate where a 1.5 cm diameter region of interest is identified.

Blood-based biomarkers

Any potential pharmacological consequences of DGAT2i and DGAT2i+ACCi are being studied via blood-based biomarkers summarised in table 1.

Sparse blood sampling for pharmacokinetic analyses over the 48-week dosing period is included to estimate drug exposure and help describe the relationship between dose, concentration, and efficacy/safety of DGAT2i and DGAT2i+ACCi via population pharmacokinetics and pharmacokinetic/pharmacodynamic analyses. Additionally, blood samples are collected for exploration of other plasma- or serum- biomarkers and single nucleotide polymorphisms linked to NASH.

Table 1. Blood-based biomarkers assessed in MIRNA. Additional samples collected for exploratory biomarker analysis are listed in supplementary table 4.

Parameter	Biomarker
Liver function tests	• Alanine aminotransferase • Aspartate aminotransferase • Alkaline phosphatase • γ-glutamyl transferase • Total bilirubin
NASH-related	• 3-parameter derived enhanced liver fibrosis™ score (marker of liver fibrosis used to track disease progression) • Cytokeratin-18-M30 fragment (marker of apoptotic activity) • Cytokeratin-18-M65 fragment (marker of necrotic activity) • N-terminal propeptide of procollagen type III (marker of fibrinogenesis) • C-terminal fragment of α3 chain of procollagen type VI (marker of fibrinolysis)

Fasting lipid parameters/markers of target engagement	• Fasting serum lipid panel:
	  ○ Total cholesterol
	  ○ Triglycerides
	  ○ High density lipoprotein cholesterol
	  ○ Direct low density lipoprotein cholesterol
	  ○ Direct very low density lipoprotein cholesterol
	• Fasting serum apolipoproteins:
	  ○ A1
	  ○ B_{total}
	  ○ B_{100}
	  ○ B_{48}
	  ○ C3
	  ○ E
	• High-sensitivity C-reactive protein
	• Proprotein convertase subtilisin/kexin type 9

Glycaemic	• HbA1c • Fasting plasma glucose

- Fasting plasma insulin
- Homeostatic model assessment of insulin resistance
- Adiponectin

HbA1c, glycated haemoglobin; MIRNA, Metabolic Interventions to Resolve NASH with Fibrosis;

NASH, nonalcoholic steatohepatitis.

Statistical considerations

Sample size

Sample size estimation is driven by the characterisation of dose–response and drug effect using a Bayesian maximum effect of drug (E_{max}) study design and modelling approach, which utilises weakly informative priors for model parameters. This approach increases the precision in drug/dose comparisons (supplementary table 5) and enables the required sample size to be reduced by almost half compared with conventional pairwise comparisons. Nonetheless, MIRNA is over-enrolling by approximately 20% (450 participants with 50 per arm) to minimise the risk of an underpowered study due to a lack of primary endpoint data. Anticipated reasons for insufficient primary endpoint data include nonevaluable biopsies, participant withdrawal, and inconsistencies in scoring/grading digitised slides when determining eligibility and pairwise (second screening and end-of-dosing period visits) blinded review.[40] The decision to over-enrol in MIRNA was informed by learnings from a previous trial, which reported statistically significant improvements in some secondary endpoints (glucose, HbA1c, fasting plasma insulin, liver enzymes, and NAS), but not primary and secondary histological endpoints after treatment with an insulin sensitiser for NASH, due to issues with interpretation of liver biopsies.[41]

Priors for E_{max} model parameters were evaluated and for DGAT2i, ED_{50} was estimated to be approximately 30 mg BID (based on the projected EC_{50}) and the placebo responder rate (E_0) was estimated to be 16%. Based on the above assumptions, at the theoretical E_{max} of 0.6 (i.e. a 60% responder rate) and an estimated sample size of 450 participants, there is enough precision to show a >24% difference in the primary endpoint responder rate between placebo and the second-highest DGAT2i dose, 150 mg BID, with a probability of ≥89%. In addition, a sample size of 450 participants provides 75% power to demonstrate a 24% difference in the primary endpoint responder rates between QD doses and placebo, and adequate precision to assess whether DGAT2i+ACCi provides a higher responder rate than DGAT2i with a probability of 82% if the true effect size is at least 6%.

Inter and intra-pathologist variability

Variability is assessed quarterly on a randomly selected sample comprising 10% of screening biopsies and Week 48/discontinuation biopsies from randomised participants. Pathologists review the same biopsy images ≥3 months apart, to ascertain if the same levels of calibre are maintained over time (intra-pathologist variability). Reviews by the pathologists are compared to NASH-CRN peers using weighted kappa statistics (inter-pathologist variability).

Statistical models

In assessing the primary objective, a Bayesian dose–response model will characterise the dose–response across all DGAT2i BID arms, to estimate the proportion of responders (and 95% confidence intervals [CI]) for each dose, and to estimate the placebo-adjusted proportion of responders for each dose (with 95% CI). The Bayesian estimation of the E_{max} dose–response model uses prior distributions on the placebo response (E_0), as well as the ED_{50} (30 mg BID) and E_{max} parameters. A normal prior distribution for the logit of the placebo response centred at logit (0.16) with a prior standard deviation of 2.0 (logistic scale) is planned to be used, and similarly, the prior for the E_{max} parameter will be centred at logit (0.6) with a prior standard deviation of 2.0. These are diffuse parameters on the logistic scale, which will ensure that the data collected in this study are not overly influenced by these prior distributions, while ensuring convergence of the Bayesian dose–response model. If an E_{max} dose–response model cannot be fitted to the data, other models that allow dose–response to be estimated will be fitted (i.e. linear, log-linear, or exponential).

Similar Bayesian dose–response models will be utilised for the secondary objectives of achievement of different responder definitions based on histological outcomes. Other comparisons (DGAT2i QD doses vs placebo, and DGAT2i+ACCi BID doses vs placebo and vs corresponding DGAT2i BID doses) will be analysed using logistic regression models to estimate the proportion of responders in each arm and odds ratio (95% CI) for each comparison.

For the secondary objective of percent change from baseline in liver fat, all drug effect contrasts will be based on a hypothetical estimand strategy, which assumes that all participants remained in the trial for 48 weeks and received study drug(s) as planned without withdrawal. Any available MRI data for all participants is included, including those who withdrew from study drug(s) due to lack of efficacy or toleration. If the Week 48 response is missing, this is imputed using a model-based analysis based on the treatment arm assigned at randomisation. This will follow the average treatment effect as observed in the same assigned treatment arm. A Bayesian E_{max} dose–response model for the DGAT2i BID doses will also be utilised. Other dose group comparisons will use an analysis of covariance (ANCOVA) performed on log-transformed relative change from baseline, with dose group and baseline fibrosis stage (F2 or F3) as factors and log-transformed baseline liver fat value as a covariate. Estimates of the mean relative changes for each dose comparison and 95% CI will be obtained from the model and will be exponentiated to provide estimates of the percent change. For comparisons of DGAT2i+ACCi BID doses versus corresponding DGAT2i BID doses, 50% CI will also be calculated. No adjustment for multiple comparisons will be made. Safety analyses will be summarised descriptively.

Ethics and dissemination

MIRNA is conducted in accordance with ethical principles derived from the Declaration of Helsinki and CIOMS International Ethical Guidelines, applicable International Council for Harmonisation Good Clinical Practice guidelines, and applicable laws and regulations, including privacy laws. Before the study is initiated, the protocol, protocol amendments (if any), informed consent and other forms are reviewed and approved by local independent review board/ethics committees (IRB/ECs): central IRB, WCB IRB tracking number 20200277, for sites initiated in the USA. Local approvals are ongoing, and a full list of IRB/ECs will be disclosed with the study results upon completion. Study participants provide written informed consent to investigators at pre-qualification and separately when entering the main study at the first screening visit, with additional consent required for the imaging substudy.

Participants may withdraw from the study at any time. Given a screening population without biopsy-confirmed NASH, the triage approach necessitates screening many prospective participants. Only those participants with biopsy-confirmed NASH and fibrosis receive study-specific information to minimise anxiety in those who do not have NASH with fibrosis. Information provided at pre-qualification incorporates education about NAFLD and NASH including risk factors. All parties are required to comply with all applicable laws, including laws regarding the implementation of organisational and technical measures to ensure protection of participant data. All participant data relating to the study will be recorded on printed or electronic case report forms (CRFs) unless transmitted to the sponsor or designee electronically (e.g. laboratory data). The investigator is responsible for verifying that data entries are accurate, maintaining accurate documentation (source data) that supports information entered into the CRFs and ensuring that the CRFs are securely stored at the study site in encrypted electronic form, password protected to prevent access by unauthorised third parties. The investigator must permit study-related monitoring, audits, IRB/EC review and regulatory agency inspections and provide direct access to source data documents. The sponsor or designee is responsible for the data management of this study, including quality checking of the data.

As per patient feedback that was incorporated into the design of MIRNA, aggregate-level summaries of study results in lay language are to be disseminated to randomised participants; study results will be publicly disclosed 24 months after completion, through posting on www.clinicaltrials.gov, the EudraCT, and/or www.pfizer.com, and other public registries in accordance with applicable local laws/regulations. Participant-level data will be anonymised in accordance with applicable privacy laws and regulations. The results of MIRNA may be published or presented at scientific meetings by the investigators after disclosure of the overall study results or 1 year after the end of the study (or study termination), whichever comes first.

DISCUSSION

MIRNA represents the first clinical study to assess an oral DGAT2i alone and coadministered with another investigational chemical entity, ACCi, in patients with biopsy-confirmed NASH with F2 or F3.

The rationale for MIRNA is supported by nonclinical and clinical data. Reduced liver steatosis (accompanied by an increase in hepatic free fatty acids and increasing fibrosis) was observed with an antisense oligonucleotide DGAT2 inhibitor in a specific rodent model[42] but this increase in fibrosis has not been replicated with orally administered DGAT2i.[17] Furthermore, nonclinical data showed no change in fasting (4 hours) nonesterified fatty acids at Day 17 of dosing in Western-diet fed rats with DGAT2i (PF-06865571, ervogastat) alone or in combination with ACCi (unpublished data), which is consistent with previous data showing that DGAT1 rather than DGAT2 is the active DGAT isoform during stimulated lipolysis, promoting fatty acid re-esterification to protect adipocytes from lipid-induced endoplasmic reticulum stress.[43] Dose-dependent reductions in liver fat of ≤41% (vs 11% with placebo) and fasting serum triglycerides of ≤24.5 mg/dL (vs 7.0 mg/dL with placebo) were reported following oral administration of the DGAT2i ≤300 mg BID for 14 days in patients with NAFLD.[18] Moreover, preliminary data suggest that DGAT2i+ACCi could extend the efficacy of DGAT2i and also mitigate ACCi-induced increases in serum triglycerides.[23] Further data from MIRNA will help elucidate the benefit-to-risk profile of these new chemical entities, particularly when considering that hepatic fibrosis (defined by VCTE ≥8.2 kPa) is associated with several cardiometabolic disease risk factors.[44]

This study has several strengths, including a triage approach with double-confirmation prior to screening/baseline liver biopsy, coupled with central reading of all liver biopsies with consensus required to determine eligibility and assess drug(s) effects, that is designed to improve efficiency in identifying participants likely to meet histological entry criteria and robust confidence in histological findings. Assessing a wide range of DGAT2i doses via QD and BID regimens along with DGAT2i+ACCi allows for a thorough assessment of several objectives, using statistically efficient methodology to

identify potentially well-tolerated and efficacious dose(s) and dosing regimen(s) for pivotal phase III/IV trials.[45] The ongoing phase IIa trial (NCT04399538) will provide additional information on optimal doses of DGAT2i+ACCi. Furthermore, evaluating drug effects on imaging and blood-based biomarkers alongside regulatory-mandated histological endpoints during the dosing period could help identify surrogate endpoints for NASH with F2 or F3 fibrosis.[36, 46] This is particularly pertinent as European and US guidelines currently recommend striving for validated, noninvasive endpoints for NASH.[37, 47] MIRNA is designed to satisfy this recommendation with adequate statistical power to assess the primary endpoint. Enrolment above the minimum requirement, double screening by two pathologists, and collection of plasma and serum samples for future noninvasive endpoints further supports the robustness of the study design. The study is limited in that the design relies on assumptions around the translation of effects observed in earlier nonbiopsy studies to a histological endpoint; the impact of the drug(s) on clinical outcomes will need confirmation in an adequately sample-sized phase III trial.

MIRNA incorporates learnings from previous trials in patients with NASH and F2 or F3 fibrosis, and other metabolic diseases (e.g. type 2 diabetes).[48, 49] This dose-ranging, dose-finding study in patients with NASH and F2 or F3 fibrosis aims to collect robust data for histological, imaging, and blood-based biomarkers to provide confidence in the efficacy of DGAT2i and ACCi, so that the pivotal phase III trials can focus on confirming efficacy and evaluate safety in a much larger sample size.

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Competing interests

NBA, AD, DSL, MV and CY are employees of, and hold stock or stock options with, Pfizer Inc. QMA has received fees for consultancy on behalf of Newcastle University, grant funding via the EU IMI2 scheme and speaker fees from Pfizer Inc in relation to the submitted work. QMA has received grant funding from AbbVie, Allergan/Tobira, AstraZeneca, Genfit SA, GlaxoSmithKline, Glympse Bio, Intercept Pharma Europe Ltd (via the EU IMI2 scheme), Novartis Pharma AG, Pfizer Inc (via the EU IMI2 scheme); speaker fees from Bristol Myers Squibb, Gilead, Kenes, Novo Nordisk, Pfizer Inc; consultancy fees on behalf of Newcastle University from 89Bio, Allergan/Tobira, Altimmune, AstraZeneca, Axcella, BGMBio, Blade, BNN Cardio, Bristol Myers Squibb, Celgene, Cirius, CymaBay, E3Bio, EcoR1, Eli Lilly & Co., Galmed, Genentech, Genfit SA, Gilead, Grunthal, HistoIndex, Imperial Innovations, Indalo, Intercept Pharma Europe Ltd., Inventiva, IQVIA, Janssen, Madrigal, MedImmune, Metacrine, NewGene, NGMBio, North Sea Therapeutics, Novartis Pharma AG, Novo Nordisk, PathAI, Pfizer Inc, Poxel, Raptor Pharma, Servier, Terns, Viking Therapeutics, outside the submitted work. VWSW has received medical writing and article processing charges support from Pfizer Inc for the submitted work. VWSW's institution has received grant funding from Gilead and support for meetings attendance from AbbVie and Gilead; VWSW has received consulting fees and payments for participation on a Data Safety Monitoring Board or Advisory Board from 3V-Bio, AbbVie, Allergan, Boehringer Ingelheim, Center for Outcomes Research in Liver Diseases, Echosens, Gilead, Hanmi Pharmaceutical, Intercept, Inventiva, Merck, Novartis, Novo Nordisk, Perspectum Diagnostics, Pfizer Inc, ProSciento, Sagimet Biosciences, TARGET PharmaSolutions, Terns; VWSW has received payment or honoraria for lectures, presentations, speakers bureaux, manuscript writing or educational events

from Abbott, AbbVie, Bristol Myers Squibb, Echosens, Gilead; VWSV has stock or stock options in Illuminatio Medical Technology Ltd.

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MC and AN declare no competing interests.

A patent rationalising the invention of administering DGAT2i+ACCi to mitigate effects of ACCi alone has been submitted.

Contributors

- Conception and study design: NBA, AD, DSL, MV, QMA, VWSW, FT, CY
- Oversight and leadership of the study planning and execution: NBA, AD, CY
- Study conduct: NBA, AD, QMA, VWSW, FT, MV, MC, NA, AN, CY
- Drafting, revising, and final approval of the manuscript: NBA, AD, QMA, VMSM, FT, MV, DSL, MC, NA, AN, CY

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FIGURE LEGENDS

Figure 1. Effects of ACC and DGAT2 inhibition on hepatic lipid metabolism.[11, 13, 16, 27, 50]

Footnotes for Figure 1:

[a]Adaptive effects.

[b]In nonclinical models.

ACC, acetyl-coenzyme A carboxylase; ACCi, ACC inhibitor; ACS, acyl-CoA synthetase; CoA, coenzyme A; CPT1, carnitine palmitoyl transferase 1; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; DGAT2i, DGAT2 inhibitor; DNL, *de novo* lipogenesis; FAS, fatty acid synthase; FFA, free fatty acid; G-3-P, glycerol-3-phosphate; MUFA, monounsaturated fatty acid; PPARα, peroxisome proliferator-activated receptor α; SCD1, stearoyl-CoA desaturase 1; SREBP, sterol regulatory element-binding protein; TAG, triacylglycerol (also known as triglyceride); TG, triglyceride.

Figure 2. MIRNA study design.

The intervals depict the maximum time between the various periods in the study.

Footnotes for Figure 2:

[a]In addition, metformin dose reduced if dose is >1 g/day.

ACCi, acetyl-CoA carboxylase inhibitor; BID, twice-daily; DGAT2i, diacylglycerol acyltransferase 2 inhibitor; MIRNA, Metabolic Interventions to Resolve NASH with Fibrosis; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; n, target number of participants; QD, once-daily.

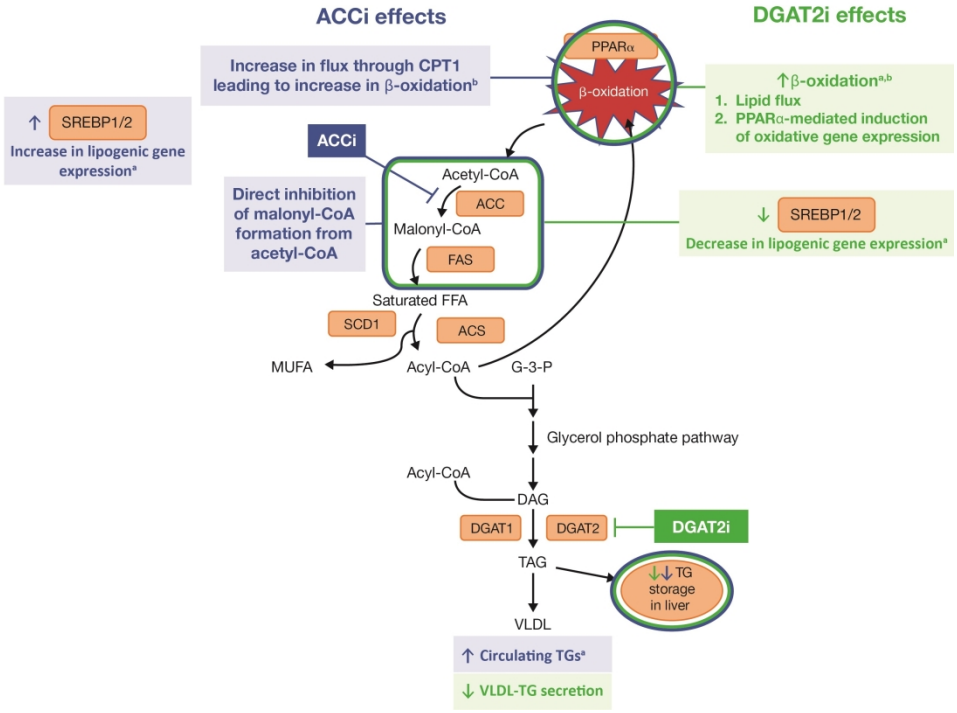
Figure 3. Prospective, centralised grading and scoring of liver biopsies at screening for eligibility using the NASH-CRN definition.

Footnotes for Figure 3:

NAFLD, nonalcoholic fatty liver disease; NASH-CRN, Nonalcoholic steatohepatitis Clinical Research Network.

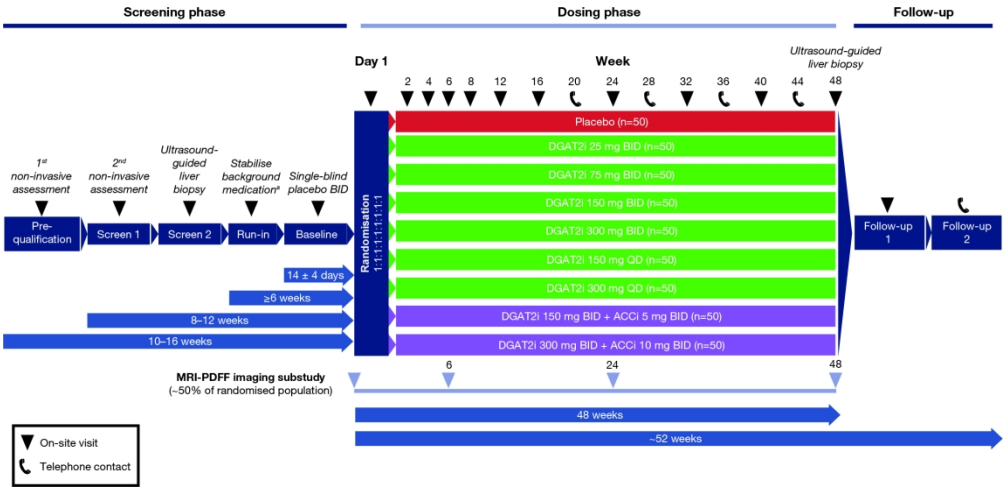
[a]Consensus review to reach agreement is not required if both pathologists agree that either the NAFLD Activity Score or fibrosis grade renders the participant ineligible

Figure 1



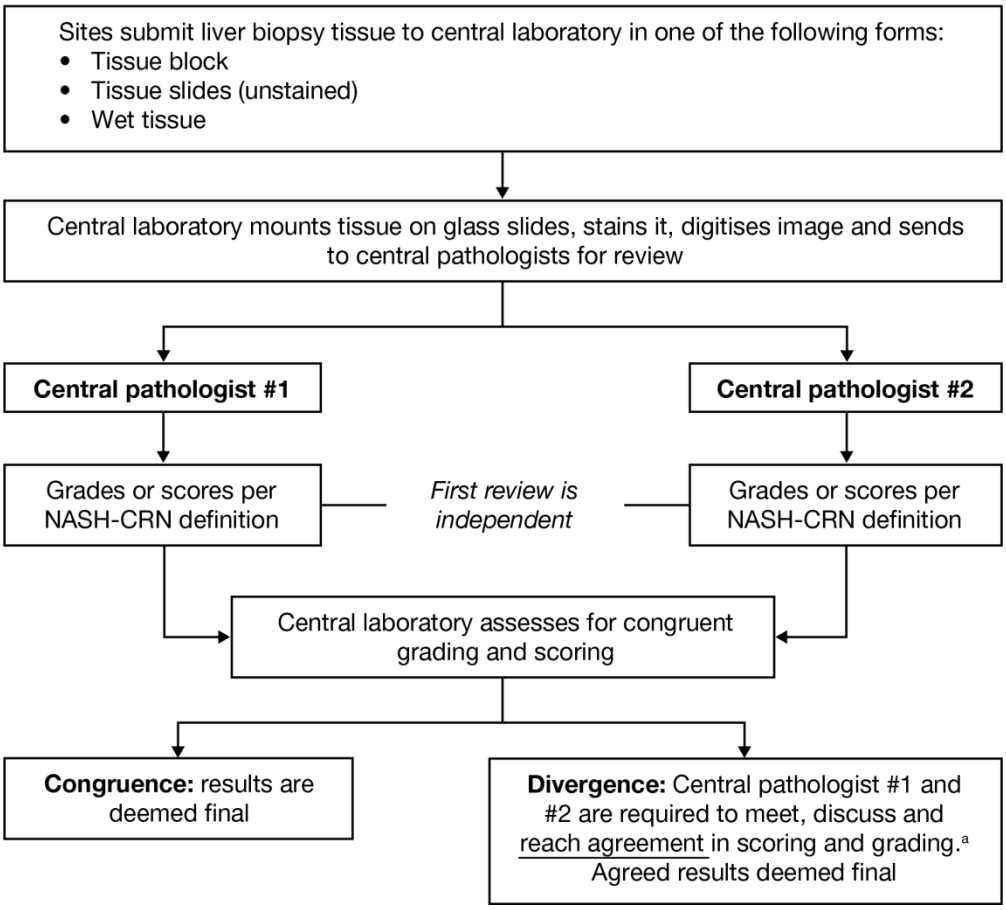
258x189mm (300 x 300 DPI)

Figure 2



193x106mm (300 x 300 DPI)

Figure 3



159x160mm (300 x 300 DPI)

Efficacy and safety of an orally administered DGAT2 inhibitor alone or coadministered with a liver-targeted ACC inhibitor in adults with nonalcoholic steatohepatitis (NASH): rationale and design of the phase II, dose-ranging, dose-finding, randomised, placebo-controlled MIRNA (Metabolic Interventions to Resolve NASH with fibrosis) study

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SUPPLEMENTARY INFORMATION

Supplementary Table 1. Collection of data during MIRNA

	Pre-qualification	Screen 1	Screen 2	Run-in	Baseline	Dosing week																Follow-up	Discontinuation
Week	–	–	–	–6	–2	0	2	4	6	8	12	16	20	24	28	32	36	40	44	48	50	52	
Procedures																							
Informed consent, demography	✓	✓																					
Medical & medication history (update)	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ultrasound-guided liver biopsy			✓																	✓			✓
Liver fat and stiffness (FibroScan®)	✓	✓			✓				✓		✓			✓		✓		✓		✓			✓
Liver MRI-PDFF (Imaging substudy)					✓				✓					✓						✓			✓
Physical exam	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓		✓	✓		✓
Alcohol intake assessed (AUDIT)	✓	✓				✓														✓			✓
Counselling on diet/exercise guidelines				✓		✓																	
Adverse events (open-ended query)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Single supine 12-lead ECG	✓	✓			✓	✓								✓						✓	✓		✓

	Pre-qualification	Screen 1	Screen 2	Run-in	Baseline	Dosing week																Follow-up	Discontinuation
Week	–	–	–	–6	–2	0	2	4	6	8	12	16	20	24	28	32	36	40	44	48	50	52	
Singled seated vitals (blood pressure, pulse rate) and body weight	✓	✓			✓	✓	✓		✓		✓			✓		✓		✓		✓	✓		✓
Study intervention taken with morning meal					✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓			
Blood collection (after overnight fast of ≥8 hours)																							
FSH (females only), HBsAg, HCVAb, HIV, α1-antitrypsin, ceruloplasmin	✓	✓																					
% carbohydrate deficient transferrin	✓	✓			✓	✓														✓			✓
Haematology, chemistry, coagulation, triglycerides, direct LDL-C, HDL-C, total cholesterol, pregnancy (females only)	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓		✓	✓	✓	✓
HbA1c, plasma glucose	✓	✓			✓	✓		✓		✓		✓		✓		✓		✓		✓	✓		
Direct VLDL, ApoA1, $ApoB_{total}$, ApoB100, ApoB48, ApoC3, ApoE, PCSK9, plasma insulin, adiponectin, CK18-M30, CK18-M65, ProC3, ProC6, enhanced liver fibrosis test, hs-CRP					✓	✓		✓		✓		✓		✓		✓		✓		✓	✓		
Pre-dose PK – DGAT2i and ACCi						✓	✓			✓	✓	✓				✓				✓			
Post-dose PK – DGAT2i and ACCi							✓			✓	✓	✓											
Spot urine collection																							

	Pre-qualification	Screen 1	Screen 2	Run-in	Baseline	Dosing week																	Follow-up	Discontinuation
Week	–	–	–	–6	–2	0	2	4	6	8	12	16	20	24	28	32	36	40	44	48	50	52		
Urine drug test	✓	✓			✓																			
Urinalysis	✓	✓			✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓		✓	✓	✓	✓	
Pregnancy test (women of child-bearing potential)					✓	✓	✓	✓	✓	✓	✓	✓		✓		✓		✓		✓	✓	✓	✓	

ACCi, acetyl-coenzyme A carboxylase inhibitor; Apo, apolipoprotein; AUDIT, Alcohol Use Disorders Identification Test; CK18-M30, cytokeratin-18-M30 fragment; CK18-M65, cytokeratin-18-M65 fragment; DGAT2i, diacylglycerol acyltransferase 2 inhibitor; ECG, electrocardiogram; FSH, follicle-stimulating hormone; HbA1c, glycated haemoglobin; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; HDL-C, high density lipoprotein-cholesterol; HIV, human immunodeficiency virus; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low density lipoprotein-cholesterol; MIRNA, Metabolic Interventions to Resolve NASH with Fibrosis; MRI-PDFF, magnetic resonance imaging-proton density fat fraction; NASH, nonalcoholic steatohepatitis; PCSK9, proprotein convertase subtilisin/kexin type 9; PK, pharmacokinetics; ProC3, N-terminal propeptide of type III procollagen; ProC6, C-terminal fragment of α3 chain of procollagen type VI; VLDL, very low density lipoprotein.

Supplementary Table 2. Inclusion and exclusion criteria in MIRNA

Inclusion Criteria

• At pre-qualification and the first screening, participants must meet ≥2 of the following:
  ○ Fasting plasma glucose ≥100 mg/dL (or taking agents to improve glycaemic control)
  ○ Fasting serum HDL-C <40 mg/dL for males and <50 mg/dL for females (or taking agents to increase HDL-C)
  ○ Fasting serum triglycerides ≥150 mg/dL (or taking agents to reduce triglycerides)
  ○ Seated blood pressure ≥130/85 mmHg (or taking agents for blood pressure control)
  ○ Waist circumference ≥40 inches for males and ≥35 inches for females

• At both the pre-qualification and the first screening, FAST™ ≥0.30

• At the second screening, ultrasound-guided liver biopsy meeting the NASH-CRN definition
  ○ Total NAS ≥4 with steatosis, inflammation, and ballooning grades all ≥1
  ○ Fibrosis scoring of F2 or F3

• Participants are willing and able to comply with all scheduled visits, dosing plan, laboratory tests, lifestyle considerations, and other study procedures including a second biopsy while in the study
• At pre-qualification and first screening, BMI ≥25 kg/m^2 or ≥22.5 kg/m^2 (Asia only) and ≤40 kg/m^2
• Demonstration of stable body weight (within 5%) for ≥12 weeks before the first screening
• Capable of giving signed informed consent
Exclusion Criteria

• At pre-qualification and first screening visit, current significant alcohol consumption defined by any of the following:
  ○ >14 or >7 drinks/week for males or females, respectively
  ○ % carbohydrate deficient transferrin ≥1.5 x ULN
  ○ Total score of ≥8 on the interview-based AUDIT questionnaire[1]

• At pre-qualification and first screening, evidence of other causes of liver disease, including:
  ○ Alcoholic steatohepatitis, compensated and decompensated cirrhosis, histological presence of cirrhosis on screening/baseline liver biopsy, HIV infection, hepatocellular carcinoma or other types of liver cancer
  ○ Active viral hepatitis B, defined by presence of HBsAg
  ○ Active viral hepatitis C, defined as presence of HCVAb
  ▪ Those cured are eligible so long as there is evidence of SVR for ≥3 years
  ○ Wilson's disease, defined as ceruloplasmin level <0.1 g/L
  ○ A1AT deficiency, defined as A1AT level <LLN
  ○ Upper gastrointestinal bleed due to oesophageal varices, liver transplant, or current MELD-Na score >12

• At pre-qualification, history of pancreatitis
• At pre-qualification, any condition possibly affecting absorption (eg. prior bariatric surgery, gastrectomy, ileal resection)

- Within 12 weeks prior to first screening, diagnosis of type 2 diabetes mellitus which requires management with >3 medications
- Within 12 weeks prior to first screening, dyslipidaemia which requires management with >3 lipid-modifying agents
- Severe hypertension (≥180 mmHg systolic and ≥105 mmHg diastolic) at pre-qualification and first screening, or management with >3 agents to control blood pressure within 12 weeks prior to first screening
- A cardiovascular event within 12 months prior to pre-qualification
- Recent (within 5 years of pre-qualification) systemically administered treatments for malignancy
- Known participation in a trial involving DGAT2i or ACCi, or previous administration with an investigational product, ≤30 days or 5 half-lives preceding the first dose of investigational product
- Any of the following diagnostic measurements, at both pre-qualification and first screening:
    - ALT <0.5x ULN or >5x ULN
    - AST >5x ULN
    - ALP >2x ULN
    - Total bilirubin >ULN and direct bilirubin >ULN
    - HbA1c >9%
    - Fasting plasma glucose >270 mg/dL
    - Fasting serum triglycerides >400 mg/dL
    - Platelet count <LLN
    - INR ≥1.3
    - Albumin <LLN
    - eGFR of <30 mL/min/1.73 m^2, using Cystatin-C and CKD-EPI equation
    - Positive urine test for illicit drugs
- Supine ECG QTc interval >480 msec or QRS interval >120 msec at pre-qualification and first screening
- Participants meeting criteria for contraindication to undergoing imaging assessments
- Investigator site staff or Pfizer employee directly involved in the conduct of the study, site staff otherwise supervised by the investigator, and their respective family members

A1AT, alpha-1-antitrypsin; ACCi, acetyl-coenzyme A carboxylase inhibitor; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUDIT, alcohol use disorders identification test; BMI, body mass index; CAP™, controlled attenuation parameter; CRN, clinical research network; DGAT2i; diacylglycerol acyltransferase 2 inhibitor; ECG, electrocardiogram; eGFR, enhanced glomerular filtration rate; FAST™, a derived score (using CAP™, VCTE™, and AST) to identify those with progressive NASH; HbA1C, glycated haemoglobin; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody; HIV, human immunodeficiency virus; HDL-C, high density lipoprotein-cholesterol; INR, international normalised ratio; LLN, lower limit of normal; MELD-Na,

model of end-stage liver disease including serum sodium, serum creatinine, total bilirubin and INR; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD Activity Score; NASH, nonalcoholic steatohepatitis; SVR, sustained virology response; ULN, upper limit of normal; VCTE™, vibration-controlled transient elastography.

Supplementary Table 3. Concomitant medications in MIRNA

Medication for glycaemic control

- Participants are permitted to be on stable doses of ≤3 agents for glycaemic control, for ≥12 weeks prior to first screening and until first on-site follow-up, across the country-specific approved classes of agents for glycaemic control. For example:
  - Biguanides
  - Dipeptidyl peptidase-IV inhibitors
  - Sodium-glucose cotransporter 2 inhibitors
  - Sulphonylureas
  - α-glucosidase inhibitors
  - Meglitinide analogues
- Those on thiazolidinediones/peroxisome proliferator-activated receptor gamma (e.g. pioglitazone) must be on a stable dose for ≥24 weeks before first screening
- Those on metformin at doses >1 g/day must decrease the dose by one-third or one-half starting at the run-in visit.[a]
  - Upward adjustment is permitted post-randomisation based on fasting plasma glucose
- Those on insulin must be on stable doses for ≥12 weeks before first screening
  - Short-term use of sliding scale insulin to manage glycaemic control during a concomitant acute medical condition is acceptable
- Those on glucagon-like peptide-1 receptor agonists must be on stable doses for ≥12 weeks before first screening

Lipid-modifying medications

- Participants are permitted to be on stable doses of ≤3 lipid-modifying oral agents, for ≥12 weeks prior to first screening and until the first on-site follow-up/week 50, across the country-specific, approved classes of agents including the following:
  - Those on selected statins which are BCRP substrates will only be permitted if on:
    - Rosuvastatin doses up to 10 mg/day
    - Atorvastatin doses up to 40 mg/day
    - Simvastatin or fluvastatin doses up to half-maximum in-country approved dose
  - Bile acid sequestrants such as cholestyramine, colestipol, as well as colesevalam
  - Fibric acid derivatives such as fenofibrate, bezafibrate, pemfibrate
  - Nicotinic acid/niacin
  - Ezetimibe
  - Participants on gemfibrozil at first screening are to be switched to another acceptable agent starting at the Run-In visit, with stable dose of the acceptable agent achieved for ≥6 weeks before day 1.

Medications for controlling blood pressure

- Participants are permitted to be on stable doses of ≤3 agents for blood pressure control, for ≥12 weeks prior to first screening and until the first on-site follow-up

Other acceptable concomitant medications

- Multi-vitamins are permitted, but vitamin E doses must be stable for ≥24 weeks before first screening

- Aspirin ≤325 mg/day
- Oral agents that alter gastric pH
- Inhaled and topical corticosteroids
    - Intercurrent treatment with systemic steroids may be permitted if treatment does not exceed 14 days
- Thyroid replacement therapy
- Postmenopausal hormone therapy
- Antipsychotic medications such as tricyclic agents, selective serotonin reuptake inhibitors, and serotonin/norepinephrine reuptake inhibitors
- Select supplements (herbal or approved agents) as a part of standard care to lower liver function markers: glutathione, glycyrrhizic acid, polyene phosphatidylcholine, silymarin, ursodeoxycholic acid
- Chronic and intermittent use of nonsteroidal anti-inflammatory drugs
- Intermittent use of acetaminophen/paracetamol at doses up to 2 g/day is acceptable.

Prohibited medications

- Use of drugs historically associated with fatty liver, taken within any interval lasting ≥4 weeks in the previous 12-months prior to first screening:
    - Amiodarone, methotrexate, systemic glucocorticoids (such as prednisone, dexamethasone, triamcinolone, budesonide, betamethasone), anabolic steroids, tetracyclines, tamoxifen, oestrogens at doses greater than those used for hormone replacement, valproic acid, other known hepatotoxins
- Use of the following medications ≤ 12 weeks prior to first screening, or likely to need these medications based on medical history at any time until first on-site follow-up:
    - Chronic use of immunosuppressants (e.g. cyclosporine and tacrolimus)
    - Agents with approved indication for weight loss (e.g. orlistat and sibutramin)
    - Over-the-counter appetite-stimulants or appetite-suppressants
- P-gp substrates with narrow therapeutic index (e.g. digoxin)
- Potent inducers and inhibitors CYP-3A
- CYP-2C9 substrates with narrow therapeutic index (e.g. warfarin or phenytoin)
- Blood thinners (e.g. apixaban, dabigatran, rivaroxaban, edoxaban, fondaparinux, heparin, and vitamin K antagonists [such as warfarin])
- Clinically significant OATP inhibitors (e.g. cyclosporine, gemfibrozil, rifampin)

[a]DGAT2i 300 mg BID was shown to increase metformin exposures approximately 2-fold (data on file).

BCRP, breast cancer resistant protein; BID, twice-daily; CYP, cytochrome P-450; DGAT2i, diacylglycerol acyltransferase 2 inhibitor; OATP, organic anion-transporting polypeptide; P-gp, P-glycoprotein.

Supplementary Table 4. Clinical laboratory tests performed in MIRNA

Haematology	Chemistry	Urinalysis	Other
• Haemoglobin • Haematocrit • Red blood cell count • Reticulocyte count (absolute) • Mean corpuscular volume • Mean corpuscular haemoglobin • Mean corpuscular haemoglobin concentration • Platelet count • White blood cell count • Total neutrophils (absolute) • Eosinophils (absolute) • Monocytes (absolute) • Basophils (absolute) • Lymphocytes (absolute)	• Blood urea nitrogen • Creatinine • Plasma glucose • Calcium • Sodium • Potassium • Chloride • Total carbon dioxide (bicarbonate) • Aspartate aminotransferase • Alanine aminotransferase • Alkaline phosphatase • γ-glutamyl transferase • Total bilirubin • Direct (conjugated) bilirubin • Indirect (unconjugated) bilirubin • Total bile acids • Creatine kinase • Uric acid • Albumin • Total protein	• pH • Glucose • Protein • Blood • Ketones • Nitrites • Leukocyte esterase • Urobilinogen • Urine bilirubin • Microscopy[a]	• Cystatin-C (and enhanced glomerular filtration rate using Chronic Kidney Disease-Epidemiology Collaboration equation-Cystatin-C) • Plasma activated partial thromboplastin time, prothrombin time, and international normalised ratio • Serum follicle-stimulating hormone[b] • Serum and urine pregnancy test • Urine drug test[c] • α1-antitrypsin[d] • Ceruloplasmin[d] • Serology:[d] hepatitis B surface antigen, hepatitis C virus antibody (and if positive, reflex hepatitis C virus ribonucleic acid), human immunodeficiency virus • % carbohydrate deficient transferrin relative to total transferrin[e] • Glycated haemoglobin • Fasting serum lipid panel[f] • Adiponectin
Additional exploratory biomarker assessments[g] include:			

- Serum apolipoprotein A1, B (total), B100, B48, C3, E and direct very low density lipoprotein
- Plasma insulin
- High-sensitivity C-reactive protein
- Cytokeratin-18-M30 fragment; cytokeratin-18-M65 fragment
- N-terminal propeptide of type III procollagen
- C-terminal fragment of α3 chain of procollagen type VI
- Plasma proprotein convertase subtilisin/kexin type 9
- Enhanced liver fibrosis test

[a]Only if urine dipstick is positive for blood, protein, nitrites, or leukocyte esterase

[b]In females, at pre-qualification, and first screening, only

[c]At pre-qualification, first screening, and baseline only; minimum requirement for urine drug test include cocaine, opiates/opioids, benzodiazepines, and amphetamines; this test not permitted to be repeated at scheduled visits.

[d]At pre-qualification and first screening only

[e]At pre-qualification, first screening, baseline, day 1, week 48, and when study intervention is prematurely stopped (with participant remaining in study or permanently withdrawn)

[f]Includes triglycerides, high density lipoprotein-cholesterol, direct low density lipoprotein-cholesterol, and total cholesterol

[g]At selected visits starting from baseline to first on-site follow-up

Supplementary Table 5. Summary of the probability of meeting decision criteria for drug/dose comparisons, in order to establish sample size

Arm	Dose group	Comparator	Δ	Analysis method	Criteria	Probability of meeting criteria	N evaluable per group
Placebo	Placebo	–	–	–	–		40
DGAT2i	25 mg BID	Placebo	24%	E_{max} DR modelling	≥95% certainty of ≥0% Δ vs placebo and ≥67% certainty of ≥24% Δ vs placebo	0.004[a]	40
	75 mg BID	Placebo	24%	E_{max} DR modelling	≥95% certainty of ≥0% Δ vs placebo and ≥67% certainty of ≥24% Δ vs placebo	0.626[a]	40
	150 mg BID	Placebo	24%	E_{max} DR modelling	≥95% certainty of ≥0% Δ vs placebo and ≥67% certainty of ≥24% Δ vs placebo	0.892[a]	40
	300 mg BID	Placebo	24%	E_{max} DR modelling	≥95% certainty of ≥0% Δ vs placebo and ≥67% certainty of ≥24% Δ vs placebo	0.945[a]	40
	150 mg QD	Placebo	24%	Pairwise/ER modelling	Power for 24% Δ vs placebo	0.75 (power)	40
	300 mg QD	Placebo	24%	Pairwise/ER modelling	Power for 24% Δ vs placebo	0.75 (power)	40
DGAT2i+ACCi	150 mg BID + 5 mg BID	150 mg BID	(3%)	Pairwise/linear DR modelling	≥75% certainty of ≥0% Δ vs 150 mg BID	0.67	40
	300 mg + 10 mg BID	300 mg BID	(6%)	Pairwise/linear DR modelling	≥75% certainty of ≥0% Δ vs 300 mg BID	0.82	40

[a]Assuming an E_{max} = 0.6.

DR, dose response; E_{max}, maximum effect of drug; ER, exposure–response.

References

1. Saunders JB, Aasland OG, Babor TF*, et al.* Development of the alcohol use disorders identification test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption-II. *Addiction* 1993;88:791–804.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Manuscript: 1 Protocol: 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Manuscript: 4, 7 Protocol: 1
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	Manuscript: 12 Protocol: 2
Funding	4	Sources and types of financial, material, and other support	Manuscript: 25
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	See Authors: manuscript: 1, 26
	5b	Name and contact information for the trial sponsor	Manuscript: 24 (corresponding author: 1)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Manuscript: 24
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Manuscript: 8, 15 Protocol: 88
Introduction			

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Manuscript: 5-7 Protocol: 17-19
	6b	Explanation for choice of comparators	Manuscript: 6-7 Protocol: 19, 25, 28
Objectives	7	Specific objectives or hypotheses	Manuscript: 7, 13-14 Protocol: 25-26
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Manuscript: 8-12, Figure 2 Protocol: 26-27
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Manuscript: 8 Protocol: 45
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Manuscript: 8-10, Supplementary Table 2 Protocol: 36-42, 60
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Manuscript: 10-11 Protocol: 31, 35-36, 43
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Manuscript: 14-15 Protocol: 55-56
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Manuscript: 10, 11 Protocol: 49-50
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Manuscript: 11-12, Supplementary Table 3 Protocol: 50

Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Manuscript: 13-14 Protocol: 25-26
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Manuscript: Figure 2, Supplementary Table 1 Protocol: 27
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Manuscript: 19 Protocol: 79
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Manuscript: 10 Protocol: 48-49
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Manuscript: 11 Protocol: 48-49
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Manuscript: 11 Protocol: 49
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Manuscript: 9-11, 14, 15 Protocol: 11, 49, 98

	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	Manuscript, 11 Protocol: 49
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	Manuscript: 9, 14-15, Figure 3 Protocol: 58-60
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Manuscript: 9, 13 Protocol: 55
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Manuscript: 22 Protocol: 92-93
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Manuscript: 19-21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Manuscript: 11, 15 Protocol: 59
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Manuscript: 20
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	Manuscript: 14-15 Protocol: 87-88

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Manuscript: 15 Protocol: 87
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Manuscript: 14-15, Suppl Table 1 Protocol: 63-72
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Manuscript: 21-22 Protocol: 93
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Manuscript: 20-21 Protocol: 89
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Manuscript: 20-21 Protocol: 90
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Manuscript: 20 Protocol: 90-91
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Manuscript: 21-22 Protocol: 91
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	-
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Manuscript: 22 Protocol: 91-92
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Manuscript: 22
	31b	Authorship eligibility guidelines and any intended use of professional writers	-
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Manuscript: 4, 22 Protocol: 92
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Manuscript: 16, Table 1, Suppl Table 1, Suppl Table 4

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.