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: o Biguanides o Dipeptidyl peptidase-IV inhibitors o Sodium-glucose cotransporter 2 inhibitors o Sulphonylureas o α-glucosidase inhibitors o 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] o 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 o 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: o 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 o Bile acid sequestrants such as cholestyramine, colestipol, as well as colesevalam o Fibric acid derivatives such as fenofibrate, bezafibrate, pemfibrate o Nicotinic acid/niacin o Ezetimibe o 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.