PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Amin, Neeta; Darekar, Amanda; Anstee, Quentin; Wong, Vincent; Tacke, Franck; Vourvahis, Manoli; Lee, Douglas; Charlton, Michael; Alkhouri, Naim; Nakajima, Atsushi; Yunis, Carla

VERSION 1 – REVIEW

REVIEWER	Billeter, Adrian University of Heidelberg
REVIEW RETURNED	30-Oct-2021

GENERAL COMMENTS	Well written study protocol. There are a lot of treatment arms making any firm conclusions difficult. However, this design allows to address one of the main study aims of finding the appropriate dose. One could argue whether a lifestyle intervention with the aim to loose weight would be a useful comparison. However, due to the high failure rate of lifestyle change for weight loss,

REVIEWER	Brown, Emily University of Liverpool Faculty of Health and Life Sciences, Department of cardiovascular and metabolic health
REVIEW RETURNED	14-Nov-2021

GENERAL COMMENTS	Comments to the Author 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. MIRNA is also supplemented by a concurrent, short-term 6-week dose finding study (NCT04399538) that aims to identify the lowest dose of DGAT2i that can mitigate the well reported ACCi-induced adverse effects on serum lipids. DGAT2i are in early clinical development and the authors discuss the limited research in this area. MIRNA is envisioned to add to the body of scientific evidence by assessing histological endpoints, such as NAFLD Activity Score (NAS), and liver fibrosis, and consider optimal dosing. Some of the study population participate in an imaging substudy to characterise effect on liver steatosis. Major comments

	1. I have some reservations about using a FAST score cut-off of ≥0.30 and not ≥0.35 to identify participants as per most validation studies. What is the rule-out (sensitivity) at this value? For the identification of patients with NASH≥4 and F3 the cut-offs would be even higher not lower. 2. The main deficit in the present protocol is the lack of a study arm treated with ACCi alone. Thus, it will not be possible to put together what the single intervention (ACCi or DGAT2i) does in comparison to the combination. 3. The choice of 48 weeks for measuring the change needs explanation. 4. Page 13, Line 15, “Some participants are enrolled in an imaging substudy…”. The protocol does not outline the numbers (and the selection) of patients planned to take part in the substudy. 5. The randomisation process needs to be more explicit on how the investigators will actually randomise patients (IVRS, IWRS, other ?), and whether the randomisation is adequately concealed. 6. The authors should specify in more detail who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts). The authors should also specify that unblinded members of the trial have no role in the follow-up or evaluation of patients in the study, and explain how this can be achieved. 7. Currently oversight of the study is only described in the protocol. Can Data Monitoring and Independent oversight of the trial be included in the main manuscript paper? 8. The study is evaluating drug effects on blood-based biomarkers. The authors should highlight that full blood count, specifically platelet counts, will be incorporated into this analysis. This is only mentioned in the supplementary tables. Minor comments 1. Formatting Amendments (where applicable): • Reference 23 needs updating when available.

REVIEWER	Liesa, Marc David Geffen School of Medicine at UCLA
REVIEW RETURNED	22-Nov-2021

GENERAL COMMENTS	The protocol aims to define the efficacy of ACCi and DGAT2i in humans with NASH in a phase II randomized study. It is well written and outcomes are defined. A concern about study is the absence of NEFA measurements in the plasma or liver homogenates. As the authors discuss, in some preclinical models, DGAT2 inhibition can lead to fibrosis and this could be expected to be mediated by NEFA. It is a possibility that some of the non responders could be done explained by an excessive increase in NEFA. This should be discussed.

VERSION 1 – AUTHOR RESPONSE

Response to reviewers' comments

Reviewers' comments to the authors:	Response/amends
Reviewer 1	
Well written study protocol. There are a lot of treatment arms making any firm conclusions difficult. However, this design allows to address one of the main study aims of finding the appropriate dose. One could argue whether a lifestyle intervention with the aim to loose weight would be a useful comparison. However, due to the high failure rate of lifestyle change for weight loss,	We thank the reviewer for their favourable feedback and taking the time to review our manuscript. We agree that the high failure rate of lifestyle change alone makes this impractical as a comparator arm. Instead, diet/lifestyle guidelines are advocated across all arms/participants to permit evaluation of the study drugs on top of basic guidelines appropriate for the concomitant medical conditions in this population.
Reviewer 2	
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. MIRNA is also supplemented by a concurrent, short-term 6-week dose finding study (NCT04399538) that aims to identify the lowest dose of DGAT2i that can mitigate the well reported ACCi-induced adverse effects on serum lipids. DGAT2i are in early clinical development and the authors discuss the limited research in this area. MIRNA is envisioned to add to the body of scientific evidence by assessing histological endpoints, such as NAFLD Activity Score (NAS), and liver fibrosis, and consider optimal dosing. Some of the study population participate in an	We thank the reviewer for taking the time to review our manuscript and respond to their individual comments below.

Reviewers' comments to the authors:	Response/amends
imaging substudy to characterise effect on liver steatosis.	
Major comments 1. I have some reservations about using a FAST score cut-off of ≥0.30 and not ≥0.35 to identify participants as per most validation studies. What is the rule-out (sensitivity) at this value? For the identification of patients with NASH≥4 and F3 the cut-offs would be even higher not lower.	Based on validation studies (Newsome PN et al. Lancet Gastroenterol Hepatol 2020;5:362-73), the lower cut-off would be expected to result in a higher screen fail rate, but with a lower missed case rate (fewer false negatives) for detecting participants with NASH + NAFLD activity score ≥4 + fibrosis grade ≥2. Using the data from the validation studies, FAST score ≥0.30 offers a missed case rate of 9.7% (vs a missed case rate of 12.1% with FAST score ≥0.35) and a screen fail rate of 49.5% (vs a screen fail rate of 46.0% with FAST score ≥0.35). The slightly lower cut-off of FAST score ≥0.30 is used in MIRNA as this threshold needs to be met twice, once at the pre-qualification and again at the first screening visits, before confirmation of eligibility by liver biopsy.
2. The main deficit in the present protocol is the lack of a study arm treated with ACCi alone. Thus, it will not be possible to put together what the single intervention (ACCi or DGAT2i) does in comparison to the combination.	The results of a phase IIa dose-ranging study (NCT03248882) of ACCi PF-05221304 administered alone for 16 weeks in participants with NAFLD, including a subpopulation with diagnosed or presumed NASH, were recently published in Nature Medicine (Calle RA et al. Nat Med 2021;27:1836-48). ACCi treatment reduced liver steatosis and improved NASH-related biomarkers. However, asymptomatic elevations in serum triglycerides at higher doses raise questions over the long-term use of this class as a monotherapy, while coadministration of ACCi and DGAT2i induced beneficial effects on steatosis and mitigated the limiting effects of ACCi monotherapy. The current study assesses the longer-term effects of ACCi and DGAT2i coadministration on NASH resolution over 48 weeks to permit an assessment of histological endpoints. Dosing with ACCi monotherapy for this duration was deemed unethical given the risk demonstrated with 16 weeks of dosing.
3. The choice of 48 weeks for measuring the change needs explanation.	The duration of dosing with study interventions is proposed as 48 weeks, in line with multiple regulatory guidance documents (including The Center for Drug Evaluation and Research) for agents in development for the treatment of NASH with liver fibrosis. This detail has now been included in the revised manuscript on page 11 as follows: 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]

Reviewers' comments to the authors:	Response/amends
4. Page 13, Line 15, "Some participants are enrolled in an imaging substudy…". The protocol does not outline the numbers (and the selection) of patients planned to take part in the substudy.	It is planned that approximately half of study participants will be enrolled in the imaging substudy, which is being conducted in sites in North America. This was noted in the header to this section and has now been clarified on page 11 of the revised manuscript as follows: Approximately ~~Half~~ half of the total sample size are participating in an imaging substudy to characterise the effect on liver steatosis and liver volume over time ~~Some~~ Approximately 50% of participants are ~~enrolled~~ forecast to be enrolled in an imaging substudy…
5. The randomisation process needs to be more explicit on how the investigators will actually randomise patients (IVRS, IWRS, other ?), and whether the randomisation is adequately concealed.	This has now been clarified in the revised manuscript on page 11 as follows: 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.
6. The authors should specify in more detail who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts). The authors should also specify that unblinded members of the trial have no role in the follow-up or evaluation of patients in the study, and explain how this can be achieved.	This information is provided in the revised manuscript as follows: Page 9, 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. Page 11, Participants are randomly allocated to treatment groups by blinded investigators using an interactive response technology system (interactive web response) programmed with instructions for breaking the blind 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] Page 11, Participants and all persons involved in trial conduct, participant interactions and data analysis are blinded to treatment assignment. Page 12, 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. Page 14, 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. Page 15, In addition, an independent adjudication committee consisting of external experts will perform blinded review of all potential fatal events,

Reviewers' comments to the authors:	Response/amends
	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. Page 15, MRI-PDFF image analyses are performed by ~~a central~~ a blinded external vendor~~, 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.
7. Currently oversight of the study is only described in the protocol. Can Data Monitoring and Independent oversight of the trial be included in the main manuscript paper?	A statement on the data monitoring and independent adjudication committees is now included in the revised manuscript on page 15 as follows: 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.
8. The study is evaluating drug effects on blood-based biomarkers. The authors should highlight that full blood count, specifically platelet counts, will be incorporated into this analysis. This is only mentioned in the supplementary tables.	This has now been clarified in the revised manuscript on page 14 as follows: ~~safety~~ 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 ~~up~~ to at least Week ~~48~~ 50.
Minor comments 1. Formatting Amendments (where applicable): • Reference 23 needs updating when available.	Reference 23 has now been updated with the published citation: Calle RA, Amin NB, Carvajal-Gonzalez S, et al. ACC inhibitor alone or co-administered with a DGAT2 inhibitor in patients with non-alcoholic fatty liver disease: two parallel, placebo-controlled, randomized phase 2a trials. Nat Med 2021;27:1836-48.
Reviewer 3	

Reviewers' comments to the authors:	Response/amends
The protocol aims to define the efficacy of ACCi and DGAT2i in humans with NASH in a phase II randomized study. It is well written and outcomes are defined.	We thank the reviewer for their useful feedback.
A concern about study is the absence of NEFA measurements in the plasma or liver homogenates. As the authors discuss, in some preclinical models, DGAT2 inhibition can lead to fibrosis and this could be expected to be mediated by NEFA. It is a possibility that some of the non responders could be done explained by an excessive increase in NEFA. This should be discussed.	Data from one specific study of DGAT2 inhibition by antisense oligonucleotide in a murine model showed reduced steatosis with an increase in liver fibrosis that has not been replicated by us or others. While the reason for this is not clear, one could speculate that this could be related to the use of the methionine- and choline-deficient diet, which produces more severe liver damage (including hepatic fibrosis) than a Western diet (Machado MV et al. PLoS One 2015;10:e0127991), or to the use of antisense oligonucleotides, which themselves can cause hepatotoxicity (Kamola PJ et al. Mol Ther Nucl Acids 2017;8:383-94). It is possible that increased NEFAs may lead to increased fibrosis; however, endpoints in the current study include a collection of exploratory blood-based NASH-related biomarkers, including the three-parameter-derived enhanced liver fibrosis™ score, N-terminal propeptide of procollagen type III and the C-terminal fragment of the α3 chain of procollagen type VI (markers of fibrinogenesis and fibrinolysis, respectively). Unpublished data from studies recently reported in Calle RA et al. Nat Med 2021;27:1836-48 showed no change in fasting (4 hours) NEFAs at Day 17 of dosing in Western-diet fed rats with DGAT2i alone or in combination with ACCi. This 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 (Chitraju C et al. Cell Metab 2017;26:407-18.e3). In this study, inhibition of DGAT1, but not DGAT2, led to an increase in free fatty acids. Therefore, we would not expect to see changes in plasma NEFA in the current clinical study. Pending results from this study, exploratory work to understand the reason for failure (if indeed encountered) via highly specialised mechanistic studies specifically designed to dissect this, can be determined in due course. The Discussion on page 23 has been revised to address this point as follows: 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

Reviewers' comments to the authors:	Response/amends
	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]

VERSION 2 – REVIEW

REVIEWER	Brown, Emily University of Liverpool Faculty of Health and Life Sciences, Department of cardiovascular and metabolic health
REVIEW RETURNED	21-Feb-2022

GENERAL COMMENTS	All of my concerns have been addressed. No further comments