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Effect of phospholipid curcumin Meriva® on liver histology and kidney disease in nonalcoholic steatohepatitis a randomized, double-blind, placebo-controlled trial

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Effect of phospholipid curcumin Meriva® on liver histology and kidney disease in Nonalcoholic Steatohepatitis A randomized, double-blind, placebo-controlled trial

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SCHOLARONE™
Manuscripts

Phospholipid curcumin Meriva® for Nonalcoholic Steatohepatitis

Biopsy - proven NASH (n=52)

Placebo

n=26

Meriva®

(phospholipid curcumin)

2 g/die, n=26

72 weeks

N=3 (12%)	NASH resolution (p=0.003)	N=16 (62%)
N=2 (8%)	Fibrosis improvement by ≥1 stage (p=0.008)	N=13 (50%)
N=0 (0%)	F≥2 stage fibrosis improvement (p=0.02)	N=11 (42%)
N=0 (0%)	Chronic kidney disease regression (p=0.004)	N=13 (50%)

Meriva is safe, well-tolerated and improves liver and kidney health

Effect of phospholipid curcumin Meriva® on liver histology and kidney disease in Nonalcoholic Steatohepatitis

A randomized, double-blind, placebo-controlled trial

RUNNING TITLE: phospholipid curcumin Meriva® for NASH

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Author contribution

Giovanni Musso: designed the trial, acquired and analyzed data, drafted the work, approved the final version, agreed to be accountable for all aspects of the work

Silvia Pinach: gave substantial contributions to the study design and data acquisition, revised the work critically for important intellectual content; approved the final version to be published; agreed to be accountable for all aspects of the work.

Filippo Mariano: gave substantial contributions to the study design and data acquisition, revised the work critically for important intellectual content; approved the final version to be published; agreed to be accountable for all aspects of the work

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KEY WORDS: steatohepatitis, histology, chronic kidney disease, NF-kB, Phytosome, Meriva

Abstract

Background & Aims. Nonalcoholic steatohepatitis (NASH) confers an increased liver-related and kidney morbidity. Phospholipid curcumin (Meriva®) is a phospholipid formulation with ameliorated systemic curcumin absorption and delivery. We assessed safety and efficacy of Meriva® in NASH.

Approach. In this double-blind trial, 52 biopsy-proven NASH patients(71% with stage ≥F2 fibrosis, 58% with stage A2-G2/A2-G3a CKD) were randomized 1:1 to receive Meriva®2 g/day or placebo for 72 weeks.

Primary end-point was NASH resolution with no worsening of fibrosis. Secondary end-points included: a ≥1 stage liver fibrosis improvement with no NASH worsening; regression of significant(i.e. stage≥F2) fibrosis and of chronic kidney disease(CKD); improvement in renal, glucose, lipid and inflammatory parameters. We also explored treatment effect on hepatic activation of Nuclear Factor(NF)-kB, a key proinflammatory transcription factor and a major target of curcumin.

Results

Fifty-one patients(26 on Meriva® and 25 on placebo) completed the trial.

Sixteen(62%) patients on Meriva® vs. three(12%) patients on placebo had NASH resolution(RR=5.33[95%CI=1.76–12.13]; p=0.003).

Thirteen(50%) patients on Meriva® vs. 2(8%) patients on placebo had ≥1 stage fibrosis improvement(RR=6.50[(1.63-21.20]; p=0.008).

Eleven(42%) patients on Meriva® vs. 0(0%) on placebo had regression of significant liver fibrosis(RR=18.01[1.43-36.07]; p=0.02). Hepatic NF-kB inhibition predicted NASH resolution(AUC=0.90,95%CI=0.84-0.95) and fibrosis improvement(AUC=0.89,95%CI=0.82-0.96).

Thirteen(50%) patients on Meriva® vs. 0(0%) on placebo had CKD regression(RR=10.71[1.94-17.99)]; p=0.004).

Compared with placebo, Meriva® improved eGFR(difference in adjusted eGFR change: +3.59[2.96-4.11] ml/min/1.73 m^2/year, p=0.009), fasting glucose(-17 mg/dL;95%CI=-22, -12), HbA1c(-0.62%;95%CI=-0.87%, -0.37%), LDL-C(-39 mg/dL; 95%CI= -45, -33), triglycerides(-36 mg/dL, 95%CI= -46, -26), HDL-C(+10 mg/dL; 95%CI=+8, +11) and inflammatory markers.

Adverse events were rare, mild and evenly distributed.

Conclusion. In NASH patients, Meriva® administration for 72 weeks was safe, well-tolerated, improved liver histology, possibly through NF-kB inhibition, kidney disease, and metabolic profile.

Trial registration ID: ISRCTN27793187.

Keywords: steatohepatitis, histology, chronic kidney disease, NF-κB, Phytosome, Meriva

INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is a common cause of chronic liver disease, characterized by hepatic fat accumulation (steatosis), hepatocyte injury, and inflammation. NASH can be considered a multisystem disease and is associated with fibrosis, cirrhosis, and an increased risk of hepatocellular carcinoma, cardiovascular disease, chronic kidney disease, and death[1,2,3,4].

NASH is the most rapidly growing indication for liver and simultaneous liver-kidney transplantation[5,6]. There is no approved pharmacological treatment for NASH.

Curcumin is a natural lipophilic polyphenol with prominent antioxidant, anti-inflammatory, and antifibrotic properties, which targets multiple molecular pathways involved in NASH pathogenesis and progression , including the key pro-inflammatory transcription factor Nuclear Factor-kappa B (NF-kB)[7,8,9].

Dietary curcumin supplementation ameliorated NASH in preclinical models[8,10,11] and improved surrogate markers of liver disease and of metabolic risk in NAFLD patients[12].

Meriva® is a patented formulation of *Curcuma longa* extract with phospholipids leveraging Phytosome™ technology which greatly improved oral bioavailability of curcumin metabolites[13], allowing greater systemic concentration and delivery to target organs, including the liver, kidney and adipose tissue[14,15,16,17,18].

We evaluated the safety and efficacy of Meriva on liver histology and on renal and metabolic parameters in biopsy-proven NASH patients.

METHODS

Trial design and oversight

This phase 2 randomized double blind, placebo-controlled trial was conducted at two medical Centres (HUMANITAS Gradenigo, Turin, Italy, and Città della Salute e della Scienza, Turin, Italy). between Dec 21st, 2021, and Nov 26th, 2023. The trial was conducted in accordance with the principles of both the Declarations of Helsinki and Istanbul, was funded by Indena Spa, Milan, was

approved by local Ethics Committee and is registered with ISRCTN clinical trial registry (study ID: ISRCTN27793187). All subjects provided written informed consent to participate. All authors had access to the study data and reviewed and approved the final manuscript. Details on study design and full protocol are provided in **Appendix 1.**

Population

The trial entry criteria were based: on age 18-70 years, on exclusion of competing causes of liver disease and on a diagnosis of 'definite' NASH on a centrally read liver biopsy obtained at screening or within the preceding 6 months (in the latter case, participants had to have a stable body weight, defined as no more than a 5% loss of initial body weight between the time of biopsy and screening, and not to take any medications reported to be inducers or potential therapies for NASH). Additional eligibility, inclusion and exclusion criteria are detailed in **Appendix 1**.

Randomization and Masking

Participants were randomly assigned in a 1:1 ratio to receive 2000 mg daily of phospholipid curcuminoids (from now on: "Meriva") or placebo for 72 weeks

Meriva dose was selected based on the highest dose of this curcumin formulation used in previous trials[14,15,16,17,18] (see **Appendix 1**), and treatment duration was chosen following current recommendations of regulatory agencies for development of NASH pharmacological agents[19,20].

Randomization was computer-generated and stratified according to diabetes status (present/absent), liver fibrosis stage (F0-2 vs. F3-4), CKD status (present/absent), obesity status (present/absent). Trial participants were allocated a unique trial identification number to preserve patient confidentiality and allocation concealment. Participants, investigators, trial site staff, and the histopathologist remained blinded to treatment assignment throughout the trial. All screening, baseline and EOT liver biopsies were assessed centrally by one liver histopathologist (RP) at

Humanitas Gradenigo, blinded to study treatment allocation, clinical or laboratory information, and each other's assessments.

Placebo tablets and active comparator were identically appearing, colorless, tasteless tablets packaged and administered in an identical manner, of identical composition, except for the active substance (phospholipid curcumin).

All participants received standard recommendations on lifestyle modifications, including exercise, weight reduction, and dietary modification (see **Appendix 1**).

Procedures

The study comprised four stages and 9 visits: (1) screening, enrollment, randomization and baseline investigations (visits 1-2, over a maximum period of 2 weeks), (2) Meriva/placebo treatment (visits 3–7, over 72 weeks), (3) end-of-treatment (EOT) primary endpoint assessment including liver biopsy (visit 8) and (4) post-treatment follow-up assessment (visit 9, 12 weeks after EOT).

The complete trial schedule is summarized in **Appendix 2 Figure 1.**

Outcomes

Primary outcome was histological NASH resolution (defined by the NASH Clinical Research Network as no more than mild residual inflammatory cells [score of 0 or 1] and no hepatocyte ballooning [score of 0]), **and** no worsening of liver fibrosis (with worsening defined as an increase of at least one stage on the Kleiner fibrosis classification scale) after 72 weeks (18 months), in line with regulatory perspectives[19,20.21].

Secondary liver histological outcomes were:

1) a ≥1 stage improvement in fibrosis and no worsening of NASH after 72 weeks, with worsening defined as an increase of ≥1 point in either the lobular inflammation score or the hepatocyte ballooning score according to the NASH Clinical Research Network criteria.

2) NASH resolution and a ≥1 stage improvement in liver fibrosis

3) regression of clinically significant (i.e. stage≥2) liver fibrosis[1,22], defined by the proportion of subjects with baseline fibrosis stage 2-4 who had fibrosis stage 0-1 at EOT, without worsening of NASH.

4) regression of advanced (i.e. stage≥3) fibrosis, defined by the proportion of subjects with baseline fibrosis stage 3-4 who have fibrosis stage 0-2 at EOT, without worsening of NASH.

5) a ≥2 point improvement in NAFLD activity score (NAS), with at least 1 point improvement in either ballooning or lobular inflammation score;

6) improvement in individual components of the NAFLD activity score (steatosis, hepatocyte ballooning, lobular inflammation).

Secondary liver-centered non-histological outcomes included: changes in serum liver enzymes, changes in noninvasive biomarkers of liver disease severity (computerized sonographic hepato-renal ratio for steatosis[23,**24**] , serum cytokeratin-18 fragments for NASH, FIB-4 Index, APRI and serum lumican[25)](see **Appendix 1 page 17-18**).

Secondary non liver-centered outcomes included:

Chronic kidney disease (CKD) regression. CKD was defined according to Kidney Disease Improving Global Outcomes (KDIGO) guidelines[26] as sustained (i.e., documented on 2 occasions 3 month apart) reduction of eGFR (i.e. <90 mL/min/1.73 m^2, assessed from serum creatinine using the CKD-EPI equation) **and/or** increased urinary albumin excretion rate (i.e., AER≥30 mg/d)]. CKD regression was defined as sustained (i.e., documented 3 month apart, on both follow-up visit 7 and visit 8) normalization of eGFR (i.e. ≥90 mL/min/1.73 $m^{2)}$ **and** of urinary AER (i.e., <30 mg/d)].

- change in absolute values and in KDIGO categories of eGFR and of AER

- difference between groups in adjusted mean annual rates of change in eGFR from baseline (total slope)[27](detailed in Statistics section);

- changes in fasting lipids, glycemic control (fasting plasma glucose, HbA1c), insulin resistance (homeostasis model of assessment of insulin resistance [HOMA-IR], anthropometry (body weight, BMI, waist circumference), serum inflammatory markers, daily dietary intake and physical activity.

Exploratory outcome was the effect of treatment on NF-kB activation in liver cells (**Appendix 1 page 19-21**). NF-kB is a key proinflammatory transcription factor involved in NASH development and progression and a known molecular target of curcumin

Safety outcomes included adverse events after the start of treatment, biochemical assessments, and clinical assessments from baseline until follow-up visit 9.

A complete list of trial outcomes is provided in **Appendix 1**.

Statistical analysis

Based on previous trials showing an improvement in surrogate markers of NAFLD (i.e., transaminase normalization or ultrasonographic steatosis regression) with curcumin in up to 50% of subjects , vs. 15% with lifestyle intervention[28], allowing for a 10% dropout rate, with a significance level of 0.05 (type I error) and power of 0.90 (type II error) [29], the design required at least 25 subjects per arm to detect a significant difference in the primary outcome**.**

All eligible patients were analyzed on an intention-to-treat basis. All individuals who had undergone randomization received at least one dose of the assigned regimen, and no subject was excluded from the full analysis population.

The primary analysis used a Cochran–Mantel–Haenszel test based on all randomized participants for the in-trial period with subjects with missing data considered as non-responders.

The common risk ratio (RR) between Meriva and placebo was estimated along with exact 95% CI.

A sensitivity analysis, in which missing data were alternatively handled by multiple

imputation from the placebo group, was performed (detailed in **Appendix 2**). Adjustments for multiplicity testing in the primary analysis were performed with the Benjamini-Hochberg procedure.

Two-sided P values <0.05 were considered statistically significant.

An unpowered, preplanned secondary analysis of categorical outcome measures was done using the $\chi 2$ test or Fisher exact test, as appropriate.

A preplanned logistic regression analysis was undertaken to determine the treatment effect when adjusted for stratification variables (diabetes status, obesity status, fibrosis stage, CKD status) and trial site. Adjusted relative risks were calculated using the Mantel-Haenszel test.

Continuous secondary outcome measures were compared between groups using linear regression, adjusting for baseline values and allocated treatment (as model covariates, equivalent to ANCOVA), with multilevel modelling for key continuous outcome measures to account for repeated measures within each patient.

For eGFR, we calculated the difference between treatment groups in the slope of annual eGFR change, adjusted for variables *a priori* known to be associated with CKD progression (age, sex, baseline CKD stage, baseline eGFR, baseline urinary AER, baseline liver fibrosis stage, diabetes, history of cardiovascular disease, systolic blood pressure, angiotensin-converting enzyme/angiotensin receptor blocker use, HbA1c changes[27] (**Appendix 2**).

We also explored the existence of a threshold in hepatic NF-kB activation change predicting histological response (NASH resolution, fibrosis improvement by ≥1 stage) using the area under receiver operating characteristic curve (AUROC) analysis and Youden index.

The following subgroup analyses were planned to assess the effect of patient characteristics on main efficacy and safety outcomes:

- trial site
- diabetes status, obesity status, CKD status, fibrosis stage (F3-4 vs F0-2)

- per protocol analysis (only subjectswho completed treatment, EOT liver biopsy and no major protocol deviations were included).

All analyses were carried out with Easy R ver1.61 Software, Saitama, Japan[30].

RESULTS

Population

Participants flow-chart through the study is reported in **Figure 1**. Between Dec 21st, 2021, and Nov 26th, 2023, 52 subjects with histologically confirmed definite NASH were randomized to receive Meriva 2g/day (n=26) or placebo (n=26). Fifty-one patients received assigned treatment and had post-treatment liver biopsy and completed the trial: one patient in the placebo group withdrew from the study at week 69 and missed the post-treatment biopsy.

Baseline demographic, clinical, laboratory, and histological features were similar in the two groups (**Table 1**): 36 (69%) participants were obese, 25 (48%) had type 2 diabetes mellitus (T2DM), 37 (71%) had clinically significant fibrosis [21 (40%) had stage 2 fibrosis, 13 (25%) had stage 3 fibrosis, 3 (6%) had stage 4 fibrosis (cirrhosis)]. Mean NAFLD activity score (NAS) was 5.6 (±0.8). Thirty-two (62%) participants had CKD; 22 (42%) had stage A2 G2 CKD, 10 (19%) had stage A2 G3a CKD.

Efficacy: liver outcomes

Sixteen (61%) of the 26 patients in the Meriva group vs. 3 (12%) patients in the placebo group had NASH resolution with no worsening of fibrosis (relative risk [RR] 5.33[95%CI 1.76–12.13, p=0·003], thereby meeting the primary end-point (**Table 2**).

Thirteen (50%) of the 26 subjects in the Meriva group vs. 2(8%) in the placebo group had ≥1 stage fibrosis improvement with no worsening of NASH (RR 6.50, 95%CI: 1.63–21.20, p=0·008).

Thirteen (50%) of the 26 subjects in the Meriva group vs. 1 (4%) in the placebo group had NASH resolution and a ≥1 stage fibrosis improvement (RR 7.31, 95%CI: 1.95–23.12, p=0·004).

Eleven (42%) of the 26 subjects in the Meriva group vs. 0 (0%) in the placebo group had regression of clinically significant (i.e. stage F2-4) fibrosis (RR 18.01, 95%CI: 1.33–36.07, p=0.02).

Five (19%) subjects in the Meriva group vs. 0 (00%) in the placebo group had regression of advanced (stage 3-4) fibrosis (p=0.10, **Table 2**), of whom one subject had cirrhosis regression (**Appendix 2 Table 2**); 0 in the Meriva group vs 4 (16%) in the placebo group had worsening of fibrosis (p=0.19), of whom 2 patients had progression to cirrhosis (stage F4 fibrosis) (**Table 2, Appendix 2 Table 2**). Three (12%) subjects in the Meriva group vs. 0 in the placebo arm had a 2-stage fibrosis improvement (**Appendix 2 Table 2**).

The effect of treatment on individual histological features is detailed in **Table 2** and in **Appendix 2 Table 2.**

The observed changes in liver histology were consistent with changes in liver enzymes and in noninvasive biomarkers of liver disease severity **(Figure 2-3, Table 2)** and were related to the extent of hepatic NF-kB inhibition (**Figure 4A, Appendix 2 Figure 2**): remarkably, we could identify a threshold in NF-kB inhibition (≥20% of liver cells) predicting NASH resolution and fibrosis improvement (**Figure 4B-F**).

Efficacy: renal and metabolic outcomes

Thirteen subjects who received Meriva vs. 0 receiving placebo has regression of CKD (RR 10.71, 95%CI: 1.94–17.99, p=0·004) (**Table 3**). Subjects who received Meriva had also significant improvements in the slope of adjusted annual eGFR change (**Figure 2C**), AER stage, plasma lipids, glycemic parameters (fasting plasma glucose, HbA1c, HOMA-IR), and inflammatory markers (**Figure 2D-G, Table 3**).

We found no significant difference in dietary habits, physical activity, body weight or blood pressure between the two groups at **EOT** (**Table 3, Appendix 2 Table 4**).

Logistic regression analyses

When adjusting for baseline stratification factors and trial site in a multivariate logistic regression analysis, there was still a significant treatment effect for key histological end-points and for CKD (**Appendix 2 Table 5**). Notably, univariate analysis suggests that subjects with CKD at baseline are less likely to improve liver fibrosis in response to Meriva (the odds of response are less), and, mutually, that subjects with worse fibrosis were less likely to normalize renal function following treatment..

Sensitivity and subgroup analyses

A predefined sensitivity analysis of the primary and relevant secondary efficacy end-points in which multiple imputation was used to account for missing data (up to 16% of participants in the Meriva group and 12% in the placebo group) found similar results to the primary analysis (**Appendix 2 Table 6**)

Similarly, we found no evidence of heterogeneity ($p>0.634$) for primary and secondary outcomes when stratifying study population according to T2DM, obesity, CKD, fibrosis stage and trial site (**Appendix 2 Table 7**).

Safety

All 52 participants were included in the safety analysis. Overall, adverse events were rare (occurring in 12% of subjects in the Meriva group and in 19% in the placebo group), mild (grade 1), transient, mainly gastrointestinal, with similar incidence in the two groups for all organ classes and symptoms, and did not determine discontinuation (**Table 4**).

DISCUSSION

In the first study assessing the effect of curcumin on liver histology in NASH, we found:

1) Compared with placebo, Meriva 2 g/day for 72 weeks induced NASH resolution and improved overall fibrosis stage and clinically significant (i.e. stage ≥2) fibrosis;

2) Meriva was safe, well-tolerated, reversed early CKD, improved eGFR, glucose and lipid parameters;
3) the observed histological benefits were associated with a downregulation of hepatic NF-kB, a key proinflammatory transcription factor and a known curcumin target, thereby providing a mechanistic basis for the observed clinical benefits

NASH is a common disease associated with increased morbidity and mortality; while lifestyle modifications are not achieved or maintained by most NASH patients, side effects and long-term safety of experimental pharmacological therapies are a concern[31 ,32, 33].

We found Meriva, a formulation of *Curcuma longa* extract with phospholipids leveraging Phytosome™ technology that ameliorates systemic bioabsorption , is associated with NASH resolution and an improvement in fibrosis, the latter involving subjects with fibrosis ≥F2, i.e. the subgroup of NASH patients with a higher risk of liver-related morbidity and mortality[1].

Meriva improved also glucose, lipid and inflammatory parameters, which may be clinically useful as cardio-metabolic disorders are common in these population. Among non-liver related conditions, the relevance of CKD is being increasingly recognized, as NASH is becoming a leading indication for combined liver-kidney transplantation[5]. Remarkably, Meriva normalized renal function and reversed microalbuminuria; furthermore, while the rate of eGFR declined in the placebo group was consistent with available literature[2,34], Meriva supplementation ameliorated eGFR trajectory throughout the trial duration, as reported above. Growing evidence suggests NAFLD and CKD share numerous pathogenic mechanisms[35,36] : Meriva reduced circulating proinflammatory cytokines and chemokines and reshaped gut microbiota toward a healthy phenotype in patients with CKD[37] or temporary kidney dysfunction[38].

We also investigated potential mechanisms underlying the observed clinical benefits: the key proinflammatory and profibrogenic nuclear transcription factor NF-κB is being increasingly implicated in human[39] and experimental CKD and may thus mediate liver and kidney injury in NASH. Since NF-kB pathway activation is a promoter of hepatocellular carcinoma (HCC)[40], and

curcumin exhibits high activity against this and other different pathways involved in hepatocarcinogenesis[41,42,43], future research may explore the nephroprotective and chemo-preventive potential of novel curcumin formulations in NASH[44,45].

The thorough hepatic histological and renal assessment and the demonstration of a plausible molecular mechanism mediating the effects of curcumin are considerable strengths, while the small sample size is a limitation of this study. Long-term safety of curcumin pharmacological formulations will also have to be evaluated: currently, curcuminoids have been rated as safe as food additives by the US Food and Drug Administration (FDA)[46], the Joint United Nations and World Health Organization Expert Committee on Food Additives(JECFA)[47] and the European Food Safety Authority(EFSA) for a daily curcumin intake of up to 3 mg/kg[48], and good tolerability and safety profiles for base and previous reported bioavailable curcuminoids formulations have been shown in varied unhealthy conditions[49,50,51, 52,53].

Putting the results of this trial into clinical context, this study supports Meriva as a highly safe and well tolerated therapeutic tool to improve liver and renal disease in NASH; from a research standpoint, it justifies ongoing research efforts to ameliorate curcumin bioavailability and delivery to the liver and prompts evaluation of anti-fibrotic and chemo-preventive potential curcumin in NASH with advanced fibrosis/cirrhosis.

LEGENDS TO FIGURES

Figure 1. CONSORT diagram of patient flow through the study.

Figure 2: time course of changes in liver enzymes, metabolic and renal parameters according to each treatment group.

Panel A: serum ALT

Panel B: serum GGT

Panel C: eGFR

Panel D: plasma Triglyceride

Panel E: plasma LDL-C

Panel F: plasma HDL-C

Panel G: HbA1c(%)

For eGFR, we calculated the difference between treatment groups in the slope of annual eGFR change, adjusted for variables *a priori* known to be associated with CKD progression (age, sex, baseline CKD stage, baseline eGFR, baseline urinary AER, baseline liver fibrosis stage, diabetes, history of cardiovascular disease, systolic blood pressure, angiotensin-converting enzyme/angiotensin receptor blocker use, HbA1c changes.

Figure 3: correlation between computerized sonographic hepato/renal ratio and hepatic steatosis (expressed as % hepatocytes) on liver histology in the whole study population with available pre-post-supplementation liver biopsy (n= 51 subjects, 26 paired liver biopsies in the Meriva arm and 25 paired liver biopsies in placebo arm).

Figure 4: relationship between treatment, changes in hepatic Nuclear Factor (NF)-kB activation (expressed as percentage positive staining cells/high-power field), and changes in liver histology.

† $p<0.001$ vs baseline ‡ $p<0.001$ vs placebo.

Panel A: changes in hepatic NF-kB activation by each arm.

Panel B: correlation between changes in hepatic NF-kB activation and changes in NAFLD activity score (NAS) in the whole study population (n=51).

Panel C: correlation between changes in hepatic NF-kB activation and changes in Fibrosis stage in the whole study population (n=51).

Panel D: predictive performance of changes in hepatic NF-kB activation (% cells/HPF from baseline) for NASH resolution in the whole study population (n=51).

Panel E: predictive ability of changes in hepatic NF-kB activation (% cells/HPF from baseline) for ≥1 stage fibrosis stage improvement in the whole study population (n=51).

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Data sharing statement. Deidentified participant data are available with publication to anyone upon reasonable request to corresponding author.

REFERENCES

[1]Rinella ME, Neuschwander-Tetri BA, et al. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology. 2023;77:1797-1835

[2]Musso G, Gambino R, Tabibian JH, et al. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. PLoS Med. 2014;11:e1001680

[3]Sun DQ, Targher G, Byrne CD, et al. An international Delphi consensus statement on metabolic dysfunction-associated fatty liver disease and risk of chronic kidney disease. Hepatobiliary Surg Nutr. 2023;12:386-403

[4]de Avila L, Henry L, Paik JM, Ijaz N, Weinstein AA, Younossi ZM. Nonalcoholic Fatty Liver Disease Is Independently Associated With Higher All-Cause and Cause-Specific Mortality. Clin Gastroenterol Hepatol. 2023 ;21:2588-2596

[5]Younossi ZM, Stepanova M, Ong J, et al. Nonalcoholic steatohepatitis is the most rapidly increasing indication for liver transplantation in the United States. Clin Gastroenterol Hepatol. 2021;19:580–9

[6]Singal AK, Hasanin M, Kaif M, Wiesner R, Kuo YF. Nonalcoholic Steatohepatitis is the Most Rapidly Growing Indication for Simultaneous Liver Kidney Transplantation in the United States. Transplantation. 2016 ;100: 607-12.

[7]Musso G, Cassader M, Gambino R. Non-alcoholic steatohepatitis: emerging molecular targets and therapeutic strategies. Nat Rev Drug Discov. 2016;15:249-74

[8]Xu XY, Meng X, Li S, Gan RY, Li Y, Li HB. Bioactivity, Health Benefits, and Related Molecular Mechanisms of Curcumin: Current Progress, Challenges, and Perspectives. Nutrients. 2018;10: 1553

[9] Baker RG, Hayden MS, Ghosh S. NF-κB, inflammation, and metabolic disease. Cell Metab. 2011;13:11-22

[10]Li JM, Li YC, Kong LD, Hu QH. Curcumin Inhibits Hepatic Protein-Tyrosine Phosphatase 1B and Prevents Hypertriglyceridemia and Hepatic Steatosis in Fructose-Fed Rats. Hepatology 2010; 51: 1555-1566.

[11] Heritage M, Jaskowski L, Wilkinson AS, et al . The progression of NAFLD to NASH in a mouse model of Hfe-/-associated steatohepatitis is attenuated by coadministration of curcumin and vitamin E: 1448. Hepatology 2012; 56: 873A.

[12]Ngu MH, Norhayati MN, Rosnani Z, Zulkifli MM. Curcumin as adjuvant treatment in patients with non-alcoholic fatty liver (NAFLD) disease: A systematic review and meta-analysis. Complement Ther Med. 2022 ;68:102843.

[13] Cuomo J, Appendino G, Dern AS, et al. Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. *J Nat Prod.* 2011;74: 664–669.

[14]Hegde M, Girisa S, Bharathwaj Chetty B, Vishwa R, Kunnumakkara AB. Curcumin Formulations for Better Bioavailability: What We Learned from Clinical Trials Thus Far? ACS Omega. 2023;8:10713-10746

[15]Singh S, Sharma N, Shukla S, et al. Understanding the Potential Role of Nanotechnology in Liver Fibrosis: A Paradigm in Therapeutics. Molecules. 2023; 28:2811.

[16] Antiga E, Bonciolini V, Volpi W, Del Bianco E, Caproni M. Oral Curcumin (Meriva) Is Effective as an Adjuvant Treatment and Is Able to Reduce IL-22 Serum Levels in Patients with Psoriasis Vulgaris. Biomed Res Int. 2015; 2015:283634.

[17] Golombick T, Diamond TH, Manoharan A, Ramakrishna R. The effect of curcumin (as Meriva) on absolute lymphocyte count (ALC), NK cells and T cell populations in patients with stage 0/1 chronic lymphocytic leukemia. Journal of Cancer Therapy 2015; 6: 566-571

[18] Pastorelli D, Fabricio ASC, Giovanis P, D'Ippolito S, Fiduccia P, Soldà C, Buda A, Sperti C, Bardini R, Da Dalt G, Rainato G, Gion M, Ursini F. Phytosome complex of curcumin as

complementary therapy of advanced pancreatic cancer improves safety and efficacy of gemcitabine: Results of a prospective phase II trial. Pharmacol Res. 2018;132:72-79.

[19]Food and Drug Administration. Noncirrhotic nonalcoholic steatohepatitis with liver fibrosis: developing drugs for treatment. Guidance for industry: draft guidance. December 2018 (https://www.fda .gov/media/119044/download).

[20]European Medicines Agency. Reflection paper on regulatory requirements for the development of medicinal products for chronic non-infectious liver diseases (PBC, PSC, NASH). November 2018 (https://www.ema.europa.eu/en/documents/scientific -guideline/reflection-paper-regulatory -requirements-development-medicinal -products-chronic-non-infectious-liver_en .pdf).

[21]Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41: 1313-21.

[22]Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. Hepatology. 2017;65:1557-1565.

[23]Xia MF, Yan HM, He WY, et al. Standardized ultrasound hepatic/renal ratio and hepatic attenuation rate to quantify liver fat content: an improvement method. Obesity (Silver Spring). 2012;20:444-52.

[24]Mancini M, Prinster A, Annuzzi G, et al. Sonographic hepatic-renal ratio as indicator of hepatic steatosis: comparison with (1)H magnetic resonance spectroscopy. Metabolism. 2009;58:1724-30.

[25]Decaris ML, Li KW, Emson CL, et al. Identifying nonalcoholic fatty liver disease patients with active fibrosis by measuring extracellular matrix remodeling rates in tissue and blood. Hepatology. 2017;65:78-88.

[26]Lameire NH, Levin A, Kellum JA, et al. Conference Participants. Harmonizing acute and chronic kidney disease definition and classification: report of a Kidney Disease: Improving Global Outcomes (KDIGO) Consensus Conference. Kidney Int. 202;100:516-526

[27]Heerspink HJL, Stefánsson BV, Correa-Rotter R, et al. DAPA-CKD Trial Committees and Investigators. Dapagliflozin in Patients with Chronic Kidney Disease. N Engl J Med. 2020; 383:1436-1446.

[28]Rinella ME, Tacke F, Sanyal AJ, Anstee QM. on behalf of the participants of the AASLD/EASL Workshop. Report on the AASLD/EASL Joint Workshop on Clinical Trial Endpoints in NAFLD. Hepatology 2019; 70:1424-1436.

[29]A'Hern RP. Sample size tables for exact single-stage phase II designs. Stat Med 2001;20:859–66.

[30]Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48:452-8

[31]Younossi Z.M, Golabi P., Paik JM., Henry A., Van Dongen C., Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): A systematic review. Hepatology. 2023;77:1335–1347

[32]May 19, 2023 Meeting of the Gastrointestinal Drugs Advisory Committee- FDA Briefing Document. https://www.fda.gov/media/168215/download

[33]He L, Wang J, Ping F, et al. Association of Glucagon-Like Peptide-1 Receptor Agonist Use With Risk of Gallbladder and Biliary Diseases: A Systematic Review and Meta-analysis of Randomized Clinical Trials. *JAMA Intern Med.* 2022;182:513–519

[34]Newsome PN, Buchholtz K, Cusi K, et al. A Placebo-Controlled Trial of Subcutaneous Semaglutide in Nonalcoholic Steatohepatitis. N Engl J Med. 2021;384:1113-1124.

[35]**Musso G, Cassader M**, Cohney S, et al. Fatty Liver and Chronic Kidney Disease: Novel Mechanistic Insights and Therapeutic Opportunities. Diabetes Care. 2016;39:1830-45

[36]Musso G, De Michieli F, Bongiovanni D, et al. New Pharmacologic Agents That Target Inflammation and Fibrosis in Nonalcoholic Steatohepatitis-Related Kidney Disease. Clin Gastroenterol Hepatol. 2017;15:972-985.

[37] Pivari F, Mingione A, Piazzini G, et al. Curcumin Supplementation (Meriva®) Modulates Inflammation, Lipid Peroxidation and Gut Microbiota Composition in Chronic Kidney Disease. Nutrients. 2022;14: 231

[38] Ledda A, Belcaro G, Feragalli B, et al. Temporary kidney dysfunction: supplementation with Meriva® in initial, transient kidney micro-macro albuminuria. Panminerva Med. 2019;61:444-448

[39]Foresto-Neto O, Albino AH, Arias SCA, et al. NF-κB System Is Chronically Activated and Promotes Glomerular Injury in Experimental Type 1 Diabetic Kidney Disease. Front Physiol. 2020; 11: 84.

[40]Dai W, Wu J, Zhang S, et al. Genes directly regulated by NF-κB in human hepatocellular carcinoma HepG2. Int J Biochem Cell Biol. 2017;89:157-170.

[41] Moghe A, Ghare S, Joshi-Barve S, et al. et al. Curcumin Induces FasL Gene Expression via Promoter Chromatin Remodeling and Reduces Cell Survival and Invasiveness in Hepatocellular Carcinoma Cells. Hepatology 2012; 56: 798A.

[42] Marquardt JU, Gómez-Quiroz LE, Arreguin Camacho LO, et al.Curcumin targets stem-like liver cancer cells by NF-kB mediated inhibition of histone deacetylases. Hepatology2014, 60(S1):639A.

[43]Moghe A, Barve A, Barker D, et al. et al. Curcumin induces epigenetic modifications, reactivates silenced tumor suppressor gene-tissue factor pathway inhibitor-22 (TFPI-2) and decreases cell survival and invasiveness in hepatocellular carcinoma cells. Hepatology 2010; 52(S1):575A.

[44]Yu H, Lin L, Zhang Z. *et al.* Targeting NF-κB pathway for the therapy of diseases: mechanism and clinical study. Sig Transduct Target Ther 2020; 5: 209.

[45] Shah PA, Patil R, Harrison SA. NAFLD-related hepatocellular carcinoma: The growing challenge. Hepatology. 2023;77:323-338.

[46] https://www.fda.gov/media/132575/download

[47] https://apps.who.int/food-additives-contaminants-jecfa-database/Home/Chemical/638

[48]European Food Safety Authority (2014). Refined exposure assessment for curcumin (E 100). EFSA Journal 2014; 12: 3876.

[49]Hu S, Belcaro G, Dugall M, et al. Interaction study between antiplatelet agents, anticoagulants, thyroid replacement therapy and a bioavailable formulation of curcumin (Meriva®). Eur Rev Med Pharmacol Sci. 2018;22:5042-5046

[50]Belcaro G, Cesarone MR, Dugall M, Pellegrini L, Ledda A, Grossi MG, Togni S, Appendino G. Efficacy and safety of Meriva®, a curcumin-phosphatidylcholine complex, during extended administration in osteoarthritis patients. Altern Med Rev. 2010;15:337-44.

[51]Allegri P, Mastromarino A, Neri P. Management of chronic anterior uveitis relapses: efficacy of oral phospholipidic curcumin treatment. Long-term follow-up. Clin Ophthalmol. 2010 ;4: 1201-6.

[52]Pivari F, Mingione A, Piazzini G, et al. Curcumin Supplementation (Meriva®) Modulates Inflammation, Lipid Peroxidation and Gut Microbiota Composition in Chronic Kidney Disease. Nutrients. 2022;14: 231

[53] Lee H, Kuwabara Y, Hirose A, et al. Safety evaluation of high bioavailability curcumin in Healthy Japanese Adults: A Randomized, placebo-controlled, double-blind, parallel-group comparison study. Functional Food in Health and Disease 2023; 13; 702-716

Author names in bold designate shared co-first authorship

Figure 1: CONSORT diagram of patient flow through the study.

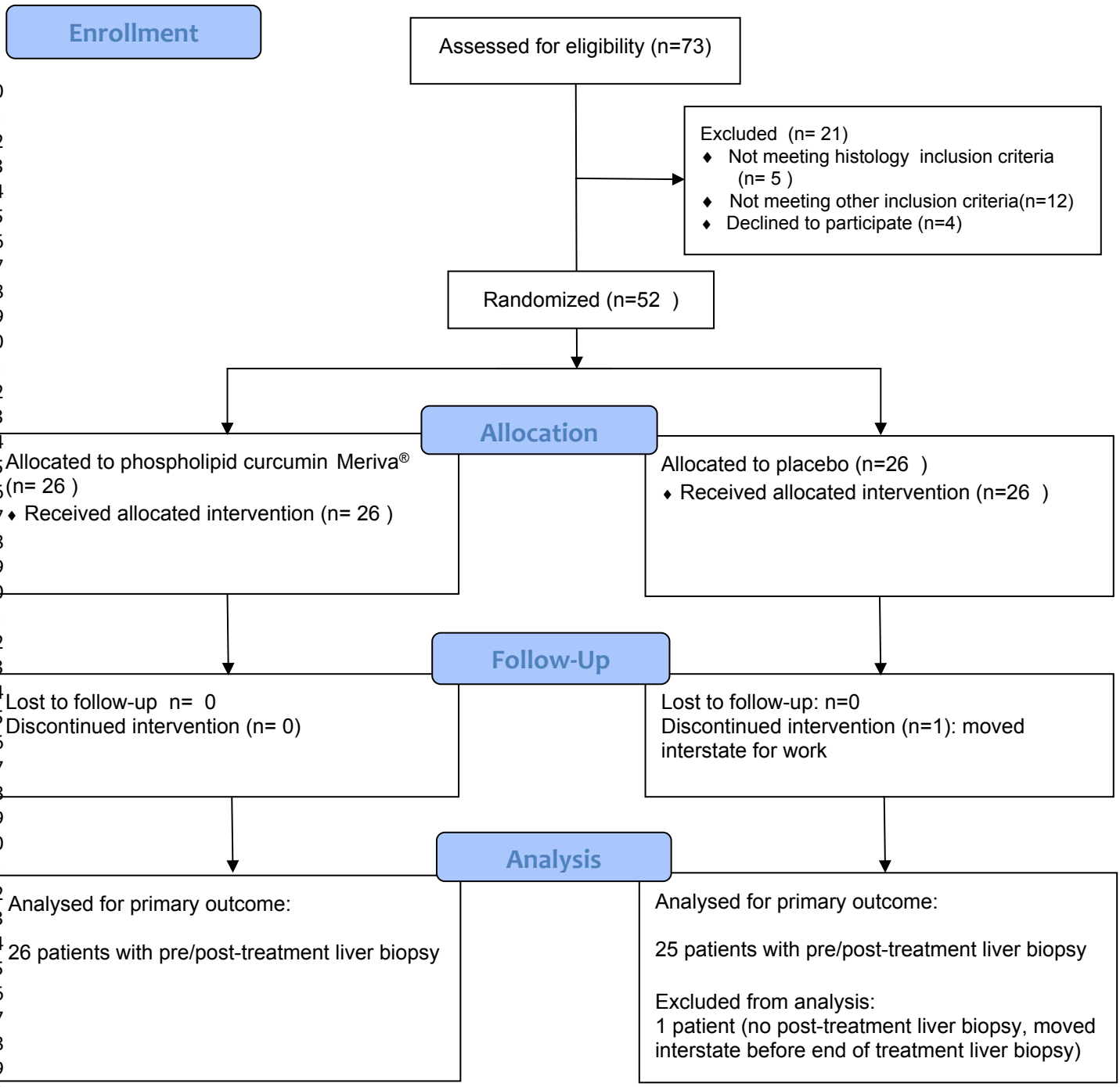
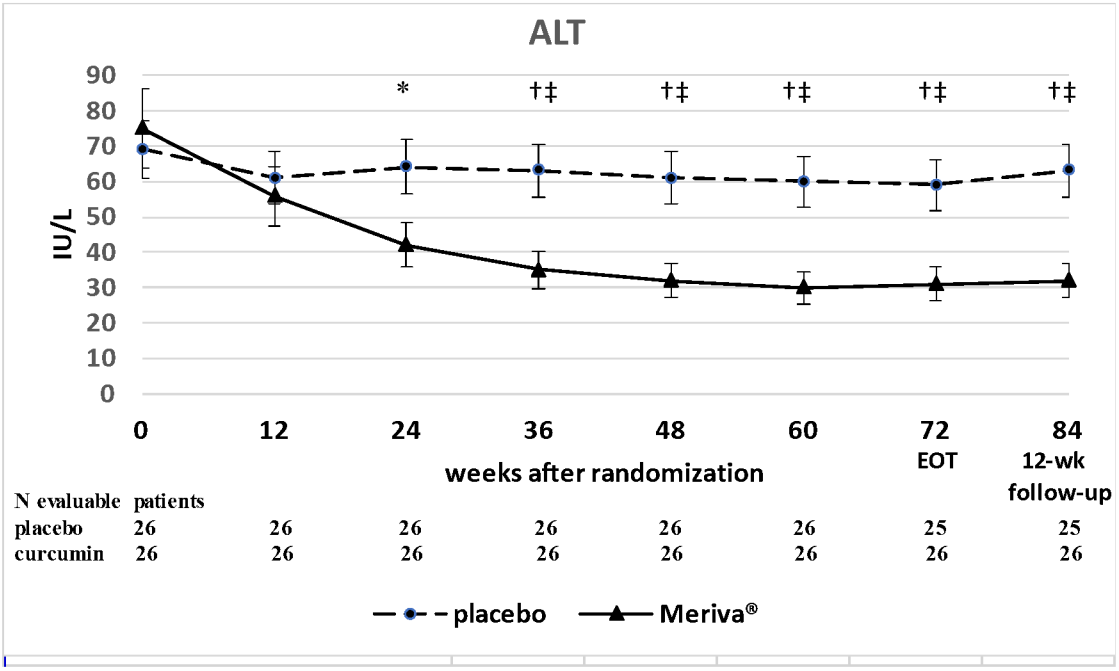


Figure 2 Panel A: serum ALT



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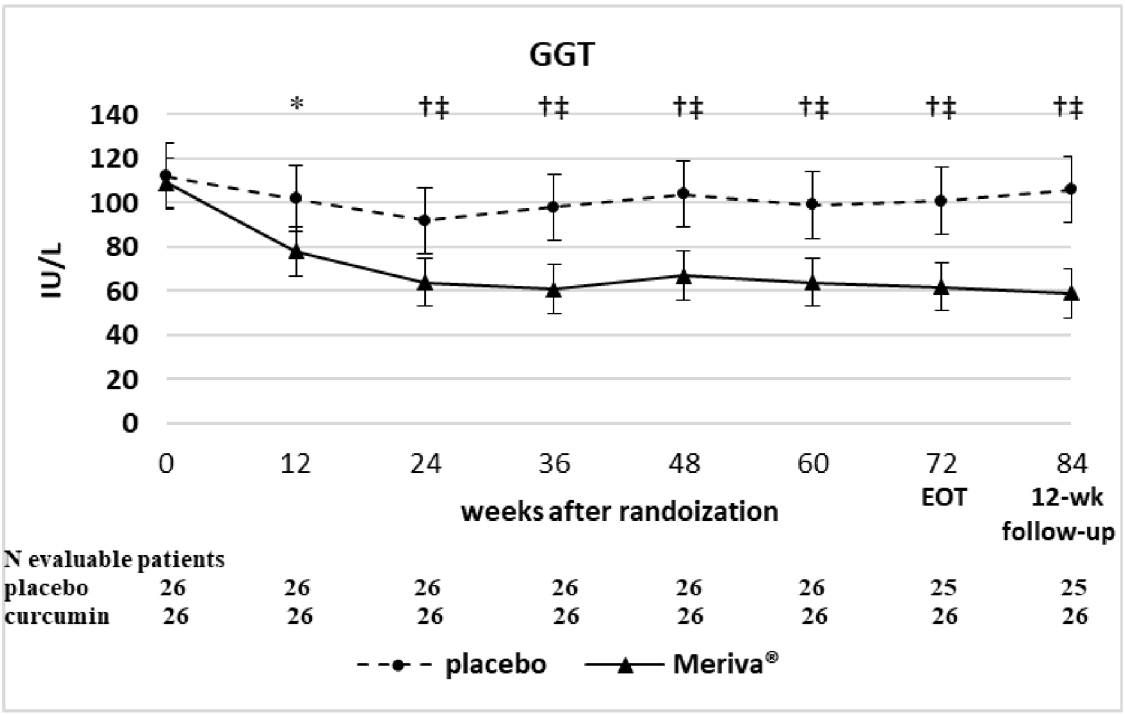


Figure 2 Panel C: eGFR

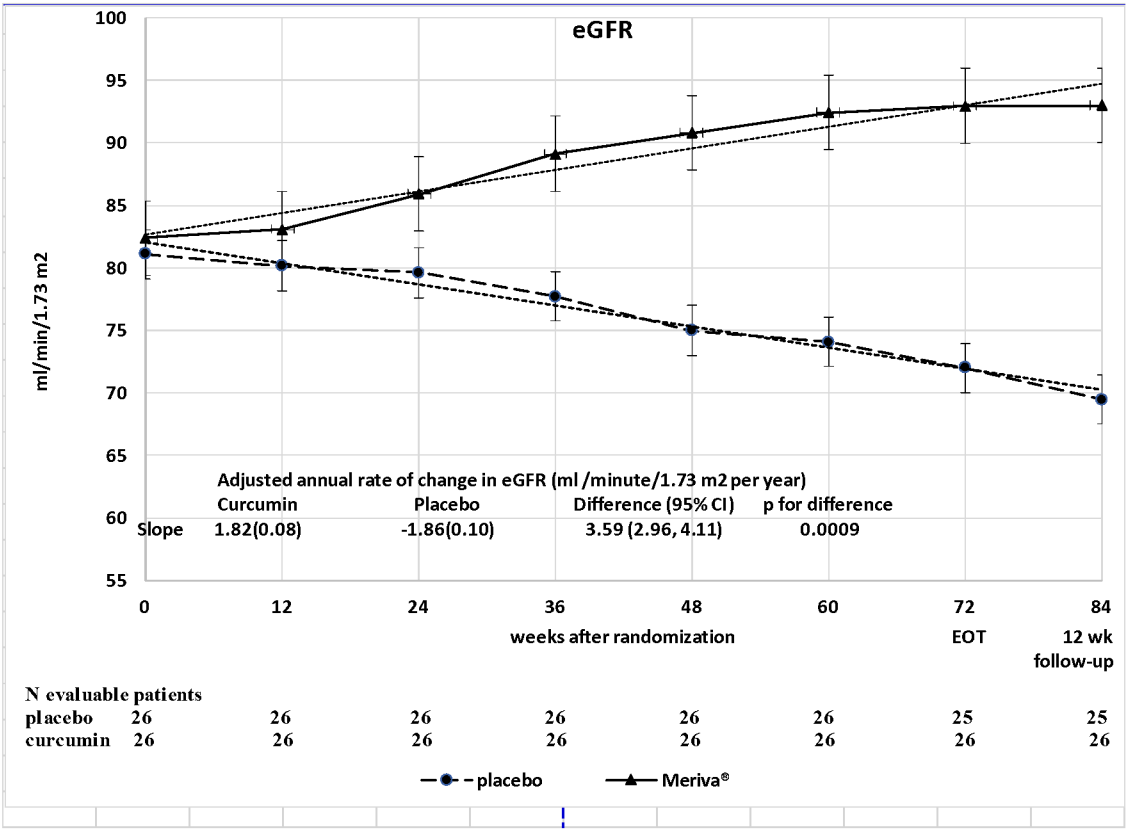


Figure 2 Panel D: plasma Triglyceride

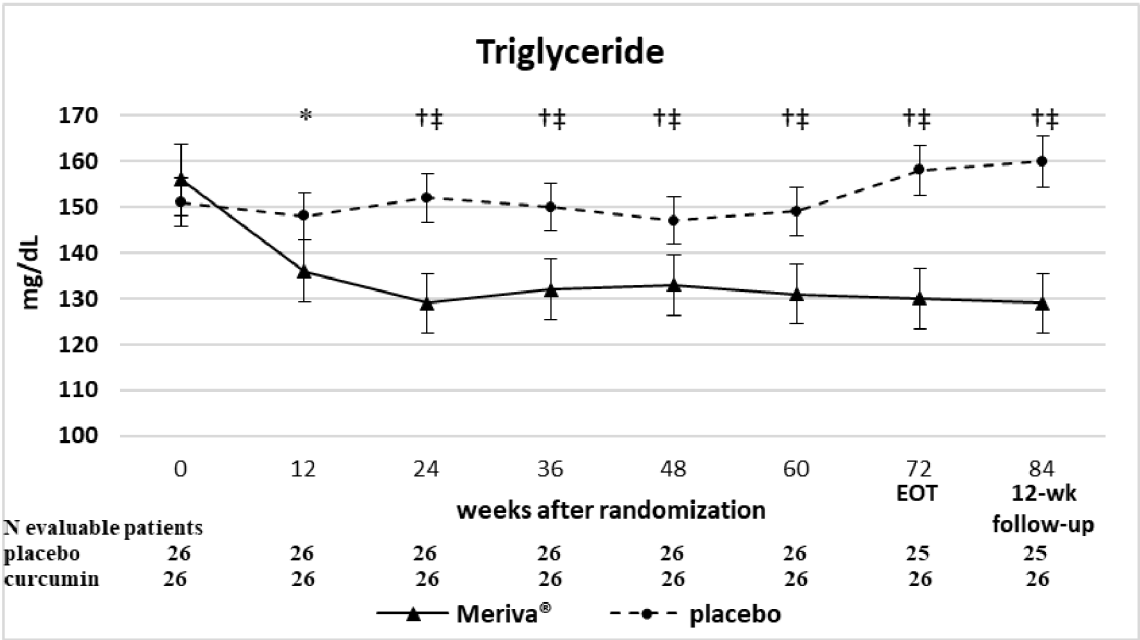
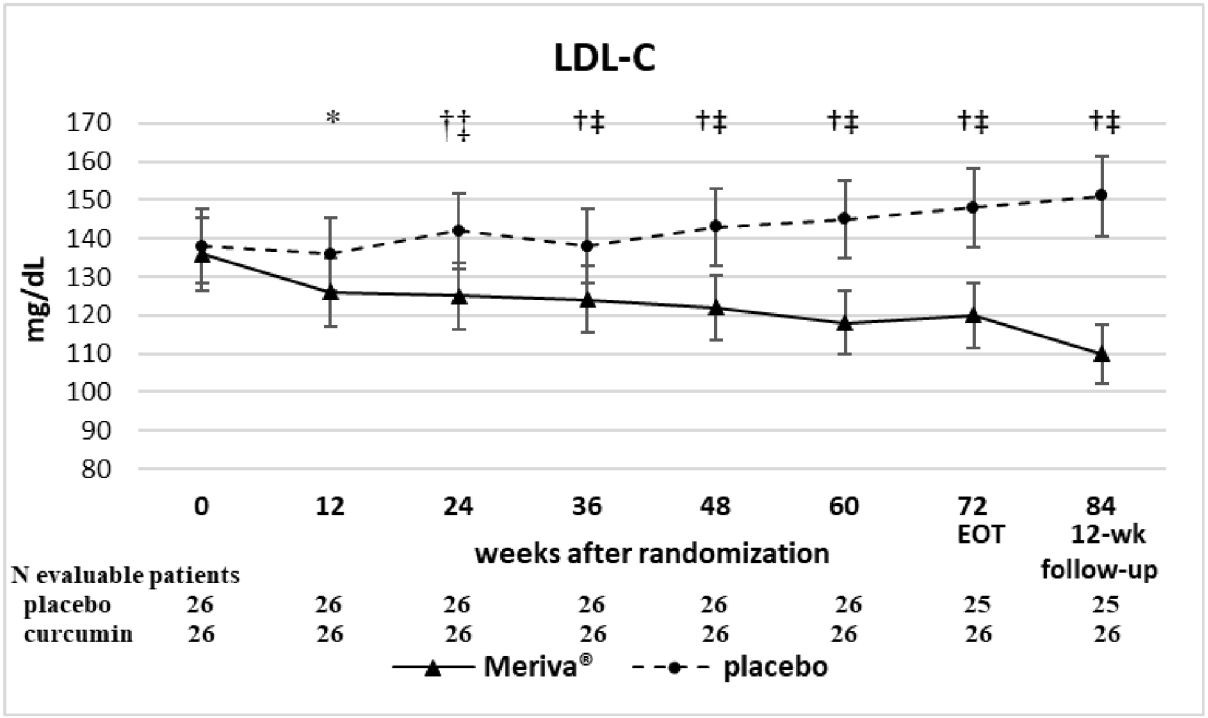


Figure 2 Panel E: plasma LDL-C



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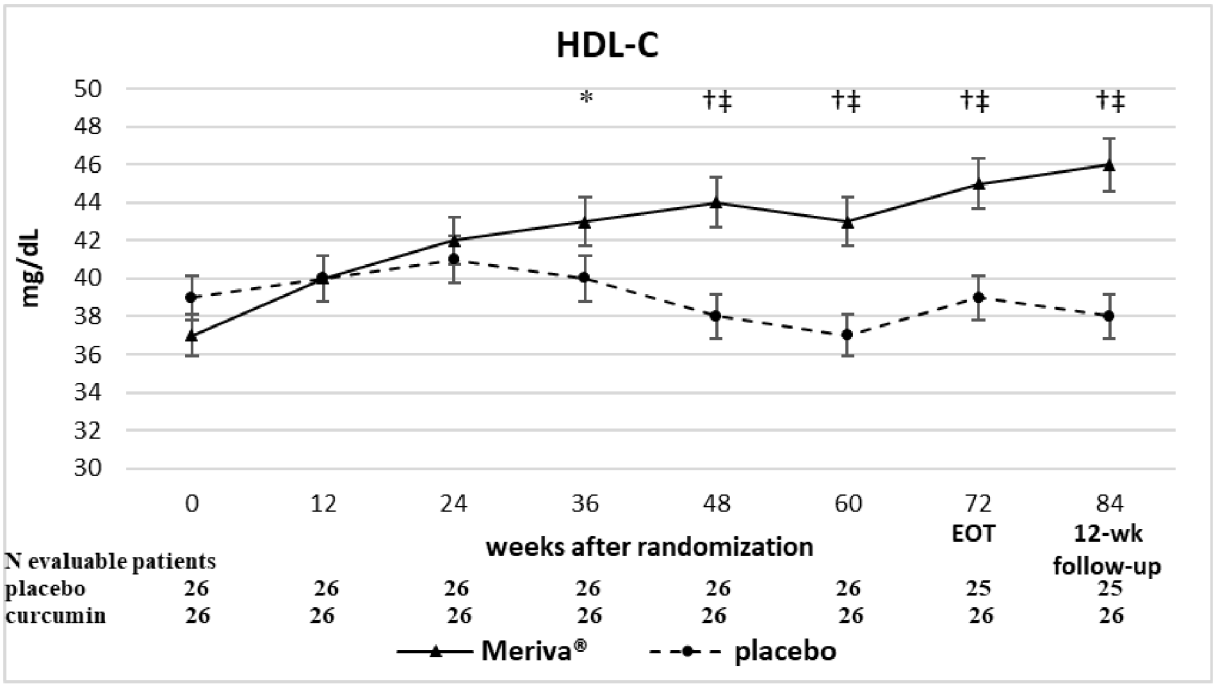


Figure 2 Panel G: HbA1c(%)

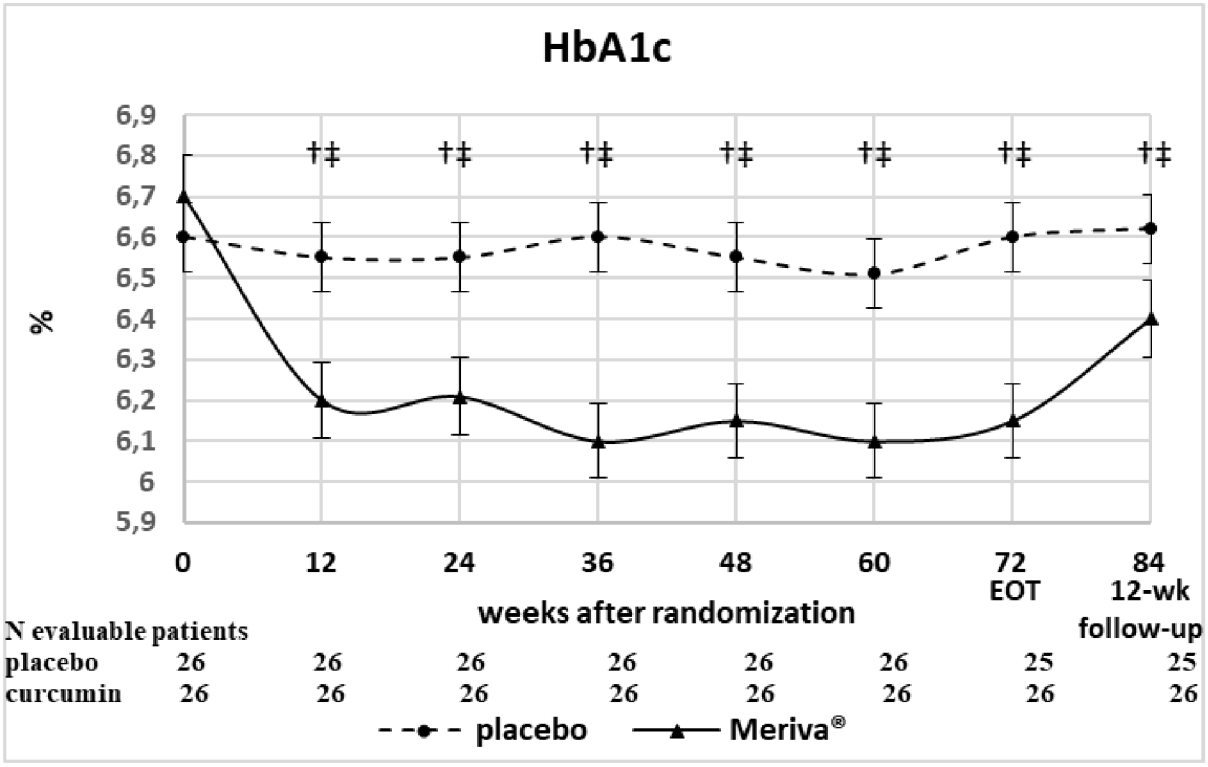
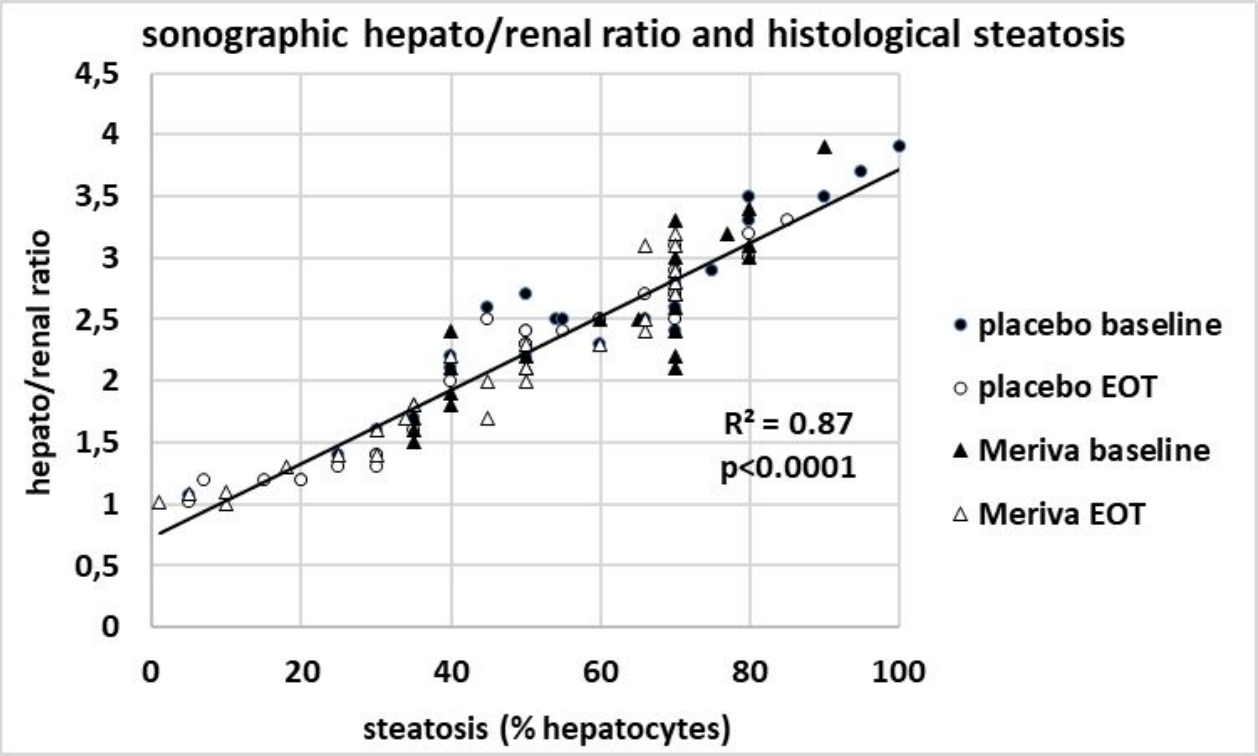
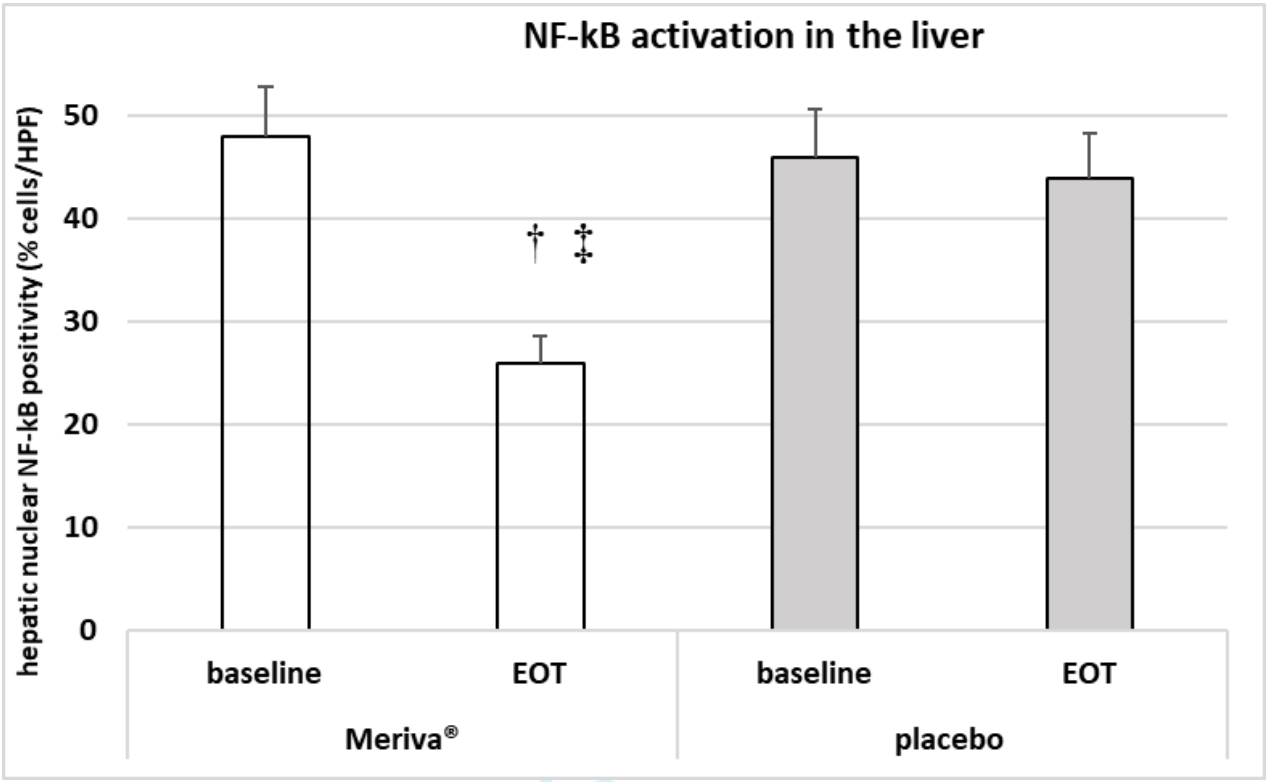


Figure 3. Sonographic hepato/renal ratio and histological hepatic steatosis (expressed as % hepatocytes) in the whole study population with available pre-post-treatment liver biopsy (n= 51)



For Review

Figure 4 Panel A



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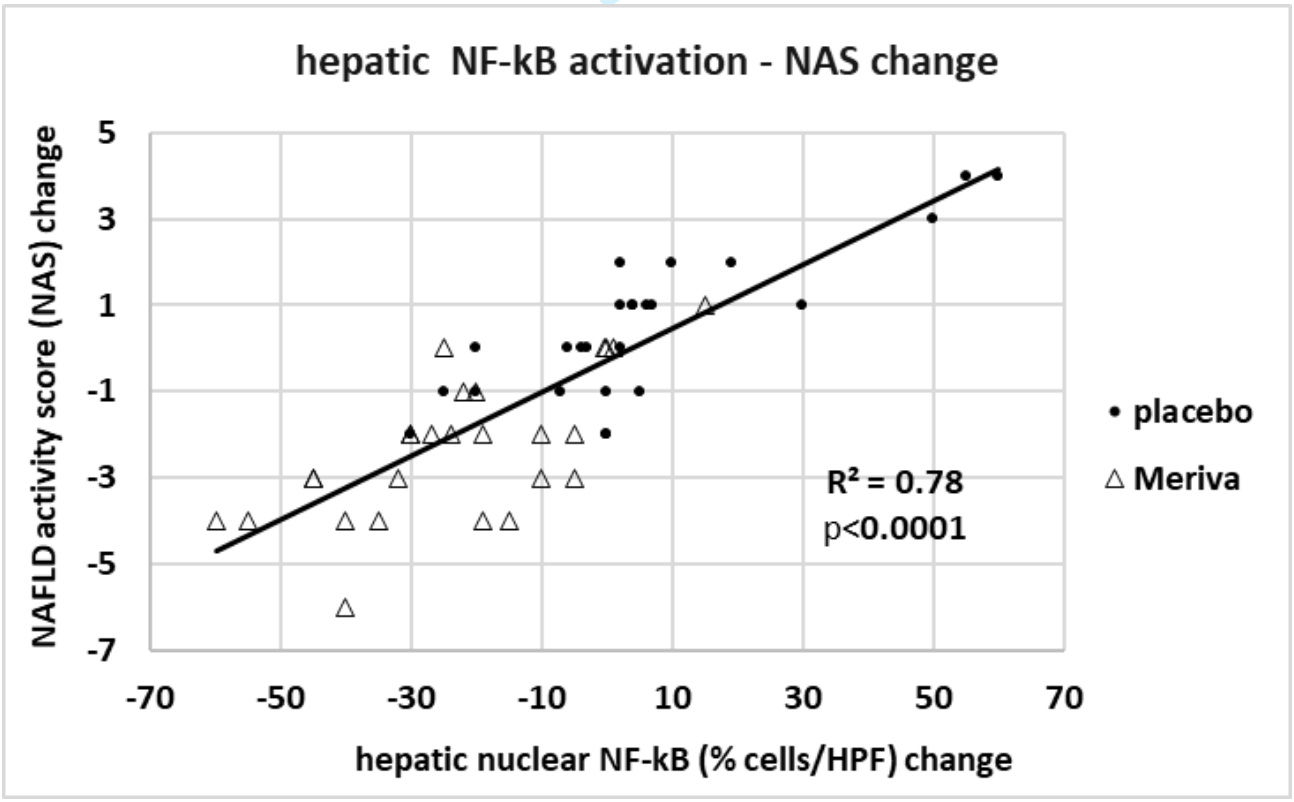

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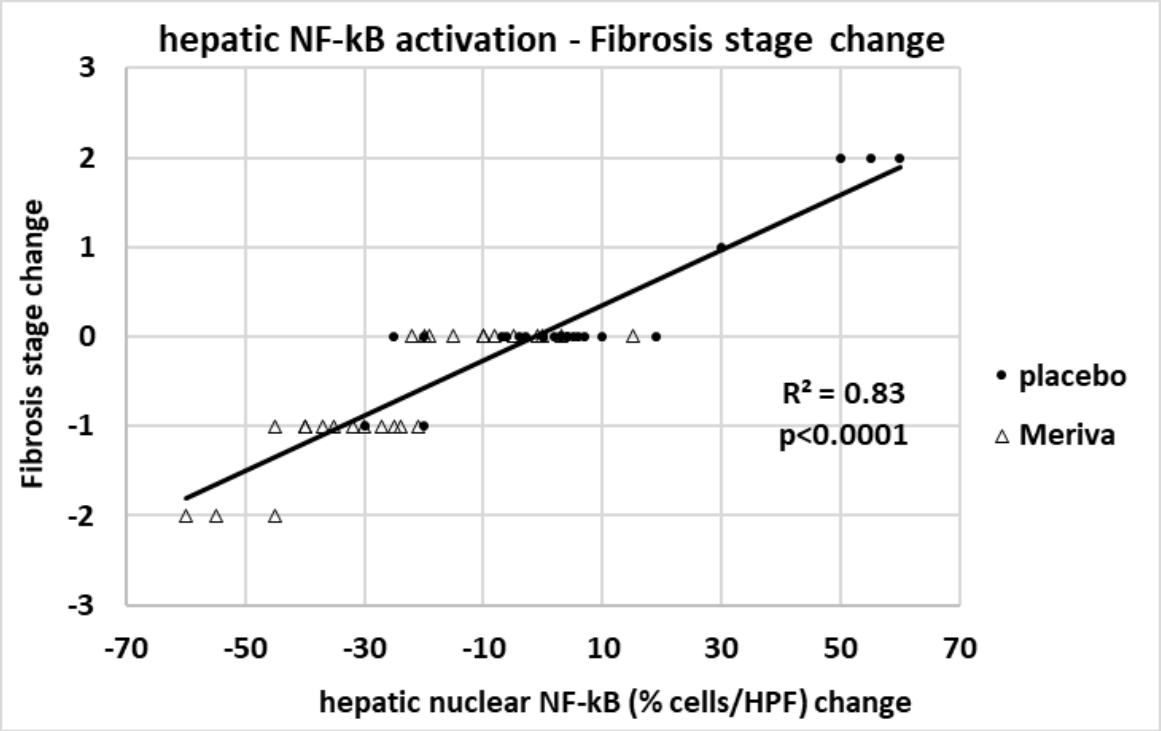


Figure 4 Panel D

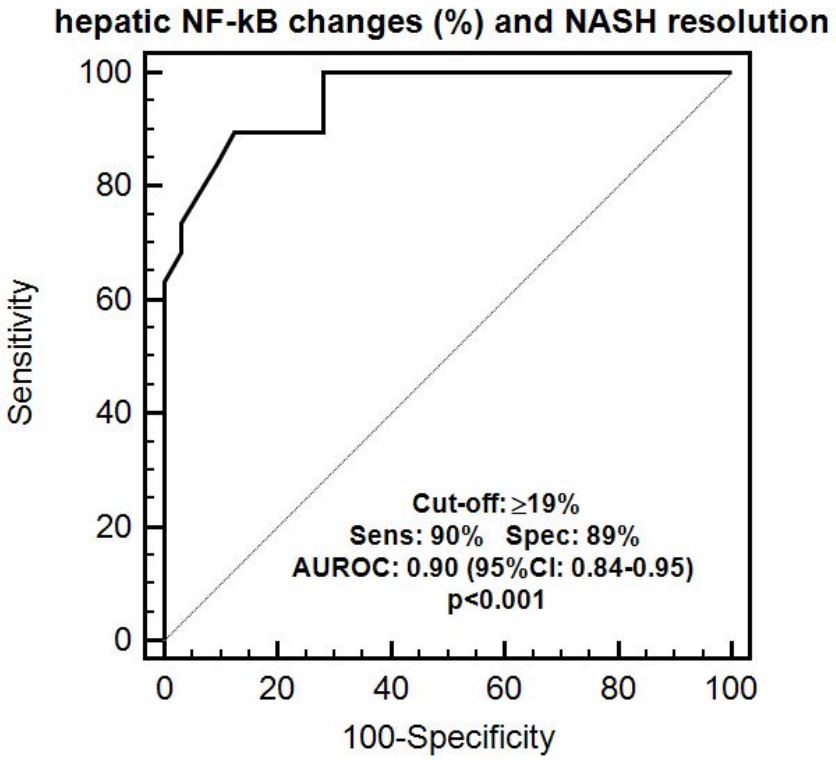
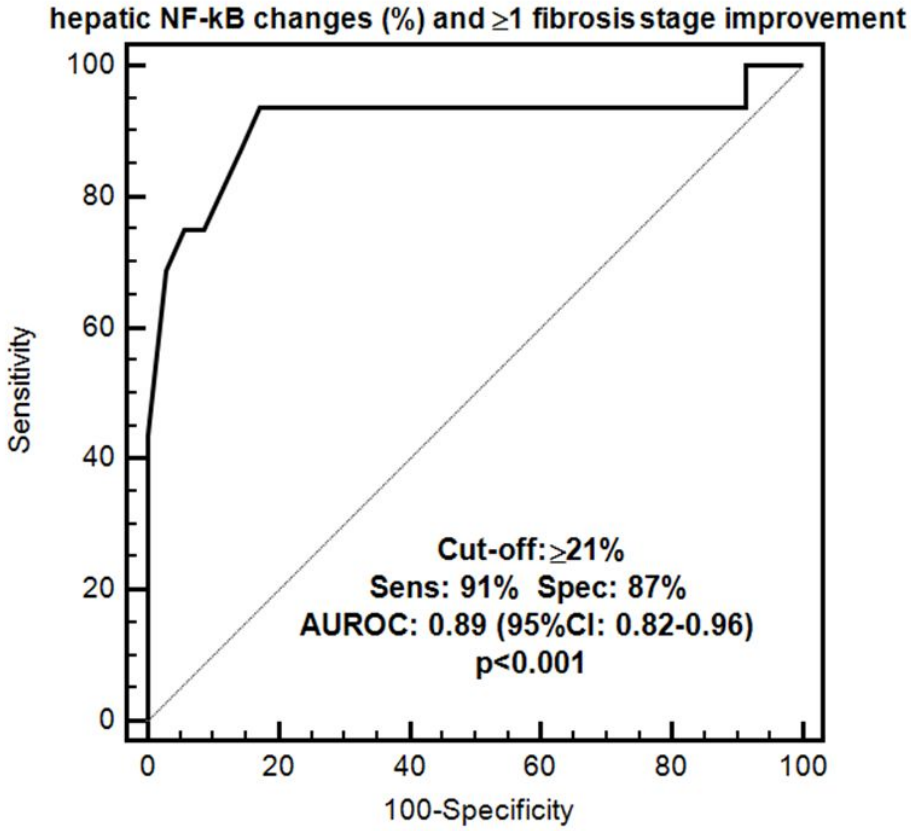


Figure 4 Panel E



eview

Table 1. Baseline demographic, clinical, biochemical and histological characteristics of included patients(n=52).

	Meriva (n=26)	Placebo (n=26)
Demographics		
Age (years)	55 (11)	53 (10)
Male	14 (53%)	12 (47%)
Caucasian white race	26 (100%)	26 (100%)
Weight status **Obesity** **Overweight**	 19 (73%) 7 (27%)	 18 (69%) 8 (31%)
Comorbidities		
Type 2 diabetes	13 (50%)	12 (48%)
Hyperlipidaemia*	15 (58%)	13 (50%)
Hypertension†	16 (62%)	14 (54%)
Cardiovascular disease	1 (4%)	1 (4%)
Current smoker	3 (12%)	4 (16%)
Ex-smoker	10 (38%)	12 (46%)
Never smoked	13 (50%)	10 (38%)
Medications		
Anti-diabetic		
Metformin	12 (48%)	12 (48%)
Sulfonylurea	1 (4%)	1 (4%)
DPP-IV inhibitors	3 (12%)	2 (8%)
Insulin	7 (27%)	8 (31%)
Anti-lipidaemic **Statins** **PUFA** **Fibrates**	10 (38%) 9 (35%) 2 (8%) 1 (4%)	9 (35%) 9 (35%) 3 (12%) 0 (0%)
Anti-hypertensive		
ACEI/ARB	14 (54%)	13 (50%)
Others	14 (54	12 (46%)
Anti-platelet	3 (12%)	3 (12%)
Dietary habits		
Total energy Intake (kcal/d)	2493 (142)	2569 (131)
Kcal/kg BW/d	31 (1)	32 (1)
Alcohol (g/d)	4.5 (0.7)	4.6 (0.8)
Fat (% kcal/d)	35.1 (0.8)	35.1 (0.9)
CHO (% kcal/d)	48.7 (1.1)	49.1 (1.1)
Fiber (g/d)	26.5 (2.2)	24.9 (1.9)
Protein (% kcal/d)	16.8 (0.7)	16.6 (0.9)

SFA (% total fat)	34.9 (0.4)	34.4 (0.9)
MUFA(% total fat)	48.9 (1.3)	47.2 (1.4)
PUFA(% total fat)	14.1 (0.8)	14.0 (0.9)
Physical activity (PA) categories		
Light PA (min/d)	171.8 (31.5)	193.4 (38.9)
Moderate PA(min/d)	98.6 (22.1)	102.4 (18.9)
Vigorous PA(min/d)	8.1 (7.3)	10.2 (8.1)
Sedentary time(min/d)	809.8 (80.4)	838.4 (91.3)
Metabolic parameters		
Weight (kg)	101.7 (19.4)	102.1 (18.3)
Body-mass index (kg/m²)	33·9 (5.1)	33.4 (4.9)
Waist circumference (cm)	107.3 (12.1)	109.1 (14.2)
HbA1c(%)	6.72 (0.81)	6.68 (0.72)
HbA1c(%) in T2DM	6.96 (0.84)	7.12 (0.86)
Fasting plasma glucose (mg/dL)	118 (28)	115 (26)
Fasting insulin (uU/mL)	26.3 (11.4)	23.8 (12.1)
Fasting NEFA (mmol/L)	0.56 (0.24)	0.52 (0.21)
HOMA-IR	7.7 (4.9)	6.8 (4.1)
Total cholesterol (mg/dL)	196 (43)	199 (41)
LDL-C(mg/dL)	138 (24)	136 (26)
HDL-C(mg/dL)	39 (4)	37 (4)
Oxidized LDL (oxLDL)(IU/L)	48.3 (17.5)	51.7 (21.8)
Non-HDL-C(mg/dL)	159 (35)	162 (39)
Triglycerides(mg/dL)	151 (33)	158 (29)
hsC-reactive protein (mg/L)	6.7 (4.1)	5.6 (3.8)
Systolic blood pressure (mm Hg)	130 (13)	133 (12)
Diastolic blood pressure (mm Hg)	79 (11)	78 (9)
Renal function		
Creatinine (mg/dL)	0.96 (0.20)	0.93 (0.17)
eGFR (mL/min/1.73 m2)	82(8)	81(12)
eGFR stage (ml/min/1.73 m²)		
G1 (≥90 ml/min/1.73 m²)	10 (38%)	10 (38%)
G2(60-89)	11 (42%)	11 (42%)
G3a(45-59)	5 (19%)	5 (19%)
G3b (30-44)	0 (0%)	0 (0%)
G4 (15-29)	0 (0%)	0 (0%)
G5(<15)	0 (0%)	0 (0%)
Albuminuria(AER)(mg/day)	172(85)	141(62)
A1 stage (<30 mg/day)	10(38%)	10(38%)
A2 stage (30-300)	16(62%)	16(62%)
A3 stage (>300)	0(0%)	0(0%)
CKD (eGFR stage≥G2 and/or albuminuria stage≥A2) n (%)	16 (62%)	16(62%)
Liver function		

Alanine aminotransferase (U/L)	76 (28)	69 (32)
Aspartate aminotransferase (U/L)	57 (21)	54 (24)
γ-glutamyl transferase (U/L)	103 (68)	112 (75)
Alkaline phosphatase (U/L)	87 (25)	86 (38)
Total bilirubin (mg/dL)	0.82 (0.29)	0.70 (0.24)
Albumin (g/dL)	3.9 (0.6)	4.0 (0.5)
Noninvasive markers of liver disease severity		
Computerized sonographic Hepato/Renal ratio	2.62(0.74)	2.60(0.60)
Cytokeratin-18 fragments M30 (U/L)	501.4(127.6)	492.2(132.1)
FIB-4	1.97/0.36)	2.01(0.33)
APRI	0.71(0.23)	0.78(0.20)
Lumican (ng/mL)	179(26)	188(27)
Liver histology		
Definite NASH	26 (100%)	26 (100%)
Total NAFLD activity score (0–8)	5.6 (0·9)	5.7 (0·9)
Hepatocyte ballooning score (0–2)	1.5 (0.4)	1.4 (0.5)
Steatosis score (0–3)	2.2 (0.7)	2.4(0.5)
Lobular inflammation score (0–3)	1.6 (0.6)	1.5 (0.4)
Kleiner fibrosis stage (F0–F4)	1.9 (1.3)	2.0 (0.9)
Kleiner fibrosis stages F0-F4 **F0** **F1** **F2** **F3** **F4**	 1 (4%) 6 (23%) 11(4%) 6 (23%) 2 (8%)	 1 (4%) 7 (26%) 10(0%) 7 (27%) 1 (4%)
Clinically significant fibrosis (stage F2-4) n (%)	19 (73%)	18 (69%)
Advanced fibrosis (stage 3-4) n (%)	8 (31%)	8 (31%)
Biopsy length (mm)	19.8 (5.1)	20.6 (4.5)
Number of portal tracts	19.1 (7.3)	17.9 (6.1)

Data are n (%) or mean (SD). HOMA-IR=homoeostasis model assessment-estimated insulin resistance. ADIPO-IR=adipose tissue insulin resistance.; DPP-IV: dipeptidyl peptidase (DPP) IV inhibitors.

*Hyperlipidemia was defined as recorded in the past medical history, as receiving lipid-lowering drugs (e.g., statin, fibrate, ezetimibe), or both. †Hypertension was defined as recorded in the past medical history, as receiving an anti–hypertensive drug, or both. ‡LDL concentration was calculated using the Friedwald formula. The non-alcoholic fatty liver disease (NAFLD) activity score is the algebraic sum of steatosis score, lobular inflammation score and hepatocyte ballooning score

estimated Glomerular Filtration Rate (eGFR) was assessed from serum creatinine using the **CKD-EPI** (Chronic Kidney Disease Epidemiology Collaboration) equation, as recommended by KDIGO guidelines. eGFR and urinary albumin excretion rate (AER) were classified according to Kidney Disease Improving Global Outcomes (KDIGO) categories.

Chronic kidney disease (CKD) was defined according to KDIGO guidelines as sustained (i.e., documented on 2 occasions at least 3 months apart) reduction in eGFR<60 mL/min/1.73 m^2 and/or sustained increase in AER≥30 mg/d.

CKD regression was defined as sustained (i.e., documented 3 month apart, on both follow-up visit 7 and visit 8) eGFR≥60 mL/min/1.73 m^2 **and** normalization of AER (i.e., <30 mg/d).

Data are n (%) or mean (SD). HOMA-IR=homoeostasis model assessment-estimated insulin resistance; *Hyperlipidemia was defined as recorded in the past medical history, as receiving lipid-lowering drugs (e.g., statin, fibrate, ezetimibe), or both. †Hypertension was defined as recorded in the past medical history, as receiving an anti–hypertensive drug, or both. ‡LDL concentration was calculated using the Friedwald formula. The non-alcoholic fatty liver disease (NAFLD) activity score and Kleiner scoring system are described in the appendix.

Table 2. Changes in liver histology after 72 weeks of treatment

	Meriva	placebo	Relative risk or mean changes (95% CI) from baseline to 72 weeks (Meriva *vs* placebo)	P
Number of patients with paired liver biopsies	26	25	-	-
Primary outcome				
Patients with NASH resolution and no worsening of fibrosis	16 (62%)	3 (12%)	5.33 (1.76 to 12.13)	**0.003**
Secondary outcomes				
NAFLD activity score (NAS)				
Change in score	-2.81 (1.6)	-0.76 (0.9)	-2.05(-2.78 to -1.34)	**<0.00001**
Patients with improvement	21 (79%)	12 (46%)	1.75(1.11 to 2.76)	**0.02**
Hepatocyte ballooning score				
Change in score	-0.77(0.7)	-0.07(0.6)	-0.70(-1.05 to -0.35)	**0.001**
Patients with improvement	18 (70%)	7 (27%)	2.57 (1.30 to 5.09)	**0.007**
Steatosis				
Change in score	-1.31 (0.72)	-0.4(0.79)	-0.91(-1.29 to -0.53)	**<0.00001**
Patients with improvement	18 (70%)	10 (37%)	1.80(1.04 to 3.12)	**0.04**
Lobular inflammation				
Change in score	-0.70 (0.51)	-0.15 (0.57)	-0.55(-0.82 to -0.29)	**<0.0001**
Patients with improvement	18 (71%)	8 (29%)	2.25(1.20 to 4.23)	**0.01**
Kleiner fibrosis stage				
Change in score	-0.79 (0.82)	0.08 (0.74)	-0.86(-1.19 to -0.35)	**0.001**
Patients with ≥1 stage fibrosis improvement and no worsening of NASH	13 (50%)	2 (8%)	6.50(1.63 to 21.20)	**0.008**
Patients with ≥1 stage fibrosis worsening	0 (0%)	4 (16%)	0.14(0.01 to 2.87)	0.19
Patients with NASH resolution and	13 (50%)	1 (4%)	7.31 (1.95 to 23.12)	**0.004**

≥1 stage fibrosis improvement				
Patients with significant (stage F2-4) fibrosis regression and no worsening of NASH *	11 (42%)	0 (0%)	18.01 (1.43 to 36.07)	**0.02**
Patients with advanced (stage F3-4) fibrosis regression and no worsening of NASH ¶	5 (19%)	0 (0%)	11.00 (0.64 to 89.31)	0.10

Data are presented as n (%) or mean (SD).

For comparison of continuous variables, p values and mean changes from baseline were calculated by linear regression analysis using the baseline characteristic score and treatment as model covariates (equivalent to ANCOVA); for categorical comparisons, p values were determined by $\chi 2$ analysis or Fisher's exact test, as appropriate.

* Patients with clinically significant fibrosis regression were defined as having stage F2-4 fibrosis at baseline and stage F0-1 fibrosis at EOT.

¶ Patients with advanced fibrosis regression were defined as having stage F3-4 fibrosis at baseline and stage F0-2 fibrosis at EOT

Table 3: Changes in anthropometry and biochemical parameters after 72 weeks of treatment

	Meriva (n=26)	Placebo (n=25)	Relative risk or mean changes (95% CI) from baseline to 72 weeks (Meriva *vs* placebo)	P
Metabolic parameters				
Glucose (mg/dL)	–12 (10)	5 (7)	–17 (–22 to –12)	**0.001**
Insulin (uU/mL)	–11.9 (8.5)	-5.4 (7.7)	–6.5(–10.9 to -2.1)	**0.004**
Fasting NEFA (mmol/L)	-0.21 (0.18)	0.06 (0.24)	-0.27 (-0.39 to -0.15)	**<0.0001**
HOMA-IR	-3.89 (2.92)	-1.35 (2.71)	–2.54 (-4.07 to -1.01)	**0.001**
Total cholesterol (mg/dL)	-39 (28)	11 (26)	-50(-66 to -35)	**<0.0001**
LDL-C (mg/dL)	-26 (11)	13 (10)	-39(-45 to -33)	**<0.0001**
OxLDL (IU/L)	-13 (24)	7 (16)	-20 (-31 to -9)	**0.0004**
HDL-C (mg/dL)	9 (3)	-1 (2)	10 (8 to 11)	**<0.0001**
Non-HDL-C (mg/dL)	-31 (35)	11 (27)	-42 (-59 to -25)	**<0.0001**
Triglycerides (mg/dL)	-27 (21)	9 (13)	-36 (-46 to -26)	**<0.0001**
hs-C-reactive protein (mg/L)	-2.1 (2.8)	0.7 (1.4)	-2.8(-4.00 TO -1.60)	**<0.0001**
HbA1c (%)	-0.63%(0.40%)	-0.01%(0.52%)	–0.62% (–0.87% to –0.37%)	**<0·0001**
HbA1c (%) in T2DM	-0.69%(0.49%)	0.11%(0.63%)	-0.80% (-1.25% to -0.35%)	**0.0005**
Absolute weight (kg)	-0.9 (2.7)	–0.7 (2.5)	–0.2 (–0.6 to 0.4)	0.780
Body-mass index (kg/m²)	–0.3 (1.1)	–0.2 (0.9)	–0.1 (–2·66 to 0·51)	0·721
Waist circumference (cm)	–2.5 (6.4)	–2.3 (7.1)	–0.2 (–3.8 to 3.5)	0·893
Systolic blood pressure (mmHg)	–4.0 (12.3)	–3.2 (14.1)	–0.8 (–7.8 to 3.6)	0.439
Diastolic blood pressure (mm Hg)	-0.6 (9.1)	0.9 (8.8)	–1.5 (–5.9 to 0.5)	0.071
Renal function				
Creatinine (mg/dL)	–0.15 (0.10)	0.09 (0.12)	-0.24 (–0.30 to -0.18)	**0·001**
eGFR (ml/min 1.73 m²)	9.88 (7.12)	-10.32 (11.24)	20.20 (15.09 to 25.31)	**<0.0001**
eGFR stage at EOT				
G1	23 (88%)	10 (38%)	11.50(2.71 to 38.67)	**0.0009**
G2	3 (12%)	11 (42%)	0.17 (0.04 to 0.70)	**0.01**
G3a	0 (0%)	5 (19%)	0.14 (0.01 to 2.67)	0.19

G3b	0 (0%)	0 (0%)	-	-
G4	0 (0%)	0 (0%)	-	-
G5	0 (0%)	0 (0%)	-	-
Albumin Excretion rate (AER) (mg/day)				
AER change (mg/day)	-149 (64)	32 (28)	-181(-207.85, to -144.15)	**<0.0001**
A1 stage (<30 mg/day) at EOT	23 (88%)	10 (38%)	11.50(2.71 to 38.67)	**0.0009**
A2 stage (30-300 mg/day) at EOT	3 (12%)	14 (54%)	0.10 (0.01 to 0.43)	**0.002**
A3 stage (>300 mg/day) at EOT	0 (0%)	2 (8%)	0.33 (0.01 to 7.82)	0.50
Patients with regression of CKD at EOT (eGFR≥90 ml/kg 1.73m^2 and AER<30 mg/d)	13 (50%)	0 (0%)	10.71 (1.94 to 17.99)	**0.004**
Liver function				
Aspartate aminotransferase (AST)(U/L)	–22 (15)	–8 (14)	–14 (–2. to -6)	**0·001**
Alanine aminotransferase (ALT) (U/L)	-41 (32)	-10 (26)	–31 (–46 to -16)	**<0.0001**
γ-glutamyl transferase (GGT)	–44 (34)	–7 (28)	–37 (–54 to –20)	**<0.0001**
Alkaline phosphatase (ALP) (U/L)	–7 (11)	–2 (19)	–5 (–13 to -3)	0·25
Total bilirubin (mg/dL)	–0.7 (0.1)	–0.1 (0.11)	–0·6 (–2.5 to 1.3)	0.51
Albumin (g/dL)	0.2 (0.7)	–0.1 (0.2)	0.3(0.0 to 0.6)	**0.04**
Non-invasive markers of liver disease severity				
Computerized sonographic hepato/renal ratio	-0.68 (0.57)	-0.25 (0.43)	-0.43 (-0.70 to -0.16)	**0.002**
Cytokeratin-18 (CK-18) fragments M30 (U/L)	-189 (70)	+27 (43)	-216.00(-254.32 to -177.68	**0.006**
FIB-4 Index	-0.3 (0.2)	0.1 (0.2)	–0.4 (–0.41 to -0·29)	**<0.0001**
APRI	-0.2 (0.1)	0.1 (0.1)	-0.2 (-0.25 to -0.15)	**<0.0001**
Lumican (ng/mL)	-47/23)	16(26)	--63 (-76 to .50)	**<0.0001**

Data are presented as n (%) or mean (SD). For comparison of continuous variables, p values and mean changes from baseline were calculated by linear regression analysis using the baseline characteristic score and treatment as model covariates (equivalent to ANCOVA), unless otherwise

specified; for categorical comparisons, p values were determined by $\chi 2$ analysis or Fisher's exact test, as appropriate.

Abbreviations: ADIPO-IR: adipose tissue insulin resistance; AER: albumin Excretion Rate; APRI: AST to platelet ratio index; eGFR: estimated Glomerular Filtration rate (calculated with CKD-EPI equation); EOT: end-of-treatment; FIB-4: Fibrosis 4 Index; HbA1c: glycated haemoglobin A1c; MCP-1: monocyte chemoattractant protein-1; NEFA: non-esterified fatty acids; OxLDL: oxidized LDL cholesterol; T2DM: type 2 diabetes mellitus,

Table 4: Adverse events (AE) by treatment allocation. Each individual patients could fulfill several criteria.

	Placebo (n=26)	**Meriva (n=26)**
Overall treatment withdrawal	**1 (4%)**	**0 (0%)**
Treatment withdrawal due to AE	**0 (0%)**	**0 (0%)**
Participants with Serious AE	**0 (0%)**	**0 (0%)**
Participants with AE	**5 (19%)**	**4 (15%)**
AE (any severity) incidence in any treatment group, by system organ class and preferred term		
Gastrointestinal disorders	**5 (19%)**	**3 (12%)**
Nausea	3 (12%)	2 (8%)
Diarrhoea	3 (12%)	3 (12%)
Abdominal pain	2 (8%)	1(4%)
Vomiting	1 (4%)	1(4%)
Constipation	2(8%)	2(8%)
Dyspepsia	3 (12%)	1 (4%)
Flatulence	2 (8%)	2 (8%)
Bloating	4 (16%)	1(4%)
Cardiac disorders	**0 (0%)**	**0(0%)**
Infections and infestations	**1 (4%)**	**1 (4%)**
Chest infection	**0 (0%)**	**0 (0%)**
Urinary tract infection	**0 (0%)**	**0 (0%)**
General disorders	**3 (12%)**	**3 (12%)**
Fatigue	3 (12%)	3 (12%)
Flu-like symptoms	2 (0%)	1 (4%)
Peripheral oedema	0 (8%)	0 (0%)
Chills	2 (8%)	2(8%)
Non-specific pain	1(4%)	0(0%)
Musculoskeletal and connective disorders	3 (12%)	1 (42%)
Back pain	0 (0%)	3 (25%)
Arthralgia	0 (0%)	1 (8%)
Muscle cramps	0 (0%)	0 (0%)
Eye disorders	1 (4%)	0 (0%)
Investigations	**2(8%)**	**2 (8%)**
Increased gamma-glutamyl transferase	2 (8%)	1 (4%)
Increased aspartate aminotransferase	2 (8%)	1 (4%)
Metabolism and nutrition disorders	**2 (8%)**	**2 (8%)**
Anorexia (loss of appetite)	3 (12%)	1(4%)
Nervous system disorders	**2(50%)**	**2(58%)**
Dizziness	1 (4%)	1(4%)
Headaches/Migraines	1 (4%)	1(4%)
Psychiatric disorders	**0 (0%)**	**0(0%)**
Renal and Urinary disorders	**1 (4%)**	**1 (4%)**
Respiratory, thoracic and mediastinal disorders	**1 (4%)**	**2 (8%)**
Cough	1 (4%)	2(8%)
Skin and soft tissue disorders	**1(1%)**	**1(4%)**

Appendix 1. Trial protocol

Effect of phospholipid curcumin Meriva® on liver histology and kidney disease in Nonalcoholic Steatohepatitis

A randomized, double-blind, placebo-controlled trial

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1. Background and Rationale

1.1. NASH: epidemiology, pathogenic mechanisms, clinical relevance and unmet therapeutic needs

Non-alcoholic fatty liver disease (NAFLD) is the commonest cause of chronic liver disease, affecting up to 30% of the general population and 70–90% of obese and type 2 diabetic patients[1, 2].

NAFLD encompasses a spectrum of histological severity ranging from simple steatosis (SS) to necroinflammation and fibrosis (nonalcoholic steatohepatitis, NASH), which can differentiated only by liver biopsy. Even though simple hepatic steatosis is a benign condition, up to 30% of patients with NAFLD have the more severe, progressive inflammatory condition known as NASH.

NASH confers an increased risk of cirrhosis, liver failure and hepatocellular carcinoma, and is projected to be the commonest indication for liver transplantation in forthcoming years.

Furthermore, emerging evidence links NAFLD to chronic kidney disease(CKD), as these two conditions share common pathogenic mechanisms[3],[4],[5]: consistently, NASH poses a 2.5-fold risk of CKD than the general population[6] and is becoming the leading cause of combined liver-kidney transplantation[7,8].

Despite this, there are no universally accepted pharmacological therapies for NASH. Therefore the need for novel, safe agents in NASH is of paramount importance to prevent disease progression and the accompanying clinical burden.

1.2 Curcumin: mechanisms of action, rationale for liver, metabolic and kidney disease treatment and challenges for NASH therapeutics

Curcumin is a lipophilic polyphenol that is the primary constituent of turmeric (*Curcuma longa*), the most common popular spice in Indian cuisine and a major ingredient of curry powders. In vitro studies showed curcumin has prominent antioxidant, anti-inflammatory, antiproliferative and antifibrotic properties, and targets multiple molecular pathways involved in the pathogenesis of NASH and of chronic kidney disease (CKD)[9,10], most prominently nuclear factor-kappa B (NF-kB)[11], a key pro-inflammatory transcription factor

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involved in metabolic and nutritional inflammation[12]. Dietary curcumin supplementation ameliorated liver disease in preclinical NASH models [13, 14,15, 16, 17, 18, 19]

Preliminary human data indicate curcumin supplementation improves surrogate markers of liver disease and of metabolic risk in NAFLD patients[20,21].

Meriva is a formulation of *Curcuma longa* extract with phospholipids (sunflower lecithin) leveraging Phytosome™ technology with a 29-fold higher oral curcuminoids absorption as compared with unformulated curcumin[22], and allows greater systemic concentrations and delivery to target organs[23,24]. Meriva®administration for up to 18 months was well-tolerated, safe[25] and improved clinical end-points and inflammatory parameters in patients with osteoarthritis, uveitis and chronic kidney disease (CKD)[26, 27,28].

2. Research Hypothesis and Aims

On the basis of above-mentioned data, we hypothesized that treatment with Meriva would result in significant improvement in liver histology in patients with NASH.

Key secondary hypotheses include tolerability and safety of Meriva and the potential to improve NASH-related CKD, systemic inflammation, glucose and lipid metabolism.

To test this hypothesis, we designed a phase II, multicentre, double-blinded, placebo-controlled randomized controlled trial (RCT).

3. Study Design setting and timeline

This is a 72-week, investigator-initiated, multicentre, randomized, double-blinded, placebo-controlled trial of treatment with Meriva 2 g daily for adults with biopsy-proven NASH. Screening was undertaken within 14 days of randomization to assess eligibility and collect baseline data. Patients who satisfied the eligibility criteria were randomly assigned (1:1) to receive oral Meriva 1 g/12 hours (total 2 g/day) (experimental) or placebo (control). After which, a 12-week washout period is scheduled.

The trial will be conducted at two University hospitals (HUMANITAS Gradenigo, Turin, Italy, and Città della Salute e della Scienza, Turin, Italy) between May 25th, 2018 and Dec 30th, 2023.

The primary outcome measure will be assessed using an intention-to-treat analysis of the proportion of evaluable patients achieving an improvement in liver histology between liver biopsies at baseline (within 6 months of screening) and after 72 weeks of treatment. Histological improvement will be defined as a

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combination of resolution of active steatohepatitis and no worsening in fibrosis (assessed by Kleiner Fibrosis score[29]; detailed in outcome section).

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4. Ethics and regulatory approval

The study is funded by Indena Spa, Milan, is conducted in accordance with the ethical principles of the Declaration of Helsinki, was approved by A.O.U San Luigi Gonzaga hospital Ethics Committee (prot. N. 0008942) on May 25th, 2018 and is registered with ISRCTN clinical trial registry (study ID: ISRCTN27793187). All patients gave written informed consent to paricipate to the study.

End of study

Study will continue until 84 days (12 weeks) after the last enrolled patient or in any case until Dec 2023, depending on levels of enrollment.

5. Methods

5.1 Eligibility criteria

Eligible adult patients (≥18 years old) will be identified and recruited at the participating trial site centres starting in Dec 2021 and ending by June 2022. Fifty-two patients will be recruited. Participating trial centres will include the Liver and Metabolic Units at the Humanitas Gradenigo Hospital (Turin) since Dec 2021), and the Metabolic Unit of Dept of Medical Sciences at the Città della Salute e della Scienza hospital(Turin) since Dec 2021).

All trial participants will give informed written consent at the beginning of the screening visit prior to undergoing any tests and procedures needed to assess eligibility.

Eligibility for the trial will be determined at screening visit 1 by standard blood tests, clinical history (including written-confirmation of drug history where necessary) and physical examination to identify other illnesses or contraindications for participation.

Patients who satisfied the eligibility criteria trial at both sites will be offered to take part in a metabolic mechanistic substudy. The substudy will involve a standard Oral Glucose tolerance Test (OGTT) and a standard Oral Fat Tolerance Test (OFTT) on three admissions (at least 24 hours apart from each other) and an indirect calorimetry test at the two participating centres on visits 2 (pretreatment) and 8 (4 weeks

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post-treatment). A detailed report of the metabolic substudy will be published separately. A patient's decision to partake or withdraw from the metabolic substudy will not affect their participation in the main 72-week trial.

A schematic view of trial schedule and design is presented in **Figure 1**.

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5.2 Inclusion criteria

The trial entry criteria will be based on a diagnosis of 'definite' NASH on liver biopsy obtained within 6 months of screening or at screening. The diagnosis was confirmed by a centrally read biopsy sample obtained at screening or in the preceding 6 months (patients whose diagnosis was not confirmed at screening had to have a stable body weight, defined as no more than a 5% loss of initial body weight between the time of biopsy and screening, and not to take any medications reported to be inducers or potential therapies for NASH.

Throughout the study, all liver biopsies will be read centrally by one single (RP) expertised pathologist from the central trial site (HUMANITAS Gradenigo), bilnded to clinical and laboratory data and to treatment allocation: prior to randomisation, the liver histopathologist will review all of the liver biopsies of the potential participants to assess whether a diagnosis of 'definite' NASH is present.

A 'definite' diagnosis of NASH iss defined if all of the following are present on biopsy: (1) macrovesicular steatosis (>5%); (2) hepatocyte ballooning (± Mallory Hyaline) and (3) lobular inflammation (mixed infiltrate, related to foci of ballooning). Only patients with 'definite' NASH will be classified as eligible. All participants are to be ≥18 to <70 years of age and have a BMI ≥25 kg/m2 at screening. Patients with type 2 diabetes mellitus (T2DM) at screening had to have stable glycaemic control (HbA1c <9%) and be managed by either diet and/or a stable dose of metformin/sulfonylurea/s. Patients without a history of T2D prior to the screening visit will undergo a 75-g standard OGTT and/or have their HbA1c measured at screening to determine their glycaemic status and will be labelled as 'non-diabetic' if one or more of the following was confirmed

-Impaired fasting glucose, defined using the European Criteria between 110 and 126 mg/dL.

-IGT, defined as 2 h plasma glucose levels between 140 and 200 mg/dL on the 75 g OGTT.

-Normal Fasting Plasma Glucose <110 mg/dL and normal 2 h plasma glucose levels <140 on the 75 g OGTT.

-HbA1c<6.5%

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5.3 Exclusion criteria

A detailed summary of the exclusion criteria is provided in **box 1**. In brief, patients with a history or current significant alcohol consumption, poor glycaemic control (HbA1c >9.0%), Child's Pugh B or C cirrhosis or other liver disease aetiology were excluded. The latter was confirmed with a full liver aetiology screen (drug induced, viral hepatitis B/C, autoimmune and genetic) at the screening visit. Past and current alcohol consumption was ascertained by a detailed review of the patient's medical, social history and by a validated, self-administered food frequency questionnaire with reference pictures to remind participants of drink equivalents (see dietary record section). Concomitant use of drugs reported to be inducers (methotrexate, amiodarone, steroids) or potential therapies for NASH (TZDs, GLP-1 analogues, vitamin E), or other known hepatotoxins were assessed during the screening visit (**box 1**).

Box 1. Exclusion criteria

1. Refusal or lacks capacity to give informed consent to participate in the trial.
2. Participation in any clinical trial of an investigational therapy or agent within 12 months of randomisation.
3. Age at screening <18 or >70 years
4. NAFLD Activity Score (NAS) <3 on liver biopsy.
5. Child's B or C cirrhosis or clinical evidence of decompensated chronic liver disease: radiological or clinical evidence of ascites, current or previous hepatic encephalopathy and evidence of portal hypertensive haemorrhage or varices on endoscopy.
6. Medical history of multiple drug allergies (defined as anaphylactoid drug reactions in >2 drug groups) or allergy to curry or curcumin-based nutraceuticals
7. Presence of any acute/chronic infections or illness that at the discretion of the chief investigator might compromise the patient's health and safety in the trial.
8. Pregnancy or breastfeeding.
9. Women, of childbearing age, who are not willing to practice effective contraception (ie, barrier, oral contraceptives, impenon or past medical history of hysterectomy) for the 72-week duration of the trial and for 1 month after the last administration of the drug.
10. Liver disease of other aetiologies (ie, drug-induced, viral hepatitis, autoimmune hepatitis, PBC, PSC, haemochromatosis, A1AT deficiency, Wilsons disease).
11. Average alcohol consumption >20 g/d(males) and >10 g/d(females) (as assessed by a validated questionnaire within the last 5 years.
12. Medical/surgery history of; gastric bypass surgery, orthotopic liver transplant (OLT) or listed for OLT, hepatocellular, pancreatic, thyroid carcinoma, acute or chronic pancreatitis and total parenteral nutrition within 6 months of randomisation.
13. Diagnosis of malignancy within the last 3 years (with the exception of treated skin malignancies).
14. Hepatocellular carcinoma: dysplastic or intermediate nodules to be excluded. Regenerative and other nodules to be included at the discretion of the chief investigator.

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15. Alanine aminotransferase or aspartate aminotransferase >10×upper limit of normal.
16. >5% weight loss since the diagnostic liver biopsy was obtained.
17. Recent (within 3 months of the diagnostic liver biopsy or screening visit) or significant change (as judged by the chief investigator) in dose of the following drugs: inducers of hepatic steatosis (steroids (oral/intravenous), methotrexate, amiodarone), orlistat and/or multivitamins/ vitamin E (containing >200% recommended daily amount; >30 mg/day).
18. Known positivity for antibody to HIV.
19. Currently being treated with renal replacement therapy (ie, haemodialysis or peritoneal dialysis).

Specific exclusion criteria for participants with T2D
1. Participants receiving thiazolidinediones (TZDs), GLP-1-based therapies, SGLT2 inhibitors
3. HbA1c ≥10%.

Patients who met any of the criteria (listed above) at the screening visit were excluded from trial participation.

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5.4 Intervention

5.4.1 Experimental arm (Meriva)

Meriva® (Indena S.p.A. Milan, Italy) 1,000 mg (active experimental group) (Indena s.p.a., Milan, Italy) was supplied was administered before meals at a dose of 1000 mg twice daily 12 hours apart (total daily dose: Meriva 2000 mg, corresponding to 400 mg curcuminoids).

Participants were encouraged to take Meriva at the same time each day, according to the most convenient time for them. All tablets were produced and sponsored by the manufacturer who otherwise had no role in the study design, data collection, data analysis, data interpretation or publication of this manuscript. Placebo and Meriva tablets contained the same ingredients except for curcuminoids. For simplicity, Meriva tablets will be referred to as curcumin in the following. Compliance will be considered acceptable if more than 75% of dosages will be ingested. Participants reported missed dosages, concomitant medication, and adverse events during the trial.

5.4.2 Control arm (placebo, inactive)

Matching placebo (Indena S.p.A. Milan, Italy)) consist of identically appearing , colourless, tasteless tablets packaged and administered in an identical manner to the active comparator,. Placebo pills and active comparator have identical composition, except for the active components in Meriva, such as curcuminoids .

5.4.3 Concomitant therapy and medications

No dose reductions of Meriva or placebo will be allowed throughout the 72-week treatment period. Previous treatment with oral antidiabetic drugs (metformin, sulfonylureas, insulin, DPP-IV inhibitors) was continued at the same dose in participants with T2DM at randomisation. The reported rate of hypoglycaemia in literature, with curcumin is very low and not different from placebo[3,4]. However In the event of recurrent major hypoglycaemic episodes (requiring medical or hospital intervention), the dose of the sulfonylurea will be reduced by 50% at the discretion of the investigators. In the event of recurrent major hypoglycaemic episodes in which no dose reduction could be undertaken (ie, not on a sulfonylurea) the participant will be withdrawn from treatment at the discretion of the chief investigator.

Glycaemic control will be assessed at each 12-weekly trial visit with self-measured plasma glucose readings and HbA1c. In the event that glycaemic control deteriorates, defined as HbA1c >9% (75 mmol/mol), the participant will be informed and counselled with regard to starting open labelled long-acting insulin detemir once daily (Levemir) or other allowed drugs in accordance with European guidelines (http://www.ema.europa.eu) to ensure that the participant's standard of diabetes care is not significantly compromised as a result of participating in the clinical trial.

The HbA1c cut-off >9% was based on the opinions of the TMG (GM and RG), consisting of expert endocrinologists and in accordance with previous clinical trial guidance[30].

In addition to study medications, participants will receive standard National Health Services (NHS) care recommendations concerning life-style modifications (ie, exercise, weight loss and dietary modification) and management of various coexisting illnesses throughout the trial.

Patients will be asked to limit alcohol consumption to less than 20 mg/day for women (ie, 14 units/week) and 30 mg/day for men (ie, 21 units/week), consistent with current guidelines[31]

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Participants will not be allowed any new prescription or over-the-counter therapies (ie, herbal remedies, milk thistle) that may improve or worsen NASH throughout the duration of the trial, including nutraceuticals of any type containing curcumin.

Potential NASH therapies that will not be allowed during the trial duration included thiazolidinediones (TZDs), GLP-1 receptor agonists, SGLT2 inhibitors, vitamin E, orlistat or other experimental therapies for NASH. Steroids (oral or intravenous), methotrexate and/or amiodarone will also not permitted based on their ability to promote hepatic steatosis.

5.5 Randomization, stratification and blinding

Randomization and stratification

Participants meeting all the eligibility criteria and providing written informed consent will be randomized centrally on a 1:1 basis to either of the two-study treatments (Meriva vs placebo) using computer generated randomization. To ensure even distribution between groups in key baseline features, randomization will be stratified into 16 blocks of 4 according to the following variables: diabetes status (present vs. absent), fibrosis stage (stage F3-4 vs. stage F0-2), CKD(present vs. absent), obesity status(present vs. absent),. Trial participants will be allocated a unique trial identification number to preserve patient confidentiality and enable the study to be double-blinded. Patients, investigators, assessors, trial site staff, and the sponsor (except for specific laboratory staff and individuals responsible for safety) remained blinded to treatment assignment throughout the trial.

Medication preparation and blinding/unblinding procedures

Both active treatment (Meriva) and placebo control will be packaged and labelled with a unique identification number (in keeping with the European Unions Good Manufacturing Practice for Medicinal Product

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guidelines) by the manufacturer (Indena S.p.A.), to the extent that the receiving trial site will be blinded to the study drug throughout the duration of the trial. Sealed parcels (containing electronic information) will be sent with each drug package for the attention of the unblinded members of the central trial management group (TMG) nominated statistician, RG and database programmer, EP, to ensure (1) safe delivery of the correct drug and (2) blinding of the treatment allocation from the remainder of the TMG and the trial patient. Unblinding of treatment will only take place if the identity of the allocated study medication will be necessary for patient safety and care. If a serious adverse event (SAE) is deemed unexpected and possibly, probably or definitely related to Meriva (ie, suspected unexpected serious adverse reaction=SUSAR), a clinical member of the TMG was unblinded to the medication to evaluate causality and subsequently label the event either as an unrelated SAE (for patients receiving placebo) or a SUSAR (for patients receiving Meriva). The latter will be reported to the MHRA and the NRES, and only if patient safety is jeopardised the study medication will be discontinued and the treating clinician/patient informed.

6. Trial schedule and data collection

The trial will involve nine patient-related visits at their nearest trial site (**Figure 1**).

The study comprises four stages: (1) screening, enrollment, randomisation and baseline investigations (visits 1 and 2, over a maximum period of 2 weeks), (2) 504 days of study treatment (visits 3–7, over 72 weeks), (3) primary endpoint assessment including liver biopsy (visit 8, within 1 day of the EOT) and (4) post-treatment follow-up assessment (visit 9, 12 weeks after EOT). If the trial investigating team or the trial participant suspected an AE, an unscheduled visit was arranged within 24 h. Each visit included patient interview, clincal examination, anthropometry and blood/urine chemistry.

The schedule for the study visits and data collection is summarised in **Figure 1**. All participants were asked to attend each visit under fasting from eating or drinking (with exception of water and due medications) for a minimum of 8 h prior to each visit. A follow-up liver biopsy (ie, primary endpoint) was obtained under ultrasound-guidance after completion of the 72-week study treatment. Wherever possible, a 16-gauge biopsy needle and a specimen length of a minimum of 15 mm are preferred. The liver tissue will bes prepared at the local trial site in preparation for histological assessment (under

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light microscopy) at the central trial site at HUMANITAS Gradenigo hospital. On receipt, the 'blinded' central histopathologist (RP) will record the size and quality of the histology slides. A minimum of four unstained slides were available for each liver biopsy to enable repeat staining (H&E, haematoxylin van Gieson, Ubiquitin) to ensure adequate quality for interpretation. Liver biopsy tissue specimens will be collected, paraffin-fixed and stored at the diagnostic archive of the Department of cellular pathology (HUMANITAS Gradenigo hospital). Fasting serum and plasma samples were collected at each of the 9 visits, will be partly used for routine biochemistry and partly stored frozen in 0.5–1.0 mL aliquots at –80°C at HUMANITAS Gradenigo Hospital. Additional blood (buffer coat) will be obtained at visits 1 and 8 for future DNA extraction and stored at –80°C.

Treatment compliance

Treatment compliance will be monitored at each visit by a review of the returned sealed envelopes and pill count and refill history per computer record. Participants were instructed to bring their medication bottles to each visit. Pill counts were calculated as the number of pills taken (the number of pills dispensed – the number of pills counted). The number of pills expected to have been taken was calculated by multiplying the daily dose by the number of days since the date dispensed. We a prior defined successful adherence on pill counts as 85–100% of the pills taken during each follow-up period . Adherence per refill history was determined by assessing the electronic pharmacy record system to quantify the number of pills dispensed relative to time. Adherence was noted if all of the patient's chronic medication refills were consistent with the number of days dispensed by the pharmacy, always within 15 days.

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Figure 1: schematic of the trial design.

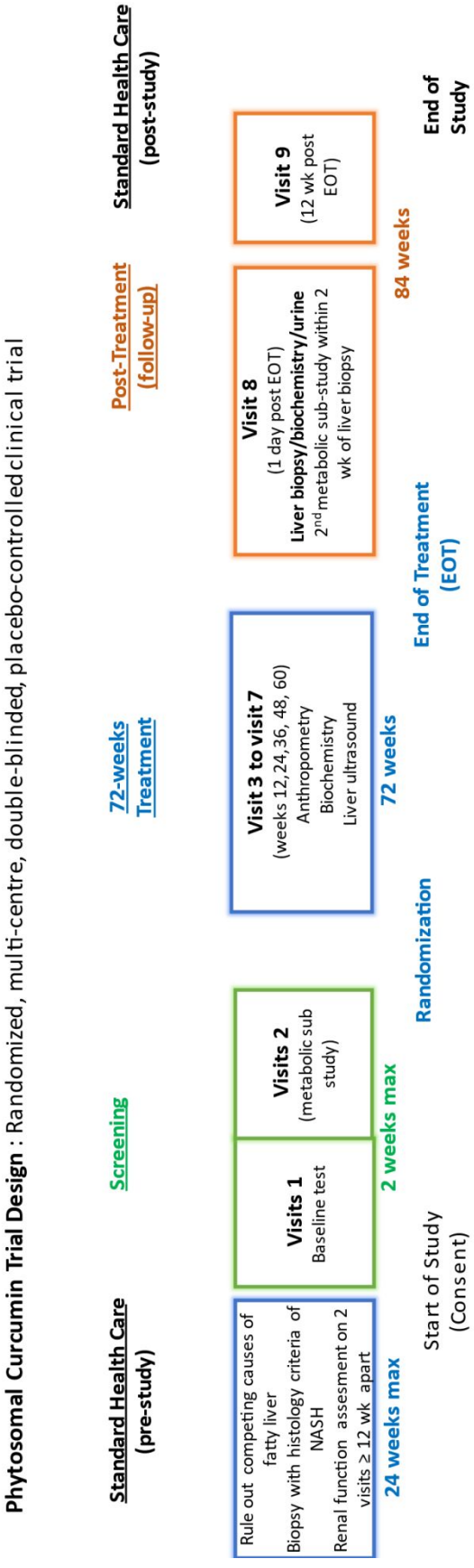

Figure 1 Schematic of phytosomal curcumin efficacy and action in non -alcoholic steatohepatitis trial design. Eligible participants are randomly assigned to a 72-week treatment of phytosomal curcumin 2 g daily or placebo control. Both the trial investigators and the participants are blinded to drug allocation.

7. Study outcome measures: primary and secondary outcomes, adverse events(AEs) reporting and analysis

Primary outcome measure

Histological NASH resolution (defined by the NASH Clinical Research Network as no more than mild residual inflammatory cells [score of 0 or 1] and no hepatocyte ballooning [score of 0]) **and** no worsening of liver fibrosis (with worsening defined as an increase of one stage or more on the Kleiner fibrosis classification scale) after 72 weeks, in line with regulatory perspectives[32,33] .

All screening, baseline and EOT liver biopsies will be assessed centrally by one c liver histopathologist (RP) at Humanitas Gradenigo, blinded to study treatment allocation, clinical or laboratory information), and each other's assessments.

Secondary outcome measures

Liver-centered histological outcomes:

1) a ≥1 stage improvement in fibrosis and no worsening of NASH (with worsening defined as an increase of ≥1 point in either the lobular inflammation score or the hepatocyte ballooning score according to the NASH Clinical Research Network criteria) after 72 weeks

2)NASH resolution **and** a ≥1 stage improvement in liver fibrosis

3)regression of clinically significant (i.e. stage≥2) fibrosis[34], defined by the proportion of patients with baseline fibrosis stage≥2 who have fibrosis stage 0-1 at EOT, without worsening of NASH.

4)regression of advanced (i.e. stage≥3) fibrosis, defined by the proportion of patients with baseline fibrosis stage 3-4 who have fibrosis stage 0-2 at EOT, without worsening of NASH.

5)a ≥2-point improvement in NAFLD activity score(NAS), with at least 1 point improvement in either ballooning or lobular inflammation score;

6)individual components of the NAFLD activity score (steatosis, hepatocyte ballooning, lobular inflammation) and the Kleiner fibrosis stage. Fibrosis stages 1a, 1b, and 1c were considered stage 1 for the purposes of analysis.

Liver-centered non-histological outcomes:

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4)changes from baseline to 72 weeks in serum liver enzyme concentrations, improvement in noninvasive markers of liver disease severity, including computerized sonographic hepato-renal ratio (which correlates closely with histologic and ^{1}H magnetic resonance spectroscopy steatosis [35,36, 37,,38,39], see **Analytical Methods subheading**), serum cytokeratin-18 fragments(a marker of NASH), NAFLD fibrosis score, FIB-4 and serum lumican (as markers of fibrosis)(see supplementary material).

The extracellular matrix proteoglycan lumican was chosen because its circulating levels have been shown to be a reliable marker not only of hepatic fibrosis but also of active hepatic fibrosis deposition, its expression is upregulated by Nf-kB, and its levels have been shown to differentiate histologically progressive, fibrosing NASH from mild, nonprogressive NAFLD[40,41,42]

Non liver-centered outcomes

-Improvement in anthropometry, physical activity and dietary parameters (body weight, BMI, waist circumference), physical activity and daily dietary consumption.

-Improvement in glycaemic control (fasting plasma glucose, HbA1c)

-Improvement in cardiovascular risk profile: fasting plasma lipids, oxidative stress markers, inflammatory markers/adipokines and chemokine MCP-1.

-Chronic kidney disease (CKD) regression. CKD will be defined according to Kidney Disease Improving Global Outcomes (KDIGO) guidelines as sustained (i.e., documented on 2 occasions at least 3 months apart) reduction in estimated Glomerular Filtration Rate [eGFR] <90 mL/min/1.73 m^2 **and/or** sustained increase in albumin excretion rate (AER)≥30 mg/d.

CKD regression will be defined as sustained (i.e., documented 3 month apart, on both follow-up visit 7 and visit 8) estimated Glomerular Filtration Rate [eGFR] ≥90 mL/min/1.73 m^2 **and** normalization of AER (i.e., <30 mg/d).

eGFR will be assessed from serum creatinine using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation, as recommended by KDIGO guidelines[43]. eGFR and AER will be classified according to KDIGO categories[44]

-Change in absolute values and in KDIGO categories of eGFR[45,41] and of albumin excretion rate(AER).

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We will also estimate mean annual rates of change in eGFR from baseline to the final follow-up visit (total slope), within each treatment allocation, after adjustments for age, baseline eGFR, baseline urinary AER, diabetes, baseline fibrosis stage, systolic blood pressure, angiotensin-converting enzyme/angiotensin receptor blocker use[46](see Statistics section)

Exploratory outcomes

We will explore the effect of treatment on Nuclear Factor(NF)-kB activation in the liver and in circulating mononuclear cell(MNCs). NF-kB is a key proinflammatory transcription factor involved in NASH development and progression and a known molecular target of curcumin (see Methods and supplementary material).

Safety outcomes

Safety end points will include adverse events after the start of treatment, biochemical assessments, and clinical assessments. Selected events (including deaths, cardiovascular events, and acute pancreatitis) were adjudicated by an independent, external event-adjudication committee, whose members were unaware of the treatment assignments.

AE reporting and analysis

The reporting period for AEs will start at screening visit 1 and will continue until follow-up visit 9. SAEs were reported until day 504 (week 72) of the trial treatment and for 84 days post-EOT. All SAEs and adverse reactions will be evaluated by the investigators and recorded. The National Cancer Institute's common terminology criteria for AEs (CTCAE, V.4.02, 2010) will be used to grade each AE.

An AE will be deemed serious if it results in hospitalization or a visit to the emergency room where an intervention is made to avert hospitalization. The nature and number of all serious adverse events was adjudicated by an independent data management committee (DMC) who is unaware of treatment assignment at the time of ascertainment.

The central trial office (CRCTU, Turin) will keep detailed records of all AEs reported (nature, onset, duration,severity, outcome) and perform an evaluation with respect to seriousness, causality and expectedness.

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Interim analysis of safety data will be performed and presented to the independent data management committee (DMC) on a 6-monthly basis. The unblinded DMC advises accordingly with regards to the trial conduct and specifically whether extra/new data monitoring will be required for the remainder of the trial. The DMC operated in accordance with a trial specific charter based on a predefined template.

8. Analytical Methods

8.1 Liver histopathology and immunohistochemistry

8.1.1 Liver histopathology

One liver histopathologist (RP) with specific expertise in NAFLD, blinded to the clinical, laboratory and study treatment allocation, will perform all the histopathological assessments at the central trial site (HUMANITAS Gradenigo) using an in-house designed proforma. The histological diagnosis of NASH will be established using H&E staining and haematoxylin van Gieson stains of formalin-fixed paraffin-embedded liver tissue. Both the baseline and end-of-treatment (72 weeks) biopsies will be reported as either 'definite NASH' 'uncertain NASH' or 'not NASH.' The histological diagnosis of 'definite NASH' is defined as a combination of >5% macrovesicular steatosis, hepatocyte ballooning (± Mallory's Hyaline) and lobular inflammation (mixed infiltrate, related to foci of ballooning)[47]. The assessment of ballooning is subjective, and thus for 'uncertain' hepatocyte ballooning, a key component of the diagnosis of NASH, ubiquitin immunohistochemistry will be used to identify material compatible with Mallory's hyaline.

To validate the quality of the biopsy specimen, the core specimen length will be measured and the number of portal tracts will be recorded.

The NAS will be calculated based on the Kleiner classification[48] The NAS is scored of 8, with 8 representing the highest activity. The NAS is the sum of scores of the 3 components of the histological scoring system, namely steatosis (0=<5%, 1=5–33%, 2=>33–66%, 3=>66%), lobular inflammation

(0=no foci, 1=<2 foci/×200, 2=2–4 foci/×200, 3=>4 foci) and hepatocyte ballooning (0=none, 1=few ballooned cells, 2=many cells/prominent ballooning). The Kleiner scoring system for NAFLD fibrosis (F0-

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F4) will be used to evaluate the stage of fibrosis in each biopsy specimen. Portal tract changes (inflammation, interface hepatitis, ductular reaction), an intrinsic feature of NASH, will also be recorded[49].

The pathologists will score each histological feature of liver biopsies and fill in separate forms.

8.1.2 Potential relevance of Nuclear Factor κB (NF-κB) activation in the liver and mononuclear cells (MNCs) for NASH pathogenesis and curcumin treatment

The pro-inflammatory transcription factor Nuclear Factor(NF)-κB is a master regulator at the crossroads of metabolic, oxidative, immune and inflammatory pathways and a known molecular target of curcumin[3,50]. NF-κB activation in all liver cell subtypes and in peripheral mononuclear cells (MNCs) plays a central role in NASH pathogenesis and progression and in NAFLD-associated metabolic abnormalities[51, 52, 53,54, 55]. We will therefore assess the effect of treatment on NF-κB activation in the liver and in circulating MNCs.

Nuclear Factor(NF)-κB activation in the liver by immunohistochemistry

The ImmunoCruz® Staining System will be used in deparaffinized liver tissue according to the manufacturer's instructions (Santa Cruz Biotechnology, Santa Cruz, CA). Briefly, slides will be soaked in 3% hydrogen peroxide for 5 min, washed, and incubated in serum blocking solution for 20 min. Specimens will be then incubated with primary antibodies for 2 h at 37◦ C. Tissue samples will be probed with mouse monoclonal antibodies reactive to NF-*κ*B p65 (Santa Cruz Biotechnology). After rinsing, specimens will be incubated with biotinylated secondary antibody and a horseradish peroxidase-streptavidin complex, for 30 min each. Tissue samples will be then colorized with DAB substrate, counterstained, mounted, and examined. NF-*κ*B immunoreactivity will be expressed as the % of positive cells/high-power field (×400)[56] and will also be semiquantitatively evaluated using a 4-point scoring system (0—no staining; 1—positive staining in <30% of cells/high-power field; 2— positive staining in 30–70% of cells/high-power field; 3—positive staining in >70% of cells/high-power field).

Nuclear Factor(NF)-κB activation in mononuclear cells (MNCs)

Blood samples will be collected in tubes containing Na-EDTA as an anticoagulant; 3.5 mL of the anticoagulated blood sample will be layered over 3.5 mL of the PMNL isolation medium (Robbins Scientific

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Corp, Sunnyvale, CA). Samples will be centrifuged at 450 x *g* in a swing outrotor for 30 min at 22 °C. At the end of centrifugation, 2 bands separate out at the top of the red blood cell pellet. The top band consists of monocuclear leukocytes(MNCs), whereas the bottom band consists of polymorphumuclear leukocytes(PMNs). The MNC and PMN bands will be harvested with a Pasteur pipette, repeatedly washed with Hank's balanced salt solution, and reconstituted to a concentration of 4 x 10^5 cells/mL in Hank's balanced salt solution. This method yields> 95% pure PMN and MNC suspensions57.

NF-kBp50/p65 transcription factor assay kit was purchased from Cayman Chemical (Ann Arbor MI, USA). The method detects specific transcription factor DNA binding activity in nuclear extracts and cell lysates. A specific double stranded DNA sequence containing the NF-kB response element is immobilized onto the bottom of wells of a 96 well-plate. NF-kB in nuclear or cytoplasmatic extract binds specifically to the NF-kB response element. NF-kBp50/65 is detected by addition of specific primary antibody directed against NF-kBp50/65. A secondary antibody conjugated to Horseradish peroxidase is added to provide a sensitive colorimetric readout at 450 nm.

Other clinical and laboratory data

Fasting blood samples will be analysed for full blood count, urea, creatinine and electrolytes, thyroid stimulating hormone (TSH), lipid profile (total cholesterol, high density lipoprotein, triglycerides), liver function tests, prothrombin time, international normalised ratio (INR), amylase, α-fetoprotein, C reactive protein, HbA1c, Monocyte chemoattractant protein-1(MCP-1), and plasma glucose using standard laboratory methods (Roche Modular system, Roche Ltd, Lewes, UK). Urinary creatinine and albumin will be assayed using Beckman-Coulter DxC700 clinical chemistry analysers.

Serum Insulin (Mercodia, Uppsala, Sweden), nonesterified fatty acids (Zen-Bio, Research Triangle Park, North Carolina, USA) and CK-18 M30 (M30 Apoptosense ELISA Kit; PEVIVA AB, Bromma, Sweden) lumican and adiponectin will be measured in-house using commercially available colorimetric ELISAs. Serum caspase-cleaved cytokeratin-18 (CK-18 M30) was performed at study entry to assess hepatic apoptosis. HOMA-IR, a marker of insulin resistance, will be calculated in the standard

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fashion: glucose×insulin/22.5.

Adipose tissue insulin resitance (ADIPO-IR) will be calculated (adipose IR=fasting FFA x fasting plasma insulin), as previously described and validated in diabetic and nondiabetic subjects[58, 59].

Measurements of weight (kg), height, systolic/diastolic blood pressure and waist:hip circumferences were recorded. Waist and hip circumferences were defined as

the circumferential measurements immediately above the level of the iliac crests and at the level of the greater trochanters, respectively. BMI was defined as weight in kilograms divided by the square of the height in metres (kg/m2).

Determination of plasma curcumin and curcumin metabolites

We used a validated HPLC-MS/MS method to simultaneously measure plasma curcumin metabolites from blood samples of all included patients during the oral fat tolerance test and of the oral glucose tolerance test[60, 61,62].

To investigate curcumin and curcumin metabolite kinetics after coingestion with fat or glucose, on the day of each of the two oral tolerance tests (OGTT and OFTT) at entry and at EOT patients took two pills of their curcumin/placebo formulations together with the glucoe or fat meal, and blood samples were subsequently drawn at the predefined times(see below). These data will be presented with the metabolic substudy in a separate report.

Dietary and physical activity record

Dietary record with food frequency questionnaire. Subjects will be instructed to fill in a 7-day validated and reproducible dietary questionnaire during an individual training session with a nutritionist; a list of foods is designed, for each item different portion sizes are specified according to the EPIC study([63]). The diet record was analyzed using the WinFood database (Medimatica, Teramo, Italy) according to the table of food consumption of the Italian National Institute of Nutrition and Food Composition Database for

Epidemiological Study in Italy[64]. Based on FFQ data, excessive alcohol use will be defined as .30 g/d for male individuals and .20 g/d for female individuals.

The FFQ will be completed at baseline (visit 1) and at end-of-treatment (visit 7). Analysis will report the change from baseline scores to the end-of-treatment .

Physical activity record.

Physical activity (PA) will be measured with the accelerometer SenseWear Armband (SWA), which had to be worn continuously on the nondominant arm for at least 3 consecutive days (2 working days and a week-end day) at baseline and at EOT. The SenseWear Armband (SWA) is a relatively new accelerometer device that can be used to quantify and monitor physical activity and energy expenditure (EE), which has been validated against indirect calorimetry[65] and has been shown to have acceptable reliaiability and reproducibility. The SWA is designed to be worn on the upper arm over the triceps. Its internal sensors include an accelerometer, a thermal flow sensor, a galvanic sensor that records skin response, a skin temperature sensor, and an air temperature sensor. The accelerometer in the armband has two axes and uses a microelectromechanical sensor that measures movement.

Physical activity is measured relative to gravity (1 mg=9.81 mm/sec2) with an interval of 20 ms and categorized based on intensity, according to White et al.[66] , into time spent in sedentary behavior (,48 mg) and in light (48–154 mg, e.g., walking), moderate (154–389 mg, e.g., cycling), and vigorous activities (.389 mg e.g., running). The software created by the manufacturer calculates then EE using a patented algorithm that combines acceleration, heat flow, and other parameters: technical and statistical procedures have been described in detail earlier[67, 68, 69].

PA record will be completed at baseline (visit 1) and at end-of-treatment (visit 7). Analysis will report the change from baseline scores to the end-of-treatment .

Upper abdomen ultrasound and computerized sonographic hepato-renal ratio for quantification of steatosis

All examinations will be performed at each patient visit with a GE Vivid7 ultrasound machine (GE Healthcare,

Horten, Norway) equipped with a GE 4C curved array transducer (GE H4904PC). US studies will be performed by two experienced radiologists (GM and EP) who were unaware of the patient's clinical details and laboratory findings. All the instrument settings, including "gain," "depth," and "time-gain compensation," will be preset and fixed for each measurement. For assessment of US H/R ratio, ultrasound images with both liver and right kidney clearly visualized will be obtained in the sagittal liver/right kidney view in the lateral position. US hepatic echo-intensity attenuation rate was assessed in right intercostals
view at anterior axilla line in the supine position. All images will be reviewed by one of the two radiologists involved in scanning. Analysis of digitized ultrasound images was performed by using NIHimage software (ImageJ 1.41o, National Institutes of Health, Bethesda, MD).

US hepatic/renal echo-intensity ratio

In sagittal liver/right kidney view, a region of interest (ROI) of 1.5 × 1.5 cm (1,296 pixels) in the liver parenchyma was selected. This area is large enough to calculate an average histogram value and small enough to avoid the inclusion of vessels or bile ducts in the specimen. Several ROI parameters were displayed including circumference and area, total brightness level, mean brightness level, SD, most frequent brightness level, and a histogram. Of all these
parameters, we used only the mean brightness level for each organ (liver and right kidney) in this study.
Technically, the echo intensity can be influenced by many factors, particularly by gain intensity.
To avoid confounding by factors that can modify the echo intensity and thus bias comparisons,
the mean brightness levels of both the liver and the right kidney cortex were obtained on the
same longitudinal sonographic plane. The ratio between the mean brightness level of the liver and the right kidney was calculated manually to determine the hepatorenal sonographic index.
In each case, the calculation of the hepatorenal sonographic index was repeated at least twice.
When the difference was less than 0.20, the average was calculated. If there was a greater discrepancy, a third measure was performed and the average of the two closest measurements was used.
The ROI had to be as uniform as possible, excluding blood vessels, bile ducts, and other focal hypo/hyperechogenicity. Another ROI of 0.5 × 0.5 cm (144 pixels) was identified in the right renal cortex with no large vessels, renal sinus or medulla. To avoid the interference of depth-depended echo-intensity attenuation and the borderline echo distorting effects, the boundary between liver and right kidney area should

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be placed near the center of the image, and the liver and right kidney ROIs were selected at the same depth of the ultrasound images. The gray scale mean value of the mixels within the two ROIs was used as measurement of echo intensity. Then we divided the average hepatic gray scale by the average renal cortex gray scale to calculate the US hepatic/renal ratio. US hepatic echo-intensity attenuation rate

In right intercostals view at anterior axilla line, a tangent line of the sector ultrasound image was drawn and the ultrasound wave transmission line was determined, starting from the point of tangency and perpendicular to the tangent line. Two ROIs of 1.5 × 1.5 cm (1,296 pixels) were selected in liver homogeneous regions along the ultrasound transmission line near the liver anterior margin (depth 4–6 cm) and the liver posterior margin, respectively. The linear distance

between the two ROIs was also measured.

The echo intensity of ultrasound wave was attenuated exponentially, shown as the following equation:

$$A\ A\ a\ f\ d$$

$$d = 0 \times e - \cdot \cdot (1)$$

Where $A0$ and Ad are ultrasound echo intensity at the sound source and the liver parenchyma at a specific depth, respectively; a is the attenuation coefficient of the liver parenchyma; f is the frequency of the ultrasound detector; d is the depth of ROI. The ratio of the average echo intensity in the liver near-field ROI to liver far-field ROI was then calculated based on equation (1):

$$A\ A\ a\ f\ f\ n$$

$$n\ f$$

$$d\ d = e \cdot \cdot (-) \ (2)$$

Where An and Af are average ultrasound echo intensity in the near-field ROI and the far-field ROI, respectively; a and f have been defined in equation (1); dn and df are the depth of liver near-field and far-field ROIs.

8.4 Metabolic substudy

ORAL FAT TOLERANCE TEST (OFTT). Within 3 months from entry, all patients will undergo a standard oral fat load test.

Participants will be encouraged to avoid strenuous physical efforts and to follow their usual diet

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during the 24 hours preceding the test. The fat load consists of a mixture of 200 ml dairy cream (35% fat) and 26 g egg yolk for a total energy content of 766 kcal.: 78.3 g fat (55.6% saturated fatty acids, 29.6% monounsaturated fatty acids, 14.8% polyunsaturated fatty acids), 595 mg cholesterol, 8.8 g protein, 7 g carbohydrate. The fat load will be consumed over 5 minutes; subjects will be kept

fasting on the test morning, and strenuous activity will be forbidden.

Samples will be drawn at 0 (baseline), 2, 4, 6, and 8 hours. Plasma total cholesterol (Chol), triglyceride (Tg), and free fatty acids (FFA) will be measured by automated enzymatic methods. Details on analytical methods and lipid measurements will be provided in a dedicated report.

ORAL GLUCOSE TOLERANCE TEST (OGTT). Within 3 months from entry into the trial, patients will undergo a standard 75-g oral-glucose-tolerance test (OGTT), with measurement of plasma glucose, insulin, C-peptide, FFA. Areas under the concentration curves (AUCs) of different parameters during the OGTT will be calculated with the trapezoidal method. A Minimal Model analysis will be undertaken to calculate parameters related to glucose homeostasis and detailed in a dedicated report.

9. Statistical analyses

9.1 Power analysis

Sample size justification. There are no data on liver histological response to curcumin.

The sample size was calculated using A'Hern's single stage phase II methodology[70] with a significance level of 0.05 (type I error) and power of 0.90 (type II error).

Based on previous trials showing an improvement in surrogate markers of NAFLD with curcumin ranging 20- 50% of patients, depending on different liver disease surrogates, curcumin dose, formulations and treatment duration vs. 15% with lifestyle intervention[71], the design required 21 evaluable patients in the Meriva group; after allowing for a 10% dropout rate, at least 25 subjects per arm were needed to detect a significant difference in the primary outcome. The study A'Hern's design stipulates that at least eight (38%) out of 21 patients in the Meriva group have to achieve histological improvement for Meriva to be deemed worthy of further investigation with a phase III trial [84]

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9.2 Methods for analysis of primary and secondary outcomes

All evaluable patients will be analysed on an intention-to-treat basis. Evaluable patients are defined as those who have had an end-of-treatment biopsy, irrespective of the amount of treatment they have received. Patients will be categorised as either achieving the primary histological endpoint (ie, NASH resolution) or not. The proportion of patients with a reported improvement in liver histology will be presented and compared across treatments descriptively with 95% CIs.

The primary analysis will be based on a Cochran–Mantel–Haenszel test based on all randomised patients for the in-trial period with missing data handled as non-responders. The common risk ratio between Meriva and placebo was estimated along with exact 95% CI. To test for superiority, the exact two-sided p-value was calculated as the sum of probabilities of outcomes having equal or lower probability than the observed outcome under the null hypothesis.

Adjustments for multiplicity testing in the primary analysis will be performed with the use of the Benjamini-Hochberg procedure; each active-treatment group was compared with the placebo group. Two-sided P values <0.05 were considered statistically significant.

An unpowered, preplanned secondary analysis of categorical outcome measures will be done using the $\chi 2$ test or Fisher exact test, as appropriate, of the difference between the proportions of patients with histological improvement in each treatment group.

A preplanned logistic regression analysis will be undertaken to determine the treatment effect when adjusted for the stratification variables (diabetes status, obesity status, fibrosis stage, CKD status) and trial site. We will calculate adjusted relative risks using the Mantel-Haenszel test. Two-sided P values<0.05 will be considered statistically significant in all analyses.

Continuous secondary outcome measures will be compared between treatment groups using linear

regression, adjusting for parameter baseline values and allocated treatment (as model covariates, equivalent to ANCOVA), with multilevel modelling for key continuous outcome measures to account for repeated measures within each patient.

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For eGFR, we will estimate mean annual rates of change in eGFR from baseline to the final follow-up visit (total slopes), by treatment allocation using multilevel modelling; adjustments will be made for variables *a priori* known to be associated with CKD progression (age, sex, baseline CKD stage, baseline fibrosis stage, baseline eGFR, baseline urinary AER, diabetes, history of cardiovascular disease, systolic blood pressure, angiotensin-converting enzyme/angiotensin receptor blocker use, HbA1c changes[72]

Albumin excretion rate(AER) will be estimated using measurements of urinary protein and creatinine at baseline and at baseline and at each visit thereafter.

AER and eGFR stage will be categorized according to the KDIGO classification system[73]

The end-stage renal disease was defined as kidney transplantation or the receipt of dialysis for at least 30 days unless death occurred.

For exploratory outcomes, we will explore the existence of a threshold in hepatic NF-kB activation change predicting histological response(NASH resolution, fibrosis improvement) using the area under receiver operating characteristic curve (AUROC) analysis and Youden index.

A sensitivity analysis will be performed in which missing data were handled by reference-based multiple imputation informed by data from placebo recipients (**see Preplanned subgroup and sensitivity analyses subheading**).

Safety will be analysed descriptively

9.3 Methods in analysis to handle missing data

The primary imputation method for missing data will the non-responder imputation for primary and secondary dichotomous outcomes. This approach does not rely on an assumption of missing at random

and is considered conservative for estimating the treatment effect.

To investigate the sensitivity of the histological results with regard to the handling of missing

data, post-hoc sensitivity analyses using multiple imputation will be conducted. These analyses

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are based on the same CMH method as for the main analyses but using a multiple imputation at random method for missing data(see following Subheading)

9.5 Prespecified additional subgroup and sensitivity analyses

As previously specified, the primary imputation method for missing data will the non-responder imputation for primary and secondary dichotomous outcomes.

To investigate the sensitivity of the results of the primary analysis with regard to the handling of missing data, a sensitivity analysis using imputation based on unconditional reference was performed for the primary and secondary outcomes. The analysis is based on the same type of non-parametric method as for the primary analysis but with missing data handled by a method of multiple imputation which assumes that the unobserved outcomes are well described by the observed outcomes of the subjects in the placebo arm with similar baseline characteristics. The imputation is done by random sampling of observed outcomes from placebo subjects with the same baseline diabetes status, obesity status, fibrosis stage and CKD status. Multiple (100) replicates of a complete data set will be generated and, for each complete data set, the log risk ratios will be estimated together with the asymptotic standard errors. The estimates and standard errors for the 100 complete data sets were pooled using Rubin's rule to obtain a single estimate of the risk ratio and a 95% confidence interval. The analysis differs from the primary analysis in that it assumes that subjects with missing week 72 data had the same chances of NASH resolution as subjects with week 72 data in the placebo group. If there will be more missing week 72 data in the Meriva group than in the placebo group, this analysis would be less conservative than the primary analysis and probably gives a better estimate of the treatment effect. The handling of missing data is analogous to the "copy reference" approach for longitudinal normal data presented by Carpenter et al.[74]

We also plan to perform the following subgroup analyses to assess the effect of the following characteristics on main efficacy and safety outcomes:

-trial site

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-diabetes status, obesity status, CKD status, fibrosis stage (F3-4 vs F0-2)

-per protocol analysis (only patients who completed treatment, had EOT liver biopsy and no major protocol deviations will be included).

Statistical heterogeneity will quantified using the I^2 statistic with values of 25%, 50%, and 75% consistent with low, moderate, and high degrees of heterogeneity, respectively[75]

All analyses will be carried out with Easy R ver1.61, Saitama, Japan[76].

10. Ethics and Dissemination

10.1 Data collection and patient consent and confidentiality

Data will be collected from patient paper sanitary record into an electronic case report form (CRF) consisting of an excel datasheet file, where each patient will be deidentified and coded by a unique, computer-generated number. CRFs included baseline/follow-up medical history and physical examinations to capture comorbidities and concomitant medications in the trial's electronic database. Other CRFs incorporated in the electronic database included: laboratory tests and questionnaire results were recorded for visit 1 (eligibility criteria) through to visit 8; safety monitoring during the treatment follow-up periods; central site histopathology reports of liver biopsy specimens; specialist non-invasive markers of liver disease; AE reporting and study drug dispensing forms for study treatment adherence and accountability.

. No patient identifiers will leave hospital unit. Patient confidentiality will be maintained by keeping data collected in the study coded. The key will be at the HUMANITAS Gradenigo Hospital, and will be managed by the principal investigator after EOT and unblinding. No Personal detail or identifying data will be transferred from the patient sanitary file to the CRF which will be available for data analysis.

Data management was undertaken according to the standard operating procedures of the CRCTU at HUMANITAS Gradenigo hospital.. The CRCTU followed the International Conference on Harmonisation Good Clinical Practice (ICH GCP). The CRCTU was responsible for monitoring the trial and providing annual reports to the sponsor. Participant identifiable data were shared only within the clinical team on a need-to-

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know basis to provide clinical care, and to ensure good and appropriate follow-up and the primary care practitioners. All participants provided specific written consent at trial entry to enable data to be shared with the above. Otherwise, confidentiality was maintained throughout the trial and thereafter. On completion of the trial, data will be transferred to a secure archiving facility at HUMANITAS Gradenigo hospital, where data will be held for a minimum of 10 years and then destroyed

10.2 Sponsorship, indemnity and monitoring

HUMANITAS Gradenigo acted as the sponsor of the trial. As sponsor Humanitas was responsible for the general conduct of the study and indemnified the trial centre against any claims, arising from any negligent act or omission by the University in fulfilling the sponsor role in respect to the study. Both on-site and off-site monitoring of the trial were performed as perthe Trial Quality Management Plan.

10.3 Role of the funding source

The manifactures funding te trial (Indena SpA) had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had access to all data in the study and had fi nal responsibility for the decision to submit for publication.

10.4 Competing interest

All participating investigators have no conflict of interest related to this study.

10.5 Dissemination

We plan to publish the results of this controlled trial to an international journal following peer review.

11. History of changes

06/01/2022: the recruitment end date was changed from 01/01/2022 to 01/06/2022 to complete patient enrollment and achieve the planned study sample size

11/09/2023: the recruitment start date was changed from 01/12/2018 to 20/12/2021 due to technical issues with patient enrollment and with performing planned study outcome measures at study sites during the COVID-19 pandemic.

For Peer Review

For Peer Review

[1]Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67:328-357.

[2]Kanwal F, Shubrook JH, Adams LA, Pfotenhauer K, Wai-Sun Wong V, Wright E, Abdelmalek MF, Harrison SA, Loomba R, Mantzoros CS, Bugianesi E, Eckel RH, Kaplan LM, El-Serag HB, Cusi K. Clinical Care Pathway for the Risk Stratification and Management of Patients With Nonalcoholic Fatty Liver Disease. Gastroenterology. 2021;161:1657-1669.

[3] Musso G, Cassader M, Cohney S, De Michieli F, Pinach S, Saba F, Gambino R. Fatty Liver and Chronic Kidney Disease: Novel Mechanistic Insights and Therapeutic Opportunities. Diabetes Care. 2016 Oct;39(10):1830-45

[4]Musso G, De Michieli F, Bongiovanni D, Parente R, Framarin L, Leone N, Berrutti M, Gambino R, Cassader M, Cohney S, Paschetta E. New Pharmacologic Agents That Target Inflammation and Fibrosis in Nonalcoholic Steatohepatitis-Related Kidney Disease. Clin Gastroenterol Hepatol. 2017;15:972-985.

[5]Musso G, Cassader M, Gambino R. PNPLA3 rs738409 and TM6SF2 rs58542926 gene variants affect renal disease and function in nonalcoholic fatty liver disease. Hepatology. 2015 Aug;62(2):658-9

[6]Musso G, Gambino R, Tabibian JH, Ekstedt M, Kechagias S, Hamaguchi M, Hultcrantz R, Hagström H, Yoon SK, Charatcharoenwitthaya P, George J, Barrera F, Hafliðadóttir S, Björnsson ES, Armstrong MJ, Hopkins LJ, Gao X, Francque S, Verrijken A, Yilmaz Y, Lindor KD, Charlton M, Haring R, Lerch MM, Rettig R, Völzke H, Ryu S, Li G, Wong LL, Machado M, Cortez-Pinto H, Yasui K, Cassader M. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. PLoS Med. 2014 Jul 22;11(7):e1001680

[7]Sun DQ, Targher G, Byrne CD, Wheeler DC, Wong VW, Fan JG, Tilg H, Yuan WJ, Wanner C, Gao X, Long MT, Kanbay M, Nguyen MH, Navaneethan SD, Yilmaz Y, Huang Y, Gani RA, Marzuillo P, Boursier J, Zhang H, Jung CY, Chai J, Valenti L, Papatheodoridis G, Musso G, Wong YJ, El-Kassas M, Méndez-Sánchez N, Sookoian S, Pavlides M, Duseja A, Holleboom AG, Shi J, Chan WK, Fouad Y, Yang J, Treeprasertsuk S, Cortez-Pinto H, Hamaguchi M, Romero-Gomez M, Al Mahtab M, Ocama P, Nakajima A, Dai C, Eslam M, Wei L, George J, Zheng MH. An international Delphi consensus statement on metabolic dysfunction-associated fatty liver disease and risk of chronic kidney disease. Hepatobiliary Surg Nutr. 2023 Jun 1;12(3):386-403

[8]Singal AK, Hasanin M, Kaif M, Wiesner R, Kuo YF. Nonalcoholic Steatohepatitis is the Most Rapidly Growing Indication for Simultaneous Liver Kidney Transplantation in the United States. Transplantation. 2016 ;100: 607-12.

[9]Musso G, Cassader M, Gambino R. Non-alcoholic steatohepatitis: emerging molecular targets and therapeutic strategies. Nat Rev Drug Discov. 2016;15:249-74

[10]Musso G, De Michieli F, Bongiovanni D, Parente R, Framarin L, Leone N, Berrutti M, Gambino R, Cassader M, Cohney S, Paschetta E. New Pharmacologic Agents That Target Inflammation and Fibrosis in Nonalcoholic Steatohepatitis-Related Kidney Disease. Clin Gastroenterol Hepatol. 2017;15:972-985.

[11]Xu XY, Meng X, Li S, Gan RY, Li Y, Li HB. Bioactivity, Health Benefits, and Related Molecular Mechanisms of Curcumin: Current Progress, Challenges, and Perspectives. Nutrients. 2018;10: 1553

[12]Baker RG, Hayden MS, Ghosh S. NF-κB, inflammation, and metabolic disease. Cell Metab. 2011;13:11-22

[13]Shehzad A, Ha T, Subhan F, Lee YS. New mechanisms and the anti-inflammatory role of curcumin in obesity and obesity-related metabolic diseases. Eur J Nutr. 2011; 50: 151-61

[14]Xu XY, Meng X, Li S, Gan RY, Li Y, Li HB. Bioactivity, Health Benefits, and Related Molecular Mechanisms of Curcumin: Current Progress, Challenges, and Perspectives. Nutrients. 2018;10: 1553.

[15]Kang Q, Chen A. Curcumin eliminates oxidized LDL roles in activating hepatic stellate cells by suppressing gene expression of lectin-like oxidized LDL receptor-1. Lab Invest. 89:1275-90, 2009

[16]Tang Y, Zheng S, Chen A. Curcumin eliminates leptin's effects on hepatic stellate cell activation via interrupting leptin signaling. Endocrinology 150:3011-20, 2009

[17]Weisberg SP, Leibel R, Tortoriello DV. Dietary curcumin significantly improves obesity-associated inflammation and diabetes in mouse models of diabesity. Endocrinology. 2008;149:3549-58.

[18]Vizzutti F, Provenzano A, Galastri S, Milani S, Delogu W, Novo E, Caligiuri A, Zamara E, Arena U, Laffi G, Parola M, Pinzani M, Marra F. Curcumin limits the fibrogenic evolution of experimental steatohepatitis.Lab Invest. 2009 Nov 9. [Epub ahead of print]PMID: 19901911

[19]Jian-Mei Li, Yu-Cheng Li, Ling-Dong Kong, and Qing-Hua Hu. Curcumin Inhibits Hepatic Protein-Tyrosine Phosphatase 1B and Prevents Hypertriglyceridemia and Hepatic Steatosis in Fructose-Fed Rats. Hepatology 2010;51:1555-1566

[20]Ngu MH, Norhayati MN, Rosnani Z, Zulkifli MM. Curcumin as adjuvant treatment in patients with non-alcoholic fatty liver (NAFLD) disease: A systematic review and meta-analysis. Complement Ther Med. 2022 ;68:102843.

[21]Wei Z, Liu N, Tantai X, Xing X, Xiao C, Chen L, Wang J. The effects of curcumin on the metabolic parameters of non-alcoholic fatty liver disease: a meta-analysis of randomized controlled trials. Hepatol Int. 2019 ;13:302-313

[22] Cuomo J, Appendino G, Dern AS, et al. Comparative absorption of a standardized curcuminoid mixture and its lecithin formulation. *J Nat Prod.* 2011;74: 664–669.

[23]Ma Z, Wang N, He H, Tang X. Pharmaceutical strategies of improving oral systemic bioavailability of curcumin for clinical application. J Control Release. 2019;316: 359-380

[24]Hegde M, Girisa S, BharathwajChetty B, Vishwa R, Kunnumakkara AB. Curcumin Formulations for Better Bioavailability: What We Learned from Clinical Trials Thus Far? ACS Omega. 2023 Mar 13;8(12):10713-10746

[25]Hu S, Belcaro G, Dugall M, Peterzan P, Hosoi M, Ledda A, Riva A, Giacomelli L, Togni S, Eggenhoffner R, Cotellese R. Interaction study between antiplatelet agents, anticoagulants, thyroid replacement therapy and a bioavailable formulation of curcumin (Meriva®). Eur Rev Med Pharmacol Sci. 2018;22:5042-5046

[26]Belcaro G, Cesarone MR, Dugall M, Pellegrini L, Ledda A, Grossi MG, Togni S, Appendino G. Efficacy and safety of Meriva®, a curcumin-phosphatidylcholine complex, during extended administration in osteoarthritis patients. Altern Med Rev. 2010;15:337-44.

[27]Allegri P, Mastromarino A, Neri Management of chronic anterior uveitis relapses: efficacy of oral phospholipidic curcumin treatment. Long-term follow-up. Clin Ophthalmol. 2010 ;4: 1201-6.

[28] Pivari F, Mingione A, Piazzini G, Ceccarani C, Ottaviano E, Brasacchio C, Dei Cas M, Vischi M, Cozzolino MG, Fogagnolo P, Riva A, Petrangolini G, Barrea L, Di Renzo L, Borghi E, Signorelli P, Paroni R, Soldati L. Curcumin Supplementation (Meriva®) Modulates Inflammation, Lipid Peroxidation and Gut Microbiota Composition in Chronic Kidney Disease. Nutrients. 2022;14: 231

[29]Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21.

[30]Health Canada. Standards for clinical trials in type 2 diabetes in Canada. 2007. http://www.hc-sc.gc.ca

[31]Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67: 328-357.

[32]Food and Drug Administration. Noncirrhotic nonalcoholic steatohepatitis with liver fibrosis: developing drugs for treatment. Guidance for industry: draft guidance. December 2018 (https://www.fda .gov/media/119044/download).

[33]European Medicines Agency. Reflection paper on regulatory requirements for the development of medicinal products for chronic non-infectious liver diseases (PBC, PSC, NASH). November 2018 (https://www .ema.europa.eu/en/documents/scientific -guideline/reflection-paper-regulatory -requirements-development-medicinal -products-chronic-non-infectious-liver_en .pdf).

[34]Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, Kleiner DE, Loomba R. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology. 2023 May 1;77(5):1797-1835

[35]Webb M, Yeshua H, Zelber-Sagi S, Santo E, Brazowski E, Halpern Z, Oren R. Diagnostic value of a computerized hepatorenal index for sonographic quantification of liver steatosis. AJR Am J Roentgenol. 2009 Apr;192(4):909-14

[36]Xia MF, Yan HM, He WY, Li XM, Li CL, Yao XZ, Li RK, Zeng MS, Gao X. Standardized ultrasound hepatic/renal ratio and hepatic attenuation rate to quantify liver fat content: an improvement method. Obesity (Silver Spring). 2012 Feb;20(2):444-52.

[37]Mancini M, Prinster A, Annuzzi G, Liuzzi R, Giacco R, Medagli C, Cremone M, Clemente G, Maurea S, Riccardi G, Rivellese AA, Salvatore M. Sonographic hepatic-renal ratio as indicator of hepatic steatosis: comparison with (1)H magnetic resonance spectroscopy. Metabolism. 2009 ;58:1724-30.

For Peer Review

[38]von Volkmann HL, Havre RF, Løberg EM, Haaland T, Immervoll H, Haukeland JW, Hausken T, Gilja OH. Quantitative measurement of ultrasound attenuation and Hepato-Renal Index in Non-Alcoholic Fatty Liver Disease. Med Ultrason. 2013 Mar;15(1):16-22.

[39]Borges VF, Diniz AL, Cotrim HP, Rocha HL, Andrade NB. Sonographic hepatorenal ratio: a noninvasive method to diagnose nonalcoholic steatosis. J Clin Ultrasound. 2013 Jan;41(1):18-25

[40]Decaris ML, Li KW, Emson CL, Gatmaitan M, Liu S, Wang Y, Nyangau E, Colangelo M, Angel TE, Beysen C, Cui J, Hernandez C, Lazaro L, Brenner DA, Turner SM, Hellerstein MK, Loomba R. Identifying nonalcoholic fatty liver disease patients with active fibrosis by measuring extracellular matrix remodeling rates in tissue and blood. Hepatology. 2017 ;65:78-88.

[41]Krishnan A, Li X, Kao WY, Viker K, Butters K, Masuoka H, Knudsen B, Gores G, Charlton M. Lumican, an extracellular matrix proteoglycan, is a novel requisite for hepatic fibrosis. Lab Invest. 2012;92:1712-25.

[42]Charlton M, Viker K, Krishnan A, Sanderson S, Veldt B, Kaalsbeek AJ, Kendrick M, Thompson G, Que F, Swain J, Sarr M. Differential expression of lumican and fatty acid binding protein-1: new insights into the histologic spectrum of nonalcoholic fatty liver disease. Hepatology. 2009;49:1375-84

[43]Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) : A new equation to estimate glomerular filtration rate. *Ann Intern Med* 150: 604–612, 2009

[44] Lameire NH, Levin A, Kellum JA, Cheung M, Jadoul M, Winkelmayer WC, Stevens PE; Conference Participants. Harmonizing acute and chronic kidney disease definition and classification: report of a Kidney Disease: Improving Global Outcomes (KDIGO) Consensus Conference. Kidney Int. 2021 Sep;100(3):516-526

[45]Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) : A new equation to estimate glomerular filtration rate. *Ann Intern Med* 150: 604–612, 2009

[46]Heerspink HJL, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou FF, Mann JFE, McMurray JJV, Lindberg M, Rossing P, Sjöström CD, Toto RD, Langkilde AM, Wheeler DC; DAPA-CKD Trial Committees and Investigators. Dapagliflozin in Patients with Chronic Kidney Disease. N Engl J Med. 2020 Oct 8;383(15):1436-1446.

[47]Sanyal AJ, Brunt EM, Kleiner DE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. Hepatology 2011;54:344–53.

[48]Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21.

[49]Brunt EM, Kleiner DE, Wilson LA, et al. Portal chronic inflammation in nonalcoholic fatty liver disease (NAFLD): a histologic marker of advanced NAFLD-clinicopathologic correlations from the nonalcoholic steatohepatitis clinical research network. Hepatology 2009;49:809–20.

For Peer Review

[50]Muñoz A, Costa M. Nutritionally mediated oxidative stress and inflammation._Oxid Med Cell Longev. 2013;2013:610950

[51]Franceschetti L, Bonomini F, Rodella LF, Rezzani R. Critical Role of NFκB in the Pathogenesis of Non-alcoholic Fatty Liver Disease: A Widespread Key Regulator. Curr Mol Med. 2021;21(6):495-505

[52]Yang B, Yang X, Tan X, Lu L, Fan W, Barbier-Torres L, Steggerda J, Liu T, Yang H. Regulatory Networks, Management Approaches, and Emerging Treatments of Nonalcoholic Fatty Liver Disease. Can J Gastroenterol Hepatol. 2022 Nov 8;2022:6799414

[53]Vonderlin J, Chavakis T, Sieweke M, Tacke F. The Multifaceted Roles of Macrophages in NAFLD Pathogenesis. Cell Mol Gastroenterol Hepatol. 2023;15(6):1311-1324.

[54]Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M. IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med. 2005 Feb;11(2):191-8

[55]Mussbacher M, Derler M, Basílio J, Schmid JA. NF-κB in monocytes and macrophages - an inflammatory master regulator in multitalented immune cells. Front Immunol. 2023 Feb 23;14:1134661

[56]Ribeiro PS, Cortez-Pinto H, Solá S, Castro RE, Ramalho RM, Baptista A, Moura MC, Camilo ME, Rodrigues CM. Hepatocyte apoptosis, expression of death receptors, and activation of NF-kappaB in the liver of nonalcoholic and alcoholic steatohepatitis patients. Am J Gastroenterol. 2004;99:1708-17.

[57]Ghanim H, Abuaysheh S, Sia CL. Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells following a high-fat high-carbohydrate meal: implications for insulin resistance. Diabetes Care 2009;32:2281–2287

[58]Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, et al. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. Gastroenterology 2007;133: 496-506.

[59]Gastaldelli A, Harrison SA, Belfort-Aguilar R, Hardies LJ, Balas B, Schenker S, Cusi K. Importance of changes in adipose tissue insulin resistance to histological response during thiazolidinedione treatment of patients with nonalcoholic steatohepatitis. Hepatology. 2009 ;50: 1087-93.

[60]Kroon MAGM, van Laarhoven HWM, Swart EL, Kemper EM, van Tellingen O. A validated HPLC-MS/MS method for simultaneously analyzing curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetra-hydrocurcumin and piperine in human plasma, urine or feces. Heliyon. 2023 Apr 20;9(5):e15540

[61]Zhang ZB, Luo DD, Xie JH, Xian YF, Lai ZQ, Liu YH, Liu WH, Chen JN, Lai XP, Lin ZX, Su ZR. Curcumin's Metabolites, Tetrahydrocurcumin and Octahydrocurcumin, Possess Superior Anti-inflammatory Effects *in vivo* Through Suppression of TAK1-NF-κB Pathway. Front Pharmacol. 2018 Oct 17;9:1181

[62]Luo DD, Chen JF, Liu JJ, Xie JH, Zhang ZB, Gu JY, Zhuo JY, Huang S, Su ZR, Sun ZH. Tetrahydrocurcumin and octahydrocurcumin, the primary and final hydrogenated metabolites of curcumin,

For Peer Review

possess superior hepatic-protective effect against acetaminophen-induced liver injury: Role of CYP2E1 and Keap1-Nrf2 pathway. Food Chem Toxicol. 2019 Jan;123:349-362.

[63] Sacerdote C, Fiorini L, Dalmasso M. Alimentazione e rischi di cancro. Indagine su un campione di 10054 volontari residenti nell'area torinese. Torino: AGAT 2000.

[64] Carnovale E, Marletta P. Food composition table. Istituto Nazionale della Nutrizione. Milano: EDRA 1997 .

[65]Mielke GI, de Almeida Mendes M, Ekelund U, Rowlands AV, Reichert FF, Crochemore-Silva I. Absolute intensity thresholds for tri-axial wrist and waist accelerometer-measured movement behaviors in adults. Scand J Med Sci Sports. 2023 Sep;33(9):1752-1764

[66]White T, Westgate K, Wareham NJ, et al. Estimation of physical activity energy expenditure during free-living from wrist accelerometry in UK adults. PLoS One 2016;11(12):e0167472.

[67]Santos-Lozano A, Hernández-Vicente A, Pérez-Isaac R, Santín-Medeiros F, Cristi-Montero C, Casajús JA, Garatachea N. Is the SenseWear Armband accurate enough to quantify and estimate energy expenditure in healthy adults? Ann Transl Med. 2017 Mar;5(5):97. doi: 10.21037/atm.2017.02.31

[68]Lopez GA, Brønd JC, Andersen LB, Dencker M, Arvidsson D. Validation of SenseWear Armband in children, adolescents, and adults. Scand J Med Sci Sports. 2018 Feb;28(2):487-495.

[69]Bhammar DM, Sawyer BJ, Tucker WJ, Lee JM, Gaesser GA. Validity of SenseWear® Armband v5.2 and v2.2 for estimating energy expenditure. J Sports Sci. 2016 Oct;34(19):1830-8.

[70]A'Hern RP. Sample size tables for exact single-stage phase II designs. Stat Med 2001;20:859–66.

[71]Ampuero J, Gallego-Durán R, Maya-Miles D, Montero R, Gato S, Rojas Á, Gil A, Muñoz R, Romero-Gómez M. Systematic review and meta-analysis: analysis of variables influencing the interpretation of clinical trial results in NAFLD. J Gastroenterol. 2022;57:357-371.

[72] Heerspink HJL, Stefánsson BV, Correa-Rotter R, Chertow GM, Greene T, Hou FF, Mann JFE, McMurray JJV, Lindberg M, Rossing P, Sjöström CD, Toto RD, Langkilde AM, Wheeler DC; DAPA-CKD Trial Committees and Investigators. Dapagliflozin in Patients with Chronic Kidney Disease. N Engl J Med. 2020;383:1436-1446.

[73]Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl. 2013:1-150.

[74]Carpenter JR, Roger KH, Kenward MG. Analysis of longitudinal trials with protocol deviation: a framework for relevant, accessible assumptions, and inference via multiple imputation, J Biopharm Statistics. 2013; 23:1352-1371.

For Peer Review

[75]Turner RM, Davey J, Clarke MJ, Thompson SG, Higgins JP. Predicting the extent of heterogeneity in meta-analysis, using empirical data from the Cochrane Database of Systematic Reviews. Int J Epidemiol. 2012; 41:818–27.

[76]Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48:452-8

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Appendix 2. Supplementary material

Effect of phospholipid curcumin Meriva® on liver histology and kidney disease in Nonalcoholic Steatohepatitis

A randomized, double-blind, placebo-controlled trial

Content

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Appendix 2 Figure 1: schematic of the trial design.

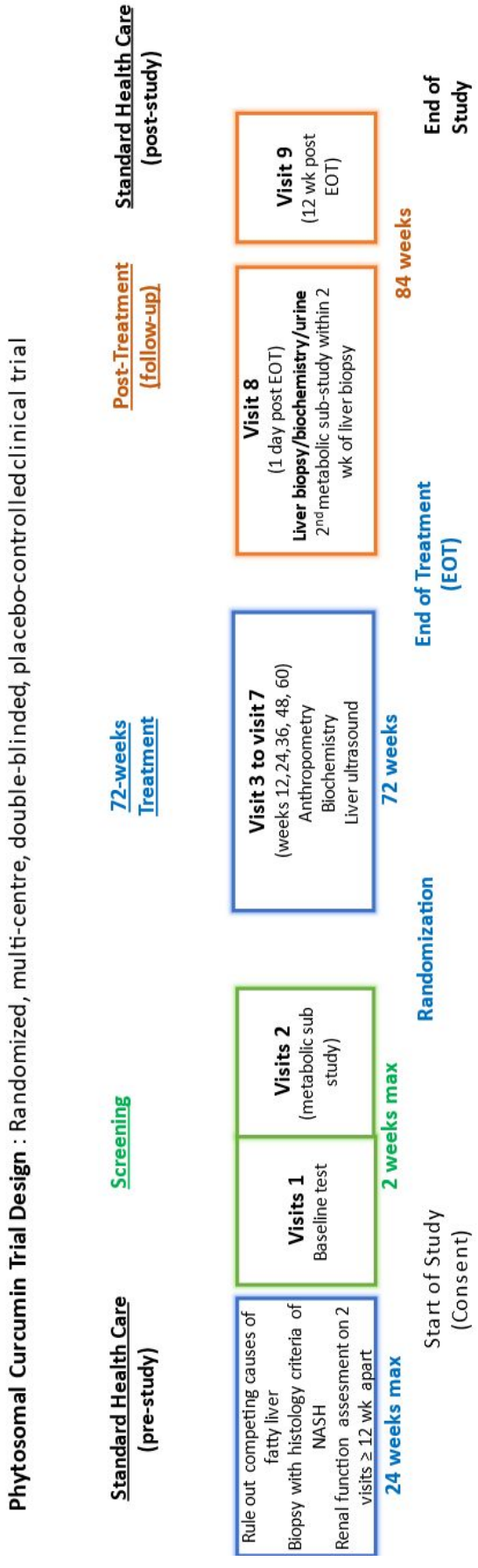
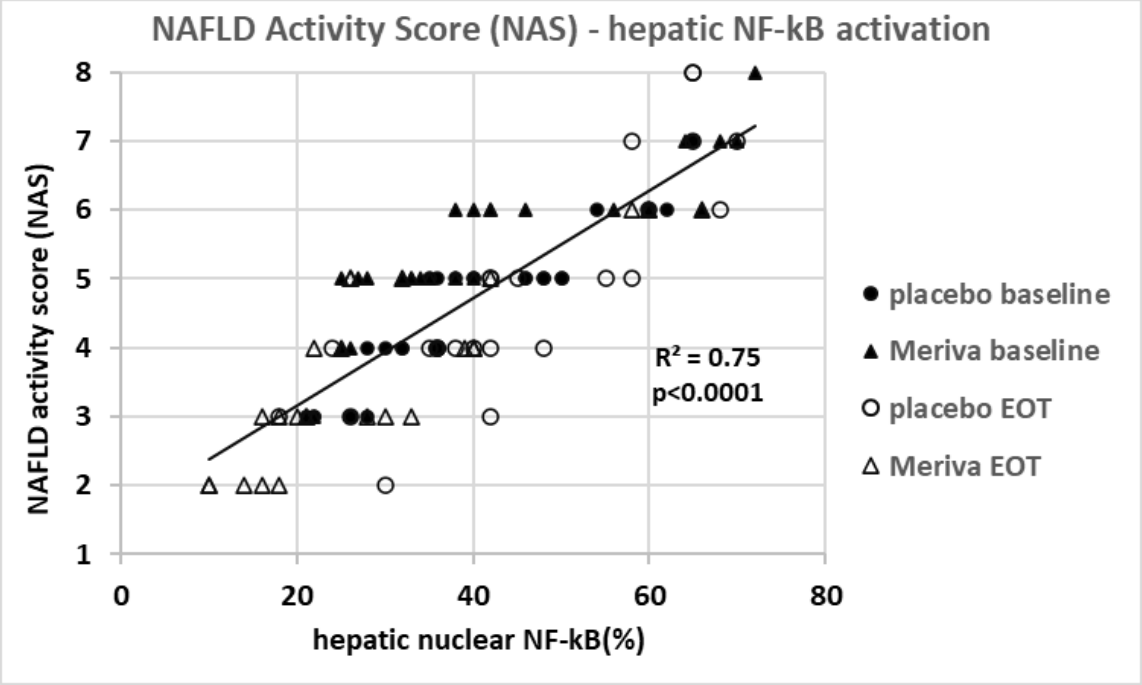


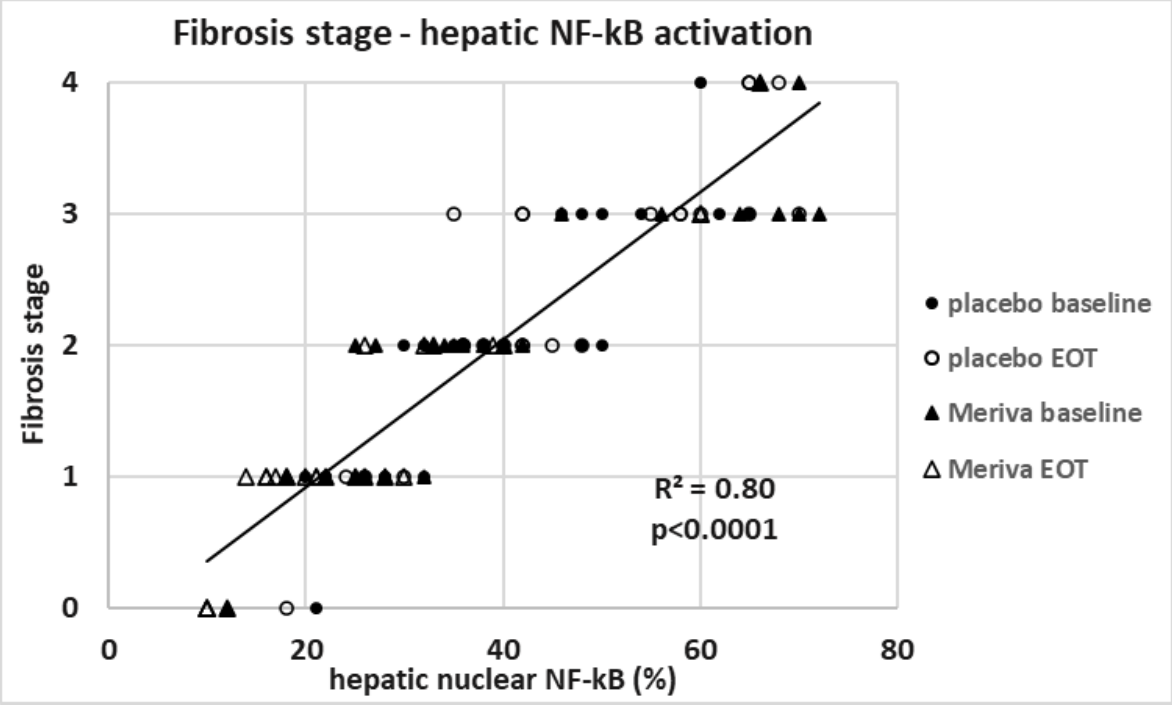
Figure 1 Schematic of phytosomal curcumin efficacy and action in non -alcoholic steatohepatitis trial design. Eligible participants are randomly assigned to a 72-week treatment of phytosomal curcumin 2 g daily or placebo control. Both the trial investigators and the participants are blinded to drug allocation.

Appendix 2 Figure 2. Correlation between hepatic NF-kB activation, NAFLD activity score(NAS) and Fibrosis stage at baseline and at end of treatment (EOT) in the whole study population (n=51).

Panel A: correlation between NAFLD activity score (NAS) and hepatic NF-kB activation



Panel B: correlation between Fibrosis stage and hepatic NF-kB activation



Appendix 2 Table 1. Exclusion criteria from the study

2

1. Refusal or lacks capacity to give informed consent to participate in the trial.
2. Participation in any clinical trial of an investigational therapy or agent within 12 months of randomisation.
3. Patient (or carer) deemed not competent at using the correct site and technique for subcutaneous injection of the trial treatment (containing
dummy drug on practice).
4. NAFLD Activity Score (NAS) <3 on liver biopsy.
5. Child's B or C cirrhosis or clinical evidence of decompensated chronic liver disease: radiological or clinical evidence of ascites, current or previous hepatic
encephalopathy and evidence of portal hypertensive haemorrhage or varices on endoscopy.
6. Medical history of multiple drug allergies (defined as anaphylactoid drug reactions in >2 drug groups) or allegry to curry or curcumin-based nutraceuticals
7. Presence of any acute/chronic infections or illness that at the discretion of the chief investigator might compromise the patient's health
and safety in the trial.
8. Pregnancy or breastfeeding.
9. Women, of childbearing age, who are not willing to practise effective contraception (ie, barrier, oral contraceptives, impenon or past
medical history of hysterectomy) for the 48-week duration of the trial and for 1 month after the last administration of the drug.
10. Liver disease of other aetiologies (ie, drug-induced, viral hepatitis, autoimmune hepatitis, PBC, PSC, haemochromatosis, A1AT deficiency, Wilsons disease).
11. Average alcohol consumption >20 g/d(males) and >10 g/d(females) (as assessed by a validated questionnaire(AUDIT-10) within the last 5 years.
12. Medical/surgery history of; gastric bypass surgery, orthotopic liver transplant (OLT) or listed for OLT, hepatocellular, pancreatic, thyroid carcinoma, acute or chronic pancreatitis and total parenteral nutrition within 6 months of randomisation.
13. Diagnosis of malignancy within the last 3 years (with the exception of treated skin malignancies).
14. Hepatocellular carcinoma: dysplastic or intermediate nodules to be excluded. Regenerative and other nodules to be included at the discretion of the chief investigator.
15. Alanine aminotransferase or aspartate aminotransferase >10×upper limit of normal.
16. >5% weight loss since the diagnostic liver biopsy was obtained.
17. Recent (within 3 months of the diagnostic liver biopsy or screening visit) or significant change (as judged by the chief investigator) in dose of the following drugs: inducers of hepatic steatosis (steroids (oral/intravenous), methotrexate, amiodarone), orlistat and/or multivitamins/
vitamin E (containing >200% recommended daily amount; >30 mg/day).
18. Known positivity for antibody to HIV.
19. Currently being treated with renal replacement therapy (ie, haemodialysis or peritoneal dialysis).
Specific exclusion criteria for participants with T2D
1. Participants receiving thiazolidinediones (TZDs), Sodium-glucose Cotransporter-2 (SGLT2) Inhibitors and GLP-1 analogue. These drugs were prohibited throughout the trial,.
3. HbA1c ≥10%.

Patients who met any of the criteria listed above at the screening visit were excluded from trial

participation.

Appendix 2 Table 2. Individual histological scores at baseline and at the end of treatment (EOT).

A Steatosis

Treatment	Baseline histology	EOT(week 72)				Mean change	% improved score
		Score 0 ()<5%	Score 1 (5-33%)	Score 2 (33-66%)	Score 3 (>66%)		
Meriva	Score 1 (5-33%)	1	4	1	0		
	Score 2 (33-66%)	1	6	1	1		
	Score 3 (>66%)	0	2	1	5		
						-1.3	70%
Placebo	Score 1 (5-33%)	0	4	3	0		
	Score 2 (33-66%)	0	4	5	1		
	Score 3 (>66%)	0	2	3	0		
						-0.4	37%
					p-value	<0.00001	0.04

B. Lobular inflammation

Treatment	Baseline histology	EOT(week 72)				Mean change	% improved score
		Score 0 (0 foci)	Score 1 (<2 foci/200x)	Score 2 (2-4 foci/200x)	Score 3 (>4 foci/200x)		
Meriva	Score 0 (0 foci)	0	0	0	0		
	Score 1 (<2 foci/200x)	5	9	4	0		
	Score 2 (2-4 foci/200x)	3	2	0	0		
	Score 3 (>4 foci/200x)	1	2	0	0		
						-0.70	71%
Placebo	Score 0 (0 foci)	0	0	0	0		
	Score 1 (<2 foci/200x)	0	13	3	0		
	Score 2 (2-4 foci/200x)	0	5	1	2		
	Score 3 (>4 foci/200x)	0	0	0	1		
						-0.15	29%
						<0.0001	0.01

2

C: Hepatocyte ballooning

Treatment	Baseline histology	EOT(week 72)			Mean change	% improved score
		Score 0 (none)	Score 1 (few cells)	Score 2 (many cells/prominent ballooning)		
Meriva	Score 0 (none)	0	0	0		
	Score 1 (few cells)	9	4	1		
	Score 2 (many cells/prominent ballooning)	7	2	3		
					-0.77	70%
Placebo	Score 0 (none)	0	0	0		
	Score 1 (few cells)	3	10	2		
	Score 2 (many cells/prominent ballooning)	0	2	8		
					-0.07	27%
					0.001	0.007

D: NAFLD Activity Score (NAS)

Treatment	Baseline histology	EOT(week 72)								Mean change	% improved score
		1	2	3	4	5	6	7	8		
Meriva	3	0	0	0	0	0	1	0	0		
	4	0	1	0	1	0	0	0	0		
	5	0	4	4	1	1	1	0	0		
	6	0	0	1	1	3	2	0	0		
	7	0	0	3	1	0	0	0	0		
	8	0	0	0	1	0	0	0	0		
										-2.81	79%
Placebo	3	0	0	2	2	1	0	0	0		
	4	0	0	1	1	1	0	1	2		
	5	0	1	1	23	3	2	2	0		
	6	0	0	0	3	0	0	0	0		
	7	0	0	0	1	0	0	0	0		
	8	0	0	0	0	0	0	0	0		
										-0.76	46%
									p-value	<0.0001	0.02

E: Kleiner Fibrosis stage

Treatment	Baseline histology	EOT(week 72)					Mean change	% improved score
		F0	F1	F2	F3	F4		
Meriva	F0	1	0	0	0	0		
	F1	3	3	0	0	0		
	F2	0	8	3	0	0		
	F3	0	3	2	1	0		
	F4	0	0	0	1	1		
							-0.79	46%
Placebo	F0	1	0	0	0	0		
	F1	0	3	2	2	0		
	F2	0	0	7	2	1		
	F3	0	0	0	5	2		
	F4	0	0	0	0	1		
							0.08	8%
					p-value		0.001	0.01

Appendix 2 Table 3. Individual stage of eGFR and of albumin excretion rate (AER) (according to KDIGO guidelines) at baseline and at the end of treatment (EOT).

2

A: eGFR stage

Treatment	eGFR stage at baseline	eGFR stage at EOT (week 72)						Mean change in eGFR	% improved
		G1	G2	G3a	G3b	G4	G5		
Meriva	G1	10	0	0	0	0	0		
	G2	10	1	0	0	0	0		
	G3a	3	2	0	0	0	0		
	G3b	0	0	0	0	0	0		
	G4	0	0	0	0	0	0		
	G5	0	0	0	0	0	0		
								+9.88	46%
Placebo	G1	10	0	0	0	0	0		
	G2	0	9	3	0	0	0		
	G3a	0	0	5	0	0	0		
	G3b	0	0	0	0	0	0		
	G4	0	0	0	0	0	0		
	G5	0	0	0	0	0	0		
								-10.32	0%
							p-value	<0.0001	0.001

B: AER stage

Treatment	AER stage at baseline	AER stage at EOT (week 72)			Mean change in AER	% improved
		A1	A2	A3		
Meriva	A1	10	0	0		
	A2	13	3	0		
	A3	0	0	0		
					-149	50%
Placebo	A1	10	1	0		
	A2	0	13	2		
	A3	0	0	0		
					+32	0%
					<0.0001	0.003

2

Appendix 2 Table 4. **Changes in daily intake of main dietary constituents and physical activity at end-of-treatment.**

	Placebo (n=25)	Meriva (n=26)
Dietary record		
Total energy Intake (kcal/d)	-193±42	-269±31
Kcal/kg BW/d	1± 1	2± 1
Fat (% kcal)	-5.1±0.8	-3.6±0.8
CHO (% kcal)	-8.7±1.1	-9.1±1.1
Fiber (g)	+6.5±2.2	+4.9±1.9
Protein (% kcal)	+1.8±0.7	+6.6±0.9
SFA (% total fat)	-4.9±0.4	-4.4±0.9
MUFA(% total fat)	-2.9±1.3	-7.2±1.4
PUFA(% total fat)	+4.1±0.8	+4.0±0.9
Physical activity(PA) categories		
Light PA (min/d)	+33.4(31.9)	+21.8(25.5)
Moderate PA(min/d)	+12.4(18.1)	+8.6(12.1)
Vigorous PA(min/d)	+3.2(8.1)	+4.1(7.3)
Sedentary time(min/d)	794.4(50.3)	789.8(60.4)

No significant difference between groups in any nutrient intake was found. Data are expressed as mean±SD.

Abbreviations: BW: body weight; CHO: carbohydrates; SFA: saturated fat; MUFA: monounsaturated fatty adics; PUFA Polyunsaturated fatty acids; P:S: polyunsaturated-to saturated fat

2

Appendix 2 Table 5: Logistic regression analysis in the whole study population with a post-treatment liver biopsy(n=51).

Outcome: NASH resolution			
Model		**Odds ratio (95% CI)**	**p-value**
Univariable analysis	Treatment (Meriva)	11.73 (2.77, 39.02)	**0.0008**
	Diabetes (Diabetic)	0.76 (0.31, 2.96)	0.841
	Obesity (obese)	0.88 (0.31, 4.96)	0.837
	CKD (present)	0.68(0.21, 1.39)	0.194
	Baseline Fibrosis (F3-4)	0.44 (0.13, 1.08)	0.091
	Trial site (HUMANITAS Gradenigo)	0.93 (0.30, 2.87)	0.871
Multivariable analysis	Treatment (Meriva) and diabetes and obesity and CKD and fibrosis(F3-4) and trial site	5.51 (1.39, 16.75)	**0.001**
Outcome: fibrosis improvement of ≥1 stage and no worsening of NASH			
Model		**Odds ratio (95% CI)**	**p-value**
Univariable analysis	Treatment (Meriva)	11.50 (2.24, 43.09)	**0.003**
	Diabetes (Diabetic)	0.78 (0.12, 4.19)	0.479
	Obesity (obese)	0.63 (0.15, 5.49)	0.639
	CKD (present)	0.59 (0.12, 4.68)	0.592
	Baseline Fibrosis (F3-4)	0.48 (0.62, 1.34)	0.214
	Trial site (HUMANITAS Gradenigo)	0.99 (0.80, 4.37)	0.871
Multivariable analysis	Treatment (Meriva) and diabetes and obesity and CKD and fibrosis(F3-4) and trial site	5.79 (1.34, 19.72)	**0.001**
Outcome: NASH resolution and fibrosis improvement of ≥1 stage			
Model		**Odds ratio (95% CI)**	**p-value**
Univariable analysis	Treatment (Meriva)	24.00 (2.82, 49.02)	**0.004**
	Diabetes (Diabetic)	0.77 (0.19, 4.13)	0.481
	Obesity (obese)	0.48 (0.09, 4.83)	0.837
	CKD (present)	0.42 (0.12-0.96)	**0.04**
	Baseline Fibrosis (F3-4)	0.48 (0.13, 1.71)	0.219
	Trial site (HUMANITAS Gradenigo)	0.98 (0.51, 3.21)	0.673
Multivariable analysis	Treatment (Meriva) and diabetes and obesity and CKD and fibrosis(F3-4) and trial site	4.28 (1.34, 6.43)	**0.009**
Outcome: regression of significant (stage F2-4 fibrosis) and no worsening of NASH			
Model		**Odds ratio (95% CI)**	**p-value**
Univariable analysis	Treatment (Meriva)	11.73 (2.77, 39.02)	**0.0008**
	Diabetes (Diabetic)	0.89 (0.48, 4.32)	0.729
	Obesity (obese)	0.68 (0.10, 6.79)	0.872
	CKD (present)	0.52(0.14-0.91)	**0.04**
	Trial site (HUMANITAS	0.99 (0.80, 4.37)	0.871

Gradenigo)			
Multivariable analysis	Treatment (Meriva) and diabetes and obesity and CKD and trial site	4.36 (1.45, 7.69)	**0.001**
Outcome: regression of CKD			
Model		**Odds ratio (95% CI)**	**p-value**
Univariable analysis	Treatment (Meriva)	51.99 (2.81, 59.02)	**0.001**
	Diabetes (Diabetic)	0.61 (0.18, 2.59)	0.692
	Obesity (obese)	0.48 (0.09, 4.83)	0.837
	Baseline Fibrosis (F3-4)	0.26 (0.07, 0.92)	**0.03**
	Trial site (HUMANITAS Gradenigo)	0.99 (0.80, 4.37)	0.871
Multivariable analysis	Treatment (Meriva) and diabetes and obesity and fibrosis(F3-4) and trial site	5.79 (1.34, 19.72)	**0.009**

Appendix 2 Table 6. Sensitivity analysis for selected, key histological, anthropometry and biochemical outcomes.

	Phytosomal Meriva (n=26)		Placebo (n=26)			
Parameter	**n (%) or mean (SD) change from baseline**	**Patients with missing values N(%)**	**n (%) or mean (SD)**	**Patients with missing values N(%)**	**Relative risks or mean changes (95% CI) from baseline to 72 weeks (Meriva *vs* placebo)**	**P between groups**
Patients with NASH resolution and no worsening of fibrosis	16 (62%)	0 (0%)	3 (12%)	1 (4%)	5.53 (1.79 to 12.95)	**0.002**
Δ NAFLD activity score (NAS)	-2.81(1.6)	0 (0%)	-0.76(0.9)	1 (4%)	-2.12(-2.92 to -1.41)	**<0.00001**
Patients with ≥1 stage fibrosis improvement and no worsening of NASH	12(46%)	0 (0%)	2 (8%)	1 (4%)	6.58(1.69 to 21.28)	**0.007**
Patients with NASH resolution and ≥1 stage fibrosis improvement	21 (79%)	0 (0%)	12 (46%)	1 (4%)	7.38 (1.99 to 23.41)	**0.002**
Patients with ≥1 stage fibrosis worsening	0	0 (0%)	4	1 (4%)	0.15 (0.01 to 2.81)	0.13
Patients with significant (stage F2-4) fibrosis regression and no worsening of NASH *	9(35%)	0 (0%)	0/26	1 (4%)	18.78 (1.51 to 35.12)	**0.018**
Patients with advanced (stage F3-4) fibrosis regression and no worsening of NASH ¶	5 (19%)	0 (0%)	0/8	1 (4%)	11.39 (0.69 to 82.12)	0.09
Δ hepatic NF-kB activation(% cells/HPF)	-21(11)	0 (0%)	-2(12)	1 (4%)	-23 (-29 to -18)	**<0.0001**
Δ ALT (IU/l)	-41 (28)	0 (0%)	-10 (26	1 (4%)	–31 (–44 to -19)	**<0.0001**
Δ Sonographic hepato/renal ratio	-0.68 (0.57)	2(8%)	-0.25 (0.43)	3(12%)	-0.46 (-0.77 to -0.23)	**0.001**
Δ CK-18 fragments(IU/l)	-189 (70)	4(16%)	+27 (43)	3(12%)	-218(-256 to -179)	**<0.0001**
Δ FIB-4	-0.3 (0.2)	1(4%)	0.1 (0.2)	1(4%)	–0.4 (–0.42 to -0·31)	**<0.0001**
Patients with CKD regression	13 (50%)	0(0%)	0 (0%)	1(4%)	10.79 (1.98 to 18.03)	**0.003**
Δ eGFR(ml/min 1.73 m²)	9.88 (7.12)	0 (0%)	-10.32 (11.24)	1(4%)	20.24 (15.12 to 25.39)	**<0.0001**
Δ LDL-C (mg/dL)	-26 (14)	0(0%)	13 (11)	1(4%)	-39(-46 to -36)	**<0.0001**

Δ HDL-C (mg/dL)	9 (3)	0(0%)	-1 (2)	1(4%)	10 (8 to 11)	**<0.0001**
Δ Triglyceride (mg/dL)	-27 (21)	0(0%)	9 (13)	1(4%)	-36 (-45 to -28)	**<0.0001**
Δ Glucose (mg/dL)	–12 (10)	0(0%)	5 (7)	1(4%)	–17 (–23 to –13)	**0.0009**
Δ HOMA-IR	-3.89 (2.92)	2(8%)	-1.32 (2.46)	3(12%)	–2.68 (-4.00 to -1.11)	**0.0008**
Δ HbA1c(%)	-0.63% (0.40%)	3(12%)	-0.03% (0.51%)	2(8%)	–0.60% (–0.85% to –0.39%)	**<0·0001**
Δ NF-kB activation in MNCs (% cells)	-19(12%)	0(0%)	-4%(9%)	1(4%)	-15 (-21 to -9)	**0.003**
Δ BMI (kg/m²)	–0.3 (1.1)	3(12%)	–0.2 (0.9)	3(12%)	–0.1 (–2·65 to 0·49)	0.639

Data are n (%) or mean (SD). Patients with missing values were defined as having at least 1 missing value in at least 1 scheduled study visit and/or at EOT. This method of sensitivity analysis is based on the same type of non-parametric method as for the primary analysis but with missing data handled by multiple imputation, which assumes that the unobserved outcomes are well described by the observed outcomes of the subjects in the placebo arm with similar baseline characteristics. The analysis differs from the primary analysis in that it assumes that subjects with missing week 72 data had the same chances of NASH resolution as subjects with week 72 data in the placebo group. If there will be more missing week 72 data in the Meriva group than in the placebo group, this analysis would be less conservative than the primary analysis and may give a better estimate of the treatment effect.

Δ: change from baseline

* Patients with clinically significant fibrosis regression were defined as having stage F2-4 fibrosis at baseline and stage F0-1 fibrosis at EOT.

¶ Patients with advanced fibrosis regression were defined as having stage F3-4 fibrosis at baseline and stage F0-2 fibrosis at EOT

Appendix 2 Table 7. Subgroup analyses of changes in primary and selected relevant secondary outcomes after 72 weeks of treatment for patients with or without type 2 DM (T2DM) at baseline

	T2DM present (n=25)				T2DM absent (n=27)			
Outcome	**Meriva (n=13)**	**Placebo (n=12)**	**RR or mean changes (95%CI)**	**P**	**Meriva (n=13)**	**Placebo (n=14)**	**RR or mean changes (95%CI)**	P
NASH resolution n(%)	8(62%)	1(8%)	7.38(1.08-24.68)	0.04	8(62%)	2(14%)	4.31(1.11-16.67)	0.03
Δ NAS	-2.39 (1.8)	-0.37 (0.7)	-2.01(-2.45, -1.47)	0.001	-2.84(1.7)	-0.42 (1.5)	-2.42(-2.98, -2.03	0.0009
≥1 stage fibrosis improvement n(%)	6	1	7.01(1.10-38.4)	0.04	7	1	7.00(1.01-39.7)	0.03
NASH resolution and ≥1 stage fibrosis improvement n(%)	7	0	7.43 (1.07-51.42)	0.04	6	1	7.54 (1.07-53.23)	0.04
Significant (stage F2-F4) fibrosis regression n(%)	6	0	7.00(1.01-28.54)	0.04	5	0	6.43(0.98-46.92)	0.06
Δ hepatic NF-kB activation (%cells/HPF)	-18(12)	-1(11)	-17(-26, -8)	0.0002	-23(14)	-4(12)	-19(-29, -9)	0.0002
CKD regression n(%)	6	0	7.00(1.01-28.54)	0.04	7	0	8.00(1.15-55.80)	0.04
Δ eGFR (ml/min/1.73 m2)	7.32(6.43)	-9.81 (7.47)	18.01 (6.13, 24.36)	0.0002	9.79(8.11)	-6.58 (6.32)	15.42 (9.49, 22.31)	0.003
Δ LDL-C (mg/dL	-24(13)	15(12)	-39(-48, -31)	0.0002	-28(14)	7(12)	-35(-46, -34)	0.0004
Δ HDL-C (mg/dL)	7(2)	-3(3)	10(10, 7)	0.009	11(13, 9)	-5(6)	16(19, 12)	0.002
Δ Tg (mg/dL)	-21(9)	11/10)	-31(-42, -22)	0.005	-29(13)	8(12)	-48(-55, -40)	0.004
Δ FPG (mg/dL)	-15(11)	8(9)	-23(-30, -7)	0.001	-9(7)	3(7)	-11(-16, -7)	0.002
Δ HbA1c (%)	-0.69(0.49)	0.11 (0.63)	-0.80(-1,25, -0.35)	0.0005	-0.58(0.40)	-0.03 (0.41)	-0.55(-0.68, 0.43)	-0.009
Δ HOMA–IR	-3.31(2.13)	-0.97 (1.73)	-2,28 (-3.89, -1.53)	0.003	-3.98(2.02)	-1.34 (1.62)	-2.54(-3.01, -2.09)	0.003
Δ BMI(kg/m²)	-0.2(1.2)	-0.1(1.1)	-0.1(-0.4, 0.20)	0.215	-0.7(0.9)	-0.5(1.0)	-0.2(-0.4, 0.2)	0.415
	Obesity present (n=37)				**Obesity absent (n=15)**			
Outcome	**Meriva (n=19)**	**Placebo (n=18)**	**RR or mean changes (95%CI)**	**P**	**Meriva (n=7)**	**Placebo (n=8)**	**RR or mean changes (95%CI)**	P
NASH resolution n(%)	9	2	4.50(1.13-17.99)	0.03	7	1	7.00(1.10-34.11)	0.04
Δ NAS	-2.45 (1.9)	-0.33 (0.8)	-2.21(-2.52, -1.52)	0.0008	-2.48(1.4)	-0.36 (1.4)	-2.12(-2.73, -2.00	0.0002
≥1 stage fibrosis improvement n(%)	7	1	8.00(1.11-47.77)	0.04	6	1	15.00(1.00-49.12)	0.04
NASH resolution and ≥1 stage fibrosis improvement n(%)	7	0	7.43 (1.07-51.42)	0.04	6	1	7.54 (1.07-53.23)	0.04
Significant (stage F2-F4) fibrosis regression n(%)	6	0	7.00(1.01-28.54)	0.04	5	0	6.43(0.98-46.92)	0.06
Δ hepatic NF-kB activation (%cells/HPF)	-18(12)	-1(11)	-17(-26, -8)	0.0002	-23(14)	-4(12)	-19(-29, -9)	0.0002
CKD regression n(%)	7	0	8.00(1.15-46.5)	0.04	6	0	7.00(1.10-34.51)	0.04
Δ eGFR (ml/min/1.73 m2)	7.32(6.43)	-9.81 (7.47)	18.01 (6.13, 24.36)	0.0002	9.79(8.11)	-6.58 (6.32)	15.42 (9.49, 22.31)	0.003
Δ LDL-C (mg/dL	-24(13)	15(12)	-39(-48, -31)	0.0002	-28(14)	7(12)	-35(-46, -34)	0.0004
Δ HDL-C (mg/dL)	7(2)	-3(3)	10(10, 7)	0.009	11(13, 9)	-5(6)	16(19, 12)	0.002
Δ Tg (mg/dL)	-21(9)	11/10)	-31(-42, -22)	0.005	-29(13)	8(12)	-48(-55, -40)	0.004
Δ FPG (mg/dL)	-15(11)	8(9)	-23(-30, -7)	0.001	-9(7)	3(7)	-11(-16, -7)	0.002
Δ HbA1c (%)	-0.69(0.49)	0.11	-0.80(-1,25,	0.0005	-0.58(0.40)	-0.03	-0.55(-0.68,	-0.009

		(0.63)	-0.35)			(0.41)	0.43)	
Δ HOMA–IR	-3.31(2.13)	-0.97 (1.73)	-2,28 (-3.89, -1.53)	0.003	-3.98(2.02)	-1.34 (1.62)	-2.54(-3.01, -2.09)	0.003
Δ BMI(kg/m²)	-0.2(1.2)	-0.1(1.1)	-0.1(-0.4, 0.20)	0.215	-0.7(0.9)	-0.5(1.0)	-0.2(-0.4, 0.2)	0.415
	CKD present (n=32)				**CKD absent (n=20)**			
Outcome	**Meriva (n=16)**	**Placebo (n=16)**	**RR or mean changes (95%CI)**	**P**	**Meriva (n=10)**	**Placebo (n=10)**	**RR or mean changes (95%CI)**	P
NASH resolution n(%)	8	2	4.00(1.01-15.81)	0.04	8	1	8.00(1.19-53.67)	0.03
Δ NAS	-2.45 (1.9)	-0.33 (0.8)	-2.21(-2.52, -1.52)	0.0008	-2.48(1.4)	-0.36 (1.4)	-2.12(-2.73, -2.00	0.0002
≥1 stage fibrosis improvementn(%)	7	1	7.00(1.14-50.42)	0.04	6	1	7.00(1.00-31.95)	0.05
NASH resolution and ≥1 stage fibrosis improvement n(%)	7	1	7.00(1.14-50.42) 7.00(1.14-50.42)	0.04	6	0	7.00 (1.02-47.81)	0.04
Significant (stage F2-F4) fibrosis regression n(%)	6	0	7.00(0.98-28.54)	0.05	5	0	6.00(0.98-46.92)	0.06
Δ hepatic NF-kB activation (%cells/HPF)	-18(12)	-1(11)	-17(-26, -8)	0.0002	-23(14)	-4(12)	-19(-29, -9)	0.0002
Δ eGFR (ml/min/1.73 m2)	7.32(6.43)	-9.81 (7.47)	18.01 (6.13, 24.36)	0.0002	9.79(8.11)	-6.58 (6.32)	15.42 (9.49, 22.31)	0.003
Δ LDL-C (mg/dL	-24(13)	15(12)	-39(-48, -31)	0.0002	-28(14)	7(12)	-35(-46, -34)	0.0004
Δ HDL-C (mg/dL)	7(2)	-3(3)	10(10, 7)	0.009	11(13, 9)	-5(6)	16(19, 12)	0.002
Δ Tg (mg/dL)	-21(9)	11/10)	-31(-42, -22)	0.005	-29(13)	8(12)	-48(-55, -40)	0.004
Δ FPG (mg/dL)	-15(11)	8(9)	-23(-30, -7)	0.001	-9(7)	3(7)	-11(-16, -7)	0.002
Δ HbA1c (%)	-0.69(0.49)	0.11 (0.63)	-0.80(-1,25, -0.35)	0.0005	-0.58(0.40)	-0.03 (0.41)	-0.55(-0.68, 0.43)	-0.009
Δ HOMA–IR	-3.31(2.13)	-0.97 (1.73)	-2,28 (-3.89, -1.53)	0.003	-3.98(2.02)	-1.34 (1.62)	-2.54(-3.01, -2.09)	0.003
Δ BMI(kg/m²)	-0.2(1.2)	-0.1(1.1)	-0.1(-0.4, 0.20)	0.215	-0.7(0.9)	-0.5(1.0)	-0.2(-0.4, 0.2)	0.415

	Fibrosis F0-2 (n=36)				**Fibrosis F3-4 (n=16)**			
Outcome	**Meriva (n=18)**	**Placebo (n=18)**	**RR or mean changes (95%CI)**	**P**	**Meriva (n=8)**	**Placebo (n=8)**	**RR or mean changes (95%CI)**	P
NASH resolution n(%)	9	2	4.50(1.13-17.99)	0.03	7	1	7.00(1.13-34.51)	0.04
Δ NAS	-2.39 (1.8)	-0.37 (0.7)	-2.01(-2.45, -1.47)	0.001	-2.84(1.7)	-0.42 (1.5)	-2.42(-2.98, 2.03	-0.0009
≥1 stage fibrosis improvementn(%)	7	1	7.00(1.01-31.25)	0.04	6	0	7.00(1.10-34.61)	0.04
NASH resolution and ≥1 stage fibrosis improvement n(%)	7	1	7.00(1.01-31.25)	0.04	6	0	7.00(1.10-34.61)	0.04
Significant (stage F2-F4) fibrosis regression n(%)	6	0	7.00(1.01-28.54)	0.04	5	0	6.43(0.98-46.92)	0.06
Δ hepatic NF-kB activation(%)	-22(12)	-1(11)	-21(-28, -17)	0.0001	-24(14)	-2(12)	-26(-32, -20)	0.0001
CKD regression n(%)	7	0	8.00(1.15-46.5)	0.04	6	0	7.00(1.10-34.51)	0.04
Δ eGFR (ml/min/1.73 m2)	7.32(6.43)	-9.81 (7.47)	18.01 (6.13, 24.36)	0.0002	9.79(8.11)	-6.58 (6.32)	15.42 (9.49, 22.31)	0.003
Δ LDL-C (mg/dL	-24(13)	15(12)	-39(-48, -31)	0.0002	-28(14)	7(12)	-35(-46, -34)	0.0004
Δ HDL-C (mg/dL)	7(2)	-3(3)	10(10, 7)	0.009	11(13, 9)	-5(6)	16(19, 12)	0.002
Δ Tg (mg/dL)	-21(9)	11/10)	-31(-42, -22)	0.005	-29(13)	8(12)	-48(-55, -40)	0.004
Δ FPG (mg/dL)	-15(11)	8(9)	-23(-30, -7)	0.001	-9(7)	3(7)	-11(-16, -7)	0.002
Δ HbA1c (%)	-0.69(0.49)	0.11	-0.80(-1,25,	0.0005	-0.58(0.40)	-0.03	-0.55(-0.68,	-0.009

		(0.63)	-0.35)			(0.41)	0.43)	
Δ HOMA–IR	-3.31(2.13)	-0.97 (1.73)	-2,28 (-3.89, -1.53)	0.003	-3.98(2.02)	-1.34 (1.62)	-2.54(-3.01, -2.09)	0.003
Δ BMI(kg/m²)	-0.2(1.2)	-0.1(1.1)	-0.1(-0.4, 0.20)	0.215	-0.7(0.9)	-0.5(1.0)	-0.2(-0.4, 0.2)	0.415

	HUMANITAS Gradenigo hospital (n=28)				**Citta Della Salute e Della Scienza hospital (n=24)**			
Outcome	**Meriva (n=14)**	**Placebo (n=14)**	**RR or mean changes (95%CI)**	**P**	**l Meriva (n=12)**	**Placebo (n=12)**	**RR or mean changes (95%CI)**	P
NASH resolution n(%)	8	2	6.00(1.03-15.60)	0.03	8	1	8.00(1.17-38.50)	0.03
Δ NAS	-2.65 (1.1)	-0.63 (0.8)	-2.01(-2.79, -1.72)	0.0002	-2.78(1.5)	-0.69 (1.1)	-2.09(-2.81, -1.45)	0.0001
≥1 stage fibrosis improvementn(%)	6	1	6.00(1.03-33.59)	0.04	7	1	7.00(1.11-38.54)	0.03
NASH resolution and ≥1 stage fibrosis improvement n(%)	6	0	7.50(1.05, 43,49)	0.04	5	0	6.00(0.95, 43.59)	0.06
Significant (stage F2-F4) fibrosis regression n(%)	6	0	7.50(1.05, 43,49)	0.04	5	0	6.00(0.95, 43.59)	0.06
Δ hepatic NF-kB activation(%)	-21(11)	-2(11)	-19(-26, -8)	0.0001	-25(11)	-3(12)	-22(-31, -13)	0.0001
CKD regression n(%)	7	0	7.09 (1.15, 35.80)	0.04	6	0	7.00 (1.01-38.54)	0.04
Δ eGFR (ml/min/1.73 m2)	+9.9(7.1)	-10.81 (8.1)	20.3(14.4, 26,4)	0.005	9.87(8.1)	-9.94(10.1)	19.8(9.7)	0.006
Δ LDL-C (mg/dL	-24(13)	15(12)	-39(-48, -31)	0.0002	-28(14)	7(12)	-35(-46, -34)	0.0004
Δ HDL-C (mg/dL)	7(2)	-3(3)	10(10, 7)	0.009	11(13, 9)	-5(6)	16(19, 12)	0.002
Δ Tg (mg/dL)	-21(9)	11/10)	-31(-42, -22)	0.005	-29(13)	8(12)	-48(-55, -40)	0.004
Δ FPG (mg/dL)	-15(11)	8(9)	-23(-30, -7)	0.001	-9(7)	3(7)	-11(-16, -7)	0.002
Δ HbA1c (%)	-0.69(0.49)	0.11 (0.63)	-0.80(-1,25, -0.35)	0.0005	-0.58(0.40)	-0.03 (0.41)	-0.55(-0.68, 0.43)	-0.009
Δ HOMA–IR	-3.31(2.13)	-0.97 (1.73)	-2,28 (-3.89, -1.53)	0.003	-3.98(2.02)	-1.34 (1.62)	-2.54(-3.01, -2.09)	0.003
Δ BMI(kg/m²)	-0.2(1.2)	-0.1(1.1)	-0.1(-0.4, 0.20)	0.215	-0.7(0.9)	-0.5(1.0)	-0.2(-0.4, 0.2)	0.415

Effect of phospholipid curcumin Meriva® on liver histology and kidney disease in Nonalcoholic Steatohepatitis

A randomized, double-blind, placebo-controlled trial

CONSORT CHECKLIST

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction4			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5-6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	5-6
Participants	4a	Eligibility criteria for participants	5; Appendix 1
	4b	Settings and locations where the data were collected	4-5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7, Appendix 1
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7-9
	6b	Any changes to trial outcomes after the trial commenced, with reasons	7-9
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	9
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6, Appendix 1
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	6, Appendix 1
Allocation	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers),	6, Appendix 1

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concealment mechanism		describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6, Appendix 1
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6, Appendix 1
	11b	If relevant, description of the similarity of interventions	6-7
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9-11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	10-11, Appendix 1
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Appendix eFig 2
	13b	For each group, losses and exclusions after randomisation, together with reasons	11, Appendix eFig 2
Recruitment	14a	Dates defining the periods of recruitment and follow-up	11
	14b	Why the trial ended or was stopped	11
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Tab 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Tab 2-3
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Tab 2-3
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Tab 2-3
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	12-13; appendix 2 eTab 5-8
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Etab 8
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14-15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	14-15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	14-15
Other information			

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Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	Appendix 1
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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