Menarini Group

CLINICAL STUDY PROTOCOL

Title: A randomized, double-blinded, placebo-controlled, clinical study of the effects of a nutraceutical combination on LDL cholesterol levels in subjects with sub-optimal blood cholesterol levels. (Acronym: NATCOL)

Study code: ***MEIF/16/MOF-Col/001***

Study type and design: A clinical, exploratory, monocentric, interventional, non-drug, randomized, placebo-controlled, double-blinded, parallel-group study.

Amended Protocol version n°: 4.0 **Release Date: 16.04.2018**

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SUMMARY OF CHANGES

Amendment 3 (non-substantial amendment)

Amendment rationale

The purpose of this non-substantial amendment is to no longer use a portable device (4 in 1 Mission Cholesterol Meter) for the evaluation of inclusion criteria LDL-C 115 – 190 mg/dL and TG < 400 mg/dL during Visit 2 - Baseline. The protocol originally called for the use of a portable device (4 in 1 Mission Cholesterol Meter) that provided immediate results and thus allowed the confirmation of eligibility and subsequent randomization at the same visit. The results from the portable device were however found to be inconsistent with those of the laboratory run on blood drawn during the same visit and led to patients being incorrectly excluded from the study due to screening failure. LDL-C and TG eligibility criteria will now be confirmed only through the blood sample evaluated by local laboratory at Visit 2 - Baseline. If eligibility is confirmed, the patient will be asked to return no later than 3 days after V2 for randomization and treatment dispensation.

Changes to the protocol

Changes to specific sections of the protocol are shown in the track changes version of the protocol using strike through font for deletions and underlined font for insertions.

Section 6, Section 9, Section 11, the flow chart in Section 2.2 and the Protocol Synopsis in Section 2 have been modified to reflect the changes described in the amendment rationale.

A typographic error in the eGFR value that defines chronic renal failure has also been corrected in Section 7.2 and Protocol Synopsis.

The changes in the protocol affect the Informed Consent. Therefore, a revised Informed Consent that reflects the changes described in this protocol amendment will be provided. None of the changes made are due to safety concerns and none have an impact on the conduct of the trial or alter in any way the treatment of study subjects. Based on these premises, the amendment will be submitted to the Ethics Committee as a non-substantial amendment.

Amendment 2 (non-substantial amendment)

Amendment rationale

The purpose of this non-substantial amendment was to correct errors in the “General Guidelines for a Proper Mediterranean Style Diet”.

Changes to the protocol

The “General Guidelines for a Proper Mediterranean Style Diet” in Appendix 1 were modified.

Amendment 1 (non-substantial amendment)

Amendment rationale

The purpose of this non-substantial amendment was to provide detailed diet guidelines so that the diets among patients in the two treatment groups could be as similar as possible.

Changes to the protocol

The “General Guidelines for a Proper Mediterranean Style Diet” were added to the protocol as Appendix 1.

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1. RESPONSIBILITIES

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2. PROTOCOL SYNOPSIS

Title Study Code:	A randomized, double-blinded, placebo-controlled, clinical study of the effects of a nutraceutical combination on LDL cholesterol levels in subjects with sub-optimal blood cholesterol levels. MEIF/16/MOF-Col/001
Investigational Product Reference Therapy (comparator) Dose Regimen	Phytosterols 800 mg + DIF1STAT® (containing fermented red rice titrated in 5 mg monacolin K). Placebo for oral administration identical in appearance, size, shape, weight and taste to the active product. Both administered as one tablet, per os, per day in the evening.
Study Type and Design	This is an exploratory, monocentric, interventional, non-drug, randomized, double-blinded, placebo-controlled, parallel-group study.
Background and Rationale	High cholesterol is one of the major controllable risk factors for coronary heart disease. Numerous preclinical and clinical studies have demonstrated that drugs that reduce the intestinal absorption of cholesterol (ex. ezetimibe), or block the hepatic synthesis of cholesterol (statins) or the association of both can reduce cholesterolemia and are associated with reduced rate of cardiovascular events. The search for natural alternatives to this approach includes the evaluation of the single and associated effects of nutritional inhibitors of dietary cholesterol absorption and endogenous hepatic synthesis. Phytosterols are plant sterols structurally similar to cholesterol that act in the intestine to lower cholesterol absorption and effectively reduce LDL-cholesterolemia. Since they have very low systemic absorption, increasing the intake of phytosterols may be a practical way to reduce cholesterol levels. Chinese red yeast rice is a dietary supplement made by fermenting the yeast, Monascus purpureus, over rice. Monascus yeast produces a family of substances called monacolins, including monacolin K.

	Monacolins act as reversible inhibitors of the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis. Besides the inhibition of HMG-CoA reductase, red yeast rice has been found to contain sterols, isoflavones and isoflavone glycosides, and monounsaturated fatty acids, all capable of lowering LDL cholesterol.
Aim of the Study	The purpose of this study is to evaluate the effect of a combination of phytosterols and DIF1STAT® (fermented red rice titrated in 5 mg monacolin K), on modulation of lipid profile and other biomarkers in subjects with sub-optimal blood cholesterol levels.
Primary Objective	To evaluate the effect of a nutraceutical combination over an 8-weeks period, on blood LDL-cholesterol levels in subjects with sub-optimal blood cholesterol levels.
Secondary Objectives	To evaluate the effect of a nutraceutical combination on the following parameters following 8-weeks treatment, in subjects with sub-optimal blood cholesterol levels: • Total blood cholesterol levels • Blood HDL-cholesterol levels • Blood non-HDL cholesterol levels • Blood triglyceride levels • Apolipoprotein B levels • TC/HDL and LDL-C/HDL risk ratios • Endothelial reactivity (Endocheck®) • Glycemia, ALT, AST gamma-GT, creatinine, uric acid, CPK
Number and characteristics of Patients	Ninety (90) subjects with sub-optimal blood LDL-cholesterol levels.
Number of Centers & Countries	One Italian center
Study Duration (specify different study phases)	Enrollment period: 6 months Screening period: 2 weeks Treatment period: 8 weeks The study will last 10 weeks per patient.

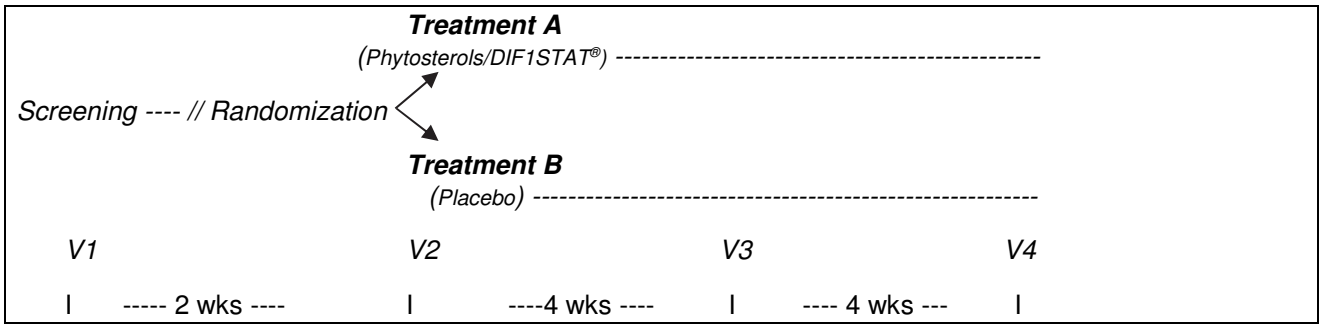
Study Procedures	The study is made up of four visits distributed over a 10-weeks period: V 1 (day -14) – Screening: Subjects will be enrolled into the study within their normal health-care program. After providing written informed consent, tests will be run in order to check the subject's eligibility for the study. Subjects will also be given suggestions regarding their diet (a Mediterranean-style diet is to be maintained for the entire duration of the study – see Appendix 1 of the protocol). V2 (baseline) and Day 0 (randomization): After confirmation of the subject's eligibility (LDL-C and TG criteria confirmed with blood test results), eligible subjects will be randomized within 3 days to one of the two treatment groups (phytosterols 800 mg + DIF1STAT® or placebo). During this visit (Visit 2 - baseline) an endothelial reactivity test (Endocheck®) will be performed. V3 (28 ±3 days after Day 0) – Intermediate: Blood will be drawn for tests and compliance with treatment will be assessed. V4 (28 ± 3 days after Visit 3) – End of study: Blood tests and an endothelial reactivity test (Endocheck®) will be performed and treatment compliance will be assessed. Weight, waist circumference, Index of Central Obesity (ICO) and Body Mass Index (BMI), Hepatic Steatosis Index (HSI) and Lipid Accumulation Product (LAP) will be measured/calculated at each visit, height at Visit 1. Heart rate and blood pressure will be measured at each visit. This study will be conducted in a double-blinded manner. Patients will be assigned to a treatment according to a computer-generated randomization list produced by the trial statistician. Code break cards will be kept at the study center and may be opened to reveal what treatment the patient is taking in case of emergency.
Inclusion Criteria	Subjects must meet all of the following inclusion criteria: • Age 30-75 years • LDL-cholesterol = 115 -190 mg/dL • Triglycerides < 400 mg/dL • Any cardiovascular therapy should be stable for type and dose for at least three months • Signed, written informed consent
Exclusion Criteria	Subjects must meet none of the following exclusion criteria:

	• Intolerance to any ingredient of dietary supplement • Patients already suffering from cardiovascular diseases or at high risk of developing cardiovascular diseases • Myopathies • Uncontrolled diabetes mellitus based on PI judgment • Chronic renal failure (defined as eGFR <60ml/min/1.73m^2) or liver failure (defined as AST and /or ALT>3 ULN) • Body Mass Index > 32 kg/m^2 • Therapy with statins or other drugs or supplements with effects on lipid metabolism • Patients with acquired immunodeficiency • Treatment with immunosuppressants • Pregnant or breastfeeding women • Women of childbearing potential not willing to use effective birth control methods • Patients participating or having participated in another clinical trial within the previous 3 months • Current or recent history of drug or alcohol addiction based on PI judgment
Study Variables	• <u>Primary Variable</u>: The primary variable of the study is the change in blood LDL cholesterol level from baseline (V2) to Week 8 (V4). • <u>Secondary Variables</u> The secondary variables of the study are defined as the change from baseline (V2) to Week 8 (V4) in the following parameters: ○ Total blood cholesterol level ○ Blood HDL cholesterol level ○ Blood non-HDL cholesterol level ○ Blood triglycerides level ○ Apolipoprotein B level

	○ Total cholesterol /HDL cholesterol ratio ○ LDL cholesterol/HDL cholesterol ratio ○ Pulse Volume (PV) waveform (endothelial reactivity) ○ Glycemia, AST, ALT, Gamma-GT, serum creatinine, serum uric acid, CPK • Other Variables ○ Change from baseline at each assessment time point for each laboratory parameter ○ Adverse Events ○ Vital Signs ○ Weight ○ Height ○ Waist Circumference ○ Index of Central Obesity (ICO) ○ Body Mass Index (BMI) ○ Hepatic Steatosis Index (HSI) ○ Lipid Accumulation Product (LAP)
Statistical Assumptions	Patients will be randomized to one of the two treatment arms in a 1:1 ratio (phytosterols 800 mg + DIF1STAT® or placebo). A patient randomization list, and its respective randomization cards, will be generated by means of a validated computerized system. Patients, investigators, site personnel, and data analysis will remain blinded to the identity of the treatment administered from the time of randomization until database lock. Unblinding will only occur in case of patient emergencies and at the conclusion of the study. **Justification of Sample Size** Considering the primary endpoint of the study as the reduction from baseline to week 8 in LDL cholesterol level and the data available from literature [Phytomedicine. 2016 Oct 15; 23(11):1113-8], a reduction of LDL level of approximately 10% is expected after the intake of the nutraceutical, the total number of patients to be evaluated should be 35 per treatment arm in a 1:1 ratio (NQuery Advisor, 7.0). Allowing for an approximate 20% dropout rate, at least 88 patients should be randomized: 44 patients in each treatment group.

	Analysis populations: Full Analysis Set (FAS): all patients to whom study treatment has been assigned by randomization and who have the assessment for LDL cholesterol at visit 4 (week 8). The primary and secondary analyses will be based on the FAS. Per Protocol (PP) Set: all patients in the FAS who have no major protocol deviations. Results of the primary analysis conducted in the PP set will be considered as supportive. Tolerability Set: all patients who received at least one dose of study medication. Patients will be analyzed according to the treatment they actually received. **Statistical analyses** Summary statistics of raw data and change from baseline at each assessment time point for LDL cholesterol level will be presented overall and by treatment group. An analysis of covariance (ANCOVA) model will be estimated on the changes from baseline to Week 8 in LDL cholesterol levels considering treatment group as factor and baseline value of LDL cholesterol as continuous covariate. Results will be reported as Least-Square Means together with associated two-tailed 95% CI and p-value. If the assumption of normality of residuals is violated, the ANCOVA model will be fitted on rank transformed data. No alpha level adjustment for multiplicity will be adopted. The same statistical models described for the analysis of the primary variable will be applied to the secondary variables. No technique for missing data imputation will be adopted.

2.1 Study Scheme:



2.2 Study Flow Chart

	Day -14	Day 0	Day 28 (± 3)	Day 56 (± 3)
	Visit 1^	Visit 2^§	Visit 3^	Visit 4^
	Screening	Baseline	Intermediate	End of study
Informed consent	✓			
Inclusion/exclusion criteria	✓	✓		
Medical history	✓			
Weight, height[&], waist circumference, Index of Central Obesity (ICO), Body Mass Index (BMI), Hepatic Steatosis Index (HSI), Lipid Accumulation Product (LAP)	✓	✓	✓	✓
Heart rate, blood pressure[#]	✓	✓	✓	✓
Hematology/clinical chemistry*	✓	✓	✓	✓
Urine Pregnancy Test (dipstick)		✓		✓
Endothelial reactivity (Endocheck)		✓		✓
Diet evaluation[+]	✓	✓	✓	✓
Randomization/Dispensation of treatment		✓°		
Compliance with treatment			✓	✓
Concomitant medications	✓	✓	✓	✓
Adverse events (AE)		✓†	✓	✓

^ Visits to take place in the morning. Participants will be asked to fast overnight 12h before the visits.
[#] Orthostatic and clinostatic, diastolic and systolic blood pressure (average of three measurements).
* Full lipid panel (total cholesterol, LDL-cholesterol, HDL and non-HDL cholesterol, triglycerides, risk ratio, B apolipoproteins, LDL-C/Apo B), glycemia, ALT, AST, gamma-GT, CPK, uric acid, creatinine, eGFR with MDRD formula.
[§] During Visit 2 – Baseline evaluation – the LDL-C and TG values will be confirmed through a blood test analysis. If the eligibility was confirmed, the patient will be asked to return no later than 3 days after V2 for randomization and treatment dispensation.
[†] Collection of AEs occurred after signature of informed consent.
[&] Height only at Visit 1.
[+] See Appendix 1 of the protocol for diet guidelines.
° Randomization and dispensation of treatment will take place within 3 days from Visit 2^ - Baseline evaluation and will be considered as “Day 0”.

3. ABBREVIATIONS

AE	Adverse Events
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
AST	Aspartate Aminotransferase
BMI	Body Mass Index
CHD	Coronary Heart Disease
CPK	Creatinine Phosphokinase
CRF	Case Report Form
CRO	Contract Research Organization
EC	Ethics Committee
eGFR	Estimated Glomerular Filtration Rate
FAS	Full Analysis Set
HDL	High-Density Lipoprotein
HSI	Hepatic Steatosis Index
ICF	Informed Consent Form
ICO	Index of Central Obesity
LAP	Lipid Accumulation Product
LDL	Low-Density Lipoprotein
LDL-C	Low-Density Lipoprotein-Cholesterol
MDRD	Modification of Diet in Renal Disease
PP	Per Protocol
PV	Pulse Volume
SAE	Serious Adverse Event
SD	Standard Deviation
TC	Total Cholesterol
TG	Triglycerides
ULN	Upper Limit of Normal

4. BACKGROUND INFORMATION

High cholesterol is one of the major controllable risk factors for coronary heart disease (CHD), heart attack and stroke. Dietary cholesterol has been associated with cardiovascular disease (1), leading the 2010 Dietary Guidelines Advisory Committee to recommend no more than 300 mg/d of cholesterol intake for healthy populations in the United States (2). The International Atherosclerosis Society also recommends decreasing dietary cholesterol as a strategy for lowering Low-Density-Lipoprotein (LDL) cholesterol (3). In contrast to dietary guidelines in the United States, other developed and developing countries do not define an upper limit on cholesterol intake but focus on controlling the intake of saturated and trans fat, which are major determinants of blood cholesterol concentrations (4).

The relationship between raised plasma cholesterol and atherosclerotic vascular disease has been clearly demonstrated and the evidence that reducing plasma cholesterol is associated with reduced CV risk is equally unequivocal. A 10% proportional reduction in all-cause mortality and 20% proportional reduction in CAD death per 1.0 mmol/L (40 mg/dL) LDL-C reduction is reported from a large number of meta-analyses. The risk of major coronary events was reduced by 23% and the risk of stroke was reduced by 17% per 1 mmol/L (40 mg/dL) LDL-C reduction. The benefits were similar in all subgroups examined. The benefits were significant within the first year but were greater in subsequent years (5).

A relatively large number of dietary supplements and nutraceuticals have been studied for their supposed or demonstrated ability to reduce cholesterolemia in humans.

Antioxidants are natural substances that exist as vitamins, minerals and other compounds in foods. They are believed to help preventing disease by fighting free radicals that cause cellular damage, leading to the oxidation of low-density lipoproteins and contributing to atherosclerosis. A meta-analysis reviewing the results from seven large randomized trials of vitamin E, alone or in combination with other antioxidants, concluded that vitamin E did not provide any benefit in lowering mortality compared to control treatments, and did not significantly decrease the risk of cardiovascular death or stroke. The lack of any beneficial effect was seen consistently regardless of the doses of vitamins used and the diversity of the patient populations (6).

Numerous preclinical and clinical studies have demonstrated that drugs that reduce the intestinal absorption of cholesterol (ex. ezetimibe), others that block the hepatic synthesis of cholesterol (statins) or the association of both besides reducing lipid profile, also reduce the rate of cardiovascular events (7).

The search for natural alternatives to this approach includes the evaluation of the single and associated effects of nutritional inhibitors of dietary cholesterol absorption and endogenous hepatic synthesis.

Phytosterols are plant sterols structurally similar to cholesterol that act in the intestine to lower cholesterol absorption and effectively reduce LDL-cholesterol. Since they have very low systemic absorption, increasing the intake of phytosterols may be a practical way to reduce cholesterol levels (8).

Chinese red yeast rice is a dietary supplement made by fermenting the yeast, Monascus purpureus, over rice. Monascus yeast produces a family of substances called monacolins, including monacolin K.

Monacolins act as reversible inhibitors of the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis. In addition to the inhibition of HMG-CoA reductase, red yeast rice has been found to contain sterols (β-sitosterol, campesterol, stigmasterol, and sapogenin), isoflavones and isoflavone glycosides, and monounsaturated fatty acids (9, 10), all capable of lowering LDL cholesterol (11).

A recent double-blinded, placebo-controlled, randomized clinical trial carried out on 25 moderately hypercholesterolemic, nonsmoking, pharmacologically untreated subjects found that a product containing 10 mg of monacolins from M. purpureus and a mix of antioxidants appears to safely reduce cholesterolemia, hs-CRP, and endothelial dysfunction in moderately hypercholesterolemic subjects (12).

5. TRIAL OBJECTIVES AND PURPOSE

The purpose of this study is to evaluate the modulation of lipids, lipoproteins, and other biomarkers using a combination of phytosterols and DIF1STAT® (fermented red rice titrated in 5 mg monacolin K).

The primary objective of this study is to evaluate the effect of this nutraceutical combination on blood LDL-cholesterol levels over an 8-week period in subjects with sub-optimal blood cholesterol levels.

The secondary objective is to evaluate in subjects with sub-optimal blood cholesterol levels the effect of this nutraceutical combination on the following parameters over an 8-week period:

- Total blood cholesterol (TC)
- Blood High Density Lipoprotein (HDL)-cholesterol
- Blood non-HDL cholesterol
- Blood triglycerides (TG)
- Apolipoprotein B
- TC/HDL and LDL-C/HDL risk ratios

- Pulse Volume (PV) waveform (Endothelial reactivity)
- Glycemia, ALT, AST, gamma-GT, serum creatinine, serum uric acid, Creatinine Phosphokinase (CPK)

6. STUDY DESIGN

This is an exploratory, monocentric, interventional, non-drug, randomized, placebo-controlled, double-blinded, parallel-group study.

The study duration will be of approximately ten weeks for each subject. Four study visits are planned and will take place in the morning; participants will be asked to fast overnight (12 hours) prior to the visit.

Visit 1 (screening) (Day -14)

Subjects will be enrolled into the study within their normal health-care program. Those who provide written informed consent will undergo screening assessments to evaluate compliance with inclusion/exclusion criteria and eligibility for the study. Patients will be assigned a patient number made up of five-digits; the first two correspond to the center number and the last three to the patient. Visit 1 includes:

Medical history
Weight, height, waist circumference, Index of Central Obesity (ICO), Body Mass Index (BMI), Hepatic Steatosis Index (HSI), Lipid Accumulation Product (LAP)
Heart rate, blood pressure (average of three measurements)
Hematology/clinical chemistry in fasting condition (total cholesterol, LDL-cholesterol, HDL and non-HDL cholesterol, triglycerides, risk ratio, B apolipoproteins, LDL-C/Apo B, glycemia, ALT, AST, gamma-GT, CPK, uric acid, creatinine, eGFR with MDRD formula
Concomitant medication

Subjects will also be given standard dietary suggestions to correct unhealthy habits (a Mediterranean-style diet is to be maintained for the entire duration of the study – see Appendix 1 for diet guidelines).

Visit 2 (baseline evaluation) and Day 0 (randomization)

During this visit, LDL-C and TG values will be confirmed through a blood test analysis in fasting condition to evaluate compliance with inclusion criteria (LDL-cholesterol = 115 -190 mg/dL, Triglycerides < 400 mg/dL).

Once inclusion/exclusion criteria are confirmed, eligible patients will be asked to return no later than 3 days after Visit 2 for randomization and treatment dispensation. The day of randomization will be considered as Day 0.

Weight, waist circumference, Index of Central Obesity (ICO), Body Mass Index (BMI), Hepatic Steatosis Index (HSI), Lipid Accumulation Product (LAP)
Heart rate, blood pressure (average of three measurements)
Hematology/clinical chemistry in fasting condition (total cholesterol, LDL-cholesterol, HDL and non-HDL cholesterol, triglycerides, risk ratio, B apolipoproteins, LDL-C/Apo B, glycemia, ALT, AST, gamma-GT, CPK, uric acid, creatinine, eGFR with MDRD formula
Endothelial reactivity test (Endocheck®)
Urine Pregnancy Test (dipstick)
Diet evaluation (see Appendix 1)
Dispensation of treatment
Concomitant medication
Adverse events

Eligible patients will be randomly assigned to one of the two groups in a 1:1 ratio (45 subjects per group) and treated with phytosterols 800 mg + DIF1STAT® or placebo, one tablet a day for 8 weeks.

Visit 3 (Day 28) (intermediate) will take place 28 ± 3 days after Day 0.

During V3, the following evaluations are performed:

Weight, waist circumference, Index of Central Obesity (ICO), Body Mass Index (BMI), Hepatic Steatosis Index (HSI), Lipid Accumulation Product (LAP)
Heart rate, blood pressure (average of three measurements)
Hematology/clinical chemistry in fasting condition (total cholesterol, LDL-cholesterol, HDL and non-HDL cholesterol, triglycerides, risk ratio, B apolipoproteins, LDL-C/Apo B, glycemia, ALT, AST, gamma-GT, CPK, uric acid, creatinine, eGFR with MDRD formula
Diet evaluation (see Appendix 1)
Concomitant medication
Accountability and compliance with treatment
Treatment tolerability evaluation (adverse events, if any)

Visit 4 (Day 56) (end of study) will take place 28 ± 3 days after V3.

During the last visit the following evaluations are performed:

Weight, waist circumference, Index of Central Obesity (ICO), Body Mass Index (BMI), Hepatic Steatosis Index (HSI), Lipid Accumulation Product (LAP)
Heart rate, blood pressure (average of three measurements)
Hematology/clinical chemistry in fasting condition (total cholesterol, LDL-cholesterol, HDL and non-HDL cholesterol, triglycerides, risk ratio, B apolipoproteins, LDL-C/Apo B, glycemia, ALT, AST, gamma-GT, CPK, uric acid, creatinine, eGFR with MDRD formula
Endothelial reactivity (Endocheck)
Urine Pregnancy Test (dipstick)
Diet evaluation (see Appendix 1)
Concomitant medication
Accountability and compliance with treatment
Treatment tolerability evaluation (adverse events, if any)

This study will be conducted in a double-blinded manner; the investigator, study staff, subjects and monitors will remain blinded to the treatment until study closure. Patients will be assigned to a treatment according to a computer-generated randomization list produced by the trial statistician. Code break cards will be kept at the study center and may be opened to reveal what treatment the patient is taking in case of emergency.

7. SELECTION AND WITHDRAWAL OF SUBJECTS

This study will randomize 90 male/female subjects, aged 30-75 years, with sub-optimal blood LDL-cholesterol levels (LDL-cholesterol = 115 -190 mg/dL and Triglycerides < 400 mg/dL. It will take place at one center located in Italy.

Informed Consent Process

Prior to the subject's enrolment in the study and before performing any study-related procedures, the Investigator or authorized delegate shall obtain the subject's written, dated and signed informed consent to participate in the study and to the confidential disclosure, processing and transfer of necessary documentation of the subject's health and personal data to the CRO, Sponsor and its Affiliates, the competent Health Authorities and any other institutions (even if located outside the European Economic Area), as legally required and in accordance with applicable privacy laws.

Institution and Investigator undertake to duly inform patients about personal data processing and relevant applicable privacy rights before their participation in the study.

Prior to submittal to the patient, the Informed Consent form must be approved in the corresponding local language and in accordance with local laws and regulations by the Ethics Committee (EC).

In the patient information section, patients will be given information and a comprehensive explanation in easily understandable terms of the study procedures, benefits, restrictions, discomforts, and risks in taking part in the study, the properties of the nutraceutical product, and the method of assignment to treatments.

Patients will also be informed about the measures taken to ensure their confidentiality according to pertinent legislation. The Investigator shall provide the patient with an emergency telephone number.

After being duly informed and interviewed by the Investigator, the patient has to date and sign the 2 copies of Informed Consent Form (ICF) before being enrolled in the study and before undergoing any study procedure. One signed copy of the ICF will be stored by the Investigator in the Investigator's File, the other will be given to the patient.

Should a protocol amendment affect the terms of the ICF, it will be revised to reflect the protocol change and be submitted to the EC for approval.

The Investigator will ensure that this new consent form is signed by both new patients subsequently entered in the study and those already enrolled.

7.1 Inclusion criteria

Subjects must meet all the following inclusion criteria:

- Age 30-75 years
- LDL-cholesterol = 115-190 mg/dL
- Triglycerides < 400 mg/dL
- Any cardiovascular therapy should be stable for type and dose for at least three months
- Signed, written informed consent

7.2 Exclusion criteria

Subjects must meet none of the following exclusion criteria:

- Intolerance to any ingredient of dietary supplement
- Patients already suffering from cardiovascular diseases or at high risk of developing cardiovascular diseases
- Myopathies

- Uncontrolled diabetes mellitus based on PI judgment
- Chronic renal failure (defined as eGFR<60ml/min/1.73m^2) or liver failure (defined as AST and /or ALT>3 Upper Limit of Normal) (ULN)
- Body Mass Index > 32 kg/m^2
- Therapy with statins or other drugs or supplements with effects on lipid metabolism
- Patients with acquired immunodeficiency
- Treatment with immunosuppressants
- Pregnant or breastfeeding women
- Women of childbearing potential not willing to use effective birth control methods
- Patients participating in or having participated in another clinical trial within the previous 3 months
- Current or recent history of drug or alcohol addiction based on PI judgment

7.3 Withdrawal criteria

The patient may withdraw from the study at any time without explanation, without losing the right to future medical care. The participation of the patient may be terminated by the Investigator at any moment, if considered appropriate.

Treatment with the study product must be terminated during the study for any of the following reasons:

- Request of the patient
- Pregnancy
- Investigator deems it to be in the best interest of the patient to discontinue
- Failure to comply adequately with the dosing, evaluations, or other requirements of the study.

If possible, and after the permanent discontinuation of treatment, the patients will be assessed using the procedures normally planned for the last visit.

If patients fail to return to the site, the Investigator should make every effort to recontact the patient and determine his/her health status. Attempts to contact such patients must be

documented in the patient's records (times and dates of attempted telephone contact, receipt for sending a registered letter).

Patients who have withdrawn from the study cannot be re-randomized (treated) in the study, and their random numbers must not be reused.

The reason for the withdrawal must be well documented in the CRF.

8. TREATMENT OF SUBJECTS

8.1 Treatments

Patients will be randomized with a 1:1 ratio to one of the following two treatment arms:

1. Phytosterols 800 mg, + DIF1STAT®. 1,300 mg film-coated tablets. Ingredients: phytosterols (61.5%) bulking agent: microcrystalline cellulose, cross-linked sodium carboxymethyl cellulose, fermented red rice titrated at 3% in monacolin K (Monascus purpureus went, seed), niacin, anti-caking agents: fatty acid magnesium salts, silicon dioxide; policosanols titrated to 60% octacosanol; coating agents: polyvinyl alcohol, talcum, polyethylene glycol, polysorbate 80; colorants: E120, E172.

   Nutritional information: each tablet contains phytosterols (800 mg), Monascus pupureus (167 mg) titrated at 3% in monacolin k (5mg), niacin (27 mg), linear aliphatic alcohols titrated to 60% octacosanol.

   One tablet per os, per day to be taken in the evening.

2. Placebo tablets for oral administration identical in appearance, size, shape, weight and taste to the active product. 1,300 mg film-coated tablets. Ingredients: bulking agent: microcrystalline cellulose, cross-linked sodium carboxymethyl cellulose; colorants: red beet, elder extract plv, anti-caking agents: fatty acid magnesium salts, silicon dioxide; coating agents: polyvinyl alcohol, talcum, polyethylene glycol, polysorbate 80; colorants: E120, E172.

   One tablet per os, per day to be taken in the evening.

Patients will be treated for 8 weeks.

8.2 Blinding procedures

Patients, Investigator staff, personnel performing the study assessments and data analysts will remain blinded to the identity of the treatment from the time of randomization until database lock using the following methods:

- The two treatments will be identical in packaging, labeling, administration schedule, appearance, taste and odor.
- Randomization data will be kept strictly confidential until the time of unblinding and will not be accessible by anyone involved in the study.

Unblinding will occur only in case of patient emergencies by opening the code break cards stored at the center, and at the conclusion of the study.

Health Authorities will be granted access to unblinded data if needed.

8.3 Method of assigning patients to treatment group

A computer-generated randomization list produced by the trial statistician will randomize patients to one of the two treatment groups (phytosterols 800 mg + DIF1STAT® or placebo) in a 1:1 ratio. Paper based randomization procedure will assign a randomization number to the patient, which will be used to link the patient to a treatment arm and will specify a unique kit number for the package of treatment to be dispensed to the patient. The randomization numbers will be generated using a procedure to ensure that treatment assignment is unbiased (further details are available in the randomization document).

8.4 Packaging, labeling and storage

Each patient will receive one bottle containing 80 film-tablets of either the study product or placebo. The product bottles should be kept in a cool, dry place. Shelf life of the product is 24 months.

Two-part labels will be computer-generated for this blinded study. One part of the label is attached to the container, whereas the other part is a tear-off portion to be removed at the time of dispensing and attached to the Drug Label Page.

The label contains the following information:

- Name of the Sponsor (A. Menarini Industrie Farmaceutiche Riunite S.r.l.).
- Name of the study: NATCOL.
- Batch number and expiration date.
- One bottle contains 80 1,300 mg film-tablets: phytosterols 800 mg + DIF1STAT® or placebo.

- Take one tablet per os per day.
- Kit number.
- Random number.
- Store in a dry, cool place. The expiration date applies to undamaged, properly stored product.
- This sample product is to be used exclusively during the clinical study. Return bottle and all unused tablets.

The Principal Investigator or other authorized persons (Pharmacists) is responsible for storing treatments in a secure and safe place in accordance with local regulations, labeling specifications, policies, and procedures.

8.5 Treatment accountability and compliance

Patients will be asked to return all unused tablets at Visit 3 and Visit 4. Compliance will be assessed at Visit 3 and Visit 4 by counting unused tablets. A subject Treatment Accountability Log for dispensed and returned treatment will be kept for each patient.

The Investigator will be responsible for ensuring that an accurate record of the treatment issued and returned is maintained through a Site Treatment Accountability Log. Destruction of all study products (used or unused) shall be carried out following the Sponsor's written authorization.

8.6 Concomitant medications

The use of any concomitant medication should be reported at each visit and reported in CRF.

The use of statins or other drugs/products that affect lipid metabolism is prohibited during the study.

9. ASSESSMENT OF PRIMARY AND SECONDARY VARIABLES

9.1 Laboratory parameters

The following laboratory parameters will be assessed at baseline and over the 8-week treatment period (Visits 2, 3 and 4):

- Blood LDL cholesterol
- Total blood cholesterol
- Blood HDL-cholesterol
- Blood non-HDL cholesterol
- Blood triglycerides
- Apolipoprotein B
- TC/HDL and LDL-C/HDL risk ratios
- Glycemia, ALT, AST, gamma-GT, creatinine, uric acid, CPK,

9.2 Endothelial reactivity (Endocheck®)

Endothelial function will be evaluated through Endocheck® (BC Biomedical Laboratories Ltd, Vancouver, BC, Canada), a non-invasive method embedded within the Vicorder® device (PRN Vascular, Fall River, MA, USA), which records brachial pulse volume (PV) waveforms, at baseline and during reactive hyperemia.

Reactive hyperemia is provoked through PV displacement, obtained by inflating a cuff positioned distally around the forearm. After a 10-minute rest, brachial blood pressure is evaluated, and PV waveforms recorded at baseline for 10 seconds. Then, the cuff is inflated to 200 mmHg for 5 minutes, and PV waveforms are recorded for 3 minutes after cuff release. PV displacement is then calculated as a percent change in the PV waveform area, comparing waveforms during and before hyperemia through the equation $\sqrt{PV2/PV1}$, where PV1 represents PV at baseline and PV2 represents PV during hyperemia (12).

The endothelial reactivity test will be performed at baseline (Visit 2) and Visit 4.

9.3 Other variables

Heart rate, blood pressure

Heart rate, diastolic and systolic blood pressure in orthostatic and clinostatic position (average of three measurements) will be measured at all visits.

Weight, height, waist circumference, Index of Central Obesity (ICO), Body Mass Index (BMI), Hepatic Steatosis Index (HSI), Lipid Accumulation Product (LAP)

Weight, Height (only at V1), Waist Circumference, Index of Central Obesity (ICO), Body Mass Index (BMI) Hepatic Steatosis Index (HSI) and Lipid Accumulation Product (LAP) will be evaluated at all visits. Here below the corresponding formulas:

- BMI = Weight (kg) / Height ^{2}(m)
- ICO = Ratio of Waist Circumference and Height (cm) (13)
- HSI = 8 x (ALT/AST ratio)+BMI (+2, if female; +2, if diabetes mellitus) (14)
- LAP for men = (waist circumference [cm] – 65) × (triglyceride concentration [mmol/l]) (15)
- LAP for women = (waist circumference [cm] – 58) × (triglyceride concentration [mmol/l] (15)

10. ASSESSMENT OF SAFETY

10.1 Adverse events (AE)

An adverse event (AE) is as any untoward medical occurrence in a patient administered the product and which does not necessarily have to have a causal relationship with the product.

Adverse events should be recorded in the Adverse Events CRF under the signs, symptoms or diagnosis associated with them accompanied by the following information.

- severity grade
  - mild: usually transient in nature and generally not interfering with normal activities
  - moderate: sufficiently discomforting to interfere with normal activities
  - severe: prevents normal activities
- Relationship to the investigational product (no/yes)
- Duration (start and end dates) or if the event is ongoing an outcome of not recovered/not resolved should be reported.
- Action taken regarding investigational treatment
- Whether other medication or therapies have been taken
- Outcome (not recovered/not resolved; recovered/resolved; recovering/resolving, recovered/resolved with sequelae; fatal; or unknown)
- Whether it constitutes a serious adverse event (SAE)

A **serious adverse event** (SAE) is any untoward medical occurrence that at any dose:

- Results in death, or
- Is life-threatening, or
- Requires inpatient hospitalization or prolongation of existing hospitalization, or
- Results in persistent or significant disability/incapacity, or

- Is a congenital anomaly/birth defect, or
- Is a medically important event.

Should an SAE occur, the Investigator or any designees must complete and send the SAE form signed and dated by fax or e-mail within 24 hours following awareness to the Pharmacovigilance Officer whose fax number and e-mail address appear below.

The Investigator should take appropriate measures to follow all AEs (both serious and not serious) until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized to ensure the safety of the patients. This may imply that observations will continue beyond the last planned visit per protocol, and that additional investigations may be requested by the monitoring team up to as noticed by the Sponsor.

Any follow-up information of SAEs should be transmitted by fax or email as described above.

CRO Pharmacovigilance Officer: Dr Riccardo Chisci, OPIS s.r.l.
Email: all_phv@opis.it
Fax: +39 0362 633622
Tel: +39 0362 633312
Mobile: +39 348 6440813

10.2 MANAGEMENT OF ANY LABORATORY ABNORMALITY

Any laboratory test abnormality that is considered by the Investigator as an AE is to be managed as detailed above (see 10.1).

10.3 Management of pregnancy exposure cases

The Investigator is expected to record any case of pregnancy exposure occurring in a female patient or in a male patient's partner during the study on the pregnancy form and send it to the Sponsor by fax to the address indicated in Section 10.1.

The Investigator is requested to follow each case of pregnancy exposure until the outcome.

11. STATISTICS

11.1 Statistical methods (blinding and randomization)

Randomization

Patients will be randomized to one of the two treatment arms in a 1:1 ratio (phytosterols 800 mg + DIF1STAT® or placebo).

The randomization numbers will be generated to ensure that treatment assignment is unbiased and concealed from patients and investigator staff. A patient randomization list, and its respective randomization cards, will be generated by the CRO by means of a validated computerized system that automates the random assignment of randomization numbers to the different treatment arms. Prior to dosing, all patients who fulfill all inclusion/exclusion criteria will be randomized, by means of randomization cards, to one of the two treatment arms. The investigator or his/her delegate will use the first available randomization card, which will assign a randomization number to the patient that will be used to link the patient to a treatment arm and will specify a unique medication number for the package of study treatment to be dispensed to the patient.

Blinding

Patients, investigators, site personnel, and data analysts will remain blinded to the identity of the treatment administered from the time of randomization until database lock. Randomization data are kept strictly confidential until the time of unblinding and will not be accessible by anyone else involved in the study. The identity of the treatments will be concealed by the use of study treatments that are all identical in packaging, labeling, schedule of administration and appearance.

Unblinding will only occur in case of patient emergencies and at the conclusion of the study.

Interim Analysis

No interim analyses are planned.

General methodology

All data collected in the study will be listed and summarized as appropriate as described below in Section 11.5. Continuous data will be summarized by means of common descriptive statistics: mean, standard deviation (SD), median, first and third quartiles, minimum and maximum. Categorical data will be presented by absolute and relative frequencies (n and %) or contingency tables.

Patients will be included in each analysis based on available assessments, after the data handling conventions have been applied. The prevalence approach, if not differently indicated, will be applied.

Unless stated otherwise, two-sided p-values <0.05 will be considered statistically significant.

All statistical tables, listings, figures and analyses will be generated using SAS® release 9.4 or later (SAS Institute Inc., Cary NC, USA).

Further details about data analysis will be provided in the Statistical Analysis Plan document.

11.2 Determination of sample size

Considering the primary endpoint of the study as the reduction from baseline to week 8 in LDL cholesterol level and the data available from literature [Phytomedicine. 2016 Oct 15; 23(11):1113-8], a reduction of LDL level of approximately 10% is expected after the intake of the nutraceutical. Therefore, a mean difference of 15 mg/dL in the reduction of LDL cholesterol level at week 8 between the nutraceutical treatment group and the placebo group is considered clinically significant.

Assuming a baseline LDL level of 145 ±19 mg/dL, a power of 90% and a 5% two-sided alpha level to detect a difference in mean change in LDL from baseline to week 8 equal to 15 mg/dL between the nutraceutical and the placebo group, the total number of patients to be evaluated should be 35 per treatment arm in a 1:1 ratio (NQuery Advisor, 7.0). Allowing for an approximate 20% dropout rate, at least 88 patients should be randomized: 44 patients in each treatment group.

11.3 Analysis populations

Patients without a valid or adequately obtained Informed Consent Form (ICF) will be excluded from any analysis population.

The following analysis populations will be defined for statistical analyses:

- **Full Analysis Set (FAS)**: All patients to whom study treatment has been assigned by randomization and who have the assessment for LDL cholesterol at visit 4 (week 8). According to the intent-to-treat principle, patients will be analyzed according to the treatment they have been assigned to during the randomization procedure. The primary and secondary analysis will be based on the FAS.
- **Per Protocol (PP) Set**: All patients in the FAS who have no major protocol deviations. All major protocol deviations leading to exclusion from the PP set will be detailed in the analysis plan. Results of the analysis of the primary variable conducted in the PP set will be considered as supportive.

- **Tolerability Set**: All patients who received at least one dose of investigational product. Patients will be analyzed according to the treatment actually received (if different from treatment assigned through randomization).

11.4 Analysis variables

- The primary variable of the study is the change from baseline to Week 8 in LDL cholesterol levels
- The secondary variables of the study are defined as change from baseline to Week 8 in:
  - Total blood cholesterol
  - Blood HDL-cholesterol
  - Blood non-HDL cholesterol
  - Blood triglycerides
  - Apolipoprotein B
  - TC/HDL and LDL-C/HDL risk ratios
  - Pulse Volume (PV) waveform (endothelial reactivity)
  - Glycemia, ALT, AST, gamma-GT, creatinine, uric acid, CPK
- Other variables are as follows:
  - Change from baseline at each assessment time point for each laboratory parameter
  - Adverse Events
  - Vital Signs
  - Weight
  - Height
  - Waist Circumference
  - Index of Central Obesity (ICO)
  - Body Mass Index (BMI)
  - Hepatic Steatosis Index (HSI)
  - Lipid Accumulation Product (LAP)

11.5 Statistical analysis

Patient demographics and other baseline characteristics

All data about patient demographics and baseline characteristics will be summarized in the FAS, overall and by treatment group, by means of summary descriptive statistics.

The number and percentages of subjects meeting all eligibility criteria at screening will be provided. The analysis populations will be described and the reasons for excluding a patient

from any particular population will be provided with the number of protocol deviators per each criterion.

A complete description of patients' disposition will be provided, overall and by treatment group specifying the number of randomized patients, the number of patients at each visit, the number of completed and discontinued patients and the reason for the discontinuation.

Medical history data will be presented by MedDRA dictionary system organ class and preferred term, overall and by treatment group.

Treatments (study treatment, concomitant therapies, compliance)

The Tolerability set will be used for the analyses on treatment administration, compliance, concomitant medications and adverse events.

Extent of exposure is defined as the difference in days between the date of last treatment administration and the date of first treatment administration plus one, regardless of unplanned intermittent discontinuations.

Overall treatment compliance is defined as the actual number of capsules taken compared to the planned treatment duration (i.e. extent of exposure).

Extent of exposure and treatment compliance will be summarized by means of descriptive statistics, overall and by treatment group. The percentage of patients per compliance level will be also summarized:

- ≤80%
- >80% and ≤120%
- >120%

A subject that has taken at least 80% and no more than 120% of the required treatment will be considered compliant.

Dose reductions and dose interruptions (including the reasons) will be listed and summarized overall and by treatment group.

Concomitant medications and significant non-drug therapies prior to and after the start of the study treatment will be presented by WHO Drug Dictionary ATC class and preferred term, overall and by treatment group.

Primary variable

The statistical analysis of the primary variable will be performed on the FAS population and on the PP population as supportive.

No alpha level adjustment for multiplicity will be adopted.

All data available will be used for analysis and no technique for missing data imputation will be adopted.

Summary statistics of raw data and change from baseline at each assessment time point for LDL cholesterol levels will be presented overall and by treatment group.

An analysis of covariance (ANCOVA) model will be estimated on the changes from baseline to Week 8 in LDL cholesterol levels considering treatment group as factor and baseline value of LDL cholesterol as continuous covariate. Results will be reported as Least-Square Means together with associated two-tailed 95% CI and p-value. If the assumption of normality of residuals is violated, the ANCOVA model will be fitted on rank transformed data.

Secondary variables

The statistical analyses of the secondary variables will be performed on the FAS population.

Given the exploratory nature of the secondary objectives of the study, no alpha level adjustment for multiplicity will be adopted.

The prevalence approach will be applied, and therefore no technique for missing data imputation will be adopted.

The same statistical models described for the analysis of the primary variable will be applied.

Other variables

All the other analyses will be performed on the Tolerability set.

No missing data handling approach will be applied.

Adverse Events

The incidence of Adverse Events (AEs) and Serious Adverse Events (SAEs) recorded throughout the study will be presented.

According to the onset date of the event, AEs will be defined as follows:

- treatment-emergent AE, those events with an onset date after treatment initiation
- non-treatment-emergent AE, those events with an onset date between informed consent and treatment initiation

Non treatment-emergent AEs will be listed only.

Treatment-emergent AEs will be summarized by MedDRA dictionary system organ class and preferred term. A summary of treatment-emergent AEs by preferred term and severity will be also provided. For analysis purposes, all treatment-emergent AEs defined as ‘‘certain”, ‘‘probable’’, ‘‘possible’’ or “unassessable” will be considered as product related. If the relationship to study treatment is missing, the treatment-emergent AE will be considered treatment-related. All related treatment-emergent AEs, treatment-emergent AEs with an outcome of death, treatment-emergent AEs leading to discontinuation of treatment will be summarized by MedDRA dictionary system organ class and preferred term and will also be listed. Serious treatment-emergent AEs will be summarized similarly. Percentages will be based on the number of subjects in the Tolerability set.

Laboratory parameters

Summary statistics of raw data and change from baseline at each assessment time point for each laboratory parameter will be presented overall and by treatment group.

Laboratory test results will be classified by the low/normal/high classification based on local laboratory normal ranges. A frequency table for low/normal/high classification will be presented overall and by treatment group for each laboratory parameter. A listing of all out of range laboratory parameters will be provided with values flagged to show the classification relative to the laboratory normal ranges.

Vital signs, weight, waist circumference, Index of Central Obesity (ICO), Body Mass Index (BMI), Hepatic Steatosis Index (HSI), Lipid Accumulation Product (LAP).

Heart rate and blood pressure will be measured in orthostatic and clinostatic position. Summary statistics of raw data and change from baseline at each assessment time point for each parameter will be presented overall and by treatment group.

Deaths

All deaths occurred will be listed together with all their details.

Pregnancy test results

Any pregnancies reported will be listed.

11.6 Subgroup analysis

No subgroup analyses are currently planned.

11.7 Protocol violations and blind review

Protocol deviations will be detected throughout the study and reported by means of a specific form. All cases of prospectively defined protocol deviations (PD) will be identified prior to clinical database lock/unblinding and included in the clinical database. Certain deviations may stipulate that data collected from the patients will be excluded from analysis population(s). All exceptional cases and the final decisions on the allocation of patients to populations will be fully defined, documented and approved by the Sponsor study team during the blind review meeting, which will be held before data base lock and before breaking the blind.

11.8 Statistical analysis plan

Further details about data analysis will be provided in the Statistical Analysis Plan.

The document will have to be finalized before the database lock. Any deviation from the original statistical plan, as reported in the study protocol, will be properly documented and justified in the Statistical Analysis Plan.

12. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator/Institution will permit trial related monitoring, audits, IRB/IEC review, and regulatory inspections, providing direct access to source data/documents.

13. QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Data Quality Control/Study Monitoring

The study will be monitored according to the details specified in the Monitoring Plan.

The Investigator will be contacted by the study monitor on a regular basis. The monitor will have the responsibility of reviewing the ongoing study with the Investigator to verify adherence to the protocol and to deal with any problems. CRFs will be checked for completeness and consistency with the source data and special attention will be dedicated to patient enrolment, obtaining signed informed consent, occurrence of AEs, product accountability, and accurate recording of variables. The confidentiality of study related documents shall be maintained at all times. The Investigator agrees to allow access to all study materials needed for the proper review of study conduct. The Investigator agrees to assist the monitor in resolving any problem that may be detected during the monitoring visit or data cleaning process.

13.2 Case Report Forms

Data collected during the study will be recorded in the Case Report Form (CRF). Data reported on the CRF have to be consistent with the source documents. The Investigator must ensure the accuracy, completeness and the consistency of the data entered in the CRF.

On the CRF, patients will be identified by a patient number, assigned at the Screening Visit. The patient number will be composed of a two-digit center number followed by a three-digit patient identification number (01-001 for the first patient).

During the conduct of the study, the CRF must be available and up-to-date so that it always reflects the latest observations on the respective patient.

The Investigator will be responsible for entering study data into the CRF in accordance with the CRF user guidelines.

13.3 Quality Assurance

An independent quality audit/inspection at the study site may take place at any time during or after the study. The independent audit/inspection can be carried out by the Sponsor's independent Quality Assurance (QA), by a Health Authorities or an Ethics Committee (EC).

14. ETHICAL ASPECTS

This study will be carried out in compliance with the study protocol, the recommendations on biomedical research on human subjects of the Declaration of Helsinki, International Conference of Harmonization – Good Clinical Practice (ICH-GCP) Guidelines, EU-Directive 2001/20 and national requirements.

All clinical work conducted under this protocol is subject to Good Clinical Practice (GCP) rules.

14.1 Ethics Committees

Before starting the study at a study site, study protocol and relevant documentation (Patient information leaflet, informed consent form) must be submitted to and approved by the Ethics Committees and the Competent Authorities.

In addition, all local national legal requirements for the conduct of a clinical study have to be met prior to the start of the study. Any amendment to the protocol will be submitted to the EC for approval before implementation, after prior discussion between the Sponsor and Principal Investigator.

The Competent Authorities and EC will be informed of any changes in the study protocol, the end of the study or premature study termination as appropriate and within the requested time.

14.2 Insurance

The Sponsor has stipulated an insurance policy for patients participating in the study in accordance with local regulatory requirements.

Details of the insurance company, the insurance policy number and conditions will be made available to patients in the information sheet and/or provided as a separate document, in accordance with national requirements.

A copy of the insurance certificate will be provided to the Investigator and will be filed in the Investigator's File at the site and in the study's Trial Master File (TMF).

The Investigator must notify the Sponsor of any claims or lawsuits immediately.

15. DATA PROTECTION LAWS COMPLIANCE

By signing the study protocol, the Institution and the Principal Investigator (including their appointed staff) acknowledge that the performance of the study will imply the processing of personal data. Personal data processing is regulated by the Sponsor's national legislation as well as local laws (i.e. the country where the study is conducted): such provisions will apply mandatorily. The Sponsor clarifies that strict compliance with the applicable data protection laws by any parties and relevant employees who take part in the study is an

essential condition for the appointment of, and the collaboration with, research institutions, investigators, Contract Research Organization (CRO), etc.

The parties shall acknowledge that according to the applicable privacy laws, Sponsor and Institution/s will act as independent data controllers, while CRO and Principal Investigator/s will act as data processors. Before the beginning of the Study, the Institution will appoint in writing its Principal Investigator as its data processor, and the Sponsor will appoint in writing the CRO as its data processor. In addition, the Institution will appoint any Investigators other than the Principal Investigator as "persons in charge of the processing of personal data" ("incaricato del trattamento"), pursuant to art. 30 of the Italian Legislative Decree 196/2003 ("*Codice in material di protezione dei dati personali*").

Principal Investigator and Institution (including their personnel) shall comply with the applicable privacy laws and Sponsor's instructions concerning the protection of personal data. Such obligations will include, by way of example:

(i) the duty to provide the subjects involved in the Study with adequate, law-compliant information notices (based on the "information notice and consent form to process personal data" supplied by the Sponsor), where the nature of the study and the personal data processing operations it implies will duly be described;

(ii) the duty to collect the informed consent of the patients involved in the Study prior to their participation; such consent will include the possibility to transfer personal data outside the EEA (including to countries which do not ensure a level of protection of personal data comparable to that afforded by EU Law);

(iii) the duty to respect the data privacy rights of any data subjects - particularly of patients- as established by applicable privacy laws (including, by way of example, the right to have one's personal data amended, corrected or deleted, and to withdraw from the study);

(iv) the duty to adopt all physical, logical, organizational, technical and IT security measures in accordance with applicable privacy laws.

Given the sensitive nature of data processed in the Study, the parties undertake to implement adequate safety measures (physical, logical, organizational, technical, etc.) to warrant that data are always processed safely and in compliance with local privacy laws, and in particular with the Italian Data Protection Authority's "*Guidelines on Personal Data Processing in the context of Clinical Trials*" ("*Linee guida per i trattamenti di dati personali nell'ambito delle sperimentazioni cliniche di medicinali*") of 24.07.2008. Specifically:

a) The CRO, Sponsor, Principal Investigator and Institution must ensure that data is safely processed and stored in a way that minimizes the risk of theft and unauthorized access. To this extent, patients' personal data shall be securely stored in restricted areas, accessible only to those individuals who need to retrieve patients' data for professional purposes and should also be password-protected

(in the case of electronic documents) or key-locked (in the case of paper documents). In addition, the password of any electronic database containing patients' personal data (including the centralized database held by the CRO) shall be at least 8 characters long and updated at least every three months, and should have adequate cryptographic protection measures in place to prevent data breaches (such as, for example, file system or database cryptography, or any other equivalent IT measure which renders data unintelligible to those who are not authorized to access them);

b) The Principal Investigator must ensure that each patient's anonymity is safeguarded and will therefore "pseudonymize" patients' personal data (i.e., the patient's name and surname shall never be reported in the study documentation; instead, only the patient number described under section 13.2 of this Protocol will be used). The Principal Investigator will keep a separate log of patients' names, addresses and telephone numbers, and of the "key" to link this information to the respective patient numbers. Such data shall be securely stored as described under point a) above and shall be kept separate from the study documents that have been "pseudonymized" as described above. The Principal Investigator will keep these records for the longest time allowed by own institution and in any case until further communication from Sponsor.

c) All the parties that transfer patients' personal data through internet and/or through the centralised database(s) used to process the study's data or to generate statistical analyses shall implement secure protocols based on cryptographic standards which make data unintelligible to unauthorized individuals; this applies also to the CRO's centralized database;

d) The centralised database held by the CRO shall be accessible to authorized users by means of authentication methods which permit access to each specific set of subjects' data only to those for whom access is essential in the context of their work for the study;

e) The CRO and Institution shall ensure that any member of staff/collaborator involved in the study have received proper training on data protection issues.

Upon request of the CRO or the Sponsor, the Institution and Investigator will provide a detailed description of the measures adopted to comply with the requirements of the applicable data protection laws. In addition, all actions related to the implementation of the afore mentioned-measures shall be provided by the Sponsor and/or the CRO to the competent authorities (including data protection authorities) and Ethics Committees if and when requested. If such authorities or the Sponsor consider the implementation of the afore-mentioned measures insufficient to guarantee an adequate level of protection of the patients' personal data, the parties undertake to adopt all the necessary activities to overcome such remarks to assure the full compliance with the data protection laws.

16. DATA HANDLING AND RECORDS KEEPING

The Investigator should keep all study-related documents, as specified in ICH/GCP Section 8 “Essential Documents for the Conduct of a Clinical Trial” and all study documents as specified by applicable regulatory requirements, in the Investigator's Trial Center File.

The Investigator will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained in a secure place as long as needed to comply with national and international regulations.

If the responsible Investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility.

The Sponsor must be notified in writing of the name and address of the new custodian.

If it becomes necessary for the Sponsor and/or a Regulatory Authority to review any documentation related to this study, the Investigator must permit access to such documentation.

Any difficulty in storing original documents should be discussed with Sponsor personnel prior to initiation of the study.

17. PUBLICATION POLICY AND RESULTS

By signing the study protocol, the Investigator (and his/her appointed staff) states that any information and all the study documents provided by the Sponsor will be maintained strictly confidential.

None of this material may be disclosed to any party not directly involved in the study without written permission from Sponsor.

The Investigator will supply the Sponsor with all the data/results from the study.

All information concerning the study, the product as well as data and results of the study are confidential and property of the Sponsor. The Sponsor will prepare the final report, including the statistical and clinical evaluations. The Investigator’s agreement and signature will be obtained and a copy will be provided to the Investigator.

Sponsor reserves the exclusive right to publish and present data and results of the present study at scientific meetings, or to submit these clinical trial data to national and international Regulatory Authorities. The Investigator may not use the results of this study for publication or presentation without written authorization from Sponsor.

18. REFERENCES

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19. Public Protocol Approval Page

Study Title: A randomized, double-blinded, placebo-controlled, clinical study of the effects of a nutraceutical combination on LDL cholesterol levels in subjects with sub-optimal blood cholesterol levels. (Acronym: NATCOL)

Code: MEIF/16/MOF-Col/001

The signers confirm that they have read and approved the protocol

Study Medical Expert: *(name in capital letters)* SUADA METO

Signature & Date: 16 / 04 / 2018

Principal Investigator: (if applicable) *(name in capital letters)* CLAUDIO BORGHI

Signature & Date: 17 / 04 / 2018

Statistician: *(name in capital letters)* CHIARA COSTANTINI

Signature & Date: 20 / 04 / 2018

Medical Director CRO *(name in capital letters)* LAURA AMBROSOLI

Signature & Date: 18 / 04 / 2018

Corporate Medical Director: MECANI LORENZO

Signature & Date: 16 / 04 / 2018

20. Investigator's approval Page

INVESTIGATOR'S APPROVAL PAGE

(original to be kept in the Trial Center File)

I understand that all information concerning the product Phytosterols 800 mg + DIF1STAT® supplied by **A.** Menarini Industrie Farmaceutiche Riunite S.r.l. in connection with this study protocol are confidential information. This information includes: Protocol, Investigator's Brochure, Case Report Form, Other documents in the context of this study.

I understand that any change in this study protocol must be approved in writing by the Sponsor the Co-ordinating Investigator and the Ethics Committee before implementation, except where necessary to eliminate apparent immediate hazard to patients.

I confirm that I will conduct the study according to this protocol (except when mutually agreed to in writing with the Sponsor, the Good Clinical Practice (GCP), the Declaration of Helsinki and laws and regulations in the Country where the study is to be conducted.

I confirm that I will record and report all adverse events occurring during the study, according to this protocol.

I confirm that I am informed about the need of data records retention, according to current regulations and that no data can be destroyed without the written consent of the Sponsor.

I confirm that I will transfer adequate ownership of my responsibilities for the trial and will inform the Sponsor, in case I retire from my PI role.

I confirm that in case the Trial Center File is stolen or anyhow damaged, I will promptly inform the Sponsor and declare it to the Competent Authorities.

Principal Investigator: _____

Signature & Date: _____

21. APPENDICES

21.1 Appendix 1: General Guidelines for a Proper Mediterranean Style Diet

GENERAL DIETARY SUGGESTIONS:

- Keep meal times constant (3 main meals, 2 snacks between meals).
- Avoid large meals and feeling full.
- Avoid associating bread and pasta/rice, bread and potatoes, pasta/rice and potatoes during a single meal.
- Try to eat pasta/rice at lunch and meat/fish at dinner.
- If possible, start your meal with vegetables, possibly raw (without limits), with an oil, lemon and vinegar dressing.
- Do not lie down immediately after meals, but do regular physical activity.

Type of food	***Avoid***	***Limit (= acceptable if not in excess)***	***Preferred***
Carbohydrates (Pasta, bread, etc.)	Egg pasta, soft wheat pasta, rice pasta, gluten-free pasta, corn pasta, toast, bread containing lard (even if whole wheat!), boiled brown rice, French fries, mashed potatoes; packaged bakery products containing transesterified fatty acids (on the label indicated as “hydrogenated fats” or “partially hydrogenated fats”)	Boiled partial whole grain rice, potatoes (even boiled and baked!), polenta (grits)	Partial whole wheat or whole wheat bread without lard, durum wheat pasta cooked until just firm (better long pasta than short pasta), pasta with added fiber, boiled partial whole grain rice cooked until just firm, soups with cereals (oats and barley)
Meat	Offal, tongue, liver, pig trotters, cotechino, capocollo (cured pork shoulder), fatty meat, lamb, canned meat, lard, sausage, salami, bologna, greaves, bacon, and in general all fatty cold cuts.	Eggs: 2 per week (including “hidden” eggs in egg pasta, cream etc.) Bresaola or cold cuts from which fat can be removed such as ham or speck	Lean meat: poultry (cooked without skin!), pork (lean cuts with fat removed before cooking; ex.: filet, shoulder), beef (lean cuts with fat removed before cooking), rabbit, guinea fowl, pheasant, quail, horse meat, goat meat (kid), frog.

			Preferred cooking is grilled with extra-virgin olive oil and spices.
Fish	Eel, roe, crustaceans (shrimp, scampi, lobster), clams, mussels, oysters and other shellfish	Fish in oil	Sardines, mackerel, sole, bass, sea bream, trout, tuna, salmon, cuttlefish squid (not fried!)
Dairy products	Medium-fat cheeses (fontina, edamer, emmenthal) and high-fat cheeses (asiago, gorgonzola, camembert, brie, bleu), and buffalo mozzarella	Seasoned cheeses (parmesan, pecorino), used on pasta or as shavings after a meal	Low-fat cheeses (ricotta, spreadable light cheeses or shavings of light cheeses, light cow mozzarella), skimmed milk.
	Whole milk, whole goat milk, cream (liquid and whipped)	Low-fat milk (2%)	
	Whole milk yogurt	Low-fat yogurt	Yogurt from skimmed milk
Dressings and seasoning	Butter, lard, margarine containing animal fat, peanut oil, palm oil, coconut oil, mixed seed oil	Mayonnaise (in moderate doses), olives, margarine 100% vegetable	Extra-virgin olive oil, corn oil, rice oil, vinegar, lemon, spices (pepper, chili peppers, saffron, curry, herbs)
Vegetables	None	Carrots, zucchini, squash (especially cooked)	All legumes, green leafy vegetables, better if raw.
Fruit	Fruit in syrup, grapes, bananas, figs, pineapple, coconuts, avocados, macadamia nuts, cashew nuts, pecans, peanuts, dried fruit (dates, dried figs), candied fruit	Unsweetened canned fruit and fruit juice	Fresh seasonal fruit with skin (not more than 200 grams per meal). Dried fruit such as almonds, walnuts or hazelnuts (not more than 20 grams shelled a day).
Beverages	Strong beer, liquor, strong wine, sweet cider, sparkling wine, sweetened beverages and fruit juice, syrups, “energy drinks,” mineral supplements containing glucose (read label!!!)	Unsweetened beverages and fruit juice (carbonated and non) (not more than one glass a day), dry cider, low alcohol beer (1 can) or wine (1 glass) with meals	Spring water, still or sparkling

Dessert	Cakes with cream, pastries, ice cream, sorbets, fruit gelatin with sugar, sweetened breakfast cereals, caramel, milk chocolate, coconut, honey, packaged bakery products containing transesterified fatty acids (on the label indicated as “hydrogenated fats” or “partially hydrogenated fats”).	2 bars of extra dark chocolate with low quantity of sugar, Uno low-fat yogurt with 2-3 nuts and one spoonful of honey, spreadable hazelnut cream without added sugar (max. 1 tablespoon)	An exception as “gratification” or special occasions is allowed within an acceptable quantity. Artificial sweeteners for coffee without milk (max. 3 times a day) and tea.

21.2 Appendix 2: Declaration of Helsinki

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value

and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group.

In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.