EFFECT OF LYCOSOME FORMULATION OF LYCOPENE CHAPERONED WITH PHOSPAHTIDYLCHOLINE ON PROGRESSION AND OUTCOMES OF NONALCOHOLIC STEATOHEPATITIS

Protocol Number: DT 01-12-16-14

Clinical Trial Phase: Phase two

Principal Investigator (PI):

Ivan M Petyaev MD, PhD

Funding Mechanism: Lycotec Ltd, Cambridge, UK

Protocol Chair/Co-Chair: Yuriy K Bashmakov MD, PhD,
Nigel H Kyle

Medical Officer/Medical Monitor: Yuriy K Bashmakov MD, PhD
Victor Klochkov MD

Investigational compound / nutraceutical: LYCOSOME FORMULATION OF LYCOPENE CHAPERONED WITH PHOSPATIDYLCHOLINE (Lycotec Ltd, UK)

SIGNATURE PAGE

We agree to conduct the study in accordance with the relevant, current protocol and will not make changes to the protocol without permission of the funding authority, except when necessary to protect the safety, rights, or welfare of study participants.

We agree to personally conduct or supervise this study.

We will ensure that the requirements relating to obtaining informed consent and Ethics Committee (EC) or Institutional Review Board (IRB) review are met.

We agree to report to the sponsor any adverse events that occur during the course of this study.

We agree to maintain adequate and accurate study records and to make those records available for inspection by any authorized representatives, and/or other applicable regulatory entities. We will ensure that an EC or IRB that complies with the requirements of Ethics in Conduct of Research will complete initial and continuing review and approval of the study. We also agree to promptly report to the EC/IRB all changes to the study and any unanticipated problems involving risks to human subjects or others. Additionally, we will not make any changes to the study without EC and IRB approval, except where necessary to eliminate apparent immediate hazards to study participants.

We agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator:

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4. KEY ROLES

Principal Investigator: Ivan M Petyaev MD, PhD

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Principal Investigator is ultimately responsible for all aspects of the project including:

A. It is the responsibility of the PI to ensure that each subject is adequately informed and freely consents to participate in the research. The PI will personally ensure that every reasonable

precaution is taken to reduce risks to the study participants, including subjects who are enrolled initially but prove to be screen failures or for whom data is incomplete, unusable, and/or otherwise eliminated during analysis.

B. It is the responsibility of the PI to delegate responsibility to the research staff in a manner that is commensurate with the staff's training and qualifications.

C. It is the responsibility of the PI to ensure that all procedures associated with the research are performed in accordance with the protocol, including the schedule of events as applicable, with the appropriate level of supervision, only by individuals who are licensed or otherwise qualified to perform them under the applicable laws, regulations, and policies.

D. The PI will ensure adherence to the study protocol and monitor the informed consent process.

E. The PI will ensure that there are appropriate facilities and resources to conduct the research.

F. It is the responsibility of the PI to be available to the research staff as needed and to regularly review research processes and address any deficiencies identified through quality improvement processes. The PI may also contact funding partners to schedule an external assessment of his or her research through an on-site visit and a regulatory audit of the protocol file. The PI is responsible for maintaining documentation of any quality improvement process for their specific studies.

G. It is the responsibility of the PI to ensure that adequate staff, resources, and professional practices and standards of care are maintained at external performance sites.

H. It is the responsibility of the PI to evaluate the intermediate and final results of the project and to take a leading role in the preparation of the final report.

Protocol Chair/Co-Chair:

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Protocol Chair and Co-Chair are ultimately responsible for all aspects of the study protocol and its implementation including:

A. Writing the protocol document.

B. Ensuring that the necessary approvals are obtained, including those of the review boards, the sponsor, and any others for the protocol and subsequent amendments.

C. Monitoring the study during its execution, which includes: reviewing each case record to confirm eligibility, reviewing each case record to determine compliance with the protocol, reporting adverse events, determining any necessary changes to the protocol and the informed consent documents and submitting them as protocol amendments to the clinical site.

Medical Officer - Victor Klochkov MD, Institute of Cardiology, 12 Chernyshevskogo Str, Saratov, Russian Federation.

Medical Officer is ultimately responsible for all aspects of the medical services related to the study protocol and provided to the volunteers enrolled in the study including:

A. Providing medical advice and guidance on the writing, review, approval, and development of the study protocol.

B. Serving as a consultant to other study participants on issues related to the health of individual volunteers enrolled in the study.

C. Leading the analysis and evaluation of medical results of the study.

D. Evaluation of complex issues related to the medical safety of the study.

Medical Monitor: Yuriy K Bashmakov MD, PhD yuriy.bashmakov@lycotec.com, Lycotec Ltd, McClintock Building,

Granta Park, Cambridge, CB21 6GP, United Kingdom. Tel (44) - 1223-42-721 Fax (44)-1223-42-72

Medical Monitor is ultimately responsible for all aspects of coordination of work and communications between Lycotec Ltd, UK and Institute of Cardiology, 12 Chernyshevskogo Str, Saratov, Russian Federation, including:

A. Verification of the Investigator's qualifications and resources (clinical base, staff, equipment, laboratory).

B. Verification of Investigator's compliance with the protocol.

C. Control and reporting of findings, processes and conduct of the study.

D. Discussion and negotiation of any adjustments to the study that may arise over the course of the study.

5. LIST OF ABBREVIATIONS

LDL- Low Density Lipoprotein

HDL- High Density Lipoprotein

VLDL- Very Low Density Lipoprotein

PI- Principal Investigator

CT- Computed Tomography

AST- Aspartate Aminotransferase

ALT- Alanine Aminotransferase

LDH- Lactate Dehydrogenase

NASH- Non Alcoholic Steatohepatitis

PTDC- Phosphatidylcholine

6. PROTOCOL SUMMARY

Full Title: **EFFECT OF LYCOSOMAL FORMULATION OF LYCOPENE CHAPERONED WITH PHOSPHATIDYLCHOLINE ON PROGRESSION AND OUTCOMES OF NONALCOHOLIC STEATOHEPATITIS**

Short Title: **EFFECT OF LYCOSOMAL FORMULATION OF LYCOPENE CHAPERONED WITH PHOSPHATIDYLCHOLINE ON NONALCOHOLIC STEATOHEPATITIS**

Sample Size

40 subjects divided into two clinical groups, 20 subjects in each group).

Study Population

Newly diagnosed and confirmed patients with NASH, males and females, aged from 40-65 years.

Participating Sites

1. Lycotec Ltd, McClintock Building, Granta Park, Cambridge, CB21 6GP, United Kingdom. Tel (44) -1223-42-721 Fax (44)-1223-42-72
2. Institute of Cardiology, 12 Chernyshevskogo Str, Saratov, Russian Federation. Tel (+7) -9633-4522.

Study Design

The study is designed as a double-blinded, interventional, randomized clinical study involving patients with NASH. A major research question is whether ingestion of lycopene stabilized with Lycosome™ formulated phosphatidylcholine chaperone can reverse clinical and laboratory manifestations of NASH. Therefore, the primary endpoint of the project is the size of the liver and parameters reflecting lipid accumulation in the hepatic tissue at the end of the interventional period. Changes in the activity of liver-specific enzymes and parameters of lipid homeostasis represent the secondary question since normalization of serum levels of hepatic enzymes represents another important criterion of liver damage and its reversal in NASH. Biochemical parameters in serum specimens will be investigated before commencing the study, at the intermediate time points of 1 month and in the end of the study after 2 months treatment. To assess changed in the liver span Ultrasonography was used before an in the end of the trial.

Subjects will be assigned to take orally once a day either capsules containing 450 mg of regular phosphatidylcholine or 450 mg of the same phospholipid in Lycosome™ formulation with 7 mg of lycopene (Cambridge, UK).

The formulations will be dispensed to the subjects during follow-up visits each month. Monthly visits are planned to determine compliance and adherence to the protocol. A basic medical check-up will be performed and subjects will be asked questions to determine adherence to the protocol during each visit.

Packaging and labeling of the study products will be carried out according to the guidelines of NIH and US Department of Health and Human Services as recommended for biologics and investigational health food products. The formulations will be shipped to the study site in specially designed containers with primary and secondary components. The container closure system will prevent exposure of the investigational products to light, moisture, temperature extremes and air. This packaging system guarantees stability of the product for intended use. Protection from microbial contamination will be provided by maintaining container integrity after the packaging system is sealed. An adequate and validated procedure was used for investigational product manufacture and packaging.

All individual packages will be labeled with a numerical code. None of the volunteers or trial personnel will be informed of study group assignment for the duration of the study. Unblinding of the results is planned to be performed by an independent statistician who will be hired and assigned to the work at the end of the study period.

Upon arrival at the clinical site the study products will be inspected by the Medical Officer and Clinical Monitor, then placed in a locked room at the clinic. Only the Medical Officer, PI and Clinical Monitor will have access to the products during the study period. Each subject will be given a one month supply of the study product at the "zero" time point of the study and then subsequently for each month of the study period following verification of compliance with the study protocol.

Total study duration is six months with a medical check-up for each volunteer at the "0" time point of the study as well as at 1st month and at the 2nd, the end of the interventional period.

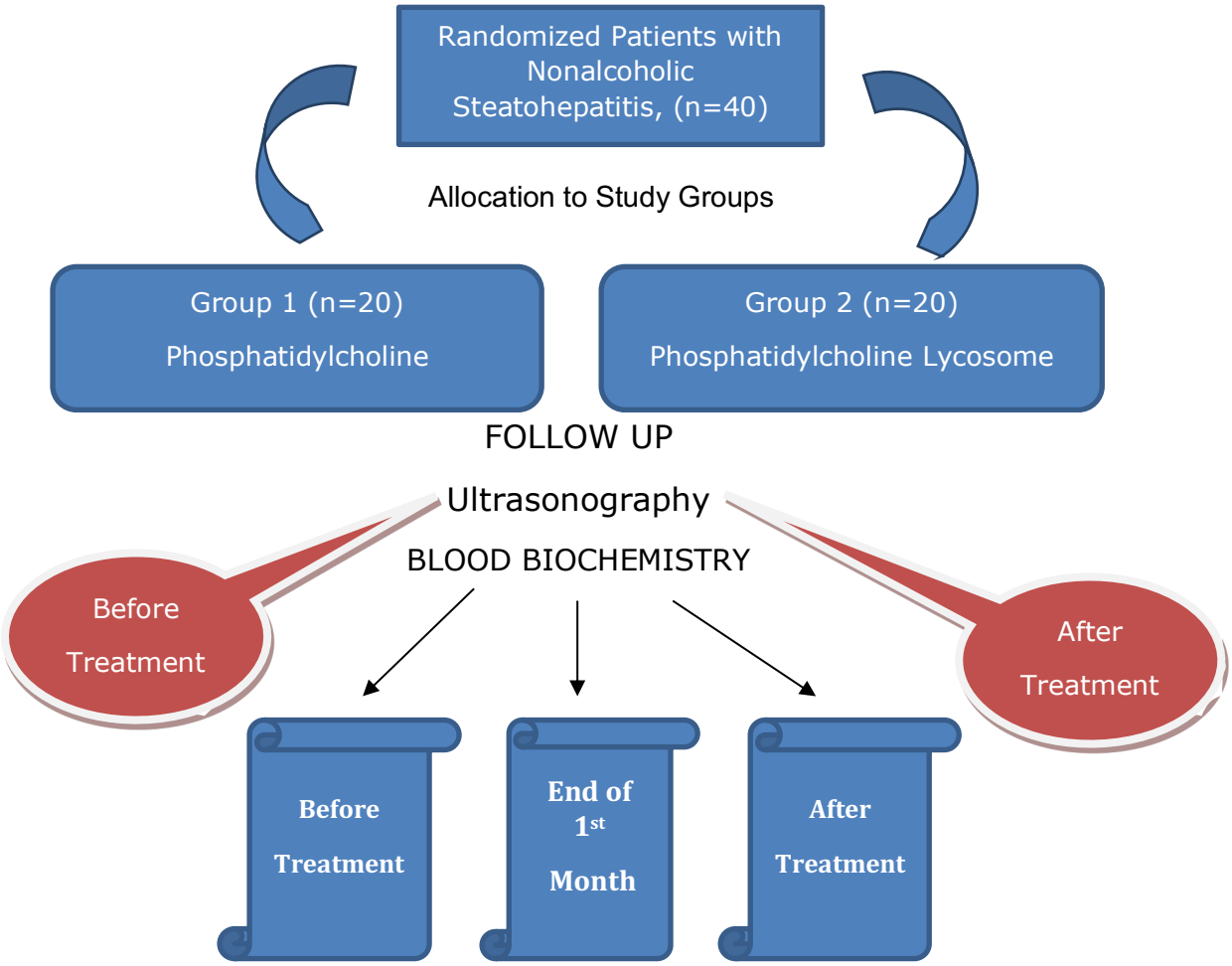
During the medical check-ups each volunteer will be subjected to thorough clinical and laboratory investigation. Clinical investigation will include reading the vital signs, determination of body mass index, anthropometry, blood pressure measurement and computed tomography (at the initial and final time points of the study). Among laboratory tests will be determination of fasting serum glucose and lipids (total cholesterol, LDL, HDL and total triglyceride

levels), determination of ALT and AST levels, alkaline phosphatase, gamma glutamyl transpeptidase, lactate dehydrogenase, total bilirubin, CRP, LDL oxidation and IOD. Moreover, serum lycopene concentration and lycopene isomer profile will be measured at the initial and final points of the interventional period. All volunteers will also be subject to a standard hematological investigation.

Results collected for each volunteer will be stored as both paper files and digital files in Excel format and will be available for access by the Medical Officer, Medical Monitor and PI. To avoid any bias each subject will be given a random computer code containing three numerical characters and three letters. Decoding of the individual results will be carried out by an independent statistician hired for the purpose.

Packages containing the study formulations will be coded and blinded for both the subjects and medical personnel conducting the study. Only the Principal Investigator will be informed of package contents and the subjects belonging to each study group.

Study Schema



Inclusion Criteria:

1. Age 18 years and above with provision of consent.
2. Patients with definite or probable nonalcoholic steatohepatitis based on the results of computed tomography and laboratory tests which were obtained no more than 90 days prior to randomization with or without type 2 diabetes mellitus and insulin resistance.

Exclusion Criteria:

1. Current alcohol use for a period of 3 consecutive months.
2. Patients with heart failure.
3. Anamnestic indication of long-term alcohol use in the past.
4. Patients with acute kidney injury at the time of enrollment
5. Patients with CKD (Chronic Kidney Disease) or with serum creatinine > 1 mg/dL.
6. Patients with platelet count <100.000/mm^3
7. Patient with severe cases of type 2 diabetes mellitus with Hb A1c >11.0 %
8. Pregnancy.
9. Patients with hepatocellular carcinoma.
10. Patients who are not willing to participate in the study.
11. Patients with any form of decompensation at the time of enrollment in the study.
12. Patients with hepatitis C as defined by presence of HCV RNA or antibodies to HCV.
13. Hepatitis B as defined by presence of HBsAg.
14. Users of medications associated with NASH – tetracycline, amiodarone, tamoxifen, glucocorticoids, anabolic steroids.

7. CLINICAL PROTOCOL

7.1 Introduction

Background

Lycopene is a polycarbon polyunsaturated red pigment from certain fruits and vegetables (tomatoes, papaya, strawberries, red bell pepper and cherries) which belongs to the carotenoid family and has extremely powerful antioxidant properties in vivo and in vitro (1). There is a growing body of clinical evidence that introduction of lycopene into the human diet prevents multiple diseases such as prostate cancer, type 2 diabetes and atherosclerosis. Recent studies indicate that lycopene supplementation deeply affects liver functions (2). In particular, as shown under experimental conditions, lycopene can prevent development of steatohepatitis in rodents (3).

Steatohepatitis, otherwise known as fatty liver disease, is a common pathological condition of liver which is characterized by accumulation of lipids in the hepatic tissue (4). Steatohepatitis accompanies multiple internal diseases such as obesity, type 2 diabetes, insulin resistance and hyperlipidemia and may finally result in liver cirrhosis (5). Although the exact number of patients with steatohepatitis in the world is not precisely defined, this number may approach 250 million patients who suffer from obesity and type 2 diabetes (6). Methods for steatohepatitis treatment are not well defined or standardized as yet. Diet, exercise and antiglycemic drugs are believed to be helpful in management of steatohepatitis, however no specific medication has been proposed as yet (7).

However, there is recent evidence that vitamin E therapy may attenuate steatohepatitis development (8). This suggests that use of antioxidants, including lycopene, may represent a novel strategy in treatment of steatohepatitis (8).

7.2 Rationale

Dietary factors are crucial for maintenance of liver health and prevention/treatment of liver disease (9). Epidemiological evidence suggests carotenoid intake, in particular lycopene intake, may normalize some hepatic functions and prevent liver disease (10). Nevertheless, the effect of lycopene on liver disease and steatohepatitis in particular remains poorly investigated. These effects are largely attributed to the antioxidant properties of carotenoids. Amongst these antioxidants lycopene represents the most powerful agent capable of neutralizing superoxide radicals, nitric oxide and hydroxyl radicals under in vitro and in vivo conditions (11). Carotenoids are known to decrease plasma

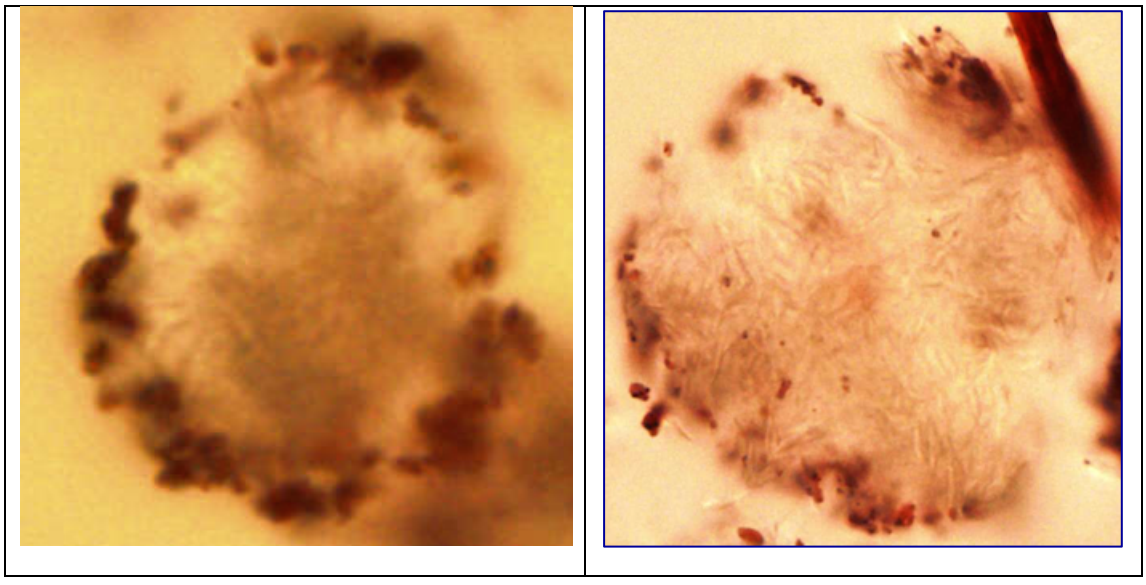
activities of liver specific enzymes including alanine aminotransferase, aspartate aminotransferase and gamma-glutamyl transpeptidase (12). Carotenoids have also been shown to stabilize bilirubin level and increase bile production (13). Each of these changes was described in experimental models of liver disease, therefore adequate clinical studies should be performed to confirm the hepatoprotective properties of carotenoids in clinical settings.

Phosphatidylcholine is an important molecules to help to scaffold lipid structure in cell membranes and in particular in hepatocytes. It has been used for treatment liver fatty disease and liver steatosis. However, the main limitation of its use is a poor compliance because to achieve therapeutic effect patients need to take 1 gram, or 2 grams, or 3 grams or even more. The main reason behind administration of such high doses is that phosphatidylcholine is very sensitive to acidic oxidation. Hence to pass the stomach acidic environment, it has been only available approach, until recently, to provide a significant excess of the phospholipid to allow its to be oxidised in order to provide a smaller part to reach the intestine.

Development of Lycosome technology, which uses acid-resistant carotenoids help to protect sensitive molecules from stomach degradation (14-16)

The lycosome phosphatidylcholine particles are composed of clusters of this lipid and surrounding lycopene aggregate structures (Figure 1).

Figure 1. Phosphatidylcholine lycopene Lycosomes
diameter from 0.5 to 5 μ – light microscopy.



LYCOSOME STRUCTURE

The lycosome is protected by an external layer containing lycopene. Moreover, the external layer contains an amphiphilic phospholipid (phosphatidylcholine) serving as a chaperone for lycopene and as an emulsifying as well as hydrophilizing agent. Lycosome particles contain only food-derived natural ingredients. Lycopene, used as a core-forming agent in lycosome particles, is derived from tomatoes, while phosphatidylcholine originates from egg yolk or soy beans. This resolves a biosafety issue. Moreover, besides an exceptional biosafety record and obvious impact on bioavailability of other nutraceuticals, lycopene inherently confers some measurable degree of cardiovascular and neoplastic protection. The cholesterol-lowering and anti-tumorigenic actions of lycopene arising from its antioxidant properties have been reported in multiple independent clinical trials.

Microencapsulation protocols based on lycosome technology open new horizons for targeted organ-specific delivery of nutraceutical and possibly pharmacological agents. Lycopene is known to be a powerful ligand for carotenoid receptors abundantly expressed in the liver, brain and certain other vital organs. Therefore, upon ingestion lycosome particles become bound and internalized in the tissues where carotenoid receptors are expressed to the highest degree creating thereby targeted traffic of nutraceuticals in the human body via the network of carotenoid receptors. Selective hepatic delivery of nutraceuticals and certain drugs is essential, since the liver is the central organ responsible for biotransformation and action of bioactive substances of foreign origin. Some nutraceuticals and drugs work exclusively through hepatic mechanisms and do not display any effect unless they are available in the liver. As we recently reported in the "Archives of Medical Sciences", lycosome formulation of simvastatin, a hepatoselective drug used for treatment of hypercholesterolemia and cardiovascular disease, has a superior cholesterol-lowering ability as compared to an unmodified formulation of the drug.

All of the reasons stated above create a rationale for the present clinical study, which is designed to investigate the effect of lycopene on progression and outcomes of nonalcoholic steatohepatitis.

7.3 Clinical Study Objectives

7.3.1 Primary objective

The primary objective of this clinical study is to verify the effect of a novel formulation of lycopene fused with phosphatidylcholine chaperone on liver size and function as well as progression and outcomes of nonalcoholic steatohepatitis (NASH).

Secondary objectives

- Investigation of the effect of a novel formulation of lycopene fused with phosphatidylcholine chaperone on tomographic parameters of liver in NASH patients.
- Evaluation of the effect of a novel formulation of lycopene fused with phosphatidylcholine chaperone on liver specific enzymes including alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transpeptidase, alkaline phosphatase and some others in NASH patients.
- Assessment of the effect of phosphatidylcholine chaperone alone on tomographic parameters of liver and plasma biochemistry values in NASH patients.

7.4 Study Design

7.4.1 Selection and randomization of volunteers

Medical personnel from the outpatient clinic of the Institute of Cardiology, Saratov, Russian Federation will be informed about launching the study, its major goal and selection criteria for volunteers. Suitable individuals will be invited for a preliminary check-up (physical and laboratory investigation) during the initial phase of enrolment. All suitable individuals will be re-screened after the wash-out period of the study before the final decision on study enrolment is made.

Randomization of volunteers in the study will be performed using widely accepted methods such as simple randomization and stratified randomization. Briefly, software containing a random number generator will be applied to the database of volunteers. The assigned group will be considered final. The groups will be balanced according to numerical age and gender with special software.

Stratified randomization will be used to enhance the statistical power of the final results and will ensure equality of groups for secondary selection criteria.

7.4.2 Schedule of Procedures/Evaluations

1. 2 months prior to study initiation. (Initial enrolment with preliminary physical and laboratory investigation).
2. 2 – 5 days prior to study initiation. Final qualifying physical and laboratory investigation.
3. Day "0" of the study. Physical and laboratory investigation to obtain baseline clinical and laboratory values. Computed tomography.
4. End of the 2^{nd} and 4^{th} months of the study. Physical and laboratory investigation to obtain mid-point clinical and laboratory values.
5. End of the 6^{th} month of the study. Concluding physical and laboratory investigation to obtain final clinical and laboratory values. Computed tomography.

7.4.3 Study Duration

Interventional period is designed to last for 6 months. Enrolment period and search for volunteers may take up to 2 months.

7.4.4 Inclusion Criteria

1. Age 18 years and above with provision of consent.

2. Patients with definite or probable nonalcoholic steatohepatitis based on the results of computed tomography and laboratory tests which have been obtained no more than 90 days prior to randomization with or without type 2 diabetes mellitus and insulin resistance.

7.4.5 Exclusion Criteria

1. Current alcohol use for a period of 3 consecutive months. Patients with heart failure.
2. Anamnestic indication of long-term alcohol use in the past.
3. Patients with acute kidney injury at the time of enrollment.
4. Patients with CKD (Chronic Kidney Disease) or with serum creatinine > 1 mg/dL.
5. Patients with platelet count <100.000/mm^3
6. Patients with severe cases of type 2 diabetes mellitus with HbA1c >11.0 %
7. Pregnancy.
8. Patients with hepatocellular carcinoma.
9. Patients who are not willing to participate in the study.
10. Patients with any form of decompensation at the time of enrolment in the study.
11. Patients with hepatitis C as defined by presence of HCV RNA or antibodies to HCV
12. Hepatitis B as defined by presence of HBsAg
13. Users of medications associated with NASH – tetracycline, amiodarone, tamoxifen, glucocorticoids, anabolic steroids.

7.4.6 Formulations and Mode of Intake

Subjects will be assigned to take orally twice a day either LycosomeTM formulated phosphatidylcholine chaperone, an essential phospholipid formulation containing 450 mg of phosphatidylcholine, or LycosomeTM formulated phosphatidylcholine chaperone (450 mg) fused with lycopene (7mg), an essential carotenoid. The latter is a novel lycosome formulation of lycopene with enhanced bioavailability developed by Lycotec Ltd (Cambridge, UK). Both formulations will be taken orally, twice a day (morning and evening) for a period of 6 months. The volunteers will be asked to take the study products twice a day after meals.

8. STUDY DRUG STORAGE AND ACCOUNTABILITY

The Principal Investigator and Medical Officer for the study are responsible for ordering, receiving, and tracking inventory; storing, dispensing, and returning study products properly; and, where necessary, labeling of study products prior to dispensing according

to protocol guidelines and good manufacturing practices (GMPs). All study products supplied for the protocol will be accounted for and tracked in a manual or electronic accountability log for the study. Accountability for the study products will be documented from the time of initial receipt through dispensation and final disposal of leftover study products. The accountability log will indicate the date, amounts, batch numbers, and condition upon receipt of all materials received from the supplier. Upon study completion remaining study products will be returned or destroyed as defined by specific Investigator instructions, or other documented instructions. The balance returned or sent for destruction will be recorded in the study accountability log. Quality assurance reviews and inspections of study product accountability documentation will be performed at intervals during the study by the Clinical Monitor. Study products will be stored at the clinical site facility in a locked cabinet.

9. STUDY DRUG COMPLIANCE AND ADHERANCE

Compliance of subjects with the protocol and adherence to product intake will be determined by asking questions of the patients during intermediate and final visits to the clinical site and by random private telephone calls. Subjects with poor adherence to the study protocol will be withdrawn from the study by the Medical Officer.

10. CONCOMITANT MEDICATIONS

Patients with concomitant intake of anti-hypertensive, lipid-lowering or any other cardio-vascular drugs will be illegible for the study. Subjects will be allowed to take prescription medication assigned by primary care physician.

11. SAFETY AND EFFECTIVENESS ASSESSMENTS

Safety assessment procedures are barely applicable to the current study since widely consumed food ingredients will be used for the study. Volunteers with intolerance or adverse reaction to phospholipids or lycopene will not be enrolled in the study.

Effectiveness assessment will be performed by measuring liver size and serum lipid levels at the intermediate and final points of the interventional period.

11.1 Safety assessments

The volunteers will be instructed to check for the appearance of skin rash, nausea and dyspepsia and to report any of these to the Medical Officer should they occur.

11.2 Effectiveness assessments

Computed tomography, measurements of blood pressure, serum activities of liver specific enzymes and concentration of total cholesterol, LDL, HDL and triglycerides will be measured as effectiveness assessment parameters.

12. ADVERSE EVENT REPORTING

Occurrence of adverse events in subjects consuming lycopene and phosphatidylcholine is very unlikely. If any do occur it is the responsibility of the Medical Officer and the PI to determine and analyze adverse event cause.

Among adverse reactions could be skin rash, dyspepsia and nausea.

Occurrence of any of these will be considered as a reason for volunteer withdrawal from study participation.

12.1 Recording/Reporting requirements

All subjects will be required to report any possible adverse effect to the Medical Officer assigned to the study, whose responsibility is to immediately inform the PI of any possible adverse reaction. Moreover, appropriate information will be immediately passed to the Local Ethical Committee. All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of study group or suspected causal relationship to the study, will be recorded in the subjects' case histories. Adverse events or abnormal test findings felt to be associated with the study will be followed up until the event (or its sequelae) or the abnormal test finding resolves or stabilizes.

For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a *serious adverse event*) and; 2) an assessment of the casual relationship between the adverse event and the study product(s).

An abnormal test finding will be classified as an *adverse event* if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms

- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy

Lycotec Ltd and Saratov Institute of Cardiology will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study product(s); and 3) if the adverse event meets the criteria for a *serious adverse event*.

If the final determination of causality is "unknown and of questionable relationship to the study product(s)", the adverse event will be classified as *associated with the use of the study product* for reporting purposes. If the Sponsor-Investigator's final determination of causality is "unknown but not related to the study product", this determination and the rationale for the determination will be documented in the respective subject's case history.

12.2 Withdrawal of subjects due to adverse events

Any subject with an adverse reaction and/or abnormal laboratory test findings will be withdrawn from the study by the Medical Officer and replaced with an eligible volunteer from the pre-screened pool of volunteers. An appropriate report to the Local Ethical Committee will be filed.

13. STATISTICAL METHODS/DATA ANALYSIS

13.1 Study endpoint

For assessment of normally distributed parameters the Shapiro-Wilk method will be used. Student's t-test will then be applied for both paired and unpaired samples. Between-group differences at one time point will be evaluated by Wilcoxon-Mann-Whitney test (continuous variables) and Fisher's Exact test (categorical variables). Data analysis will be performed using Stata SE, version 12.1. All statistical tests will be two-sided and statistical significance level alpha will be set at 0.05 for the analysis.

13.2 Sample size determination

Sample size has been determined based on preliminary results obtained in our previously published pilot clinical study and statistics obtained in our work.

Sample size has been calculated based on standard deviation values according to methods widely described elsewhere. The major statistical requirements will be pre-specified in our work as follows: significance of probability in one-tailed test will be taken to be 2.5%; the statistical power level will be chosen to be 90%.

With these requirements for sample size determination we decided to enroll a total of 80 volunteers (40 individuals per group).

13.3 Statistical effectiveness analysis

Statistical analysis of results will be performed by an independent statistician. If the statistical methods and settings listed above are not appropriate for the newly obtained results due to specificity of variant distribution, other methods of statistical analysis will be applied. Any deviations from the statistical plan described above will be explained and justified in a protocol amendment and/or in the final report submitted to the Institutional Ethics Committee.

14. QUALITY CONTROL AND QUALITY ASSURANCE

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by Institutional Ethics Committee qualified staff from Saratov Institute of Cardiology.

Saratov Institute of Cardiology will permit direct access of study monitors and appropriate regulatory authorities to the study data

and to the corresponding source data and documents to verify the accuracy of the data.

15. DATA HANDLING AND RECORD KEEPING

15.1 Data recording/Case Report Forms

A Case Report Form (CRF) will be completed for each subject enrolled onto the clinical study. The Sponsor-Investigator will review, approve and sign/date each completed CRF; the Sponsor-Investigator's signature serving as attestation of the Sponsor-Investigator's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. Where applicable, information recorded on the CRF shall match the *Source Data* recorded on the *Source Documents*.

15.2 Record maintenance and retention

The Sponsor-Investigator will maintain records in accordance with Good Clinical Practice guidelines; to include:

- Financial disclosure information (i.e., for the Sponsor-Investigator and for sub-investigators who will be involved in the administration of the study products and/or the evaluation of research subjects [i.e., who will contribute significantly to the research study data]).
- Curriculum vitae for all participants (i.e., for the Sponsor-Investigator).
- Certificates of required training; e.g., human subject protection, Good Clinical Practice, etc. (i.e., for the Sponsor-Investigator and for all sub-investigators who

will be involved in the administration of the study products and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data]).

- Listing of printed names/signatures. (i.e., for the Sponsor-Investigator and for all sub-investigators who will be involved in the administration of the study products and/or the evaluation of research subjects [i.e., who will contribute significantly to the study data]).
- Normal value(s)/range(s) for medical/laboratory/technical procedures or tests included in the clinical protocol.
- Laboratory certification information.
- Instructions for on-site preparation and handling of the investigational product(s), study treatment(s), and other study-related materials (i.e., if not addressed in the clinical protocol).
- Responsibility delegation log.
- Signed informed consent forms.
- Completed Case Report Forms; signed and dated by Sponsor-Investigator.
- Source Documents or certified copies of Source Documents.
- Monitoring visit reports.
- Copies of Sponsor-Investigator correspondence (including notifications of safety information) to sub-investigators.
- Subject screening and enrollment logs.
- Subject identification code list.
- Investigational product accountability records, including documentation of product disposal.
- Final clinical study report.
- Decoding procedures for blinded studies.
- Master randomization list.

- Retained biological specimen log.
- Interim data analysis report(s).

The Sponsor-Investigator will retain the specified records and reports for up to 5 years.

16. ETHICS

16.1 Institutional Review Board (IRB) approval

The Sponsor-Investigator will obtain from the Institutional Review Board (IRB) of Saratov Institute of Cardiology and Ethics Committee prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e. directed at potential research subjects) for study recruitment.

16.2 Ethical and scientific conduct of the clinical research study

The clinical research study will be conducted in accordance with the current IRB-approved clinical protocol; ICH GCP guidelines adopted by the FDA; and relevant policies, requirements, and regulations of the Russian Federation and Saratov Institute of Cardiology.

16.3 Subject informed consent

The Sponsor-Investigator will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed of the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The Sponsor-Investigator, or sub-investigator(s) designated by the Sponsor-Investigator, will obtain the written, signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The date and time that the subject, or the subject's authorized representative, signs the

informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The Sponsor-Investigator will retain the original copy of the signed informed consent form and a copy will be provided to the subject, or to the subject's authorized representative.

The Sponsor-Investigator will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the Sponsor-Investigator will obtain the informed consent of enrolled subjects for continued participation in the clinical study.

17. STUDY DISCONTINUATION CRITERIA

17.1 Discontinuation of individual research subjects

Individual research subjects can be withdrawn from the study due to occurrence of adverse effects, volunteer's request (regardless of reason) and non-compliance with the protocol. Volunteers withdrawn from the study will be replaced from the pre-screened pool of volunteers.

17.2 Sponsor-Investigator discontinuation of the clinical research study

The clinical study can be terminated by mutual agreement between Sponsor and Investigator for any reason. The Institutional Review Board (IRB) and Ethics Committee of Saratov Institute of Cardiology will be notified promptly of discontinuation of the entire clinical study.

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