A Phase IIb, double blind randomized, controlled clinical trial, to evaluate the efficacy and safety of two Aramchol doses versus placebo in patients with Non-Alcoholic Steatohepatitis (NASH)

ARREST study

Protocol No: 005
EUDRACT No: 2014-003107-29
IND: 79,200
Clinical Phase: IIb

[redacted]

Amendment No. 7: 03 May 2017

Principal Investigator: [redacted]

Sponsor: **Galmed Pharmaceuticals Ltd.**

[redacted]

This clinical study will be conducted in accordance with the sponsor's and/or Contract Research Organization's Standard Operating Procedures (SOPs), Current Good Clinical Practice (GCP), the provisions of ICH (International Conference on Harmonization) Guidelines and EU Directives

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SIGNATURE PAGE

Title: **A Phase IIb, double blind randomized, controlled clinical trial, to evaluate the efficacy and safety of two Aramchol doses versus placebo in patients with Non-Alcoholic Steatohepatitis (NASH)**

[redacted]

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[redacted]

Galmed Pharmaceutical

ARREST Study

Protocol No. 005

INVESTIGATOR'S AGREEMENT

I have carefully read the foregoing protocol including all appendices and agree that it contains all the necessary information for conducting the study safely.

I will conduct this study in strict accordance with this protocol and according to the current GCP guidelines and will attempt to complete the study within the time designated.

I will provide copies of the protocol and all other information relating to pre-clinical and prior clinical experience submitted by the sponsor to all personnel responsible to me who participate in the study. I will discuss this information with them to assure that they are adequately informed regarding the drug and conduct of the study.

I agree to keep records on all subject information (case report forms, shipment and drug return forms and all other information collected during the study) in accordance with the current GCP and local regulations.

_____
Principal Investigator's name

_____
Signature

_____
Date

_____
Institution

1 SYNOPSIS

Protocol Number:	005
Eudract/IND Number(s)	2014-003107-29 / 79,200
Study Title:	A Phase IIb, double blind, randomized controlled clinical trial to evaluate the efficacy and safety of two Aramchol doses versus placebo in patients with Non-Alcoholic- Steatohepatitis (NASH).
Clinical Phase:	IIb
Study Drug:	ARAMCHOL
Indication:	Aramchol is indicated for the treatment of non-alcoholic Steatohepatitis in patients with two additional features of metabolic syndrome -overweight or obesity and Diabetes Mellitus type II or pre-diabetes.
Sponsor Name and Address:	**GALMED Pharmaceuticals LTD**. [redacted]
Principal Investigator:	[redacted]
Study Duration:	Screening phase – 4 weeks (up to 45 days) Treatment Period – 52 weeks Follow up – 13 weeks
Study Population:	Males and females 18 – 75 years old diagnosed with Non-Alcoholic Steatohepatitis (NASH) who are overweight or obese and have Type II Diabetes Mellitus or pre-diabetes
Objectives	**Primary Objective:** To evaluate the efficacy on steatosis reduction as measured by NMRS of two Aramchol doses (400 mg and 600 mg), once daily for 52 weeks vs placebo. **Key Secondary Objectives:** 1. To evaluate the efficacy of Aramchol on CRN Fibrosis Score by liver biopsy. 2. To evaluate the efficacy of Aramchol on disease activity as measured by NAS and SAF activity score 3. To evaluate the efficacy of Aramchol on NASH resolution by liver biopsy. 4. To evaluate the effect of Aramchol on ALT levels. [redacted] Safety: Adverse events and serious adverse events, safety laboratory evaluations, vital signs, 12-Lead ECG and physical examinations will be assessed.

	Tolerability: Proportion of subjects (%) who prematurely discontinued from the study, reason of discontinuation and the time to withdrawal Proportion of subjects (%) who prematurely discontinued from the study due to AEs and the time to withdrawal.
Study Design:	This is a multicenter, Phase IIb, randomized, double blind, placebo-controlled study designed to evaluate the efficacy and safety of two Aramchol doses in subjects that are 18 to 75 years of age, with Non-Alcoholic Steatohepatitis (NASH) confirmed by liver biopsy performed in a period of 6 months before screening visit, with overweight or obesity and who are pre diabetic or type II diabetic. Eligible subjects will be enrolled into three treatments arms: Aramchol 400 and 600 mg tablets and placebo tablets in ratio 2:2:1. The subjects will be evaluated at study sites for 11 scheduled visits: at screening (visit 1(weeks -4 – 0)), baseline (visit 2 (day 0)), visit 3 (week 2), visit 4 (week 4), visit 5 (week 8), visit 6 (week 12), visit 7 (week 24), visit 8 (week 32), visit 9 (week 40) and visit 10 (week 52 End-of-Treatment/Early Termination visit). After completion of the study treatment period, the subjects will be followed for an additional period of 13 weeks without study medication until visit 11 (week 65). During the screening period, the severity of the disease will be evaluated with blood tests, liver biopsy and NMRS. During the study the following assessments will be performed: - Vital signs will be measured at each study visit. - A physical examination will be performed at the screening visit, 24 weeks, End-of-Treatment/Early Termination and week 65 visit. - The following blood tests will be performed: complete blood count (CBC), serum chemistry (including electrolytes, liver enzymes, direct and total bilirubin, glucose, lipid profile which include triglyceride, cholesterol, HDL, LDL and VLDL, CPK, creatinine, urea, albumin, alkaline phosphatase), ESR and urinalysis during the screening visit, baseline, week 2, 4, 8, 24, 40, 52 and 65 (end of follow up) visits. Serology (HBV, HCV and HIV) will be performed during the screening visit. Coagulation (fibrinogen, PT/INR, aPTT) will be measured at screening and baseline, week 24, End-of-Treatment/Early Termination and week 65 visits. Insulin (HOMA) will be measured at the screening, week 24 and End-of-Treatment/Early Termination visits. HbA1C will be measured at the screening, week 8, 24, 40 and End-of-Treatment/Early Termination visits. C reactive protein, Leptin, Adiponectin, [redacted] will be measured at the baseline visit and end of treatment period. The blood samples taken at these visits, will be tested for possible biomarkers [redacted]. TSH, T3 and T4 will be measured during the screening visit. β-hCG in women of childbearing potential will be performed during the screening visit. A serum sample will be collected and kept frozen until study end in case special investigation needs to be performed. This sample will be collected during the screening and visit 10/Early Termination.

	- Body weight and waist circumference will be measured at screening, baseline, week 24, end of treatment and week 65 visits. Height will be measured during the screening visit. - ECG will be performed during the screening visit, visit 7 (week 24) and End of Treatment/Early Termination visit. - All subjects will undergo two NMRS scans, at screening and end of treatment visits. - FibroMax test will be performed only if the investigator thinks it is necessary. - Liver biopsy will be conducted during the screening and end of treatment visit. The biopsy in the screening visit will be performed only if it was not done within the 6 months prior to this visit. - Metabolomics blood test will be performed at the screening, visit 7 and the End-of-Treatment/Early Termination visits. From some consenting patients (about 15) a sample from the liver biopsy will be taken for analysis. - Endothelial Function will be conducted during the baseline visit before the study treatment will be given and End-of-Treatment/Early Termination visit. This test will be conducted at selected sites. If test was not done on Visit 2 (Day 0) or needs to be repeated due to unacceptable test data, it will be done at Visit 3 or at an Unscheduled Visit before Visit 3. - Blood sample for Aramchol trough level will be collected (pre-dose) from patients in Israel at baseline (visit 2) week 4 (visit 4), week 12 (visit 6), week 24 (visit 7), week 40 (visit 9), end of treatment (visit 10) and follow up (visit 11). At selected sites in Mexico, USA, Europe and Hong Kong one blood sample will be collected (pre-dose) on visit 4 or visit 9 to test for trough Aramchol blood level differences between populations (e.g., African American, Asian, Hispanic), - Blood sample for gene analysis will be taken from all consenting patients during the baseline visit, will be kept frozen and analyzed only at the study end. - Life style changes will be monitored throughout the study - Adverse events will be monitored throughout the study. - Concomitant Medications will be monitored throughout the study. - Telephone contacts will be performed at week 16, 20, 28, 36, 44 and 48. An interim safety analysis will be conducted as soon as 120 subjects have completed 24 weeks on study treatment. An independent DSMB will analyze the safety data and recommend a continued course of action. All patients will continue to be treated under the study protocol until conclusion of the analysis will be known . Safety assessments will include frequency and severity of treatment–emergent AEs, clinically significant laboratory abnormalities, ECG changes and physical examination findings.
Number of Sites:	Approximately 75
Number of Subjects:	240 adult patients.
Drug Dosage/ Frequency:	400 mg or 600 mg administration once daily.
Route of Administration:	Oral Administration
Background and Rationale:	Fatty Liver Disease is currently an extremely common medical condition that may carry major complications while no accepted medical therapy is approved. Aramchol (3 beta arachidyl amido, 7 alpha 12 alpha dihidroxy, 5

	beta cholan-24-oic acid) is a conjugate of 2 natural substances: cholic acid, the main bile acid produced in the liver and arachidic acid, a dietary fatty acid present in many nutrients (e.g. peanuts). In earlier clinical phase-I studies, it was shown that Aramchol reaches C max within 10-12 hours and is eliminated slowly. Aramchol partially inhibits Stearoyl-Coenzyme A Desaturase1 (SCD1), an enzyme has an important role in fatty acid metabolism by converting saturated fatty acids to monounsaturated fatty acids and thus regulates the use and storage of body fat. In animal studies, inhibition of SCD1 enzyme protected against diet-induced obesity, hepatic steatosis or fatty liver and insulin resistance by instructing the body to use, rather than store, all fatty acids. Aramchol has been shown in several animal species to prevent and reduce liver fat in experimental NAFLD. Aramchol also up regulates ABCA1 transporter resulting in increased cholesterol elimination from cells. In Phase Ia & Ib studies in a total of 41 human volunteers both single daily oral doses of Aramchol of 30-900 mg were tested and subsequently 30 and 300 mg doses were given for 4 consecutive days. No serious adverse events were noted and minor and transient possible side effects were similarly distributed between placebo treated and Aramchol treated patients at various dose levels. A pharmacokinetics study was conducted in 66 healthy volunteers to evaluate the safety and tolerability, as well as the pharmacokinetics of Aramchol following single and multiple escalation doses (200 mg, 400 mg, 600 mg) and food effect. Pharmacokinetic evaluation showed that there were dose-related but less than dose-proportional increases in the mean Aramchol plasma concentration, Cmax, AUC(0-t), and AUC(inf) following administration of single and multiple doses of Aramchol. Administration of Aramchol after a high fat / high calorie meal resulted in a 2.6-fold increase in exposure. As a high fat/high calories meal is contraindicated in the NASH population, the sponsor recommendation is the administration of Aramchol after a light meal. All but 3 AEs (headache, nausea and mild anemia) were mild and unrelated to Aramchol and all AEs were transient and gave no indication of target organ toxicity. All doses of Aramchol administered during the study were well tolerated. No SAEs or deaths occurred during the study. No clinically significant abnormalities related to any Aramchol dose were noted in ECG, laboratory results, vital signs or physical examination. In a Phase IIa study conducted in 58 NAFLD and NASH patients, Aramchol demonstrated significant dose-dependent lowering effect on liver fat infiltration measured by NMRS over a three-month treatment period of once daily administration and a trend of improvement of metabolic parameters. Nevertheless, the optimal dose has not been identified, thus a confirmatory Phase IIb dose-finding study is warranted. The present Phase IIb study has been designed to evaluate efficacy and safety of two higher daily Aramchol doses administered for 52 weeks: the aim of the study is to select the safest and most efficient dose. The doses of 400mg /day and 600 mg/day, have been chosen based on the results of the Phase I and phase IIa studies, and calculated by extrapolating in vitro efficacy data on de-novo FFA synthesis by HepG2 cells, to human serum concentration of Aramchol. PK Lab tests in the previous Phase IIa study showed a positive correlation between the concentration of Aramchol and its pharmacological effect, which showed good safety and tolerability. The study population was chosen as NASH patients with overweight/obesity, Diabetes Mellitus Type II or pre-diabetes, a population

	at risk to develop NASH hepatic and extra hepatic complications, and as such, more likely to benefit from early treatment of NASH.
Inclusion Criteria	1. Male or female age 18 to 75 years. 2. BMI between 25kg/m^2 to 40kg/m^2 or waist circumference between 88cm to 200cm for women, and between 102cm to 200cm for men. If there is deviation above the upper limit, please consult the MRI center, to ensure that the machine is suitable for the patient 3. Known type II Diabetes Mellitus or pre-Diabetes according to American Diabetes Association. One of the following 3 criteria is needed for pre-Diabetes: Fasting Plasma Glucose > 100mg/dl (5.5 mmol/l) or 2hPG following 75g OGTT > 140 (7.8 mmol/l) mg/dl or HbA1c > 5.7%. HbA1c can be repeated at Investigator's discretion 4. Histologically proven Steatohepatitis on a diagnostic liver biopsy performed either during Screening, or within 6 months before screening visit, confirmed by central laboratory reading of the slides (steatosis ≥1 + inflammation ≥1 + ballooning ≥1). Total activity NAS score of 4 or more. 5. Liver fat concentration of 5.5% or more as measured by NMRS. 6. Biopsies with an activity NAS score of 4 or more. 7. Normal synthetic liver function (serum albumin >3.2g/dl, INR 0.8-1.2, conjugated bilirubin < 35 µmol/L). 8. Understanding the nature of the study and Signature of the written informed consent. 9. Negative pregnancy test at study entry for females of child bearing potential. 10. Females of child bearing potential practicing reliable contraception throughout the study period (including oral contraceptives). If barrier methods are used, it is recommended to practice two methods (e.g. male condom + female diaphragm with spermicide). For country-specific requirements (e.g. Germany) contraception failure rates (Pearl Index) should be under 1% in accordance with the recommendations of the CTFG Working Group on Contraception. 11. Hypertensive patients must be well controlled by stable dose of anti-hypertensive medication for at least 2 months prior to screening. 12. Patients previously treated with vitamin E (>400IU/day), Polyunsaturated fatty acid (>2g/day), Ursodeoxycholic acid or fish oil can be included if drugs are stopped at least 3 months prior to diagnostic liver biopsy (and are not started during the trial). These treatments-dosages are allowed if they were stable for at least 12 months prior to biopsy and can remain stable throughout the study. (Dosages less than the amounts stated above are allowed without washout- or stable-period restrictions). 13. For patients with type II Diabetes, glycaemia must be controlled (Glycosylated Hemoglobin A1c ≤ 9% while any HbA1c change should not exceed 1.5% during the 6 months prior to enrollment). Treatments with anti-diabetic medications (except for those mentioned in Exclusion 16) are permitted if glycaemia is self-monitored by the patient. HbA1c can be repeated at Investigator's discretion.
Exclusion Criteria:	1. Patients with other active (acute or chronic) liver disease other than NASH (e.g. viral hepatitis, unless eradicated at least 3 years prior to

	screening; genetic hemochromatosis; Wilson disease; alpha 1antitripsin deficiency; alcohol liver disease, drug induced liver disease) at the time of randomization. 2. Patients with clinically or histologically documented liver cirrhosis (CRN fibrosis score =4). 3. Known alcohol and/or any other drug abuse or dependence in the last five years. 4. Known history or presence of clinically significant cardiovascular, hepatic other than NASH, gastrointestinal, metabolic other than Diabetes Mellitus, neurologic, pulmonary, endocrine, psychiatric, neoplastic disorder or nephrotic syndrome, that in the opinion of the Investigator warrant exclusion from the study. 5. Patients with familial (i.e., genetic) hypertriglyceridemia and familial (i.e., genetic) hypercholesterolemia. 6. History or presence of any disease or condition known to interfere with the absorption, distribution, metabolism or excretion of drugs including bile salt metabolism (e.g. inflammatory bowel disease (IBD); previous intestinal (ileal or colonic) operation; chronic pancreatic; celiac disease or previous vagotomy. Ongoing chronic constipation. 7. Patients with heart or brain pacemaker. (i.e., implantable neurological devices). 8. Surgery within three months of screening which involved stent implantation of metal devices (e.g., knee, hip etc.) 9. Weight loss of more than 5% within 6 months prior to randomization. 10. History of bariatric surgery within 5 years of liver biopsy. 11. Uncontrolled arterial hypertension. 12. Women who are pregnant or breast feeding. 13. Diabetes Mellitus other than type II (type I, endocrinopathy, genetic syndromes etc.). 14. Patients with HIV infection. 15. Daily alcohol intake >20 g/day for women and >30 g/day for men (on average per day), as per medical history. 16. Treatment with other anti-diabetic medications: GLP-1 receptor agonists and Thiazolidinediones (TZDs) unless started at least 12 months prior to biopsy and on stable dose over 6 months. In case GLP-1 receptor agonist being stopped, it should be at least 6 months prior to biopsy, as per medical history. 17. SGLT-2 Inhibitors, Metformin, fibrates, statins, insulin, DPP-4 inhibitors and sulfonylurea unless the prescribed dose has been stable for the last 6 months prior to the biopsy. 18. Treatment with Valproic acid, Tamoxifen, Methotraxete, Amiodarone or chronic treatment with anti-cholinergic agents, corticosteroids, high dose estrogen and tetracycline within 12 months prior to the screening visit. 19. Chronic antibiotic treatment (e.g. Rifaximin). 20. Homeopathic and/or alternative treatments. Any treatment should be stopped during the screening period, at least 48 hours before randomization. 21. Uncontrolled hypothyroidism defined as Thyroid Stimulating Hormone >2X the upper limit of normal (ULN). Thyroid

	dysfunction controlled for at least 6 months prior to screening is permitted. 22. Patients with renal dysfunction eGFR< 40. 23. Unexplained serum creatine phosphokinase (CPK) >3X the upper limit of normal (ULN). Patients with an intermittent CPK elevation may have the repeated measurement prior to randomization; a CPK retest > 3X ULN leads to exclusion. 24. Patients with condition(s) that makes them unsuitable to perform the NMRS (as determined by the PI or the MRI facility). 25. Known Hypersensitivity to Aramchol or to any of the excipients in the tablets 26. Known Hypersensitivity to cholic acid or bile acid sequestrants
Efficacy Endpoints:	List of endpoints provided in this protocol is aligned with Statistical Analysis Plan (SAP) signed on 26Jan2017. **Primary endpoint**: Percent (%) change from baseline to end of study in liver triglycerides ratio as measured by NMRS. **Key Secondary endpoints:** 1. Proportion (%) of subjects with CRN fibrosis score improvement 2. Proportion (%) of subjects with NAS score improvement without worsening of CRN fibrosis score. 3. Proportion (%) of subjects with SAF activity score improvement without worsening of CRN Fibrosis Score. 4. Proportion (%) of subjects with NASH resolution without worsening of CRN fibrosis score 5. Change from baseline to Week 52/Termination in ALT (U/L) levels. [redacted]
Safety Evaluation:	**Safety and tolerability evaluations** 1. Adverse events and serious adverse events 2. Safety laboratory evaluations.

	3. Vitals signs. 4. 12-Lead ECG 5. Physical examinations 6. Drop-out rates
Pharmacokinetics	Blood samples for Aramchol blood trough level will be collected from the study population in Israel on visits 2, 4, 6, 7, 9, 10 and 11, and on visit 4 or 9 from study population at selected sites in Mexico, USA, Europe and Hong Kong. The blood sample will be collected during the visit before the daily dose is taken.
Statistical Methodology:	Statistical methodology provided in this protocol is aligned with Statistical Analysis Plan (SAP) signed on 26Jan2017. **Sample Size and Power Consideration** The planned sample size is 215 subjects, 86 in each of the two active groups and 43 in the placebo group. [redacted] The total sample size is therefore 240 (96 in each of the two active groups and 48 in the placebo group). **Randomization** The ratio of 1:2:2 between the placebo and the two active groups has been chosen in order to minimize the numbers of subjects undergoing two liver biopsies, while taking a placebo. The randomization list was generated prior to the study initiation, using a computer-generated randomization list produced by a Sintesi statistician on behalf of the Sponsor. The randomization was performed in blocks, 48 blocks containing 5 subjects each in a 2:2:1 ratio (2 subjects for each of the active groups and 1 placebo). **Significance Level and Multiplicity Adjustment** One (1) primary endpoint and 5 secondary end-points are pre-defined for each of the 2 Aramchol doses (400mg and 600mg) studied. The overall significance level for this study will be 5% using two-tailed tests utilizing the hierarchical gate keeping approach to control the overall Type-I error rate. Since two doses of Aramchol will be tested vs. placebo, there will be a total of 12 comparisons (contrasts) for the primary and secondary endpoints altogether. **Primary Efficacy Endpoint and Principal Statistical Analysis** The primary endpoint of the study is the percent (%) change from baseline to end of study in liver triglycerides ratio as measured by NMRS. The Full Analysis Set (FAS) will be used as the primary analysis set for efficacy analysis and inference. Data for this analysis will be retrieved from the central MRI reading center dataset specifically designed to capture multiple locations measurements of liver triglycerides ratios done for each MRI scan. For each MRI scan, liver triglycerides ratios were reported as taken from one or several locations of the liver as deemed necessary by the MRI reading specialist. Furthermore, in some cases replications within liver location sites were also been performed to improve precision. These within location replications will be averaged, and the percent (%) change from baseline for

	matched locations will be calculated having in the analysis repeated measures (repeated per matched locations). The statistical model will be a Mixed Model (SAS® MIXED procedure) with random intercept subcommand. The model will include the following covariates: treatment group, CGR1, age (1df), sex, baseline liver triglycerides ratio (1df) and baseline BMI (1df). The model will be a Random Intercept model. The REML estimation method will be used and degrees of freedom will be adjusted using the Kenward-Roger method. The calculated percent change from baseline for each subject's liver location will be used as response in the model and differences between the treatments groups will be estimated using contrasts. **Safety Analysis**: 1. Adverse events and serious adverse events 2. Safety laboratory evaluations. 3. Vitals signs. 4. 12-Lead ECG 5. Physical examinations 6. Drop-out rates **Interim safety analysis:** An independent DSMB will analyze the safety data collected during the first 6 months for the 120 subjects and recommend a continued course of action. All patients will continue to be treated under the study protocol as done during the 6 months prior the cutoff date, till conclusion of the analysis will be known.

2 LIST OF ABBREVIATIONS

AASLD	American Association for the Study of Liver Disease
ABCA1	ATP binding cassette transported A1
ADA	American Diabetes Association
AE	Adverse Event
ALT	Alanine Aminotransferase/GPT
APO A1	Apo lipoprotein A1
aPPT	activated Partial Thromboplastin Time
AST	Aspartate aminotransferase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BMI	Body Mass Index
CBC	Complete Blood Count
CK-18	Cytokeratin 18 fragment (M30 and M65)
CPK	Creatine phosphokinase
CI	Confidential interval
CRF	Case Report/Record Form
CRN	Clinical Research Network
CRO	Contract Research Organization
DMC	Data Monitoring Committee
EDTA	Ethylene diamine tetraacetic acid
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
eCRF	electronic case report form
eGFR	estimated Glomerular Filtration Rate
EC	Ethics Committee
ECG	Electrocardiogram
ET	Early Termination
EoT	End-of-Treatment
ESR	Erythrocyte sedimentation rate
EU	European Union
FABACs	Fatty acid – bile acid conjugates
FDA	Food and Drug Administration
FFA	Free Fatty Acid
FGF-19	Fibroblast Growth Factor 19
FLI	Fatty Liver Index
FMD	Flow Mediated Dilation
GCP	Good Clinical Practice
GDF15	Growth differentiation factor 15
GLP-1	Glucagon-like peptide-1
GMP	Good Manufacturing Practice
Hb	Hemoglobin
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus

HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HOMA	Homeostasis Model Assessment
hs-CRP	High Sensitivity C-reactive protein
IBD	Inflammatory Bowel Disease
ICH	International Conference on Harmonization
IMP	Investigational medical product
IL-6	Interleukin-6
IRB	Institutional Review Board
IWRS	Interactive Web Response System
LAR	Leptin Adiponectin Ratio
LDL	Low density lipoprotein
µM	Micromole
MedDRA	Medical Dictionary for Regulatory Activity
MRI	Magnetic Resonance Imaging
NAFLD	Non Alcoholic Fatty Liver Disease
NFS	NAFLD Fibrosis Score
NAS	NAFLD Activity Score
NASH	Non Alcoholic Steatohepatitis
NMRS	Nuclear Magnetic Resonance Spectroscopy
NOEL	No Observed Effect Level
OGTT	Oral Glucose Tolerance Test
PAI-1	Plasminogen Activator Inhibitor 1
PI	Principle Investigator
PK	Pharmacokinetic
P.O	Per Os/by mouth/orally
PT/INR	Prothrombin Time/International Normalized Ratio
QA	Quality Assurance
RNA	Ribonucleic Acid
SAB	Safety Advisory Board
SAE	Serious Adverse Event
SGOT	Glutamic-oxaloacetic transferase
SCD1	Stearoyl Coenzyme A Desaturase 1
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{½}$	Elimination half life
TNFα	Tumor necrosis factor α
TSH	Thyroid Stimulated Hormone
UDCA	Ursodeoxychlic
ULN	Upper Limit of Normal
VLDL	Very Low Density Lipoprotein
TZD	Thiazolidine

3 INTRODUCTION

3.1 Background Information

3.1.1 Non-Alcoholic Fatty Liver Disease and Steatohepatitis

Non-alcoholic fatty liver disease (NAFLD) is a major cause for chronic liver -morbidity all over the world (1, 27). The clinical and histological presentation may range widely from macro vesicular steatosis only (NAFLD) to steatohepatitis (NASH): with necro-inflammatory activity and fibrosis – that may progress further to cirrhosis, liver failure and hepatocellular carcinoma (1, 2). The reported prevalence of NAFLD and NASH in the general population is 15-39% and 1-5% respectively (3, 4, 28). Higher prevalence is expected in the future, facing the worldwide epidemic of obesity and its close linkage to metabolic syndrome and NAFLD/NASH. In fact, it is expected that by 2020 NASH will become the leading cause for liver transplantation in the US (5). Natural history of fatty liver disease is associated with liver histology at the time of presentation: patients with steatosis only most probably will have a benign course, while 26-37% of untreated NASH patients will demonstrate fibrosis progression over time with up to 9% progression to cirrhosis (6, 7).

The exact pathogenesis of NASH is not definitively understood, but is believed to involve at least two steps. In the initial step, excessive accumulation of triglycerides in the liver occurs. This step, sometimes designated as a “hit,” actually may be an adaptive or protective response diverting accumulating fatty acids from pathological pathways (29). The accumulation of fat in the liver is due to an imbalance in hepatic fat buildup and removal that can result from increased delivery of fatty acids, increased fatty acid synthesis, decreased triglyceride and cholesterol export, and reduced beta oxidation. The second step, characterized by steatohepatitis and/or fibrosis, depends on a complex interplay of several factors including accumulation of free fatty acids (FFA), the production of inflammatory cytokines and adipokines, oxidative stress, mitochondrial dysfunction, bacterial overgrowth and genetic predisposition (30). An increase of oxidative stress may also inhibit hepatocyte replication, leading to impaired proliferation and tissue regeneration resulting in more advanced progression of the disease (30).

3.1.2 Current treatment for NAFLD/NASH

There are currently no drugs approved by regulatory authorities for the treatment of NASH. Certain drugs, such as insulin sensitizers and antihyperlipidemic agents, are prescribed off-label for some NASH patients. However, they are not approved for the treatment of NASH and their efficacy has not been proven in well-designed clinical studies.

Modulation of the mechanisms involved in lipid accumulation in the liver and inflammation could provide useful targets for treatment of NAFLD or NASH. Thus, current strategies for treatment are directed towards improving metabolic parameters (e.g. insulin resistance,

dislipidemia, cardiovascular disease, and obesity) which are known to contribute to disease pathogenesis, mainly through lifestyle modification (28).

Thus, current existing pharmacological therapies for NASH are limited and controversial.

The recent published American Association for the Study of Liver Disease (AASLD) guidelines recommend that treatment with anti-oxidants such as vitamin E can be considered for non-diabetic NASH proven biopsy patients only (8). One concern with vitamin E as was pointed out in some meta-analysis is the suspected relation between high doses vitamin E and all-cause mortality (9). Other concerns are on increased risk of prostate cancer in male older than 50 years and an increase in hemorrhagic stroke.

Thiazolidinedione (TZD's) such as Pioglitazone improves biochemical and histological findings in diabetic and non-diabetic NASH patients (10) and can be used to treat biopsy proven NASH (8). Never the less, because there is a considerable debate about the long term safety and efficacy of TZD's, including cardiovascular complications (11), their use in non-diabetic NASH patients is controversial.
Ursodeoxycholic acid (UDCA) or Metformin failed to reveal any significant effect on liver histology therefore they are not recommended for the treatment of NAFLD/NASH (8).

3.1.3 The Test Substance

Aramchol (3 beta arachidyl amido, 7 alpha 12 alpha dihidroxy, 5 beta cholan-24-oic acid) is a synthetic molecule which conjugate by amid bond two natural based substances: Cholic acid (the main bile acid produced by the liver) and arachidic acid (a dietary fatty acid present in many nutrients, e.g. peanuts) (12). Aramchol is a lipid, only marginally soluble in water, never the less, it is absorbed following oral administration (at doses of 30-900 mg/day as tested during phase I), while amid bond provides stability against intestinal degradation. It reaches C max within 10-12 hours and is slowly but completely excreted in the feces, mostly by the biliary route. At 24 hours the levels in blood are still high, permitting once a day dosage.

3.1.4 Experimental and Clinical Data

Aramchol, like other 'fatty acid-bile acid conjugates' (FABACs), was developed to prevent formation of gallstones, but was found to have a significant metabolic and anti steatogenic activity.
Aramchol affects two different key liver enzymes: Stearoyl Coenzyme A Desaturase 1 (SCD1) and ATP Binding Cassette A1 (ABCA1).

1) SCD1 is a key enzyme involved in regulating lipid metabolism, specifically catalyzing the formation of monounsaturated fatty acids (MUFA) from SFAs (31, 32). MUFA are major components of cellular membranes, triglycerides, and cholesterol esters (29). Inhibition of the enzyme provides protection from obesity, insulin resistance and fatty liver resulting in an overall improvement in metabolic parameters. This has been

confirmed in a variety of animal models 35(13, 32). Interestingly, complete inhibition of the enzyme may lead to accumulation of saturated fatty acids, which possess pro-inflammatory effects (13). Aramchol was found to partially inhibit the activity of SCD1. This has been confirmed in human liver microsomes and animal studies, showing that following treatment there is a reduction in the ration of 16:1/16:0 and 18:1/18:0 fatty acids, surrogate markers of SCD1 activity (14,15). The inhibitory effect of Aramchol on SCD1 is partial (can reach 70-83%) is most likely involves a direct effect rather than upstream regulation. Unlike other SDC1 inhibitors, it was shown that Aramchol's effects are non-atherogenic.

2) ABCA1 is a cellular membrane protein that mediates the transport of cholesterol and phospholipids from cells to apolipoprotein A-I (apo A-I) generating high density lipoprotein particles which results in extracellular lipid transport (33). In addition to modifying lipid transport, ABCA1 is involved in the regulation of hepatic lipid storage (22). Over-expression of ABCA1 in HepG2 cells produced a decrease in cellular fatty acids and triglycerides while suppression of ABCA1 (by an ABCA1 interference RNA plasmid) increased both cellular fatty acids and triglycerides. Moreover, ABCA1 levels and fatty acid synthesis have been found to be inversely correlated: over expression of ABCA1 leads to a reduction in fatty acid synthesis whereas an increase in the level of unsaturated fatty acids suppresses ABCA1 protein levels and promotes its degradation (33, 22). Yang and coworkers also determined that reduced levels of ABCA1 protein were found in the hepatocytes of rats exhibiting NASH. Thus, these findings suggest a possible role for ABCA1 in NASH by decreasing lipid storage, modifying extracellular hepatic lipid transport and inhibiting fatty acid synthesis.

   Aramchol has been shown to induce cholesterol efflux via up-regulation of ABCA1 protein. Aramchol increased ABCA1 protein levels in human fibroblasts, liver cell lines of human origin, murine hepatocytes and macrophages by 2- to 4-fold without affecting ABCA1 expression (15, 16). In addition, Aramchol treatment was associated with up regulation of CYP7A, a rate limiting enzyme in bile acid synthesis from cholesterol, and down regulation of HMG-CoA enzyme, an enzyme which catalyzes the production of hepatic cholesterol (14).

   The physiological effects of Aramchol were studied in various animal models of NAFLD. Aramchol treatment was associated with:

- Reductions in liver fat (mostly in triglyceride and diglyceride components), plasma triglycerides and cholesterol levels in a dose dependent manner to levels comparable to those of animals fed a regular diet, and hepatic cholesterol levels,
- Increase in cholesterol efflux, the ratio of fecal bile acids/neutral sterols, and total fecal sterol excretion, and
- Enhancement in bile acid synthesis.

3.1.5 Potential Risks and Benefits

Trials in animals and humans have demonstrated that Aramchol is safe following oral administration. In maximum tolerated dose trials in rats, doses of up to 2000 mg/kg caused no mortality or obvious morbidity. In 28 day, formal toxicity studies in rats and dogs the NOEL dose of 1000 mg/kg/day was determined, although a minor transient transaminase elevation was observed in rats. There were no findings on liver histology and these findings were not reproduced in any other subsequent formal toxicity tests. In 3 months toxicity trials in rats and dogs using Aramchol 50-500 mg/kg/day of a 3 times more bioavailable modified formulation no side effects were found and the NOEL level was determined to be 500mg/kg/day.
In human, Phase I (single and repeated) trails of once daily Aramchol dose of 30, 100, 300, and 900 mg, no notable changes were found in biochemical, hematological, cardiovascular or other safety parameter among 41 healthy male volunteers.

A pharmacokinetics study was conducted in 66 healthy volunteers to evaluate the safety and tolerability, as well as the pharmacokinetics of Aramchol following single and multiple escalation doses (200mg, 400mg, 600mg) and food effect. Pharmacokinetic evaluation showed that there were dose-related but less than dose-proportional increases in the mean Aramchol plasma concentration, Cmax, AUC(0-t), and AUC(inf) following administration of single and multiple doses of Aramchol. Administration of Aramchol after a high fat / high calorie meal resulted in a 2.6-fold increase in exposure. As a high fat/high calories meal is contraindicated in the NASH population, the sponsor recommendation is the administration of Aramchol after a light meal. All but 3 AEs (headache, nausea and mild anemia) were mild and unrelated to Aramchol and all AEs were transient and gave no indication of target organ toxicity. All doses of Aramchol administered during the study were well tolerated. No SAEs or deaths occurred during the study. No clinically significant abnormalities related to any Aramchol dose were noted in ECG, laboratory results, vital signs or physical examination.

Phase IIa study included 58 NAFLD/NASH patients treated for 3 months with an additional one month follow-up period with 100 mg vs 300 mg vs placebo. No severe drug related adverse events were observed during the 3-months treatment and subsequent recovery periods. In this phase IIa study there was a significant reduction in liver fat in a dose dependent manner measured by NMRS after 3 months of Aramchol treatment. Improvement of inflammatory markers and metabolic indexes was also observed, although failed to reach statistical significance.

As a result of all of the above, Aramchol is considered a potential therapeutic agent for the reduction of liver fat and inflammation in patients with fatty liver due to NAFLD, NASH and other causes. The present trial aims to test this effect for the first time in patients with NASH with glucose intolerance and overweight/obesity.

4 STUDY OBJECTIVES

4.1 Primary Objective

To evaluate the efficacy on steatosis reduction as measured by NMRS of two Aramchol doses (400 mg and 600 mg), once daily for 52 weeks vs placebo.

4.2 Key Secondary Objectives

1. To evaluate the efficacy of Aramchol on CRN Fibrosis Score by liver biopsy.
2. To evaluate the efficacy of Aramchol on disease activity as measured by NAS and SAF activity score.
3. To evaluate the efficacy of Aramchol on NASH resolution by liver biopsy.
4. To evaluate the effect of Aramchol on ALT levels.

[redacted]

4.3 Safety and Tolerability

4.3.1 Safety

- Adverse events
- Vital signs
- ECG findings
- Clinical laboratory parameters
- Physical examination findings

4.3.2 Tolerability

Proportion of subjects (%) who prematurely discontinued from the study, reason of discontinuation and the time to withdrawal.
Proportion of subjects (%) who prematurely discontinued from the study due to AEs and the time to withdrawal.

4.3.3 Efficacy Endpoints

List of endpoints provided in this protocol is aligned with Statistical Analysis Plan (SAP) signed on 26Jan2017.

4.3.4 Primary Endpoint

Percent (%) change from baseline to end of study in liver triglycerides ratio as measured by NMRS.

4.3.5 Key Secondary Endpoints

1. Proportion (%) of subjects with CRN fibrosis score improvement
2. Proportion (%) of subjects with NAS score improvement without worsening of CRN fibrosis score..
3. Proportion (%) of subjects with SAF activity score improvement without worsening of CRN Fibrosis Score.
4. Proportion (%) of subjects with NASH resolution without worsening of CRN fibrosis score
5. Change from baseline to Week 52/Termination in ALT (U/L).
   level

[redacted]

5 STUDY DESIGN

5.1 Over view and plan

This is a multicenter, phase IIb, randomized, double blind, placebo controlled study designed to evaluate the efficacy and safety of two Aramchol doses (400 mg and 600 mg) in subjects that

are 18 to 75 years of age, with Non-Alcoholic Steatohepatitis (NASH) and type II DM or elevated fasting glucose and overweight/obesity (type II diabetic or pre diabetic).
Eligible subjects will be enrolled in three treatments arms:

- Aramchol 400 mg tablets
- Aramchol 600 mg tablets
- Placebo for Aramchol

The subjects will be evaluated at study sites for 11 scheduled visits: visit 1 (screening), visit 2 (baseline), visit 3 (2 weeks), visit 4 (4 weeks), visit 5 (8 weeks), visit 6 (12 weeks), visit 7 (24 weeks), visit 8 (32 weeks), visit 9 (40 weeks), visit 10 (End-of-Treatment/Early Termination) (52 weeks) and visit 11 (follow up) (65 weeks).
The subjects will be treated for a period of 52 weeks. After completion of the study treatment period, the subjects will be followed up for an additional period of 13 weeks without study medication
During the study the following assessments will be performed:

- Vital signs will be measured at each study visit.
- A physical examination will be performed at the screening visit, 24 weeks, End-of-Treatment/Early Termination and week 65 visits.
- The following safety blood tests will be performed: complete blood count (CBC), serum chemistry (including electrolytes, liver enzymes, direct and total bilirubin, glucose, lipid profile which include triglyceride, cholesterol, HDL, LDL and VLDL, CPK, creatinine, urea, albumin, alkaline phosphatase), ESR and during the screening visit, baseline, week 2, 4, 8, 24, 40, 52 and 65 (end of follow up) visits. Serology (HBV, HCV and HIV) and urinalysis will be performed during the screening visit. Coagulation (fibrinogen, PT/INR, aPTT) will be performed during the screening, baseline, week 24, End-of-Treatment/Early Termination and week 65 visits. Insulin (HOMA) will be measured in the screening, week 24 and End-of-Treatment/Early Termination visits. HbA1c will be measured in the screening, week 8, 24, 40 and End-of-Treatment/Early Termination visits. C reactive protein, Leptin, Adiponectin, [redacted] will be measured in baseline visit and end of treatment period (week 52, visit 10). The blood samples taken at these visits, will be tested for possible biomarkers [redacted].
  TSH, T3 and T4 will be measured during the screening visit. β-hCG in women of child bearing potential will be performed during the screening visit. A serum sample will be collected and kept frozen until study end in case special investigation will be needed to be performed. This sample will collected during the screening and visit 10/Early Termination.
- Body weight and waist circumference will be measured in screening, baseline, week 24, End- of-Treatment/Early Termination and week 65 visits. Height will be measured during the screening visit.

- ECG will be performed during the screening visit, visit 7 (week 24) and End-of-Treatment/Early Termination visit.
- All subjects will undergo two NMRS scans, at screening and end of treatment visits.
- FibroMax test will be performed only if investigator will think it is necessary.
- Endothelial Function test will conducted at baseline visit before the patient will take the first dose of study medication and during the End-of-Treatment/Early Termination visit. This test will be conducted at selected sites. If test was not done on Visit 2 (Day 0) or needs to be repeated due to unacceptable test data, it will be done at Visit 3 (two weeks after Visit 2) – or at an Unscheduled Visit before Visit 3.
- Liver biopsy will be conducted during the screening and end of treatment visit. The biopsy in the screening visit will be performed only if it was not done within the 6 months prior this visit.
- Metabolomics blood test will be performed at the screening, visit 7 and at End-of-Treatment/Early Termination visits. From some consenting patients (about 15) a sample from the liver biopsy will be taken for analysis.
- Blood sample for Aramchol trough level will be collected (pre dose) from patients in Israel during visit 2 (baseline), visit 4 (4 weeks), visit 6 (12 weeks), visit 7 (24 weeks), visit 9 (40 weeks), visit 10 (End-of-Termination/Early Termination) (52 weeks) and visit 11 (follow up) (65 weeks). At selected sites in Mexico, USA, Europe and Hong Kong one blood sample will be collected (pre-dose) on visit 4 or visit 9.
- Blood sample for gene expression will be taken from all consenting patients during the baseline visit(will be kept frozen and analyzed only at the study end).
- Life style changes will be monitored throughout the study. Life style questionnaire (appendix E) may be used as a guideline, but is not mandatory
- Adverse events will be monitored throughout the study.
- Concomitant Medications will be monitored throughout the study.
- Telephone contacts will be performed on week 16, 20, 28, 36, 44 and 48.

An interim safety analysis will be conducted as soon as 120 subjects have completed a period of 6 months on study treatment. An independent DSMB will analyze the safety data and recommend a continued course of action. All patients will continue to be treated under the study protocol as done during the 6 months prior to the cutoff date, until conclusion of the analysis will be known.
Safety assessment will include frequency and severity of treatment–emergent AEs, clinically significant laboratory abnormalities, ECG changes and physical examination findings.

5.2 Rationale for study design, dose and population

Aramchol is effective in reducing liver fat in a dose dependent manner as was pointed out in the previous Phase IIa study. Three months of daily treatment of 300mg Aramchol vs. placebo significantly reduced liver fat in patients with NASH, and thus the primary endpoint of this study was achieved. The same study also revealed a non-significant dose dependent improvement of insulin resistance parameters such as the adiponectin levels. Current

understanding of the pathophysiology of NASH reveals that the reduction of liver fat alone is not sufficient for disease improvement. Therefore pharmacological interventions showed also aim at improving liver inflammation and all injury. The Galmed Phase IIb study is designed to ascertain the effect of Aramchol on liver inflammation in addition to liver fat reduction. The sponsor's position is that a study of 12 month duration with higher doses of Aramchol is warranted in order to achieve an optimal benefit from the treatment. The national UK, French and German Authorities, as well as the FDA have endorsed this view.

The principle endpoint of the study is the reduction in the amount of fat, measured by NMRS. We hypothesize that the reduction of fat will induce reduction of the inflammatory process, therefore the secondary endpoint of the study is the relative improvement in inflammation, as measured by two liver biopsies at screening (before starting the treatment) and after 12 months of treatment. The sponsor aims at correlating the NMRS and the biopsy findings, thus validating NMRS as a future non-invasive surrogate marker for Aramchol's efficacy.

Additional secondary endpoints were designed to confirm the trends in improvement in metabolic parameters occurring in the Phase IIa Study.

[redacted]

Population: The study population targets NASH patients with type II DM or pre diabetes according to the American Diabetes Association (23) and overweight/obesity. This population is considered at higher risk for developing cirrhosis and metabolic syndrome related complications in the future, and as such, most likely to benefit from early intervention.

Aramchol Dosage: The trial is designed to study the safety of the 400 mg and 600 mg, doses of Aramchol and the election of the safest and most effective dose to be taken forward in pivotal phase III studies. Those doses were chosen based on the safety profile of Aramchol from previous Phase I and Phase IIa studies and calculated by extrapolating in vitro efficacy data on de-novo FFA synthesis by HepG2 cells, to human serum concentration of Aramchol.

PK parameters showed a positive correlation between the concentration of Aramchol and its pharmacological effect. [redacted]

[redacted]

[redacted]

Sample size consideration: The planned evaluable sample size is 215 subjects, 86 in each of the two active groups and 43 in the placebo group. [redacted]

[redacted]

The total sample size is 240.

The ratio of 1:2:2 between the placebo and the two active groups has been chosen in order to minimize the numbers of subjects undergoing two liver biopsies, while taking a placebo. This was considered necessary both for ethical and practical reasons, in order to facilitate the study's approval and the rate of recruitment.

6 STUDY POPULATION

240 subjects who will meet the eligibility criteria will be enrolled to the study.
Dropouts will not be replaced.

6.1 Inclusion Criteria

1. Male or female aged 18-75 years.
2. BMI between 25 Kg/m2 to 40Kg/m2, or waist circumference between 88cm to 200cm for women, and between 102cm to 200cm for men. If there is deviation above the upper limit, please consult the MRI center, to ensure that the machine is suitable for the patient.
3. Known type II Diabetes Mellitus or pre-diabetes according to American Diabetic Association. One of the following 3 criteria is needed for pre-Diabetic: Fasting Plasma Glucose > 100mg/dl (5.5 mmol/l) or 2hPG following 75g OGTT >140 mg/dl (7.8 mmol/l) or HbA1c > 5.7%. HbA1c can be repeated at Investigator's discretion.
4. Histologically proven Steatohepatitis on a diagnostic liver biopsy performed either during screening or within 6 months before screening visit, confirmed by central laboratory reading of the slides (steatosis ≥1 + inflammation ≥1 + ballooning ≥1). total activity NAS score of 4 or more

5. Liver fat concentration of 5.5% or more as measured by NMRS.
6. Biopsies with an activity NAS score of 4 or more.
7. Normal synthetic liver function (serum albumin >3.2g/dl, INR 0.8-1.2, conjugated bilirubin < 35 μmol/L).
8. Understanding the nature of the study and signature of the written informed consent.
9. Negative pregnancy test at study entry for females of child bearing potential.
10. Females of child bearing potential practicing reliable contraception throughout the study period (including oral contraceptive). If barrier methods are used, it is recommended to practice two methods (e.g. male condom + female diaphragm with spermicide). For country-specific requirements (e.g. Germany) contraception failure rates (Pearl Index) should be under 1% in accordance with the recommendations of the CTFG Working Group on Contraception.
11. Hypertensive patients must be controlled by stable dose of anti-hypertensive medication for at least 2 months prior to screening.
12. Patients previously treated with vitamin E (>400IU/day), Polyunsaturated fatty acid (>2g/day), Ursodeoxycholic acid or fish oil can be included if drugs are stopped at least 3 months prior to diagnostic liver biopsy (and are not started during the trial). These treatments-dosages are allowed if they were stable for at least 12 months prior to biopsy and can remain stable throughout the study. (Dosages less than the amounts stated above are allowed without washout- or stable-period restrictions.)
13. For patients with type II Diabetes, glycaemia must be controlled (Glycosylated Hemoglobin A1c ≤9%) while any HbA1c change should not exceed 1.5% during 6 months prior to enrolment). Treatments with anti-diabetic medications (except for those mentioned in Exclusion 16) are permitted if glycaemia is self-monitored by the patient. HbA1c can be repeated at Investigator's discretion

6.2 Exclusion Criteria

1. Patients with other active (acute or chronic) liver disease other than NASH (e.g. viral hepatitis, unless eradicated at least 3 years prior to screening; genetic hemochromatosis; Wilson disease; alpha 1antitripsin deficiency; alcohol liver disease; drug-induced liver disease) at the time of randomization.
2. Patients with clinically or histologically documented liver cirrhosis (CRN fibrosis score =4).
3. Known alcohol and/or any other drug abuse or dependence in the last five years.
4. Known history or presence of clinically significant cardiovascular, gastrointestinal, metabolic other than Diabetes Mellitus, neurologic, pulmonary, endocrine, psychiatric, neoplastic disorder or nephrotic syndrome, that in the opinion of the Investigator warrant exclusion from the study.
5. Patients with familial (i.e., genetic) hypertriglyceridemia and familial (i.e., genetic) hypercholesterolemia.

6. History or presence of any disease or condition known to interfere with the absorption distribution, metabolism or excretion of drugs including bile salt metabolites (e.g. inflammatory bowel disease (IBD)), previous intestinal (ileal or colonic) operation, chronic pancreatitis, celiac disease or previous vagotomy. Ongoing Chronic constipation
7. Patients with heart or brain pacemaker (i.e., implantable neurological devices).
8. Surgery during the last three months before screening which involved stent implantation of metal devices (e.g. knee, hip etc.)
9. Weight loss of more than 5% within 6 months prior to randomization.
10. History of bariatric surgery within 5 years of liver biopsy.
11. Uncontrolled arterial hypertension.
12. Women who are pregnant and breast feeding.
13. Diabetes Mellitus other than type II (type I, endocrinopathy, genetic syndromes etc.).
14. Patients with HIV infection.
15. Daily alcohol intake >20 g/day for women and >30 g/day for men (on average per day) as per medical history.
16. Treatment with other anti-diabetic medications:
    GLP-1 receptor agonists and Thiazolidinediones (TZDs), unless started at least 12 months prior to biopsy and on stable dose for 6 months. In case of GLP-1 receptor agonists stopped, it should be at least 6 months before biopsy as per medical history.
17. SGLT-2 Inhibitors, Metformin, fibrates, statins, insulin, DPP-4 inhibitors and sulfonylurea unless prescribed dose has been stable for the last 6 months prior to the biopsy.
18. Treatment with Valproic acid, Tamoxifen, Methotrexate, Amiodarone or chronic treatment with anti-cholinergic agents, corticosteroids, high dose estrogen and tetracycline within 12 months prior to the screening visit.
19. Chronic treatment with antibiotics (e.g. Rifaximin).
20. Homeopathic and/or alternative treatments. Any treatment should be stopped during the screening period at least 48 hours before randomization.
21. Uncontrolled hypothyroidism defined as Thyroid Stimulating hormone >2X the upper limit of normal (ULN). Thyroid dysfunction controlled for at least 6 months prior to screening is permitted.
22. Patients with renal dysfunction eGFR< 40.
23. Unexplained serum creatine phosphokinase (CPK) >3X the upper limit of normal (UNL). Patients with a reason for CPK elevation may have the measurement repeated prior to randomization; a CPK retest > 3X ULN leads to exclusion.
24. Patients with condition(s) that makes them unsuitable to perform the NMRS (as determined by the PI or the MRI facility).
25. Known Hypersensitivity to Aramchol or to any of the excipients in the tablets
26. Known Hypersensitivity to cholic acid or bile acid sequestrants

7 MEDICATIONS/THERAPIES – ALLOWED AND DISALLOWED

7.1 Allowed concomitant medications / therapies during the study

Any medications, excluding those mentioned in Section 7.2 may be given concomitantly as needed for the subject's welfare.

- For patients that met inclusion/exclusion criteria, continued treatment with stable doses of metformin, sulfonylurea and insulin, DPP-4, GLP-1, SGLT-2 Inhibitors is permitted if glycaemia is self-monitored by the patient.
- For patients that met inclusion/exclusion criteria, continued treatment with Vitamin E (>400IU/day), Polyunsaturated fatty acid (>2g/day), Ursodeoxycholic acid or fish oil is allowed.
- Steroids, if given, are allowed for no more than 3 consecutive days.
- Non-chronic use of anti-cholinergic agents (e.g. anti-histamines) is allowed.

Administration of all medications, including indication, dose, frequency, and route of administration will be recorded in the source documentation file and in the electronic Case Report Form (eCRF). All concomitant medications (including anti-diabetic medications) will be discussed and documented at each visit. The study investigator will ensure that anti-diabetic therapy is initiated and monitored.

7.2 Disallowed concomitant medications / therapies during the study

The following medications are disallowed to be introduced during study:

- Treatment with Vitamin E (>400IU/day), Polyunsaturated fatty acid (>2g/day), Ursodeoxycholic acid or fish oil.
- Alternative treatments and/or investigational products are disallowed during the study
- To ensure adequate exposure to Aramchol, co-administration (for more than 3 consecutive days) of enzyme inducers such as rifampicin, carbamazepine, phenytoin, enzalutamide and St John's wort should be avoided

The following medications should not be introduced during the study. If need to be introduced, subjects will be discontinued from the study.

- Medications causing hepatic steatosis such as Valproic acid, Tamoxifen, Methotrexete, or anti-cholinergic agents.

If the following medications need to be introduced during the study (and no alternative exists), inform the Sponsor.

- GLP-1 receptor agonists, Thiazolidinediones (TZDs), GLP-1, SGLT-2, Metformin, Insulin, Sulfonylurea, DPP-4.
- Fibrates and statins

Continuing participation of patients that will require a change/prescription of the above medication will be considered by sponsor.

7.3 Rescue treatment and stopping treatment criteria

Patients who present with newly onset Diabetes Mellitus during study and require treatment according to clinical and laboratory data as recommended by the American Diabetes Association (26) will start treatment with metformin without being excluded from the study. Patients who fail to control diabetes within 3 months (further increment of HbA1c of>1.5% despite metformin treatment) will be withdrawn from study.

Diabetic patients already treated with hypoglycemic agent who fail to maintain their individualized HbA1c target level will be allowed to improve treatment by adding one of the above medications (metformin, sulfonylurea or insulin) or adjusting their insulin level (according to ADA or other international guidelines) (26). If 3 months later, they still fail to reach their individualized HbA1c target level they will be withdrawn from study.

Subjects may also be discontinued when, in the opinion of the Investigator, intensification of their anti-diabetic therapy (pursuant to guidelines) is not possible.

Pregnancy:
Pregnancy should be avoided during study and all females of child bearing potential should practice reliable contraception throughout the study period (including oral contraceptives). If barrier methods are used, it is recommended to practice two methods (e.g. male condom + female diaphragm with spermicide). For country-specific requirements (e.g. Germany) contraception failure rates (Pearl Index) should be under 1% in accordance with the recommendations of the CTFG Working Group on Contraception.

Any subject who becomes pregnant during the study period must not receive additional doses of investigational product and will be withdrawn from the study. If the subject requests to know which treatment she received, this information will be provided to her.

Liver Toxicity:
Patients with suspected or confirmed hepatotoxicity should be discussed with the Sponsor and discontinued from the study.

7.4 Drug interactions

No drug interaction studies have yet been performed. A simulation PBPK fit for purpose model predicted increased exposure to drugs that are substrates for CYP3A4 metabolism when administered concomitantly with Aramchol. The interaction ratios for both AUC0-24 and Cmax were 1.3 for midazolam and 1.9 for simvastatin in the presence of Aramchol 600 mg.
Investigators should note that Aramchol may cause an increase in plasma concentrations of some statins (e.g. simvastatin, lovastatin). Therefore, caution should be exercised in treating patients receiving such highly susceptible substrates and other CYP3A4 substrates with narrow therapeutic range such as alfentanil, fentanyl and cyclosporin. No significant interaction potential was observed with the CYP2B6 and BCRP substrates.
Studies have shown that, while Aramchol does not undergo extensive metabolism, CYP3A4 activity does contribute to the clearance of Aramchol, which could therefore be affected by inducers or inhibitors of CYP3A4. To ensure adequate exposure to Aramchol, co-administration (for more than 3 consecutive days) of enzyme inducers such as rifampicin, carbamazepine, phenytoin, enzalutamide and St John's wort should be avoided. Conversely, concentrations of Aramachol could be increased in the presence of strong CYP3A4 inhibitors. To avoid such interactions, co-administration of clarithromycin, verapamil, diltiazem, itraconazole and most other conazole antifungal agents, ciprofloxacin, grapefruit juice and a number of anti-HIV agents e.g. ritonavir, should be used with caution if required.

8 STUDY CONDUCT

8.1 Study Period

The study assessments will be performed according to the summary of the Visit Schedule and Assessments (see table 1).
A month in the treatment period is defined as 28 days ± 7 days.
The study period consists of the following phases:
Screening period: up to 4 weeks. In case of delay in NMRS scans performed and/or interpretation, screening period may be extended up to 45 days.
Double blind treatment period (treatment phase): 52 weeks of once-daily oral administration of Aramchol 400 mg/day or 600 mg/day or matching placebo.
Follow up period: 13 weeks after end of active treatment, without study medication.

Table 1 – Visit schedule and Assessments

Week	**-4 to 0 weeks**	**0** day 1	**2**	**4**	**8**	**12**	**16&20**	**24**	**28**	**32**	**36**	**40**	**44 & 48**	**52**	**65**	**Un schedu led**
	Pre-trial visit	**B/L** day 1												**ET/ EoT**	**F/U**	
Visit N°	**1**	**2**	**3**	**4**	**5**	**6**	**T1&2**	**7**	**T3**	**8**	**T4**	**9**	**T5& 6**	**10**	**11**	
Informed Consent	X															
Eligibility Criteria	X	X														
Demographic Data	X															
Medical History	X															
Disease History	X															
Current Disease Status	X															
Physical examination	X							X						X	X	X
Vital Signs	X	X	X	X	X	X		X		X		X		X	X	X
Body Weight	X	X						X						X	X	X
Height	X	X														
BMI	X	X														
Waist circumference	X	X						X						X	X	X
Imaging - NMRS	X													X		
ECG	X							X						X		X
Liver Biopsy	X[1]													X		
Hematology	X	X	X[7]	X[7]	X[7]			X				X[7]		X	X	X
Coagulation[6]	X	X						X						X	X	
ESR[8]	X[8]	X[8]	X[8]	X[8]	X[8]			X[8]				X[8]		X[8]	X[8]	X[8]
Blood chemistry	X	X	X[7]	X[7]	X[7]			X				X[7]		X	X	X
Urinalysis	X	X	X[7]	X[7]	X[7]			X				X[7]		X	X	X
Insulin (HOMA)	X							X						X		X
HbV and HCV Ab	X															
HIV 1 & 2 antibodies	X															
HbA1c	X				X			X				X		X		X
Leptin		X												X		
Adiponectin		X												X		
CK-18 (M30 and M65)		X												X		
C-reactive protein (hs-CRP)		X												X		
Ferritin		X												X		
PAI-1		X												X		
IL-6		X												X		
TNF-α		X												X		
FGF-19		X												X		
C4		X												X		
Pool Serum Bile Acids		X												X		
B-hydroxybutirate		X												X		
Free Fatty Acids		X												X		
Serum Sample[10]	X													X		
Fatty liver index = FLI		X												X		
Fibromax	X[5]															
Blood Metabolomics	X							X						X		
Endothelial Function[9]		X												X		
TSH, T_3, T_4	X															
Pregnancy test	X															X
NFS		X												X		
Gene Analysis[11]		X														
Aramchol trough level[3]		X[2]		X[2,4]		X[2]		X[2]				X[2,4]		X[2]	X[2]	
Lifestyle changes		X	X	X	X	X		X		X		X		X	X	X
Telephone contact							X		X		X		X			

Concomitant Medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
IMP Dispensing		X		X	X	X		X		X		X				X
Drug accountability			X	X	X	X		X		X		X		X		

1. A biopsy will be performed if not done within 6 months prior to the screening visit.
2. Will be conducted only in Israel
3. Blood samples for Aramchol trough level will be taken on the visit day before the daily dose is taken.
4. Will be conducted at selected sites in Mexico, USA, Europe and Hong Kong at visit 4 or 9.
5. Only if investigator thinks it is necessary
6. Coagulation = fibrinogen, PT/INR, aPTT.
7. Lab samples will be done locally at site or central lab per country.
8. ESR performed by site; according to the lab manual
9. Baseline test will be conducted before first dose is administrated. (If the Endothelial Function test was not done on Visit 2 or needs to be repeated due to unacceptable test data, it will be done at Visit 3 or at an Unscheduled Visit before Visit 3)
10. Serum sample for investigational test will be collected and kept frozen.
11. This sample can be collected at any visit during the study if it was not approved by the IRB prior to the baseline visit.

8.2 Detailed Study Plan

8.2.1 Visit 1 (-4 to 0 weeks) – screening period

Please note that the patient should fast for 8-12 hours before attending the visit.
If the patient is taking any anticoagulant it should be stopped sufficient time before biopsy is taken. Taking NSAIDs should be prohibited 48 hours prior to conducting biopsy.

- Prior to performing any study activities/evaluations, the subject must be thoroughly informed about all aspects of the study, including scheduled study visits and activities, and must sign the informed consent form. A separate consent form will be signed for the blood sample which will be taken for genetic parameters. Signed copies of the informed consent forms should be given to the subject.
- Assign a screening number. The subject will be allocated a screening number by the investigator or designee using the Interactive Web Response System (IWRS). The subject will then be assessed for eligibility criteria.
- Demographic Data (date of birth, sex, childbearing potential and smoking status).
- Medical history (including drug and alcohol use) disease history and current disease status (including staging, diagnosis information, previous anti fatty liver treatments).
- Recording of concomitant medication.
- Physical examination.
- Vital signs including sitting blood pressure, heart rate, respiratory rate and temperature.
- Measurement of body weight, height and waist circumference. BMI will be calculated.
- Checking of Eligibility Criteria.
- Safety laboratory assessments Chemistry, Hematology, ESR, Coagulation and Urinalysis. **Please note that Coagulation results should be within normal ranges before the Biopsy is performed (see Section 9.2).**
- Serum blood sample will be collected and kept frozen in case special test to clarify safety issue will be required and in case special investigation will be needed to be performed.
- Serological evaluation of Hepatitis B, C and HIV.

- Blood samples for insulin and fasting plasma glucose (HOMA).
- HbA1c.
- Blood sample for TSH,T_3 and T_4.
- Pregnancy test to child bearing potential women.
- ECG (12 leads) evaluation.
- Fibromax only if investigator thinks it is necessary.
- Metabolomics blood tests (from all patients) as well as part of the liver biopsy (from approximately 15 consenting patients, world-wide) and kept frozen.
- Liver biopsy (to confirm NASH) will be obtained if not performed within the last 6 months before screening entry.
- Liver NMRS examination. NMRS examination should be done no more than 4 weeks before starting first dose.
- Lifestyle discussion (importance of diet and exercise in weight management).

The procedures should proceed in the following order. First blood tests should be taken; if the results show that the subject is eligible, *liver biopsy should be performed if not done in the 6 months period prior to this visit. NMRS should be performed if all other tests show that the subject is eligible to participate in the study.
**** If the subject has tattoo(s) consult your MRI facility prior to biopsy***

The screening period will be up to 4 weeks however if it is not possible to complete all tests and receive results within this period, the screening period can be extended to 45 days.

8.2.2 Re-Screening Visit

Re-Screening may be allowed upon sponsor approval. An informed consent form should be re-signed if more than 45 days have elapsed from previous signature (or the ICF was revised in the interim). A new screening number will be assigned to the subject.

8.2.3 Visit 2 (Baseline (day 1)) – Randomization

Please note that the patient should fast for 8-12 hours before attending the visit.

- Eligibility Criteria (to ensure no changes occurred since screening visit).
- Vital signs including sitting blood pressure, heart rate, respiratory rate and body temperature.
- Concomitant medication.
- Adverse Events that occurred since the last visit will be recorded.
- Measurement of body weight, height and waist circumference. BMI will be calculated
- Safety laboratory assessments: Chemistry, Hematology, Coagulation, ESR and Urinalysis.
- Blood samples for Adiponectin, Leptin, CK-18, hs-CRP, Ferritin, PAI-I, IL-6, TNF-α, FGF-19, C4, Pool Serum Bile Acids, Ferritine, B-hydroxbutyrate, Free Fatty Acids. The blood samples will be tested for possible biomarkers, including, but not limited to, Fetuine A and GDF15.

- Blood sample for Aramchol trough blood level. (only in Israel- the patients will be asked to take the daily tablet at the clinic after blood sample are taken and to record the time).
- Endothelial Function will be performed prior first dose is taken. This test will be conducted at selected sites. If the test was not done on Visit 2 (Day 0) or needs to be repeated due to unacceptable test data, it will be done at Visit 3, or at an Unscheduled Visit before Visit 3.
- Fatty Liver Index (FLI).
- NFS.
- Lifestyle changes (including importance of diet and exercise in weight management). Life style Questionnaire (appendix E) may be used as a guideline, but is not mandatory.
- Assign the subject a randomization number*.
- Study medication dispensing after calling the IWRS to obtain medication kit number.
- Blood sample for gene analysis for consenting subjects (will be kept frozen and analyzed only at the study end).

* Randomization will be determined according to a computer-generated randomization list produced by the CRO's statistician on behalf of the sponsor. The IWRS procedure will be used to allocate the subject to the treatment group. On the day of randomization, each subject will be assigned a subject number according to the randomization list. This number, allocated via the IWRS in sequential chronological order will replace the screening number. In addition to the subject number, the IWRS will assign a pack number. The subject will be supplied and treated throughout the study with IMP/study drug labeled with the pack number assigned at each visit.

8.2.4 Visits 3 (2 weeks); 4 (4 weeks); 5 (8 weeks); 6 (12 weeks); 7 (24 weeks); 8 (32 weeks) and 9 (40 weeks)

Please note that the patient should fast for 8-12 hours before attending the visit, where labs are drawn

Israel: Visits 3, 4, 5, 6, 7, 9

All other countries: Visits 3, 4, 5, 7, 9

- Physical examination, weight and waist circumference (only at visit 7 (24 weeks)).
  Vital signs including sitting blood pressure, heart rate, respiratory rate and body temperature.
- Concomitant medication.
- Adverse Events that occurred since the last visit will be recorded.
  Safety laboratory assessments: Chemistry, Hematology, ESR and Urinalysis (to be taken at visits 3 (2 weeks); 4 (4 weeks); 5 (8 weeks); 7 (24 week) and 9 (40 weeks). The laboratory samples will be analyzed locally at the site or in some countries in one central lab per the country. The samples collected at visit 7 will be tested in the central lab (CRL)
- HbA1c will be assessed at visits 5 (8 weeks), 7 (24 weeks) and 9 (40 weeks). The samples will be sent to the central lab.
- Blood sample for Coagulation only at visit 7.
- Blood Metabolomics blood tests only at visit 7.

- Blood samples for insulin (HOMA) only at visit 7.
- ECG (12 leads) evaluation will be performed only at visit 7.
- Lifestyle changes (including importance of diet and exercise in weight management. Life style Questionnaire (appendix E) may be used as a guideline, but is not mandatory.
- Study medication dispensing (with the exception of visit 3) after calling the IWRS to obtain medication kit number.
- Drug accountability.
- Blood samples for Aramchol trough blood level – one sample will be taken at visits 4, 6, 7 & 9 in Israel. One sample will be taken on visit 4 or visit 9 at selected sites in Mexico, USA, Europe and Hong Kong . The patients will be asked to take the daily tablet at the clinic after blood sample are taken and to record the time.

8.2.5 Telephone contact (week 16, 20, 28, 36, 44 and 48)

- Telephone call.
- Adverse events that occurred since last visit at site or telephone call.
- Record of any change in concomitant medication, including verify study drug compliance and record each event of study drug interruption in the subject's source document and appropriate eCRF page, if applicable

8.2.6 Visit 10 (week 52) – End of treatment /Early Termination

Please note that the patient should fast for 8-12 hours before attending the visit.
If the patient is taking any anticoagulant it should be stopped sufficient time before biopsy is taken. Taking NSAIDs should be prohibited 48 hours prior to conducting biopsy

- Physical examination.
- Vital signs including sitting blood pressure, heart rate, respiratory rate and temperature.
- Measuring body weight and waist circumference.
- Safety laboratory assessments: Chemistry Hematology, ESR, Coagulation and Urinalysis. Please note that Coagulation results should be within normal ranges before Biopsy performed.
- Blood samples for insulin (HOMA).
- Blood samples for Adiponectin, Leptin, [redacted]. The blood samples taken at these visits, will be tested for possible biomarkers [redacted].
- Serum blood sample will be collected and kept frozen in case special test to clarify safety issue will be required and in case special investigation will be needed to be performed.
- HbA1c.
- Endothelial Function. This test will be conducted at selected sites.
- ECG (12 leads) evaluation.
- Liver NMRS examination.

- Liver biopsy which should be reviewed by the recruiting investigator and confirmed by the study pathologist.
- Fatty liver index (FLI).
- Metabolomics blood tests (from all patients) as well as part of the liver biopsy (from approximately 15 patients, the same patients who had not revoked consent and samples collected from at the screening visit) will be taken for the test and kept frozen.
- NFS
- Recording of concomitant medication.
- Adverse Events that occurred since the last visit will be recorded.
- Lifestyle changes (including importance of diet and exercise in weight management. Life style Questionnaire (appendix E) may be used as a guideline, but is not mandatory .
- Blood sample for Aramchol blood trough level (only in Israel- The patients will be asked to take the daily tablet at the clinic after blood sample are taken and to record the time).
- Drug accountability.

MRI will be recommended to patients that completed at least 24 weeks in the study (visit 7).
Biopsy will be recommended to patients that completed at least 40 weeks in the study (visit 9).

8.2.7 Visit 11 (65 week) - Follow-up

Please note that the patient should fast for 8-12 hours before attending the visit.

- Physical examination, weight and waist circumference.
- Vital signs including sitting blood pressure, heart rate, respiratory rate and body temperature.
- Blood sample for Aramchol blood trough level. (only in Israel-the patients will be asked to take the daily tablet at the clinic after blood sample are taken and to record the time).
- Concomitant medication.
- Adverse Events that occurred since the last visit will be recorded.
- Safety laboratory assessments: Chemistry Hematology, ESR, Coagulation and Urinalysis.
- Lifestyle changes (including importance of diet and exercise in weight management). Life style Questionnaire (appendix E) may be used as a guideline, but is not mandatory.

8.3 Unscheduled Visit

An unscheduled visit may be performed at any time during the study at the subject's request or as deemed necessary by the investigator. The date and reason for the unscheduled visit will be recorded in the subject’s source documents and eCRF.

The reasons for the unscheduled visit could include (but are not limited to):

- Appearance of a new AE or an AE follow-up.
- Change in concomitant medications.
- Laboratory follow-up.
- IMP dispensing, accountability and/or replacement after calling the IWRS to obtain medication kit number.

- Subject compliance.
- Other, specify.

It is mandatory to take or collect the following information in each visit:
- Vital Signs.
- Adverse Event.
- Changes in concomitant medication.
- Additional analyses may be performed by the investigator and stated in the source documentation.

8.4 Early Termination

An early termination visit should be completed for all subjects who have been randomized and prematurely discontinue from the study drug.
Every attempt should be made to have subjects return to the clinic for their Early Termination Visit. In case of early termination due to ongoing AE, manifestation of a severe degree of intolerance to IMP and/or disease worsening, the subject should remain under medical observation and followed until the medical condition returns to baseline or is considered stable or chronic.

8.4.1 Criteria for Early Termination

A subject may withdraw or be withdrawn from the study for the following reason.
- Subject withdrew consent.
- Sponsor requested subject to be withdrawn.
- Request of primary care physician or investigator.
- Protocol violation/ Non-compliance.
- Lost to follow-up/failure to return.
- Adverse Event (specify primary AE in the AE log).
- Marked disease progression as defined at any time during study drug therapy.
- The occurrence of marked progressive liver disease
- Unresolved or recurrent grade III or IV toxicity.
- Pregnancy .
- Death.
- Other.

8.5 Temporary Discontinuation of Study Drug Treatment

Temporary discontinuation is defined as missing more than three consecutive doses.
The reasons for temporary IMP/study drug discontinuation should be recorded in the subject source document and the study drug administration and interruption eCRF pages and the local clinical management should be notified**.**

The subject will report any temporary discontinuation to the investigator and will be instructed by the investigator regarding continuation of treatment.

8.6 Investigational Medicinal Products/Study Drugs

8.6.1 Treatments Administration

Subjects will be administered Aramchol as follows:

- One tablet of Aramchol 400 mg and one tablet of matching placebo for Aramchol.
- One tablet of Aramchol 400 mg and one tablet of Aramchol 200 mg.
- Two tablet of Aramchol matching placebo.

The tablets should be taken orally in the morning within 30 minutes after breakfast with a glass of water (250 ml).

Subjects are allowed to omit study drugs up to 3 consecutive days during the study.

8.6.2 Method of Assigning Subjects to Treatment Groups

There will be a screening period of 4 weeks (with an option to extend it up to 45 days), during which the subject will be assigned a screening number through the Interactive Web Response System (IWRS). At the baseline visit, subjects who are deemed eligible will be assigned to one of three possible treatment groups by the IWRS according to the randomization scheme produced by the CRO's statistician on behalf of Galmed Pharmaceutical Ltd. Each subject will be allocated a unique randomization number in addition to their screening number. The IWRS will keep track of subjects' visits and status.

A randomization list will be generated prior to the study initiation, using a computerized algorithm. The randomization will be performed in blocks, 48 blocks containing 5 subjects each in a 2:2:1 ratio (2 subjects for each of the active groups and 1 placebo).

8.6.3 Blinding

The investigators, the sponsor and any personnel involved in subjects' assessments, monitoring, analysis and data management (excluding the designated Clinical Supplies Unit's personnel), are blinded to the subject assignment.

8.6.4 Emergency Code Breaking

In case of a serious adverse event or pregnancy, when the study drug assignment is needed to make treatment decisions for the subject, the investigator may unblind the subject's drug assignment. The sponsor should be notified of the event prior to breaking the code, if possible. If this is not possible, the sponsor should be notified immediately afterwards, and the subject's drug code assignment should not be revealed. The circumstances leading to the breaking of the code should be fully documented, in the investigator's study files and in the subject's source

documentation. Treatment assignment should not be recorded in any study documents or source document.

In studies conducted in the EU for AEs that are defined as: Suspected, Unexpected, Serious, Adverse Reaction (SUSAR), the Global Drug Safety & Pharmacovigilance (GDSP) may break the subject's code for possible regulatory submission.

8.6.5 Description of Investigational Medicinal Products/Study Drugs

Aramchol tablets 200 mg, 400 mg and placebo for Aramchol tablets are manufactured in compliance with current Good Manufacturing Practice (GMP) standards and guidelines applicable to IMPs [redacted]. The color of Aramchol 200 mg, 400 mg and their matching placebo tablets are of identical appearance, gray non-coated tablets, to maintain study blinding. Aramchol 200 mg and its matching placebo are round and Aramchol 400 mg and its matching placebo are oval.

8.6.6 Packaging and Labeling

The study drugs are packaged in HDPE 100ML Duma Twist Off containers. Each container will contain 35 tablets. Each kit will contain 2 bottles. The bottles are capped with PP Duma Twist off Caps.

[redacted]

The label on the bottles will include a fixed information (i.e. product name, dosage, storage conditions, instructions etc.) and the kit number.

Each patient monthly kit will include 2 bottles. The label on the kit will include fixed information (i.e. product name, dosage, storage conditions, instructions etc.) and a variable information section. The variable information section will include 2 parts: 1 detachable label, which will be attached to the subject's file upon bottle dispensing and 1 label to remain on the bottle. The variable information section will include the following variable data: batch code, bottle number, and expiration date (all to be pre-printed) and blank fields for investigator name, visit number and subject number. These blank fields will be manually filled-in by the Investigator or designee upon provision of the kit to the subject.

8.6.7 Distribution and Shipment

A sub-contracted Vendor, according to the distribution guidelines, will perform distribution and shipment of IMP.

The IMP bottles will be packed and shipped at room temperature in appropriate storage boxes. If the IMP supplies appear to be damaged/missing upon arrival at the investigational site, the sponsor should be contacted immediately.

Each shipment of IMP supplies for the study will contain a shipment form describing the content of the shipment. This form will assist the site in maintaining current and accurate inventory records. When a shipment is received, the site investigator/[redacted] coordinator/pharmacist will acknowledge receipt of the IMP supply by sending a "Confirm Shipment" form to the sending center.

When a shipment is received at a depot, acknowledgement of receipt of the IMP supply will be coordinated by the sending center.

Importing of the IMP by an EU member will be carried out according to the provisions of Directive 2001/20/EC, Article 13. Importing to any other participating country will be done according to the local regulations.

8.6.8 Storage, Dispensing and Return

IMP supplies will be kept in a secure, limited-access, temperature-controlled storage area .

Only authorized personnel will have access to the IMP. The study site personnel at each site will be responsible for correct storage and handling of the IMP .

All IMP bottles must be stored at room temperature.

The IMP kits will be dispensed to the subject at the study site at visits 2, 4 – 9 & unscheduled visits (as needed). The bottles will be assigned to subjects according to the randomization list provided by the CRO. At dispensing visits, the site personnel should fill in the subject's number, the visit number and the Investigator's name on the label of the dispensed bottle as well as on the detachable label, which is adhered to the subject's file.

At each visit during the treatment period the subject will receive 1 - 3 kits of IMP according to the period between the visits. The dispensed total should include an amount that will suffice until the following scheduled dispensing visit. The subject should finish all of the tablets in each kit before moving to the next kit.

Subjects will be instructed to store IMP at room-temperature and to return all used empty bottles and unused IMP at each visit. The site investigator/site coordinator is responsible for performing IMP accountability at the site. The monitor is responsible for the accountability of the returned IMPs.

8.6.9 Verification of Compliance with Treatment Regimen

At each study visit, the investigator/site coordinator will assess the subject's compliance with the prescribed regimen for the study medication. This will include checks of protocol

compliance and use of study drug in order to assess the reliability of subject-generated data. Subjects who fail to comply with the study requirements may be withdrawn from the study, following consultation with the sponsor.

Compliance with the dosing regimen will be determined by performing IMP accountability of returned bottles of the IMP used and unused at each visit. Site personnel will record the number of used, unused and lost tablets on the Accountability Log. Percent compliance will be calculated as the number of used capsules divided by the number of total capsules expected to be used, multiplied by 100. Subjects with less than 75% compliance between previous visit to the clinic and the current one will be warned. If a second incident of non-compliance occurs, the subject will be withdrawn from the study**.**

8.6.10 Accountability and Compliance

IMP accountability records must be maintained at the site at all times.

Upon Galmed's (or Galmed's designee) monitor visit at the site, accountability of the returned IMPs should be performed and recorded by the monitor. The subject number, the date, batch code, bottle (kit) number and quantity of IMP returned by the subject will be checked for correctness and recorded on the appropriate accountability form.

At the end of the study, the monitor must return all IMP (used and unused bottles) and the corresponding accountability forms to the sponsor or sponsor's designee (CRO) for reconciliation and destruction. A photocopy of these records must be kept at the study sites. If needed, IMP can be returned during the study upon sponsor approval.

9 ASSESSMENTS METHODS

9.1 NMRS evaluation

All subjects will have two NMRS evaluations at the beginning of the study (Visit 1) and at visit 10 (Early Termination/End-of-Treatment). The End-of Treatment NMRS should occur 0 – 7 days prior to visit 10, before the subject stops taking study medication or within 7 days after the visit. For patients that early terminated from the study, MRI will be recommended to patients that completed at least 24 weeks in the study (visit 7).

All subjects should maintain a low fat high carbohydrate iso-caloric diet within 48 hours prior to MRI scan. Any alcohol consumption is not allowed during this period. Fasting for 6 hours before the MR scan is required (only water allowed).

All NMRS scans will be evaluated locally for any clinical abnormalities. The clinical report should be forwarded to the investigator within 48-72 hours. If abnormalities are found during the screening NMRS, it is up to the investigator's discretion whether to include the patient in the study, to include him after further exploration the findings or exclude the patient.

The primary efficacy parameter to be assessed will be liver triglyceride concentration: Liver-fat/water ratio in the liver. This will be evaluated in the MRI unit at Tel Aviv Sourasky Medical Center (Appendix A) who will act as a central reading center.

9.2 Liver Biopsy

Biopsies will be performed at the screening visit (if not performed within 6 months prior) and at visit 10 (End-of Treatment/Early Termination. The End-of-Treatment biopsy should occur 0 – 7 days prior to visit 10, before subject stops taking study medication or within 7 days after it. For patients that early terminated from the study, biopsy will be recommended to patients that completed at least 40 weeks in the study (visit 9).

Coagulation results should be within normal ranges before biopsies are performed. Coagulation values outside the normal range should be evaluated by the Investigator to determine subject eligibility and the scheduling/timing of the biopsy. If the subject is taking any anti-coagulant drug, it should be stopped or specifically antagonized in sufficient time before biopsy is taken. Investigators should take into account the pharmacokinetic properties (e.g., half-life) of the anti-coagulant or antagonist drug to allow sufficient time for desired effect. Taking NSAIDs should be prohibited 48 hours prior to conducting biopsy.

Slides will be prepared at each site (Appendix B) and will be sent to the Institute of Pathology, Medical University of Graz Auenbuggerplatz 25 A-8036 Graz, Austria for evaluation. This center will act as the central reading center for the study.

Liver biopsy may be recommended for definitive diagnosis of any rapidly progressive liver disease or unusual phenomena which may be suspicious of alteration in the disease, or suspicious for another disease affecting the liver.

9.3 NAFLD Fibrosis Score (NFS):

The score will be calculated for based on the following formula:
-1.675+0.037Xage (years) + 0.094xBMI (kg/m2) + 1.13XIFG/diabetes (yes=1, no=0)
+0.99XAST/ALT ratio – 0.013Xplatelet(x109/l) – 0.66Xalbumin (g/l)

9.4 HOMA

The Homeostasis Model Assessment (HOMA) estimates steady state beta cell function (%B) and insulin sensitivity (%S), as percentages of a normal reference population. These measures correspond well, but are not necessarily equivalent, to non-steady state estimates of beta cell function and insulin sensitivity.

This parameter will be calculated based on plasma glucose level and insulin, which will be tested among other lab tests .
HOMA –IR calculation: [glucose (mmol/L)*insulin (µU/mL)/22.5], using fasting values.
HOMA-Beta = 20*[Insulin(µU/mL)] / ([Glucose mmol/L] – 3.5) %,.

9.5 Fatty liver Index (FLI)

Fatty Liver Index will calculated by the following equation (24):

FLI = (e 0.953*loge (triglycerides) + 0.139*BMI + 0.718*loge (ggt) + 0.053*waist circumference - 15.745) / (1 + e 0.953*loge (triglycerides) + 0.139*BMI + 0.718*loge (ggt) + 0.053*waist circumference - 15.745) * 100

9.6 Blood Metabolomics

The assessment will be done on week -4 (screening visit), visit 7 and week 52 (End-of-Treatment/Early Termination) visit will be collected from all patients.
Small part of the liver biopsy will be collected from about 15 consenting patients (the same patients) during the screening and the termination visits.
Serum samples as well as biopsy samples will be prepared and stored as described in Appendix C. Samples will be analyzed at OWL (One Way Liver Genomics, S.L.), Parque Tecnologico de Bizkaia, edif. 502, 48160 DERIO (Bizkaia) Spain.

9.7 Endothelial Function

The assessment will be done during the baseline visit before first dose is taken and at visit 10 (End-of Treatment/Early Termination visit) This test will be conducted at selected sites. If the Endothelial Function test was not done on Visit 2 (Day 0) or needs to be repeated due to unacceptable test data, it will be done at Visit 3 (two weeks after Visit 2) – or at an Unscheduled Visit before Visit 3.
The examination will be conducted as describe in Appendix D. The data collected will be analyzed by Itamar Medical Ltd. 9 Halamish St., Caesarea, 38900, Israel.

9.8 Aramchol blood trough level

Blood samples for trough Aramchol blood level will be collected from subjects participating in the study in Israel at visits 2 (baseline), 4, 6, 7, 9, 10 and 11.
One blood sample will be collected on visit 4 or 9 at selected sites in Mexico, USA, Europe and Hong Kong to test for trough Aramchol blood level differences between populations (e.g., African American, Asian, Hispanic).

In these visits the subjects will be asked not to take the study medication before attending the visit at the clinic but only after the blood sample for trough Aramchol blood level will be taken. The date and time of the blood sample, as well as the date and time of the last study drug dose prior to the sample will be recorded on the e-CRF.

The blood samples will be collected and transferred into pre-labeled pre-chilled (ice-based water bath (4^0C)) EDTA-K2 tubes. Following each collection, the tube will be gently inverted several times to assure complete mixing with the potassium EDTA and should then be placed on ice pending centrifugation.

Plasma will be separated by centrifugation for 10 min within 45 minutes of collection at approximately 2000g (2600-3000 rpm) and +4°C. Deviations from the blood sampling times will be recorded in the appropriate eCRF. Plasma samples will be divided into duplicate labeled Cryotubes (at least 600 µl per tube) by the site and sent frozen on dry ice to the central laboratory for storage at -70°C. Samples for blood trough levels evaluation will be sent periodically on dry ice to Analyst Research Laboratories, Rehovot, Israel. All shipments will be accompanied by a detailed sample inventory list, and the bio-analytical laboratory will be notified in advance by telephone, fax or e-mail before the shipment of study samples. Aramchol plasma concentrations will be determined using a validated LC-MS/MS method.

9.9 Changes in Lifestyle

The subjects will be counselled on the importance of diet and exercise in proper weight management and asked if any change took place in their lifestyle between visits to the clinic. These changes can be in:

- Their diet (meal composition or what they are eating).
- Their physical exercise.

Life style Questionnaire (appendix E) may be used as a guideline, but is not mandatory.

9.10 Gene Analysis

The assessment of possible relations between the genetic background (including but not limited to PNPLA3 I148M variant) and response to Aramchol will be performed. Evaluations will be based on results from the subjects as a group. Individual subjects will not be identified. Data will be kept confidential and stored separately. Subjects will have to give a separate consent to the sampling and storage. This analysis will be performed in the pooled population of the program. The samples will be collected from all subjects who will consent to the test. In case a patient decides to withdraw his/hers consent the sample will be destroyed and only will be tested the parameters that the patient has previously consented and that are reported in the Informed Consent form. The samples will be destroyed 2 years after the study conclusion .

Our study will enroll 240 NASH patients and one of the exploratory endpoints will be on the correlation of the response to Aramchol treatment and the PNPLA3 genotype. As part of the study, the subjects will undergo two liver biopsies, for the diagnosis of hepatic steatosis and for monitoring disease severity. Clinical characteristics of the patients and severity of NASH will be correlated with the PNPLA3 rs738409 C>G genotype, encoding for the I148M protein sequence variant. This study will try to determine if there is an association between PNPLA3 rs738409 genotype and the response of the patients to Aramchol treatment.

Nonalcoholic fatty liver disease is characterized by the excess accumulation of lipid in the liver (hepatic steatosis). The intrahepatic lipid balance is maintained by different processes including receptor-mediated uptake of lipoproteins from the blood, hepatic oxidation of lipids and de novo synthesis of lipids, secretion of lipoproteins and hepatic storage of lipids (17). Recent genome-wide association studies have suggested that variations in the adiponutrin/patatin-like

phospholipase-3 (PNPLA3) gene contribute to differences in hepatic lipid content and the susceptibility to NAFLD (18-20). The PNPLA3 I148M variant is a cytosine to guanine exploratory substitution that transforms codon 148 from isoleucine to methionine. The 148M variant has been found to be more common in Hispanic populations, who are known to be at an increased risk for NASH, contributing to ethnic and inter-individual differences in hepatic fat content and susceptibility to nonalcoholic fatty liver disease (NAFLD) (19). Importantly, the I148M SNP has been reported to influence not only liver fat content and the predisposition to develop NAFLD, but also the development of steatohepatitis (NASH), and the progression of hepatic fibrosis and hepatocellular carcinoma development (34).

The samples will be analyzed as follows: PNPLA3 (I148M variant) polimorphism rs738409 C>G, by 5'-nuclease (Taqman) assay, on DNA extracted from the peripheral blood. (35).

Only one blood sample is required to determine the PNPLA3 rs738409 (I148M) genotype. It will be collected from each participating subject who consents for it, during the baseline visit before the first dose taken. If the sample is not taken during the baseline visit by mistake, it could be taken at any subsequent visit. The samples will be kept frozen till the end of the study Laboratorio Malattie Metaboliche del Fegato UO Medicina Interna ad Indirizzo Metabolico, Fondazione IRCCS Ca' Granda Ospedale Policlinico Milano, Department of Pathophysiology and Transplantation, Università degli Studi di Milano under the responsibility of Prof. Luca Valenti. After the treatment codes will be open, the frozen blood samples of the subjects who received active treatment will be assessed for the gene expression. The assessment will be performed by Laboratorio Malattie Metaboliche del Fegato UO Medicina Interna ad Indirizzo Metabolico, Fondazione IRCCS Ca' Granda Ospedale Policlinico Milano Department of Pathophysiology and Transplantation, Università degli Studi di Milano under the responsibility of Prof. Luca Valenti.

For each sample, it is required 7cc K3-EDTA frozen whole blood. Appropriate tubes will be provided by the CRL. The samples can be stored in a -20°-80° freezer. The samples of all patients will be stored at CRL and from time to time will be sent to the examining Lab .

The unutilized genetic material will be destroyed at the central lab and a destruction certificate will be issued.

[redacted]

[redacted]

[redacted]

10 SAFETY ASSESSMENTS

10.1 Adverse Events

Adverse events will be recorded from when a subject has signed the Informed Consent Form and throughout the study, including the follow-up period. They should be reviewed and updated at each subsequent visit and during any phone contact with the subject.

10.2 Safety Laboratory Evaluations

All laboratory testing will be performed by a central laboratory facility and/or affiliated laboratories which work under the central laboratory regulations and within its tests normal ranges. On visits 3, 4, 5 and 9 those tests can be performed at site's local lab or at a central lab. The lab manual should be referred to for specific instructions.

The following tests will be performed:

- Serum Chemistry
  - Fasting Plasma Glucose
  - Creatinine
  - Creatinine Clearance
  - Bilirubin (direct and total)
  - Urea
  - AST (SGOT)
  - ALT (SGPT)
  - GammaGT (GGT)
  - Lipid profile- once in the study either at screening or any other visit; 8-12 hours fasting is mandatory: Total cholesterol, LDL cholesterol, HDL cholesterol, VLDL and triglycerides.
  - Albumin
  - CPK
  - Alkaline Phosphatase
  - hs-CRP
- Electrolytes
  - Sodium
  - Potassium

- Chloride
- Calcium
- Phosphorous

- Coagulation
  - Fibrinogen
  - PT/INR
  - aPTT
- Serology (only at screening)
  - HBV
  - HCV
  - HIV
- Hematology
  - Hemoglobin
  - MCV
  - Hematocrit
  - Red Blood Cells count (RBC)
  - White Blood Cells count + differential
  - Platelet count
  - Basophils
  - Eosinophils
  - Lymphocytes
  - Monocytes
  - Neutrophils
  - Large Unstained Cells
- Urinalysis
  - pH
  - Blood
  - Glucose
  - Ketones
  - Erythrocytes
  - Protein
  - Specific Gravity (USG)
  - Nitrite
  - Leukocytes
  - Bilirubin
  - Urobilinogen
- ESR

Measurement of Complete Blood Counts, INR and chemistry are in fact regarded as current clinical practice for fatty liver patients, and should therefore be collected and analysis performed by the Central Laboratory according to the Visit Schedules.

The sponsor must be provided with a copy of the laboratory's certification, and a tabulation of the normal ranges for each parameter required. Additionally, if at any time a patient has laboratory parameters obtained from a different outside laboratory, the sponsor must be provided with a copy of the certification and a tabulation of the normal ranges for that laboratory. At any time during the study, abnormal laboratory parameters which are clinically relevant (e.g. require dose modification and/or interruption of study drug, lead to clinical symptoms or signs or require therapeutic intervention), whether specifically requested in the protocol or not, must be recorded on the appropriate Comment CRF page in addition to the appropriate laboratory CRF page. When abnormal laboratory values or test results constitute an adverse event (i.e., induces clinical signs/ symptoms or requires therapy) they must be recorded on the Adverse Events CRF.

When, in the opinion of the investigator, other clinical laboratory evaluations may be relevant for assessing the patient's status, they will be completed and entered into the database as appropriate.

Special tests:

- Total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides will be specifically determined as probable safety parameters in this study, which affect lipid metabolism.
- See Section 9.11 for additional specialty test (Exploratory Laboratory tests and other Laboratory tests needed)

10.3 ECG

ECGs will be performed at weeks -4 – 0 (screening) visit (additional recording, up to 30 minutes apart will be performed if QTc is >450msec according to the machine output), week 24, week 52 (End-of-Treatment/Early Termination) and at any other time the investigator thinks it is necessary. Print-outs of the ECGs should be kept with the subject source document.

The subject should rest for at least 10 minutes before measurement is taken. Twelve-lead ECG should be performed following the subject being in a supine position for 5 minutes.

The ECG will be evaluated by the investigator at time of performance (signed and dated) and the printout should be kept in the source documentation file. These printouts may be sent by the Sponsor (or sites) to a central reading center at a later stage. When potentially clinically significant findings are detected by the Site Investigator, a cardiologist should be consulted for a definitive interpretation. All communications and diagnoses should be filed in the source documentation file.

The final decision whether the ECG findings are of clinical significance is under investigator responsibility/local cardiologist. The investigator's interpretation of the ECG will be recorded in the eCRF.

10.4 Pregnancy test

Pregnancy should be avoided during the study. Pregnancy test will be done to child bearing potential women. This test will be performed during the week -4 -0 (screening) visit and any other visit the investigator thinks it will be necessary.
Urine test maybe done at the Investigator's discretion (after the screening test) should only be recorded in the source documentation, unless the test results are positive (see Section 11.6).

10.5 Vital Signs

Vital signs (temperature, heart rate and blood pressure) will be completed at all scheduled and unscheduled visits.
Blood pressure and pulse will be recorded in a sitting position after resting for 5 minutes .
All measurements will be recorded in the source documents.
Weight and waist circumference will be measured at screening, week 24 (visit 7), week 52 (End-of Treatment/Early Termination) and week 65 (follow up visit).
Height will be measured at week -4 - 0 (screening) visit only.

Information about the vital signs must be presented in the subject source documentation at the study site. Significant findings present prior to the start of the study medication treatment must be included in the relevant medical history or current medical history condition in the eCRF. Significant findings after the start of the study which meet the definition of an adverse event must be recorded in appropriate place in the eCRF.

10.6 Physical Examinations

Physical examination will be performed at weeks -4 - 1 (screening), visit 7, week 52 (End-of-Treatment/Early Termination) and at any time the investigator thinks it is necessary for the safety of the subject.

Information about the physical examination must be presented in the subject source documentation at the study site. Significant findings present prior to the start of the study medication treatment must be included in the relevant medical history or current medical history condition in the eCRF. Significant findings after the start of the study which meet the definition of an adverse event must be recorded in appropriate place in the eCRF.

11 SAFETY ASSESSMENTS AND PHARMACOVIGILANCE

Safety assessments will consist of evaluating relevant medical history, adverse events and serious adverse events, laboratory parameters including hematology, chemistry, vital signs, physical examinations, and documentation of all concomitant medications and/or therapies.

11.1 Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with the treatment.

In the study, any event occurring after the clinical trial subject has signed the study Informed Consent should be recorded and reported as an AE.

An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

A new condition or the worsening of a pre-existing condition will be considered an AE. Stable chronic conditions such as arthritis that is present prior to study entry and do not worsen during the study will not be considered AE.

Worsening of the disease under study will be measured by laboratory test and clinical parameters should only be recorded as an AE if the outcome is more serious than would normally be expected from the normal course of the disease in a particular subject.

An abnormal result of diagnostic procedures including abnormal laboratory findings will be considered an AE if it:

- Results in subject's withdrawal by the investigator.
- Is associated with a serious adverse event (SAE).
- Is associated with clinical signs or symptoms.
- Is considered by the physician to be of clinical significance.

The intensity or severity of the AE will be characterized as:

Mild: AE which is easily tolerated.
Moderate: AE sufficiently discomforting to interfere with daily activity.
Severe: AE which prevents normal daily activities.

Unlabeled/ Unexpected AE - A reaction which is not included in the Adverse Reaction section of the relevant reference, including **specificity, severity, outcome or frequency.**
The safety reference of this study is the Investigator's Brochure (IB).

The relationship of an AE to the study drug is characterized as:

TERM	DEFINITION	CLARIFICATION
Unrelated	This category applies to those adverse events which, after careful consideration, are	

TERM	DEFINITION	CLARIFICATION
	clearly due to extraneous causes (disease, environment, etc.)	
Unlikely	In general, this category can be considered applicable to those adverse events, which after careful medical consideration at the time they are evaluated, are judged to be unrelated to the test drug.	An adverse experience may be considered unlikely related if or when (must have two): • It does not follow a reasonable temporal sequence from the administration of the test drug. • It could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject. • It does not follow a known pattern of response to the test drug. • It does not reappear or worsen when the drug is re-administered.
Possibly	This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with the test drug administration appears unlikely but cannot be ruled out with certainty.	An adverse experience may be considered possibly related if or when (at least two of the following): • It follows a reasonable temporal sequence from administration of the drug. • It could not readily have been produced by the Subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the Subject. • It follows a known pattern of response to the test drug.
Probably	This category applies to those adverse events which, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the test drug.	An adverse experience may be considered probably related if or when (at least three of the following): • It follows a reasonable temporal sequence from administration of the drug. • It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors or other modes of

TERM	DEFINITION	CLARIFICATION
		therapy administered to the subject. • It disappears or decreases on cessation or reduction in dose. There are important exceptions when an adverse event does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists. • It follows a known pattern of response to the test drug.

The date of onset, a description of the AE, severity, seriousness, action taken, relationship to the study drug, outcome of the event and date of resolution will be recorded.

11.2 Serious Adverse Event

A Serious Adverse Event (SAE) is defined as an AE that results in any of the following:

- Death.
- Life-threatening.
- Requires hospitalization or prolongs existing inpatients' hospitalization.
- Results in persistent or significant disability or incapacity.
- Results in a congenital abnormality or birth defect.
- An important medical event which requires medical intervention to prevent any of the above outcomes.

Important medical events are those which may not be immediately life-threatening, but may jeopardize the subject and may require intervention to prevent one of the other serious outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; resulting in an adverse event will normally be considered serious by this criterion.

Inpatient **hospitalization** or prolongation of existing hospitalization means that hospital inpatient admission and/or prolongation of hospital stay were required for treatment of AE, or that they occurred as a consequence of the event. It does not refer to pre-planned elective hospital admission for treatment of a pre-existing condition that has not significantly worsened, or to diagnostic procedure.

The term **"life-threatening"** in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

Any new SAE that occurs after the study period and is considered to be related (possibly/probably) to the IMP or study participation should be recorded and reported immediately. The study period for the purpose of SAE reporting is defined as the period from the subject's signature on the informed consent form until the end of the follow-up period.

Events NOT considered to be SAEs are hospitalizations which:

1. Were planned before entry into the study.
2. Are for elective treatment of a condition unrelated to the indication or its treatment.
3. Occur on an emergency, outpatient basis and do not result in admission (unless fulfilling the criteria outlined above for a SAE).
4. Are part of the normal treatment or monitoring of the indication and not associated with any deterioration in condition .
5. Social reasons and respite care in the absence of any deterioration in the patient's general condition.

11.3 SAE Reporting

In order to satisfy regulatory requirements, any Serious Adverse Event, whether deemed IMP-related or not, must be reported to the Local Clinical Management (CRA or sponsor representative) as soon as possible after the investigator or coordinator has become aware of its occurrence. The SAE form completion and reporting must not be delayed even if all of the information is not available at the time of the initial contact.

The SAE should be submitted within 24 hours of becoming aware of the event to the Local Clinical Management. The local Clinical Management will forward the report to [redacted] which will handle all the safety reports.

Galmed have delegated responsibility for the safety management for this study to [redacted]

SAE originated in this study should be sent to:

[redacted]

Additional information (follow-up) about any SAE unavailable at the initial reporting should be forwarded by the site within 24 hours of the information becoming available to the Local Clinical Management.

The following information should be provided on the SAE report form to accurately and completely record the event:

1. Investigator Name and Center Number.
2. Subject Number.
3. Subject initials.
4. Subject Demographics.
5. Clinical Event.
   1) Description.
   2) Date of onset.
   3) Severity.
   4) Treatment.
   5) Relationship to study drug (causality).
   6) Action taken regarding study drug.
6. If the AE result in Death.
   1) Cause of death (whether or not the death was related to study drug).
   2) Autopsy findings (if available).
7. Medical History case report form (copy).
8. Concomitant Medication case report form (copy).
9. Any relevant reports (laboratory, discharge, X-ray, etc).

Related SAEs ***MUST*** be collected and reported regardless of the time elapsed from the last study drug administration, even if the study has been closed.

Unrelated SAEs must be collected and reported during the study and up to the follow-up assessment.

For both initial and follow-up SAE reports the Local Clinical Management (CRA or sponsor representative) forwards this information to Diamond PV Services within 48 hours.

The blinding will be maintained for the people who are involved directly in the study, therefore in case of a SUSAR un-blinded information will not be forward to local study management or Galmed personnel.

This information should be sent to the Local Clinical Management who will forward the information to Diamond PV Services.

11.4 Safety reporting to Investigators, Institutional Review Boards or Independent Ethics Committees, and Regulatory Authorities.

The sponsor, via Diamond PV Services Ltd. is responsible for reporting all applicable SAEs to Regulatory authorities, Investigators, and IRBs/IECs, as applicable, in accordance with national regulations in the countries where the study is conducted.

For all investigators located in the European Economic Area, the sponsor via Diamond PV Services Ltd. will be responsible for reporting suspected unexpected adverse reactions (SUSARs) and any other applicable SAEs to regulatory authorities including the European Medicines Agency, Investigators, and IRB/IECs, as applicable, in accordance with national

regulations in the countries where the study is conducted. The SUSARs will be submitted within 7 days for fatal and life-threatening events and within 15 days for other serious events.

In non-EU countries, SAEs will be reported to the regulatory authorities in accordance with local requirements by Diamond PV Services Ltd. SAEs should be reported by the site to their local EC/IRB as dictated by their board's policies and procedures.

Subjects who have had an SAE during the treatment period must be followed clinically until all parameters (including laboratory) have either returned to normal or have stabilized or are otherwise explained .

Any newly emergent SAEs after treatment is discontinued or the subject has completed the study and is considered to be related to the IMP or study participation should be recorded and reported immediately. The post-study period for the purpose of SAE reporting is routinely for up to 30 days following last visit of the study or until SAE is resolved or stabilized. In certain cases, such as where the drug has a long half-life, a longer follow-up period may be required.

11.5 Treatment and Follow-up of Adverse Events

Adverse events, especially those for which the relationship to the study drug is possible, probable or remote, should be followed up until they have returned to baseline status or stabilised. If at follow-up, return to baseline status or stabilisation cannot be established an explanation should be recorded on the CRF.

Any newly emergent SAEs after treatment is discontinued or the subject has completed the study and is considered to be related to the IMP or study participation should be recorded and reported immediately. The post-study period for the purpose of SAE reporting is routinely for up to 30 days following last visit of the study or until SAE is resolved or stabilized. In certain cases, such as where the drug has a long half-life, a longer follow-up period may be required.

11.6 Pregnancy reports

A female clinical trial subject must be instructed to inform the Investigator immediately if she becomes pregnant during the study. Pregnancies occurring up to 90 days after the completion of the study drug must also be reported to the Investigator.

The Investigator should report all pregnancies in female clinical trial subjects to [redacted] within one working day of becoming aware of them using a clinical trial pregnancy reporting form.

All pregnancy reports will be captured in the safety database. This includes normal pregnancies without an AE.

Any subject who becomes pregnant during the study period must not receive additional doses of investigational product and will be withdrawn from the study. If the subject requests to know which treatment she received, this information will be provided to her.

The pregnancy should be followed for outcome of the mother and the child, including any premature terminations, and should be reported to [redacted] for any outcome.

12 STATISTICAL METHODOLOGY

A detailed Statistical Analysis Plan (SAP) which describes in more details the planned analysis and reporting for this study was signed on 26Jan2017. When differences exist in descriptions, explanations or definitions provided in the Study Protocol and the SAP, the SAP prevails.

12.1 Original Protocol Sample Size and Power Consideration

The planned sample size is 215 subjects, 86 in each of the two active groups and 43 in the placebo group. [redacted]

The total sample size is therefore 240 (96 in each of the two active groups and 48 in the placebo group).

12.2 Randomization

The ratio of 1:2:2 between the placebo and the two active groups has been chosen in order to minimize the numbers of subjects undergoing two liver biopsies, while taking a placebo.

The randomization list was generated prior to the study initiation, using a computer-generated randomization list produced by a Sintesi statistician on behalf of the Sponsor. The randomization was performed in blocks, 48 blocks containing 5 subjects each in a 2:2:1 ratio (2 subjects for each of the active groups and 1 placebo).

The Interactive Web Response System (IWRS) procedure was used to allocate the subject to the treatment group. On the day of randomization, each subject was assigned a randomization number according to the randomization list. This number, allocated via the IWRS in sequential chronological order will replace the screening number. In addition to the subject number, the IWRS assigned a kit number. The subject was to be supplied and treated throughout the study with IMP/study drug labeled with the kit number assigned at each visit.

Please note that randomization was stratified by countries.

12.3 Data Analyses Sets

The following data analysis sets are defined for this study:

- **Intention-to-Treat Analysis Set (ITT)**: In accordance with the intention-to-treat principle, all randomized participants will be included in this analysis set, according to the treatment group to which they were originally assigned to.

- **Safety Analysis Set (ST)**: The Safety Analysis Set will include all randomized participants who took at least one dose of the study drug according to the treatment actually received. The ST analysis set will serve as the principal analysis set for safety assessments.
- **Full Analysis Set (FAS)**: The Full Analysis Set (FAS) will include all patients of the ITT population who have baseline and at least 1 post baseline efficacy assessment. The FAS analysis set will include efficacy observations that were collected up to, including Visit 10 (Week 52) and all NMRS measurements. Note that efficacy analyses for the liver biopsy derived parameters will use only the FAS described below.
- **Full Analysis Set for Liver Biopsy Data (FAS_{biopsy})**: The Full Analysis Set for Liver Biopsy Data (FAS_{biopsy}) will include all patients of the ITT population who underwent the baseline and the post baseline biopsies. This analysis set is defined as the vast majority of Israeli participants are lacking the pair of biopsies due to regulatory limitation.
- **Completers Analysis Set (CO)**: The Completers analysis set (CO) will consist of all randomized subjects who normally completed Visit 10 (end of treatment) according to the treatment actually received and underwent both pre-treatment and post-treatment NMRS assessments.
- **Per Protocol Analysis Set (PP)**: The Per-Protocol analysis set (PP) will consist of all randomized subjects who normally completed Visit 10 (end of treatment), underwent both pre-treatment and post-treatment NMRS assessments without any major protocol violations according to the treatment actually received.

12.4 Study Population Summary

The ITT analysis set will be used for the description of study population. Descriptive statistics of these data will be provided by treatment group and overall.

12.4.1 Subject Disposition

Data from subjects who are screened, randomized, subjects in the pre-defined analysis sets, subjects who complete the study, subjects underwent two MRI scans, subjects underwent two liver biopsies, and subjects who early terminated the study prior to week 52 and between weeks 52-65 will be summarized using descriptive statistics. Termination reasons will also be summarized by reason using descriptive statistics. The denominator for calculating percentages will be the set of the ITT.

12.4.2 Study Inclusion and Exclusion Criteria

The number and proportion (%) of the ITT analysis set subjects who failed to meet study inclusions/exclusion criteria will be reported.

12.4.3 Demographics and Baseline Characteristics

Baseline characteristics including demography, underlying disease characteristics, baseline physical examination and baseline efficacy endpoints will be provided in summary tables broken down by treatment groups and overall. Summary of these data will use the ITT Analysis Set.

For continuous variables, descriptive statistics (number [n], mean, standard deviation, standard error, median, minimum, and maximum) will be provided. For categorical variables, subject

counts and percentages will be provided. Categories for missing data will be presented if necessary.

12.4.4 Medical History

The incidence (no. of patients) will be provided when broken down by SOC and by SOC and Preferred Term according to MedDRA dictionary Version 19. Subjects with at least 1 abnormal finding and abnormal findings for each category will be summarized using descriptive statistics. Subjects will be counted only once in each category. Summary of these data will use the ITT Analysis Set.

12.4.5 Prior and Concomitant Medications

All prior and concomitant medications will be coded using the WHODRUG Sep 2016 DDE. The incidence of prior medications and separately those consumed concomitantly, from randomization day and onwards, to study will be summarized using descriptive statistics by therapeutic main group (ATC Level 2) and preferred term. Subjects will only be counted once in each Therapeutic Main Group, and only once in each Preferred Term category. Summary of these data will use the ITT Analysis Set.

12.4.6 Pre-Study Medications

Analyses will include only coded medications that were initiated prior to randomization date. Medications in which start date was not reported in database are also considered as pre-study medications. Incidence table including subject counts (no. of subjects) and percentages broken down by Therapeutic Main Group and Preferred Term as well as by treatment group will be generated.

12.4.7 Concomitant Medications

Analyses will include only coded medications that were consumed on randomization date or afterwards. Medications in which start date was not reported in database are also considered as concomitant medications. Incidence table including subject counts (no. of subjects) and percentages broken down by Therapeutic Main Group and Preferred Term as well as by treatment group will be generated.

12.4.8 Disallowed Medications Use

Disallowed medications will be listed. Summary table including subject counts (no. of subjects) and relative percentages when broken down by these two periods and overall will be displayed.

12.4.9 Physical Examination at Baseline

The number and proportion (%) of subjects' physical examination results at baseline will be tabulated.

12.4.10Protocol Violations/Deviations

Protocol deviations and violations are recorded in an Excel file maintained by the Sponsor.

An incidence table displaying the number and proportion (%) of subjects with at least 1 protocol violation in each category will be summarized using descriptive statistics. Individual subjects listings will also be provided.

12.5 Significance Level and Multiplicity Adjustment

One (1) primary endpoint and 5 secondary end-points are pre-defined for each of the 2 Aramchol doses (400mg and 600mg) studied. The overall significance level for this study will be 5% using two-tailed tests utilizing the hierarchical gate keeping approach to control the overall Type-I error rate. Since two doses of Aramchol will be tested vs. placebo, there will be a total of 12 comparisons (contrasts) for the primary and secondary endpoints altogether.

According to the gate keeping approach, the 1st contrast (600mg vs. Placebo in the primary endpoint) will be tested using a two-tailed 5% significance level. If, the 1st contrast will statistically significantly favor the 600mg vs. Placebo in the primary endpoint, then the 2nd contrast (600mg vs. Placebo in the key secondary endpoint; Proportion (%) of Subjects with CRN Fibrosis Score Improvement) will be tested using a two-tailed 5% significance level. In the case the 1st contrast will fail to statistically significantly favor the 600mg vs. Placebo in the primary endpoint then significance testing will be ended failing to demonstrate statistically significant evidence in favor of both Aramchol doses (400mg and 600mg).

In the case that the 1st contrast will statistically significantly favor the 600mg vs. Placebo comparison, then the study will be considered successful as primary objective was met.

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted] Placebo Contrast.

Nominal p values will be reported in the CSR in the case of failure to reach the above defined statistical significance.

12.6 Primary Efficacy Endpoint and Principal Statistical Analysis

The primary endpoint of the study is the percent (%) change from baseline to end of study in liver triglycerides ratio as measured by NMRS.

The Full Analysis Set (FAS) will be used as the primary analysis set for efficacy analysis and inference.

Data for this analysis will be retrieved from the central MRI reading center dataset specifically designed to capture multiple locations measurements of liver triglycerides ratios done for each MRI scan.

For each MRI scan, liver triglycerides ratios were reported as taken from one or several locations of the liver as deemed necessary by the MRI reading specialist. Furthermore, in some cases replications within liver location sites were also been performed to improve precision. These within location replications will be averaged, and the percent (%) change from baseline for matched locations will be calculated having in the analysis repeated measures (repeated per matched locations).

The statistical model will be a Mixed Model (SAS® MIXED procedure) with random intercept subcommand. The model will include the following covariates: treatment group, CGR1, age (1df), sex, baseline liver triglycerides ratio (1df) and baseline BMI (1df).

The model will be a Random Intercept model. The REML estimation method will be used and degrees of freedom will be adjusted using the Kenward-Roger method. The calculated percent change from baseline for each subject's liver location will be used as response in the model and differences between the treatments groups will be estimated using contrasts.

[redacted]

[redacted]

[redacted]

12.7 Key Secondary Endpoints and Analyses

Key secondary endpoints, with the exception of the liver biopsy derived endpoints, will be tested for the Full Analysis Set (FAS). Liver biopsy derived endpoints will be tested using the

Full Analysis Set for Liver Biopsy Data (FAS_{biopsy}). Controlling for multiplicity will be performed as detailed in Section 12.5.

As for liver biopsies derived endpoints, please note that biopsies were originally planned to be taken at screening and at study termination. However, pairs of biopsies will not be available for the vast majority of study participants from Israel as a second biopsy was not approved by regulatory authorities until September 2016. Also note that whenever CRN Fibrosis Score is mentioned in description of liver biopsies derived endpoints, the reference is made to database variable name "CRN Fibrosis Score (NAS)".

The key secondary endpoints for this study are provided below in the hierarchy order of analysis in employing the gate keeping approach:

12.7.1 Proportion (%) of Subjects with CRN Fibrosis Score Improvement

This endpoint will be derived from the liver biopsies dataset and analysis will use the Full Analysis Set for Liver Biopsy Data (FAS_{biopsy}).

A subject will be defined as a treatment responder in the case that the CRN Fibrosis Score is decreased as compared to screening by 1 point (stage) or more and there is no worsening of NASH defined by an increase of Inflammation (NAS) and or Ballooning (NAS) grades.

Baseline adjusted logistic regression (SAS® LOGISTIC procedure) stratified by CGR_2 using the STRATA sub-command with the following effects: treatment group, baseline CRN Fibrosis Score (1df) and NAS Score (1df) will be used to test the between the active groups and placebo contrasts.

12.7.2 Proportion (%) of Subjects with NAS Score Improvement without Worsening of CRN Fibrosis Score

This endpoint will be derived from the liver biopsies dataset and analysis will use the Full Analysis Set for Liver Biopsy Data (FAS_{biopsy}).

A subject will be defined as a treatment responder in the case that the NAS Score improvement (reduction as compared to baseline) will be of 2 point or more contributed by more than one of the following: Steatosis (NAS), Inflammation (NAS), Ballooning (NAS), pending of no CRN Fibrosis Score worsening.

Baseline adjusted logistic regression (SAS® LOGISTIC procedure) stratified by CGR_2 using the STRATA sub-command with the following effects: treatment group baseline CRN Fibrosis Score (1df) and NAS Score (1df) will be used to test the between the active groups and placebo contrasts.

12.7.3 Proportion (%) of Subjects with SAF Activity Score Improvement without Worsening of CRN Fibrosis Score

This endpoint will be derived from the liver biopsies dataset and analysis will use the Full Analysis Set for Liver Biopsy Data (FAS_{biopsy}).

A subject will be defined as a treatment responder in the case that the SAF Activity Score improvement will be of 2 point or more pending of no CRN Fibrosis Score worsening.

Baseline adjusted logistic regression (SAS® LOGISTIC procedure) stratified by CGR_2 using the STRATA sub-command with the following effects: treatment group, baseline CRN Fibrosis Score (1df) and NAS Score (1df) will be used to test the between the active groups and placebo contrasts.

12.7.4 Proportion (%) of Subjects with NASH Resolution without Worsening of CRN Fibrosis Score

This endpoint will be derived from the liver biopsies dataset and analysis will use the Full Analysis Set for Liver Biopsy Data (FAS_{biopsy}).

A subject will be defined as a treatment responder in the case that all of the below criteria are met:

- No worsening in CRN Fibrosis Score.
- NAS Ballooning Score equals 0 at termination.
- NAS Inflammation Score equals 0 or 1 at termination.

Baseline adjusted logistic regression (SAS® LOGISTIC procedure) stratified by CGR_2 using the STRATA sub-command with the following effects: treatment group, baseline CRN Fibrosis Score (1df) and NAS Score (1df) will be used to test the between the active groups and placebo contrasts.

12.7.5 Change from Baseline to Week 52/Termination in ALT (U/L)

This endpoint will be derived from the central laboratory dataset. Analysis will include data obtained until Week 52/Termination. The Full Analysis Set (FAS) will be used for the analysis of this endpoint.

The statistical model will be a Mixed Model for Repeated Measures (MMRM) (SAS® MIXED procedure with REPEATED sub-command). The model will include the following fixed effects: categorical week in trial by treatment interaction, CGR_1 and baseline ALT value (1df). The model will use the unstructured covariance structure and the REML estimation method and degrees of freedom will be adjusted using the Kenward-Roger method. Data from all changes form baseline to scheduled post-baseline visits will be used as response in the model and differences between the treatments groups at Week 52/Termination will be estimated using contrasts.

In case that the model will not converge, the Maximum-Likelihood (ML) estimation method will be used instead of the default Restricted ML (REML). If the model still does not converge, then a simpler covariance structure with less parameters will be used, according to the following order: Heterogeneous Autoregressive(1) [ARH(1)], Heterogeneous Compound Symmetry (CSH), Autoregressive(1) [AR(1)], and Compound Symmetry (CS).

[redacted]

[redacted]

12.9 Safety and Tolerability Analyses

Safety analyses will be performed for the Safety Analysis Set.

12.9.1 Adverse Events

An adverse event is any untoward medical occurrence in a patient administered a pharmaceutical product, regardless of whether it has a causal relationship with this treatment. In this study, any adverse event occurring after the clinical study patient has signed the informed consent form should be recorded and reported as an adverse event.

The MedDRA dictionary Version 19 will be used to standardize the terms used by the investigator to describe the Adverse Events (AEs).

The following analyses are pre-planned for adverse events:

- The incidence (no. of patients) and frequency (no. of events) of most frequent TEAEs (>5% of subjects in at least one study arm) by Preferred Term.
- The incidence (no. of patients) and frequency (no. of events) of TEAEs when broken down by SOC and by SOC and Preferred Term according to MedDRA dictionary.
- The incidence (no. of patients) and frequency (no. of events) of TEAEs started during the off-drug study follow-up period (from week 52 to week 65) when broken down by SOC and by SOC and Preferred Term according to MedDRA dictionary.
- The incidence (no. of patients) and frequency (no. of events) of TEAEs by MedDRA System Organ Class and Preferred Term in Subjects Early Terminated from Study/Treatment.
- The incidence (no. of patients) and frequency (no. of events) of serious TEAEs by MedDRA System Organ Class and Preferred Term, data listing as well as summary table of SAEs when broken down by study trimesters (90 days each).
- The incidence (no. of patients) and frequency (no. of events) of TEAEs when broken down by severity.
- The incidence (no. of patients) and frequency (no. of events) of TEAEs when broken down by relationship to study IP.

- The incidence (no. of patients) and frequency (no. of events) of TEAEs when broken down by action taken.
- The incidence (no. of patients) and frequency (no. of events) of TEAEs when broken down by event outcome.
- Adverse Events dictionary used to code Investigator's verbatim terms will also be provided.

Individual subject listings of TEAEs and non-TEAEs.

12.9.2 Laboratory Data

Analyses of safety central laboratory data will be performed in the following manner:

- The below table displays the quantitative criteria used to define the potentially clinically significant (PCS) abnormal laboratory values. Measurements used in the analysis are those taken only at post randomization day visits. The incidence tables of PCS lab values as well as the individual subject listing will be provided. Please note that the denominator to be used for calculating percentages is the number of subjects with at least one post randomization day observation.

Criteria for Potentially Clinically Significant Laboratory Values

Test Group	Parameter	Lower Normal SI	Upper Normal SI	Lower PCS	Upper PCS
Chemistry	ALBUMIN (g/L)	32	55		<30g/l
Chemistry	ALK PHOS (U/L)	29	149		>2.5ULN
Chemistry	ALT (U/L)	.	45		>3ULN
Chemistry	AST (U/L)	.	41		>3ULN
Chemistry	CALCIUM (mmol/L)	2.14	2.62	<2mmol/l	>2.9mmol/l
Chemistry	CHOLESTEROL (mmol/L)	2.2	5.2		>7.75mmol/l
Chemistry	CPK (U/L)	26	145		>2.5ULN
Chemistry	CREATININE (umol/L)	31	106		>1.5ULN
Chemistry	GGT (U/L)	2	42		>2.5ULN
Chemistry	GLUCOSE, PLASMA (mmol/L)	3.9	6.9	<3.0mmol/l	>13.9mmol/l
Chemistry	POTASSIUM (mmol/L)	3.5	5.3	<3.5mmol/l	>5.5mmol/l
Chemistry	SODIUM (mmol/L)	135	148	<130mmol/l	>150mmol/l
Chemistry	TOTAL BILIRUBIN (umol/L)	2	21		>1.5ULN
Coagulation	APTT (secs)	22	35		>1.5ULN
Coagulation	INR	0.8	1.2		>1.5ULN
Hematology	HEMOGLOBIN (g/L)	113	156	<100g/l	
Hematology	PLATELETS (x10E9/L)	140	370	<75 x10e9 /L	
Hematology	WBC (x10E9/L)	3.5	10.5	<3.0 x 10e9 /L	>100 x 10e9 /L

ULN=upper limit of normal range.

- The incidence (no. of subjects) of abnormal values at any time after randomization day, calculated for subjects with normal values at baseline will be provided. Analysis will include, per tested parameter, those subjects with normal baseline and at least one post-randomization

day measurements. Summary table will display the number and relative percentage of subjects with at least one abnormal value (above upper and below the lower normal range) at any time post randomization days.

- Quantitative laboratory measurements were categorized with reference to the normal ranges as Low, Normal or High. Shift analysis of the categorical change form baseline to each scheduled visit and to the last observed value will be provided. Please note that the last measurement per visit will be used to represent subject's value in the analysis in case of sporadic repeated measurement within a visit.
- Box-Plots of measurements done, figures of mean values ±SEs of changes from baseline as well as descriptive statistics for all laboratory quantitative parameters and changes from baseline will be provided by scheduled visits and treatment groups. Please note that the last measurement per visit will be used to represent subject's value in the analysis in case of sporadic repeated measurement within a visit.
- Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH, Drug Saf 2011; 34 (3): 1-10) was performed according to the following:
  - ✓ Pre-study drug administration data was not included in the analysis.
  - ✓ Max ALT was defined as the maximal value of all ALT assessments measured after randomization.
  - ✓ The maximal Bilirubin (Total) value taken for the scatter plot was selected among measurements taken at the same day or after the maximum ALT value was detected.

  The eDISH figure, list of subjects with potential drug-induced serious hepatotoxicity according to this analysis, summary table of the incidence of subjects falling within each of the 4 quadrants will be provided. For subjects falling within the Cholestasis and Hy's Law Quadrants an additional individual subjects listings will be generated displaying demographics, exposure and termination reason, concomitant drug use, adverse events and selected laboratory data.

- The distribution of maximal values of AST, ALT and total bilirubin at any time after randomization day in term of the upper normal range multiples will be provided for subjects with normal AST, ALT and total bilirubin at baseline.

12.9.3 Vital Signs

Vital signs will be analyzed in the following manner:

- The below table displays the quantitative criteria used to define the potentially clinically significant (PCS) abnormal values. Measurements used in the analysis are those taken post randomization day. The incidence tables of PCS values as well as the individual subject listing will be provided. Please note that the denominator to be used for calculating percentages is the number of subjects with at least one post randomization day observation.

Potentially Clinically Significant (PCS) Vital Signs Ranges

Parameter	Criteria for PCS
High BP (mmHg)	**SBP≥160 or DBP≥100**
Low BP (mmHg)	**SBP≤80 or DBP≤50**
Change from Baseline in BP (mmHg)	**SBP≥ |30| or DBP≥|30|**
Heart Rate (Beats/Min)	**≤50 or ≥120**
Change from Baseline in Heart Rate (Beats/Min)	**≥|30|**

- Box-Plots of measurements done, figures of mean values ±SEs as well as descriptive statistics for all parameters and changes from baseline will be provided by scheduled visits and treatment groups. Please note that the last measurement per visit will be used to represent subject's value in the analysis in case of sporadic repeated measurement within a visit.

12.9.4 12-Lead ECG

The study site Investigator classified each ECG as either "normal", "abnormal not clinically significant (NCS)", or "abnormal clinically significant (CS)".

Analyses of ECG evaluations will be performed in the following manner:

- Distribution of the number of subjects by the interpretation outcome made by the Investigator by treatment group and scheduled visit.
- Shift analysis of categorical change of Investigator assessment to each scheduled visit and to last observed assessment will also be performed.
- Incidence and listings of individual subjects ECG findings of Potential Clinical Significance (PCS) following randomization date and onwards will be provided.

12.9.5 Physical Examinations

Analyses of physical examinations will be performed in the following manner:

- Distribution of the number of subjects by body system examined and result assessments made will be presented by scheduled visit.
- Shift analysis of categorical change each scheduled visit and to last observed assessment by body system will also be performed.
- Incidence and listings of individual subjects findings of Potential Clinical Significance (PCS) following randomization date and onwards will be provided.

12.9.6 Tolerability and Drop-Out Assessments

Tolerability analysis will be based on the number and percent (%) of subjects who failed to complete the 52 Weeks of the study by withdrawal reason will be presented. Time to withdrawal will also be presented by Kaplan-Meier curves.

The number and percent (%) of subjects who failed to complete the entire study duration of 65 Weeks (52 weeks of double blind treatment and additional 13 weeks of double blind off study drug follow-up) will also be reported.

12.10 Statistical Software

All data listings, summaries, and statistical analyses will be generated using SAS® Version 9.4 or higher (SAS is a registered trademark of the SAS Institute Inc., Cary, NC, USA).

12.11 Deviation from Statistical Analysis Plan (SAP)

The SAP dated 26Jan2017 formulates the final analysis plan for this study. When differences exist in descriptions, explanations or definitions provided in the Study Protocol and this SAP, the SAP prevails.

Any deviation from this Statistical Analysis Plan (SAP) will be reported in the Clinical Study Report.

Dunnett's test or other suitable multiple comparison procedures will be used to control the type I error rate of 5%.

A secondary comparison will be between the combined Aramchol arms and placebo. This comparison will only be performed if neither of the primary comparisons are statistically significant.

Descriptive statistics:
All measured variables and derived parameters will be listed individually and, if appropriate, tabulated by descriptive statistics.
For categorical variables summary tables will be provided giving sample size, frequency and proportions by study arm.
For continuous variables summary tables will be provided giving sample size, arithmetic mean, standard deviation, median, minimum and maximum and percentiles by study arm.

Primary efficacy endpoint:
An ANCOVA model will be used for testing the difference in the % change from baseline in liver triglycerides concentration at the end of study between the treatments with adjustment for baseline measurement.
The ITT population will be the primary population of interest.

Sensitivity analysis including other covariates such as country, age and gender will also be performed.

The distribution of liver triglyceride % change from baseline will be investigated. Data transformation or non-parametric analysis as appropriate will be used in case the distribution is not considered normal.

Secondary and exploratory endpoints:

The difference in improvement in NASH activity index (defined as at least two points improvement, contributed by more than one parameter without worsening of fibrosis) between the groups will be analyzed using Logistic regression with adjustment for baseline measurement.

The difference in the percent of patients who achieved resolution of Steatohepatitis between the groups will be analyzed using the Chi-square test or Fisher's Exact test or Logistic regression with adjustment for baseline measurements if appropriate.

The difference in changes from baseline to end of study between the study groups will be analyzed using ANCOVA with adjustment for baseline measurement for the following secondary and exploratory endpoints:

- Change in ALT levels.
- Change in insulin resistance, measured by HOMA score.
- Change in A1CHbA1c levels
- Change in Adiponectin level and Leptin: Adiponectin ratio (LAR).
- Change in inflammation and fibrosis biomarkers: Fibrinogen, CK-18, hs-C-reactive protein (hs-CRP), TNFα, IL-6 and fibrosis tests (NFS).
- Change in body weight and in waist circumference.
- Changes in fatty liver index (FLI)

Additional exploratory endpoints (e.g. Metabolomics and Genetic profiling) will be summarized in appropriate tables by study group.

Safety Analysis:

1. Adverse events and serious adverse events
2. Safety laboratory evaluations.
3. Vitals signs.
4. 12-Lead ECG
5. Physical examinations
6. Drop-out rates

Interim Safety Analysis:

An independent DSMB will analyze the safety data collected during the first 6 months for the 120 subjects and recommend a continued course of action. All patients will continue to be treated under the study protocol as done during the 6 months prior the cutoff date, till conclusion of the analysis will be known.

13 REGULATORY AND ETHICAL ISSUES

13.1 Compliance with Regulations Applicable to Clinical Trials

The study will be conducted according to the laws, regulations and administrative provisions relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use, as applicable by national legislation and EU Directives and US 21 CFR Part 11, 50, 54, 56, 312.

13.2 Informed Consent

The principles of Informed Consent, according to the Declaration of Helsinki and its updates, ICH guidelines on GCP, 21 CFR part 50 of the FDA regulations and/or EU Directives, will be followed. A subject should not enter a clinical study until he/she has been properly informed, has been given time to contemplate participation, and has freely given his/her consent by signing and dating the Ethics Committee/Institutional Review Board (EC/IRB) approved informed consent form. This must be done prior to performing any study related procedures.

The proposed consent form and any other documents relevant to the consent process must be submitted to the EC/IRB, together with the protocol, and must be approved prior to study start. A copy of the fully signed and dated Informed Consent Form and any other documents relevant to the consent process will be given to the subject and the original will be maintained at the site.

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB approval.

13.2.1 Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki which can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.

13.3 Ethics Committee (EC) / Institutional Review Board (IRB)

The study must have unconditional approval in writing, by an appropriate Ethics Committee/Institutional Review Board (EC/IRB). Any amendments to the protocol or subsequent changes to the informed consent form as a result of changes to the protocol and/or investigator brochure that is approved by Galmed must also be approved by the EC/IRB and documentation of this approval provided to Galmed. Records of the EC/IRB review and approval of all documents pertaining to this study must be kept on file by the investigator and are subject to the sponsor's audit and/or regulatory authority inspection, during or after completion of the study.

Serious Adverse Events (SAEs) must also be reported to the EC/IRB by the investigator or the sponsor.

Periodic status reports must be submitted to the EC/IRB as required, as well as notification of completion of the study and a final report where applicable. A copy of all reports submitted to the EC/IRB must be sent to the sponsor.

13.4 Protocol Amendments

Changes to the protocol should only be made by an approved protocol amendment. Protocol amendments must be approved by the Regulatory/Competent Authority (if applicable), Central EC/IRB (if applicable) and/or each respective site's EC/IRB (if applicable), prior to implementation.

For clinical trial sites located in EU Member States, the procedures outlined in Directive 2001/20/EC, Article 10(a), are applicable.

13.5 Declaration of the End of the Clinical Trial

For clinical trial sites located in EU, a declaration of the end of the clinical trial will be made according to the procedures outlined in Directive 2001/20/EC, Article 10(c).

For those countries outside EU local regulations will be followed.

13.6 Subject Confidentiality

All subject data will be identified only by a subject identification number and subject initials or subject dummy initials and date of birth in accordance to local regulations.

After obtaining subject's consent, the investigator will permit the study monitor, independent auditor or regulatory agency personnel to review that portion of the subject's medical record that is directly related to the study. This shall include all study relevant documentation including subject medical history to verify eligibility, laboratory tests results, admission/discharge summaries for hospital admissions occurring while the subject is on study, and autopsy reports for deaths occurring during the study (if applicable).

13.7 Liability and Insurance

A Certificate of Clinical Trials Insurance will be provided to the study centers by Galmed Pharmaceutical Ltd. where required.

14 DOCUMENTATION

14.1 Study File and Site Documents

Prior to the initiation of the study, the sponsor from the site must receive the following items:

1. Confidential Disclosure Agreement.
2. Signed protocol, and amendment(s) page(s).
3. The Principal Investigator's curriculum vitae and where required current medical license (if applicable).
4. Completed and signed FDA Form 1572 form (if applicable).
5. Signed Clinical Study Agreement.
6. Competent/Regulatory Authority written approval (if applicable) .
7. EC/IRB written approval for the protocol, amendment(s), Informed Consent Form, Subject Information Sheet (if applicable), advertisements (if applicable.)
8. EC/IRB Membership list or an official statement from the EC/IRB stating the EC/IRB is in compliance with 21 CFR part 56 and/or EU Directive on GCP.
9. Hospital Management written opinion (if applicable)
10. Financial disclosure information for all persons listed on FDA Form 1572 or from the principal investigator (if applicable).

14.2 Study Documents Supplied by the Sponsor

Documents that the sponsor will supply to the site include:

- Current version of the Investigator's Brochure
- Case Report Forms or a hard copy of the electronic CRF in PDF format (Master CRF).
- Subjects study binder, in addition to hospital clinic records (if applicable).
- User manuals for all vendors in the study
- Regulatory Binder.
- Country Model Informed Consent Form.
- Central Laboratory Manual which includes Laboratory Certification and Normal Ranges.
- Insurance Certificate.

- Operations Manual.

14.3 Maintenance and Retention of Records

It is the responsibility of the investigator to maintain a comprehensive and centralized filing system of all relevant documentation.
Investigators will be instructed to retain all study records required by Galmed and regulatory authorities in a secure and safe facility with limited access for one of the following time periods based on notification from Galmed.

A period of at least two years from last marketing authorization and notification from sponsor.

Or a period of at least two years after discontinuation of clinical development of the investigational product as confirmed by Galmed.

Or longer if required by local regulations.

The investigator will be instructed to consult with Galmed before disposal of any study records and to provide written notification to Galmed of any change in the location, disposition, or custody of the study files.

14.4 Data Handling

14.4.1 Electronic Case Report Form

Galmed will provide each study site with one hard copy of the reference template for data collection tools.

The Site Investigator, Study Coordinator or the Site Coordinator, using the validated EDC application, which provides a framework for entering clinical data on a CRF, will enter data at the site.

The electronic CRFs are used to record study data and are an integral part of the study and subsequent reports. Therefore, the CRFs must be completed for each subject screened or enrolled according to the subject's source data on a per-visit basis.

Subjects should not be identified by name. Appropriately coded identification and subject initials must be used. The Site Investigator must keep a separate log of subject names and addresses (i.e., Subject Identification Record).

Each entered CRF must be approved by the Site Investigator on a per-visit basis and verified against the subject's source documents by the Clinical Research Associate (CRA)/monitor. The CRAs, Clinical Quality Control (CQC) personnel and the Data Management personnel (DMs) will monitor each entry. Once all the CRFs for a subject have been approved, verified, reviewed and cleaned, the subject's eCRFs will be locked. Site Investigators, Site Coordinators and other relevant site's staff will be trained by authorized Galmed/CRO staff on the use of the eCRF

application before receiving authorization to use the application. The assignment of user privileges will be according to the following user roles:

- Site Coordinator (SITE): data entry.
- Study Coordinator (COOR): data entry and updating, discrepancy handling.
- Site Investigator: data entry and updating, discrepancy handling and CRF approval.
- CRA: discrepancy handling and CRF verification.
- Study Project manager

14.4.2 Discrepancy Handling

The internal consistency and data integrity of the study database are defined by a validated set of rules. A deviation from this set of pre-defined computerized rules creates a database discrepancy which can be handled using the RDC® application data management facility. Any modification in these rules requires re-validation according to departmental Standard Operating Procedures (SOPs).

There are 2 main types of discrepancies:

- Automatic discrepancies are generated automatically during data entry or following execution of logical checks.
- Manual discrepancies can be generated by a system user any time.

Note that the RDC® application discrepancy management facility is fully supported by an accepted regulatory audit trail of procedures and logs.

14.4.3 Data Correction

Data correction can also be performed using the validated RDC® application update facility. For each instance of data modification, the system requires the reason and justification for change. The system keeps a full audit trail of the data values, date and time of modification, and the electronic signature of the user who performed the change.

14.4.4 Source Documents

Prescription forms, label logs, laboratory test results, ECG strips and central reading facility interpretations and all other source documents should be maintained and kept at the study site in the subject study binder.

14.4.5 Electronic CRF

At the end of the study, the sponsor will supply the site investigator a copy of the site's subjects electronic CRF in PDF format or on a disk.

14.4.6 Additional Documents and Records

1. Subject Screening and Assignment Log – A listing of all subjects who signed the informed consent and were screened.
2. Subject Identification Log - This allows linking of the enrolled subject to the study. Information should include, but is not limited to: subjects' name, date of birth and contact

information and subject non-identifiable initials (if applicable). This confidential list will be maintained by the investigational site in a sealed envelope and should not be shared or forwarded to the sponsor.

3. Drug Accountability Log - This form documents the total amount of study drug dispensed to and returned by each subject.

15 QUALITY ASSURANCE AUDITS

15.1 Good Clinical Practice

The study described in this protocol will be carried out according to the local regulatory requirements and ICH accepted standards of Good Clinical Practice. All procedures described in this protocol will be performed according to approved written Standard Operating Procedures unless otherwise stated.

15.2 Quality Laboratory Standards

Laboratory tests/evaluations described in this protocol will be conducted in accordance with quality laboratory standards. See central laboratory Manual unless otherwise stated.

15.3 Quality Assurance Program

This clinical trial may be audited according to the Galmed Quality Assurance (QA) program.

The purpose of these audits is to determine whether the study is being conducted and monitored in compliance with the protocol as well as recognized GCP guidelines and local regulations. These audits will also increase the likelihood that the study data and all other study documentation can withstand a subsequent inspection by any regulatory authority. Such audits, if necessary, will be pre-arranged with the site and conducted within a reasonable time frame.

Data from the CRFs are entered into the study database by Contract Research Organization staff following their own internal standard operating procedures that have been reviewed and approved by the sponsor.

Subsequently, the entered data are systematically checked by Data Management staff, using error messages printed from validation programs and database listings. Obvious errors are corrected by Data Management personnel. Other errors or omissions are entered on Data Query Forms, which are returned to the investigational site for resolution. The signed original and resolved Data Query Forms are kept with the CRFs at the investigator site, and a copy is sent to the sponsor so the resolutions can be entered into the database. Quality control audits of all key safety and efficacy data in the database are made prior to locking the database.

15.4 Regulatory Inspections

The study may be inspected by regulatory agencies. These inspections may take place at any time during or after the study and are based on the national regulations, as well as ICH guidelines.

16 STUDY MONITORING

16.1 Monitors/CRAs and Monitoring Visits

The Study Monitor/CRA will be responsible for ensuring adherence to FDA, EU Directives, ICH guidelines and the sponsor's Standard Operating Procedures. The sponsor will provide study Monitors for this trial. The monitors will follow the current "Guideline for the Monitoring of Clinical Investigators" supplied by the FDA or will operate according to the EU Directives and in compliance with ICH guidelines.

Regular monitoring of study data at each site will be performed as defined by the study specific monitoring plan. Individual sites will be monitored to verify that enrollment rate, data recording, and protocol adherence are satisfactory. The frequency of monitoring individual sites may fluctuate depending upon enrollment rate, quantity of data collected and the complexity of the study, and will be described in the monitoring plan.

These monitoring visits will be performed for the purposes of verifying adherence to the protocol and the completeness and accuracy of data entered on the CRF. The study monitor will verify CRF entries by comparing them with the primary source documents (hospital/clinic/office records), which will be made available for this purpose. The monitor will review the maintenance of regulatory documentation and drug accountability. The monitor will review the progress of the study with the investigator and other site personnel on a regular basis. Case report form sections may be collected during these visits. At the end of the study, a close-out monitoring visit will be performed. Monitoring visits will be arranged in advance with site personnel at a mutually acceptable time. Sufficient time must be allowed by the site personnel for the monitor to review CRFs and relevant source documents. The coordinator and/or investigator should be available to answer questions or resolve data clarifications. Adequate time and space for these visits will be made available by the investigator.

16.2 Primary Source Documents

The investigator must maintain primary source documents to support CRF data entries. These documents, which are considered "source data", may include but are not limited to:

- Demographic information
- Evidence supporting the diagnosis/condition for which the subject is being studied.
- General information supporting the subject's participation in the study
- Medical history and physical findings.
- Hospitalization or Emergency Room records (if applicable).
- Each study visit by date, including any relevant findings/notes by the investigator(s), occurrence (or lack) of adverse events, and changes in medication usage, including the date the study drug was commenced and stopped.
- Any additional visits during the study.

- Any relevant telephone conversations with the subject regarding the study or possible adverse events.
- Original, signed informed consent forms for study participation.

The investigator must also retain all subject specific printouts/reports of tests/ procedures performed as a requirement of the study. During monitoring visits the monitor will need to verify data in the eCRFs against these source data.

17 USE OF INFORMATION AND PUBLICATION

17.1 Confidential Information

All information supplied by Galmed in connection with this study and not previously published, is considered confidential information. This information includes, but is not limited to, the Investigators' Brochure, clinical protocol, case report forms and other scientific data. Any data collected during the study are also considered confidential. This confidential information shall remain the sole property of Galmed, shall not be disclosed to others without the written consent of Galmed, and shall not be used except in the performance of this study.

The information developed during the conduct of this clinical study is also considered confidential, and will be used by Galmed in connection with the development of the drug. The information may be disclosed as deemed necessary by Galmed. To allow the use of the information derived from this clinical study, the investigator is obliged to provide Galmed with complete test results and all data developed in this study.

The sponsor has full ownership of the original case report forms completed as part of the study.

By signing the clinical study protocol, the investigator agrees that the results of the study may be used for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. The authorities will be notified of the investigator's name, address, qualifications, and extent of involvement.

The information obtained during this study may be made available to other investigators who are conducting similar studies.

It is agreed that, consistent with scientific standards, publication of the results of the study shall be made only as part of a publication of the results obtained by all sites performing the protocol.

Should the investigator wish to publish the results of this study, the investigator agrees to provide Galmed with a manuscript for review 60 (sixty) days prior to submission for publication.

Galmed retains the right to delete from the manuscript information that is confidential and proprietary and to object suggested publication and/or its timing (at the Company's sole discretion).

In the event that Galmed chooses to publish the data from this study a copy will be provided to the investigator at least 30 days prior to the expected date of submission to the intended publisher.

18 STUDY PERSONNEL and COMMETTEES

18.1 Investigative Site

18.1.1 The Principal Investigator

At each investigational center, a physician will be appointed to serve as a principal investigator and will have overall responsibility to lead the site study team. The Principal Investigator will oversee the accrual of appropriate subjects, the conduct of the study according to the trial protocol and the collection of required data.

18.1.2 The NMRS Radiologist and Technician

The radiologist will be responsible for determining the subject's capability to undergo NMRS scans; the performance of the NMRS scan according to the protocol guidelines for the entire duration of the study; and the preparation of adequate electronic material. He/she will also be responsible for the quality of such material as well as for providing adequate storage and back-ups of the primary data at the site. The NMRS scans will be transferred in electronic format only to the MRI Center at Tel Aviv Sourasky Medical Centre, in Israel**.** In addition, the radiologist will be responsible to evaluate the scan for clinical abnormalities and to provide a report to the investigator.
The NMRS technician is responsible for performing the NMRS scans according to the protocol; preparing the electronic copies; and performing the required back-ups and documentation of such procedures.

18.2 The Sponsor

18.2.1 Data Management & Programing and Biostatistics

Biostatistics and Data Management & Programming units are responsible for the randomization scheme, adequate performance of the data management application; the conduct of the routine data management procedures; and the performance of the statistical analysis as defined in this protocol. All procedures will be performed according to the common units' SOPs in line with ICH regulations.

At the discretion of the sponsor Biostatistics, Data Management & Programming may be performed by a CRO or other vendor.

18.2.2 Clinical Research Associate/Monitor

The Clinical Research Associate (CRA) is responsible for the local activities of the study and to ensure that the sponsor supplies adequate resources to provide high quality study management, monitoring and data management. The CRA is responsible for submitting all safety reports to local regulatory authorities and to investigators, and to EC/IRB where required.

The Clinical Research Associate (CRA)/monitor is responsible for monitoring the conduct of the study at the study centers. Monitoring visits will be arranged in advance, at a mutually acceptable time, with site personnel.

At the discretion of the sponsor, the monitoring and study management may be performed by a CRO.

18.2.3 Drug Safety and Pharmacovigilance

Drug Safety & Pharmacovigilance is responsible for: reviewing any safety issues that arise during the study; assessing and evaluating the SAEs occurring during the study; and submission of relevant SAEs to health authorities/CA as per regulations. The Drug Safety unit will assist in the approval and preparation of safety data as required and will act as liaison with the external data monitoring committee (DMC).

At the discretion of the sponsor the Drug Safety and Pharmacovigilance may be performed by a CRO.

18.2.4 Medical Monitor

The Medical Monitor is responsible for: reviewing and all safety clinical data in an ongoing basis through the course of the study. The medical monitor is reviewing individual safety subject's safety data as well as listings and tabulated safety data.
The medical monitor is responsible to evaluate any emerging data driven safety issues during the course of the study, and alert the sponsor, study operational and clinical manager(s), the relevant CRA and notify the DMC.

The action plan of any safety emerged issues will be coordinated by the medical monitor and study operational manager. The medical monitor will prepare the narratives of any safety issues with medical importance.

The medical monitor is also responsible to follow up the safety emerged issues through the study, their capture within the eCRF, and SAE reports (if necessary), and the execution of the action plan/follow up of a subject as recommended by safety officer and DMC.

The medical monitor is responsible to prepare the safety information periodical reports for the DMC meetings and present the data during the open session of the DMC meetings.

[redacted]

18.2.5 Operational and Clinical Manger(s)

The Clinical Manager will be responsible for ensuring good conduct and adequate resources; for providing high-quality study management and data management and for the coordination of all study committees.

The Operational Manager will manage the operations of the trial and will also be responsible for, coordinating and handling all subject study data from the investigational sites.

At the discretion of the sponsor the operational and clinical issue can be managed by one person and may be performed by a CRO.

18.3 Study Committees

18.3.1 Steering and Advisory Committee

The Steering and Advisory Committee may offer expert opinion as to clinical aspects of the protocol design and may be consulted during the course of the study. Periodical teleconferences will be held to assess and discuss clinical and operative progress of the study. Protocol amendments, safety issues and potential publications will be discussed with the committee and recommendations may be made to the sponsor as appropriate. The Steering Committee will include designees from the participating countries/regions Principal Investigators**.**

18.3.2 Data Monitoring Committee

The Data Monitoring Committee (DMC) will be composed of external independent physicians with expertise Liver Disease, internal medicine, cardiology, and specialist in hepatic diseases, specialist in inflammatory diseases, and a statistician. Ad-hoc consultations may be made with additional specialists as will be deemed necessary during the course of the study. A DMC charter will be established which will govern the role, function and operational procedures of the DMC and its members.

The DMC will review the progress of the trial and the accumulation of data, both periodically and on an ad-hoc basis, in order to ensure subjects' welfare.

The DMC will be responsible for overseeing the emerging safety data of the study.

The DMC will be responsible for review of efficacy data, as deemed necessary.

The DMC, based on data review, will be responsible for assessing whether there is any basis upon which to recommend modification or premature termination of the study.

The DMC will be able, in line with the DMC and the sponsor's SOPs, to review un-blinded data, following a specific notification to the sponsor, made by the DMC chair.

It is the DMC responsibility to preserve confidentiality of both blinded and un-blinded results and conclusions from the Sponsor personnel and any other party.

The DMC chairperson is responsible for all DMC interactions with the Sponsor.

The DMC chairperson is responsible for reporting un-blinded data-driven conclusions/decisions to the independent statistician.

The chair of the DMC will interact with the sponsor's un-blinded statistician. Within this work-frame, the study un-blinded statistician, will produce and distribute blinded (and un-blinded only upon request) reports to the DMC members.

The Drug Safety and Pharmacovigilance is responsible for addressing safety issues identified by the DMC. He/she will report to the DMC about the risk management plan established by the Sponsor. As all other clinical team, the Director of Global Clinical Safety will remain blinded to the treatment assignments during the course of the study.

The statistician of the DMC will address any statistical question raised by the DMC during data review, and will perform data analyzes according to the DMC's request.

A DMC plan/charter will be prepared in the first meetings of the DMC, and signed by the DMC members and sponsor's designees. The charter describes the function and manner of operation of the DMC.

DMC meetings will take place at a set schedule, according to study characteristics, needs, and progress.

Meetings may be in a teleconference or face to face, according to study needs.

In case of need for an urgent discussion of a safety issue, the DMC chair or the sponsor may call an unscheduled meeting.

Participants of the meetings are: DMC chair, DMC members, safety officer, clinical manager, study medical monitor, and sponsors' DMC statistician.

The meetings will be divided into two parts: open session with the presence of sponsor's members, and closed session without the presence of the sponsor's members. At this session un-blinded data may be discussion and the recommendation of the DMC members will be composed.

DMC meeting minutes and conclusions are a part of the study's documents and risk management plan.

All DMC open session reports should be available upon request to regulatory and ethical authorities.

Closed session reports should be collected from the DMC chairman at the end of the study, following un-blinding of study results.

The Sponsor will work closely with the committee to provide the necessary data for review. The list of DMC members will be detailed in the DMC plan/charter.

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Appendix A - MR Scan

Magnetic Resonance Spectroscopy (MRS) for Accurate Quantification of Liver Fat Concentration

Background and Study Motivation

Nonalcoholic fatty liver disease (NAFLD) comprises a spectrum of conditions extending from simple hepatic steatosis (fatty infiltration of the liver) to end-stage liver disease [1, 2]. The prevalence of non-alcoholic fatty liver disease ranges from 9% to 36.9% of the population in different parts of the world [3, 4]. Non-alcoholic steatohepatitis (NASH) is the most extreme form of NAFLD, and is regarded as a major cause of cirrhosis of the liver in cryptogenic cases [5]. NASH is a progressive disease: over a 10-year period, up to 20% of patients with NASH will develop cirrhosis of the liver, and 10% will die from liver disease or its complications [6].

Despite the increasing prevalence of NAFLD, the criteria used to diagnose the disorder remain poorly defined. Liver biopsy is considered to be the gold standard, and although it is a relatively safe procedure when performed by experienced clinicians, it has poor patient acceptance, is not risk free and is difficult to repeat. MRI has become an increasingly important imaging modality for the investigation of patients with chronic liver disease, particularly advanced methods including diffusion, perfusion, MR Elastography (MRE) and 1H MR spectroscopy (MRS). MRS is considered to be a non-invasive gold standard method for measuring hepatic fat content, with many studies showing strong correlations between MRS and histologic grade of steatosis [1, 7-10].

Although MRS is a sensitive and accurate method, its accuracy is highly dependent on the acquisition and analysis parameters used, and system configurations. Therefore, this protocol aims to standardize the MRI/MRS protocol and to perform dummy runs in order to implement and validate optimal use of MRS, as part of preparation beforehand, and quality assurance scans during the ARREST study, a multi-center study, that will be performed by Galmed Pharmaceutical Ltd.

This appendix is related to the main protocol – MRI/MRS manual, provided by AIL, which is updated routinely according to the study needs. Therefore, the MRI/MRS manual is the definitive document.

The referred scanner for this trial follows. AIL will evaluate the use of other scanner on a case-by-case basis.

Scanner [redacted]

[redacted]

The same scanner must be used for each subject for the entire duration of the study. Software and hardware upgrades are NOT allowed along the study. In special circumstances AIL may provide specific exception to this requirement.

Scanner must be able to create a backup copy of all subject scans performed (both DICOM and raw data). Electronic backups of all scans must be archived at each site.

Subject Preparation :

1. Avoidance of red meat one day before the MR scan .
2. Avoidance of alcohol one day before the MR scan.
3. Fasting for 6 hours before the MR scan.

Positioning the subject

Given the importance of careful repositioning for serial MRS fat quantification, great care should be taken when positioning the subjects in the scanner, and the location of the coil must be centered on the subject's liver. The same coil and MR system should be used throughout the entire study.

The following rules apply to all sites:

1. Check that the subject has no contra-indications to MRI examination. Each site must use his roles/procedures for safety.
2. Enter the subject data into the scanner console[redacted]

[redacted]

[redacted]

Explain the scanning procedure to the subject and train her/him for breath hold during EXPIRATION, **before** entry into the scanner .
Positioning: head first, supine .
Make sure that breath monitor is connected correctly, and breathing monitoring is recorded on the screen.

MRI/MRS Protocol

Detailed information about the protocol is provided in the Main protocol – MRI/MRS manual.

Please read carefully the MRI/MRS manual before proceeding with sequence set up.

While running the protocol, a **Data Quality Report** should be filled out. At the end of the scan the report should be sent to AIL together with all the required data (DICOM and rda files) .

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

MRI scan parameters

For each site, a specific protocol suited for its system specification will be sent to be installed directly on the system

MRS Sequences:

Magnetic resonance spectroscopy (MRS) provides an *in vivo* method for quantification of the fats within the liver. Point resolved spectroscopy (PRESS) single voxel spectroscopy will be acquired in three different locations within the liver (acquired twice in each location, total of 6 spectra). Acquisition will be performed during breath-hold-EXHALATION using point resolved spectroscopy (PRESS), single voxel spectroscopy (while monitoring, to make sure the subject is holding her/his breath).

Please note that since Fat/Water concentration is required, **NO WATER SUPPRESSION** is performed in this sequence.

[redacted]

[redacted]

Make sure that ALL water suppression buttons are NOT checked.

Anonymized MRI data, including anatomical images MRS raw data, DICOM showing voxel locations (in RBG format) and Data Quality Report will be send to Advanced Imaging Lab (AIL) at the Functional Brain Center at Tel Aviv Sourasky Medical Center. AIL service is limited to the MRS analysis only, and clinical evaluation and all of the MR scans will be evaluated in accordance with local clinical practice by local radiologist. Any pathological finding(s) not related to fatty liver will be reported by the responsible radiologist to the Principal Investigator consistent with existing local procedures.

Data Transmission

All data (DICOM files and DICOM images with the voxel location), MRS raw data (rda files) and the Data Quality Report form will be sent to AIL. Data transfer can be performed by Internet Media Transfer (IMT, preferable), flash memory (USB) or by CD.

The local site will keep electronic backups for all MRI data (images, MRS raw data and Reference images of voxel localizations).

Four steps must be performed prior to approval and initiation of trial scanning at each site. Approval by AIL is required after each step before continuing to the next step (it should be noted that changes may take place in the sequence of the step):

1) Installing the MRI/MRS protocols sent by AIL and sending the pdf file of the acquisition protocol installed on your system.
2) Acquiring a phantom scan, with a phantom supplied by AIL/GALMED, using the protocol installed on your system (see Main protocol – MRI/MRS manual).
3) Performing a dummy run on a subject with fatty liver (see Main protocol – MRI/MRS manual).
4) Participating in a training session performed by AIL including oral presentation and hands-on session in the magnet, performing a phantom run and dummy scan on a subject with fatty liver. In special circumstances AIL may provide specific exception to this requirement.

Approval by Advanced Imaging Lab (AIL) is required before scanning of subjects within the study can begin at the MRI site. AIL will only evaluate scans for the purpose of performing quantitative measurements of liver fat.

MRS Phantom Procedure

Reproducibility and accuracy of the method will be evaluated by test-retest scans (using a phantom). The phantom run procedure will be required by each MRI site participating in the Phase IIB, ARREST Clinical Trial.
The results of this procedure allow verification that: 1) the data from a given site is of sufficient quality to support the quantitative analysis required by this study; 2) the methods used by the site to acquire the data are consistent and adequate to obtain reproducible data; 3) the acquisition parameters required by the protocol are stored and transferred properly.

A phantom provided by Galmed will be scanned according to the MRI/MRS protocol specific to the phantom.

[redacted]

**The Study Reading Center (AIL) will evaluate the consistency and adequacy of the study scanning procedures.
**Any deviation from the MRS protocol requirements must be discussed with and approved by the Study Reading Center (AIL).
**Only three attempts of a phantom scanning is allowed.
(For the detailed procedure of MRI/MRS run please refer to Main protocol MRI/MRS manual)
**Upon request from the Study Reading Center, AIL, each site may be required to acquire additional phantom scans throughout the study, to monitor reproducibility, up to once a month.

MRS Dummy Run Procedure

Reproducibility and accuracy of the method will be evaluated by test-retest scans of a volunteer believed to have fatty liver (preferably >5.5%).The dummy run procedure will be required by each MRI site participating in the Phase IIB, ARREST Clinical Trial, following approval of the phantom scan.
The results of this procedure allow verification that:
1) the data from a given site is of sufficient quality to support the quantitative analysis required by this study

2) the methods used by the site to acquire the data are consistent and adequate to obtain reproducible data
3) the acquisition parameters required by the protocol are stored and transferred properly.

Following approval of the phantom run, a dummy scan of a volunteer believed to have fatty liver will be performed. The "dummy run" protocol will be similar to that used in the Study BUT will be repeated twice on the same day, according to the protocol detailed in the Main protocol - MRI/MRS manual. The two consecutive scans will be performed with an interval of about one hour, ON FASTING VOLUNTEERS.

Dummy run electronic data must be sent to Study Reading Center upon scan completion.
Approval of the dummy run is crucial for site eligibility. Only sites with approved dummy run scan will be permitted to perform MRS scans in subjects participating in the study.
The Study Reading Center will evaluate the consistency and adequacy of the study scanning procedures.
Any deviation from the MRS protocol requirement must be discussed with and approved by the Study Reading Center.
Only three attempts of the dummy run scan are allowed.
(For detailed procedure of MRS dummy run please refer to Main protocol - MRI/MRS maual)

In the second run, please try to prescribe voxel locations as similar as possible to the location prescribed in the first run, as seen on the reference DICOM images (with the voxel locations).
For more information on voxel location, please refer to the MRI/MRS manual.

Volunteers for Dummy Runs
To control for differences within and across sites, about three volunteers (or as many s deemed necessary by the Study Reading Center) with suspected fatty liver may be scanned at each site These volunteer(s) will be considerd under a separate consent from limited to this procedure. If volunteer(s) subsequently choose to participate in the clinical trial, they must undergo full screening procedures, including consenting for the clinical trial and undergoing the MRS scans required in the trial.
The dummy run should be performed on a subject likely to have fatty live (preferable >5.5%), with the **same** protocol used in the trial (see below).
HOWEVER, the dummy run will be performed *twice* on the same volunteer, on the same day, with an interval of up to one hour (while the subject is still fasting).
In the second scan, please try to position the voxels as close as possible to the location of the voxels prescribed in scan 1, in all orientations.

1. Exclusion criteria: any contraindication to MRI scan.
2. The subject should be properly positioned in the scanner.
3. Technician should use the same acquisition parameters defined by the protocol – exactly the same as those used in the Study.

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Appendix B- Liver Biopsy

The biopsy can be performed under the site procedures.

The recommendations are that after local anesthesia, percutaneous liver biopsy will be performed under ultrasound guidance via transcostal approach.

Diagnosis, grading and staging non neoplastic, diffuse parenchymal liver disease is dependent on an adequate size biopsy, a biopsy of ≥ 20mm in length. [redacted]

The biopsy should contain at least 11 complete portal tracts to ensure sufficient quality of the sample and minimize sampling error. There are cases in which smaller specimen can show the features of steatohepatitis but in most cases insufficient sample size will have an impact on the reliability/quality of all comparisons of histological findings between the first and the follow up biopsies.

The cuts should be prepared according to the standard process of the local pathological laboratory.

At least 10 cuts should be prepared.

It is preferred that all 10 slides will be sent unstained to the reading center. But if the site decides to stain the cuts, 4 cuts will be used for the following staining procedures and at least 6 should be stored unstained on slides suited for immunohistochemistry for further analysis.

All slides should be labeled with the case number of the study and be shipped in plastic boxes or stable wrappings to avoid damage to the slides on transport.

The following stains should be performed:

- For histological interpretation: Hematoxyline & Eosine (H&E), Chromotrope Aniline blue (connective tissue) and Prussian blue (iron storage).

- For morphometric analysis of steatosis or fibrosis: Sirius red .

Appendix C– Metabolomics

Endogenous metabolic profiles of serum samples will be taken from patients and will be studies using ultra performance liquid chromatography – mass spectrometry (UPLC®-MS). The general aim of the project will be to evaluate potential metabolic differences between both treatment groups of samples, and determine possible phenotypical biomarkers by statistical data analysis.

Metabolite extraction is accomplished by fractionating the samples into pools of species with similar physicochemical properties, using appropriate combinations of organic solvents. Three separate ultra-performance liquid chromatography – mass spectrometry (UPLC-MS) based platform is used to perform optimal profiling of:

- Fatty acyls, bile acids and lysoglycerophospholipids;
- Amino acids;
- Glycerolipids, cholesterol esters, sphinogolipids and glycerophospholipids

5 ML blood samples should be collected under fasting conditions. The sample will be collected into vacutainer 5 ML SST tube without anticoagulant and with a separating gel.

The blood samples will be centrifuged for 10 min at 1500g (about 3000 RPM). Once the plasma has separated, two samples of serum will be taken into Eppendorf-type tubes, 500µl in each. The samples will be frozen immediately at -80^0 C.

Each tube will be marked with a label which will include the subject number, the visit number and the date of collection.

For approximately 15 subjects for the entire trial, a small part of the biopsy samples will be stored and shipped to CRL according to CRL (lab) manual. The sponsor will notify sites when the required number of samples have been received by CRL.

Blood samples and biopsy samples (when collected) will be shipped to CRL on dry ice and stored there under -80^0C conditions.

Upon sponsor's request samples will be shipped on dry ice to OWL.

Appendix D- Endothelial Function

The Endothelial Function test will be performed according to instruction given by Itamar Medical Ltd. Following is a short description of the endothelial function test procedure. This test will be conducted at selected sites.

EndoPAT measures endothelium-mediated changes in vascular tone via bio- sensors placed on the fingertips. It provides clinicians with a reliable and reproducible index of endothelial function in a non-invasive, 15-minute, office-based test. EndoPAT is FDA-cleared (since 2003). It is used in numerous clinical institutions, research centers, and clinical phase trials in over 40 countries and reported in nearly 400 peer reviewed publications. It is increasingly being recognized for its ease of use, user independence, and immediately generated clinical test reports.

The EndoPAT system is based on the newly developed proprietary Peripheral arterial Tone (PAT) signal technology, a non-invasive technology measuring pulsatile volume changes in the digital arterialbed. The EndoPAT system features a measurement apparatus employing a pair of plethysmography-based PAT probes that impart a uniform pressure field to the distal two-thirds of the fingers, including their tips, for a more sensitive and accurate measurement during a reactive hyperemia test procedure.

The EndoPAT test can be easily performed on any patient in both the office and hospital settings by a physician or physician's assistant, according to minimal testing pre- requisites (e.g., morning test without breakfast or after 4 hours of light fasting, and avoiding pre-test consumption of caffeine, alcohol and smoking). It quantifies the endothelium-mediated changes in vascular tone elicited by a 5-minute occlusion of the brachial artery (using a standard blood pressure cuff inflated to supra-systolic pressure). When the cuff is released, the surge in blood flow causes an endothelium-dependent flow mediated dilatation (FMD), manifested as reactive hyperemia1. This is captured by the EndoPAT as an increase in the PAT signal amplitude.

Preparation of the study subject

- Patient should refrain from smoking at least the evening before of the test
- No alcohol intake at least the evening before the test.
- Fasting at least 4 hours prior to the test
- Maintain regular medication regime (besides short acting nitrates, L- Arginine, and the
- like) Vitamin C Refrain from any high dose vitamin C and fruit juice with vitamin C
- If the patient is acutely ill (e.g., flu) they should postpone the test
- Testing should be done prior to taking blood samples
- Blood pressure measurement, if required, should be performed on the non-occluded arm and, as much as possible, prior to the test
- Patient should be acclimated as least for 20 minutes in the clinic (adjust to temperature and relax)
- Patient should wear comfortable clothes and take off jewelry, rings, watch, etc.
- Mobile phone switched off

The test procedure should be explained to the patient in details with emphasis on being relaxed and keeping fingers motionless. Patients should read the Instruction Card.

Test conditions
- Temperature: Room temperature should be kept as thermo-neutral as possible (22 – 24 C°)
- Ambience: Patient should be covered and feel comfortable (cold fingers are indicative of feeling too cold)
- Lighting: Light should be dimmed and the staff should be silent as possible
- Quiet: No telephones and staff should refrain from talking to the patient other than instructions required to perform the procedure.
- Patient should also turn off mobile phones.

What the patient should know

What is the EndoPAT test? The purpose of this test is to assess the health of the body's arteries. Blood flow is measured non-invasively using two simple fingertip monitors worn (one) on each hand. Blood flow is measured both at rest, after a blood pressure cuff is inflated on one arm (occlusion) and after its' release for precisely 5 minutes consecutively (total time 15 min.). Comparing the blood flow values from each arm allows an assessment of endothelial function.

The test is useful for assessing cardiovascular response to prescribed therapies and important lifestyle changes.

If the subject has had breast cancer surgery the blood pressure cuff should be inflated on the side opposite to the surgical treatment.

The day of the test the subject should feel free to ask questions and it is best to remain relaxed, quiet and peaceful during the testing period.

The subject should be asked not to eat for 4 hours prior to coming for the test. This time will vary depending upon the investigator's preferences, the time of day of the test and the subject medical condition - such as diabetes. All medicines should be taken with a little water, as usual, unless specifically instructed otherwise. No caffeine containing products should be consumed for at least 4 hours prior to the test. This includes coffee, tea, soft drinks containing caffeine, and chocolate or orange juice as examples.

The subject should wear comfortable clothes though there are no specific clothing recommendations.

Appendix E- Life Style

Patient's number: _____
Date: _____
Visit number: _____

Life style questions:

1. Have any change occurred in your life style since your last visit at the clinic?
   □ Yes □ No
   If yes what is the change _____.

2. Have any changes taken place in your nutrition since your last visit at the clinic?
   □ Yes □ No
   If yes, is it in
   the amount of the food you are eating? _____.
   the composition of your meals? _____.

3. Have any changes taken place in your physical exercise?
   □ Yes □ No
   Are you doing any:
   - Aerobic activity? _____.
   - Running? _____.
   - Walking? _____.
   - Swimming? _____.

   How many times per week? _____.
   Are you doing it in a Gym? _____.
   Other place? _____.
   How many times per week? _____.