國鼎生物科技股份有限公司
Golden Biotechnology Corp.

# CLINICAL STUDY PROTOCOL

## A Prospective, Double Blind, Randomized, Placebo-controlled Trial of Antroquinonol in Patients with Hypercholesterolemia and Hyperlipidemia

**Protocol Number: GHLIP-2-001**

**Study Product: Antroquinonol (Hocena®)**

| | |
|---|---|
| Phase of Development: | Research (Phase II) |
| Indication: | Hyperlipidemia |
| Principal Investigator: | Fu-Tien Chiang, M.D. |
| Sponsor: | Golden Biotechnology Corporation |
| Version Number: | **V2.1** |
| Date | **03-Feb-2017** |

# AMENDMENT HISTORY

| Amendment No. | Version No. | Version Date | Authors of changes | Summary of Revisions |
|---|---|---|---|---|
| 1 | V1.2 | 07-Dec-2015 | Sponsor | Revise criteria based on TFDA's comments |
| 2 | V2.0 | 05-Feb-2016 | Sponsor | 1. open for sites join<br>2. revise criteria<br>3. revise cfPWV to baPWV<br>4. add ABI measurement |
| **3** | **V2.1** | **03-Feb-2017** | **Sponsor** | **1. Remove NSAID as prohibited medication**<br>**2. Accept laboratory data obtained within 3 days from screening visit.**<br>**3. Accept Doppler color flow mapping and liver CT scan images obtained within 14 days from visit 2** |
| | | | | |
| | | | | |

# SIGNATURE PAGES

## PRINCIPAL INVESTIGATOR SIGNATURE PAGE

The study will be carried out in accordance with the protocol, the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), and in accordance to local legal and regulatory requirements. All personnel involved in the conduct of this study have completed human subject protection training.

| **Study Site:** | **Investigator:** | **Date:** |
|---|---|---|
| _____ | _____ | _____ (yyyy/mm/dd) |

# SPONSOR SIGNATURE PAGE

The study will be carried out in accordance with the protocol, the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), and in accordance to local legal and regulatory requirements. All personnel involved in the conduct of this study have completed human subject protection training.

| **Representative:** | **Signature:** | **Date:** |
|---|---|---|
| *Sheng-Yung Liu, CEO* | | (yyyy/mm/dd) |
| *Name, Title* | | |

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# TABLE OF CONTENT

AMENDMENT HISTORY ..... 2
SIGNATURE PAGES ..... 3
CONTACT INFORMATION OF KEY ROLES ..... 5
TABLE OF CONTENT ..... 6
LIST OF ABBREVIATIONS ..... 9
PROTOCOL SUMMARY ..... 11
1. INTRODUCTION ..... 17
1.1. Background Information ..... 17
1.2. Study Rationale ..... 18
1.3. Potential Risks and Benefits ..... 18
1.3.1. Potential risk ..... 18
1.3.2. Potential benefits ..... 18
2. STUDY OBJECTIVES ..... 19
2.1. Primary objective ..... 19
2.2. Secondary objectives ..... 19
3. STUDY DESIGNS ..... 20
3.1. General Design ..... 20
3.2. Primary Study Endpoints ..... 21
3.3. Secondary Study Endpoints ..... 21
3.4. Safety Endpoints ..... 22
4. SUBJECTS SELECTION AND WITHDRAWAL ..... 23
4.1. Number of Subjects ..... 23
4.2. Inclusion Criteria ..... 23
4.3. Exclusion Criteria ..... 24
4.4. Strategies for Recruitment and Retention ..... 25
4.5. Treatment Assignment Procedures ..... 25
4.5.1. Randomization procedures ..... 25
4.5.2. Blinding procedures ..... 25
4.5.3. Subject identifier ..... 26
4.5.4. Reasons for withdrawal ..... 27
4.5.5. Handling of withdrawals ..... 27
4.5.6. Termination of study ..... 28
5. STUDY DRUG/INTERVENTION ..... 29

5.1. Description of Study Drug....29
5.1.1. Drugs manufacture and supplier....29
5.1.2. Formulation, packaging and labeling....29
5.1.3. Product storage and stability....29
5.2. Dosage, Preparation and Administration of Study Drug....30
5.3. Modification of Study Drug for a Subject....30
5.4. Accountability Procedures and Assessment of Subject Compliance for the Study Drug(s)....30
5.5. Treatment for Study Drug Overdose....31
5.6. Concomitant Medications/Treatments....31
5.6.1 Prohibited medications/treatments....31
5.6.2 Permitted medications/treatments....31
6. STUDY PROCEDURES....32
6.1. Flow Chart....32
6.2. Study Procedures....35
6.2.1. Screening (Day -14)....35
6.2.2. Enrollment/Randomization (Day 1)....35
6.2.3. On treatment visits (Day 29±3, Day 57±3, Day 85±3)....36
6.2.4. Final study visit (Day 99±3)....37
6.2.5. Early termination visit /Withdrawal visit....38
6.2.6. Unscheduled Visit....39
7. STUDY EVALUATIONS....40
7.1. Informed Consent....40
7.2. Eligibility Assessments....40
7.3. Demography Data and Medical History....41
7.4. Urine Pregnancy Test/Contraception Methods....42
7.4.1. Urine pregnancy test....42
7.4.2. Contraception methods....42
7.5. Laboratory Evaluations....43
7.5.1 Clinical laboratory evaluations....43
7.5.2 Specimen preparation, handling, and storage....44
7.6. Adverse event....44
7.7. Concomitant medications....45
8. ASSESSMENT OF SAFETY....46
8.1. Adverse Events (AE)....46
8.2. Expected Adverse Reactions....48
8.3. Serious Adverse Events (SAE)....48

8.4. Reporting Procedures ..... 49
8.4.1. Reporting procedures for AEs and SAEs ..... 49
8.4.2. Reporting pregnancy ..... 50
9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN ..... 52
9.1. Study Hypothesis ..... 52
9.2. Sample Size Considerations ..... 52
9.3. Analyzed Population ..... 52
9.4. Analysis Plan ..... 53
9.5. Premature Termination and Missing Values ..... 54
10. DATA HANDLING AND RECORD KEEPING ..... 55
10.1. Data Management Responsibilities ..... 55
10.2. Investigator's File/Retention of Documents ..... 55
10.3. Source Documentation and Background Data ..... 55
10.4. Case Report Form (CRF) ..... 56
10.5. Data Capture Methods ..... 56
10.6. Study Records Retention ..... 56
10.7. Protocol Deviations ..... 56
11. STUDY MONITORING, AUDIT AND INSPECTION ..... 58
11.1. Study Monitoring ..... 58
11.2. Study Audit and Inspection ..... 58
12. QUALITY CONTROL AND QUALITY ASSURANCE ..... 59
13. ETHICAL CONSIDERATIONS ..... 60
13.1. Ethical Standard ..... 60
13.2. Research Ethics Committees (REC) ..... 60
13.3. Informed Consent Process ..... 60
13.4. Subject Confidentiality ..... 61
13.5. Protocol Amendments ..... 61
14. PUBLICATION POLICY ..... 62
15. APPENDIX ..... **錯誤! 尚未定義書籤。**
Appendix 1. TFDA approval letter ..... **錯誤! 尚未定義書籤。**
Appendix 2. TSGH IRB Approval Letter ..... **錯誤! 尚未定義書籤。**
16. REFERENCES ..... 62

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ABI | Ankle-brachial index |
| AE | Adverse event |
| ALT | Alanine aminotransferase |
| ANCOVA | Analysis of covariance |
| AST | Aspartate aminotransferase |
| baPWV | Brachial-ankle pulse wave velocity |
| BMI | Body mass index |
| BP | Blood pressure |
| BUN | Blood urea nitrogen |
| CAD | Coronary artery disease |
| CCR | Creatinine clearance rate |
| CPK | Creatine phosphokinase |
| CRF | Case report form[s] |
| CRO | Contract research organization |
| CT | Computer-assisted tomography |
| CVD | Cardiovascular disease |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| EENT | Eyes, ears, nose, throat |
| eGFR | Estimated glomerular filtration rate |
| HDL-C | High density lipoprotein cholesterol |
| HF | Heart failure |
| hsCRP | High sensitivity assay of C-reactive protein |
| HU | Hounsfield unit |
| IB | Investigator Brochure |
| ICH | International Conference on Harmonization |
| IL-1 | Interleukin 1 |
| IL-6 | Interleukin 6 |
| ITT | Intent to treat |
| LDL-C | Low density lipoprotein cholesterol |
| LVAD | Left ventricular assist device |
| MCH | Mean corpuscular hemoglobin |

| | |
|---|---|
| MCHC | Mean corpuscular hemoglobin concentration |
| MCV | Mean corpuscular volume |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MI | Myocardial infarction |
| MTD | Maximum tolerated dose |
| NSCLC | Non-small cell lung cancer |
| PK | Pharmacokinetic |
| PR | Pulse rate |
| REC | Research ethics committee |
| RBC | Red blood cell |
| SAE | Serious adverse event |
| TC | Total cholesterol |
| TG | Triglycerides |
| TNF-α | Tumor necrosis factor alpha |
| ULN | Upper limit of normal range |
| US | United States |
| WBC | White blood cell |

# PROTOCOL SUMMARY

| | |
|---|---|
| **Title:** | A Prospective, Double Blind, Randomized, Placebo-controlled Trial of Antroquinonol in Patients with Hypercholesterolemia and Hyperlipidemia |
| **Study Phase:** | Research (Phase II) |
| **Sponsor:** | Golden Biotechnology Corporation |
| **Chief Principal Investigator:** | Fu-Tien Chiang, M.D. 江福田 教授<br>National Taiwan University Hospital |
| **Indication:** | Hypercholesterolemia and Hyperlipidemia |
| **Study product:** | Antroquinonol (Hocena®) |
| **Dose/Route:** | Antroquinonol 150 mg, Antroquinonol 100 mg, Antroquinonol 50 mg, placebo, once daily/oral administration |
| **Objectives:** | The primary efficacy objective is to demonstrate the reduction of triglyceride (TG) by Antroquinonol, in comparison with placebo, after 12 weeks of treatment in patients with hypercholesterolemia and hyperlipidemia. Secondary objectives include the evaluation of the effects of Antroquinonol in comparison with placebo on other lipid parameters after 12 weeks of treatment and the effects of Antroquinonol on left ventricular diastolic function, arterial stiffness and fatty liver. The safety and tolerability of Antroquinonol will be monitored as well. |
| **Population:** | Patients with hypercholesterolemia and hyperlipidemia |
| **Sample Size:** | 120 enrolments to achieve 96 completed subjects (drop-out rate: 20%) |
| **Study Design:** | A multi-center, prospective, double blind, randomized, placebo-controlled trial |
| **Study Duration:** | Approximately 24 months |
| **Subject Participation Duration:** | 16 weeks including a two-week screening visit, a 12-week study treatment and a 2-week follow-up<br>The end of the study is defined as all patients complete the |

study treatment OR all patients are withdrawn and have been followed for two weeks, depending on which comes earlier.

**Estimated Time for Recruitment:** The recruitment period of this study will be approximately 12 months.

**Eligibility Criteria:**

Inclusion criteria:

1. Adults of either sex 20 to 75 years of age, inclusive, with a diagnosis of nonfamilial hypercholesterolemia or mixed hyperlipidemia as one of the following:
   - TG between 150 mg/dL and 500 mg/dL, and cholesterol between 160 mg/dL and 250 mg/dL;
   - TG between 150 mg/dL and 500 mg/dL, and LDL-C > 130 mg/dL;
2. Subject must be free of any clinically significant disease, other than nonfamilial hypercholesterolemia or mixed hyperlipidemia that would knowingly interfere with study evaluations;
3. A wash-out period of 2 weeks will be applied to patients prior treated with lipid-lowering medication;
4. Subject must be willing to adhere to protocol requirements, and provide written informed consent;
5. Female of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation.

Exclusion criteria:

1. Patients with secondary dyslipidemia caused by diabetes mellitus, hypothyroidism, obstructive liver disease, chronic renal failure or drugs which can increase LDL-C level (e.g. retinoids, cyclosporine A and phenothiazines) or decrease HDL-C level (e.g. progestins, androgens, $\beta$ -blockers, probucol and anabolic steroid);
2. Patients with lifestyle that may interfere treatment efficacy, such as alcoholism or drinking habits more than 3 times per week, late dinner, late night supper, frequent

oversea business traveler, frequent social gathering, and patients who cannot anticipate a diet control and lifestyle changes;

3. Patients with diabetes or history of coronary artery disease (has had myocardial infarction, cardiac intervention, cerebrovascular accident/stroke or transient ischemic attack less than 6 months prior to Visit 1);
4. Patients with hypertension that is uncontrolled defined as 2 consecutive measurements of sitting blood pressure of systolic >140 mmHg or diastolic > 90 mmHg at Visit 1;
5. Patient has a known hypersensitivity to Antroquinonol or related compounds;
6. Patient with uncontrolled intercurrent illness including, but not limited to, acute decompensated HF (exacerbation of chronic HF manifested by signs and symptoms that may require IV therapy), right heart failure due to severe pulmonary disease, diagnosed peripartum or chemotherapy induced cardiomyopathy within the 12 months prior to visit 1, or psychiatric illness/social situations that would limit compliance with study requirements;
7. Patients with a history of heart transplant or who are on a transplant list or with left ventricular assistance device (LVAD device);
8. Patients with documented ventricular arrhythmia with syncopal episodes within the past 3 months prior to visit 1 that remained untreated;
9. Patients with confirmed severe primary pulmonary, renal (eGFR<30 ml/min/1.73 $m^2$) or hepatic (Child-Pugh B/C classification) disease;
10. Patients who can't stop current lipid lowering drug treatments based on investigator's judgement;
11. Patients with any malignancy, treated or untreated, within the past 5 years of Visit 1 whether or not there is

evidence of local recurrence or metastases, with the exception of localized basal cell carcinoma of the skin or carcinoma in situ of the cervix;

12. Female patient during pregnancy, lactation or breastfeeding;
13. Patient has any other life-threatening complications;
14. Patient who is considered unreliable as to medication compliance or adherence to scheduled appointments, or inappropriate for inclusion determined by the investigators;
15. Any other reasons addressed by the investigators.

**Endpoints:**

Primary endpoint:

The primary efficacy endpoint is the percentage change from baseline of TG by Antroquinonol, in comparison with placebo, after 12 weeks of treatment in patients with hypercholesterolemia and hyperlipidemia.

Secondary endpoints:

Secondary endpoints include the evaluation of the effect of Antroquinonol in comparison with placebo on total cholesterol, LDL-C, HDL-C, LDL/HDL ratio after 12 weeks of treatment; the effect of Antroquinonol on the ratio of mitral velocity to early diastolic velocity of the mitral annulus (E/E' ratio) with tissue Doppler imaging and brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI) via a non-invasive arterial stiffness measurement; in addition, the evaluation of the effect of Antroquinonol on fatty liver by using CT scan.

Safety endpoints:

- Changes in laboratory examination including hematological, biochemical parameters and urinary analysis
- Changes in electrocardiogram test, vital signs, and physical

examinations

- Compliance and tolerability of the study drug
- Analysis of adverse events

**Statistical Methodology:** There will be three populations in this study, Intent-To Treat (ITT) population, Per-Protocol (PP) population and Safety population for statistical analysis.

- The ITT population includes all randomized patients who meet all inclusion criteria and exclusion criteria, take at least one study medication and have at least one post-baseline measurement
- The PP population is a subset of the ITT population and includes all randomized patients who complete 12 weeks of treatment without major protocol violation.
- All subjects who received at least one study medication will be included in the safety analysis population.

- Primary efficacy analysis will be performed in ITT and PP population, secondary efficacy analyses will be performed in ITT population and safety analyses will be performed using safety population.
- Last observation carried forward (LOCF) will be used to approach the missing data of primary endpoint in ITT population. No missing data will be imputed for the secondary efficacy or safety analyses.
- Descriptive statistics will be summarized as n, mean, standard deviation, median and range for continuous parameters, and will be tabulated as frequency and percentage for categorical parameters.
- To compare TG percentage change from baseline to week 12 between 3 treatment groups, the ANOVA with

Bonferroni adjustment will be performed, or if assumption of normality is not met, then Kruskal-Wallis Test will be performed. If the differences in baseline characteristics, such as gender, age, weight etc., are observed, the ANCOVA will adjust for the significant variables in the primary analysis. The within-group change from baseline in each treatment group will be assessed using the paired-T test. If the normal assumption is violated, Wilcoxon signed-rank test will replace the paired-T test.

- Other continuous variable at each visit and change at post-baseline visits will be summarized by descriptive statistics and be analyzed by means of ANOVA with Bonferroni adjustment. Paired T-Test will be used to compare the change from baseline within treatment groups. If the normal assumption is violated, non-parametric statistical methods such as Kruskal-Wallis Test or Wilcoxon Signed-Rank Test will be used.
- Categorical variables will be tabulated with related frequency and percentages and be compared by using Chi-square or Fisher's exact test.
- AEs, laboratory parameters, and vital signs will be reported descriptively, based on the safety population (all randomized patients who received at least one dose or partial dose of study treatment). Safety will also be assessed by a review of all safety endpoints including adverse events (AEs), laboratory examinations, and electrocardiogram test.

Unless otherwise specified, all statistical assessments will be two-sided under significant level of 0.05.

# 1. INTRODUCTION

## 1.1. Background Information

Dyslipidemia is a health risk, and epidemiologic studies have shown a link between total cholesterol levels and the risk of cardiovascular diseases (CVD), in particular coronary artery disease (CAD). Studies have shown that lowering the levels of total and low-density lipoprotein cholesterol (LDL-C) can result in a decrease in cardiac morbidity and mortality, whilst epidemiological evidence consistently indicate moderate and highly significant associations between triglyceride (TG) values and coronary heart disease risk [1]. CVD is influenced by many potential factors, including gender, age, genetics, smoking, high blood pressure, diabetes, and altered plasma lipids. Specifically, disturbances in plasma lipoprotein profiles, including elevations of plasma total and low density lipoprotein cholesterol (LDL-C), elevated plasma TG, and decreased high density lipoprotein cholesterol (HDL-C), have all been linked to an increased risk of developing CVD. Furthermore, LDL-C and HDL-C contents have been shown to be closely related to the occurrence of CVD. Therefore, lowering blood cholesterol level is an important way to reduce the chances of suffering CVDs. While statins are the treatment of choice for lowering LDL-C in the majority of patients, many patients retain a high CVD risk despite achieving the recommended LDL-C targets with statins, mainly due to elevated TG and low HDL-C levels. Following statin therapy optimisation additional pharmacotherapy, addition of a fibrate or niacin, should be considered as part of a multifaceted approach to risk reduction [2]. Antroquinonol is a new chemical entity isolated from the mycelium of Antrodia camphorata, which showed interesting anticancer and anti-inflammatory activities [3,4,5]. Previous studies have indicated that signaling molecules, such as PI3K, AMPK, and mTOR, participate in Antroquinonol-induced cancer cell death, whereas Nrf2 and NF-kB are involved in the anti-inflammatory effects of Antroquinonol [4,5,6]. Recently, we also provide evidence that Antroquinonol plays a role in the inhibition of Ras and Ras-related small GTP-binding protein functions through the inhibition of protein isoprenyl transferase activity in cancer cells [7]. Studies on lipid-lowering effects of Antroquinonol® has been conducted and the results indicated that Antroquinonol® enhances the expression of low-density lipoprotein receptor (LDLR) genes in liver cells responsible for the removal of cholesterol in low-density lipoprotein cholesterol (LDL-C) from the blood stream. In a diet-induced hyperlipidemic rat model, Antroquinonol® significantly increased high-density lipoprotein cholesterol (HDL-C) level ($p<0.001$) in plasma compared to vehicle control. Additionally, in a carotid artery ligation mouse model, the neotima-media area ratio was significantly reduced ($p<0.001$) compared with control. These results demonstrate the potential applications of Antroquinonol® in treating hyperlipidemia and prevent associated cardio vascular diseases.

## 1.2. Study Rationale

Statins-cholesterol lowering drugs are the first-choice treatments for the primary and secondary prevention of CVD, if the patient still has elevated TG and/or low HDL-C despite intensive lifestyle intervention, and addressing compliance with pharmacotherapy and secondary causes of dyslipidemia, additional lipid-modifying therapy may be considered. Along with the long-term treatment with statins in combination with other hypolipidemic drugs or alone, the safety has drawn a particular attention in clinic, such as the elevation of transaminase and rhabdomyolysis, which have raised an idea of developing the other types of lipid-lowering agents from botanic materials.

The primary hypothesis is that Antroquinonol treatment will be well tolerated and will result in greater lowering of TG at week 12 than placebo in patients with hypercholesterolemia and hyperlipidemia. The primary goal is to determine if Antroquinonol will provide greater TG lowering effect than placebo.

## 1.3. Potential Risks and Benefits

### 1.3.1. Potential risk

The phase I study determining MTD and evaluating PK, safety/tolerability and efficacy profiles of Hocena® in NSCLC subjects (NCT01134016) results showed that the study drug may be accompanied by gastrointestinal disorders such as diarrhea, vomiting, and nausea.

### 1.3.2. Potential benefits

The possible benefit of treating patients with hyperlipidemia with the trial is that subjects' health will be closely monitored and the investigator will provide the support of health care when patients take part in the study. In addition, this study expects to observe the effects on TG lowering in patients with hypercholesterolemia and hyperlipidemia.

# 2. STUDY OBJECTIVES

The primary efficacy objective is to demonstrate the reduction of TG by Antroquinonol, in comparison with placebo, after 12 weeks of treatment in patients with hypercholesterolemia and hyperlipidemia. Secondary objectives include the evaluation of the effects of Antroquinonol, in comparison with placebo, on other lipid parameters, left ventricular diastolic function, arterial stiffness, and fatty liver after a 12-week treatment. The safety and tolerability of Antroquinonol will be monitored as well.

## 2.1. Primary objective

To evaluate the effect of 12-week oral administration of Antroquinonol on the percentage change in TG from baseline as lipid-lowering therapy compared with placebo.

## 2.2. Secondary objectives

Secondary objectives are:

(1) To evaluate the effects of 12 weeks of oral administration Antroquinonol compared with placebo on percentage change from baseline in other lipid parameters as lipid-lowering therapy.

(2) To evaluate the effects of Antroquinonol on left ventricular diastolic function and arterial stiffness.

(3) To evaluate the effect of Antroquinonol on fatty liver via computed tomography (CT) scanning.

(4) To evaluate the safety and tolerability of 12 weeks of oral administration of Antroquinonol compared with placebo in subjects with hypercholesterolemia and hyperlipidemia

# 3. STUDY DESIGNS

## 3.1. General Design

This is a multi-center, phase 2, double blind, randomized, placebo-controlled trial of Antroquinonol in patients with hypercholesterolemia and hyperlipidemia. Subjects with a diagnosis of primary hypercholesterolemia (nonfamilial) or mixed hyperlipidemia (TG between 150 mg/dL and 500 mg/dL, and cholesterol between 160 mg/dL and 250 mg/dL or LDL-C > 130 mg/dL) and meet all eligible criteria will be randomized to 4 groups:

1. Antroquinonol 150 mg PO QD
2. Antroquinonol 100 mg PO QD
3. Antroquinonol 50 mg PO QD
4. Placebo

After the end of 12 weeks treatment period, subjects will be followed up for an additional 2 weeks.

Study visits will occur every 4 weeks. During these visits study drug will be administered at the site, new AEs/SAEs as well as follow-up for AEs and SAEs that have not been resolved will be recorded, changes to concomitant medications will be noted, vital signs will be taken, and efficacy evaluations will be performed as well. The studies include collection of biomarker samples, brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI), E/E', and CT scan of the liver will be performed. The end-of-treatment visit and the last efficacy assessments will occur at week 13 (visit 5) for all subjects. In addition, a 2-week follow-up final visit will be at week 15 (visit 6) in order to collect safety data after the last study drug administration. Subjects will be encouraged to complete all planned visits regardless of their adherence to study drug administration.

Schematic diagram:

The study design (**Figure 3-1**) is shown as follows:

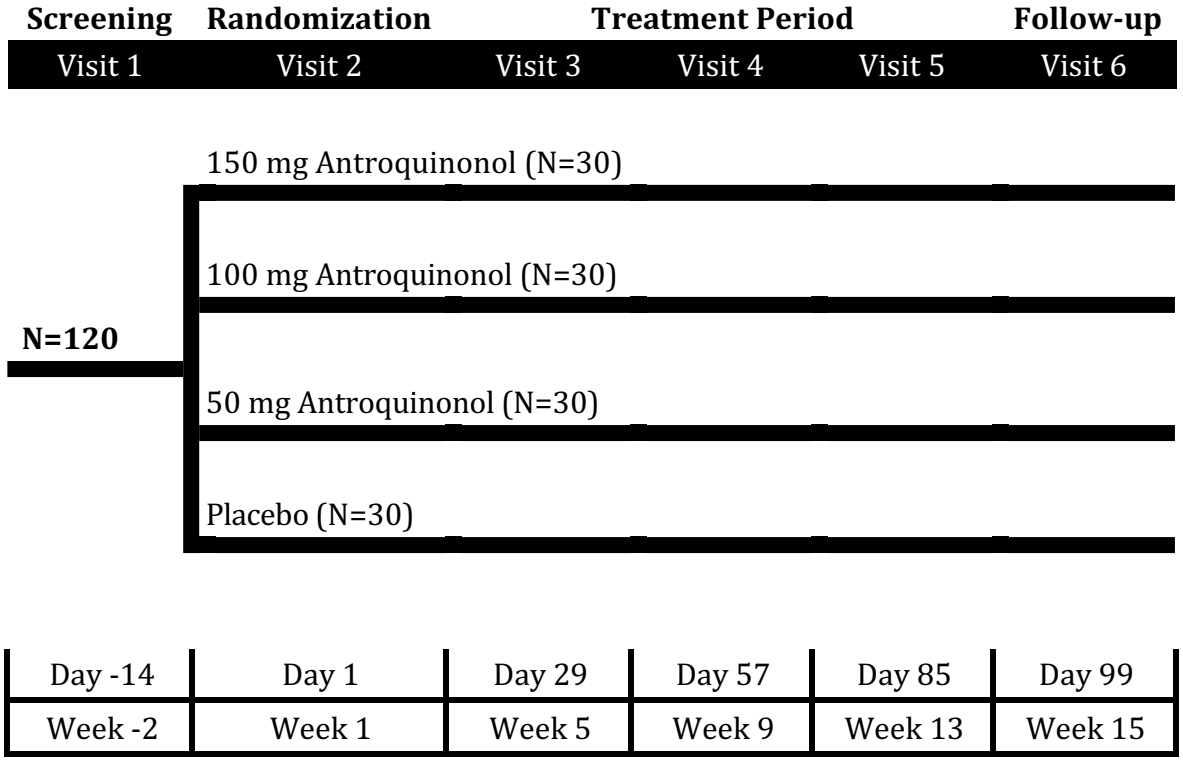


**Figure 3- 1 Schematic diagram of the study design**

## 3.2. Primary Study Endpoints

The primary efficacy endpoint is the percentage change of TG by Antroquinonol, in comparison with placebo, after 12 weeks of treatment in patients with hypercholesterolemia and hyperlipidemia.

## 3.3. Secondary Study Endpoints

Secondary endpoints include:

(1) To evaluate the effect of 12-week oral administration of Antroquinonol compared with placebo, on percentage change in total cholesterol, HDL-C, LDL-C, LDL/HDL ratio from baseline as lipid-lowering therapy.

(2) To evaluate the effect of Antroquinonol on the ratio of mitral velocity to early diastolic velocity of the mitral annulus (E/E' ratio) via tissue Doppler imaging and baPWV and ABI via a non-invasive arterial stiffness measurement.

(3) To evaluate the effect of Antroquinonol on fatty liver via computed tomography (CT)

scanning.

## 3.4. Safety Endpoints

Safety endpoints include the compliance and tolerability of the study drug, incidence of treatment emergent adverse events, safety laboratory values, vital signs, physical examinations, ECG, and the frequency and severity of muscle symptoms at each scheduled visit.

# 4. SUBJECTS SELECTION AND WITHDRAWAL

The investigators or designee must ensure that all enrolled patients meet all the following inclusion criteria and none of the exclusion criteria for the study enrollment. The inform consent will be obtained from each subject after the nature of the study is explained. A subject who has been entered (signed a inform consent) into the study is potentially eligible to be enrolled in the study, however, only he/she who meets all criteria for enrollment specified in the protocol will be randomized (assigned to a treatment group) and receive the study intervention. No additional exclusion should be applied by the investigator, in order that the study population will be representative of all eligible patients.

## 4.1. Number of Subjects

Considering an estimated dropout rate of 20%, 120 subjects will be projected to provide 96 evaluable subjects. Competitive recruitment will be applied in the present study. In average, each site may screen approximately 30-40 patients, and enroll around 25-30 subjects.

## 4.2. Inclusion Criteria

Subjects are eligible if the following inclusion criteria are satisfied:

(1) Adults of either sex 20 to 75 years of age, inclusive, with a diagnosis of nonfamilial hypercholesterolemia or mixed hyperlipidemia as one of the following:
- TG between 150 mg/dL and 500 mg/dL, and cholesterol between 160 mg/dL and 250 mg/dL
- TG between 150 mg/dL and 500 mg/dL and LDL-C > 130 mg/dL);

(2) Subject must be free of any clinically significant disease, other than nonfamilial hypercholesterolemia or mixed hyperlipidemia that would knowingly interfere with study evaluations;

(3) A wash-out period of 2 weeks will be applied to patients prior treated with lipid-lowering medication;

(4) Subject must be willing to adhere to protocol requirements, and provide written informed consent;

(5) Female of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation.

## 4.3. Exclusion Criteria

Subjects are eligible if no exclusion criterion applies:

(1) Patients with secondary dyslipidemia caused by diabetes mellitus, hypothyroidism, obstructive liver disease, chronic renal failure or drugs which can increase LDL-C level (e.g. retinoids, cyclosporine A and phenothiazines) or decrease HDL-C level (e.g. progestins, androgens, β-blockers, probucol and anabolic steroid)

(2) Patients with lifestyle that may interfere treatment efficacy, such as alcoholism or drinking habits more than 3 times per week, late dinner, late night supper, frequent oversea business traveler, frequent social gathering, and patients who cannot anticipate a diet control and lifestyle changes;

(3) Patients with diabetes or history of coronary artery disease (has had myocardial infarction, cardiac intervention, cerebrovascular accident/stroke or transient ischemic attack less than 6 months prior to Visit 1);

(4) Patients with hypertension that is uncontrolled defined as 2 consecutive measurements of sitting blood pressure of systolic >140 mmHg or diastolic > 90 mmHg at Visit 1;

(5) Patient has a known hypersensitivity to Antroquinonol or related compounds;

(6) Patient with uncontrolled intercurrent illness including, but not limited to, acute decompensated HF (exacerbation of chronic HF manifested by signs and symptoms that may require IV therapy), right heart failure due to severe pulmonary disease, diagnosed peripartum or chemotherapy induced cardiomyopathy within the 12 months prior to visit 1, or psychiatric illness/social situations that would limit compliance with study requirements;

(7) Patients with a history of heart transplant or who are on a transplant list or with left ventricular assistance device (LVAD device);

(8) Patients with documented ventricular arrhythmia with syncopal episodes within the past 3 months prior to visit 1 that remained untreated;

(9) Patients with confirmed severe primary pulmonary, renal (eGFR<30 ml/min/1.73 $m^2$) or hepatic (Child-Pugh B/C classification) disease;

(10) Patients who can't stop current lipid lowering drug treatments based on investigator's judgement;

(11) Patients with any malignancy, treated or untreated, within the past 5 years of Visit 1 whether or not there is evidence of local recurrence or metastases, with the exception of localized basal cell carcinoma of the skin or carcinoma in situ of the cervix;

(12) Female patient during pregnancy, lactation or breastfeeding;

(13) Patient has any other life-threatening complications;

(14) Patient who is considered unreliable as to medication compliance or adherence to scheduled appointments, or inappropriate for inclusion determined by the investigators;

(15) Any other reasons addressed by the investigators.

## 4.4. Strategies for Recruitment and Retention

Most subjects will be recruited from out-patients of the investigator's specialized clinic for cardiology. Other potential subjects could also be patients who referred from other clinicians.

The time of subjects' participation in the present study will be a fixed duration for 16 weeks. Only subjects who benefit from study treatment will be expected to retain in this study. If subject fail to show response to study drug, he/she will be withdrawn by investigator's determination. No further intervention will be conducted to enhance subject retention.

## 4.5. Treatment Assignment Procedures

### 4.5.1. Randomization procedures

A permuted block randomization method with 1: 1: 1 :1 ratio will be employed to allocate subjects into one of the four treatment arms as follows (1) Antroquinonol 150 mg PO QD; (2) Antroquinonol 100 mg PO QD; (3) Antroquinonol 50 mg PO QD; or (4) Placebo arm. Randomization code list will be generated by the CRO and provided to Golden Biotechnology Corporation in order to pack and label the study drugs. Randomization data will not be accessible by anyone else involved in the study. The corresponding study group of individual subjects will be recorded in a sealed envelope and should be kept strictly confidential until the time of data lock.

For this multi-center study, centralized randomization will be applied. The investigator confirms the subject fulfills the inclusion and exclusion criteria and informs the randomization center. The sponsor's independent staff will then provide the randomization number in order. The study drugs labeled with randomization number will be provided according to the request from each site.

### 4.5.2. Blinding procedures

After enrollment, subjects will be randomly assigned to receive one of the study treatments in a double-blind fashion. The identity of the treatments will be concealed. The placebo will be masked by the same appearance, odor and taste as the study drug (Antroquinonol). Subjects,

investigator, site staff, persons performing study assessments and data analysts will remain blind to the treatment assignment from the time of randomization until data lock.

All patients enrolled should take 3 capsules within 15 minutes after the first meal every day. In the study group, subjects will be treated with (1) 3* Antroquinonol 50 mg capsule; (2) 2* Antroquinonol 50 mg capsule and 1 capsule of placebo; (3) 1* Antroquinonol 50 mg capsule and 2 capsules of placebo and (4) in the control group, subjects will be treated with 3 capsules of placebo.

The blinding during the study is maintained by administration of matching placebo capsules to patients in the placebo group. Furthermore, the project teams of Golden Biotechnology Corporation and the CRO as well as the investigator and his/her study personnel will be blinded with respect to patients' assignment to one of the four treatment groups.

Treatment codes should not be broken except in the case of emergency situations. If the investigator wishes to know the identity of the treatment given to study patients for any other purpose, this request should first be discussed with Golden Biotechnology Corporation.

As per regulatory reporting requirement, Golden Biotechnology Corporation will unblind the identity of the study medication for all unexpected (as per the Investigator Brochure [IB]) serious adverse events that are considered by the investigator to be related to study drug. Details of patients who are unblinded during the study will be included in the Clinical Study Report.

All other individuals directly involved in this study will remain blinded until the final analysis of the primary parameter.

### 4.5.3. Subject identifier

Each subject will be assigned a consecutive numbers as he/she enters the study. In the study, the screening and randomization numbers are used as subject identifiers. The subject who signed the informed consent form will be assigned a sequential screening number starting with an alphabet "S" and following with 3 digits at visit 1 (Screening visit) by each site. The subject who meet the eligible criteria will be enrolled and granted a random number by randomization process before visit 2. The random number is composed of 3 digits and an initial alphabet "R". Once assigned to a subject, the screening numbers at each site and randomization numbers will not be reused. This random number will be used to identify the subject throughout the clinical study and must be used on all study documentation related to that subject. A two-digit site number will also be indicated on case report form as well as related working sheets. In that case, National Taiwan University Hospital will be coded as site "01". Once a new institution participates in the present study, the site number will be increased sequentially, e.g. site "02" for the second institution.

### 4.5.4. Reasons for withdrawal

Subjects may be discontinued from study drug at the discretion of the investigator of any untoward effects occur or for one of the following reasons:

(1) Subject who cannot comply with the medical practices and the schedule of follow-up visit;
(2) Subjects (or their legally acceptable representatives, if applicable) decide to withdraw their inform consent forms;
(3) Investigator considers that the subject is no longer physically or psychologically feasible to be included in this study;
(4) Adverse effects occur in subjects that the investigator considers a permanent cessation of the study treatment is necessary;
(5) Violation of enrollment criteria which is not recognized at the time of enrollment;
(6) Lost to follow-up;
(7) Lack of response to study treatment and the investigator considers that the subject shall be withdrawn to receive other treatments.

Subjects who are withdrawn because of lost to follow-up will be contacted by telephone, and the information will be record on the "Study Termination" of Case Report Form (CRF), including the date of the event and the reasons for not returning to the clinic by study staff.

### 4.5.5. Handling of withdrawals

Subjects have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study in the event of intercurrent illness, adverse events, protocol violations, administrative reasons or other reasons. An excessive rate of withdrawals can render the study un-interpretable; therefore, unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw, all efforts will be made to complete and report the observations prior to withdrawal as thoroughly as possible.

The investigator should contact the subject or a responsible relative either by telephone or through a personal visit to establish as completely as possible the reason for the withdrawal. A complete final evaluation at the time of the subject's withdrawal should be made with an explanation of why the subject is withdrawing from the study. If the reason for removal of a subject from the study is an adverse event, the principal specific event will be recorded on the case report form (CRF).

Subjects withdrawing from the study should be followed for occurrence of adverse events unless the patient refuses to give further information.

Any subjects withdraw from the study, the study staff shall inform the sponsor and CRO within 5 working days.

### 4.5.6. Termination of study

This study may be prematurely terminated if, in the opinion of the investigator, the sponsor or due to health authority's review, there is sufficient reasonable cause. Written notification, documenting the reason for study termination, will be provided to the investigator or sponsor by the terminating party.

# 5. STUDY DRUG/INTERVENTION

## 5.1. Description of Study Drug

### 5.1.1. Drugs manufacture and supplier

The study drug Antroquinonol is provided and manufactured by Golden Biotechnology Corporation. The matching placebo of Antroquinonol used during the study will also be supplied by Golden Biotechnology Corporation.

### 5.1.2. Formulation, packaging and labeling

Antroquinonol will be provided as a 50 mg capsule. Study drug is capsule-shaped, identical in appearance to placebo. The study drug and matching placebo will be packaged as Antroquinonol with appearance identical in all aspects. The study drug will be packaged in a light-proof polypropylene pill box and will be labeled with following information:

- Sponsor identification
- Manufacturer
- Protocol number
- Randomization number
- Quantity of contents
- Prescription instructions
- Storage conditions
- Expiration date
- Caution statements:

  "**FOR CLINICAL TRIAL USE ONLY**"

  "**KEEP OUT OF REACH OF CHILDREN**"
- Blank spaces to write the data while dispensing.

Detailed instructions and training for the administration of study supplies will be provided by investigator or designees of the study site.

### 5.1.3. Product storage and stability

The study drugs will be handled by the investigator or the designated pharmacist for management and dispensation. The storage area for study drugs at the site must be monitored by the site staff for temperature consistency with the acceptable storage temperature range specified in this protocol or in the product label. Documentation of temperature monitoring should be maintained. Importantly, the study drug supplied for this study must be maintained under adequate security and stored under the conditions specified on the label until dispensed for subject use or returned to Golden Biotechnology Corporation.

The optimal storage condition for study drugs is described as below:
The individually labeled double-blind study medication kits should be kept under 28℃, away from moisture and heat in a cool place away from light. The study drug supplies should be stored in a locked and secure area with limited access.

## 5.2. Dosage, Preparation and Administration of Study Drug

Participants will receive medication supplies at scheduled visits in sufficient quantity until the next scheduled visit. Patients will take their daily dose of Antroquinonol and/or placebo as follow: in the study group, subjects will be treated with (1) 3* Antroquinonol 50 mg capsule; (2) 2* Antroquinonol 50 mg capsule and 1 capsule of placebo; (3) 1* Antroquinonol 50 mg capsule and 2 capsules of placebo within 15 minutes after first meal every day; and in the control group, subjects will be treated with 3 capsules of placebo within 15 minutes after meal every day.

## 5.3. Modification of Study Drug for a Subject

No modification of dose will be conducted in the present study. If any drug toxicity occurs to the subject, he/she may be withdrawn from the study by the investigator's determination.

## 5.4. Accountability Procedures and Assessment of Subject Compliance for the Study Drug(s)

Accountability and subject compliance will be assessed by maintaining adequate drug dispensing and return records.
Patients will be asked to return all used and unused drug supply containers from visit 3 to visit 5 as a measure of compliance.
Drug record section on CRF and drug dispensing logs must be kept current and should contain the following information:

- the identification of the subject to whom the study medication was dispensed
- the date[s], quantity of the study medication dispensed to the subject
- the date[s] and quantity of the study medication returned by the subject

This inventory must be available for inspection by the Monitor. All supplies, including partially used or empty containers and the dispensing logs, must be returned to the Golden Biotechnology Corporation or Golden Biotechnology Corporation designee at the end of the study. The investigational staff must record quantity of medication dispensed and returned on

CRF. In addition, a dispensing log for each subject shall also be completed by the staff. This dispensing log will be supplied to the institution before study initiation. The log will include the subject's identification, drug lot/batch number, dispensed and returned date and quantity, signature of site staff, comments which is used to record any unusual situation and verification section by CRO,

## 5.5. Treatment for Study Drug Overdose

The effects of overdose of study drug are under investigation. Per the phase I and ongoing phase II study in oncology patients, no single event of overdose is recorded. In the event of overdose, standard measures to remove any unabsorbed drug should be considered. Symptomatic and supportive treatment is recommended.

## 5.6. Concomitant Medications/Treatments

### 5.6.1 Prohibited medications/treatments

The following treatments are not permitted during the study

- Statin therapy,
- Steroids,
- Fibrates,
- Anti-lipid medication

Patients will also be informed that they should avoid foods including broccoli, Brussels sprouts, charbroiled food, cruciferous vegetables, star fruit, and grapefruit juice within four hours after taking Antroquinonol. Cigarette smoking is also prohibited during the study.

### 5.6.2 Permitted medications/treatments

Throughout the study, investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care which does not affect serum lipid level except for those listed in Section 5.6.1.

# 6. STUDY PROCEDURES

This is a, randomized, double-blind, placebo-controlled study composed of the following:
(1) a screening visit including a wash-out period of 2 weeks will be applied to patients prior to the treatment of Antroquinonol;
(2) randomization of subjects who meet the eligibility criteria in 1: 1: 1 :1 ratio into the four treatment groups (Antroquinonol 150 mg, Antroquinonol 100 mg, Antroquinonol 50 mg or placebo);
(3) three on-treatment visits scheduled at four-week interval during which efficacy and safety assessments will be performed;
(4) and a follow-up visit after the end of treatment, during which safety assessments will be performed.

## 6.1. Flow Chart

Scheduled examination components are shown by visit in Tables below, all of the assessments are indicated with an “X” at the visits when they will be performed.

**STUDY SCHEDULE:**

| Procedure | Screening * | Randomization | Treatment Period | | | Follow-up |
|---|---|---|---|---|---|---|
| Visit | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 |
| Interval | Day -14 | Day 1 (Week 1) | Day 29 (Week 5) | Day 57 (Week 9) | Day 85 (Week 13) | Day 99 (Week 15) |
| Allow Window | - | - | ±3 | ±3 | ±3 | ±3 |
| Informed Consent | X | | | | | |
| Assessment of Eligibility Criteria | X | | | | | |
| Medical History | X | | | | | |
| Demographic | X | | | | | |
| Vital Signs [1] | X | X | X | X | X | X |
| Anthropometric measures [2] | X | X | X | X | X | X |
| Electrocardiogram | | X | | | X | |
| Physical Examination | X | X | X | X | X | X |
| Urine Pregnancy Test | X | | | | | |
| Randomization [3] | | X | | | | |
| Drug Dispense | | X | X | X | | |
| Drug Return | | | X | X | X | |
| Efficacy Assessment | | | | | | |
| Serum Lipid [4] | X | X | X | X | X | X |
| baPWV and ABI | | X | | | X | |
| Image Monitoring [5] | | X | | | X | |
| Biomarker Assessment | | | | | | |

| Procedure | Screening * | Randomization | Treatment Period | | | Follow-up |
|---|---|---|---|---|---|---|
| Visit | Visit 1 | Visit 2 | Visit 3 | Visit 4 | Visit 5 | Visit 6 |
| Interval | Day -14 | Day 1 (Week 1) | Day 29 (Week 5) | Day 57 (Week 9) | Day 85 (Week 13) | Day 99 (Week 15) |
| Allow Window | - | - | ±3 | ±3 | ±3 | ±3 |
| CRP and Cytokine [6] | | X | | | X | X |
| Safety Assessment | | | | | | |
| CBC and WBC differential [7] | | X | | | X | X |
| Biochemistry [8] | X | X | X | X | X | X |
| Urinary Exam [9] | | X | | | X | X |
| Concomitant Therapy | X | X | X | X | X | X |
| Adverse Event | | X | X | X | X | X |

* A wash-out period of 2 weeks will not be applied to patients prior treated with medication which does not affect serum lipid level.

1. Vital signs: pulse rate, body temperature, respiratory rate, and blood pressure (including systolic blood pressure (SBP) and diastolic blood pressure (DBP)).
2. Including height, weight, waist circumference, and BMI (calculated by $kg/m^2$)
3. Subjects who meet the eligibility criteria will be randomized into treatment arms. If the eligibility is confirmed and a wash-out period is not needed for the subject, based on investigator's judgements the blood test required at V2 can be replaced by the data obtained 14 days before V2.
4. Serum lipids: total cholesterol, triglycerides, HDL-C, LDL-C, LDL/HDL ratio. **Serum lipid results obtained 3 days prior to V1 is acceptable.**
5. Doppler color flow mapping and CT scan of the liver. CT scan will not be scheduled at visit 5 on subjects without fatty liver. **Results of Doppler color flow mapping and CT scan of the liver obtained between V1 and V2 are acceptable.**
6. hsCRP, IL-1, IL-6, and TNF-alpha.
7. WBC, RBC, Hb, Hct, RDW, platelet, MCV, MCH, MCHC, neutrophil-band and segment, lymphocyte, monocyte, eosinophil, and basophil.
8. Biochemical exam: AST, ALT, CPK, BUN and serum creatinine: visit 2 to visit 6; albumin, total bilirubin, direct bilirubin, ALP, total protein: V2, V5 and V6. At screening visit, serum creatinine shall be run for estimating glomerular filtration rate (GFR) **and data obtained 3 days prior to V1 is acceptable**.
9. Urinary exam: routine urine test (pH, protein, glucose, ketone, blood, urobilinogen, bilirubin, nitrite, leukocyte) and clinical microscopy.

## 6.2. Study Procedures

### 6.2.1. Screening (Day -14)

All patients must provide written informed consent before any study specific assessments or procedures are performed. A subject enrollment form documenting the investigator's assessment of each screened subject with regard to the protocol's inclusion and exclusion criteria has to be completed by the investigator.

Based on the results of Visit 1 assessments (see below 'Screening Assessments at Visit 1'), potentially eligible patients will enter a wash-out period as necessary. Blood test data generated by the study site or central laboratory 3 days prior to Visit 1 can be accepted.

**Screening Assessments at Visit 1**

- Demographics, medical history, including previous and concomitant diseases and treatments
- Physical examination
- Anthropometric measures (Height, Weight, Waist circumference, BMI)
- Vital signs (pulse rate, body temperature, respiratory rate, and blood pressure)
- Urine Pregnancy Test
- Blood samples for
  - lipid profile (total cholesterol, TG, HDL-C, LDL-C and LDL/HDL ratio)
  - biochemistry (serum creatinine for estimating GFR)

Patients will be instructed to come in fasted state for all subsequent study visits. Patients should receive information on a heart healthy diet and counseling on lifestyle changes as appropriate.

### 6.2.2. Enrollment/Randomization (Day 1)

**Randomization Assessments at Visit 2**

Once a patient has fulfilled the entry criteria, he/she will be randomized into either Antroquinonol 150 mg, Antroquinonol 100 mg, Antroquinonol 50 mg or placebo treatment group using a permuted block randomization method with 1: 1: 1: 1 ratio. The subject will be registered with a randomization number, and the study drug will be administered. The importance of compliance with study drug should be emphasized.

The assessments listed below will be performed. If the eligibility is confirmed and a wash-out period is not needed for the subject, based on investigator's judgements, the blood test, CT Scan, and Doppler color flow mapping required at visit 2 can be replaced by data obtained 14 days before V2.

- Physical examination
- Anthropometric measures (Height, weight, waist circumference, BMI)
- Vital signs (pulse rate, body temperature, respiratory rate, and blood pressure)

- Blood samples for
  - lipid profile (total cholesterol, TG, HDL-C, LDL-C and HDL-C /LDL-C ratio)
  - hematology (WBC, RBC, Hb, Hct, RDW, platelet, MCV, MCH, MCHC, neutrophil-band and segment, lymphocyte, monocyte, eosinophil, basophil)
  - serum chemistry (AST, ALT, CPK, BUN, serum creatinine, albumin, total bilirubin, direct bilirubin, ALP, total protein)
  - biomarkers (hsCRP, IL-1, IL-6, and TNF-alpha)
- 12-lead electrocardiogram
- Brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI)
- Doppler color flow mapping, and CT scan of the liver
- Urinalysis
  - routine urine test (pH, protein, glucose, ketone, blood, urobilinogen, bilirubin, nitrite, leukocyte)
  - clinical microscopy

In addition, information on adverse events during the wash-out period and new concomitant medications will be collected.

### 6.2.3. On treatment visits (Day 29±3, Day 57±3, Day 85±3)

During the treatment period, subjects will visit the clinic every 4 weeks. Subjects have to come in fasted state for all visits. Therefore, subjects will be instructed not to take study drug in the morning of study visits but to wait until all study assessments have been completed. Subjects will receive new drug supply at each visit and will be required to bring in all unused study drugs for assessment of compliance.

**Treatment Period Assessments at Visit 3 and 4 ( ± 3 days)**

The following evaluations will be performed at each visit throughout treatment:

- Physical examination
- Anthropometric measures (Height, weight, waist circumference, BMI)
- Vital signs (pulse rate, body temperature, respiratory rate, and blood pressure)
- Blood samples for
  - lipid profile (total cholesterol, TG, HDL-C, LDL-C and LDL/HDL ratio)
  - serum chemistry (AST, ALT, CPK, BUN, serum creatinine)
- Information on adverse events
- Information on new concomitant medications
- Record of drug dispense and drug return

**End of Treatment Assessments at Visit 5 ( ± 3 days)**

The following evaluations will be performed:

- Physical examination
- Anthropometric measures (Height, weight, waist circumference, BMI)
- Vital signs (pulse rate, body temperature, respiratory rate, and blood pressure)
- Blood samples for
  - lipid profile (total cholesterol, TG, HDL-C, LDL-C and LDL/HDL ratio)
  - hematology (WBC, RBC, Hb, Hct, RDW, platelet, MCV, MCH, MCHC, neutrophil-band and segment, lymphocyte, monocyte, eosinophil, basophil)
  - serum chemistry (AST, ALT, CPK, BUN, serum creatinine, albumin, total bilirubin, direct bilirubin, ALP, total protein)
  - biomarkers (hsCRP, IL-1, IL-6, and TNF-alpha)
- 12-lead electrocardiogram
- Brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI)
- Doppler color flow mapping, and CT scan of the liver for patients with confirmed fatty liver at Visit 2
- Urinalysis
  - routine urine test (pH, protein, glucose, ketone, blood, urobilinogen, bilirubin, nitrite, leukocyte)
  - clinical microscopy
- Information on adverse events
- Information on new concomitant medications
- Record of drug return

### 6.2.4. Final study visit (Day 99±3)

**Safety Follow-Up Assessments at Visit 6 ( ± 3 days)**

A safety follow-up for all patients will be conducted 2 weeks after the end of treatment visit.

The following assessments will be performed:

- Physical examination
- Anthropometric measures (Height, weight, waist circumference, BMI)
- Vital signs (pulse rate, body temperature, respiratory rate, and blood pressure)
- Blood samples for
  - lipid profile (total cholesterol, TG, HDL-C, LDL-C and LDL/HDL ratio)
  - hematology (WBC, RBC, Hb, Hct, RDW, platelet, MCV, MCH, MCHC, neutrophil-band and segment, lymphocyte, monocyte, eosinophil, basophil)
  - serum chemistry (AST, ALT, CPK, BUN, serum creatinine, albumin, total bilirubin, direct bilirubin, ALP, total protein)

  - biomarkers (hsCRP, IL-1, IL-6, and TNF-alpha)
- Urinalysis
  - routine urine test (pH, protein, glucose, ketone, blood, urobilinogen, bilirubin, nitrite, leukocyte)
  - clinical microscopy
- Information on adverse events
- Information on new concomitant medications
- Provide final instructions to subject

### 6.2.5. Early termination visit /Withdrawal visit

For subjects who prematurely withdraw from treatment, the same procedures as the one at the end of the treatment will be applied. An end of treatment visit should be scheduled as soon as possible and the assessments listed below should be performed. Subjects will be required to bring in all study drugs for assessment of compliance. If the decision of discontinuation is being taken while the subject is in the clinic for a visit, the end of treatment assessments may be performed on that day.

- Physical examination
- Anthropometric measures (Height, weight, waist circumference, BMI)
- Vital signs (pulse rate, body temperature, respiratory rate, and blood pressure)
- Blood samples for
  - lipid profile (total cholesterol, TG, HDL-C, LDL-C and LDL/HDL ratio)
  - hematology (WBC, RBC, Hb, Hct, RDW, platelet, MCV, MCH, MCHC, neutrophil-band and segment, lymphocyte, monocyte, eosinophil, basophil)
  - serum chemistry (AST, ALT, CPK, BUN, serum creatinine, albumin, total bilirubin, direct bilirubin, ALP, total protein)
  - biomarkers (hsCRP, IL-1, IL-6, and TNF-alpha)
- 12-lead electrocardiogram
- Brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI)
- CT scan of the liver for patients with confirmed fatty liver at Visit 2
- Urinalysis
  - routine urine test (pH, protein, glucose, ketone, blood, urobilinogen, bilirubin, nitrite, leukocyte)
  - clinical microscopy
- Information on adverse events
- Information on new concomitant medications

A safety follow-up should be conducted for subjects who prematurely withdraw from treatment

2 weeks after the end of treatment visit. A clinic visit is recommended unless the subject refused to return. If so, the safety follow-up may be conducted via telephone.
Subjects willing to provide information should be followed for assessments of adverse events until the end of the trial. At a minimum, the investigator should make every effort to obtain information on whether or not the subject is alive or dead at the end of the study.

### 6.2.6. 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 site investigator. The date and reason for the unscheduled visit will be recorded in the source documentation and CRFs. Following procedures/assessments will be performed at unscheduled visits if applicable:

- Physical examination
- Anthropometric measures (Height, weight, waist circumference, BMI)
- Vital signs (pulse rate, body temperature, respiratory rate, and blood pressure)
- Blood samples for
  - lipid profile (total cholesterol, TG, HDL-C, LDL-C and LDL/HDL ratio)
  - serum chemistry (AST, ALT, CPK, BUN, serum creatinine, albumin, total bilirubin, direct bilirubin, ALP, total protein)
- 12-lead electrocardiogram
- Information on adverse events
- Information on new concomitant medications

# 7. STUDY EVALUATIONS

A detailed scheduled examination components are shown by visit in Table 6.1.

## 7.1. Informed Consent

Consent to participate in a research study includes the elements listed below. The consent process will include all elements.

- Participants must be advised that the study involves research. Staff must explain the purposes of the research, the expected duration of participation, and a description of the procedures to be followed, including identification of experimental procedures.
- Anticipated benefits of the trial must be explained to the participant.
- Attendant discomforts and risks "reasonably to be expected" must be described.
- Appropriate alternative procedures that might be advantageous for the participant must be disclosed.
- The extent, if any, to which confidentiality of records identifying the participant will be maintained must be described.
- Prospective participants must be advised of the availability or non-availability of medical treatment or compensation for physical injuries incurred as a result of participation in the study, and if available, what they consist of, or where further information can be obtained.
- Persons responsible for the study must explain whom a participant can contact for answers to pertinent questions about the research and his or her rights, and whom to contact in the event of a research-related injury.
- Participants must be told that participation is voluntary, refusal to participate will involve no penalty or loss of benefits to which the participant is otherwise entitled, and the participant may discontinue participation at any time without penalty or loss of benefits to which he or she is otherwise entitled.

## 7.2. Eligibility Assessments

The objective of setting inclusion/exclusion criteria is to identify a trial population that will ensure adequate event rates for statistical power, provide maximum generalizability, and maximize safety. Inclusion criteria is defined in section 4.2, and exclusion criteria selected to enhance safety and adherence are defined in section 4.3. The following assessments are performed at screening visit to determine eligibility:

**(1) Check Patients' Medical History:** Eligible patients must have confirmed diagnoses of

hyperlipidemia according to European guidelines on cardiovascular disease prevention in clinical practice.

**(2) Vital Signs:** Examination items include pulse rate, body temperature, respiratory rate, and blood pressure (including systolic blood pressure (SBP) and diastolic blood pressure (DBP)).

**(3) Physical Examinations:** A physical examination will be completed and recorded in the source documents at Visit 1 prior to any study-related procedures. This evaluation will include an examination of general appearance, HEENT (head, eyes, ears, nose, and throat), mouth, skin, neck (including thyroid), lymph nodes, spine, cardiovascular system, respiratory system, gastro-intestinal system, nervous system, musculoskeletal system, blood and blood forming organs, mental status, and other body systems if applicable for describing the status of subject's health. Anthropometric measures gathered include standing height, weight, BMI, and waist circumference. Height is measured for the subject not wearing shoes at Visit 1. The measurement of height will be rounded to the nearest tenth of a centimeter. Body weight is measured for the subject not wearing shoes but wearing light clothing at Visit 1. The measurement of weight will also be rounded to the nearest tenth of a kilogram (if this procedure, measuring the height and weight, cannot be performed, obtaining this information from patients' medical chart (within 1 month) is acceptable). Other anthropometric measures include waist circumference and body mass index (BMI, calculated as $kg/m^2$) has been shown to be predictive of cardiovascular disease.

**(4) Check Patients' Drug Records:** Check record of use of lipid-lowering drugs within 2 months. Patients taking lipid-lowering drugs shall have a 14-day wash-out period. In addition, patients shall not take any prohibited drugs mentioned in section 5.6.1 during the study period. If a subject is currently taking lipid-lowering drugs, the investigators shall determine whether discontinuation of these drugs will have impacts on his/her health status. If so, he/she is not recommended to enroll in the study.

## 7.3. Demography Data and Medical History

**(1) Date of Birth**

**(2) Gender:** Male or Female

**(3) Race**

**(4) Medical History:** Review any diagnosis of liver or renal impairment, skin disorders or other severe diseases. Medical history data are collected at baseline in the form of a detailed initial medical history and collected at specified follow-up visits in the form of an abbreviated interval history. The medical history shall be reviewed thoroughly to ensure

that eligible patients don't have any symptoms/diseases specified in exclusion criteria (Section 4.3). Important aspects of the medical history include eligibility criteria, allergies, cardiovascular disease, smoking status, and diabetes. Any disease occurred within one year before visit 1 shall be recorded in CRF.

**(5) Drug Records:** Review and record drug medication within 2 months before patient enrollment till the end of the study. Information regarding the participants' concomitant medication therapy is collected and documented at baseline and then reviewed and revised at follow-up visits. Appropriate sources for obtaining this information include participant (significant other) report, current pharmacy action profiles, and verification of medications documented in the medical record. Although data are collected on all standing therapies, emphasis is placed on concurrent antihypertensive, glycemic and lipid-lowering therapy as well as background risk reduction (e.g., aspirin) therapy.

## 7.4. Urine Pregnancy Test/Contraception Methods

### 7.4.1. Urine pregnancy test

All the female subjects with childbearing potential must perform urine pregnancy test (or serum pregnancy test if urine is not available) at the screening visit, and they should avoid pregnancy during this trial. Subjects must be permanently discontinued if pregnancy is confirmed by a positive urine (or serum) pregnancy test. Notably, subject shall avoid pregnancy using an efficient contraception method (recommendation listed in **Section 7.4.2**, including but not limited to) during the entire 16 weeks of the study. Once any pregnancy event is known during the study, this pregnant subject must be withdrawn from the study immediately, and will be followed up. Pregnancies and pregnancy follow-up should be reported on the Adverse Event Form. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, the presence or absence of any congenital abnormalities, birth defect, maternal or newborn complications. Monitoring of the patient should continue until conclusion of the pregnancy.

### 7.4.2. Contraception methods

Non-pregnant, non-breast-feeding women may be enrolled if they are considered highly unlikely to conceive. A highly effective method of birth control is defined as one that results in a low failure rate (i.e., less than 1 percent per year) when used consistently and correctly, such as implants, injectables, combined oral contraceptives, some intrauterine devices, sexual abstinence, or a vasectomized partner. For subjects using a hormonal contraceptive method, information regarding the product under evaluation and its potential effect on the

contraceptive should be addressed.

A female patient who is not of reproductive potential and therefore eligible to participate in this study without requiring the use of contraception is defined as: one who has either (1) reached natural menopause (defined as ≥6 months of spontaneous amenorrhea with serum follicle-stimulating hormone levels in the postmenopausal range as determined by the laboratory, or ≥12 months of spontaneous amenorrhea), or (2) ≥6 weeks post surgical hysterectomy, or bilateral oophorectomy with or without hysterectomy, or (3) bilateral tubal ligation.

The following are considered adequate barrier methods of contraception (including but not limited to): intrauterine device, diaphragm with spermicide, contraceptive sponge, condom, or vasectomy. For the purposes of this study, use of oral contraceptive pills are permitted but are not to be considered one of the two forms of birth control required. Patients should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study and agree that in order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study.

## 7.5. Laboratory Evaluations

### 7.5.1 Clinical laboratory evaluations

Laboratory tests (including hematology, biochemistry, and urinalysis) will be scheduled as below. The examination items are included in **Table 7- 1.**

**Lipid Profile:** Blood samples to assess total cholesterol, triglycerides, HDL-C, LDL-C, LDL/HDL ratio will be obtained from visit 1 to visit 5.

**Hematology:** Blood samples to assess WBC, RBC, Hb, Hct, RDW, platelet, MCV, MCH, MCHC, neutrophil-band and segment, lymphocyte, monocyte, eosinophil, basophil will be obtained at visit 2 and visit 5.

**Serum Biochemistry:** Blood samples to assess AST, ALT, CPK, BUN and serum creatinine will be obtained from visit 2 to visit 5, albumin, total bilirubin, direct bilirubin, ALP, total protein will be obtained at visit 2 and visit 5. At screening visit, serum creatinine shall be run for estimating glomerular filtration rate (GFR).

**Urinalysis:** Assessments of urine pH, protein, glucose, ketone, blood, urobilinogen, bilirubin, nitrite, leukocyte and clinical microscopy will be tested at visit 2 and visit 5.

**Pregnancy Test:** Urine HCG test will be performed at visit 1.

**12-lead ECG:** ECG will be performed in accordance with the standard procedures at the site at visit 2 and visit 5.

**baPWV and ABI measurement:** baPWV is used to assess arterial stiffness and will be

obtained using VP 1000 or VP 1000 plus (Omron) at visit 2 and visit 5/early termination visit. ABI is a surrogate marker of atherosclerosis and will be obtained using the same device at visit 2 and visit 5/early termination visit.

**Echocardiography:** Values for S', E', and A' will be measured and averaged over three consecutive cardiac cycles. A composite mean for each of these parameters is then formed by taking the average of the values from the septal, lateral, and inferior wall. The ratio of the transmitral Doppler E wave velocity and the composite mean of E' will then be used to calculate the E/E' ratio. E/E' ratio will be obtained at visit 2 and visit 5.

**CT scan of the liver:** Each participant will undergo non-enhanced liver computed tomography (CT) scans at visit 2. The Hounsfield Unit (HU) attenuation of liver on CT scans will be utilized for determination of fatty liver (L/S ratio <1.0 and liver attenuation <40HU). Subjects who are confirmed with fatty liver will undergo liver CT scans again at visit 5.

**Table 7- 1** Laboratory tests planned to be performed

| Hematology | Biochemistry | Urinalysis | Others |
|---|---|---|---|
| WBC, RBC, Hb, Hct, RDW, platelet, MCV, MCH, MCHC, neutrophil band and segment, lymphocyte, monocyte, eosinophil, basophil | AST, ALT, CPK, BUN, serum creatinine, albumin, total bilirubin, direct bilirubin, ALP, total protein<br><br>eGFR will be counted based on MDRD (Modification of Diet in Renal Disease) formulation:<br>GFR (mL/min/1.73 $m^2$)= 186 x Scr -1.154 x Age -0.203 x 0.742 (if female) | pH, protein, glucose, ketone, blood, urobilinogen, bilirubin, nitrite, leukocyte, and clinical microscopy | Lipid Profile: total cholesterol, triglycerides, HDL-C, LDL-C, LDL/HDL ratio<br>Biomarker: hsCRP, IL-1, IL-6, TNF-α |

### 7.5.2 Specimen preparation, handling, and storage

The blood and urine samples will be examined at Union Clinical Laboratory in line with the SOPs during the study. **The blood and urine samples will be examined at the site laboratory as well only for eligibility measurement at screening visit.**

## 7.6. Adverse event

The subject will be asked to report adverse event (AE) voluntarily and an investigator will also examine a subject for identifying adverse event at each visit during the study, starting from Visit 2 until the end of the trial. The methods and procedures for recording, assessing and reporting AEs are described in Section 8.

## 7.7. Concomitant medications

All concomitant medication used, either prescription or over-the-counter, and over-the-counter supplements (vitamins and herbal supplements) throughout the study period will be recorded.

# 8. ASSESSMENT OF SAFETY

## 8.1. Adverse Events (AE)

An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

All AEs including local and systemic reactions not meeting the criteria for "serious adverse events" will be captured on the appropriate AE page of CRF. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis) and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution which means subjects experiencing AEs shall be followed until recovery, recovery with residual effects, continuing, death or lost to follow-up and be recorded on CRF AE page.

Pre-existing conditions will be recorded as baseline on the "Medical History" of CRF. If the pre-existing condition does not change, it does not have to be reported as an AE on subsequent cycles. However, if it deteriorates at any time during the study, it will be recorded as an AE.

All AEs must be graded for **severity** and **relationship** to study product. The adverse event toxicity grading scale used will be the CTCAE v4.03 grading score.

**Severity of AEs**

All AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity.

| | |
|---|---|
| Grade 1 | Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. |
| Grade 2 | Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL. |
| Grade 3 | Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL. |
| Grade 4 | Life-threatening consequences; urgent intervention indicated |
| Grade 5 | Death related to AE. |

ADL: activities of Daily Living

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

**Relationship to Study Drugs**

The clinician's assessment of an AE's relationship to test article (study drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs must have their relationship to study product assessed using the terms defined as follows. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

**Causality**

- **Certain:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs within a reasonable timeframe after study procedure(s) and cannot be explained by concurrent disease or other drugs or chemicals.
- **Probable:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after study procedure(s), is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response to study events.
- **Possible:** There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after study procedure(s)). However, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse event may be judged only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely:** A clinical event, including an abnormal laboratory test result, whose temporal relationship to study procedure(s) makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after study procedure(s)) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Unrelated:** The AE is completely independent of study procedure(s), and/or evidence exists that the event is definitely related to another cause. There must be an alternative, definitive cause documented by the clinician.

## 8.2. Expected Adverse Reactions

In clinical trials, observed adverse effects of Antroquinonol are summarized as following:

- Diarrhea (92.31%)
- Vomiting (69.23 %)
- Nausea (53.85 %)

If a GI event occurs, adequate drugs/therapies could be administered based on investigator's judgements. If the event is judged as grade 3 according to CTCAE v4.03, this event will be seen as a serious adverse event.

## 8.3. Serious Adverse Events (SAE)

An SAE is any adverse event/experience occurring at any study drug dose that results in any of the following outcomes:

- Death
- Life-threatening (defined as an event in which the subject is at immediate risk of death at the time of the event; life-threatening does not refer to an event that hypothetically might have caused death if it were more severe, resulting in permanent or significant disability/incapacity)
- Requires inpatient hospitalization or prolongation of existing inpatient hospitalization (excluding the hospitalization for the protocol specified image taking)
- Results in congenital anomaly or birth defect
- Results in persistent or significant disability or incapacity
- Events requiring medical and/or surgical intervention to prevent permanent impairment of function or permanent damage to a body structure

All SAEs will be:

- recorded on the appropriate SAE CRF
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician

An adverse event would meet the criterion of "requires hospitalization", if the event necessitated an admission to a health care facility (e.g., overnight stay). Hospitalization does not include the following:

- Rehabilitation facilities
- Hospice facilities
- Respite care (e.g., caregiver relief)

- Skilled nursing facilities
- Nursing homes

Hospitalization or prolongation of hospitalization is not in itself an SAE. Examples include:

- Admission for treatment of a preexisting condition not associated with the development of a new adverse event or with a worsening of the preexisting condition (e.g., for work-up of persistent pretreatment lab abnormality)
- Administrative admission (e.g., for yearly physical exam)
- Protocol-specified admission during a clinical trial (e.g., for a procedure required by the trial protocol)
- Preplanned treatments or surgical procedures should be noted in the baseline documentation for the entire protocol and/or for the individual subject

The study will adhere to the full requirements of the ICH Guideline for Clinical Safety Data Management, Definitions and Standards for Expedited Reporting, Topic E2 and comply with local regulatory requirements.

## 8.4. Reporting Procedures

### 8.4.1. Reporting procedures for AEs and SAEs

All AEs and SAEs must be documented in the source documents and the relevant CRF and ADR form when applicable. The investigator may be asked to provide photocopies of the medical records for completion of AE or SAE report. The subjects' names on the medical records submitted to the relevant parties will be concealed. It is the investigator's responsibility to record AEs and SAEs by diagnosis terminologies, if possible. When the diagnosis is not possible for AE or SAE recording/reporting, signs and symptoms will be used for reporting instead.

According the amended provision 106 of "Good Clinical Practice" (2010, by Department of Health, Taiwan), serious adverse events that are unexpected (i.e., SAEs that are not identified or recorded on protocol Section 8.3, investigator brochure and ICF) and related or possibly related to study drugs must be immediately reported to the "National Reporting System of Adverse Drug Reaction in Taiwan" using Adverse Drug Reaction (ADR) forms, which will be completed by the investigator. A copy of the ADR form will be filed in the site files. Those SAEs that are unexpected and related or possible related to study drugs should also be reported to local Research Ethics Committees (REC) according to its regulations.

When any mentioned event happens to a subject between the times a participant signs the informed consent form and the time a subject departs the study at the end of the final visit should be reported to ADR center and local REC. All new serious adverse events occurring

beyond this time frame and coming to the attention of the investigator must be recorded only if they are considered (based on the investigator's opinion) causally-related to the study drug.
It is very important that the investigator should take information of underlying diseases, concomitant drugs, temporal relationship of the onset of the event to the time of dosing the study drugs, and re-challenging outcomes, into account when the investigator is making a causal relation decision (as described in **Section 8.1**).
It is also investigator's responsibility to proactively follow the outcome of each AE/SAE until resolution or stabilization of the condition or loss of follow-up. **Serious, alarming and/or unusual adverse** events must be reported to local Research Ethics Committees (REC) within 24 hours of the investigator's knowledge of the event.

An ADR form should be completed for all SAEs that are **unexpected and related or possibly related** to study drugs (SUSARs, suspected unexpected serious adverse reactions) and forwarded to the ADR center and the REC in according to the requirements of the institutions. Investigators should follow subjects as far as possible until an outcome of the events is known. The investigator will notify health authority of all the SAEs that are "death" or "life-threatening" in 7 calendar days, followed by a report within additional 8 calendar days. All of the remaining SAE events should be reported to health authority within 15 calendar days. The copy of each report will be kept in investigator's files. Also, the investigator must inform SAE to local REC within the time frame regulated by REC.
Lastly, all AEs, regardless of severity, will be followed by the investigator until the resolution is satisfactory. All the information must be collected on the action taken with the study drug.
**Serious, alarming and/or unusual adverse** events must be reported to the following individuals within 24 hours of the investigator's knowledge of the event:

| AFFILIATION | DEPARTMENT | TELEPHONE NUMBER | FAX NUMBER |
|---|---|---|---|
| Bestat Pharmaservices Corp. | Clinical Research | +886-2-25955590 | +886-2-25955290 |

### 8.4.2. Reporting pregnancy

If a pregnancy occurs in a female subject while the subject is taking protocol-required therapies, subject pregnancy must be reported by the investigational staff within 24 hours of their knowledge. If a pregnancy occurs in a female partner of a male subject, while the subject is taking protocol-required therapies, it will be reported by the investigational staff within 24

hours of their knowledge. Any subject who becomes pregnant during the study must be promptly withdrawn from the study. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required. The investigational staff shall record pregnancy on adverse event page on CRF, fill in an ADR form and inform both Sponsor and CRO designee aforementioned.

# 9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

## 9.1. Study Hypothesis

Several hypotheses will be tested regarding the lipid change to determine if the benefits of Antroquinonol in the context of desirable levels of primary endpoint TG, and secondary endpoint TC, HDL-C, LDL-C, and LDL/HDL ratio. As well as the effect of Antroquinonol on E/E', baPWV and ABI and HU parameters.

## 9.2. Sample Size Considerations

The primary efficacy endpoint in this trial is "to compare the percentage change of triglyceride (TG) from baseline following a 12-week regimen of Antroquinonol versus placebo." The objective of this trial is to assess the efficacy of the study treatment compared with control treatment. According to the literature review (FDA statistical reviews of Fibrate), the TG reduction percentage change of Fibrate from baseline to last visit was 36.6% for subjects who were treated with the study treatment, and 0.7% increased for the control treatment. According to prior published clinical reference, it is assumed that the mean TG percentage reduction is 27.5% and standard deviation is 40. The evaluable patient number should be at least 24 patients for each group under one-sided significance level 0.025 and power 90%. Considering a 20% drop-out rate, a sample size of 30 patients should be enrolled to each group, and a total of 120 patients will be recruited into this study. The sample size calculation was derived using PASS 2008.

## 9.3. Analyzed Population

There will be three populations in this study, Intent-To Treat (ITT) population, Per-Protocol (PP) population and Safety populations for statistical analysis. Primary efficacy analysis will be performed in both ITT and PP population, while secondary efficacy analyses will be performed in ITT population only. Safety population will be used in analysis of safety variables. The populations for analysis applied into this study are defined as following:

**Intent to Treat Population (ITT):**
The ITT population includes all randomized patients who meet all inclusion criteria and exclusion criteria, take at least one study medication and have at least one post-baseline

measurement.

**Per-Protocol Population (PP):**
The PP population is a subset of the ITT population and includes all randomized patients who complete 12 weeks of treatment without major protocol violation.
The definition of major protocol violations include (1) violate the enrollment criteria, (2) use prohibited medications indicated to treat dyslipidemia, and (3) drug compliance less than 70%.

**Safety Analysis Population:**
All subjects who received at least one study medication will be included in the safety analysis population.

## 9.4. Analysis Plan

General data analysis
For continuous endpoints, descriptive statistics including number, mean, median, standard deviation, minimum, maximum, and 95% confidence intervals will be presented. Continuous variables will be analyzed by means of ANOVA with Bonferroni adjustment. Paired T-Test will be used to compare the change from baseline within treatment groups. If the data strongly indicate a violation of the normal assumption, the non-parametric method of Kruskall Wallis test or Wilcoxon Signed-Rank Test will be applied for analysis.
For categorical endpoints, the count and percentages will be used to summarize the categorical data. The Chi-square test will be performed to test the difference between groups while Fisher's exact test will be applied for any counting of the frequency is less than 5.
All statistical analyses will be conducted using SAS software.

Endpoints
The primary efficacy endpoint for assessing the superiority of Antroquinonol to placebo will be analyzed using analysis of covariance (ANCOVA) model and baseline measurement as covariates, if the differences in baseline characteristics, such as gender, age, weight etc., are observed, for comparing the effectiveness between the treatment groups.
Analysis of other secondary efficacy endpoints (TC, HDL-C, LDL-C, and LDL/HDL ratio as well as the effect of Antroquinonol on E/E', baPWV and ABI and HU parameters) will be similar to the primary analysis of the primary endpoint. All tests will be based on a significance level of 0.05, without adjustment for multiple endpoints.
For the safety assessments, the information of adverse events will be described in detail. Count

and percentage will be tabulated for the number of patients with AEs, number of total AEs, number of patients with AEs associated to study dosing and intensity. Adverse events will be coded by the Medical Dictionary for Regulatory Activities (MedDRA®) adverse event dictionary. All the AEs will be categorized by each represented Preferred Terms (PT) and System Organ Class (SOC), which will be tabulated by body system with related incidence rates. The clinical laboratory tests and electrocardiogram tests will be analyzed to separate subjects into normal and abnormal categories, with number of subjects and percentage of subjects in each category, and McNemar's test or Fisher's exact test will then be applied to test the changes between each visit.

## 9.5. Premature Termination and Missing Values

Subjects may be withdrawn from the study prematurely for lost to follow-up, withdrawal the consent or other conditions as mentioned in Section 4.5.4. The reason of premature termination will be summarized. For drop-out subjects, the listing table will list the detailed reason and date for patients' withdrawals. For subjects who terminate study drug early, values measured after termination of study drug will be censored.

Last observation carried forward (LOCF) will be used to approach the missing data of primary endpoint in ITT population. No missing data will be imputed for the secondary efficacy or safety analyses.

# 10. DATA HANDLING AND RECORD KEEPING

## 10.1. Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. Unanticipated problems and adverse events must be reviewed by the investigator or designee.

The assigned CRO will be responsible for data management, quality review, analysis, and reporting of the study data.

## 10.2. Investigator's File/Retention of Documents

A file for each subject must be maintained that includes the signed informed consent form and copies of all source documentation related to that subject. For each subject treated with the study drug(s), the Principal Investigator is required to prepare and maintain case histories that include all observations and other data pertinent to the investigation. This will include all source documents needed to verify the accuracy of all observations and other data contained in the Case Report Forms (CRFs) on each study patient.

The Investigator or his/her designee is required to retain the records related to the trial for a period of 2 years following the date of marketing application is approved for the indication of being investigated. If no application is to be filed or if the application is not approved for such indication, the records must still be retained until 2 years after the investigation is discontinued and the regulatory agencies are notified.

The Investigator shall retain study drug disposition records and source documents for the maximum period required by the country and the institution in which the study has been conducted, or for the period specified by the Sponsor, whichever is longer. The Investigator must contact the Sponsor prior to destroying any records associated with the study.

## 10.3. Source Documentation and Background Data

The investigator shall supply the REC and the authority(ies) on request with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental queries or requests for audit inspections, it is also necessary to have access to the complete study records, providing protection of subject's confidentiality.

## 10.4. Case Report Form (CRF)

CRFs will be used for recording all data from each subject. CRFs must be typewritten or printed legibly using black ballpoint pen or completed electronically. The investigator or his/her designee is responsible for recording all data relating to the trial on the CRFs. The investigator must verify that all data entries on the CRFs are accurate and correct by signing and dating the CRFs on the designated pages.
If an item is not available or is not applicable, it should be documented as such; no blank spaces should be left on a CRF.

## 10.5. Data Capture Methods

Case report form (CRF) is the method for recording the data of this study. Clinical data will be written to case report forms (CRFs). All entries on the CRF are made in English and are supported by the source data.

## 10.6. Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. These documents should be retained for a longer period, however, if required by local regulations.

## 10.7. Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify, record deviations in the “Protocol Deviation Form”.

All deviations from the protocol must be addressed in study subject source documents. A completed copy of the “Protocol Deviation Form” must be maintained in the regulatory file, as well as in the subject’s source document. Protocol deviations must be sent to the local REC per

their guidelines. The site PI and study staff are responsible for knowing and adhering to their REC requirements.

# 11. STUDY MONITORING, AUDIT AND INSPECTION

## 11.1. Study Monitoring

All aspect of the study will be conducted under ICH, GCP (Good Clinical Practice) guidelines and government regulations. Monitoring (by phone, fax, or on site) will be done by a representative of the study monitor designated by the sponsor. If the monitoring is done on site the monitor will check the case report forms and questionnaires for completeness and clarity, and crosscheck them with source documents. In addition to the monitoring visits, frequent communications (letter, telephone, and fax), by the study monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements. Study closeout (by phone, fax or on site) will be performed by the study monitor upon closure of the study. The investigator agrees to all these monitors access to the clinical supplies, dispensing, and storage area, and to the clinical files of the study subjects, and if requested, agrees to assist the monitor.

A separate monitoring plan document should be developed to describe who will conduct the monitoring, at what frequency monitoring will be done, and at what level of detail monitoring will be conducted.

## 11.2. Study Audit and Inspection

In accordance with the principles of GCP, the study may be subjected to internal audits. Domestic authorities and the REC may request access to all source documents, case report forms, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities. Medical records and other study documents may be copied during audit or inspection. Subject names are obliterated on the copies to ensure confidentiality. The purpose of the audits should be to evaluate study conduct and compliance with protocol, SOPs, GCP, and the applicable regulatory requirements. The observations and findings should be documented.

# 12. QUALITY CONTROL AND QUALITY ASSURANCE

Quality control is the ongoing, concurrent review of data collection forms for completion and logic. Quality assurance is a comprehensive, retrospective review of all components of research records to assess adherence to protocol, standard operation procedures, and regulations, and to evaluate the accuracy of the records. Quality management is the process of assessing the quality of processes within a system and encompasses quality assurance and quality control.

For the assurance of the data accuracy, completeness, and reliability, the investigator or the study staff assigned by the investigator will evaluate the CRFs by visual review and standard computer editing to detect errors in data collection will be performed by the assigned CRO.

To protect the safety of the subjects and to ensure the data accuracy, completeness, and reliability, the research staff would preserve documented data from all sources on CRF, including lab test results, chart records, treatment conditions, physical examination, concomitant medication, and any adverse events.

# 13. ETHICAL CONSIDERATIONS

## 13.1. Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979), the Declaration of Helsinki (2013), the ICH E6, and local regulations.

## 13.2. Research Ethics Committees (REC)

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the REC for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the REC before the changes are implemented in the study.

## 13.3. Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to subjects and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the subject. Consent forms will be REC-approved, and the subject is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the subject and answer any questions that may arise. The subject will sign the informed consent document prior to any study-related assessments or procedures. Subjects will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.
The consent process will be documented in the clinical or research record.

## 13.4. Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects. All patients will be identified by the subject number, initials, date of birth and gender in the CRFs. Subject's name will only be maintained at site. The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. The subjects should be informed about the possibility of audits by authorized representatives of the company and regulatory authorities when audit or review is required.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the investigator.

The study monitor or other authorized representatives of the investigator may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

## 13.5. Protocol Amendments

Any change of this protocol that affect study objectives, study design, study procedures, patient population, or significant administrative procedures will require a formal amendment to the protocol. Any proposed protocol amendments must be sent in written form to the REC/TFDA. Prior to implementation, an amendment must be approved by the Sponsor, the Investigator and the REC/TFDA.

# 14. PUBLICATION POLICY

Following completion of the study, the investigator is expected to publish the results of this research in a scientific journal. Member journals of The International Committee of Medical Journal Editors have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. For grants and cooperative agreements, it is the Golden Biotechnology Corporation's responsibility to register the trial in an acceptable registry.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from registering trials in a public registry such as ClinicalTrials.gov. The sponsor shall have the right to publish or present any results or information, including the study results. Once if the sponsor or the institution, investigator or the staff intend to publish the study results, mutual agreement shall be obtained at least thirty (30) business days prior to the submission for publication or presentation.

# 15. REFERENCES

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