Title: Exploratory study of the effect of omega-3-acid ethyl esters on vascular endothelial function in patients with hyperlipidemia by flow mediated dilation

NCT Number: NCT02824432

Protocol Approve Date: 07-Jul-2016

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- Proprietary information, such as scales or coding systems, which are considered confidential information under prior agreements with license holder.
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Note; This document was translated into English as the language on original version was Japanese.

PROTOCOL

Exploratory study of the effect of omega-3-acid ethyl esters on vascular endothelial function in patients with hyperlipidemia by flow mediated dilation

(Oasis Flow)

Sponsor	Takeda Pharmaceutical Company Limited 12-10 Nihonbashi 2-chome, Chuo-ku, Tokyo
Protocol number	TAK-085-4001
Version number/Revision number	2nd edition
Study drug:	Omega-3-acid ethyl esters
Creation date	July 7, 2016

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1.0 STUDY ADMINISTRATIVE INFORMATION AND CLINICAL STUDY PRINCIPLES

1.1 Clinical study principles

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- Ethical Guideline for Clinical Research (the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare, December 22, 2014).
- Good Clinical Practice: Consolidated Guideline (ICH: International Conference on Harmonization of Technical Requirement for Registration on Pharmaceuticals for Human Use. E6)
- All applicable laws and regulations, including, without limitation, data privacy laws and conflict of interest guidelines.

1.2 Clinical study implementation system

This study will be conducted in accordance with the requirements of this clinical study protocol designed and prepared by the sponsor and also in accordance with the following:

Sponsor

Takeda Pharmaceutical Company Limited

Japan Pharma Business Unit

Strategic Medical Research Planning Group, Medical Affairs Department

The sponsor shall be responsible for matters related to planning/preparation, implementation/operation, and results/reporting in this clinical study. Methods of supervision of the contractor entrusted with the services related to this clinical study will be described in the procedure to be prepared separately.

Expenses* required for the operation of this clinical study will be paid by the sponsor.

* : Based on the "Consignment Service Contract," expenses incurred for the services of Office of Clinical Research, monitoring, registration/allocation center, statistical processing, and audit

shall be paid to the contractor entrusted with services related to this clinical study. Expenses agreed by the study site shall be paid to the site based on the "Research Expense Standard."

Chair of the Clinical Study Steering Committee:

PPD

The Chair of the Clinical Research Steering Committee shall supervise implementation and reporting of the clinical research, secure medical guidance of a high degree of professionalism and a high-level scientific quality, and revise the research protocol appropriately.

Terms in this protocol are defined as follows:

Study site:

A corporation, governmental agency and sole proprietor conducting the study, excluding cases where only a part of the services related to storage of samples/information, statistical processing and other studies is entrusted.

Collaborative study site:

A study site that conducts collaborative research in accordance with the protocol, including a study site that obtains new samples/information from research subjects and provides other study sites.

Researchers, etc:

Principal investigators and other parties involved in conduction of the study (including operations at institutions involved in collection/distribution of samples/information). Those involved only in providing existing samples/information outside the study sites and those engaged in part of the entrusted operations related to the study are excluded.

Principal Investigator:

A researcher who is engaged in implementation of the study and integrates the operations involved in this study at an affiliated study site.

Chief executive of the study site

A representative of a corporation, head of a governmental agency, or a sole proprietor

Research subject:

A subject (including a dead subject) who meets any of the following:

1. Subjects being studied (including those who have been asked to be studied)
2. Subjects from whom existing samples/information to be used in the study have been obtained.

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2.0 STUDY SUMMARY

<table>
<tr><td>Sponsor:
Takeda Pharmaceutical Company Limited</td><td>Study drug:
Omega-3-acid ethyl esters</td></tr>
<tr><td colspan="2">Study title: Exploratory study of the effect of omega-3-acid ethyl esters on vascular endothelial function in patients with hyperlipidemia</td></tr>
<tr><td colspan="2">Protocol number: TAK-085-4001</td></tr>
<tr><td colspan="2">Clinical research design:
This is a multicenter, collaborative, randomized, open-label study designed to explore the effects of administration of omega-3-acid ethyl esters [2 g (2 g PO QD) or 4 g (2 g PO BID) for 8 weeks] on vascular endothelial function, as measured by flow-mediated dilation (FMD), in patients receiving a hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor and have concurrent hypertriglyceridemia (hereinafter referred to as TG).
Considering the potential bias by factors that affect FMD between treatment groups, stratified allocation will be performed with fasting TG level as a factor.</td></tr>
<tr><td colspan="2">Objectives:
To explore the effects of omega-3-acid ethyl esters on vascular endothelial function when administered for 8 weeks, as measured by FMD, in patients with hyperlipidemia.</td></tr>
<tr><td colspan="2">Subjects: Patients with hyperlipidemia</td></tr>
<tr><td>Planned number of research subjects:
40 patients evaluable for the primary endpoint
2 g group : 20 subjects
4 g group : 20 subjects
Exploratory biomarkers will be measured only for the research subjects from whom blood is taken during the observation period after approval of the revised protocol (the 2nd edition) by the committee such as the IEC.</td><td>Number of study sites:
5 to 10 institutions</td></tr>
<tr><td>Dose and method of administration:
A dose of 2 g of omega-3-acid ethyl esters will be orally administered once or twice a day immediately after meal.</td><td>Route of administration:
Oral</td></tr>
</table>

Duration of treatment: 8 weeks	**Duration of evaluation:** Screening period: 4 weeks Treatment period: 8 weeks Total: 12 weeks
Main criteria for inclusion: 1. Patients with the diagnosis of hyperlipidemia and receiving instructions for lifestyle improvement 2. Patients with a fasting triglyceride (TG) level of 150 -499 mg/dL at Visit 1 after informed consent (Day -29 to Day -1 before start of study drug administration) 3. Patients receiving a stable dose of HMG-CoA reductase inhibitor therapy continuously for at least 4 weeks before informed consent at Visit 1 (Day -29 to Day -1 before start of study drug administration) 4. Male or postmenopausal female patients 5. Patients who, in the opinion of the principal investigator or the investigator, are capable of understanding the content of the clinical research and complying with the research protocol requirements. 6. Patients who can provide written informed consent prior to the conduction of the clinical research procedures 7. Patients aged ≥20 years at the time of informed consent at Visit 1(Day -28 to Day 0 before the start of study drug administration)	
Main criteria for exclusion: 1. Patients with a history of revascularization or those have had coronary artery disease (a definitive diagnosis of myocardial infarction, angina) within 24 weeks before informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) 2. Patients who have undergo aortic aneurysmectomy within 24 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) or those with concurrent aortic aneurysm 3. Patients who have had clinically significant hemorrhagic disorders (e.g., hemophilia, capillary fragility, gastrointestinal ulcer, urinary tract hemorrhage, hemoptysis, and vitreous hemorrhage) within 24 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) or those who concurrently have the above disorders 4. Patient with a fasting FMD level of 0% measured at the start of study drug administration at	

Visit 2 (Day -15 to Day -1 before the start of study drug administration)

5. Patients in whom the type and dosage of HMG-CoA reductase inhibitors, antidiabetic drugs and antihypertensive drugs have been changed within 4 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration)
6. Patients who have started anti dyslipidemic agents within 4 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration)
7. Patients requiring a change in the dosage of dyslipidemia therapeutic, antidiabetic, or antihypertensive drugs during the period between informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) and the start of study drug administration at Visit 2 (Day -15 to Day -1 before the start of study drug administration)
8. Patients with severe hepatic dysfunction
9. Patients with severe renal dysfunction (as an indicator, CKD category ≥G3b, equivalent to an A3)
10. Patients who have been diagnosed with pancreatitis
11. Patients who have been diagnosed with lipoprotein lipase deficiency, apoprotein C-II deficiency, familial hypercholesterolemia, familial combined hyperlipidemia, or familial type III hyperlipidemia
12. Patients with concurrent Cushing's syndrome, uremia, systemic lupus erythematosus (SLE), serum dysproteinemia, or hypothyroidism
13. Patients with symptomatic Peripheral Arterial Disease (PAD)
14. Patients with concurrent hypertension of grade II or higher [Note 1)]

Note 1: Patients with systolic blood pressure of ≥160 mm Hg or diastolic BP of ≥100 mm Hg regardless of treatment with antihypertensive drugs

15. Patients who are habitual drinkers drinking an average of over 100 mL per day (expressed in terms of quantity of alcohol) or patients with, or with a history of drug abuse or addiction
16. Patients with a history of hypersensitivity or allergy for omega-3-acid ethyl esters
17. Patients who smoke
18. Patients participating in other clinical studies
19. Patients who have been determined to be ineligible as subjects in the study by the principal investigator or the investigator

Endpoints:

< Primary endpoint >

%FMD (fasting)

Secondary endpoints

(1) %FMD (4 h postprandial)

(2) TG level (fasting)

(3) TG level (4 h postprandial)

(4) Plasma fatty acid fraction

<Other endpoints>

Efficacy endpoint:

Total cholesterolLDL-C, HDL-C, Remnant-like particle (RLP) cholesterol , Apoprotein B-48, C reaction protein (CRP), determination of 8-epi-PGF2α (in urine)

Exploratory endpoints:

Exploratory biomarker evaluation

(1) Panel of blood proteins
PAI1, TFPI, TNF-α, IL-1, IL-6, IL-8, IL-10, INFγ, MCP-1, ICAM1, VCAM1, eSelectin, serum amyloid A (SAA), pentraxin 3 (PTX3), FABP4, leptin, adiponectin

(2) Panel of unsaturated fatty acid metabolites
EPA metabolites (18-HEPE, 5-HEPE, Resolvin E1)
DHA metabolites [17-HDHA (17-HDoHE), 7-HDHA (7-HDoHE), Resolvin D1, Resolvin D2, Resolvin D3, Maresin 1]
Arachidonic acid metabolites (PGE2, PGD2, PGF2α, TXB2, 6-keto-PGF1α, LTB4)

(3) Panel of lipids
Phospholipids (glycerophospholipid (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, etc.), sphingophospholipids (sphingomyelin, etc.)), sphingolipid (ceramide, ganglioside, sulfatides, etc.), neutral lipids (monoacylglycerol, diacylglycerol, triacylglycerol, cholesteryl esters, dolichol, etc.), fatty acids (free fatty acids), acylcarnitine, bile acids, ubiquinone, and molecular species related to these biosynthesis and metabolism

<Safety endpoint>

Adverse events, body weight, blood pressure in the sitting position, pulse in the sitting position, laboratory tests [fasting plasma glucose (FPG)]

Statistical methods:

(1) Analysis set

Two analysis sets, "Full Analysis Set (FAS)" and "Safety Analysis Set (SAS)" are used in this study.

Define FAS as the population of enrolled subjects who satisfy the following criterion:

- Research subjects who were randomized and given at least one dose of the study drug.

Define SAS as the population of enrolled research subjects who satisfy the following criterion:

- Research subjects who were given at least one dose of the study drug during this clinical study.

(2) Efficacy analysis

[Primary endpoints]

%FMD (fasting)

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point and the same analysis as in 1) will be performed.

3) Using fasting TG level at the start of treatment, a stratified analysis on summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be performed.

[Secondary endpoints]

(1) %FMD (4 h postprandial)

(2) TG level (fasting)

(3) TG level (4 h postprandial)

(4) Plasma fatty acid fraction

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.

3) Using fasting TG level at the start of treatment, a stratified analysis on summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be performed.

[Other endpoints]

Efficacy endpoints:

Total cholesterolLDL-C, HDL-C, Remnant-like particle (RLP) cholesterol , Apoprotein B-48, C reaction protein (CRP), determination of 8-epi-PGF2α (in urine)

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point and the same analysis as in 1) will be performed.

Exploratory endpoints:

Exploratory biomarker evaluation

1) Panel of blood proteins

PAI1, TFPI, TNF-α, IL-1, IL-6, IL-8, IL-10, INFγ, MCP-1, ICAM1, VCAM1, eSelectin, serum amyloid A (SAA), pentraxin 3 (PTX3), FABP4, leptin, adiponectin

2) Panel of unsaturated fatty acid metabolites

EPA metabolites (18-HEPE, 5-HEPE, Resolvin E1), DHA metabolites [17-HDHA (17-HDoHE), 7-HDHA (7-HDoHE), Resolvin D1, Resolvin D2, Resolvin D3, Maresin 1], and Arachidonic acid metabolites (PGE2, PGD2, PGF2α, TXB2, 6-keto-PGF1α, LTB4)

[Analytical method]

1. Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the observation and treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.
2. The change for each treatment group (Treatment Period Visit 2, Visit 3 or Visit 4 - Treatment Period Visit 1) will be calculated at each evaluation time point during the observation and treatment period and the same analysis as in 1) will be performed.
3. The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed. Moreover, the correlation coefficient will be calculated with the change of % FMD for each treatment group, and scatter plots will be drawn.

3) Panel of lipids (fasting)

Phospholipids (glycerophospholipid (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, etc.), sphingophospholipids (sphingomyelin, etc.)), sphingolipid (ceramide, ganglioside, sulfatides, etc.), neutral lipids (monoacylglycerol, diacylglycerol, triacylglycerol, cholesteryl esters, dolichol, etc.), fatty acids (free fatty acids), acylcarnitine, bile acids, ubiquinone, and molecular species related to these biosynthesis and metabolism

[Analytical method]

1. Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the observation and treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.
2. The change for each treatment group (Treatment Period Visit 2, Visit 3 or Visit 4 - Treatment Period Visit 1) will be calculated at each evaluation time point during the

observation and treatment period and the same analysis as in 1) will be performed.

3. The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed. Moreover, the correlation coefficient will be calculated with the change of % FMD for each treatment group, and scatter plots will be drawn.

4) Panel of lipids (4 h postprandial)

Phospholipids (glycerophospholipid (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, etc.), sphingophospholipids (sphingomyelin, etc.)), sphingolipid (ceramide, ganglioside, sulfatides, etc.), neutral lipids (monoacylglycerol, diacylglycerol, triacylglycerol, cholesteryl esters, dolichol, etc.), fatty acids (free fatty acids), acylcarnitine, bile acids, ubiquinone, and molecular species related to these biosynthesis and metabolism

[Analytical method]

1. Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the observation and treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2. The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed. Moreover, the correlation coefficient will be calculated with the change of % FMD for each treatment group, and scatter plots will be drawn.

(3) Analysis of safety endpoints

[Adverse events]

The following analyses will be performed for each treatment group. Adverse events will be coded using MedDRA and summarized by System Organ Class (SOC) and Preferred Term (PT).

- Tabulation of frequency of all adverse events

- Tabulation of frequency of adverse events with a causal relationship to the study drug
- Tabulation of frequency of all adverse events by severity
- Tabulation of frequency of adverse events with a causal relationship to the study drug by severity
- Tabulation of frequency of adverse events leading to study drug discontinuation
- Tabulation of frequency of serious adverse events
- Tabulation of frequency of all adverse events by time of onset

[Body weight, blood pressure in the sitting position, pulse in the sitting position, laboratory tests [fasting plasma glucose (FPG)]

1) Summary statistics of measurements by treatment group at each evaluation time point during the treatment period will be calculated and a diagram of the change of individual data will be created.
2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.
3) Shift table will be created for assessment results based on reference values for Treatment Period Visit 2 and each evaluation time point (Visit 3, Visit 4) during the treatment period.

Rationale for the number of planned research subjects

The planned sample size is based on the requierment to investigate explore atively the effects of omega-3-acid ethyl esters on vascular endothelial function considering the feasibility of this study.

3.0 ABBREVIATION

AE	adverse event
BMI	body mass index
CKD	chronic kidney disease
COI	conflict of interest
CRO	contract research organization
DHA	docosahexaenoic acid
EDC	electronic data capture
EPA	eicosapentaenoic acid
FAS	full analysis set
FMD	flow mediated dilation
GCP	Good Clinical Practice
HDL-C	high density lipoprotein-cholesterol
ICH	International Conference on Harmonisation
JAPIC	Japan Pharmaceutical Information Center
LDL-C	low density lipoprotein-cholesterol
MedDRA	Medical Dictionary for Regulatory Activities
PT	Preferred Term
SAS	safety analysis set
SLE	systemic lupus erythematosus
SOC	System Organ Class
TG	triacylglycerol

4.0 INTRODUCTION

4.1 Background

The efficacy of LDL-lowering regimen using HMG-CoA reductase inhibitor designed to control cardiovascular events has been already established, which leads to primary prevention, secondary prevention, and risk reduction in high-risk treatment groups.[1),2)] However, postprandial hypertriglyceridemia and the quality of LDL have been receiving attention as an atherosclerotic risk factor that remains after the treatment with HMG-CoA reductase inhibitors.

Although there had been no consensus regarding the significance of triglycerides (TG) as a cardiovascular risk, Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases (2012) stated that "a number of epidemiological studies suggest that elevated triglyceride levels are associated with increased incidence of cardiovascular diseases." TG levels reach the highest 3 to 4 h after meal. Especially, remnant-like lipoprotein (RLP) plays an important role to cause the elevation of TG level, and RLP particles contain a greater amount of cholesterol, as well as TG, than LDL particles. It has been reported that RLP enhances foaming of macrophage and inflammatory reaction[3)], and TG levels at 2 to 4 h after meals are more important than those before meals as a heart disease risk factor. [4)]

As insulin resistance associated with obesity, etc. increases, qualitative changes in LDL, as well as elevated levels of TG, are observed. The increase observed in small dense LDL (sdLDL) of small particle diameters is considered to enhance the formation of atherosclerotic plaque since this sdLDL is easily taken up by macrophages. Therefore, in order to aim at further inhibition of cardiovascular events after achieving the lowering of LDL-C with HMG-CoA reductase inhibitors, it is considered especially important to lower TG levels/decrease sdLDL. Omega-3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been receiving attention in recent years as the drugs having such activities. Since omega 3 fatty acids are not synthesized in the body, supplementation from meals (fish) is necessary. EPA and DHA have TG-lowering and antiatherosclerosis activities but the mechanism has not been completely elucidated. Omega-3-acid ethyl esters (EPA/DHA preparations, Lotriga®) released in 2013 lower TG levels in a dose-dependent manner[5)] and are expected to lead to lowering of RLP, increase in the LDL particle size, FMD, and lowering of IL-6 levels.[6)]

In JELIS Studies, lowering of TG levels and reduction in major coronary events have been demonstrated in the HMG-CoA reductase inhibitor and EPA combination group compared to the HMG-CoA reductase inhibitor alone group.[7)] This clinical study aims at exploring the effects of Lotriga® (EPA/DHA preparations) on vascular endothelial function and postprandial hypertriglyceridemia assessed by FMD, etc. in patients receiving a hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor and have concurrent hypertriglyceridemia (TG).

Much remains unknown in terms of the changes in endothelial cell-derived cytokines which are well known to correlate with progression of arteriosclerosis, as well as the activities of inflammatory cells as a chronic inflammation lesion in a cytokine assay panel. In addition, an unfinished work is to elucidate an additional mechanism of action by analyzing the in vivo changes in the whole lipids after treatment with Lotriga® (EPA/DHA preparations). Thus, blood proteins and lipids/unsaturated fatty acid metabolites which are associated with blood vessel inflammation will be measured to search for potential biomarkers which may correlate with the change in FMD, and to explore the mechanism of TG-lowering and antiatherosclerotic activities by the combination of HMG-CoA reductase inhibitor and Lotriga® (EPA/DHA preparations).

4.2 Rationale for the proposed research

This clinical study was planned to contribute to the prevention of arteriosclerotic disease by exploring the effects of omega-3-acid ethyl esters on vascular endothelial in patients with hyperlipidemia. This study was also planned to support the elucidation of mechanism for development and prevention of arteriosclerotic disease from the viewpoint of fundamental research, by searching for potential biomarkers for endothelial functions after treatment with omega-3-acid ethyl esters, and by exploring the mechanism of TG-lowering and antiatherosclerotic activities by the combination of HMG-CoA reductase inhibitor and omega-3-acid ethyl esters.

5.0 RESEARCH OBJECTIVES AND ENDPOINTS

5.1 Objectives

The objective of this study is to explore the effects of omega-3-acid ethyl esters on vascular endothelial function when administered for 8 weeks, as measured by FMD, in patients with hyperlipidemia.

5.2 Definition of endpoints

5.2.1 Primary endpoints

%FMD (fasting)

5.2.2 Secondary endpoints

(1) %FMD (4 h postprandial)

(2) TG level (fasting)

(3) TG level (4 h postprandial)

(4) Plasma fatty acid fraction

5.2.3 Other endpoints

Efficacy endpoints:

total cholesterol, LDL-C, HDL-C, Remnant-like particle (RLP) cholesterol , apoprotein B-48, C-reactive protein (CRP), determination of 8-epi-PGF2α (in urine)

Exploratory endpoints:

Exploratory biomarker evaluation

(1) Panel of blood proteins

PAI1, TFPI, TNF-α, IL-1, IL-6, IL-8, IL-10, INFγ, MCP-1, ICAM1, VCAM1, eSelectin, serum amyloid A (SAA), pentraxin 3 (PTX3), FABP4, leptin, adiponectin

(2) Panel of unsaturated fatty acid metabolites

EPA metabolites (18-HEPE, 5-HEPE, Resolvin E1)

DHA metabolites [17-HDHA (17-HDoHE), 7-HDHA (7-HDoHE), Resolvin D1, Resolvin D2, Resolvin D3, Maresin 1]

Arachidonic acid metabolites (PGE2, PGD2, PGF2α, TXB2, 6-keto-PGF1α, LTB4)

(3) Panel of lipids

Phospholipids (glycerophospholipid (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, etc.), sphingophospholipids (sphingomyelin, etc.)), sphingolipid (ceramide, ganglioside, sulfatides, etc.), neutral lipids (monoacylglycerol, diacylglycerol, triacylglycerol, cholesteryl esters, dolichol, etc.), fatty acids (free fatty acids), acylcarnitine, bile acids, ubiquinone, and molecular species related to these biosynthesis and metabolism

5.2.4 Safety endpoints

Adverse events, body weight, blood pressure in the sitting position, pulse in the sitting position, laboratory tests [fasting plasma glucose (FPG)]

6.0 CLINICAL RESEARCH DESIGN

6.1 Clinical research design

< Clinical research design >

This is a multicenter, collaborative, randomized, open-label study designed to explore the effects of administration of omega-3-acid ethyl esters [2 g (2 g PO QD) or 4 g (2 g PO BID) for 8 weeks] on vascular endothelial function, as measured by FMD, in patients receiving a hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor and have concurrent hypertriglyceridemia.

Considering the potential bias by factors that affect FMD between treatment groups, stratified allocation will be performed with fasting TG as a factor.

Research TREATMENT

(1) Study drug:

Omega-3-acid ethyl esters

(2) Dosage regimen

Omega-3-acid ethyl esters will be orally administered [2 g (2 g PO QD) or 4 g (2 g PO BID)] immediately after meal for 8 weeks in patients with hyperlipidemia .

(3) Duration of treatment:

8 weeks

Planned number of research subjects:

40 patients evaluable for the primary endpoint

2 g group: 20 subjects

4 g group: 20 subjects

Number of study sites:

5 to 10 institutions

< Duration of the treatment and number of visits of by subjects >

(1) Duration of treatment:

The duration of the treatment period is 8 weeks. Research subjects should visit the hospital at the time of informed consent at Visit 1 (Day-29 to Day -1 before the start of study drug) and from the start of study drug administration at Visit 2 (Day -15 to Day -1 from the start of study drug administration) to completion of study drug administration [Visit 4 (Week 8)].

(2) Number of visits

Observation period: 1 visit

Treatment period: 3 to 6 visits

The FMD measurement during the treatment period will be performed by a physician or laboratory technician who completed the FMD measurement training. Therefore, only for the FMD measurement, subjects are expected to go to a testing laboratory other than the one they normally visit for examinations. In that case, a total of 3 visits for the FMD measurement will be added to the total of 3 visits for examinations from Treatment Period Visit 2 (Day -15 to Day -1 before the start of study drug administration) to Visit 4 (Week 8). Therefore, the number of visits during the treatment period will be a minimum of 3 and a maximum of 6.

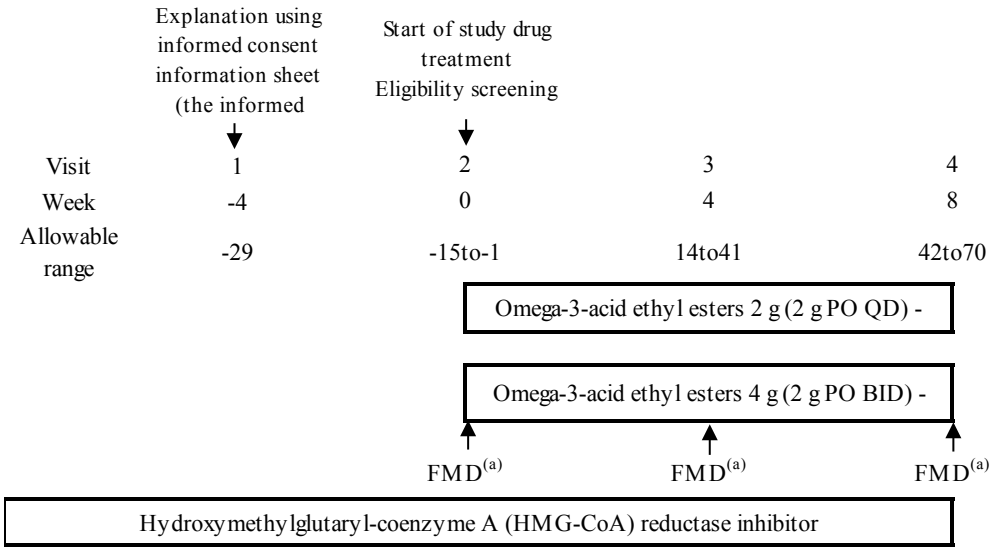
Figure 6 (a) shows an outline of the clinical research design. Refer to Appendix A for schedule of examinations, observations, and assessments.

Figure 6.a Outline of clinical research design

< Outline of clinical research>

Duration of treatment:8 weeks

Number of visits: 4 to 7 visits



(a) FMD measurement will be performed by a physician or laboratory technician who completed the FMD measurement training.

6.2 Rationale for the clinical research design

<Rationale>

Since this study aims at exploring the effects of oral administration of omega-3-acid ethyl esters in clinical practice (2 g once a day or twice a day for 8 weeks) on vascular endothelial function, the study was designed as an open-label comparing FMD before and after omega-3-acid administration without including control groups.

< Rationale for the dose and dosing regimen >

In accordance with the approved dosage and administration of omega-3-acid ethyl esters, dose and dosing regimen of omega-3-acid ethyl esters were set at 2 g (2 g PO QD) and 4 g (2 g PO BID) in this study.

< Rationale for the duration of study >

Since improvement of FMD was observed after a 2-month administration of omega-3-acid ethyl esters in the studies conducted outside Japan investigating the effect of omega-3-acid ethyl esters on FMD,[8] it was considered that the effect of omega-3-acid ethyl esters on FMD can be examined with an 8-week administration and the duration of the treatment period was, therefore, set at 8 weeks in this clinical study.

< Rationale for the planned sample size >

See Section 13.3.

6.3 Premature termination of entire clinical research or premature termination of clinical research at a study site

6.3.1 Premature termination criteria of entire clinical research

The sponsor should immediately discontinue the study when at least one of the following criteria is applicable:

- When new information or other evaluation on the safety or efficacy of the study drug becomes available that shows a change in the known risk/benefit profile of the concerned compound, and risks/benefits are no longer tolerable for research subject participation in the study.
- When there is serious deviation from Ethical Guidelines or ICH-GCP for medical and health research involving human subjects.

6.3.2 Criteria for premature termination of study sites

Termination of involvement of a study site in the study may be requested prematurely at the discretion of the sponsor if the entity (e.g., principal investigator) is found in significant violation of the ethical guidelines, protocol, or contractual agreement for medical and health research involving human subjects, or is unable to ensure proper conduct of the research.

6.3.3 Procedures of clinical study suspension and premature termination of entire clinical study or study at a study site

In the event that the sponsor or a study site committee such as an ethics review committee decides to prematurely suspend or terminate the entire clinical study or clinical study at a study site, a study-specific procedure shall be provided by the sponsor. The procedure shall be followed by applicable study sites during the course of clinical study suspension or premature termination.

6.4 Procedures for protocol revision

If the protocol needs to be revised, the sponsor shall consider and decide whether to revise the protocol.

The principal investigator of each study site shall be informed of the details of each protocol revision. Principal investigators shall confirm the content of the revision of the protocol and submit a letter of agreement to the sponsor as evidence of agreement with the protocol revision.

Upon notification, the principal investigator at each study site shall submit the revised contents to committees such as the IEC, as necessary according to institutional regulations for review, and obtain approval from the director of the entity.

7.0 SELECTION AND WITHDRAWAL CRITERIA OF RESEARCH SUBJECTS

The principal investigator or investigator shall check for all the inclusion/exclusion criteria including the test results prior to randomization.

7.1 Inclusion criteria

Eligibility of research subjects shall be determined in accordance with the following criteria.

1. Patients with the diagnosis of hyperlipidemia and receiving instructions for lifestyle improvement
2. Patients with a fasting triglyceride (TG) level of 150 – 499 mg/dL at Visit 1 after informed consent (Day -29 to Day -1 before start of study drug administration)
3. Patients receiving a stable dose of HMG-CoA reductase inhibitor therapy continuously for at least 4 weeks at the time of informed consent at Visit 1 (Day -29 to Day -1 before start of study drug administration)
4. Male or postmenopausal female patients
5. Patients who, in the opinion of the principal investigator or the investigator, are capable of understanding the content of the clinical research and complying with the research protocol requirements.
6. Patients who can provide written informed consent prior to the conduction of the clinical research procedures
7. Patients aged ≥20 years at the time of informed consent at Visit 1(Day -29 to Day -1 before the start of study drug administration)

[Rationale for the inclusion criteria]

1. This was set as a target condition in this clinical study.

2. This was set according to the diagnostic criteria for hypertriglyceridemia in "Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2012."

3. This was set for an accurate assessment of the efficacy.

4. This was set in consideration of the effect of female hormones on FMD.

5.6. These were set as fundamental items for the research.

7. This was set considering the age ≥20 years as the age where patients are capable of making a decision to participate in this clinical study.

7.2 Exclusion criteria

Research subjects meeting any of the criteria below shall not be included in this research.

1. Patients with a history of revascularization or those have had coronary artery disease (a definitive diagnosis of myocardial infarction, angina) within 24 weeks before informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) （
2. Patients who have undergo aortic aneurysmectomy within 24 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) or those with concurrent aortic aneurysm
3. Patients who have had clinically significant hemorrhagic disorders (e.g., hemophilia, capillary fragility, gastrointestinal ulcer, urinary tract hemorrhage, hemoptysis, and vitreous hemorrhage) within 24 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) or those who concurrently have the above disorders
4. Patient with a fasting FMD level of 0% measured at the start of study drug administration at Visit 2 (Day -15 to Day -1 before the start of study drug administration)
5. Patients in whom the type and dosage of HMG-CoA reductase inhibitors, antidiabetic drugs and antihypertensive drugs have been changed within 4 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration)
6. Patients who have started anti dyslipidemic agents within 4 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration)
7. Patients requiring a change in the dosage of dyslipidemia therapeutic, antidiabetic, or antihypertensive drugs during the period between informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) and the start of study drug administration at Visit 2 (Day -15 to Day -1 before the start of study drug administration)
8. Patients with severe hepatic dysfunction
9. Patients with severe renal dysfunction (as an indicator, CKD category ≥G3b, equivalent to an A3)
10. Patients who have been diagnosed with pancreatitis
11. Patients who have been diagnosed with lipoprotein lipase deficiency, apoprotein C-II deficiency, familial hypercholesterolemia, familial combined hyperlipidemia, or familial type III hyperlipidemia

12. Patients with concurrent Cushing's syndrome, uremia, systemic lupus erythematosus (SLE), serum dysproteinemia, or hypothyroidism
13. Patients with symptomatic Peripheral Arterial Disease (PAD)
14. Patients with concurrent hypertension of grade II or higher [Note 1)]

Note 1: Patients with systolic blood pressure of ≥160 mm Hg or diastolic BP of ≥100 mm Hg regardless of treatment with antihypertensive drugs

15. Patients who are habitual drinkers drinking an average of over 100 mL per day (expressed in terms of quantity of alcohol) or patients with, or with a history of drug abuse or addiction [Note 2)]
16. Patients with a history of hypersensitivity or allergy for omega 3-acid ethyl esters -
17. Patients who smoke
18. Patients participating in other clinical studies
19. Patients who have been determined to be ineligible as subjects in the study by the principal investigator or the investigator

Note 2: Alcohol conversion table (reference)

Alcohol	Type	Alcohol content	Amount corresponding to 100 mL alcohol
Brewage	Sake	15%	670 mL (approx. 3 cups)
	Beer	5%	2,000 mL (approx. 3 large bottles)
	Sparkling liquor	5%	2,000 mL
	Wine	12%	830 mL
	Chinese rice wine	18%	560 mL
Distilled liquor	Ko-rui Shochu	35%	290 mL
	Otsu-rui Shochu	25%	400 mL
	Whiskey	40%	250 mL (approx. 3 doubles)
	Brandy	40%	250 mL (approx. 3 doubles)
	Vodka	40%	250 mL(approx. 3 doubles)
Mixed liquor	Plum wine	13%	770 mL
	Synthetic sake	16%	630 mL

[Rationale for the exclusion criteria]

1.2.8.16 These were set in consideration of safety of the research subjects.

3 This was set because EPA-E, a component of the study drug, has an antiplatelet action and may impair hemostasis in patients.

4.5.6.7.9.14. 17 These were set because it was considered that drug efficacy evaluation would be affected.

10. This was set because acute pancreatitis may occur in patients with significant hypertriglyceridemia.

11. This was set to exclude patients with hypertriglyceridemia attributed to a genetic predisposition.

12. This was set to exclude an underline condition commonly seen in patients with secondary hypertriglyceridemia.

13.15 These were set in consideration of safety of the research subjects and for an accurate assessment of the efficacy.

18.19 These were set as fundamental items for the research.

7.3 Prohibited concomitant drugs and permitted concomitant drugs

7.3.1 Prohibited concomitant drugs

[Prohibited concomitant drugs]

The concomitant use of the following drugs* will be prohibited during the clinical study [Visit 1 (Day -29 to Day -1 before the start of study drug administration) to Visit 6 (Week 8)].

(*: Concomitant drugs prohibited in the package insert of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor taken at the time of informed consent are included.)

1. Eicosapentaenoic acid (EPA) preparations or EPA/DHA preparations (including supplements)
2. Antidyslipidemic agents (except for HMG-CoA reductase inhibitor taken at the time of the informed consent)

    Anion exchange resin

    Fibrates

    Ezetimibe

    Nicotinic acid derivatives

    Probucol

    Phytosterols

    Evolocumab

Others (elastase ES, dextran sulfate sodium, polyenephosphatidylcholine, pantethine)

3. Pancreatic hormones

   Insulin preparation

4. Androgens

   Testosterone, methyltestosterone

5. Follicle hormone and luteinizing hormone

   Estrogen and progestogen

6. Systemic steroids

<Rationale>

1to 6 These were set because it was considered that efficacy evaluation of the study drug would be affected by these drugs.

7.3.2 Permitted concomitant drugs

Drugs other than those prohibited for concomitant use may be taken during the study period. However, for HMG-CoA reductase inhibitors, antidiabetic drugs (except for insulin) and antihypertensive drugs, dosage change, addition of new treatment drugs, or change of treatment drugs shall not be allowed unless the principal investigator or investigator considers it necessary due to adverse events, etc.

7.4 Research subject management

The principal investigator or investigator shall instruct research subjects regarding the following:

(1) Guidance for research subjects for improving lifestyle shall be made constant during the clinical study and its content shall not be changed. Research subjects shall be instructed to avoid excessive exercise as much as possible during the study period.

(2) Research subjects shall be instructed not to consume alcohol after 9:00 pm two days before the hospital visit, and not to consume food after 9:00 pm on the day before the hospital visit. Although water can be taken as desired on the day of blood collection, research subjects shall be instructed to visit the hospital in a fasting state in order that fasting morning samples can be collected.

(3) Research subjects shall be instructed to avoid eating/drinking to excess, extreme change in dietary content (eating a high-fat meal, etc.) or excessive exercise, and spend time on a routine basis the day before the hospital visit.

(4) Research subjects shall be instructed to visit the hospital according to the schedule and take the test as prescribed during the study period.

(5) Research subjects shall be instructed to comply with the instructions or restrictions (taking the study drug, prohibited drugs for concomitant use, those allowed for concomitant use, etc.)

(6) Upon concomitant use of anticoagulants (warfarin potassium, etc.) or antiplatelet drugs (aspirin, etc.) listed under Precautions for Coadministration in the package insert of the study drug, research subjects shall be instructed to promptly report in the event of an adverse drug reaction, such as bleeding.

(7) Research subjects must notify the principal investigator or investigator in advance if they receive treatment from other physicians. If treated by other physicians, the research subjects shall be instructed to promptly report the details of the treatment.

7.5 Criteria for discontinuation or withdrawal of a research subject

The principal investigator or investigator shall record the main reason for discontinuation of protocol treatment on the case report form according to the classification described below. Refer to Section 9.1.11 for research subjects who withdraw from the research before randomization.

1. Pretreatment Event (PTE) or adverse event

When the research subject had a pretreatment event (PTE) or an adverse event that requires withdrawal of the research subject from the study because continued participation in the study would impose an unacceptable risk to the research subject's health, or when the research subject is unwilling to continue study participation because of the PTE or adverse event.

2. Major protocol deviation

When it is discovered after randomization that a research subject does not meet the eligibility criteria or is not adhering to the protocol, and continued participation in the research would impose an unacceptable risk to the research subject's health.

3. Lost to follow-up

When the research subject failed to make visits and could not be contacted despite the attempts to contact the research subject.

4. Voluntary termination

When the research subject wishes to withdraw from the research. The reason for discontinuation shall be obtained to the extent possible.

5. Research termination

When the sponsor or a committee such as the IEC or regulatory authority has decided to terminate the study. Refer to Section 6.3.1 for details.

6. Lack of efficacy

When efficacy of the study drug is not evident and continuation of the research may pose an unacceptable risk to the research subjects in the opinion of the principal investigator or investigator.

7. Others

When the principal investigator or investigator determined to terminate the study for other reasons.

The specific reasons should be recorded on the CRF.

7.6 Procedures for discontinuation of individual research subjects

The principal investigator or investigator shall terminate a research subject's research participation when the research subject meets the criteria described in Section 7.5. Individual research subjects may discontinue their research participation without giving a reason at any time during the research. Should a research subject's participation be discontinued, the primary reason for termination shall be recorded on the CRF by the principal investigator or investigator. In addition, efforts shall be made to perform all tests/observations/evaluations scheduled at the time of discontinuation.

8.0 RESEARCH TREATMENT

8.1 Study drug

Generic name: Omega-3-acid ethyl esters

Chemical name: Ethyl icosapentate;

Ethyl (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoate

Docosahexaenoic acid ethyl ester;

Ethyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoate

8.1.1 Dose and administration method

Omega-3-acid ethyl esters will be orally administered [2 g (2 g PO QD) or 4 g (2 g PO BID)] immediately after meal. In principle, the dosage prescribed to the research subject should not be changed until the end of the study. Since the research subject should in a fasting state at the time of visit, the drug to be taken after breakfast should be taken immediately after the first meal after completion of the prescribed test.

Medication should be started the day after completion of all the tests at Week 0.

8.1.2 Concomitant drug

HMG-CoA reductase inhibitors, antidiabetic drugs (except for insulin) or antihypertensive drugs taken at the time of informed consent should continue to be taken without a change in dosage and administration during the study period.

8.1.3 Overdose of the study drug

Overdose is defined as intentional or accidental administration of the study drug at a higher dose than that specified in the protocol, either by a health professional or by the research subject.

To consistently collect important safety information about overdose, the principal investigator or investigator(s) shall record all cases of overdose on the “Overdose” page of the CRF, irrespective of the presence or absence of accompanying adverse event. Adverse events associated with overdose shall be recorded on the “Adverse events” page of the CRF, in accordance with the procedures described in Section 10.0, “Pretreatment Event and Adverse Events.”P

In addition, serious adverse events associated with overdose shall be recorded in accordance with the procedures described in Section 10.2.2, “Collection and reporting of SAEs.”

In the event of overdose, the principal investigator or investigator shall treat the subject as required based on symptoms.

8.2 Allocation of the study drug and administration procedure

The principal investigator or investigator shall access the Case Registration Web System to allocate the research subjects. The principal investigator or the designee shall notify the information required for allocation in addition to the research subject identification code. Then, drugs that should be administered to each research subject will be notified through the Case Registration Web System. The principal investigator or investigator shall prescribe the study drug 2 g [(2 g PO QD) or 4 g (2 g PO BID)] according to the notification, and record the dose administered for each research subject in the CRF.

8.3 Preparation and storage of allocation list

The allocation responsible person (designated by the sponsor) shall create an allocation procedure. Information on the allocation shall be kept in a safe place and shall not be available to anyone other than authorized persons, to secure independency from the clinical research.

Using fasting TG values as stratified factors, stratified allocation shall be performed at the registration center at the start of treatment period. For allocation, the Registration Center shall use the Allocation Procedure for Stratified Allocation prepared by the allocation responsible person.

9.0 CLINICAL STUDY PROTOCOL

9.1 Research procedures

The principal investigator or investigator shall collect data in accordance with the procedure below. In principle, tests other than FMD measurement, observations, and evaluations of research subjects shall be performed by the same principal investigator or investigator. FMD measurement shall be performed by a physician or laboratory technician who has completed the FMD measurement training. The study schedule is provided in Appendix A.

9.1.1 Informed consent procedure

The procedures for obtaining informed consent are described in Section 15.3.
Consent shall be obtained from the research subject before initiation of research procedures.
Research subject code is given to each research subject from whom informed consent was acquired and who was randomized. The research subject ID code shall be used throughout the research period and shall not be changed.

9.1.2 Demographic data, medical history, and previous therapeutic drugs

Demographic data shall be collected regarding date of birth, gender, time (year/month) of onset of dyslipidemia (or diagnosis), last menstrual period (year), frequency of consumption of fish (almost every day, once/2 days, 1 to 2 times/week, almost never), smoking and drinking history, and vessel diameter.

Medical history data shall be collected regarding clinically problematic diseases or symptoms that disappeared or were terminated within 1 year from informed consent. When the symptoms or disease continues, it shall be considered as a concurrent disease (Refer to Section 9.1.7).
Previous therapeutic drug data shall be collected regarding all drugs used within 4 weeks before the informed consent and that are related to criteria for eligibility and assessment of efficacy.

9.1.3 Physical examination

All subsequent physical examinations after the start of study drug administration shall be assessed for clinically significant changes from the baseline examination.

9.1.4 Weight, height, and BMI

Body weight and height shall be measured. The sponsor shall calculate the BMI using the following formula.
Body Mass Index: BMI=body weight (kg)/[height(m)]^2
Height shall be measured to the nearest whole number in centimeters. Body weight shall be measured to one decimal place in kilograms. The result of BMI shall be shown to one decimal place.
Example:

Height = 176 cm, weight = 79.2 kg, BMI = $79.2/1.76^2 = 25.6$ kg/m^2

9.1.5 Vital signs

For vital signs, blood pressure in {sitting position (after resting for 5 minutes) and a pulse rate (bpm) shall be measured.
When timing for the vital signs measurement overlaps with blood collection, priority shall be given to blood collection, and the vital signs shall be measured within 30 minutes before or after the blood collection.

9.1.6 Concomitant drugs

Concomitant drugs are all drugs to be given in addition to the study drug. Drugs prescribed by doctors or the over-the-counter medicines purchased by the research subjects shall be included. At every hospital visit of the research subject, the status of use (drug name, route of administration) of the drugs (including vitamin compound, over-the-counter medication, and Chinese medicine) used, other than the study drug, from the time of informed consent to the completion of the clinical research shall be monitored.

9.1.7 Concurrent disease

A concurrent disease shall be defined as a disease or symptom that is present at the time of the research subject's informed consent Clinically problematic laboratory test data, ECG findings, and abnormal physical examination findings observed immediately after the acquisition of the informed consent and examination shall be considered as a concurrent disease at the discretion of the principal investigator or investigator. The content of concurrent disease (diagnosis) shall be investigated.

9.1.8 Checking food and drink consumption before the visit

At each study visit, the principal investigator, investigator, etc., physician for FMD measurement, or laboratory technician for FMD measurement will check the food and drink consumption of the research subject before the visit.

- Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of the fasting test, and food should not be consumed from 9:00 pm on the day before to the time of the fasting test.
- Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the fasting test.

9.1.9 Laboratory tests

The following laboratory tests shall be performed according to the observation schedule (Appendix A). Samples will be taken under ≥10-h fasting for the fasting test and at 4 h after consumption of a prescribed meal (allowable range ± 30 min) for the 4-h postprandial test.

The principal investigator or investigator shall evaluate and keep the reported laboratory test results.

Table 9-a Laboratory tests

Serum chemistry (fasting)	Serum chemistry (4 h postprandial)	Urinalysis (fasting)
TG	TG	Determination of 8-epi-PGF2α
Total cholesterol	Total cholesterol	
LDL-C	LDL-C	
HDL-C	HDL-C	
Remnant-like particle (RLP) cholesterol	Remnant-like particle (RLP) cholesterol	
Apoprotein B-48	Apoprotein B-48	
high-sensitivity C-reactive protein (hs-CRP)		
Fasting plasma glucose (FPG)		
Plasma fatty acid fraction (Dihomo-gamma-linolenic acid、Arachidonic acid、Eicosapentaenoic acid、Docosahexaenoic acid、EPA/AAratio、DHA/AAratio)		

The principal investigator shall keep laboratory test reference values, including the historical data.

9.1.10 Sample collection for exploratory biomarker evaluation

Evaluation of the following biomarkers shall be performed in Integrated Technology Research Laboratories, Takeda Pharmaceutical Company Limited, according to the observation schedule (Appendix A). Samples will be taken under ≥10-h fasting for the fasting test and at 4 h after consumption of a prescribed meal (allowable range ± 30 min) for the 4-h postprandial test.

The member (exploratory biomarker evaluation) of Steering Committeewill evaluate the results from panels of blood proteins, lipids, and unsaturated fatty acid metabolites.

Table 9-b Exploratory biomarker evaluation

(fasting)	(4 h postprandial)

Panel of blood proteins: PAI1, TFPI, TNF-α, IL-1, IL-6, IL-8, IL-10, INFγ, MCP-1, ICAM1, VCAM1, eSelectin, serum amyloid A (SAA), pentraxin 3 (PTX3), FABP4, leptin, adiponectin **Panel of unsaturated fatty acid metabolites:** EPA metabolites (18-HEPE, 5-HEPE, Resolvin E1) DHA metabolites [17-HDHA (17-HDoHE), 7-HDHA (7-HDoHE), Resolvin D1, Resolvin D2, Resolvin D3, Maresin 1] Arachidonic acid metabolites (PGE2, PGD2, PGF2α, TXB2, 6-keto-PGF1α, LTB4) **Panel of lipids:** Phospholipids (glycerophospholipid (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, etc.), sphingophospholipids (sphingomyelin, etc.)), sphingolipid (ceramide, ganglioside, sulfatides, etc.), neutral lipids (monoacylglycerol, diacylglycerol, triacylglycerol, cholesteryl esters, dolichol, etc.), fatty acids (free fatty acids), acylcarnitine, bile acids, ubiquinone, and molecular species related to these biosynthesis and metabolism	**Panel of lipids:** Phospholipids (glycerophospholipid (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, etc.), sphingophospholipids (sphingomyelin, etc.)), sphingolipid (ceramide, ganglioside, sulfatides, etc.), neutral lipids (monoacylglycerol, diacylglycerol, triacylglycerol, cholesteryl esters, dolichol, etc.), fatty acids (free fatty acids), acylcarnitine, bile acids, ubiquinone, and molecular species related to these biosynthesis and metabolism

9.1.11 Flow-mediated dilation (FMD) test

FMD tests shall be performed according to the observation schedule (Appendix A) based on the Procedure for FMD Measurement provided separately. The fasting test will be performed under ≥10-h fasting and the 4-h postprandial test will be performed 4 h after consumption of a prescribed meal (allowable range ±30 min). FMD measurement will be performed by a physician or laboratory technician who completed the FMD measurement training.

9.1.12 Record of research subjects who are withdrawn before randomization

A CFR shall be created for all research subjects who have signed the consent form and withdrawn before randomization.

The following items are to be described on the CRF.

- Date of consent obtainment
- Date of birth
- Sex
- Eligibility
- PTE/adverse events (with details, if any)
- Reason for discontinuation

The primary reason for withdrawal before randomization shall be recorded on the CRF according to the following classification:

- PTE adverse events

- Not satisfying at least one of the inclusion criteria or meeting any of the exclusion criteria
- Serious deviation from the protocol
- Lost to follow-up
- Voluntary discontinuation (specify the reason)
- Discontinuation of the entire study
- Others (specify the reason)

Research subject ID numbers assigned to research subjects withdrawn from the research before {randomization shall not be reused.

9.1.13 Record of randomization

Research subjects to be randomized shall meet all of the inclusion criteria and shall not meet any of the exclusion criteria according to Section 8.2. The principal investigator or investigator shall specify the reason why the subject cannot be randomized / transferred to the treatment period.

9.2 Drug-taking status of the research subjects

The principal investigator or investigator shall confirm the drug-taking status of the study drug of the research subject at every visit. Throughout the clinical research period, drug-taking status will be checked through interview for two categories, “compliance of at least 50% of the prescribed dose or compliance of < 50% of the prescribed dose.” If poor compliance with study treatment (e.g., < 50% of the prescribed dose) has been found through the interview and does not improve, the research subject may be withdrawn from the research if appropriate for the circumstances.

9.3 Implementation time point of the test and observation items

The schedule for all tests, observations, and evaluations is shown in Appendix A. The principal investigator or investigator shall perform the tests, observations, and evaluations at the time points shown below.

9.3.1 Observation period (Visit 1)

At Visit 1 (Day -29 to Day -1 before study drug administration), the study shall be explained using the informed consent form to the research subjects who meet the inclusion criteria (except for 2) in Section 7.1 and do not meet any of the exclusion criteria (except for 4 and 7) in Section 7.2, and consent shall be obtained in accordance with Section 15.3. - .

Tests, observations, and evaluations to be performed at Visit 1 (Day -29 to Day -1 before study drug administration) are shown below.

- Informed consent procedure
- Demographic data, medical history, previous treatment drug(s)
- Physical examination

- Height, weight and BMI
- Vital signs
- Concomitant drugs
- Concurrent disease
- Laboratory tests (fasting)
- Exploratory biomarker evaluation (fasting)

Items to be checked: Food and drink consumption before visit:

- Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of visit and food should not be consumed from 9:00 pm on the day before to the time of visit.
- Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

9.3.2 Treatment period (Visit 2)

1. Laboratory tests/FMD measurement

Research subjects shall take the following tests at a designated medical institution for FMD measurement.

- Laboratory tests [fasting and 4-h postprandial (allowable range ±30 min)]
- Exploratory biomarker evaluation [fasting and 4-h postprandial (allowable range ±30 min)]
- FMD test [fasting and 4-h postprandial (allowable range ±30 min)]

Items to be checked: Food and drink consumption before visit

- Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of visit and food should not be consumed from 9:00 pm on the day before to the time of visit.
- Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

Research subjects shall visit the study site after undergoing prescribed tests at a designated medical institution for FMD measurements.

2. Implementation of examination

Based on the results of the tests/observations at Visit 1 (Day -29 to Day -1 before the start of study drug administration) and the FMD test results (fasting) at Visit 2 (Day -15 to Day -1 before the start of study drug administration), the principal investigator or the investigator shall perform the following tests, observations, and evaluations in research subjects who meet the inclusion criteria and do not meet any of the exclusion criteria.

- Physical examination
- Vital signs
- Concomitant drugs
- Pretreatment Event

The principal investigator or the investigator shall give notification to the registration center (accessing to the Case Registration Web System) regarding research subjects in whom all the tests at Visit 2 (Day -15 to Day -1 before study drug administration), observation results, and evaluation have been completed and study drug administration was considered possible.
The principal investigator or the investigator shall prescribe the study drug at a dose notified from the registration center and tell the research subject to take the drug starting from the next day.

The research subject with a fasting FMD level of 0% shall be withdrawn from this study based on Section 7.2.
Refer to Section 9.1.11 for preparation of records relating to the research subjects withdrawn before randomization.

9.3.3 Treatment period (Visit 3)

1. Research subjects shall undergo the following tests at Visit 3 (Day 14 to Day 41 after the study drug administration) at a designated medical institution for FMD measurements.
   - FMD test (fasting)
   - Laboratory tests [fasting and 4-h postprandial (allowable range ± 30 min)]
   - Exploratory biomarker evaluation [fasting and 4-h postprandial (allowable range ±30 min)]

   Food and drink consumption before the visit will be checked.
   - Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of visit and food should not be consumed from 9:00 pm on the day before to the time of visit.
   - Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

2. The principal investigator and the investigator shall perform the following tests, observations, and evaluations at Visit 3 (Day 14 to Day 41 after the study drug administration).
   - Physical examination
   - Vital signs
   - Concomitant drugs

- Study drug compliance
- Adverse Events

Tests, observations, and evaluations of (1) and (2) at Visit 3 may be performed in any order [so long as the completion of all the contents falls within the allowable range (Day 14 to Day 41 after the study drug administration)].

9.3.4 Completion (Visit 4) or discontinuation

1. Visit 4 shall be the last VISIT, and research subjects shall undergo the following tests at the designated medical institution for FMD measurement within the allowable range of Visit 4 (42 to 70 days after the study drug administration). If the study drug administration is discontinued, research subjects should, to the extent possible, undergo tests within 3 days from the last dose taken.
   - FMD test [fasting and 4-h postprandial (allowable range ± 30 min)]
   - Laboratory tests [fasting and 4-h postprandial (allowable range ± 30 min)]
   - Exploratory biomarker evaluation [fasting and 4-h postprandial (allowable range ±30 min)]

   Food and drink consumption before the visit will be checked.
   - Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of visit and food should not be consumed from 9:00 pm on the day before to the time of visit.
   - Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

2. The principal investigator and the investigator shall perform the following tests, observations, and evaluations at Visit 4 (Day 42 to Day 70 days after the study drug administration). If the study drug administration is discontinued, research subjects should, to the extent possible, undergo tests, observations, and evaluations required for the last visit within 3 days from the last dose taken.
   - Physical examination
   - Vital signs
   - Concomitant drugs
   - Study drug compliance
   - Adverse Events

Since the principal investigator or the investigator is required to confirm safety of the tests, observations, and evaluations at Visit 4 or at discontinuation, FMD test and laboratory tests in (1) shall be performed first, and tests, observations, and evaluations in (2) shall be performed last.

At completion of the clinical research, the status of all research subjects administered the study drug shall be recorded on the CRF.

10.0 PRETREATMENT EVENT AND ADVERSE EVENTS

10.1 Definitions

10.1.1 When pretreatment events are collected

A Pretreatment Event (PTE) is defined as any unfavorable medical event that occurs to a research subject prior to administration of study drug but after the acquisition of informed consent. PTEs are not limited to events with clear causal relationship with treatment with the concerned drug.

10.1.2 Adverse events

An adverse event is defined as any untoward medical occurrence in a patient or a research subject receiving a pharmaceutical product (including the study drug). It does not necessarily have an apparent causal relationship with this pharmaceutical product (including study drug).

An adverse event can therefore be any unfavorable and unintended sign (e.g., a clinically significant laboratory abnormality), symptom, or disease temporally associated with the use of a pharmaceutical product (including the study drug), regardless of whether it is considered relate d to the pharmaceutical product (including the study drug) or not.

10.1.3 Considerations for PTEs and adverse events

Generally unfavorable findings are described below:

- Newly diagnosed disease or unexpected aggravation of existing symptom (intermittent event of an existing symptom is not considered a PTE or an adverse event)
- Requiring action or medical practice
- Requiring invasive diagnostic treatment
- Requiring discontinuation or a change in the dose of the study drug or a concomitant medication
- Considered unfavorable by the principal investigator or the investigator

Diagnosis name and signs/symptoms:

PTEs or adverse events shall be recorded by diagnosis name. Accompanying signs (including abnormal laboratory values) and symptoms shall not be recorded as adverse events. If an adverse event could not be expressed by a diagnosis name, the signs or symptoms shall be recorded as the adverse event.

Laboratory test values:

Abnormal laboratory values shall be recorded as adverse events when the principal investigator or the investigator judges the results are clinically problematic (in other words, when certain action or medical practice is required, or when the principal investigator or the investigator judges the change has exceeded the normal physiological variation range of the research subject). Retest and/or continued monitoring of an abnormality are not considered medical practice. Also, repeated or additional conduction of non-invasive tests for verification, evaluation, and monitoring of an abnormality are not considered medical practice.

However, when abnormal laboratory values are the accompanying symptoms of a disease diagnosed as an adverse event (e.g., increased creatinine due to renal dysfunction, etc.), the adverse event shall be handled by its diagnosis name.

Pre-existing conditions (a disease or symptoms that had been present since before before the informed consent) : A disease or symptoms that had been present since before the acquisition of informed consent shall be regarded as a concurrent disease and not an adverse event. When a concurrent medical condition is aggravated, the aggravation shall be determined as an adverse event and the principal investigator or the investigator shall record on the CRF that the adverse event is an aggravation of the concurrent disease (e.g., “aggravation of hypertension,” etc.).

If a research subject has a pre-existing episodic condition (e.g., asthma, epilepsy), each episode shall be recorded as an adverse event if the episodes become more frequent, serious, or severe in nature. If a research subject has a chronic concurrent condition (e.g., cataracts, rheumatoid arthritis), worsening of the condition shall be recorded as adverse event if the degree of the worsening exceeds that which would be expected. The principal investigator or the investigator shall ensure that the adverse event term to be reported represents the change in the condition from baseline (e.g. “worsening of…”).

Worsening of adverse events:
If a research subject experiences a worsening of the adverse event after a change of the study drug, or secondary signs and symptoms are caused by the adverse event, the worsening or the secondary signs and symptoms shall be recorded as a new adverse event on the CRF. The principal investigator or the investigator shall use an adverse event term that explicitly means a change of the condition (e.g., “worsening of…”).

Change of severity of adverse events / severity of PTEs:
If the research subject experiences changes in the severity of an adverse event / a severity of PTE, the event shall be recorded once, at its peak severity.

Previously planned surgery or treatment:
Preplanned surgeries or interventions that were scheduled before study treatment shall not be

considered adverse events. However, when the existing symptom is aggravated to a degree requiring emergency surgery or treatment, the condition or the event shall be considered an adverse event. A concurrent disease that resulted from previously planned surgery shall be reported as an adverse event.

Non-urgent surgery or treatment:
Non-urgent surgery or treatment that does not induce a change in the condition of a research subject (cosmetic surgery, etc.) shall not be considered an adverse event; However, it shall be recorded in the source documents. Concurrent diseases due to a non-urgent surgery shall be reported as an adverse event.

The Insufficient clinical response (lack of efficacy): Insufficient clinical response, efficacy, or pharmacological action shall not be recorded as an adverse event.
The principal investigator or the investigator shall make the distinction between worsening of a pre-existing condition and lack of therapeutic efficacy.

Overdose:
Overdose of any medication without manifested symptoms shall not be recorded as an adverse event, but the overdose shall be recorded on the "Overdose" page of the CRF. Any manifested symptoms shall also be recorded as adverse events on the "Adverse events" of the CRF.

10.1.4 Serious adverse event

Of all the unfavorable medical events that develop with administration of a pharmaceutical product (including study drug) (irrespective of dose), a serious adverse event is an event that:

1. results in death,
2. is life threatening*,
3. requires inpatient hospitalization or prolongation of existing hospitalization,
4. results in persistent or significant disability/incapacity,
5. leads to a congenital anomaly/birth defect, or
6. other medically significant condition: a medically important event that causes a risk to a research subject even if it is not immediately life-threatening and does not result in death or hospitalization, or requires an action or treatment to prevent the results described in 1 to 5 above. In addition, points described in the Takeda Medically Significant Adverse Event List (Table 10 (a)) are included in this section.

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

Table 10-a Takeda Medically Significant AE List

Acute respiratory failure/acute respiratory distress syndrome (ARDS)	Hepatic necrosis
Torsades de pointes/ ventricular fibrillation/ventricular tachycardia	Acute hepatic failure
Malignant hypertension	Anaphylactic shock
Convulsive seizure (including convulsion and epilepsy)	Acute renal failure
Agranulocytosis	Pulmonary hypertension
Aplastic anemia	Pulmonary fibrosis (including interstitial pneumonia)
Toxic epidermal necrolysis/ Oculomucocutaneous syndrome (Stevens-Johnson syndrome)	Neuroleptic malignant syndrome/ malignant hyperpyrexia
	Spontaneous abortion/ stillbirth and fetal death
	Confirmed or suspected transmission of infection by a medicinal product
	Confirmed or suspected endotoxin shock

When a PTE meets any of the above criteria for SAEs, it shall be reported in accordance with a procedure similar to that for serious adverse events (Refer to Section 10.2.2 and 10.3).

10.1.5 Severity of pretreatment events and adverse events

The severity of adverse events shall be classified and defined as shown below.

Mild	The event is transient and easily tolerated by the subject.
Moderate	The event interrupts the subject's usual activities.
Severe	The event causes considerable interference with the subject's usual activities.

10.1.6 Causality of adverse events

The causal relationship of each PTE and adverse event to the study drug shall be classified and defined as shown below.

Related	An adverse event that follows a temporal sequence (including clinical course after discontinuation), or an adverse event in which there is at least a reasonable probability that a causal relationship to the study drug cannot be ruled out, although other factors such as underlying disease, concurrent diseases, or concomitant drugs/treatment are also suspected.
Not	An adverse event that does not follow a temporal sequence from administration of

related	the study drug. Very likely due to other factors such as underlying disease, concurrent diseases, or concomitant drugs/treatment.

10.1.7 Relationship to study procedures

The relationship shall be recorded as "Yes" if the principal investigator or the investigator considers that there is reasonable possibility that an adverse event is due to a study procedure. Otherwise, the relationship shall be recorded as "No."

10.1.8 Date of onset

The date of onset of adverse event shall be determined according to the following rules:

Adverse event, etc.	Date of onset
Signs, symptoms, diseases (diagnoses)	The date on which the first signs/symptoms were noted by the research subject and/or the principal investigator or investigator.
Asymptomatic diseases	The date on which a diagnosis was confirmed through a test(s). The date on which a diagnosis was confirmed, even when the test results indicate an old sign(s) of the disease or an approximate time of its onset.
Exacerbation of concurrent diseases or PTE	The date on which the first worsening of diseases/symptoms was noted by the research subject and/or the principal investigator or investigator.
Onset of a test abnormality after the start of the study drug administration (in case of PTE) (in case of AE)	The date on which a clinically significant laboratory abnormality was detected.
Worsening of a baseline test abnormality after initiation of study treatment	The date on which a clear increase/decrease in a laboratory parameter was clinically confirmed based on the time profile of the parameter.

10.1.9 Date of resolution

The date of resolution of an adverse event, etc. is the date on which the research subject recovered (including resolution with sequelae). If a research subject died due to the adverse event, etc. concerned, it shall be the date of death. The adverse event shall be recorded as "ongoing" if the research subject has not yet recovered by the end of the study.

10.1.10 Actions taken for the study drug

Actions taken for the study drug shall be classified or defined as shown below.

Drug withdrawn	The study drug is discontinued because of an adverse event (including withdrawal by the research subject at his/her own discretion). The case in which the study drug administration is continued after withdrawal from the study shall be categorized into "Dose not changed."
Dose not changed	The dose was not changed after the onset of the adverse event. The study drug was discontinued, reduced, or increased because of another adverse event. The study drug was discontinued or reduced for a reason other than the adverse event, e.g., inadvertence of the research subject.
Unknown	It has not been possible to determine what action has been taken because the research subject is lost to follow-up.
Not Applicable	The administration of the study drug had already been completed or discontinued before the onset of the adverse event.

10.1.11 Outcome

Outcome of adverse events is classified as follows:

Category	Criteria
Recovered	Disappearance or recovery of symptoms and findings Laboratory values returned to normal or baseline
Improved	The intensity is lowered by one or more stages Symptoms or findings mostly disappeared Laboratory values improved, but have not returned to normal or baseline The research subject died from a cause other than the concerned adverse event while the condition was resolving (recording of the date of death unnecessary)
Not recovered	No change in symptoms, findings, or laboratory data The symptoms, findings, or laboratory data on the final day of observable period were aggravated compared with the date of onset Irreversible congenital anomaly The research subject died from another cause before resolution of the concerned adverse event (recording of the date of death unnecessary)
Recovered with sequelae	Disability that disturbs daily life
Death	Direct relationship between death and the concerned adverse event "Direct relationship" means that the concerned adverse event was the cause of death, or the concerned adverse event was clearly responsible for death. Outcome of an adverse event which was not determined (judged, presumed) a direct cause of death observed in the same research subject is not considered as death. The date of death shall be recorded.
Unknown	Follow-up specified in the protocol after the date of onset was not possible due to

	change of hospitals or relocation, etc.

10.2 Procedures

10.2.1 Collection and reporting of adverse events

10.2.1.1 Adverse event collection period

The collection of PTEs shall start after the acquisition of informed consent and continue until the start of the study drug administration at Visit 2 (Day -15 to Day -1 before the start of study drug administration). If withdrawal was decided before the start of administration of the study drug, the collection of PTEs shall be terminated at that point.

Collection of the adverse events shall commence at the start of administration of the study drug to research subjects [at Visit 2 (Day -15 to Day -1 before the start of study drug administration)] and continue until completion of the treatment period [Visit 4 (Week 8)] or at discontinuation.

10.2.1.2 Reporting of adverse events

At each study visit, the principal investigator or investigator shall check for the presence of any onset of subjective symptoms. A neutral question, such as “How have you been feeling since your last visit?” may be asked to collect any adverse events that occurred between the previous and present visits.

When a PTE corresponds to a serious criterion, the principal investigator or investigator shall follow up all research subjects experiencing the PTE until the symptom resolve, any clinically significant abnormal laboratory values have returned to the baseline after acquisition of informed consent, or there is a satisfactory explanation for the change (permanent and irreversible PTEs). When the PTE does not correspond to a serious criterion, irrespective of the causal relationship with the procedure, follow-up of the research subject is not required by the protocol.

The principal investigator or investigator shall follow up all research subjects experiencing an adverse event irrespective of the causal relationship with the study drug, until the symptom resolve, or any clinically significant abnormal laboratory values have returned to baseline or there is a satisfactory explanation for the change (permanent and irreversible adverse events, etc.). All adverse events shall be recorded in the CRF. Name of adverse event, date of onset, date of resolution, grade, causal relationship with the study drug (i.e. "Unrelated" or "Related"), action taken for the study drug, outcome, relationship to study procedures, and severity shall be recorded.

Follow-up period of PTEs meeting any of the criteria for AEs and SAEs shall be until recovery of the adverse events, or the time when the principal investigator or investigator judges that further follow-up would be unnecessary.

10.2.2 Collection and reporting of serious adverse events

When a serious adverse event develops during the period of collecting adverse events, it shall be reported according to the following procedures. When a serious PTE develops, it shall also be reported in accordance with a procedure similar to that for serious adverse events.

At the time of onset of a serious adverse event or after notification of the onset by the research subject, the principal investigator shall report the serious adverse event to the head of the study site immediately, and the sponsor or CRO to whom the sponsor has entrusted responsibility shall notify the principal investigator of the study site.

The principal investigator shall then report the serious adverse event to the sponsor (for the contact information, refer to the attachment) within 1 working day of after notification of the onset. Further, the investigator shall submit a formal report within 10 calendar days to the sponsor.

Furthermore, it shall be mandatory to include the contents below in the report to be submitted to the sponsor within 1 working day, and other items shall be reported as far as possible.

- Brief description of adverse event and the reason for why it was determined as serious
- Research subject ID number
- Name of investigator or the subinvestigator
- Name of the study drug
- Determined causal relationship

10.2.3 Reporting of additional information concerning adverse events

If the sponsor requests provision of additional information concerning adverse events for reporting to regulatory authorities, the investigator or the subinvestigator shall confirm the necessary additional information and enter in the EDC system or submit a report within the period specified by the sponsor.

10.3 Follow-up of serious adverse events

When information that was not included in the detailed report was obtained later, the investigator or subinvestigator shall state it in the copy of the report on serious adverse events, or create another document and submit it to the contact address shown on the attached sheet. Relevant data collected

at the research implementing entity (e.g., ECG charts, laboratory test values, discharge summary, postmortem results) shall be sent to the sponsor or the committee such as the IEC upon request.

The investigator or the subinvestigator shall follow-up all serious adverse events, etc., until recovery is confirmed, or the final outcome is determined. In addition, serious PTEs shall also be followed, in accordance with procedure similar to that for serious adverse events.

10.3.1 Reporting of serious adverse events, etc., to Ethics Review Committee, etc., and regulatory authorities

When the head of study site receives a report of a serious adverse event from the principal investigator, the head of study site shall consult the Ethics Review Committee, etc., and notify the study sites that are conducting the clinical research through the sponsor or the CRO consigned by the sponsor.

When the principal investigator reported a serious adverse event for which a causal relationship to the research (study drug) cannot be ruled out and is unexpected, the head of the study site shall prepare a written report of the unexpected serious adverse event containing the information reported by the principal investigator plus the information below, and submit the report to the Minister of Health, Labour and Welfare, and notify other study sites conducting the clinical research. (The chief executive of the research implementing entity may report it to the Minister of Health, Labour and Welfare via the sponsor, and notify it to other clinical research implementing entities via the sponsor.) Serious PTEs shall also be reported in accordance with the same procedure as that for the serious adverse events. (No need to report to the Minister of Health, Labour and Welfare.)

- Actions taken for serious adverse events (discontinuation of new enrollment, revision of informed consent form, re-consents to other research subjects, etc.)
- Date of review, summery of review, result, necessary action, etc., related to Ethics Review Committee, etc.
- Notification to other research implementing entities

The sponsor shall report, in accordance with regulations, unexpected serious adverse drug reactions and other serious adverse events that are subject to emergency reporting to regulatory authorities, the investigators, and the head of study site.

From the time point of first acknowledging the event or receiving additional information, the sponsor or the CRO consigned by the sponsor shall comply with regulatory required time frames for reporting, and make emergency reports concerning unexpected serious adverse drug reactions and expected serious adverse drug reactions to regulatory authorities. Also, the sponsor shall, in the same way, make an emergency report of other critical safety information that may have a major effect on

the study drug risk-benefit, continuation of study drug administration, or continuation of clinical research. The research implementing entity shall submit copies of emergency report documents to the Ethics Review Committee, etc.

11.0 COMMITTEES ESTABLISHED FOR THIS STUDY

11.1 Clinical Endpoint Committee

The Central Assessment Committee members shall be independent specialists who have appropriate experience in the assessment of the parameters set as the endpoints of this research.

REGARDING THE PRIMARY ENDPOINT “%FMD (FASTING)” AND THE SECONDARY ENDPOINT “%FMD (4 H POSTPRANDIAL)”,THE CENTRAL ASSESSMENT COMMITTEE SHALL CONFIRM THE VALIDITY OF THE ASSESSMENT OF THE MEASUREMENT RESULTS ACCORDING TO THE PROCEDURE defined separately on the measured images reported from the FMD measuring study sites or measuring institutes.

The assessment results from the committee members shall be entered into the clinical research database and used for the analysis of endpoints.

12.0 DATA MANAGEMENT AND STORAGE OF RECORDS

Data management operations shall be performed according to the standard operating procedure by the data management department of the sponsor independent from the medical affairs department. Adverse events, PTE, medical history and concurrent disease shall be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Drugs shall be coded using the WHO (World Health Organization) Drug Dictionary.

12.1 Case report form

The principal investigator or investigator shall complete a CRF for each research subject who has signed the informed consent form.

The sponsor or the designee shall provide access rights to the electric CRF system to the study site. Before use of the electric CRF system, the sponsor shall provide training to the principal investigator, investigators, and study collaborators. The CRF shall be used to report the information collected during the study period to the sponsor. CRF must be completed in Japanese. Data shall be directly entered in preparing the CRF.

A change or correction of the CRF shall be recorded as an audit trail that records the information before and after the change or correction, the person who made the change or correction, date of change or correction, and its reason.

The investigator the designee shall ensure the accuracy and completeness of the CRF, and provide an electronic signature on the relevant page of the case report form. The principal investigator shall bear full responsibility for the accuracy and reliability of all data entered on the CRF.

The following data shall be recorded on the CRF directly (except for the data included in the source documents).

- Eligibility, completioin status, reason for discontinuation, the seriousness, severity, relation to study drug and study procedures outcome

The following data shall not be recorded directly into the CRFs.

- Laboratory test values
- FMD/Vessel caliber measurement results
- Measurement results from panels of blood proteins, unsaturated fatty acid metabolites, and lipids

When the principal investigator or the investigator makes a change or correction in the data entered on the CRF after fixation of clinical data base, a record (Data Clarification Form) of change or correction on the CRF provided by the sponsor shall be used. The principal investigator shall

confirm that the record of change or correction on the CRF is accurate and complete, and sign or write name/affix a seal, and date it.

The sponsor or the designee shall confirm that the CRFs are completed appropriately according to the procedures set by research. The sponsor or the designee shall have access to the medical records of the research subjects and in-house records to ensure the accuracy of the CRF as necessary. The completed CRF is the property of the sponsor, and the principal investigator or investigator shall not disclose the information to a third party without a written permission from the sponsor.

12.2 Timing of data entry into the electronic CRF system

The sponsor or the designee shall request the principal investigator and investigator to promptly enter data into the EDC following enrolment of the research subject, each visit during study treatment, completion/discontinuation of the study, and follow-up period.

12.3 Storage of records

The principal investigator or the chief executive of the study site shall store the following materials, including those specified in Section 12.1, and study-specific documents to be investigated or audited by the regulatory authority and the sponsor or the designee. The documents include research subject ID code, medical records, clinical study worksheets (if used), original signed and dated informed consent forms, the record of change or correction on the CRF (copy), and electric copies of electronic CRF including audit trail. The principal investigator and the chief executive of the study site shall appropriately retain the material/information related to this study for at least 5 years from the date of reporting the end of the research by the principal investigator, or for 3 years from the date of reporting final publication of the study result, whichever date is later. However, when the sponsor requires a longer storage period, the chief executive of the study site shall discuss the period and methods of storage with the sponsor.

13.0 STATISTICAL ANALYSIS METHODS

The person in charge of analysis and the designee [analysis personnel, who belongs to contract research organization (CRO) independent from the sponsor] shall perform the statistical analysis. The sponsor will not be involved in the statistical analysis.

13.1 Statistical and analytical plans

The analysis personnel shall prepare a statistical analysis plan before the acquisition of the informed consent of the earliest research subject, and issue the first edition. Detailed definition of endpoints and analytical methods should be specified in the SAP to deal with all the purposes of the research.

13.1.1 Analysis set

Two analysis sets, “Full Analysis Set；FAS” and “Safety Analysis Set；SAS” are used in this research. The FAS primarily used for efficacy analysis will be defined as “research subjects who have been randomized and received the study drug at least once”, and the SAS as “research subjects who have received the study drug at least once during the clinical research”.

13.1.2 Analysis of demographic and other baseline characteristics

From “SAS” primary research subject background items will be tabulated.

13.1.3 Efficacy analysis

From FAS the following shall be analyzed.

13.1.3.1 Analysis of primary endpoints

%FMD(fasting)

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.
2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.
3) Using fasting TG at the start of treatment, a stratified analysis on summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be performed.

13.1.3.2 Analysis of secondary endpoints

① %FMD (4 h postprandial)

② TG level (fasting)

③ TG level (4 h postprandial)

④ Plasma fatty acid fraction

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.

3) Using fasting TG at the start of treatment, a stratified analysis on summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be performed.

13.1.3.3 Analysis of other endpoints

Efficacy endpoints:

Total cholesterol, LDL-C, LDL-C, Remnant-like particle (RLP) cholesterol , Apoprotein B-48, C-reactive protein (CRP), 8-epi-PGF2α quantitative (urinary)

Analytical method

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.

Exploratory endpoints:

Exploratory biomarker evaluation

(1) Panel of blood proteins

PAI1, TFPI, TNF-α, IL-1, IL-6, IL-8, IL-10, INFγ, MCP-1, ICAM1, VCAM1, eSelectin, serum amyloid A (SAA), pentraxin 3 (PTX3), FABP4, leptin, adiponectin

(2) Panel of unsaturated fatty acid metabolites

EPA metabolites (18-HEPE, 5-HEPE, Resolvin E1), DHA metabolites [17-HDHA (17-HDoHE), 7-HDHA (7-HDoHE), Resolvin D1, Resolvin D2, Resolvin D3, Maresin 1], and Arachidonic acid metabolites (PGE2, PGD2, PGF2α, TXB2, 6-keto-PGF1α, LTB4)

Analytical method

1. Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the observation and treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.
2. The change for each treatment group (Treatment Period Visit 2, Visit 3 or Visit 4 - Treatment Period Visit 1) will be calculated at each evaluation time point during the observation and treatment period and the same analysis as in 1) will be performed.
3. The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed. Moreover, the correlation coefficient will be calculated with the change of % FMD for each treatment group and drawn a scatter plot.

(3) Panel of lipids (fasting)

Phospholipids (glycerophospholipid (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, etc.), sphingophospholipids (sphingomyelin, etc.)), sphingolipid (ceramide, ganglioside, sulfatides, etc.), neutral lipids (monoacylglycerol, diacylglycerol, triacylglycerol, cholesteryl esters, dolichol, etc.), fatty acids (free fatty acids), acylcarnitine, bile acids, ubiquinone, and molecular species related to these biosynthesis and metabolism

Analytical method

1. Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the observation and treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.
2. The change for each treatment group (Treatment Period Visit 2, Visit 3 or Visit 4 - Treatment Period Visit 1) will be calculated at each evaluation time point during the observation and treatment period and the same analysis as in 1) will be performed.
3. The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed. Moreover, the correlation coefficient will be calculated with the change of % FMD for each treatment group, and scatter plots will be drawn.

(4) Panel of lipids (4 h postprandial)

Phospholipids (glycerophospholipid (phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, etc.), sphingophospholipids (sphingomyelin, etc.)), sphingolipid (ceramide, ganglioside, sulfatides, etc.), neutral lipids (monoacylglycerol, diacylglycerol, triacylglycerol, cholesteryl esters, dolichol, etc.), fatty acids (free fatty acids), acylcarnitine, bile acids, ubiquinone, and molecular species related to these biosynthesis and metabolism

Analytical method

1. Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the observation and treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2. The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed. Moreover, the correlation coefficient will be calculated with the change of % FMD for each treatment group, and scatter plots will be drawn.

13.1.4 Safety analysis

From Safety Analysis Set (SAS) the following will be analyzed.

(1) Adverse events

The following analysis will be performed for each treatment group. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by System Organ Class and Preferred Term.

- Tabulation of frequencies of all adverse events
- Tabulation of frequency of adverse events with a causal relationship to the study drug
- Tabulation of frequency of all adverse events by severity
- Tabulation of frequency of adverse events with a causal relationship to the study drug by severity
- Tabulation of frequency of adverse events leading to study drug discontinuation
- Tabulation of frequency of serious adverse events
- Tabulation of frequency of all adverse events by time of onset

(2) Body weight, blood pressure in the sitting position, pulse in the sitting position, laboratory tests [fasting plasma glucose (FPG)]

1) Summary statistics of measurements by treatment group at each evaluation time point during the treatment period will be calculated and a diagram of the change of individual data will be created.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.

3) Regarding evaluation results based on standard values, shift tables of treatment period Visit 2 and assessment time points of treatment period (Visit 3, Visit 4) will be prepared.

13.2 Criteria for interim analysis and premature discontinuation

No interim analysis is planned.

13.3 Determination of the number of planned research subject

40 patients evaluable for the primary endpoint

(Omega-3-acid ethyl esters 2 g: 20 patients, Omega-3-acid ethyl esters 4 g: 20 patients)

Exploratory biomarkers will be measured only for the research subjects from whom blood is taken during the observation period after approval of the revised protocol (the 2nd edition) by the committee such as the IEC.

[Rationale for the number of planned research subjects]

The planned sample size is based on the feasibility to explore the effects of omega-3-acid ethyl esters on vascular endothelial function.

14.0 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring of the study site

The sponsor or the designee shall perform periodic monitoring of the study site during the research to confirm that the research is conducted in accordance with all specifications in the research protocol. In the monitoring, the data recorded on the CRF will be checked by comparing them with those in the source documents. Source documents are the original documents, data and records. The principal investigator and the chief executive of the study site shall ensure that the sponsor or the designee and the Ethics Review Committee, etc., have access to the source documents.

The sponsor or the designee shall access the records, including the list of research subject ID numbers, medical records of the research subjects, and signed and dated original consent forms to confirm that the research is appropriately conducted in compliance with the research protocol. Also, confirm the consistency between CRF and the related source documents. The principal investigator, investigator, and other personnel involved in the research shall spare sufficient time to facilitate monitoring procedures during visits to the study site.

Detailed procedures for monitoring shall be described separately in the procedure.

14.2 Deviation from the Ethical Guidelines for Medical and Health Research Involving Human Subjects and the research protocol.

The principal investigator or investigator shall record all deviations from Ethical Guidelines for Medical and Health Research Involving Human Subjects, and research protocol.

If any deviation is found, the principal investigator shall promptly notify the chief executive of the study site for the clinical research and the sponsor. As necessary, the principal investigator will discuss protocol revisions with the sponsor to reach agreement. For protocol revisions, draft revisions should be submitted as early as possible to the study site for approval of the committee such as the IEC.

14.3 Quality assurance audits and regulatory agency inspections

The study site may be research subject to audits by the sponsor or the designee. In such a case, the auditor designated by the sponsor shall contact the study site in advance to determine the date of audit. The auditor may ask to visit the facilities where laboratory specimens are collected and any other facilities used during the clinical research. In addition, this research may be inspected by regulatory agencies, including those of foreign governments (e.g., the Food and Drug Administration [FDA], the United Kingdom Medicines and Healthcare products Regulatory Agency [MHRA]). If the study site is contacted for an inspection by a regulatory body, the sponsor should be notified

promptly. The principal investigator and the chief executive of the study site shall ensure that the auditor has access to all the research-related source documents.

15.0 ETHICAL CONDUCT OF CLINICAL RESEARCH

This research shall be conducted with the highest respect for the individual participants (i.e., research subjects) according to the research protocol, and the ethical principles that have their origin in the Declaration of Helsinki, Ethical Guidelines for Medical and Health Research Involving Human Subjects. Each principal investigator will conduct the study according to regulatory requirements and in accordance with "Responsibilities of the Investigator" in Appendix B.

15.1 Approval of the Ethical Review Board, etc.

The Ethical Review Board, etc., shall be constituted in accordance with the regulations.

The sponsor or the designee should obtain the document listing the name and title of each committee member. When a committee member directly participates in this clinical research, the document describing that he/she is not participating in deliberation or voting for the study will be obtained.

The sponsor or the designee shall supply relevant documents for submission to study site committee such as the Ethics Review Committee for the protocol's review and approval. In addition to the research protocol, a copy of the informed consent form and information sheet, written materials related to research subject recruitment, advertisement, and other documents required by regulations, when necessary, shall be submitted to the central committee or a study site committee such as the Ethics Review Committee to obtain approval. The sponsor or the designee must obtain written approval of the protocol and subject informed consent from the study site committee such as the Ethics Review Committee before commencement of the study. The study site committee such as the Ethics Review Committee's approval must refer to the study by exact protocol title, number and version date; identify versions of other documents (e.g., informed consent form) reviewed; and state the approval date. The sponsor shall notify the study site, the principal investigator, and investigator after confirming the validity of the regulatory documents of the study site. Protocol procedures such as obtainment of consent shall not be started until the study site, the principal investigator, and investigator receive notification.

The study site shall observe all requirements that the Ethical Review Board, etc. prescribe. The requirements may include notifications to committees such as the IEC, for example, revision of the protocol, revision of the informed consent form and information sheet, revision of materials related to research subject recruitment, reports on safety in accordance with the regulatory requirements, reports on status of implementation of the research at intervals determined by a study site committee such as the Ethics Review Committee, and submission of the study completion report. The sponsor or the designee shall obtain written approval from the Ethics Review Committee, etc. related to the above mentioned items and all related materials.

15.2 Conflict of interest

These clinical studies shall be conducted with the support of the research client.

Prior to the conduction of this clinical research, the principal investigators involved in this clinical research shall ensure appropriate management of any conflicts (COI) in the conduct of the research in accordance with the rules of the study site.[8)-12)]

The study site shall observe all requirements that the Ethical Review Board, etc. prescribe. This will include self-declaration of COI, clinical research protocol, informed consent and information sheet.

15.3 Informed consent and information sheet, and the agreement of the research subjects

The informed consent and information sheet form shall contain specific requirements of the Declaration of Helsinki, Ethical Guidelines for Medical and Health Research Involving Human Subjects and all applicable laws and regulations. The informed consent form and information sheet shall specify the use of personal information and medical information of research subjects in this clinical research (both in and outside Japan: supply to a third party), and disclosure. The informed consent form and the information sheet will explain in detail the nature of the research, its objectives, and potential risks and benefits. The informed consent form and the information sheet will detail the requirements of the participant and the fact that research subject is free to withdraw at any time without giving a reason and without any negative effect on further medical care.

The principal investigator is responsible for the preparation, contents, and approval of the informed consent form and research subject information sheet by the committee such as the IEC. The informed consent form and information sheet must be approved by the committee prior to use.

The informed consent form and information sheet shall be written in language that can be easily understood by the potential research subjects. The principal investigator or investigator shall be responsible for providing detailed explanation of the informed consent form and information sheet to the potential subjects. Information should be given in both oral and written form whenever possible and in manner deemed appropriate by the committee such as the IEC.

The principal investigator or investigator must (1) give the opportunity to ask questions and (2) sufficient time to consider whether to participate in the study to the potential subjects. If the potential subject decides to participate, the informed consent form must be signed and dated by the potential subject prior to entering into the research. The principal investigator or investigator shall instruct the potential subject or representative to sign using their legal names, not nicknames, using a blue or black ball point ink pen. Also the principal investigator or investigator shall sign and date the informed consent form prior to entering into the research.

Once signed, the original informed consent form shall be retained by the principal investigator or investigator. The principal investigator or investigator shall record the date that the potential research subject signed the informed consent form in the subject's medical record. A copy of the signed informed consent form shall be given to the research subject.

The principal investigator or investigator shall follow the same procedure as for obtaining the initial consent when newly obtaining re-consent from the concerned research subject when the informed consent form is revised. The date of obtaining new consent shall be recorded in the research subject's medical record, and a copy of the revised consent form shall be provided to the research subject.

Exploratory biomarkers will be measured only for the research subjects from whom blood is taken during the observation period after approval of the revised protocol (the 2nd edition) and the revised informed consent form by the committee such as the IEC. Exploratory biomarkers should not be measured for the research subjects from whom blood collection is completed before the approval of the revised protocol (the 2nd edition) and the revised informed consent form by the committee such as the IEC, even if a re-written informed consent is obtained from them.

15.4 Personal information of the research subjects

The sponsor or the designee shall affirm the principle of the protection of research subjects' private/personal information. Throughout this study, research subject ID numbers shall be used to link the subject's source data to the sponsor's research database and research-related documents. Limited information on research subjects such as gender, age, and date of birth may be used within the scope of all applicable laws and regulations for identification of research subjects and confirmation of accuracy of research subject ID number.

For verification of the conduct of the research in compliance with this protocol and the Ethical Guidelines for Medical and Health Research Involving Human Subjects, the sponsor shall require the principal investigator to provide the research sponsor's designee, representatives of regulatory authorities, designated auditors, and committees such as the Ethical Review Board direct access to research subjects' original medical records (source data or documents), including laboratory test results, admission and discharge records during a subject's research participation, and autopsy reports. The principal investigator or investigator shall obtain specific authorization of the research subject as part of the informed consent process for access to research subject's original medical records by research sponsor's designee and representatives of regulatory authorities (see Section 15.3).

When providing a copy of source documents to the sponsor, the principal investigator or investigator shall delete information that may lead to identification of an individual (name and address of research subject, other personal information not recorded on the CRF of the research subject).

15.5 Consultation windows for the research subjects or persons related to the research concerned

The principal investigator shall establish a contact service to respond to inquiries concerning this clinical research from research subjects or concerned people. Details of the contacts for inquiries will be described in the ICF.

15.6 Financial burden or reward to the research subjects

Of the expenses for this clinical research, the sponsor shall offer compensation for medical treatment not covered by health insurance as research expenses. The research subjects shall pay expenses for medical treatment covered by ordinary health insurance.

In addition, the principal investigator shall pay expenses such as transportation expenses for participation in this clinical research to the research subjects at each visit from the research funds. Details of the financial burden on the research subjects and rewards shall be described in the informed consent form and information sheet.

15.7 Benefits and inconveniences to the research subjects

15.7.1 Benefits to research subjects

By participating in this clinical research, the research subjects may understand one's own condition of vascular endothelial function in detail.

15.7.2 Inconveniences to research subjects

By participating in this clinical research the burden of the research subject may increase as number of visits will increase compared to daily medical care.

15.8 Attribution of research results and access rights

15.8.1 Attribution of research results

The research results and data obtained from this research shall belong to the sponsor.

15.8.2 Data access rights

Access rights for all data and information generated from this research will be given to personnel approved by the sponsor. In addition, secondary use (meta-analysis, etc.) of the data obtained in this

clinical research may be possible if used in such a way that the data shall not be linked to personal identification information.

15.9 Reporting of results, Publication, disclosure, and clinical research registration policy

15.9.1 Reporting of results, publication and disclosure

The principal investigator shall report a written summary of results of the research to the chief executive of the study site and provide the sponsor with all the results and data obtained from the research. Only the sponsor may disclose the research information to other principal investigators, investigators or regulatory authorities during the research period, except when required by laws and regulations. The sponsor shall be responsible for publication of the research protocol and research-related results (including the public web site) except for other cases permitted in the research contract.

During research period and after the end of research, the sponsor or the designee should promptly summarize the results and present it to medical journals and academic conferences, etc. The sponsor may publish any data or information obtained from the research (including data and information provided by the principal investigator) without obtaining agreement of the principal investigator.

The investigator or the subinvestigator should obtain the prior written approval from the sponsor when publishing the information obtained in this research at an academic conference, etc.

15.9.2 Clinical research registration

To ensure that information on clinical research is made accessible to the public in a timely manner and to comply with applicable laws, regulations, and guidelines, Takeda Pharmaceutical Company Limited shall register all clinical research being conducted in patients around the world at public trial registration sites, including at least the website(s) of ClinicalTrials.gov (and) Japan Pharmaceutical Information Center Clinical Trials Information (JAPIC), before initiation of the clinical research. On such websites, the research location (city, country), subject recruitment status, and contact information for Takeda Pharmaceutical Company Limited are open to the public.

15.9.3 Clinical trial results disclosure

Takeda Pharmaceutical Company Limited shall post the research results, irrespective of the nature of the results, at the public trial registration site(s) of Clinical Trials.gov (and) JAPIC in accordance with applicable laws and regulations.

15.10 Insurance and compensation for injury

In case of injuries, each research subject in the clinical research must be insured in accordance with the regulations applicable to the study site where the subject is participating. The sponsor or the designee shall buy an insurance policy to compensate for health injury in research subjects.

Healthy injury in a research subject will be compensated as specified in the study contract. Compensation-related questions by the principal investigator or investigators should be made to the sponsor or the designee.

16.0 REFERENCES

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9. Guidelines for determining the conflict of interest polity for clinical research (Review group for ethical aspects of clinical research and conflict of interest, March 2006)

10. Guidelines on the Management of COI in Health and Labor Scirences Research (No. 0331001, science, issued on March 31, 2008, and approved by the Chief of the Health Sciences Division)

11. Guidelines for management of COI in medical research (COI Committee of Japan Association of Medical Sciences, February 2011)

12. Common guidelines for conflict of interest (COI) in clinical research (Japanese Society of Internal Medicine, Japan Society of Hematology, Japanese Circulation Society, Japan Endocrine Society, Japan Diabetes Society, Japanese Respiratory Society, Japanese Society of Hematology, Japanese Society of Allergology, Japanese Association for Infectious Diseases, Aug 2011)

Appendix A Schedule for Research Procedures

		Observation period	Treatment period			Discontinuation [g]
Time of visit	Week	-4	0	4	8	
	Day	-29	-1[f]	28	56	
Allowable range (Day)		-29 to -1	-15 to -1	14 to 41	42 to 70	Up to 3 days after the last dose
VISIT Number		1	2	3	4	
Informed consent procedure		X				
Inclusion/Exclusion criteria		X	X			
Demographic data, medical history, previous therapeutic drugs		X	X[h]			
Physical examination		X[d]	X	X	X	X
Confirmation of food and drink consumption before visit [a]		X	X	X	X	X
Height		X				
Body weight		X	X	X	X	X
BMI		X				
Vital signs		X[d]	X	X	X	X
Concomitant drugs [b]		X[d]	X	X	X	X
Concurrent disease		X[d]				
Laboratory tests						
Serum chemistry (fasting)		X[d]	X	X	X	X
Serum chemistry (4 h postprandial)[c]			X	X	X	X
Urinalysis (fasting)		X[d]	X	X	X	X
Exploratory biomarker evaluation						
Panel of blood proteins		X[d]	X	X	X	X
Panel of unsaturated fatty acid metabolites		X[d]	X	X	X	X
Panel of lipids (fasting)		X[d]	X	X	X	X
Panel of lipids (4 h postprandial)[c]			X	X	X	X
FMD						
FMD (fasting)			X[e]	X[e]	X[e]	X[e]
FMD (4 h postprandial) [c]			X[e]		X[e]	X[e]
Prescription of the study drug			X	X		
Drug compliance				X	X	X
PTE and adverse events assessment		(X)	←X→	←X→	←X	←X

(a) Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

(b) All concomitant drugs will be recorded.

(c) Time window ± 30 minutes

(d)To be performed before test at Visit 2

(e) To be performed by the physician or the clinical laboratory technician who has finished the FMD measurement training

(f) To be performed after consent has been obtained. Medication will be started the day after the completion of all the tests of Week 0. The start date of medication will be referred to as day 1.

(g) To be performed to the extent possible

(h) To be investigated only for "FMD/Vessel caliber measurement results" obtained from the FMP measurement before fasting

Appendix B Responsibilities of the Principal Investigator

1. Appropriately To appropriately conduct the clinical research in compliance with this research protocol and the Ethical Guidelines for Medical and Health Research Involving Human Subjects and with the highest respect for human rights, safety, and welfare of research subjects.
2. Prepare To prepare a list of any other investigators and/or research collaborators when certain important research-related activities are divided by investigators and/or research collaborators, and submit the list to the sponsor as required.
3. To prepare the informed consent form and revise it as necessary.
4. To check the contents of the study contract.
5. To provide sufficient information on the protocol, drug and duties of each personnel to subinvestigators and study collaborators, and give guidance and supervision.
6. To select research subjects who satisfy the inclusion criteria, give explanation using written information, and obtain consent in writing.
7. To be responsible for all medical judgments related to the research.
8. Corresponding to request from the chief executive of the study site, to report the latest progress status at least once a year to the chief executive of the study site.
9. To confirm and comprehended the most update status regarding the COI of the investigators participating in the clinical research according to the study site.
10. To ensure, together with the chief executive of the study site, that sufficient medical care is provided to research subjects for all research-related clinically problematic adverse events throughout the period of subjects' research participation and thereafter.
11. When a research subject is treated at another medical institution or department, to inform the acting physician at the medical institution or department in writing of the research subject's study participation and research completion/discontinuation after obtaining the research subject's consent, and prepare a record.
12. When emergency reporting of serious adverse events, etc., is required, to immediately report it in writing to the chief executive of the study site and the sponsor.
13. Prepare correct and complete CRFs, and submit them to the sponsor with an electronic signature.
14. To verify any entries on the CRFs prepared by the investigator or transcribed by the collaborator from source data, {electronically sign/ sign or write name/affix a seal, and submit them to the sponsor.
15. To discuss a revision of the protocol, etc., when proposed by the sponsor.
16. To report the research completion in writing to the chief executive of the study site.

PROTOCOL

Exploratory study of the effect of omega-3-acid ethyl esters on vascular endothelial function in patients with hyperlipidemia by flow mediated dilation

(Oasis Flow)

Sponsor	Takeda Pharmaceutical Company Limited 12-10 Nihonbashi 2-chome, Chuo-ku, Tokyo
Protocol number	TAK-085-4001
Version number/Revision number	First edition
Study drug:	Omega-3-acid ethyl esters
Creation date	March 29, 2016

CONFIDENTIAL PROPERTY

This document is a confidential communication of Takeda. Acceptance of this document constitutes agreement by the potential recipient of the drug to be administered that no information contained herein will be published or disclosed without written authorization from Takeda except to the extent necessary to obtain informed consent from those research subjects to whom the drug may be administered. Furthermore, the information is only intended for review and compliance by the recipient, his or her staff, and applicable institutional review committee and regulatory agencies to enable conduction of the study.

1.0 STUDY ADMINISTRATIVE INFORMATION AND CLINICAL STUDY PRINCIPLES

1.1 Clinical study principles

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- Ethical Guideline for Clinical Research (the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare, December 22, 2014).
- Good Clinical Practice: Consolidated Guideline (ICH: International Conference on Harmonization of Technical Requirement for Registration on Pharmaceuticals for Human Use. E6)
- All applicable laws and regulations, including, without limitation, data privacy laws and conflict of interest guidelines.

1.2 Clinical study implementation system

This study will be conducted in accordance with the requirements of this clinical study protocol designed and prepared by the sponsor and also in accordance with the following:

Sponsor

Takeda Pharmaceutical Company Limited

Japan Pharma Business Unit

Strategic Medical Research Planning Group, Medical Affairs Department

The sponsor shall be responsible for matters related to planning/preparation, implementation/operation, and results/reporting in this clinical study. Methods of supervision of the contractor entrusted with the services related to this clinical study will be described in the procedure to be prepared separately.

Expenses* required for the operation of this clinical study will be paid by the sponsor.

* : Based on the “Consignment Service Contract,” expenses incurred for the services of Office of Clinical Research, monitoring, registration/allocation center, statistical processing, and audit

shall be paid to the contractor entrusted with services related to this clinical study. Expenses agreed by the study site shall be paid to the site based on the "Research Expense Standard."

Chair of the Clinical Study Steering Committee:

PPD

The Chair of the Clinical Research Steering Committee shall supervise implementation and reporting of the clinical research, secure medical guidance of a high degree of professionalism and a high-level scientific quality, and revise the research protocol appropriately.

Terms in this protocol are defined as follows:

Study site:

A corporation, governmental agency and sole proprietor conducting the study, excluding cases where only a part of the services related to storage of samples/information, statistical processing and other studies is entrusted.

Collaborative study site:

A study site that conducts collaborative research in accordance with the protocol, including a study site that obtains new samples/information from research subjects and provides other study sites.

Researchers, etc:

Principal investigators and other parties involved in conduction of the study (including operations at institutions involved in collection/distribution of samples/information). Those involved only in providing existing samples/information outside the study sites and those engaged in part of the entrusted operations related to the study are excluded.

Principal Investigator:

A researcher who is engaged in implementation of the study and integrates the operations involved in this study at an affiliated study site.

Chief executive of the study site

A representative of a corporation, head of a governmental agency, or a sole proprietor

Research subject:

A subject (including a dead subject) who meets any of the following:

1. Subjects being studied (including those who have been asked to be studied)
2. Subjects from whom existing samples/information to be used in the study have been obtained.

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2.0 STUDY SUMMARY

<table>
<tr><td>Sponsor:
Takeda Pharmaceutical Company Limited</td><td>Study drug:
Omega-3-acid ethyl esters</td></tr>
<tr><td colspan="2">Study title: Exploratory study of the effect of omega-3-acid ethyl esters on vascular endothelial function in patients with hyperlipidemia</td></tr>
<tr><td colspan="2">Protocol number: TAK-085-4001</td></tr>
<tr><td colspan="2">Clinical research design:
This is a multicenter, collaborative, randomized, open-label study designed to explore the effects of administration of omega-3-acid ethyl esters [2 g (2 g PO QD) or 4 g (2 g PO BID) for 8 weeks] on vascular endothelial function, as measured by flow-mediated dilation (FMD), in patients receiving a hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor and have concurrent hypertriglyceridemia (hereinafter referred to as TG).
Considering the potential bias by factors that affect FMD between treatment groups, stratified allocation will be performed with fasting TG level as a factor.</td></tr>
<tr><td colspan="2">Objectives:
To explore the effects of omega-3-acid ethyl esters on vascular endothelial function when administered for 8 weeks, as measured by FMD, in patients with hyperlipidemia.</td></tr>
<tr><td colspan="2">Subjects: Patients with hyperlipidemia</td></tr>
<tr><td>Planned number of research subjects:
40 patients evaluable for the primary endpoint
2 g group : 20 subjects
4 g group : 20 subjects</td><td>Number of study sites:
5 to 10 institutions</td></tr>
<tr><td>Dose and method of administration:
A dose of 2 g of omega-3-acid ethyl esters will be orally administered once or twice a day immediately after meal.</td><td>Route of administration:
Oral</td></tr>
<tr><td>Duration of treatment:
8 weeks</td><td>Duration of evaluation:
Screening period: 4 weeks
Treatment period: 8 weeks
Total: 12 weeks</td></tr>
</table>

Main criteria for inclusion:

1. Patients with the diagnosis of hyperlipidemia and receiving instructions for lifestyle improvement
2. Patients with a fasting triglyceride (TG) level of 150 -499 mg/dL at Visit 1 after informed consent (Day -29 to Day -1 before start of study drug administration)
3. Patients receiving a stable dose of HMG-CoA reductase inhibitor therapy continuously for at least 4 weeks before informed consent at Visit 1 (Day -29 to Day -1 before start of study drug administration)
4. Male or postmenopausal female patients
5. Patients who, in the opinion of the principal investigator or the investigator, are capable of understanding the content of the clinical research and complying with the research protocol requirements.
6. Patients who can provide written informed consent prior to the conduction of the clinical research procedures
7. Patients aged ≥20 years at the time of informed consent at Visit 1(Day -28 to Day 0 before the start of study drug administration)

Main criteria for exclusion:

1. Patients with a history of revascularization or those have had coronary artery disease (a definitive diagnosis of myocardial infarction, angina) within 24 weeks before informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration)
2. Patients who have undergo aortic aneurysmectomy within 24 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) or those with concurrent aortic aneurysm
3. Patients who have had clinically significant hemorrhagic disorders (e.g., hemophilia, capillary fragility, gastrointestinal ulcer, urinary tract hemorrhage, hemoptysis, and vitreous hemorrhage) within 24 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) or those who concurrently have the above disorders
4. Patient with a fasting FMD level of 0% measured at the start of study drug administration at Visit 2 (Day -15 to Day -1 before the start of study drug administration)
5. Patients in whom the type and dosage of HMG-CoA reductase inhibitors, antidiabetic drugs and antihypertensive drugs have been changed within 4 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration)
6. Patients who have started anti dyslipidemic agents within 4 weeks prior to informed consent at

Visit 1 (Day -29 to Day -1 before the start of study drug administration)

7. Patients requiring a change in the dose of dyslipidemia therapeutic, antidiabetic, or antihypertensive drugs during the period between informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) and the start of study drug administration at Visit 2 (Day -15 to Day -1 before the start of study drug administration)
8. Patients with severe hepatic dysfunction
9. Patients with severe renal dysfunction (as an indicator, CKD category ≥G3b, equivalent to an A3)
10. Patients who have been diagnosed with pancreatitis
11. Patients who have been diagnosed with lipoprotein lipase deficiency, apoprotein C-II deficiency, familial hypercholesterolemia, familial combined hyperlipidemia, or familial type III hyperlipidemia
12. Patients with concurrent Cushing's syndrome, uremia, systemic lupus erythematosus (SLE), serum dysproteinemia, or hypothyroidism
13. Patients with symptomatic Peripheral Arterial Disease (PAD)
14. Patients with concurrent hypertension of grade II or higher [Note 1)]

Note 1: Patients with systolic blood pressure of ≥160 mm Hg or diastolic BP of ≥100 mm Hg regardless of treatment with antihypertensive drugs

15. Patients who are habitual drinkers drinking an average of over 100 mL per day (expressed in terms of quantity of alcohol) or patients with, or with a history of drug abuse or addiction [Note 2)]
16. Patients with a history of hypersensitivity or allergy for omega-3-acid ethyl esters
17. Patients who smoke
18. Patients participating in other clinical studies
19. Patients who have been determined to be ineligible as subjects in the study by the principal investigator or the investigator

Endpoints:

< Primary endpoint >

%FMD (fasting)

Secondary endpoints

(1) %FMD (4 h postprandial)

(2) TG level (fasting)

(3) TG level (4 h postprandial)

(4) Plasma fatty acid fraction

<Safety endpoint>

Adverse events, body weight, blood pressure in the sitting position, pulse in the sitting position, laboratory tests [fasting plasma glucose (FPG)]

<Other endpoints>

Efficacy endpoint:

(1) Total cholesterolLDL-C, HDL-C, Remnant-like particle (RLP) cholesterol , Apoprotein B-48, C reaction protein (CRP), determination of 8-epi-PGF2α (in urine)

(2)

Statistical methods:

(1) Analysis set

Two analysis sets, "Full Analysis Set (FAS)" and "Safety Analysis Set (SAS)" are used in this study.

Define FAS as the population of enrolled subjects who satisfy the following criterion:

- Research subjects who were randomized and given at least one dose of the study drug.

Define SAS as the population of enrolled research subjects who satisfy the following criterion:

- Research subjects who were given at least one dose of the study drug during this clinical study.

(2) Efficacy analysis

[Primary endpoints]

%FMD (fasting)

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point and the same analysis as in 1) will be performed.

3) Using fasting TG level at the start of treatment, a stratified analysis on summary

statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be performed.

[Secondary endpoints]

(1) %FMD (4 h postprandial)

(2) TG level (fasting)

(3) TG level (4 h postprandial)

(4) Plasma fatty acid fraction

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.

3) Using fasting TG level at the start of treatment, a stratified analysis on summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be performed.

[Other endpoints]

Efficacy endpoints:

Total cholesterolLDL-C, HDL-C, Remnant-like particle (RLP) cholesterol , Apoprotein B-48, C reaction protein (CRP), determination of 8-epi-PGF2α (in urine)

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point and the same analysis as in 1) will be performed.

1. Exploratory endpoints:

(1) Panel of blood proteins

PAI1, TFPI, TNF-α, IL-1, IL-6, IL-8, IL-10, INFγ, MCP-1, ICAM1, VCAM1, eSelectin, SAA, PTX3, FABP4, leptin, adiponectin

(1) Panel of lipids

Phospholipids, sphingolipids, neutral lipids, fatty acids (free fatty acids), acylcarnitine, bile acids

(2) Panel of unsaturated fatty acid metabolites

EPA metabolites (18-HEPE, 5-HEPE, Resolvin E1)

DHA metabolites [17-HDHA (17-HDoHE), 7-HDHA (7-HDoHE), Resolvin D1, Resolvin D2, Resolvin D3, Maresin 1]

Arachidonic acid metabolites (PGE2, PGD2, PGF2α, TXB2, 6-keto-PGF1α, LTB4)

[Analytical method]

Summary statistics by treatment group (number of subjects, mean, SD, minimum

(3) Analysis of safety endpoints

[Adverse events]

The following analyses will be performed for each treatment group. Adverse events will be coded using MedDRA and summarized by System Organ Class (SOC) and Preferred Term (PT).

- Tabulation of frequency of all adverse events
- Tabulation of frequency of adverse events with a causal relationship to the study drug
- Tabulation of frequency of all adverse events by severity
- Tabulation of frequency of adverse events with a causal relationship to the study drug by severity
- Tabulation of frequency of adverse events leading to study drug discontinuation
- Tabulation of frequency of serious adverse events
- Tabulation of frequency of all adverse events by time of onset

[Body weight, blood pressure in the sitting position, pulse in the sitting position, laboratory

tests [fasting plasma glucose (FPG)]

1) Summary statistics of measurements by treatment group at each evaluation time point during the treatment period will be calculated and a diagram of the change of individual data will be created.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.

3) Shift table will be created for assessment results based on reference values for Treatment Period Visit 2 and each evaluation time point (Visit 3, Visit 4) during the treatment period.

Rationale for the number of planned research subjects

The planned sample size is based on the requierment to investigate explore atively the effects of omega-3-acid ethyl esters on vascular endothelial function considering the feasibility of this study.

3.0 ABBREVIATION

AE	adverse event
BMI	body mass index
CKD	chronic kidney disease
COI	conflict of interest
CRO	contract research organization
DHA	docosahexaenoic acid
EDC	electronic data capture
EPA	eicosapentaenoic acid
FAS	full analysis set
FMD	flow mediated dilation
GCP	Good Clinical Practice
HDL-C	high density lipoprotein-cholesterol
ICH	International Conference on Harmonisation
JAPIC	Japan Pharmaceutical Information Center
LDL-C	low density lipoprotein-cholesterol
MedDRA	Medical Dictionary for Regulatory Activities
PT	Preferred Term
SAS	safety analysis set
SLE	systemic lupus erythematosus
SOC	System Organ Class
TG	triacylglycerol

4.0 INTRODUCTION

4.1 Background

The efficacy of LDL-lowering regimen using HMG-CoA reductase inhibitor designed to control cardiovascular events has been already established, which leads to primary prevention, secondary prevention, and risk reduction in high-risk treatment groups.[1),2)] However, postprandial hypertriglyceridemia and the quality of LDL have been receiving attention as an atherosclerotic risk factor that remains after the treatment with HMG-CoA reductase inhibitors.

Although there had been no consensus regarding the significance of triglycerides (TG) as a cardiovascular risk, Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases (2012) stated that "a number of epidemiological studies suggest that elevated triglyceride levels are associated with increased incidence of cardiovascular diseases." TG levels reach the highest 3 to 4 h after meal. Especially, remnant-like lipoprotein (RLP) plays an important role to cause the elevation of TG level, and RLP particles contain a greater amount of cholesterol, as well as TG, than LDL particles. It has been reported that RLP enhances foaming of macrophage and inflammatory reaction[3)], and TG levels at 2 to 4 h after meals are more important than those before meals as a heart disease risk factor. [4)]

As insulin resistance associated with obesity, etc. increases, qualitative changes in LDL, as well as elevated levels of TG, are observed. The increase observed in small dense LDL (sdLDL) of small particle diameters is considered to enhance the formation of atherosclerotic plaque since this sdLDL is easily taken up by macrophages. Therefore, in order to aim at further inhibition of cardiovascular events after achieving the lowering of LDL-C with HMG-CoA reductase inhibitors, it is considered especially important to lower TG levels/decrease sdLDL. Omega-3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been receiving attention in recent years as the drugs having such activities. Since omega 3 fatty acids are not synthesized in the body, supplementation from meals (fish) is necessary. EPA and DHA have TG-lowering and antiatherosclerosis activities but the mechanism has not been completely elucidated. Omega-3-acid ethyl esters (EPA/DHA preparations, Lotriga®) released in 2013 lower TG levels in a dose-dependent manner[5)] and are expected to lead to lowering of RLP, increase in the LDL particle size, FMD, and lowering of IL-6 levels.[6)]

In JELIS Studies, lowering of TG levels and reduction in major coronary events have been demonstrated in the HMG-CoA reductase inhibitor and EPA combination group compared to the HMG-CoA reductase inhibitor alone group.[7)] This clinical study aims at exploring the effects of Lotriga® (EPA/DHA preparations) on vascular endothelial function and postprandial hypertriglyceridemia assessed by FMD, etc. in patients receiving a hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor and have concurrent hypertriglyceridemia (TG).

4.2 Rationale for the proposed research

This clinical study was planned to contribute to the prevention of arteriosclerotic disease by exploring the effects of omega-3-acid ethyl esters on vascular endothelial in patients with hyperlipidemia.

5.0 RESEARCH OBJECTIVES AND ENDPOINTS

5.1 Objectives

The objective of this study is to explore the effects of omega-3-acid ethyl esters on vascular endothelial function when administered for 8 weeks, as measured by FMD, in patients with hyperlipidemia.

5.2 Definition of endpoints

5.2.1 Primary endpoints

%FMD (fasting)

5.2.2 Secondary endpoints

(1) %FMD (4 h postprandial)

(2) TG level (fasting)

(3) TG level (4 h postprandial)

(4) Plasma fatty acid fraction

5.2.3 Other endpoints

Efficacy endpoints:

total cholesterol, LDL-C, HDL-C, Remnant-like particle (RLP) cholesterol , apoprotein B-48, C-reactive protein (CRP), determination of 8-epi-PGF2α (in urine)

5.2.4 Safety endpoints

Adverse events, body weight, blood pressure in the sitting position, pulse in the sitting position, laboratory tests [fasting plasma glucose (FPG)]

6.0 CLINICAL RESEARCH DESIGN

6.1 Clinical research design

< Clinical research design >

This is a multicenter, collaborative, randomized, open-label study designed to explore the effects of administration of omega-3-acid ethyl esters [2 g (2 g PO QD) or 4 g (2 g PO BID) for 8 weeks] on vascular endothelial function, as measured by FMD, in patients receiving a hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor and have concurrent hypertriglyceridemia.

Considering the potential bias by factors that affect FMD between treatment groups, stratified allocation will be performed with fasting TG as a factor.

Research TREATMENT

(1) Study drug:

Omega-3-acid ethyl esters

(2) Dosage regimen

Omega-3-acid ethyl esters will be orally administered [2 g (2 g PO QD) or 4 g (2 g PO BID)] immediately after meal for 8 weeks in patients with hyperlipidemia .

(3) Duration of treatment:

8 weeks

Planned number of research subjects:

40 patients evaluable for the primary endpoint

2 g group: 20 subjects

4 g group: 20 subjects

Number of study sites:

5 to 10 institutions

< Duration of the treatment and number of visits of by subjects >

(1) Duration of treatment:

The duration of the treatment period is 8 weeks. Research subjects should visit the hospital at the time of informed consent at Visit 1 (Day-29 to Day -1 before the start of study drug) and from the start of study drug administration at Visit 2 (Day -15 to Day -1 from the start of study drug administration) to completion of study drug administration [Visit 4 (Week 8)].

(2) Number of visits

Observation period: 1 visit

Treatment period: 3 to 6 visits

The FMD measurement during the treatment period will be performed by a physician or laboratory technician who completed the FMD measurement training. Therefore, only for the FMD measurement, subjects are expected to go to a testing laboratory other than the one they normally visit for examinations. In that case, a total of 3 visits for the FMD measurement will be added to the total of 3 visits for examinations from Treatment Period Visit 2 (Day -15 to Day -1 before the start of study drug administration) to Visit 4 (Week 8). Therefore, the number of visits during the treatment period will be a minimum of 3 and a maximum of 6.

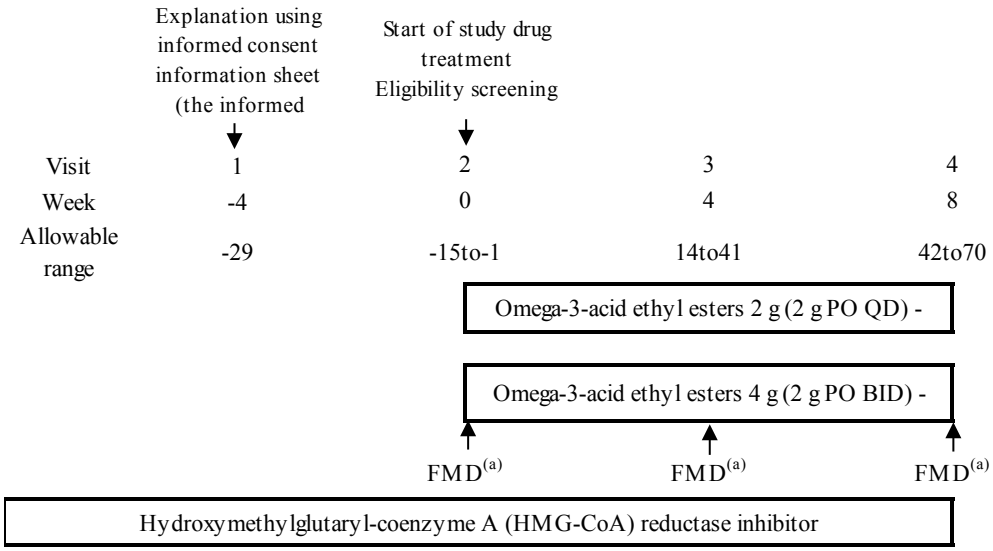
Figure 6 (a) shows an outline of the clinical research design. Refer to Appendix A for schedule of examinations, observations, and assessments.

Figure 6.a Outline of clinical research design

< Outline of clinical research>

Duration of treatment:8 weeks

Number of visits: 4 to 7 visits



(a) FMD measurement will be performed by a physician or laboratory technician who completed the FMD measurement training.

6.2 Rationale for the clinical research design

<Rationale>

Since this study aims at exploring the effects of oral administration of omega-3-acid ethyl esters in clinical practice (2 g once a day or twice a day for 8 weeks) on vascular endothelial function, the study was designed as an open-label comparing FMD before and after omega-3-acid administration without including control groups.

< Rationale for the dose and dosing regimen >

In accordance with the approved dosage and administration of omega-3-acid ethyl esters, dose and dosing regimen of omega-3-acid ethyl esters were set at 2 g (2 g PO QD) and 4 g (2 g PO BID) in this study.

< Rationale for the duration of study >

Since improvement of FMD was observed after a 2-month administration of omega-3-acid ethyl esters in the studies conducted outside Japan investigating the effect of omega-3-acid ethyl esters on FMD,[8] it was considered that the effect of omega-3-acid ethyl esters on FMD can be examined with an 8-week administration and the duration of the treatment period was, therefore, set at 8 weeks in this clinical study.

< Rationale for the planned sample size >

See Section 13.3.

6.3 Premature termination of entire clinical research or premature termination of clinical research at a study site

6.3.1 Premature termination criteria of entire clinical research

The sponsor should immediately discontinue the study when at least one of the following criteria is applicable:

- When new information or other evaluation on the safety or efficacy of the study drug becomes available that shows a change in the known risk/benefit profile of the concerned compound, and risks/benefits are no longer tolerable for research subject participation in the study.
- When there is serious deviation from Ethical Guidelines or ICH-GCP for medical and health research involving human subjects.

6.3.2 Criteria for premature termination of study sites

Termination of involvement of a study site in the study may be requested prematurely at the discretion of the sponsor if the entity (e.g., principal investigator) is found in significant violation of the ethical guidelines, protocol, or contractual agreement for medical and health research involving human subjects, or is unable to ensure proper conduct of the research.

6.3.3 Procedures of clinical study suspension and premature termination of entire clinical study or study at a study site

In the event that the sponsor or a study site committee such as an ethics review committee decides to prematurely suspend or terminate the entire clinical study or clinical study at a study site, a study-specific procedure shall be provided by the sponsor. The procedure shall be followed by applicable study sites during the course of clinical study suspension or premature termination.

6.4 Procedures for protocol revision

If the protocol needs to be revised, the sponsor shall consider and decide whether to revise the protocol.

The principal investigator of each study site shall be informed of the details of each protocol revision. Principal investigators shall confirm the content of the revision of the protocol and submit a letter of agreement to the sponsor as evidence of agreement with the protocol revision.

Upon notification, the principal investigator at each study site shall submit the revised contents to committees such as the IEC, as necessary according to institutional regulations for review, and obtain approval from the director of the entity.

7.0 SELECTION AND WITHDRAWAL CRITERIA OF RESEARCH SUBJECTS

The principal investigator or investigator shall check for all the inclusion/exclusion criteria including the test results prior to randomization.

7.1 Inclusion criteria

Eligibility of research subjects shall be determined in accordance with the following criteria.

1. Patients with the diagnosis of hyperlipidemia and receiving instructions for lifestyle improvement
2. Patients with a fasting triglyceride (TG) level of 150 – 499 mg/dL at Visit 1 after informed consent (Day -29 to Day -1 before start of study drug administration)
3. Patients receiving a stable dose of HMG-CoA reductase inhibitor therapy continuously for at least 4 weeks at the time of informed consent at Visit 1 (Day -29 to Day -1 before start of study drug administration)
4. Male or postmenopausal female patients
5. Patients who, in the opinion of the principal investigator or the investigator, are capable of understanding the content of the clinical research and complying with the research protocol requirements.
6. Patients who can provide written informed consent prior to the conduction of the clinical research procedures
7. Patients aged ≥20 years at the time of informed consent at Visit 1(Day -29 to Day -1 before the start of study drug administration)

[Rationale for the inclusion criteria]

1. This was set as a target condition in this clinical study.

2. This was set according to the diagnostic criteria for hypertriglyceridemia in "Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2012."

3. This was set for an accurate assessment of the efficacy.

4. This was set in consideration of the effect of female hormones on FMD.

5.6. These were set as fundamental items for the research.

7. This was set considering the age ≥20 years as the age where patients are capable of making a decision to participate in this clinical study.

7.2 Exclusion criteria

Research subjects meeting any of the criteria below shall not be included in this research.

1. Patients with a history of revascularization or those have had coronary artery disease (a definitive diagnosis of myocardial infarction, angina) within 24 weeks before informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) （
2. Patients who have undergo aortic aneurysmectomy within 24 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) or those with concurrent aortic aneurysm
3. Patients who have had clinically significant hemorrhagic disorders (e.g., hemophilia, capillary fragility, gastrointestinal ulcer, urinary tract hemorrhage, hemoptysis, and vitreous hemorrhage) within 24 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) or those who concurrently have the above disorders
4. Patient with a fasting FMD level of 0% measured at the start of study drug administration at Visit 2 (Day -15 to Day -1 before the start of study drug administration)
5. Patients in whom the type and dosage of HMG-CoA reductase inhibitors, antidiabetic drugs and antihypertensive drugs have been changed within 4 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration)
6. Patients who have started anti dyslipidemic agents within 4 weeks prior to informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration)
7. Patients requiring a change in the dose of dyslipidemia therapeutic, antidiabetic, or antihypertensive drugs during the period between informed consent at Visit 1 (Day -29 to Day -1 before the start of study drug administration) and the start of study drug administration at Visit 2 (Day -15 to Day -1 before the start of study drug administration)
8. Patients with severe hepatic dysfunction
9. Patients with severe renal dysfunction (as an indicator, CKD category ≥G3b, equivalent to an A3)
10. Patients who have been diagnosed with pancreatitis
11. Patients who have been diagnosed with lipoprotein lipase deficiency, apoprotein C-II deficiency, familial hypercholesterolemia, familial combined hyperlipidemia, or familial type III hyperlipidemia

12. Patients with concurrent Cushing's syndrome, uremia, systemic lupus erythematosus (SLE), serum dysproteinemia, or hypothyroidism
13. Patients with symptomatic Peripheral Arterial Disease (PAD)
14. Patients with concurrent hypertension of grade II or higher [Note 1)]

Note 1: Patients with systolic blood pressure of ≥160 mm Hg or diastolic BP of ≥100 mm Hg regardless of treatment with antihypertensive drugs

15. Patients who are habitual drinkers drinking an average of over 100 mL per day (expressed in terms of quantity of alcohol) or patients with, or with a history of drug abuse or addiction [Note 2)]
16. Patients with a history of hypersensitivity or allergy for omega 3-acid ethyl esters -
17. Patients who smoke
18. Patients participating in other clinical studies
19. Patients who have been determined to be ineligible as subjects in the study by the principal investigator or the investigator

Note 2: Alcohol conversion table (reference)

Alcohol	Type	Alcohol content	Amount corresponding to 100 mL alcohol
Brewage	Sake	15%	670 mL (approx. 3 cups)
	Beer	5%	2,000 mL (approx. 3 large bottles)
	Sparkling liquor	5%	2,000 mL
	Wine	12%	830 mL
	Chinese rice wine	18%	560 mL
Distilled liquor	Ko-rui Shochu	35%	290 mL
	Otsu-rui Shochu	25%	400 mL
	Whiskey	40%	250 mL (approx. 3 doubles)
	Brandy	40%	250 mL (approx. 3 doubles)
	Vodka	40%	250 mL(approx. 3 doubles)
Mixed liquor	Plum wine	13%	770 mL
	Synthetic sake	16%	630 mL

[Rationale for the exclusion criteria]

1.2.8.16 These were set in consideration of safety of the research subjects.

3 This was set because EPA-E, a component of the study drug, has an antiplatelet action and may impair hemostasis in patients.

4.5.6.7.9.14. 17 These were set because it was considered that drug efficacy evaluation would be affected.

10. This was set because acute pancreatitis may occur in patients with significant hypertriglyceridemia.

11. This was set to exclude patients with hypertriglyceridemia attributed to a genetic predisposition.

12. This was set to exclude an underline condition commonly seen in patients with secondary hypertriglyceridemia.

13.15 These were set in consideration of safety of the research subjects and for an accurate assessment of the efficacy.

18.19 These were set as fundamental items for the research.

7.3 Prohibited concomitant drugs and permitted concomitant drugs

7.3.1 Prohibited concomitant drugs

[Prohibited concomitant drugs]

The concomitant use of the following drugs* will be prohibited during the clinical study [Visit 1 (Day -29 to Day -1 before the start of study drug administration) to Visit 6 (Week 8)].

(*: Concomitant drugs prohibited in the package insert of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor taken at the time of informed consent are included.)

1. Eicosapentaenoic acid (EPA) preparations or EPA/DHA preparations (including supplements)
2. Antidyslipidemic agents (except for HMG-CoA reductase inhibitor taken at the time of the informed consent)

    Anion exchange resin

    Fibrates

    Ezetimibe

    Nicotinic acid derivatives

    Probucol

    Phytosterols

    Others (elastase ES, dextran sulfate sodium, polyenephosphatidylcholine, pantethine)
3. Pancreatic hormones

Insulin preparation

4. Androgens

   Testosterone, methyltestosterone

5. Follicle hormone and luteinizing hormone

   Estrogen and progestogen

6. Systemic steroids

<Rationale>

1to 6 These were set because it was considered that efficacy evaluation of the study drug would be affected by these drugs.

7.3.2 Permitted concomitant drugs

Drugs other than those prohibited for concomitant use may be taken during the study period. However, for HMG-CoA reductase inhibitors, antidiabetic drugs (except for insulin) and antihypertensive drugs, dose change, addition of new treatment drugs, or change of treatment drugs shall not be allowed unless the principal investigator or investigator considers it necessary due to adverse events, etc.

7.4 Research subject management

The principal investigator or investigator shall instruct research subjects regarding the following:

(1) Guidance for research subjects for improving lifestyle shall be made constant during the clinical study and its content shall not be changed. Research subjects shall be instructed to avoid excessive exercise as much as possible during the study period.

(2) Research subjects shall be instructed not to consume alcohol after 9:00 pm two days before the hospital visit, and not to consume food after 9:00 pm on the day before the hospital visit. Although water can be taken as desired on the day of blood collection, research subjects shall be instructed to visit the hospital in a fasting state in order that fasting morning samples can be collected.

(3) Research subjects shall be instructed to avoid eating/drinking to excess, extreme change in dietary content (eating a high-fat meal, etc.) or excessive exercise, and spend time on a routine basis the day before the hospital visit.

(4) Research subjects shall be instructed to visit the hospital according to the schedule and take the test as prescribed during the study period.

(5) Research subjects shall be instructed to comply with the instructions or restrictions (taking the study drug, prohibited drugs for concomitant use, those allowed for concomitant use, etc.)

(6) Upon concomitant use of anticoagulants (warfarin potassium, etc.) or antiplatelet drugs (aspirin, etc.) listed under Precautions for Coadministration in the package insert of the study drug, research subjects shall be instructed to promptly report in the event of an adverse drug reaction, such as bleeding.

(7) Research subjects must notify the principal investigator or investigator in advance if they receive treatment from other physicians. If treated by other physicians, the research subjects shall be instructed to promptly report the details of the treatment.

7.5 Criteria for discontinuation or withdrawal of a research subject

The principal investigator or investigator shall record the main reason for discontinuation of protocol treatment on the case report form according to the classification described below. Refer to Section 9.1.11 for research subjects who withdraw from the research before randomization.

1. Pretreatment Event (PTE) or adverse event

When the research subject had a pretreatment event (PTE) or an adverse event that requires withdrawal of the research subject from the study because continued participation in the study would impose an unacceptable risk to the research subject's health, or when the research subject is unwilling to continue study participation because of the PTE or adverse event.

2. Major protocol deviation

When it is discovered after randomization that a research subject does not meet the eligibility criteria or is not adhering to the protocol, and continued participation in the research would impose an unacceptable risk to the research subject's health.

3. Lost to follow-up

When the research subject failed to make visits and could not be contacted despite the attempts to contact the research subject.

4. Voluntary termination

When the research subject wishes to withdraw from the research. The reason for discontinuation shall be obtained to the extent possible.

5. Research termination

When the sponsor or a committee such as the IEC or regulatory authority has decided to terminate the study. Refer to Section 6.3.1 for details.

6. Lack of efficacy

When efficacy of the study drug is not evident and continuation of the research may pose an

unacceptable risk to the research subjects in the opinion of the principal investigator or investigator.

7. Others

When the principal investigator or investigator determined to terminate the study for other reasons.

The specific reasons should be recorded on the CRF.

7.6 Procedures for discontinuation of individual research subjects

The principal investigator or investigator shall terminate a research subject's research participation when the research subject meets the criteria described in Section 7.5. Individual research subjects may discontinue their research participation without giving a reason at any time during the research. Should a research subject's participation be discontinued, the primary reason for termination shall be recorded on the CRF by the principal investigator or investigator. In addition, efforts shall be made to perform all tests/observations/evaluations scheduled at the time of discontinuation.

8.0 RESEARCH TREATMENT

8.1 Study drug

Generic name: Omega-3-acid ethyl esters

Chemical name: Ethyl icosapentate;

Ethyl (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoate

Docosahexaenoic acid ethyl ester;

Ethyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoate

8.1.1 Dose and administration method

Omega-3-acid ethyl esters will be orally administered [2 g (2 g PO QD) or 4 g (2 g PO BID)] immediately after meal. In principle, the dosage prescribed to the research subject should not be changed until the end of the study. Since the research subject should in a fasting state at the time of visit, the drug to be taken after breakfast should be taken immediately after the first meal after completion of the prescribed test.

Medication should be started the day after completion of all the tests at Week 0.

8.1.2 Concomitant drug

HMG-CoA reductase inhibitors, antidiabetic drugs (except for insulin) or antihypertensive drugs taken at the time of informed consent should continue to be taken without a change in dosage and administration during the study period.

8.1.3 Overdose of the study drug

Overdose is defined as intentional or accidental administration of the study drug at a higher dose than that specified in the protocol, either by a health professional or by the research subject.

To consistently collect important safety information about overdose, the principal investigator or investigator(s) shall record all cases of overdose on the “Overdose” page of the CRF, irrespective of the presence or absence of accompanying adverse event. Adverse events associated with overdose shall be recorded on the “Adverse events” page of the CRF, in accordance with the procedures described in Section 10.0, “Pretreatment Event and Adverse Events.”P

In addition, serious adverse events associated with overdose shall be recorded in accordance with the procedures described in Section 10.2.2, “Collection and reporting of SAEs.”

In the event of overdose, the principal investigator or investigator shall treat the subject as required based on symptoms.

8.2 Allocation of the study drug and administration procedure

The principal investigator or investigator shall access the Case Registration Web System to allocate the research subjects. The principal investigator or the designee shall notify the information required for allocation in addition to the research subject identification code. Then, drugs that should be administered to each research subject will be notified through the Case Registration Web System. The principal investigator or investigator shall prescribe the study drug 2 g [(2 g PO QD) or 4 g (2 g PO BID)] according to the notification, and record the dose administered for each research subject in the CRF.

8.3 Preparation and storage of allocation list

The allocation responsible person (designated by the sponsor) shall create an allocation procedure. Information on the allocation shall be kept in a safe place and shall not be available to anyone other than authorized persons, to secure independency from the clinical research.

Using fasting TG values as stratified factors, stratified allocation shall be performed at the registration center at the start of treatment period. For allocation, the Registration Center shall use the Allocation Procedure for Stratified Allocation prepared by the allocation responsible person.

9.0 CLINICAL STUDY PROTOCOL

9.1 Research procedures

The principal investigator or investigator shall collect data in accordance with the procedure below. In principle, tests other than FMD measurement, observations, and evaluations of research subjects shall be performed by the same principal investigator or investigator. FMD measurement shall be performed by a physician or laboratory technician who has completed the FMD measurement training. The study schedule is provided in Appendix A.

9.1.1 Informed consent procedure

The procedures for obtaining informed consent are described in Section 15.3.
Consent shall be obtained from the research subject before initiation of research procedures.
Research subject code is given to each research subject from whom informed consent was acquired and who was randomized. The research subject ID code shall be used throughout the research period and shall not be changed.

9.1.2 Demographic data, medical history, and previous therapeutic drugs

Demographic data shall be collected regarding date of birth, gender, time (year/month) of onset of dyslipidemia (or diagnosis), last menstrual period (year), frequency of consumption of fish (almost every day, once/2 days, 1 to 2 times/week, almost never), smoking and drinking history, and vessel diameter.

Medical history data shall be collected regarding clinically problematic diseases or symptoms that disappeared or were terminated within 1 year from informed consent. When the symptoms or disease continues, it shall be considered as a concurrent disease (Refer to Section 9.1.7).
Previous therapeutic drug data shall be collected regarding all drugs used within 4 weeks before the informed consent and that are related to criteria for eligibility and assessment of efficacy.

9.1.3 Physical examination

All subsequent physical examinations after the start of study drug administration shall be assessed for clinically significant changes from the baseline examination.

9.1.4 Weight, height, and BMI

Body weight and height shall be measured. The sponsor shall calculate the BMI using the following formula.
Body Mass Index: BMI=body weight (kg)/[height(m)]^2
Height shall be measured to the nearest whole number in centimeters. Body weight shall be measured to one decimal place in kilograms. The result of BMI shall be shown to one decimal place.
Example:

Height = 176 cm, weight = 79.2 kg, BMI = $79.2/1.76^2 = 25.6$ kg/m^2

9.1.5 Vital signs

For vital signs, blood pressure in {sitting position (after resting for 5 minutes) and a pulse rate (bpm) shall be measured.
When timing for the vital signs measurement overlaps with blood collection, priority shall be given to blood collection, and the vital signs shall be measured within 30 minutes before or after the blood collection.

9.1.6 Concomitant drugs

Concomitant drugs are all drugs to be given in addition to the study drug. Drugs prescribed by doctors or the over-the-counter medicines purchased by the research subjects shall be included. At every hospital visit of the research subject, the status of use (drug name, route of administration) of the drugs (including vitamin compound, over-the-counter medication, and Chinese medicine) used, other than the study drug, from the time of informed consent to the completion of the clinical research shall be monitored.

9.1.7 Concurrent disease

A concurrent disease shall be defined as a disease or symptom that is present at the time of the research subject's informed consent Clinically problematic laboratory test data, ECG findings, and abnormal physical examination findings observed immediately after the acquisition of the informed consent and examination shall be considered as a concurrent disease at the discretion of the principal investigator or investigator. The content of concurrent disease (diagnosis) shall be investigated.

9.1.8 Checking food and drink consumption before the visit

At each study visit, the principal investigator, investigator, etc., physician for FMD measurement, or laboratory technician for FMD measurement will check the food and drink consumption of the research subject before the visit.

- Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of the fasting test, and food should not be consumed from 9:00 pm on the day before to the time of the fasting test.
- Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the fasting test.

9.1.9 Laboratory tests

The following laboratory tests shall be performed according to the observation schedule (Appendix A). The fasting test will be performed under ≥10-h fasting and the 4-h postprandial test will be performed 4 h after consumption of a prescribed meal (allowable range ± 30 min).

The principal investigator or investigator shall evaluate and keep the reported laboratory test results.

Table 9.a Laboratory tests

Serum chemistry (fasting)	Serum chemistry (4 h postprandial)	Urinalysis (fasting)
TG Total cholesterol LDL-C HDL-C Remnant-like particle (RLP) cholesterol Apoprotein B-48 high-sensitivity C-reactive protein (hs-CRP) Fasting plasma glucose (FPG) Plasma fatty acid fraction	TG Total cholesterol LDL-C HDL-C Remnant-like particle (RLP) cholesterol Apoprotein B-48	Determination of 8-epi-PGF2α

The principal investigator shall keep laboratory test reference values, including the historical data.

9.1.10 Flow-mediated dilation (FMD) test

FMD tests shall be performed according to the observation schedule (Appendix A) based on the Procedure for FMD Measurement provided separately. The fasting test will be performed under ≥10-h fasting and the 4-h postprandial test will be performed 4 h after consumption of a prescribed meal (allowable range ±30 min). FMD measurement will be performed by a physician or laboratory technician who completed the FMD measurement training.

9.1.11 Record of research subjects who are withdrawn before randomization

A CFR shall be created for all research subjects who have signed the consent form and withdrawn before randomization.

The following items are to be described on the CRF.

- Date of consent obtainment
- Date of birth
- Sex
- Eligibility
- PTE/adverse events (with details, if any)

- Reason for discontinuation

The primary reason for withdrawal before randomization shall be recorded on the CRF according to the following classification:

- PTE adverse events
- Not satisfying at least one of the inclusion criteria or meeting any of the exclusion criteria
- Serious deviation from the protocol
- Lost to follow-up
- Voluntary discontinuation (specify the reason)
- Discontinuation of the entire study
- Others (specify the reason)

Research subject ID numbers assigned to research subjects withdrawn from the research before {randomization shall not be reused.

9.1.12 Record of randomization

Research subjects to be randomized shall meet all of the inclusion criteria and shall not meet any of the exclusion criteria according to Section 8.2. The principal investigator or investigator shall specify the reason why the subject cannot be randomized / transferred to the treatment period.

9.2 Drug-taking status of the research subjects

The principal investigator or investigator shall confirm the drug-taking status of the study drug of the research subject at every visit. Throughout the clinical research period, drug-taking status will be checked through interview for two categories, “compliance of at least 50% of the prescribed dose or compliance of < 50% of the prescribed dose.” If poor compliance with study treatment (e.g., < 50% of the prescribed dose) has been found through the interview and does not improve, the research subject may be withdrawn from the research if appropriate for the circumstances.

9.3 Implementation time point of the test and observation items

The schedule for all tests, observations, and evaluations is shown in Appendix A. The principal investigator or investigator shall perform the tests, observations, and evaluations at the time points shown below.

9.3.1 Observation period (Visit 1)

At Visit 1 (Day -29 to Day -1 before study drug administration), the study shall be explained using the informed consent form to the research subjects who meet the inclusion criteria (except for 2) in Section 7.1 and do not meet any of the exclusion criteria (except for 4 and 7) in Section 7.2, and consent shall be obtained in accordance with Section 15.3. - .

Tests, observations, and evaluations to be performed at Visit 1 (Day -29 to Day -1 before study drug administration) are shown below.

- Informed consent procedure
- Demographic data, medical history, previous treatment drug(s)
- Physical examination
- Height, weight and BMI
- Vital signs
- Concomitant drugs
- Concurrent disease

Laboratory tests (fasting)Items to be checked: Food and drink consumption before visit:

- Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of visit and food should not be consumed from 9:00 pm on the day before to the time of visit.
- Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

9.3.2 Treatment period (Visit 2)

1. Laboratory tests/FMD measurement

Research subjects shall take the following tests at a designated medical institution for FMD measurement.

- Laboratory tests [fasting and 4-h postprandial (allowable range ±30 min)] FMD test [fasting and 4-h postprandial (allowable range ±30 min)]

Items to be checked: Food and drink consumption before visit

- Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of visit and food should not be consumed from 9:00 pm on the day before to the time of visit.
- Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

Research subjects shall visit the study site after undergoing prescribed tests at a designated medical institution for FMD measurements.

2. Implementation of examination

Based on the results of the tests/observations at Visit 1 (Day -29 to Day -1 before the start of study drug administration) and the FMD test results (fasting) at Visit 2 (Day -15 to Day -1 before the start of study drug administration), the principal investigator or the investigator shall perform the

following tests, observations, and evaluations in research subjects who meet the inclusion criteria and do not meet any of the exclusion criteria.

- Physical examination
- Vital signs
- Concomitant drugs
- Pretreatment Event

The principal investigator or the investigator shall give notification to the registration center (accessing to the Case Registration Web System) regarding research subjects in whom all the tests at Visit 2 (Day -15 to Day -1 before study drug administration), observation results, and evaluation have been completed and study drug administration was considered possible.
The principal investigator or the investigator shall prescribe the study drug at a dose notified from the registration center and tell the research subject to take the drug starting from the next day.

The research subject with a fasting FMD level of 0% shall be withdrawn from this study based on Section 7.2.
Refer to Section 9.1.11 for preparation of records relating to the research subjects withdrawn before randomization.

9.3.3 Treatment period (Visit 3)

1. Research subjects shall undergo the following tests at Visit 3 (Day 14 to Day 41 after the study drug administration) at a designated medical institution for FMD measurements.
   - FMD test (fasting)
     Laboratory tests [fasting and 4-h postprandial (allowable range ± 30 min)]

   Food and drink consumption before the visit will be checked.
   - Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of visit and food should not be consumed from 9:00 pm on the day before to the time of visit.
   - Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

2. The principal investigator and the investigator shall perform the following tests, observations, and evaluations at Visit 3 (Day 14 to Day 41 after the study drug administration).
   - Physical examination
   - Vital signs
   - Concomitant drugs

- Study drug compliance
- Adverse Events

Tests, observations, and evaluations of (1) and (2) at Visit 3 may be performed in any order [so long as the completion of all the contents falls within the allowable range (Day 14 to Day 41 after the study drug administration)].

9.3.4 Completion (Visit 4) or discontinuation

1. Visit 4 shall be the last VISIT, and research subjects shall undergo the following tests at the designated medical institution for FMD measurement within the allowable range of Visit 4 (42 to 70 days after the study drug administration). If the study drug administration is discontinued, research subjects should, to the extent possible, undergo tests within 3 days from the last dose taken.
   - FMD test [fasting and 4-h postprandial (allowable range ± 30 min)]
     Laboratory tests [fasting and 4-h postprandial (allowable range ± 30 min)]

   Food and drink consumption before the visit will be checked.
   - Alcohol should not be consumed from 9:00 pm two days before the hospital visit to the time of visit and food should not be consumed from 9:00 pm on the day before to the time of visit.
   - Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

2. The principal investigator and the investigator shall perform the following tests, observations, and evaluations at Visit 4 (Day 42 to Day 70 days after the study drug administration). If the study drug administration is discontinued, research subjects should, to the extent possible, undergo tests, observations, and evaluations required for the last visit within 3 days from the last dose taken.
   - Physical examination
   - Vital signs
   - Concomitant drugs
   - Study drug compliance
   - Adverse Events

Since the principal investigator or the investigator is required to confirm safety of the tests, observations, and evaluations at Visit 4 or at discontinuation, FMD test and laboratory tests in (1) shall be performed first, and tests, observations, and evaluations in (2) shall be performed last.
At completion of the clinical research, the status of all research subjects administered the study drug shall be recorded on the CRF.

10.0 PRETREATMENT EVENT AND ADVERSE EVENTS

10.1 Definitions

10.1.1 When pretreatment events are collected

A Pretreatment Event (PTE) is defined as any unfavorable medical event that occurs to a research subject prior to administration of study drug but after the acquisition of informed consent. PTEs are not limited to events with clear causal relationship with treatment with the concerned drug.

10.1.2 Adverse events

An adverse event is defined as any untoward medical occurrence in a patient or a research subject receiving a pharmaceutical product (including the study drug). It does not necessarily have an apparent causal relationship with this pharmaceutical product (including study drug).

An adverse event can therefore be any unfavorable and unintended sign (e.g., a clinically significant laboratory abnormality), symptom, or disease temporally associated with the use of a pharmaceutical product (including the study drug), regardless of whether it is considered relate d to the pharmaceutical product (including the study drug) or not.

10.1.3 Considerations for PTEs and adverse events

Generally unfavorable findings are described below:

- Newly diagnosed disease or unexpected aggravation of existing symptom (intermittent event of an existing symptom is not considered a PTE or an adverse event)
- Requiring action or medical practice
- Requiring invasive diagnostic treatment
- Requiring discontinuation or a change in the dose of the study drug or a concomitant medication
- Considered unfavorable by the principal investigator or the investigator

Diagnosis name and signs/symptoms:

PTEs or adverse events shall be recorded by diagnosis name. Accompanying signs (including abnormal laboratory values) and symptoms shall not be recorded as adverse events. If an adverse event could not be expressed by a diagnosis name, the signs or symptoms shall be recorded as the adverse event.

Laboratory test values:

Abnormal laboratory values shall be recorded as adverse events when the principal investigator or the investigator judges the results are clinically problematic (in other words, when certain action or medical practice is required, or when the principal investigator or the investigator judges the change has exceeded the normal physiological variation range of the research subject). Retest and/or continued monitoring of an abnormality are not considered medical practice. Also, repeated or additional conduction of non-invasive tests for verification, evaluation, and monitoring of an abnormality are not considered medical practice.

However, when abnormal laboratory values are the accompanying symptoms of a disease diagnosed as an adverse event (e.g., increased creatinine due to renal dysfunction, etc.), the adverse event shall be handled by its diagnosis name.

Pre-existing conditions (a disease or symptoms that had been present since before before the informed consent) : A disease or symptoms that had been present since before the acquisition of informed consent shall be regarded as a concurrent disease and not an adverse event. When a concurrent medical condition is aggravated, the aggravation shall be determined as an adverse event and the principal investigator or the investigator shall record on the CRF that the adverse event is an aggravation of the concurrent disease (e.g., “aggravation of hypertension,” etc.).

If a research subject has a pre-existing episodic condition (e.g., asthma, epilepsy), each episode shall be recorded as an adverse event if the episodes become more frequent, serious, or severe in nature. If a research subject has a chronic concurrent condition (e.g., cataracts, rheumatoid arthritis), worsening of the condition shall be recorded as adverse event if the degree of the worsening exceeds that which would be expected. The principal investigator or the investigator shall ensure that the adverse event term to be reported represents the change in the condition from baseline (e.g. “worsening of…”).

Worsening of adverse events:
If a research subject experiences a worsening of the adverse event after a change of the study drug, or secondary signs and symptoms are caused by the adverse event, the worsening or the secondary signs and symptoms shall be recorded as a new adverse event on the CRF. The principal investigator or the investigator shall use an adverse event term that explicitly means a change of the condition (e.g., “worsening of…”).

Change of severity of adverse events / severity of PTEs:
If the research subject experiences changes in the severity of an adverse event / a severity of PTE, the event shall be recorded once, at its peak severity.

Previously planned surgery or treatment:
Preplanned surgeries or interventions that were scheduled before study treatment shall not be

considered adverse events. However, when the existing symptom is aggravated to a degree requiring emergency surgery or treatment, the condition or the event shall be considered an adverse event. A concurrent disease that resulted from previously planned surgery shall be reported as an adverse event.

Non-urgent surgery or treatment:
Non-urgent surgery or treatment that does not induce a change in the condition of a research subject (cosmetic surgery, etc.) shall not be considered an adverse event; However, it shall be recorded in the source documents. Concurrent diseases due to a non-urgent surgery shall be reported as an adverse event.

The Insufficient clinical response (lack of efficacy): Insufficient clinical response, efficacy, or pharmacological action shall not be recorded as an adverse event.
The principal investigator or the investigator shall make the distinction between worsening of a pre-existing condition and lack of therapeutic efficacy.

Overdose:
Overdose of any medication without manifested symptoms shall not be recorded as an adverse event, but the overdose shall be recorded on the "Overdose" page of the CRF. Any manifested symptoms shall also be recorded as adverse events on the "Adverse events" of the CRF.

10.1.4 Serious adverse event

Of all the unfavorable medical events that develop with administration of a pharmaceutical product (including study drug) (irrespective of dose), a serious adverse event is an event that:

1. results in death,
2. is life threatening*,
3. requires inpatient hospitalization or prolongation of existing hospitalization,
4. results in persistent or significant disability/incapacity,
5. leads to a congenital anomaly/birth defect, or
6. other medically significant condition: a medically important event that causes a risk to a research subject even if it is not immediately life-threatening and does not result in death or hospitalization, or requires an action or treatment to prevent the results described in 1 to 5 above. In addition, points described in the Takeda Medically Significant Adverse Event List (Table 10 (a)) are included in this section.

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

Table 10.a Takeda Medically Significant AE List

Acute respiratory failure/acute respiratory distress syndrome (ARDS)	Hepatic necrosis
Torsades de pointes/ ventricular fibrillation/ventricular tachycardia	Acute hepatic failure
Malignant hypertension	Anaphylactic shock
Convulsive seizure (including convulsion and epilepsy)	Acute renal failure
Agranulocytosis	Pulmonary hypertension
Aplastic anemia	Pulmonary fibrosis (including interstitial pneumonia)
Toxic epidermal necrolysis/ Oculomucocutaneous syndrome (Stevens-Johnson syndrome)	Neuroleptic malignant syndrome/ malignant hyperpyrexia
	Spontaneous abortion/ stillbirth and fetal death
	Confirmed or suspected transmission of infection by a medicinal product
	Confirmed or suspected endotoxin shock

When a PTE meets any of the above criteria for SAEs, it shall be reported in accordance with a procedure similar to that for serious adverse events (Refer to Section 10.2.2 and 10.3).

10.1.5 Severity of pretreatment events and adverse events

The severity of adverse events shall be classified and defined as shown below.

Mild	The event is transient and easily tolerated by the subject.
Moderate	The event interrupts the subject's usual activities.
Severe	The event causes considerable interference with the subject's usual activities.

10.1.6 Causality of adverse events

The causal relationship of each PTE and adverse event to the study drug shall be classified and defined as shown below.

Related	An adverse event that follows a temporal sequence (including clinical course after discontinuation), or an adverse event in which there is at least a reasonable probability that a causal relationship to the study drug cannot be ruled out, although other factors such as underlying disease, concurrent diseases, or concomitant drugs/treatment are also suspected.
Not	An adverse event that does not follow a temporal sequence from administration of

related	the study drug. Very likely due to other factors such as underlying disease, concurrent diseases, or concomitant drugs/treatment.

10.1.7 Relationship to study procedures

The relationship shall be recorded as "Yes" if the principal investigator or the investigator considers that there is reasonable possibility that an adverse event is due to a study procedure. Otherwise, the relationship shall be recorded as "No."

10.1.8 Date of onset

The date of onset of adverse event shall be determined according to the following rules:

Adverse event, etc.	Date of onset
Signs, symptoms, diseases (diagnoses)	The date on which the first signs/symptoms were noted by the research subject and/or the principal investigator or investigator.
Asymptomatic diseases	The date on which a diagnosis was confirmed through a test(s). The date on which a diagnosis was confirmed, even when the test results indicate an old sign(s) of the disease or an approximate time of its onset.
Exacerbation of concurrent diseases or PTE	The date on which the first worsening of diseases/symptoms was noted by the research subject and/or the principal investigator or investigator.
Onset of a test abnormality after the start of the study drug administration (in case of PTE) (in case of AE)	The date on which a clinically significant laboratory abnormality was detected.
Worsening of a baseline test abnormality after initiation of study treatment	The date on which a clear increase/decrease in a laboratory parameter was clinically confirmed based on the time profile of the parameter.

10.1.9 Date of resolution

The date of resolution of an adverse event, etc. is the date on which the research subject recovered (including resolution with sequelae). If a research subject died due to the adverse event, etc. concerned, it shall be the date of death. The adverse event shall be recorded as "ongoing" if the research subject has not yet recovered by the end of the study.

10.1.10 Actions taken for the study drug

Actions taken for the study drug shall be classified or defined as shown below.

Drug withdrawn	The study drug is discontinued because of an adverse event (including withdrawal by the research subject at his/her own discretion). The case in which the study drug administration is continued after withdrawal from the study shall be categorized into "Dose not changed."
Dose not changed	The dose was not changed after the onset of the adverse event. The study drug was discontinued, reduced, or increased because of another adverse event. The study drug was discontinued or reduced for a reason other than the adverse event, e.g., inadvertence of the research subject.
Unknown	It has not been possible to determine what action has been taken because the research subject is lost to follow-up.
Not Applicable	The administration of the study drug had already been completed or discontinued before the onset of the adverse event.

10.1.11 Outcome

Outcome of adverse events is classified as follows:

Category	Criteria
Recovered	Disappearance or recovery of symptoms and findings Laboratory values returned to normal or baseline
Improved	The intensity is lowered by one or more stages Symptoms or findings mostly disappeared Laboratory values improved, but have not returned to normal or baseline The research subject died from a cause other than the concerned adverse event while the condition was resolving (recording of the date of death unnecessary)
Not recovered	No change in symptoms, findings, or laboratory data The symptoms, findings, or laboratory data on the final day of observable period were aggravated compared with the date of onset Irreversible congenital anomaly The research subject died from another cause before resolution of the concerned adverse event (recording of the date of death unnecessary)
Recovered with sequelae	Disability that disturbs daily life
Death	Direct relationship between death and the concerned adverse event "Direct relationship" means that the concerned adverse event was the cause of death, or the concerned adverse event was clearly responsible for death. Outcome of an adverse event which was not determined (judged, presumed) a direct cause of death observed in the same research subject is not considered as death. The date of death shall be recorded.
Unknown	Follow-up specified in the protocol after the date of onset was not possible due to

	change of hospitals or relocation, etc.

10.2 Procedures

10.2.1 Collection and reporting of adverse events

10.2.1.1 Adverse event collection period

The collection of PTEs shall start after the acquisition of informed consent and continue until the start of the study drug administration at Visit 2 (Day -15 to Day -1 before the start of study drug administration). If withdrawal was decided before the start of administration of the study drug, the collection of PTEs shall be terminated at that point.

Collection of the adverse events shall commence at the start of administration of the study drug to research subjects [at Visit 2 (Day -15 to Day -1 before the start of study drug administration)] and continue until completion of the treatment period [Visit 4 (Week 8)] or at discontinuation.

10.2.1.2 Reporting of adverse events

At each study visit, the principal investigator or investigator shall check for the presence of any onset of subjective symptoms. A neutral question, such as “How have you been feeling since your last visit?” may be asked to collect any adverse events that occurred between the previous and present visits.

When a PTE corresponds to a serious criterion, the principal investigator or investigator shall follow up all research subjects experiencing the PTE until the symptom resolve, any clinically significant abnormal laboratory values have returned to the baseline after acquisition of informed consent, or there is a satisfactory explanation for the change (permanent and irreversible PTEs). When the PTE does not correspond to a serious criterion, irrespective of the causal relationship with the procedure, follow-up of the research subject is not required by the protocol.

The principal investigator or investigator shall follow up all research subjects experiencing an adverse event irrespective of the causal relationship with the study drug, until the symptom resolve, or any clinically significant abnormal laboratory values have returned to baseline or there is a satisfactory explanation for the change (permanent and irreversible adverse events, etc.). All adverse events shall be recorded in the CRF. Name of adverse event, date of onset, date of resolution, grade, causal relationship with the study drug (i.e. "Unrelated" or "Related"), action taken for the study drug, outcome, relationship to study procedures, and severity shall be recorded.

Follow-up period of PTEs meeting any of the criteria for AEs and SAEs shall be until recovery of the adverse events, or the time when the principal investigator or investigator judges that further follow-up would be unnecessary.

10.2.2 Collection and reporting of serious adverse events

When a serious adverse event develops during the period of collecting adverse events, it shall be reported according to the following procedures. When a serious PTE develops, it shall also be reported in accordance with a procedure similar to that for serious adverse events.

At the time of onset of a serious adverse event or after notification of the onset by the research subject, the principal investigator shall report the serious adverse event to the head of the study site immediately, and the sponsor or CRO to whom the sponsor has entrusted responsibility shall notify the principal investigator of the study site.

The principal investigator shall then report the serious adverse event to the sponsor (for the contact information, refer to the attachment) within 1 working day of after notification of the onset. Further, the investigator shall submit a formal report within 10 calendar days to the sponsor.

Furthermore, it shall be mandatory to include the contents below in the report to be submitted to the sponsor within 1 working day, and other items shall be reported as far as possible.

- Brief description of adverse event and the reason for why it was determined as serious
- Research subject ID number
- Name of investigator or the subinvestigator
- Name of the study drug
- Determined causal relationship

10.2.3 Reporting of additional information concerning adverse events

If the sponsor requests provision of additional information concerning adverse events for reporting to regulatory authorities, the investigator or the subinvestigator shall confirm the necessary additional information and enter in the EDC system or submit a report within the period specified by the sponsor.

10.3 Follow-up of serious adverse events

When information that was not included in the detailed report was obtained later, the investigator or subinvestigator shall state it in the copy of the report on serious adverse events, or create another document and submit it to the contact address shown on the attached sheet. Relevant data collected

at the research implementing entity (e.g., ECG charts, laboratory test values, discharge summary, postmortem results) shall be sent to the sponsor or the committee such as the IEC upon request.

The investigator or the subinvestigator shall follow-up all serious adverse events, etc., until recovery is confirmed, or the final outcome is determined. In addition, serious PTEs shall also be followed, in accordance with procedure similar to that for serious adverse events.

10.3.1 Reporting of serious adverse events, etc., to Ethics Review Committee, etc., and regulatory authorities

When the head of study site receives a report of a serious adverse event from the principal investigator, the head of study site shall consult the Ethics Review Committee, etc., and notify the study sites that are conducting the clinical research through the sponsor or the CRO consigned by the sponsor.

When the principal investigator reported a serious adverse event for which a causal relationship to the research (study drug) cannot be ruled out and is unexpected, the head of the study site shall prepare a written report of the unexpected serious adverse event containing the information reported by the principal investigator plus the information below, and submit the report to the Minister of Health, Labour and Welfare, and notify other study sites conducting the clinical research. (The chief executive of the research implementing entity may report it to the Minister of Health, Labour and Welfare via the sponsor, and notify it to other clinical research implementing entities via the sponsor.) Serious PTEs shall also be reported in accordance with the same procedure as that for the serious adverse events. (No need to report to the Minister of Health, Labour and Welfare.)

- Actions taken for serious adverse events (discontinuation of new enrollment, revision of informed consent form, re-consents to other research subjects, etc.)
- Date of review, summery of review, result, necessary action, etc., related to Ethics Review Committee, etc.
- Notification to other research implementing entities

The sponsor shall report, in accordance with regulations, unexpected serious adverse drug reactions and other serious adverse events that are subject to emergency reporting to regulatory authorities, the investigators, and the head of study site.

From the time point of first acknowledging the event or receiving additional information, the sponsor or the CRO consigned by the sponsor shall comply with regulatory required time frames for reporting, and make emergency reports concerning unexpected serious adverse drug reactions and expected serious adverse drug reactions to regulatory authorities. Also, the sponsor shall, in the same way, make an emergency report of other critical safety information that may have a major effect on

the study drug risk-benefit, continuation of study drug administration, or continuation of clinical research. The research implementing entity shall submit copies of emergency report documents to the Ethics Review Committee, etc.

11.0 COMMITTEES ESTABLISHED FOR THIS STUDY

11.1 Clinical Endpoint Committee

The Central Assessment Committee members shall be independent specialists who have appropriate experience in the assessment of the parameters set as the endpoints of this research.

REGARDING THE PRIMARY ENDPOINT “%FMD (FASTING)” AND THE SECONDARY ENDPOINT “%FMD (4 H POSTPRANDIAL)”,THE CENTRAL ASSESSMENT COMMITTEE SHALL CONFIRM THE VALIDITY OF THE ASSESSMENT OF THE MEASUREMENT RESULTS ACCORDING TO THE PROCEDURE defined separately on the measured images reported from the FMD measuring study sites or measuring institutes.

The assessment results from the committee members shall be entered into the clinical research database and used for the analysis of endpoints.

12.0 DATA MANAGEMENT AND STORAGE OF RECORDS

Data management operations shall be performed according to the standard operating procedure by the data management department of the sponsor independent from the medical affairs department. Adverse events, PTE, medical history and concurrent disease shall be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Drugs shall be coded using the WHO (World Health Organization) Drug Dictionary.

12.1 Case report form

The principal investigator or investigator shall complete a CRF for each research subject who has signed the informed consent form.

The sponsor or the designee shall provide access rights to the electric CRF system to the study site. Before use of the electric CRF system, the sponsor shall provide training to the principal investigator, investigators, and study collaborators. The CRF shall be used to report the information collected during the study period to the sponsor. CRF must be completed in Japanese. Data shall be directly entered in preparing the CRF.

A change or correction of the CRF shall be recorded as an audit trail that records the information before and after the change or correction, the person who made the change or correction, date of change or correction, and its reason.

The investigator the designee shall ensure the accuracy and completeness of the CRF, and provide an electronic signature on the relevant page of the case report form. The principal investigator shall bear full responsibility for the accuracy and reliability of all data entered on the CRF.

The following data shall be recorded on the CRF directly (except for the data included in the source documents).

- Eligibility, completioin status, reason for discontinuation, the seriousness, severity, relation to study drug and study procedures outcome

The following data shall not be recorded directly into the CRFs.

- Laboratory test values

FMD/Vessel caliber measurement results When the principal investigator or the investigator makes a change or correction in the data entered on the CRF after fixation of clinical data base, a record (Data Clarification Form) of change or correction on the CRF provided by the sponsor shall be used. The principal investigator shall confirm that the record of change or correction on the CRF is accurate and complete, and sign or write name/affix a seal, and date it.

The sponsor or the designee shall confirm that the CRFs are completed appropriately according to the procedures set by research. The sponsor or the designee shall have access to the medical records of the research subjects and in-house records to ensure the accuracy of the CRF as necessary. The completed CRF is the property of the sponsor, and the principal investigator or investigator shall not disclose the information to a third party without a written permission from the sponsor.

12.2 Timing of data entry into the electronic CRF system

The sponsor or the designee shall request the principal investigator and investigator to promptly enter data into the EDC following enrolment of the research subject, each visit during study treatment, completion/discontinuation of the study, and follow-up period.

12.3 Storage of records

The principal investigator or the chief executive of the study site shall store the following materials, including those specified in Section 12.1, and study-specific documents to be investigated or audited by the regulatory authority and the sponsor or the designee. The documents include research subject ID code, medical records, clinical study worksheets (if used), original signed and dated informed consent forms, the record of change or correction on the CRF (copy), and electric copies of electronic CRF including audit trail. The principal investigator and the chief executive of the study site shall appropriately retain the material/information related to this study for at least 5 years from the date of reporting the end of the research by the principal investigator, or for 3 years from the date of reporting final publication of the study result, whichever date is later. However, when the sponsor requires a longer storage period, the chief executive of the study site shall discuss the period and methods of storage with the sponsor.

13.0 STATISTICAL ANALYSIS METHODS

The person in charge of analysis and the designee [analysis personnel, who belongs to contract research organization (CRO) independent from the sponsor] shall perform the statistical analysis. The sponsor will not be involved in the statistical analysis.

13.1 Statistical and analytical plans

The analysis personnel shall prepare a statistical analysis plan before the acquisition of the informed consent of the earliest research subject, and issue the first edition. Detailed definition of endpoints and analytical methods should be specified in the SAP to deal with all the purposes of the research.

13.1.1 Analysis set

Two analysis sets, “Full Analysis Set；FAS” and “Safety Analysis Set；SAS” are used in this research. The FAS primarily used for efficacy analysis will be defined as “research subjects who have been randomized and received the study drug at least once”, and the SAS as “research subjects who have received the study drug at least once during the clinical research”.

13.1.2 Analysis of demographic and other baseline characteristics

From “SAS” primary research subject background items will be tabulated.

13.1.3 Efficacy analysis

From FAS the following shall be analyzed.

13.1.3.1 Analysis of primary endpoints

%FMD(fasting)

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.
2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.
3) Using fasting TG at the start of treatment, a stratified analysis on summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be performed.

13.1.3.2 Analysis of secondary endpoints

① %FMD (4 h postprandial)

② TG level (fasting)

③ TG level (4 h postprandial)

④ Plasma fatty acid fraction

[Analytical method]

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.

3) Using fasting TG at the start of treatment, a stratified analysis on summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be performed.

13.1.3.3 Analysis of other endpoints

Efficacy endpoints:

Total cholesterol, LDL-C, LDL-C, Remnant-like particle (RLP) cholesterol , Apoprotein B-48, C-reactive protein (CRP), 8-epi-PGF2α quantitative (urinary)

Analytical method

1) Summary statistics by treatment group (number of subjects, mean, SD, minimum value, maximum value, quartiles) and a two-sided 95% confidence interval for the mean will be calculated at each evaluation time point during the treatment period, and a diagram illustrating the change in the mean ± SD will be prepared.

The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.

13.1.4 Safety analysis

From Safety Analysis Set (SAS) the following will be analyzed.

(1) Adverse events

The following analysis will be performed for each treatment group. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by System Organ Class and Preferred Term.

- Tabulation of frequencies of all adverse events
- Tabulation of frequency of adverse events with a causal relationship to the study drug
- Tabulation of frequency of all adverse events by severity
- Tabulation of frequency of adverse events with a causal relationship to the study drug by severity
- Tabulation of frequency of adverse events leading to study drug discontinuation
- Tabulation of frequency of serious adverse events
- Tabulation of frequency of all adverse events by time of onset

(2) Body weight, blood pressure in the sitting position, pulse in the sitting position, laboratory tests [fasting plasma glucose (FPG)]

1) Summary statistics of measurements by treatment group at each evaluation time point during the treatment period will be calculated and a diagram of the change of individual data will be created.

2) The change for each treatment group (Treatment Period Visit 3 or Visit 4 - Treatment Period Visit 2) will be calculated at each evaluation time point during the treatment period and the same analysis as in 1) will be performed.

3) Regarding evaluation results based on standard values, shift tables of treatment period Visit 2 and assessment time points of treatment period (Visit 3, Visit 4) will be prepared.

13.2 Criteria for interim analysis and premature discontinuation

No interim analysis is planned.

13.3 Determination of the number of planned research subject

40 patients evaluable for the primary endpoint

(Omega-3-acid ethyl esters 2 g: 20 patients, Omega-3-acid ethyl esters 4 g: 20 patients)

[Rationale for the number of planned research subjects]

The planned sample size is based on the feasibility to explore the effects of omega-3-acid ethyl esters on vascular endothelial function.

14.0 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring of the study site

The sponsor or the designee shall perform periodic monitoring of the study site during the research to confirm that the research is conducted in accordance with all specifications in the research protocol. In the monitoring, the data recorded on the CRF will be checked by comparing them with those in the source documents. Source documents are the original documents, data and records. The principal investigator and the chief executive of the study site shall ensure that the sponsor or the designee and the Ethics Review Committee, etc., have access to the source documents.

The sponsor or the designee shall access the records, including the list of research subject ID numbers, medical records of the research subjects, and signed and dated original consent forms to confirm that the research is appropriately conducted in compliance with the research protocol. Also, confirm the consistency between CRF and the related source documents. The principal investigator, investigator, and other personnel involved in the research shall spare sufficient time to facilitate monitoring procedures during visits to the study site.

Detailed procedures for monitoring shall be described separately in the procedure.

14.2 Deviation from the Ethical Guidelines for Medical and Health Research Involving Human Subjects and the research protocol.

The principal investigator or investigator shall record all deviations from Ethical Guidelines for Medical and Health Research Involving Human Subjects, and research protocol.

If any deviation is found, the principal investigator shall promptly notify the chief executive of the study site for the clinical research and the sponsor. As necessary, the principal investigator will discuss protocol revisions with the sponsor to reach agreement. For protocol revisions, draft revisions should be submitted as early as possible to the study site for approval of the committee such as the IEC.

14.3 Quality assurance audits and regulatory agency inspections

The study site may be research subject to audits by the sponsor or the designee. In such a case, the auditor designated by the sponsor shall contact the study site in advance to determine the date of audit. The auditor may ask to visit the facilities where laboratory specimens are collected and any other facilities used during the clinical research. In addition, this research may be inspected by regulatory agencies, including those of foreign governments (e.g., the Food and Drug Administration [FDA], the United Kingdom Medicines and Healthcare products Regulatory Agency [MHRA]). If the study site is contacted for an inspection by a regulatory body, the sponsor should be notified

promptly. The principal investigator and the chief executive of the study site shall ensure that the auditor has access to all the research-related source documents.

15.0 ETHICAL CONDUCT OF CLINICAL RESEARCH

This research shall be conducted with the highest respect for the individual participants (i.e., research subjects) according to the research protocol, and the ethical principles that have their origin in the Declaration of Helsinki, Ethical Guidelines for Medical and Health Research Involving Human Subjects. Each principal investigator will conduct the study according to regulatory requirements and in accordance with "Responsibilities of the Investigator" in Appendix B.

15.1 Approval of the Ethical Review Board, etc.

The Ethical Review Board, etc., shall be constituted in accordance with the regulations.

The sponsor or the designee should obtain the document listing the name and title of each committee member. When a committee member directly participates in this clinical research, the document describing that he/she is not participating in deliberation or voting for the study will be obtained.

The sponsor or the designee shall supply relevant documents for submission to study site committee such as the Ethics Review Committee for the protocol's review and approval. In addition to the research protocol, a copy of the informed consent form and information sheet, written materials related to research subject recruitment, advertisement, and other documents required by regulations, when necessary, shall be submitted to the central committee or a study site committee such as the Ethics Review Committee to obtain approval. The sponsor or the designee must obtain written approval of the protocol and subject informed consent from the study site committee such as the Ethics Review Committee before commencement of the study. The study site committee such as the Ethics Review Committee's approval must refer to the study by exact protocol title, number and version date; identify versions of other documents (e.g., informed consent form) reviewed; and state the approval date. The sponsor shall notify the study site, the principal investigator, and investigator after confirming the validity of the regulatory documents of the study site. Protocol procedures such as obtainment of consent shall not be started until the study site, the principal investigator, and investigator receive notification.

The study site shall observe all requirements that the Ethical Review Board, etc. prescribe. The requirements may include notifications to committees such as the IEC, for example, revision of the protocol, revision of the informed consent form and information sheet, revision of materials related to research subject recruitment, reports on safety in accordance with the regulatory requirements, reports on status of implementation of the research at intervals determined by a study site committee such as the Ethics Review Committee, and submission of the study completion report. The sponsor or the designee shall obtain written approval from the Ethics Review Committee, etc. related to the above mentioned items and all related materials.

15.2 Conflict of interest

These clinical studies shall be conducted with the support of the research client.

Prior to the conduction of this clinical research, the principal investigators involved in this clinical research shall ensure appropriate management of any conflicts (COI) in the conduct of the research in accordance with the rules of the study site.[8)-12)]

The study site shall observe all requirements that the Ethical Review Board, etc. prescribe. This will include self-declaration of COI, clinical research protocol, informed consent and information sheet.

15.3 Informed consent and information sheet, and the agreement of the research subjects

The informed consent and information sheet form shall contain specific requirements of the Declaration of Helsinki, Ethical Guidelines for Medical and Health Research Involving Human Subjects and all applicable laws and regulations. The informed consent form and information sheet shall specify the use of personal information and medical information of research subjects in this clinical research (both in and outside Japan: supply to a third party), and disclosure. The informed consent form and the information sheet will explain in detail the nature of the research, its objectives, and potential risks and benefits. The informed consent form and the information sheet will detail the requirements of the participant and the fact that research subject is free to withdraw at any time without giving a reason and without any negative effect on further medical care.

The principal investigator is responsible for the preparation, contents, and approval of the informed consent form and research subject information sheet by the committee such as the IEC. The informed consent form and information sheet must be approved by the committee prior to use.

The informed consent form and information sheet shall be written in language that can be easily understood by the potential research subjects. The principal investigator or investigator shall be responsible for providing detailed explanation of the informed consent form and information sheet to the potential subjects. Information should be given in both oral and written form whenever possible and in manner deemed appropriate by the committee such as the IEC.

The principal investigator or investigator must (1) give the opportunity to ask questions and (2) sufficient time to consider whether to participate in the study to the potential subjects. If the potential subject decides to participate, the informed consent form must be signed and dated by the potential subject prior to entering into the research. The principal investigator or investigator shall instruct the potential subject or representative to sign using their legal names, not nicknames, using a blue or black ball point ink pen. Also the principal investigator or investigator shall sign and date the informed consent form prior to entering into the research.

Once signed, the original informed consent form shall be retained by the principal investigator or investigator. The principal investigator or investigator shall record the date that the potential research subject signed the informed consent form in the subject's medical record. A copy of the signed informed consent form shall be given to the research subject.

The principal investigator or investigator shall follow the same procedure as for obtaining the initial consent when newly obtaining re-consent from the concerned research subject when the informed consent form is revised. The date of obtaining new consent shall be recorded in the research subject's medical record, and a copy of the revised consent form shall be provided to the research subject.

15.4 Personal information of the research subjects

The sponsor or the designee shall affirm the principle of the protection of research subjects' private/personal information. Throughout this study, research subject ID numbers shall be used to link the subject's source data to the sponsor's research database and research-related documents. Limited information on research subjects such as gender, age, and date of birth may be used within the scope of all applicable laws and regulations for identification of research subjects and confirmation of accuracy of research subject ID number.

For verification of the conduct of the research in compliance with this protocol and the Ethical Guidelines for Medical and Health Research Involving Human Subjects, the sponsor shall require the principal investigator to provide the research sponsor's designee, representatives of regulatory authorities, designated auditors, and committees such as the Ethical Review Board direct access to research subjects' original medical records (source data or documents), including laboratory test results, admission and discharge records during a subject's research participation, and autopsy reports. The principal investigator or investigator shall obtain specific authorization of the research subject as part of the informed consent process for access to research subject's original medical records by research sponsor's designee and representatives of regulatory authorities (see Section 15.3).

When providing a copy of source documents to the sponsor, the principal investigator or investigator shall delete information that may lead to identification of an individual (name and address of research subject, other personal information not recorded on the CRF of the research subject).

15.5 Consultation windows for the research subjects or persons related to the research concerned

The principal investigator shall establish a contact service to respond to inquiries concerning this clinical research from research subjects or concerned people. Details of the contacts for inquiries will be described in the ICF.

15.6 Financial burden or reward to the research subjects

Of the expenses for this clinical research, the sponsor shall offer compensation for medical treatment not covered by health insurance as research expenses. The research subjects shall pay expenses for medical treatment covered by ordinary health insurance.

In addition, the principal investigator shall pay expenses such as transportation expenses for participation in this clinical research to the research subjects at each visit from the research funds. Details of the financial burden on the research subjects and rewards shall be described in the informed consent form and information sheet.

15.7 Benefits and inconveniences to the research subjects

15.7.1 Benefits to research subjects

By participating in this clinical research, the research subjects may understand one's own condition of vascular endothelial function in detail.

15.7.2 Inconveniences to research subjects

By participating in this clinical research the burden of the research subject may increase as number of visits will increase compared to daily medical care.

15.8 Attribution of research results and access rights

15.8.1 Attribution of research results

The research results and data obtained from this research shall belong to the sponsor.

15.8.2 Data access rights

Access rights for all data and information generated from this research will be given to personnel approved by the sponsor. In addition, secondary use (meta-analysis, etc.) of the data obtained in this clinical research may be possible if used in such a way that the data shall not be linked to personal identification information.

15.9 Reporting of results, Publication, disclosure, and clinical research registration policy

15.9.1 Reporting of results, publication and disclosure

The principal investigator shall report a written summary of results of the research to the chief executive of the study site and provide the sponsor with all the results and data obtained from the research. Only the sponsor may disclose the research information to other principal investigators, investigators or regulatory authorities during the research period, except when required by laws and

regulations. The sponsor shall be responsible for publication of the research protocol and research-related results (including the public web site) except for other cases permitted in the research contract.

During research period and after the end of research, the sponsor or the designee should promptly summarize the results and present it to medical journals and academic conferences, etc. The sponsor may publish any data or information obtained from the research (including data and information provided by the principal investigator) without obtaining agreement of the principal investigator.

The investigator or the subinvestigator should obtain the prior written approval from the sponsor when publishing the information obtained in this research at an academic conference, etc.

15.9.2 Clinical research registration

To ensure that information on clinical research is made accessible to the public in a timely manner and to comply with applicable laws, regulations, and guidelines, Takeda Pharmaceutical Company Limited shall register all clinical research being conducted in patients around the world at public trial registration sites, including at least the website(s) of ClinicalTrials.gov (and) Japan Pharmaceutical Information Center Clinical Trials Information (JAPIC), before initiation of the clinical research. On such websites, the research location (city, country), subject recruitment status, and contact information for Takeda Pharmaceutical Company Limited are open to the public.

15.9.3 Clinical trial results disclosure

Takeda Pharmaceutical Company Limited shall post the research results, irrespective of the nature of the results, at the public trial registration site(s) of Clinical Trials.gov (and) JAPIC in accordance with applicable laws and regulations.

15.10 Insurance and compensation for injury

In case of injuries, each research subject in the clinical research must be insured in accordance with the regulations applicable to the study site where the subject is participating. The sponsor or the designee shall buy an insurance policy to compensate for health injury in research subjects.

Healthy injury in a research subject will be compensated as specified in the study contract. Compensation-related questions by the principal investigator or investigators should be made to the sponsor or the designee.

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Appendix A Schedule for Research Procedures

		Observation period	Treatment period			Discontinuation (h)
Time of visit	Week	-4	0	4	8	
	Day	-29	-1(g)	28	56	
Allowable range (Day)		-29 to -1	-15 to -1	14 to 41	42 to 70	Up to 3 days after the last dose
VISIT Number		1	2	3	4	
Informed consent procedure		X				
Inclusion/Exclusion criteria		X	X			
Demographic data, medical history, previous therapeutic drugs		X	X(i)			
Physical examination		X(e)	X	X	X	X
Confirmation of food and drink consumption before visit (a)		X	X	X	X	X
Height		X				
Body weight		X				
BMI		X				
Vital signs		X(e)	X	X	X	X
Concomitant drugs (b)		X(e)	X	X	X	X
Concurrent disease		X(e)	X			
Laboratory tests (c)						
Serum chemistry (fasting)		X(e)	X	X	X	X
Serum chemistry (4 h postprandial)(d)			X	X	X	X
Urinalysis (fasting)		X(e)	X	X	X	X
Other tests			X	X	X	X
FMD						
FMD (fasting)			X(f)	X(f)	X(f)	X(f)
FMD (4 h postprandial) (d)			X(f)		X(f)	X(f)
Prescription of the study drug			X	X		
Drug compliance				X	X	X
PTE and adverse events assessment		(X)	←X→	←X→	←X	←X

(a) Eating/drinking to excess and extreme change in dietary content should be avoided on the day before the hospital visit.

(b) All concomitant drugs will be recorded.

(c) Hematology, urinalysis, and other details will be entered below:

- Hematology
  (Fasting): TG, total cholesterol, LDL-cholesterol, HDL-cholesterol , Remnant-like particle (RLP) cholesterol , apoprotein B-48, C-reactive protein (CRP), fasting plasma glucose (FPG), plasma fatty acid fraction
  (4 hours postprandial): TG, total cholesterol, LDL-C, HDL-cholesterol, Remnant-like particle (RLP) cholesterol , apoprotein B-48
- Urinalysis: Quantitative 8-epi-PGF2α

(d) Time window ± 30 minutes

(e) To be performed before test at Visit 2

(f) To be performed by the physician or the clinical laboratory technician who has finished the FMD measurement training

(g) To be performed after consent has been obtained. Medication will be started the day after the completion of all the tests of Week 0. The start date of medication will be referred to as day 1.

(h) To be performed to the extent possible

Appendix B Responsibilities of the Principal Investigator

1. Appropriately To appropriately conduct the clinical research in compliance with this research protocol and the Ethical Guidelines for Medical and Health Research Involving Human Subjects and with the highest respect for human rights, safety, and welfare of research subjects.
2. Prepare To prepare a list of any other investigators and/or research collaborators when certain important research-related activities are divided by investigators and/or research collaborators, and submit the list to the sponsor as required.
3. To prepare the informed consent form and revise it as necessary.
4. To check the contents of the study contract.
5. To provide sufficient information on the protocol, drug and duties of each personnel to subinvestigators and study collaborators, and give guidance and supervision.
6. To select research subjects who satisfy the inclusion criteria, give explanation using written information, and obtain consent in writing.
7. To be responsible for all medical judgments related to the research.
8. Corresponding to request from the chief executive of the study site, to report the latest progress status at least once a year to the chief executive of the study site.
9. To confirm and comprehended the most update status regarding the COI of the investigators participating in the clinical research according to the study site.
10. To ensure, together with the chief executive of the study site, that sufficient medical care is provided to research subjects for all research-related clinically problematic adverse events throughout the period of subjects' research participation and thereafter.
11. When a research subject is treated at another medical institution or department, to inform the acting physician at the medical institution or department in writing of the research subject's study participation and research completion/discontinuation after obtaining the research subject's consent, and prepare a record.
12. When emergency reporting of serious adverse events, etc., is required, to immediately report it in writing to the chief executive of the study site and the sponsor.
13. Prepare correct and complete CRFs, and submit them to the sponsor with an electronic signature.
14. To verify any entries on the CRFs prepared by the investigator or transcribed by the collaborator from source data, {electronically sign/ sign or write name/affix a seal, and submit them to the sponsor.
15. To discuss a revision of the protocol, etc., when proposed by the sponsor.
16. To report the research completion in writing to the chief executive of the study site.