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

Statistical analysis plan Approve Date: 14-Nov-2017

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Note; This document was translated into English as the language on original version was Japanese.

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

(Protocol number: TAK-085-4001)

Statistical Analysis Plan

(Ver.4.0: 14 Nov 2017)

Sponsor: Takeda Pharmaceutical Company Limited

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1. DEFINITIONS of TERMS

- Summary Statistics: Number of subjects, mean, SDs, maximum values, minimum values, and quartiles
- Treatment Group: Omega-3-acid ethyl esters 2g, Omega-3-acid ethyl esters 4g

2. TIME WINDOW

For each inspection, observation and evaluation item, evaluable data is handled according to the following table. When there are multiple data that can be evaluated at the same visit, the one with the closest inspection date, observation, and evaluation date to the reference date is adopted, and if the difference from the reference date is the same, the later data is adopted.

< FMD (Fasting, 4h postprandial) >

Visit	Reference implementation date	Time Allowance
		Days after the Administration
Start of Treatment Period	Days after the Administration: -1	-15 to -1
Treatment period 4 week	Days after the Administration: 28	1 to 41
Treatment period 8 week	Days after the Administration: 56	42 to 70

< Other than FMD >

Visit	Reference implementation date	Time Allowance
		Days after the Administration
Start of Treatment Period	Days after the Administration: -1	-29 to -1
Treatment period 4 week	Days after the Administration: 28	1 to 41
Treatment period 8 week	Days after the Administration: 56	42 to 70

- The reference implementation date and the number of days after administration in the treatment period are indicated as “Day -1” for the day before study drug administration and “Day 1” for the administration day. As the administration is started from the day after the visit, 4 weeks of the treatment period and 8 weeks of the treatment period are 28 days and 56 days after the study drug administration.

3. ANALYSIS SET

- Full Analysis Set

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

- Safety Analysis Set

  The subjects who are given at least one dose of the study drug.

4. CONSIDERATIONS for ANALYSIS

- Confidence coefficient

  95% (two-sided estimation)

- Display digit

  [Mean, Confidence coefficient, Quartiles]

  Round statistics off to the 1 digits lower than significant digits of the data.

  [Standard Deviation]

  Round statistics off to the 2 digits lower than significant digits of the data.

  [Minimum and Maximum Values]

  Display the data at the significant digits.

  [Proportion, Percentage]

  Round statistics off to 1 decimal places.

5. OTHER DATA HANDLING

[Data Handling for Study Drug]

- Duration of Treatment

  Duration of Treatment = Date of the Last Dose - Date of the First Dose + 1

[Data Handling for Adverse Event]

- Adverse Events

  An adverse event is defined as any untoward medical occurrence in a patient or a 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).

- Time to Occurrence of Adverse Event

  Time to Occurrence of Adverse Event = Onset Date of Adverse Event - Date of the First Dose + 1

- Non-serious Adverse Events

  Adverse events, excluding serious adverse events (protocol 10.1.4), shall be non-serious adverse events in the case of an incidence of over 5% in at least one treatment group.

[Data Handling for Duration of Dyslipidemia (year)]

- Duration of Dyslipidemia (year)

Duration of Dyslipidemia (year) = (Date of Informed Consent (year/month) – Onset Date of Dyslipidemia (year/month)) / 12 (rounded off to two decimal places)
If only the month of the onset of dyslipidemia is unknown, the month of the onset of dyslipidemia is regarded as “January”.

6. SUBJECTS, DEMOGRAPHIC and OTHER BASELINE CHARACTERISTICS

6.1. Subject Disposition

6.1.1. Study Information

Analysis Set: All subjects who were obtained informed consent

Analysis Variables: The earliest date of informed consent
The latest date of the last date of administration
Version of MedDRA
Version of SAS

Analysis Methods: For the above analysis variables, the following analysis is performed.
(1) Show above items.

6.1.2. Eligibility of Subjects

Analysis Set: All subjects who were obtained informed consent

Analysis Variables: Randomization into the treatment period of the study
[Yes, No (and the reason)]

Analysis Methods: For the above analysis variables, the following analysis is performed.
(1) Frequency Count

6.1.3. Subject Disposition

6.1.3.1. Study completion status

Analysis Set: Randomized subjects

Analysis Variables: Study completion status
[Complete, Incomplete (and the reason)]

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group and all subjects in the analysis set.
(1) Frequency Count

6.1.4. Protocol Deviations and Analysis Datasets

6.1.4.1. Protocol Deviations

Analysis Set: Randomized subjects

Analysis Variables: Protocol Deviations

[Major GCP Violations, Deviations of Protocol Entry Criteria, Deviations of Discontinuation Criteria, Deviations Related to Treatment Procedure or Dose, Deviations Concerning Excluded Medication or Therapy, Deviations to Avoid Emergency Risk, Other Deviations]

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group and all subjects in the analysis set.

(1) Frequency Count

Summarize the number of subjects who have deviated from the protocol, classify the deviations into above category, and show the breakdown of deviations. Subjects applicable for multiple categories will be counted once in each category.

6.1.4.2. Datasets Analyzed

Analysis Set: Randomized subjects

Analysis Variables: Subjects excluded from analysis datasets

[Reason of exclusion]

Full Analysis Set [Adopted]

Safety Analysis Set [Adopted]

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group (both (1) and (2)) and all subjects (for (2)) in the analysis set. For analysis (1), Subjects applicable for multiple categories will be counted once in each category.

(1) Frequency count in each analysis set about handling for subjects

(2) Frequency count in each analysis set of adopted subjects.

6.2. Demographics and Other Baseline Characteristics

6.2.1. Distribution of Demographics

Analysis Set: Safety Analysis Set

Analysis Variables:

Age (years old) [Min<= - <65, 65<= - <75, 75<= - <=Max]

Sex [Male, Female]

Height (Observation period) (cm)

[Min<= - <150, 150<= - <160,

160<= - <170, 170<= - <=Max]

Weight (Observation period) (kg)

[Min<= - <50.0, 50.0<= - <60.0,

60.0<= - <70.0, 70.0<= - <80.0,

80.0<= - <=Max]

BMI (Observation period) (kg/m^2)

[Min<= - <18.5, 18.5<= - <25.0,

25.0<= - <=Max]

Duration of Dyslipidemia (year)

[Min<5, 5<=Max, Unknown]

Last Menstrual Period

[<2 years, 2 years <=, Not Applicable]

Frequency of Consumption of Fish

[Almost every day, About Every Two Days, About Once or Twice Per Week, Rarely]

Smoking Classification

[Never Smoked, Current Smoker, Ex-Smoker]

Drink Alcohol Almost Every Day?

[Yes, No]

TG level (fasting) (Start of Treatment Period) (mg/dL)

[Min<= - <200, 200<= - <500, 500<= - <=Max]

TG level (4h postprandial) (Start of Treatment Period) (mg/dL)

[Min<= - <200, 200<= - <500, 500<= - <=Max]

Plasma Fatty acid Fraction EPA/AA ratio (Start of Treatment Period)

[Min<0.3, 0.3<=Max]

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group and all subjects in the analysis set.

(1) Frequency count for discrete variables and summary statistics for continuous variables

6.2.2. Medical History and Concurrent Disease

Analysis Set: Safety Analysis Set

Analysis Variables: Medical history, Concurrent disease

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group. Analysis variables will be coded using the MedDRA dictionary and be summarized into SOC and PT. SOCs will be sorted in alphabetical order, then PTs will be sorted in frequency order.

(1) Frequency of medical history by SOC/PT

(2) Frequency of concurrent disease by SOC/PT

The method of accounting for the frequency is as follows.

[Number of Subjects]

Within each summary, subjects with one or more events within a level of SOC term is counted only once in that level. Similarly, subjects with one or more events within a level of PT term is counted only once in that level.

6.2.3. Prior and Concomitant Medication

Analysis Set: Safety Analysis Set

Analysis Variables: Prior medication

Concomitant medication

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group. Analysis variables will be coded using the WHO (World Health Organization) Drug. Coded medications will be sorted in frequency order. Medications used more than once within a subject will be counted only once for the subject.

(1) Frequency of prior medication

(2) Frequency of concomitant medication which was completed administration before study drug administration

(3) Frequency of concomitant medication which was started administration before study drug administration and was continued administered after start of study drug administration

(4) Frequency of concomitant medication which was started administration after start of study drug administration

(5) Frequency of concomitant medication which was administered study drug administration period.

6.3. Treatment Compliance

6.3.1. Study Medication Compliance

Analysis Set: Safety Analysis Set

Analysis Variables: Study medication compliance

[Min<= - <50.0%,

50.0%<= - <=Max]

Visit: Treatment Period 4 week and 8 week

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group and all subjects in the analysis set by visit.

(1) Frequency Count

6.3.2. Food and Drink Consumption before the Visit

Analysis Set: Safety Analysis Set

Analysis Variables: Did you consume alcohol from 9:00 pm two days before the hospital visit to the time of the fasting test, or consume food from 9:00 pm on the day before to the time of the fasting test? [Yes, No]

Did you experience excess and extreme change of dietary content (Eating/Drinking) on the day before the fasting test. [Yes, No]

Visit: Treatment Period 4 week and 8 week

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group and all subjects in the analysis set by visit.

(1) Frequency Count

6.3.3. Study Medication Exposure

Analysis Set: All Subjects Administered

Analysis Variables: Duration of exposure (days)

[1<= - <29, 29<= - <57, 57<= - <=Max]

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group and all subjects in the

analysis set.

(1) Frequency count for discrete variables and summary statistics for continuous variables

7. EFFICACY EVALUATIONS

7.1. Primary Endpoint and the Analytical Methods

Analysis Set: Full Analysis Set

Analysis Variables: %FMD (fasting)

Visit: Start of Treatment Period, Treatment Period 4 week and 8 week

Stratification Factor: Fasting TG at Observation Period

[150<= - <200, 200<= - <500]

Analysis Methods: For the above analysis variables, the following analysis is performed.

(1) Summary statistics by treatment group and a two-sided 95% confidence interval for the mean will be calculated at each visit 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 4 week/8 week – Start of Treatment Period) will be calculated at each visit during the treatment period and the same analysis as in (1) will be performed.

(3) Summary statistics using stratification factor and two-sided 95% confidence interval (CI) of mean value by visit will be calculated by treatment group.

(4) Using stratification factor, the change for each treatment group (Treatment Period 4 week/8 week – Start of Treatment Period) will be calculated at each visit during the treatment period and the same analysis as in (3) will be performed.

(5) Analyses same as (1) - (4) are performed, excluding subjects with missing of %FMD (fasting) data at any visit as sensibility analysis.

7.2. Secondary Endpoints and the Analytical Methods

Analysis Set: Full Analysis Set

Analysis Variables: %FMD (4h postprandial)
TG level (fasting)
TG level (4h postprandial)
Plasma fatty acid fraction
Visit: Start of Treatment Period, Treatment Period 4 week and 8 week
* Treatment period 4 is excluded about %FMD (4h postprandial)
Stratification Factor: Fasting TG at Observation Period
[150<= - <200, 200<= - <500]
Analysis Methods: For the above analysis variables, the same analyses describes in 7.1 (1) - (4) are performed by visit.

7.3. Analysis of other endpoints

7.3.1. Efficacy Endpoints

Analysis Set: Full Analysis Set
Analysis Variables: Total cholesterol (fasting and 4h postprandial)
LDL-C(fasting and 4h postprandial)
HDL-C(fasting and 4h postprandial)
Remnant-like particle (RLP) cholesterol (fasting and 4h postprandial)
Apoprotein B-48 (fasting and 4h postprandial)
C-reactive protein (CRP) (fasting)
8-epi-PGF2α quantitative (urinary) (fasting)
Visit: Start of Treatment Period, Treatment Period 4 week and 8 week
Analysis Methods: For the above analysis variables, the same analyses describes in 7.1 (1) and (2) are performed by visit.

8. SAFETY EVALUATION

8.1. Frequency of Adverse Event Occurrence

8.1.1. Brief Summary of Adverse Events

Analysis Set: Safety Analysis Set
Analysis Variables: Adverse Event
Category Classification:
Causal Relationship with the Study Drug [Related, Unrelated]
Severity [Mild, Moderate, Severe]
Time of Onset [1<= - <29,
29<= - <57,

57<= - <=Max]

Analysis Methods: For the above analysis variables, the following analysis is performed for each treatment group.

(1) Tabulation of frequencies of all adverse events
(2) Tabulation of frequency of adverse events with a causal relationship to the study drug
(3) Tabulation of frequency of all adverse events by severity
(4) Tabulation of frequency of adverse events with a causal relationship to the study drug by severity
(5) Tabulation of frequency of adverse events leading to study drug discontinuation
(6) Tabulation of frequency of serious adverse events
(7) Tabulation of frequency of Non-serious adverse events
(8) Tabulation of frequency of serious adverse events with a causal relationship to the study drug
(9) Tabulation of frequency of serious adverse events leading to study drug discontinuation
(10) Tabulation of frequency of adverse events leading to death
(11) Tabulation of frequency of all adverse events by time of onset

Incidence rates will be calculated as following on each analysis.

[Frequency of Subjects]

・Frequency by Severity

Subjects with one or more adverse events within a level of MedDRA term is counted only once in that level using the most severe incident. The denominator when calculating the incidence of adverse events is the number of subjects of safety analysis set.

・Frequency by Time of Onset

Subjects with one or more adverse events within a level of MedDRA term is counted only once in that category of time of onset. The denominator when calculating the incidence of adverse events is the number of subjects “whose study drug administration has been continued at that time point and later” or “who experienced an onset of adverse events at that time point or later” in safety analysis set and numerator is the number of subjects “experienced an onset of adverse events at that time point”.

・Analyses Other Than the Above

Subjects with one or more adverse events within a level of MedDRA term is counted only once for that MedDRA term. The denominator when calculating the incidence of adverse events is the number of subjects of safety analysis set.

8.1.2. Display of Adverse Event

Analysis Set: Safety Analysis Set

Analysis Variables: Adverse Event

Category Classification:

Causal Relationship with the Study Drug [Related, Unrelated]

Severity [Mild, Moderate, Severe]

Time of Onset [1<= - <29,
29<= - <57,
57<= - <=Max]

Analysis Methods: For the above analysis items, the following analysis is performed for each treatment group. Analysis variables will be coded using the MedDRA dictionary and be summarized into SOC and PT. SOCs will be sorted in alphabetical order, then PTs will be sorted in frequency order.

(1) Tabulation of frequencies of all adverse events (by SOC/PT)
(2) Tabulation of frequency of adverse events with a causal relationship to the study drug (by SOC/PT)
(3) Tabulation of frequency of all adverse events by severity (by SOC/PT)
(4) Tabulation of frequency of adverse events with a causal relationship to the study drug by severity (by SOC/PT)
(5) Tabulation of frequency of adverse events leading to study drug discontinuation (by SOC/PT)
(6) Tabulation of frequency of serious adverse events (by SOC/PT)
(7) Tabulation of frequency of Non-serious adverse events (by SOC/PT)
(8) Tabulation of frequency of serious adverse events with a causal relationship to the study drug (by SOC/PT)

(9) Tabulation of frequency of serious adverse events leading to study drug discontinuation (by SOC/PT)

(10) Tabulation of frequency of adverse events leading to death (by SOC/PT)

(11) Tabulation of frequency of all adverse events by time of onset (by SOC/PT)

Incidence rates will be calculated as following on each analysis.

[Frequency of Subjects]

・Frequency (by SOC/PT)

Within each summary, subjects with one or more adverse events within a level of SOC term is counted only once in that level. Similarly, subjects with one or more adverse events within a level of PT term is counted only once in that level. The denominator when calculating the incidence of adverse events is the number of subjects of safety analysis set.

・Frequency by Severity (by SOC/PT)

Subjects with one or more adverse events within a level of SOC/PT term is counted only once in that level using the most severe incident. The denominator when calculating the incidence of adverse events is the number of subjects of safety analysis set.

・Frequency by Time of Onset (by SOC/PT)

Subjects with one or more adverse events within a level of SOC/PT term is counted only once in that category of time of onset. The denominator when calculating the incidence of adverse events is the number of subjects “whose study drug administration has been continued at that time point and later” or “who experienced an onset of adverse events at that time point or later” in safety analysis set and numerator is the number of subjects “experienced an onset of adverse events at that time point”.

8.2. Other Endpoints and the Analytical Methods

8.2.1. Vital Sign

Analysis Set: Safety Analysis Set

Analysis Variables: Body Weight, Blood Pressure in the Sitting Position (Systolic, Diastolic), Pulse in the Sitting Position

Visit: Start of Treatment Period, Treatment Period 4 week and 8 week

Analysis Methods: The following analysis is performed for Safety Analysis Set.

(1) Summary statistics of measurements by treatment group at each visit 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 4 week/8 week – Start of Treatment Period) will be calculated at each visit 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 start of treatment period and each visit of treatment period 4 week and 8 week will be prepared.

8.2.2. Laboratory Test

Analysis Set: Safety Analysis Set

Analysis Variables: Fasting Plasma Glucose (FPG)

Visit: Start of Treatment Period, Treatment Period 4 week and 8 week

Analysis Methods: The following analysis is performed for Safety Analysis Set.

(1) Summary statistics of measurements by treatment group at each visit 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 4 week/8 week – Start of Treatment Period) will be calculated at each visit 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 start of treatment period and each visit of treatment period 4 week and 8 week will be prepared.

9. LISTING

Following lists will be create for randomized subjects.

- Demographics
- Discontinuation
- Protocol Deviation
- Subjects Excluded from Analyses

・Compliance of Study Drug Administration
・Adverse Event
・FMD Test
・Laboratory Tests (Blood biochemical examination including 4h postprandial)
・Laboratory Tests (Blood biochemical examination and urine test excluding 4h postprandial)

10. CONSIDERATIONS on STATISTICAL ANALYSIS

10.1. Adjustments for Covariates

In the analysis for primary endpoint for Full Analysis Set, stratified analysis of %FMD (fasting) is performed with Fasting TG value (150 <= - <200, 200 <= - <500) at the start of treatment as a layer. Details are described in 7.1 (3) and (4).

10.2. Handling of Dropouts or Missing Data

For laboratory test, values below quantitation limit are treated as zero.

10.3. Criteria for Interim Analysis and Early Discontinuation

Interim analysis will not be performed

10.4. Multicenter Studies

Analyses for consideration of medical institution will not be performed.

10.5. Multiple Comparisons/Multiplicity

It does not adjust multiplicity.

10.6. Examination of Subgroups

Subgroup analysis will not be performed.

11. REVISION HISTORY

Ver.	Date	Author	Revised Content	Reason for Revision
1.0	23 May 2016	PPD	-	
2.0	4 Aug 2016	PPD	[2.TIME WINDOW] Add <Exploratory biomarker evaluation> Add 7.3.2. Exploratory evaluation item (exploratory biomarker evaluation)	For exploratory evaluation item addition
3.0	29 Aug 2017	PPD	[Title page] Modify affiliation of approver and biostatistics manager.	For organization change and company integration
			[2.TIME WINDOW] Delete <Exploratory biomarker evaluation> Delete 7.3.2. Exploratory evaluation item (exploratory biomarker evaluation)	Analysis of exploratory biomarker is performed after March in 2018
			[5 OTHER DATA HANDLING] Add “Non-serious Adverse Event”	As required by Clinical Trial.gov
			[6.1.3 Subject Disposition] Change of Analysis Variables (“Exit Status of Stud Drug” → “Exit status from the study”	For mistake correction
			[7.1. Primary Endpoint and the Analytical Methods] Add analysis of Complete Case	To study as sensitivity analysis
			[8.1.1. Brief Summary of Adverse Events] [8.1.2. Display of Adverse Event] Add “Non-serious Adverse Event”	As required by Clinical Trial.gov

			[10.2. Handling of Dropouts or Missing Data] Add handling of values below quantitation limit in laboratory test	To clarify the definition of handling below quantitation limit
4.0	14 Nov 2017	PPD	[Title page] Modify affiliation of biostatistics manager.	For organization change
			[5. OTHER DATA HANDLING] Add < Data Handling for Duration of Dyslipidemia (year)>	To clarify the specification
			[General] Correct stratification factor from " Fasting TG at the Start of Treatment" to "Fasting TG at Observation Period"	Due to the data review, stratification factor was found to be " Fasting TG at Observation Period "
			[7.2. Secondary Endpoints and the Analytical Methods] “the same analyses describes in 7.1 are performed by visit” → “the same analyses describes in 7.1 (1) - (4) are performed by visit”	Analysis for complete case is performed only for primary endpoint.
			[9. LISTING] Add list to be created	For creation of lists