Study Title

Evaluation of immunity enhancement by ingestion of a Salacia plant extract-containing supplement

Study number: FJFR-13916
Study period: September 17, 2013 – October 29, 2013

[Draft history]
July 29, 2013 Draft protocol Version 1 prepared

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### 1. Study Outline

| **Title** | Evaluation of immunity enhancement by ingestion of a Salacia plant extract-containing supplement |
|-----------|---------------------------------------------------------------------------------------------------|
| **Name of products tested** | Salacia-containing supplement and placebo |
| **Objective** | The objective is to determine effects of a "Salacia plant extract-containing supplement" on immunological functions in adult males who were conscious of being prone to tiredness and ingested the supplement for 4 weeks. A placebo-controlled, double-blind study is conducted to evaluate the immunity-enhancing effect of the Salacia plant extract-containing supplement ingested, and blood pressure/pulse measurement, blood testing, immunity measurement, cytokine production measurement, blood gene expression analysis, blood bile acid measurement, intestinal flora analysis, and a questionnaire survey are conducted. |
| **Subjects** | Number of subjects: 30 (Group 1: 15, Group 2: 15) |
| **Entry criteria (based on subjects' self-reporting)** | 1) Japanese males who are at least 50 years and less than 60 years of age at the time of consenting  
※ "Japanese" herein refers to individuals with Japanese genetic background, regardless of Japanese citizenship.  
2) Individuals who have a feeling of tiredness on a daily basis and a feeling of not recovering from tiredness readily.  
3) Day-shift, sedentary workers  
4) Individuals with a regular life rhythm |
| **Screening criteria** | 1) Selection is made by the sponsor (immunological scores, etc.) |
| **Screening items** | 1) Body height measurement  
2) Body structure measurement (body measurement)  
3) Blood pressure/pulse measurement  
4) Blood test  
5) Blood sample submission  
6) Fecal sample submission |
| **Exclusion criteria** | 1) Individuals who have ever medically treated for a heart disease, a renal disorder, a hepatic disorder, a gastrointestinal disease, or some other conditions.  
2) Exclusion for diseases, etc. [individuals being treated for atrial fibrillation, arrhythmia, hepatic disorder (especially history or current illness of hepatitis), renal disorder, cerebrovascular disorder, rheumatism, diabetes, dyslipidemia, hypertension, and other chronic diseases  
3) Individuals with a gastrointestinal disease (such as extreme constipation and frequent diarrhea)  
4) Individuals with severe anemia  
5) Individuals taking an medicine (including kampo formulations) or a supplement on a regular basis  
6) Individuals who are allergic (to test food-related food/drug)  
7) Individuals taking a food/drug with a digestive conditioning effect on a daily basis over the last one month  
8) Individuals consuming alcohol excessively  
9) Smokers  
10) Individuals with diabetes  
11) Individuals who have participated within the last 3 months or is currently participating in any other clinical study  
12) Individuals with any abnormality found in the screening study  
13) Individuals determined to be ineligible by the Principal Investigator |
| **Subject allocation method** | Subjects shall be grouped in a manner such that the resulting groups are as close as possible to each other in the mean immunological score. |
| **Study design** | Placebo-controlled, double-blind |
| **Observation time points** | Before ingestion, after 4 weeks of ingestion |
|----------------------------|--------------------------------------------|
| **Number of observation**  | 2 times                                     |
| **Method/amount of ingestion** | Method of ingestion: See main text.  
Amount of ingestion at a time: See main text. |
| **Endpoints**              | · Body structure measurement (body measurement)  
· Blood pressure/pulse measurement  
· Blood test  
· Blood sample submission  
· Fecal sample submission  
· Questionnaire survey |
| **Statistical treatment**  | 1. For items/methods for statistical analysis, see Attachment 1.  
2. The significance level shall be 5% by a two-tailed test. |
| **Reporting method**       | A report shall be prepared based on the data tabulated and statistically analyzed at the Institution (Data attachment to the study report). |
| **Institution**            | SOUKEN Co., Ltd. (Clinical Study Office)  
1-9-10 Hamamatsu-cho, DaiwaA Hamamatsu-cho Building 6F, Minato-ku, Tokyo 105-0013  
TEL : 03-5408-1557 ／ FAX : 03-5408-1556 |
| **Principal investigator** | Takashi Koikeda, Director, Shiba Palace Clinic  
1-9-10 Hamamatsu-cho, DaiwaA Hamamatsu-cho Building 6F, Minato-ku, Tokyo 105-0013  
TEL : 03-5408-1599 ／ FAX : 03-5408-0059 |
| **Sponsor**                | Fujifilm Corporation  
577 Ushijima, Kaiseimachi, Ashigarakamigun, Kanagawa 258-8577  
TEL : 0465-86-1159 ／ FAX : 0465-86-1019  
Person-in-charge: Yuriko Oda |
| **Ethical considerations** | This study shall be conducted in accordance with the Declaration of Helsinki (revised at the Seoul general assembly in 2008), with due ethical considerations. |
2. **Study Title and Study Number**
   
   Study title: Evaluation of immunity enhancement by ingestion of a Salacia plant extract-containing supplement
   
   Study number: FJFR-13916

3. **Study Organization**

1) **Sponsor**
   
   Fujifilm Corporation
   
   577 Ushijima, Kaiseimachi, Ashigarakamigun, Kanagawa 258-8577
   
   TEL: 0465-86-1159/FAX: 0465-86-1019
   
   Person-in-charge: Yuriko Oda

2) **Entrustee**
   
   SOUKEN Co., Ltd. (Secretariat)
   
   1-9-10 Hamamatsu-cho, DaiwaA Hamamatsu-cho Building 6F, Minato-ku, Tokyo 105-0013
   
   TEL: 03-5408-1555/FAX: 03-5408-1556
   
   Person-in-charge: Yasushi Masuda

3) **Principal investigator**
   
   Takashi Koikeda, Director, Shiba Palace Clinic
   
   1-9-10 Hamamatsu-cho, DaiwaA Hamamatsu-cho Building 6F, Minato-ku, Tokyo 105-0013
   
   TEL: 03-5408-1599/FAX: 03-5408-0059

4) **Institution**
   
   SOUKEN Co., Ltd. (Clinical Study Office)
   
   1-9-10 Hamamatsu-cho, DaiwaA Hamamatsu-cho Building 6F, Minato-ku, Tokyo 105-0013
   
   TEL: 03-5408-1557/FAX: 03-5408-1556

5) **Investigators**
   
   In charge of Secretariat Yasushi Masuda SOUKEN Co., Ltd.
   Analysis Division Masahiro Yagibashi Same as above
   Academic Division Soichiro Matsuse Same as above

6) **Consultation service**
   
   Shiba Palace Clinic
   
   1-9-10 Hamamatsu-cho, DaiwaA Hamamatsu-cho Building 6F, Minato-ku, Tokyo 105-0013
   
   TEL: 03-5408-1599/FAX: 03-5408-0059
   
   Director, Takashi Koikeda

7) **Blood/urine tests subcontractor**
   
   BML Inc.
   
   1-34-5 Koenjiminami, Suginami-ku, Tokyo 166-0003
   The Second Tokyo Sales Office, Tokyo Sales Division, Clinical Laboratory Sales Department I
   
   TEL: 03-3316-0111/FAX: 03-3316-4429
4. **Background**

Plants of the genus Salacia have been used for generations as Ayurvedic medicinal plants for prevention of diabetes and obesity. Moreover, recent studies have shown that Salacia plants extracts improved effects on intestinal environment/flora, in addition to the already known improving effects on the blood sugar level and obesity.

In addition, various immunological effects have been demonstrated in animals that received Salacia plant extracts, including enhanced expression of Th1 cell-associated genes, increased growth of intestinal bacteria considered to be involved in immunity, and symptomatic improvement of influenza infection, and immunostimulatory effects of Salacia plant extracts are also expected for humans.

However, effects of Salacia plant extracts on human immune functions have not been determined in any previous studies, and remain to be studied.

5. **Objective**

The objective is to determine effects of a “Salacia plant extract-containing supplement” on immunological functions in adult males who were conscious of being prone to tiredness and ingested the supplement for 4 weeks. A placebo-controlled, double-blind study is conducted to evaluate the immunity-enhancing effect of the Salacia plant extract-containing supplement ingested, and blood pressure/pulse measurement, blood testing, immunity measurement, cytokine production measurement, blood gene expression analysis, blood bile acid measurement, intestinal flora analysis, and a questionnaire survey are conducted.

6. **Subjects**

※ "Japanese" herein refers to individuals with Japanese genetic background, regardless of Japanese citizenship.

Of SOUKEN registrants who have undergone a questionnaire survey at the time of recruitment, those who self-report to meet the following "Entry criteria (based on subjects’ self-reporting)” and not to meet the "Exclusion criteria" are subjected to a screening survey, and 30 of those who meet the "Screening criteria” and were considered appropriate to participate in this study are selected as subjects in this study.

Even if test results, etc. of any selected subject are found contradictory to those self-reported earlier ("Entry criteria (based on subjects’ self-reporting)” “Exclusion criteria”), the subject shall be deemed to be approved to participate in the study as long as the subject has not completed the screening survey, and shall not be excluded from the analysis set unless there is a special reason to do so.

1) **Entry criteria (based on subjects’ self-reporting)**
   1) Japanese males who are at least 50 years and less than 60 years of age at the time of consenting
   ※ "Japanese” herein refers to individuals with Japanese genetic background, regardless of Japanese citizenship.
   2) Individuals who have a feeling of tiredness on a daily basis and a feeling of not recovering from tiredness readily.
   3) Day-shift, sedentary workers
   4) Individuals with a regular life rhythm

2) **Screening criteria**
   1) Selection is made by the sponsor (immunological scores, etc.)

3) **Screening items**
   1) Body height measurement
   2) Body structure measurement (body measurement)
   3) Blood pressure/pulse measurement
   4) Blood test
   5) Blood sample submission
   6) Fecal sample submission

4) **Exclusion criteria**
1) Individuals who have ever medically treated for a heart disease, a renal disorder, a hepatic disorder, a gastrointestinal disease, or some other conditions.
2) Exclusion for diseases, etc. [individuals being treated for atrial fibrillation, arrhythmia, hepatic disorder (especially history or current illness of hepatitis), renal disorder, cerebrovascular disorder, rheumatism, diabetes, dyslipidemia, hypertension, and other chronic diseases
3) Individuals with a gastrointestinal disease (such as extreme constipation and frequent diarrhea)
4) Individuals with severe anemia
5) Individuals taking an medicine (including kampo formulations) or a supplement on a regular basis
6) Individuals who are allergic (to test food-related food/drug)
7) Individuals taking a food/drug with a digestive conditioning effect on a daily basis over the last one month
8) Individuals consuming alcohol excessively
9) Smokers
10) Individuals with diabetes
11) Individuals who have participated within the last 3 months or is currently participating in any other clinical study
12) Individuals with any abnormality found in the screening study
13) Individuals determined to be ineligible by the Principal Investigator

7. Consent from Subjects
   ① Objective and methods of this study
   ② Explanation about the test products, their effects, and adverse effects predicted to occur
   ③ Subjects are kept under adequate control by the Principal Investigator during the study.
   ④ Subjects are free from any penalty even if they do not consent to participate in the study.
   ⑤ Even after formally consenting to participate in the study, subjects can withdraw it any time.
   ⑥ Appropriate procedures and therapies that subjects are entitled to receive in case of a health damage related to this study
   ⑦ Subjects shall receive information that potentially effects subjects' intention for continued participation in this study as soon as such information is obtained.
   ⑧ Other necessary matters concerning protection of subjects' human rights and disclosure of subjects' information
   ⑨ What subjects should do and do not
   ⑩ Establishment of the consultation service at the institution that subjects should contact for more information about this study and subjects' rights or in case of health damages related to this study
   ⑪ About cooperation expenditures to be paid to subjects

8. Outline of the Test Products
   Test products are those supplied by Fujifilm Corporation (a Salacia-containing supplement and a placebo).

   1) Origin and the course of development
      The functional food "Metabarrier", which has been released in 2008 from Fujifilm Corporation, contains useful components such as green tea extracts and red wine polyphenols as well as the Salacia plant extract. A test food comprising the Salacia plant extract as the only useful component was prepared to clarify functions of the Salacia plant extract alone.

   2) Safety/efficacy data
      The test product used in this study is prepared from commercially available Metabarrier by removing some components therein, and safety/efficacy information available for Metabarrier may be used as a guide.
      No serious adverse event was reported in the results of the human use study conducted by SOUKEN before Metabarrier was launched. Furthermore, no serious health damage has been
reported to be caused by Metabarrier components since its release in 2008 through to now. Therefore, the safety of the test product appears to be sufficient.

In addition, the test product is considered to have a mild effect of reducing intestinal sugar absorption via inhibition of the α-glucosidase activity, since it contains the Salacia plant extract as does Metabarrier.

3) **Amount to be ingested and underlying rationale**

The amount of the Salacia plant extract in commercially available Metabarrier is 240 mg as a daily intake. In this study, the test product is formulated in tablets containing the Salacia plant extract in the amount corresponding to that ingested daily as Metabarrier. It should be noted that up to 1000 mg/day can be ingested safely according to the guidelines issued by the Ministry of Health, Labour and Welfare.

4) **Method of ingestion of the test products**

| Test product name | Time of the day | Time | Amount to be ingested | Method of ingestion |
|-------------------|-----------------|------|-----------------------|---------------------|
| Salacia plant extract-containing tablets | Morning, midday, evening | Before every meal | 1 tablet before breakfast, 1 tablet before lunch, 2 tablets before dinner | Tablets shall be ingested with water or plain hot water before meals. |
| Placebo tablets | Morning, midday, evening | Before every meal | 1 tablet before breakfast, 1 tablet before lunch, 2 tablets before dinner | Tablets shall be ingested with water or plain hot water before meals. |

5) **Storage method and expiration date**

| Test product name | Form | Taste/smell | Storage method | Expiration date |
|-------------------|------|-------------|----------------|----------------|
| Salacia plant extract-containing tablets | Tablet | Slight bitterness, woody aroma | Store at normal temperature away from direct sunlight, high temperature and high humidity | 1 year |
| Placebo tablets | Tablet | Slight bitterness, odorless | Store at normal temperature away from direct sunlight, high temperature and high humidity | 1 year |

6) **Ingredients of the test products and their contents**

| Test product name | Content |
|-------------------|---------|
| Salacia plant extract-containing tablets |  |
| Salacia extract powder | 60.00 mg |
| Calcium carbonate | 6.00 mg |
| Crystalline cellulose | 172.75 mg |
| Sucrose fatty acid esters | 7.50 mg |
| Particulate silicon dioxide | 3.75 mg |
| Particulate silicon dioxide | Content |
| Placebo tablets |  |
| Crystalline cellulose | 175.00 mg |
| Erythritol | 50.00 mg |
| Particulate silicon dioxide | 2.50 mg |
| Calcium stearate | 2.50 mg |
| Color adjusting agent | 20.00 mg |

9. **Study Methods**

1) **Study design**
Placebo-controlled, double-blind

2) Study schedule
The study shall be conducted in the following schedule.
  ・ Number of observation: 2
  ・ Observation time points: Before ingestion, after 4 weeks of ingestion
  ・ Ingestion period: 28 days
① Acquisition of written consent
② Main study
  [Number of subjects in the main study] Number of subject: 30 (Group 1: 15, Group 2: 15)
※ For items to be carried out in the main study, see Attachment 1.

3) Subject allocation
Subjects shall be grouped in a manner such that the resulting groups are as close as possible to each other in the mean immunological score.

10. Endpoints (various tests, measurement items, and survey methods)
Items set forth in Attachment 1 are performed.

11. Test Product Management
1) Persons in charge of the test products
   Fujifilm Corporation Yuriko Oda
   SOUKEN Co., Ltd. Kohei Miyawaki

2) Test product delivery/retrieval

| Test product name                         | Delivery date           | Subject return | Return to Sponsor | Date to return         | Return to:                |
|-------------------------------------------|-------------------------|----------------|-------------------|------------------------|--------------------------|
| Salacia plant extract-containing tablets  | Friday, September 20, 2013 | Required  | Required          | Friday, November 1, 2013 | Fujifilm Corporation Oda |
| Placebo tablets                           | Friday, September 20, 2013 | Required  | Required          | Friday, November 1, 2013 | Fujifilm Corporation Oda |

3) Test product management and storage
Test products are appropriately stored by the Institution (SOUKEN Co., Ltd.).

12. Subject Management Matters
① Subjects undergo fasting for at least 6 hours before the time of visit. (Example: Subjects scheduled to visit the clinic at 7 am may not eat after 4 am on the day) Water (mineral water) should be taken sufficiently. Subjects are not allowed to consume alcohol and should avoid excessive exercise on the day before a test.
② During the study, subjects are not allowed to take any supplement, except the test product and control product, stated to have immunological/digestive conditioning effects, or food containing an ingredient with the digestive conditioning effect (e.g., Easyfiber/Weider jelly fiber-in/Allbran/Oligo-no-okage/Calpis and the like/Kirin Supli/Kore-cut/Fibe-mini/Any green juice with FSHU stamp/Nisshin oishisa plus psyllium series/apple-flavored black vinegar/Lactic acid bacteria beverage (Yakult; Pirukuru; Mirumiru; Joa; Laurie Ace; etc.)/Yogurt; Yogurt drink (R-1; LG21; Bifidus; Meiji Bulgaria yogurt series; Soful; etc.)).
③ During the study (from 1 week before the screening day to the test day after 4-week ingestion), subjects should avoid irregular ways of living (such as lack of sleep, over-eating/drinking). For
diet and exercise, subjects should maintain the same quantity and quality as in daily lives before the start of the study.  
④ During the study, drugs considered to affect test results (such as laxatives, intestinal remedies) are prohibited in principle. Record it in web diary if any of these must be taken.  
⑤ During the ingestion period, subjects are required to record whether the test food is ingested in the web diary specified by the Institution every day.  
⑥ In case of any change in physical conditions during the study, contact the entity managing the study immediately, and follow its instructions on subsequent necessary actions.  
※ Although the items listed above on subject management must be complied in principle, they do not apply in cases medically required to do otherwise or other cases in which risks to the body or life are foreseen. If any of the items listed above is violated, the subject should contact the Institution promptly.  

13. Statistical Analysis  
① The significance level shall be 5% by a two-tailed test.  
② For items/methods for statistical analysis, see Attachment 1.  

14. Final Report  
1) A report shall be prepared based on the data tabulated and statistically analyzed at the Institution (Data attachment to the study report). Schedule until the report is prepared (plan)  
Work is done according to the following schedule. ※ However, if it takes more than 7 days to obtain a test result, the subsequent schedule is postponed for a considerable period of time (on the following day if it is a holiday or in weekend).  

| Expected submission date (only business days are counted) | Data to be submitted                                           |
|----------------------------------------------------------|---------------------------------------------------------------|
| Within 10 days after the end of the study                | Case conference                                              |
| 10 days after the end of the study                       | Submission of a draft data attachment to the study report    |
| Within 14 days after submission of a draft data          | The data attachment to the study report to be finalized      |
| attachment to the study report                           |                                                               |
| 25 days after the end of the study                       | Key open                                                     |
| Within 14 days after the data attachment to the study    | Submission of a draft study report                           |
| report has been finalized                                |                                                               |
| Within 14 days after submission of a draft study report  | The study report to be finalized                             |  

15. Criteria for discontinuation of the study and drop-out cases  
In case of any of the following, the study is terminated on the basis of Principal Investigator's medical and ethical judgment. Except for some special circumstances, appropriate medical care is provided to subjects to ensure the safety of the subjects.  
① When a serious adverse reaction, subjective/objective symptom, etc. has emerged  
② When the subject is difficult to continue the study because of complication with any other disease or progression of a complication  
③ When tests are extremely difficult to perform  
④ When the whole study is discontinued  
⑤ When the Principal Investigator finds the study necessary to be discontinued  

16. Compensation to Subjects  
If a damage to a subject has occurred due to the study during the study, or if a subject has filed a law suit seeking compensation for a damage caused by this study, the Principal Investigator shall promptly
inform the Sponsor of it.

The Institution shall be responsible for compensations for health damages caused intentionally or negligently by the Institution, while the sponsor shall be entirely responsible for compensations in cases where health damages have occurred due to the test products. However, this does not apply if the health damage was falsely reported or intentionally caused by the subject.

17. Criteria for Exclusion from Analysis (Reporting) Set

Subjects who meet any of the following criteria are discussed in the case conference and shall be excluded from the analysis (reporting) set unless there is a special reason to do otherwise.

① Subjects found to have substantially deviated from instructions pertinent to subject management matters during the study.
② Subjects with data of which reliability is compromised in a major manner due to, for example, troubles in testing
③ Subjects who have missed ingestion (the amount ingested does not meet the daily dose) in more than 15% of the days scheduled for ingestion
④ Subjects found not to meet entry criteria and to meet exclusion criteria
⑤ Subjects found appropriate to treat as drop-out cases for evident reasons

18. Note on Completing the Survey Form

A black-ink ballpoint pen shall be used to complete the survey form, and corrections shall be made with double lines.

19. Modification/Absence of Test Data

If a delay or cancellation of measurement is unavoidable for subject's health conditions or request, subject's health conditions and request shall be given priority in accordance with the spirit of the Declaration of Helsinki. If some data are impossible to collect for these reasons or other unavoidable reasons, they shall be treated as missing data.

20. Protection of Subjects’ Privacy

All personnel involved in this study shall give full considerations to handling of information that can be used to identify individuals. In other records obtained in the course of this study, numbers shall replace names to prevent individuals from being identified. The test subcontractor is not allowed to obtain subjects' personal information under any circumstance.

21. Ethics

1) Compliance matters

This study shall be conducted in accordance with the Declaration of Helsinki (revised at the Seoul general assembly in 2008), with due ethical considerations.

2) Shiba Palace Clinic Ethical Review Board

Members of the Shiba Palace Clinic Ethical Review Board meet and discuss ethics and validity of the protocol for this study. The study must be conducted based on the protocol approved by the Shiba Palace Clinic Ethical Review Board. Any substantive deviation from the protocol must be approved by the Board.

22. Attachments

Attachment 1. Endpoints
Attachment 2. Blood/fecal endpoints
September 2, 2013

Sponsor: Fujifilm Corporation
577 Ushijima, Kaiseimachi, Ashigarakamigun, Kanagawa 258-8577
Pharmaceutical/Healthcare Research Laboratories Fumitaka Ueda

Entrustee: SOUKEN Co., Ltd.
1-9-10 Hamamatsu-cho, DaiwaA Hamamatsu-cho Building 6F, Minato-ku, Tokyo
105-0013
Representative director Kazuo Shirai

Entrustee: Shiba Palace Clinic
1-9-10 Hamamatsu-cho, DaiwaA Hamamatsu-cho Building 6F, Minato-ku, Tokyo
105-0013
Director Takashi Koikeda
| ID | Test Description | Test Method | Test Description | S | Before Ingestion | Four-week after Ingestion |
|----|------------------|-------------|------------------|---|-----------------|--------------------------|
| 0  | Body height measurement | Description | Body height measurement. (Measurement items) Body height Total 1 item ○ The last value is used when measured multiple times. | -- | ○ -- -- | ○ -- -- |
|    | Data processing | Tabulation only |                        |            |                |                          |
| 1  | Body structure measurement | Description | (Measurement items) Muscle mass/body fat amount/body weight/body fat percentage/BMI Total 5 items ○ To be measured in a hospital gown The last value is used when measured multiple times. | Intern report ○ | ○ -- (S value adopted) ○ | ○ -- (S value adopted) |
|    | Instrument for measurement | InBody3.2/Biospace Co., Ltd. |                        |            |                |                          |
|    | Data processing | Tabulation/statistical analysis | Parametric |            |                |                          |
|    | Statistical analysis | See below | Statistical analysis method |            |                |                          |
|    | Comparison over time | Before ingestion vs 4-week after ingestion | Paired t test |            |                |                          |
|    | Inter-group comparison | Between the groups before ingestion and 4-week after ingestion | Unpaired t test |            |                |                          |
| 6  | Blood pressure/pulse measurement | Description | At least two measurements are made in a resting state. (Measurement sites) Left arm Total 1 site (Measurement items) Systolic blood pressure/diastolic blood pressure/pulse Total 3 items ○ The last values are used ○ If blood pressure cannot be measured with an automated sphygmomanometer because, for example, the arm does not fit in the sphygmomanometer, a mercury sphygmomanometer (Kenzmedico Co., Ltd.) may be used. | Intern report ○ | ○ -- (S value adopted) ○ | ○ -- (S value adopted) |
|    | Instrument for measurement | Automated blood pressure monitor Kentaro (HBP-9020)/Omrorn Healthcare Co., Ltd. |                        |            |                |                          |
|    | Data processing | Tabulation/statistical analysis | Parametric |            |                |                          |
|    | Statistical analysis | See below | Statistical analysis method |            |                |                          |
|    | Comparison over time | Before ingestion vs 4-week after ingestion | Paired t test |            |                |                          |
|    | Inter-group comparison | Between the groups before ingestion and 4-week after ingestion | Unpaired t test |            |                |                          |
| 9  | Blood test | Description | White blood cell image, peripheral blood general test, HbA1c,NGSP, total bilirubin, direct/indirect bilirubin, ZTT, AST (GOT), ALT (GPT), ALP, LDH, γ-GTP, cholesterol, LAP, CK, total protein, ceramine, urea nitrogen, uric acid, total cholesterol, TG (triglycerides), calcium, serum iron, serum amylase, HDL-cholesterol, free fatty acids, glucose, LDL-cholesterol, glycated albumin, NK cell activity. | Intern report ○ | ○ -- ○ | ○ -- ○ |
|    | Instrument for measurement | A designated blood testing company |                        |            |                |                          |
|    | Data processing | Tabulation/statistical analysis | Parametric |            |                |                          |
|    | Statistical analysis | See below | Statistical analysis method |            |                |                          |
|    | Comparison over time | Before ingestion vs 4-week after ingestion | Paired t test |            |                |                          |
|    | Inter-group comparison | Between the groups before ingestion and 4-week after ingestion | Unpaired t test |            |                |                          |
| 1040 | Blood sample submission | Contents | See Attachment 2 |            |                |                          |
|    | Instrument for measurement | Shiba Palace Clinic |                        |            |                |                          |
|    | Data processing | Sampling/collection |                        |            |                |                          |
| 1066 | Fecal sample submission | Contents | See Attachment 2 |            |                |                          |
|    | Instrument for measurement | Shiba Palace Clinic |                        |            |                |                          |
|    | Data processing | Sampling/collection |                        |            |                |                          |
| 9061 | Questionnaire survey | Description | A questionnaire survey (6-point scale) on subjective symptoms is conducted. | Intern report ○ | -- ○ | ○ -- ○ |
|    | Instrument for measurement | Self-administered by subjects |                        |            |                |                          |
|    | Data processing | Tabulation/statistical analysis | Non-parametric |            |                |                          |
|    | Statistical analysis | See below | Statistical analysis method |            |                |                          |
|    | Comparison over time | Before ingestion vs 4-week after ingestion | Wilcoxon’s signed rank test |            |                |                          |
|    | Inter-group comparison | Between the groups before ingestion and 4-week after ingestion | Mann-Whitney U test |            |                |                          |
## Attachment 2. Blood/Fecal endpoint

| ID | Test Description | Test Method | Test Description | S | Before ingestion | After 4-week ingestion | Sample (storage method) | Testing institution |
|----|------------------|-------------|------------------|---|------------------|------------------------|-------------------------|---------------------|
| 0  | Immunity tests (Peace-of-mind course) | Measurement description | Immunity score, T lymphocyte age, monocyte/lymphocyte age, T cell count, CD4+/CD8+ T cell ratio, naive T cell count, naive/memory T cell ratio, B cell count, NK cell count, CD8+CD20+ T cell count, and T cell proliferation index are measured. | ○ | ○ | Before ingestion vs 4-week after ingestion | Paired t test | Institute for Health and Life Science Co., Ltd. |
|    |                  | Data processing | Tabulation/statistical analysis | Parametric | See below | Unpaired t test | |
|    |                  | Statistical analysis | | | | | |
|    |                  | Comparison over time | Before ingestion vs 4-week after ingestion | Paired t test | | | |
|    |                  | Inter-group comparison | Between the groups before ingestion and 4-week after ingestion | Unpaired t test | | | |
| 1  | Treg cell counting | Measurement description | Treg cells are counted by flow cytometry. | ○ | ○ | Before ingestion vs 4-week after ingestion | Paired t test | Institute for Health and Life Science Co., Ltd. |
|    |                  | Data processing | Tabulation/statistical analysis | Parametric | See below | Unpaired t test | |
|    |                  | Statistical analysis | | | | Statistical analysis method | |
|    |                  | Comparison over time | Before ingestion vs 4-week after ingestion | Paired t test | | | |
|    |                  | Inter-group comparison | Between the groups before ingestion and 4-week after ingestion | Unpaired t test | | | |
| 2  | Cytokine measurement | Measurement description | IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, TNF-α, TNF-β, and IL-17 produced in the culture supernatant are measured (stimulus: PMA + Ionomysin). | ○ | ○ | Before ingestion vs 4-week after ingestion | Paired t test | Institute for Health and Life Science Co., Ltd. |
|    |                  | Data processing | Tabulation/statistical analysis | Parametric | See below | Unpaired t test | |
|    |                  | Statistical analysis | | | | Statistical analysis method | |
|    |                  | Comparison over time | Before ingestion vs 4-week after ingestion | Paired t test | | | |
|    |                  | Inter-group comparison | Between the groups before ingestion and 4-week after ingestion | Unpaired t test | | | |
| 3  | Gene expression analysis | Measurement description | Microarray analysis of mRNA expression levels in blood. | ○ | ○ | Analysis is performed using the analysis software R. | Blood (stored in dedicated tubes; frozen after being let stand at normal temperature for 2 hours) | Kanto Industries Ltd., SRL Inc., KAST, Fujifilm |
|    |                  | Data processing | Tabulation/statistical analysis | Parametric | See below | | |
|    |                  | Statistical analysis | | | | Statistical analysis method | |
|    |                  | Comparison over time | Before ingestion vs 4-week after ingestion | | | | |
| 4  | Bile acid measurement | Measurement description | Primary and secondary bile acids in the serum are measured. | ○ | ○ | Serum (2.5 ml) is stored in a dedicated tube and frozen. | SRL Inc. |
|    |                  | Data processing | Tabulation/statistical analysis | Parametric | See below | | |
|    |                  | Statistical analysis | | | | Statistical analysis method | |
|    |                  | Comparison over time | Before ingestion vs 4-week after ingestion | Paired t test | | | |
|    |                  | Inter-group comparison | Between the groups before ingestion and 4-week after ingestion | Unpaired t test | | | |
| 5  | Intestinal flora | Measurement description | Intestinal flora in feces is measured with the T-RFLP (Nagashima) method. | ○ | ○ | Feces (stored with a sampling kit and shipped) | TechnolSuruga Laboratory Co., Ltd. |
|    |                  | Data processing | Tabulation/statistical analysis | Parametric | See below | | |
|    |                  | Statistical analysis | | | | Statistical analysis method | |
|    |                  | Comparison over time | Before ingestion vs 4-week after ingestion | Paired t test | | | |
|    |                  | Inter-group comparison | Between the groups before ingestion and 4-week after ingestion | Unpaired t test | | | |

Criteria for exclusion from analysis set (reporting) (S value adopted)

1. If an observation is delayed for the predetermined number of days or more (before ingestion: no visit/ 4-week after ingestion: 7 days)
2. If the ingestion is missed (the amount ingested does not meet daily dose) in more than 15% of the days scheduled for ingestion