STUDY TITLE: OPEN LABEL POSTMARKET EVALUATION OF ORALLY DOSED ALMEGA PL® ON CHOLESTEROL AND CARDIO-METABOLIC PARAMETERS.

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Open label post-market evaluation of orally dosed Almega PL on cholesterol and cardio-metabolic parameters.

Trial Protocol ALM-CVH-22/ NCT05267301

Statistical Analysis Plan (SAP)

Author: Principal Investigator, Dr. Eneko Ganuza

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List of abbreviations

AE	Adverse Event
CSP	Clinical Study Protocol
CSR	Clinical study report
FAS	Full Analysis Set
GCP	Good Clinical Practice
GLU	Glucose
HbA1c	Glycated hemoglobin
HDL	High-Density Lipoprotein
Hs-CRP	High sensitivity C-reactive Protein
ITT	Intention-To-Treat Population
LDL	Low-density Lipoprotein
N	Sample size
N/A	Not applicable
Non-HDL-C	Non-High-density lipoprotein cholesterol
PP	Per protocol population
SAE	Serious Adverse Event
TC	Total cholesterol
TC: HDL	Total cholesterol, high-density lipoprotein ratio
TG	Triglycerides
U.S. FDA	the U.S. Food and Drug Administration
US	United States
VLDL	Very-low density lipoprotein

1. Introduction

This statistical analysis plan (SAP) describes all planned analyses for the Clinical Study Report (CSR) of study **ALM-CVH-22**, a multi-center, single arm, open-label post-market evaluation of orally dosed Almega PL on cholesterol and cardiometabolic parameters.

The content of this SAP is based on protocol **ALM-CVH-22** Amendment version 2. All decisions regarding final analysis, as defined in the SAP document, have been made prior to database lock of the study data.

1.1. Study design

EPA is a type of omega-3 essential fatty acid known to play a beneficial role in protection against cardiovascular disease. Almega®PL is the only natural source containing EPA as sole n-3 HUFA that is available over the counter for human consumption. We **hypothesized** that AlmegaPL supplementation improves blood markers associated with heart health and inflammation. This post-market open label single armed trial involving 200-300 male and female participants, that will be monitored during the first 6 months following their subscription to Almega®PL.

As part of the **screening procedure**, participants will complete a short questionnaire to make sure they are new iwi users. Any participant who is already using iwi will not be considered in the study. Any participant that has a serious condition (including but not limited to kidney, neurological, immunological, liver, and gastrointestinal disease, any heart condition, or diabetes) will also be disqualified for the program and directed to their doctor. Any participant attempting conception, pregnant or breastfeeding will also be disqualified. All other participants will be accepted into the limited study based on their time of application until the quota is completed. Participation in this study is purely voluntary and participants can withdraw at any point without prejudice.

Participants will be asked to take the allocated product according to the **dose** prescribed (2 capsules per day containing 1000-1100 mg Almega®PL) and adherence to the protocol will be evaluated with a follow up email. Participants will be asked to maintain their usual level of physical activity and diet for the duration of the study. The dose used in this trial is in agreement with the commercial dose communicated to the FDA and comparable to doses previously used in our clinical trials.

Along with the fist supplement order shipped to the new subscriptions, participants will receive the baseline hearth health test kit from Imaware™. Participants will be contacted 3 and 6 months after and they will be sent subsequent kits once the confirmed adherence to the supplement. The test kits will monitor blood lipid levels across a supplementation period (baseline, month 3 and month 6) thanks to a finger prick blood drawing system. The age, height, weight, gender, fasting total cholesterol, LDL-cholesterol, HDL-cholesterol, calculated VLDL-cholesterol, triglycerides, as well as hs-CRP inflammatory marker, and glucose and HbA1c as diabetes indicator of each participant will be recorded over the study period. **Qualitas Health** will receive the aggregated data of the participants only for statistical analyses at their headquarter in Houston and store it in the company share drive with access restricted to those involved in the study. There will be no cost incurred for this analytical assessment to the participant.

Results for the per intended-to-treat (ITT) population will be first tested for normality before any other test will be conducted and baseline data was compared against month 3 and month 6 data. Based on the distribution of data, either Welch two-sample unequal variance t-tests or Wilcoxon Ranks sum tests will be used to compare two-tailed differences between groups. Differences will be considered statistically significant at a p value < 0.05, but the thresholds for the significance of the 20 clinical outcomes were adjusted for multiple variables by the Bonferroni adjustment method which decrease the significance of p-value from <0.05, down to <0.0025. Intervention

effect will also test using an analysis of covariance model (ANCOVA) adjusting for baseline parameters.

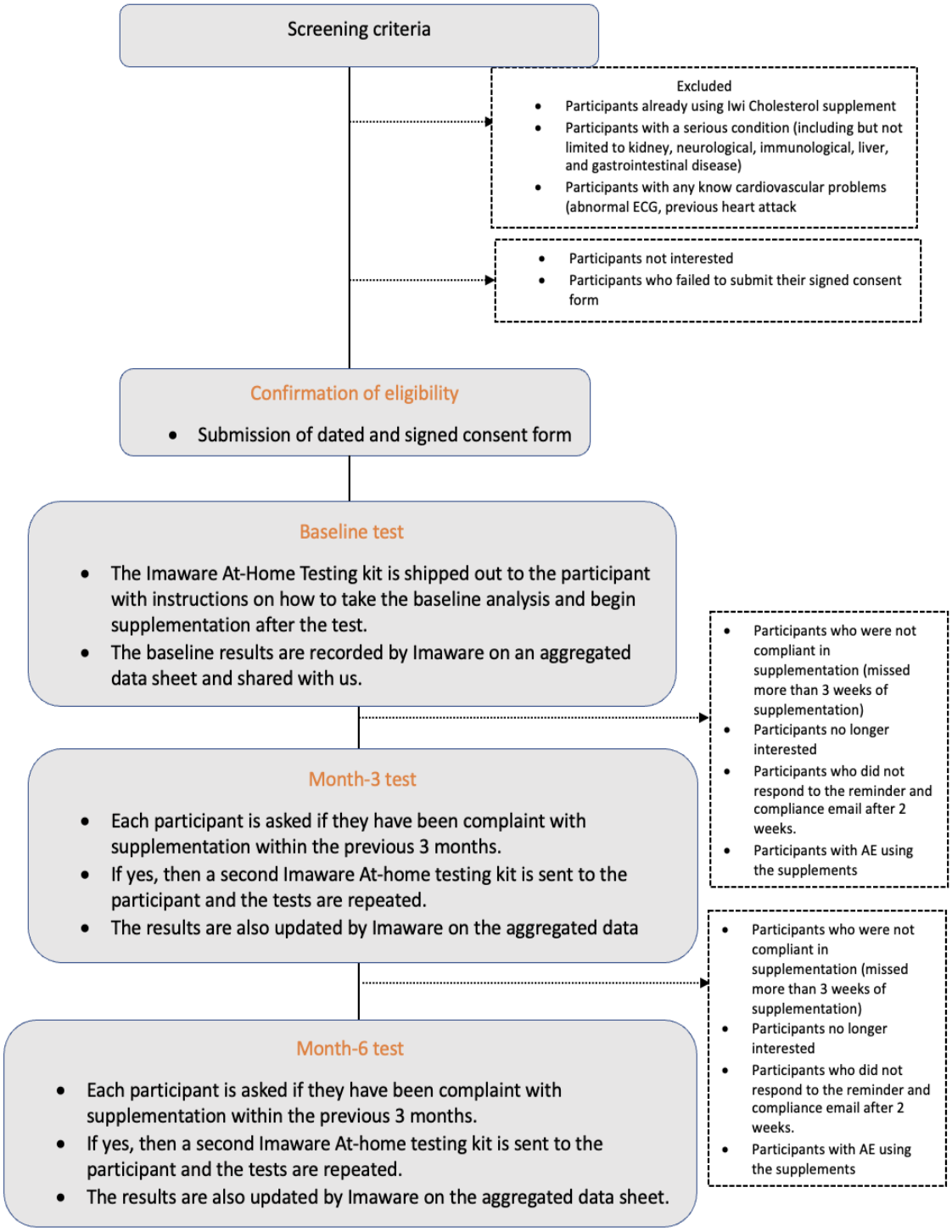

Figure 1-1 Study design

1.1. Study objectives and endpoints

The aim of this study is to assess the effectiveness of Almega®PL on improving blood markers associated with heart health of iwi customers. Specifically, we would like to confirm the decrease in cholesterol observed in our previous trial (Rao et al., 2020) while assessing the capacity to also decrease triglycerides (primary outcome). As a secondary objective we would like to understand if EPA, precursor of signalling molecules involved in the inflammatory response, could help decrease the inflammatory marker hs-CRP.

Objectives and related endpoints are described in Table 1-1 below

Table 1.1-1. Objectives and relative endpoint

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Can Almega® PL supplement decrease triacylglycerides in a generally healthy population?	Triglyceride change from baseline over 6-month period.	*Triglyceride levels is an atherogenic lipid and an important risk factor in cardiovascular disease*
Secondary		
Can Almega® PL supplement replicate the decrease in VLDL and total cholesterol we observed in our previous clinical trial (Rao et al., 2020)? *Will the results from a controlled population translate to the iwi costumers?* *Can Almega® PL supplement decrease the age-related inflammatory marker hs-CRP in a generally healthy population?*	Total Cholesterol change from baseline over 6-month period. VLDL-Cholesterol change from baseline over 6-month period. LDL-Cholesterol change from baseline over 6-month period. HDL-Cholesterol change from baseline over 6-month period. hs-CRP change from baseline over 6-month period. Fasting glucose change from baseline over 6-month period. HbA1c change from baseline over 6-month period.	*Cholesterol in plasma levels is atherogenic and a risk factor in cardiovascular disease.* *Hs-CRP is an age-related inflammatory market. Omega-3 help regulate the inflammatory response.*

2. Statistical methods

2.1. Data analysis general information

The final analysis will be performed by Qualitas Health personnel. IBM SPSS Statistics Version 29.0.0.0 (241) statistical software will be used to perform all data analyses and to generate tables, figures, and listings.

2.2. Data included in the analysis

The study data will be analyzed and reported based on all patient's data when all participants have completed at least 2 tests or discontinued the study (ITT population).

2.3. General analysis conventions

Qualitative data (e.g., gender, smoking, etc.) will be summarized by means of excel tables, a missing category will be included as applicable. Percentages will be calculated using the number of patients in the relevant population or subgroup as the denominator.
Quantitative data (e.g., age, body weight, etc.) will be summarized by appropriate descriptive statistics (i.e., mean, standard deviation, median, minimum, and maximum).

2.4. General definitions Investigational Product and study treatment

- ***Investigational Product*** refers to Iwi/Cholesterol supplement, a commercially available capsule-form herbal (algae) supplement containing Almega®PL, a *Nannochloropsis* algae-derived extract rich in eicosapentaenoic acid (EPA). EPA is a type of omega-3 essential fatty acid known to play a beneficial role in protection against cardiovascular disease. Iwi/Cholesterol is the only source of long chain omega-3 that does not contain the DHA that is present in other omega-3 sources (fish oil, krill oil, other algal oils). Almega®PL was registered at the Food and Drug Administration (FDA) as a New Dietary Ingredient (NDIN) in 2014.
- ***Study treatment*** = 1000-1100 mg/day investigational product (Almega®PL).

Table 2-1. Treatment plan

Study Stage	Specific Timing	Activity
Planning 2 Months	N/A	• Approval of protocol • Independent peer-review • Logistical arrangements • Recruitment
Pre-Study 1 Month	Upon approval and completion of recruitment	• Preliminary screening against inclusion and exclusion criteria • Pre-study interview: o Information email and gain informed consent from the participant o Enrolled participants purchase the product and receive the testing kits along with instruction regarding the study requirements.
Study Week 0-24	Baseline data collection	First test kit will be shipped to imaware
Trial Months 0-6 (Period begins at trial week 2)	Trial Period	• Participant takes product as instructed • Participant will receive and send the test kits on month 3 and 6
		• Participant completes end-trial blood test.

End-Study Month 3	Trial end interview	• Participants exit email or phone interview to address adherence.
Post-Study	N/A	• Data verification and statistical analysis • Report drafting and approval • Study debrief and review

* Please note that enrolment in the trial to receive product will only occur after all inclusion criteria have been.

2.5. Length of Treatment Time

In total, participants will be required to send the test kits 3 times throughout the total trial time of 6 months.

At the completion of month 6, participants will repeat the baseline measures including a final blood test and a required exit interview.

Clinical interviews will be undertaken by email or phone. The trial will be completed once the last participants complete the exit interview.

2.6. Baseline

For **non-efficacy assessments, Baseline** is the last available and valid assessment performed or value measured, unless otherwise stated under the related assessment section.

Patients with no data after the testing with the kit but fail to mail back the kit to Imaware or the testing kit gets lost in transit to Imaware before starting supplementation will have no baseline results documented. These participants will have to be discontinued from the study as their baseline results will be compromised once supplementation has begun.

2.7. Analysis sets

2.7.1. Per protocol set (PPS)

The Per-Protocol Set (PPS) will consist of a subset of participants in the database who complete all 3 tests (baseline, month-3, and month-6 tests).

2.7.2. Intention-To-Treat (ITT)

The Intention-To-Treat (ITT) will consist of a subset of participants in the database who complete at least 2 tests (baseline and month-3 test and/or month-6 test.

2.8. Voluntary Participation/Right to Refuse or Withdraw

There is no obligation for participants to be involved in this study. If participants decide to participate in the study and later feel they no longer wish to be part of it, participants may withdraw from the study at any time without prejudice to any current or future involvement in clinical studies held by the study investigators or sponsor. If participants withdraw their consent, all data collected up until that time will be used in the analysis of the data.

All communications will be performed by a trained iwi Qualitas employee (Study Director) or the Principal Investigator Dr. Ganuza. In the event that Dr. Ganuza does not screen the participant, Dr Ganuza will review case files before participants are enrolled. Any participant that presents a response

or result (e.g., blood test) that is outside of normal range will be directed by Imaware^{TM} to medically trained professionals with their results.

2.9. Patient disposition, disease history and medical history

2.9.1. Basic health and background data

Health and baseline disease characteristics data, as characterized by the questionnaire will be summarized and listed. Categorical data, (e.g., gender, smoking, level of physical activity, medication use, etc.) will be summarized and the percentage of participants who answer "yes" to the set questions will be calculated. Continuous data, e.g., age, weight, height, body mass index, will be summarized by descriptive statistics (N, mean, median, standard deviation, minimum and maximum). BMI (kg/m2) will be calculated as weight[kg] / (height[m]2) using weight at Baseline.

2.9.2. Diagnosis and Family disease history

Diagnosis of Cardiovascular disease and Diabetes, as well as family history of cardiovascular disease and Diabetes will be documented as categorical data. The percentage of participants with cardiovascular disease, diabetes and family history of the diseases will be calculated as well.

2.10. Termination of the Study

This research project may be stopped for a variety of reasons.

During the trial, any participant that records out-of-range analytical levels will be referred to their GP for assessment of suitability/safety for continuation in the trial.

At the discretion of the GP the participant will either be placed on standard care of cholesterol-lowering medication (and removed from the trial) or continue in the trial on the supplementation.

The participant also has the option to stop supplementation at any point.

2.11. Adverse events (AEs)

AE summaries

AEs will be documented for participants which will be available at the time of analysis.

2.12. Laboratory data

Imaware would be providing the aggregated data sheet with the results after the tests have been analyzed at their laboratories.

Table 2-2. Laboratory parameters to be investigated by the Imaware.

Lipids	Inflammatory markers	Glucose
TC	Hs-CRP	GLU
LDL		HbA1c
HDL		
VLDL		
TG		

TC = Total Cholesterol: LDL = low-density lipoprotein; HDL = high-density lipoprotein; VLDL = very low-density lipoprotein; TG = triglycerides; Hs-CRP = high sensitivity C-reactive protein; GLU = glucose; HbA1c = glycosylated hemoglobin

3. Sample size calculation

Power and sample size calculation was performed on the primary outcome (triglycerides) using G*Power 3.1 (Department of Psychology, University of Düsseldorf, Germany). Sample size calculation was based on a student t-test (two independent samples). Using an effect size of 0.5, and an allocation ratio N2/N1 of 1, the sample size was estimated to be 210 to achieve a power of 95%.

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