Alpha Omega Trial

Ancillary Statistical Analysis Plan on Cognitive Decline

Final version

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1.0 Introduction

1.1 Statistical Analysis Plan

This Statistical Analysis Plan (SAP) on the ancillary endpoint of cognitive decline ("SAP Cognitive Decline") is developed to guide the statistical analysis of the Alpha Omega trial data and the production of the statistical output. The document was developed after review of the Alpha Omega Trial protocol, including its amendments, and the Case Record Forms (CRFs), but before any trial data had been released. The SAP provides detailed information of the populations that will be analyzed, the subject characteristics and the efficacy parameters that will be evaluated, as well as the specific statistical methods that will be applied. This version of the "SAP Cognitive Decline" is designed for the results analysis, i.e. the relation between randomly allocated treatment and the ancillary study endpoint of cognitive decline.

1.2 Role of the funding source

The Alpha Omega trial was sponsored by the Netherlands Heart Foundation, US National Institutes of Health (NIH/NHLBI) and Unilever. This SAP was prepared by the Executive Committee and the independent statistician only, and none of the funding source providers had any influence or control on the planned analyses. The analyses will be conducted, and the results interpreted and reported independently of the trial sponsors.

2.0 Protocol summary

2.1 Protocol development

The Alpha Omega trial is registered at ClinicalTrials.gov as study NCT00127452 (see http://clinicaltrials.gov/ct2/show/NCT00127452). The original protocol was released on Augustus 3, 2005, and underwent several changes since then. Most importantly, because of an unexpected low incidence of the original primary endpoint (coronary heart disease [CHD]-related mortality), the Steering Committee had decided to

- redefine the primary endpoint as a composite of fatal cardiovascular disease (CVD), non-fatal myocardial infarction (MI), non-fatal cardiac arrest, non-fatal stroke, percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG);
- extend the follow-up to 40 months (original: 36 months);
- increase the sample size to a maximum of 5000 subjects (original: 4000).

This summary is based on the protocol version that is described in the design paper of the Alpha Omega Trial by Geleijnse et al, Am Heart J. 2010 Apr;159(4):539-546.[1]

2.2 Background

Dementia is a progressive neurodegenerative disease characterized by loss of function and death of nerve cells in several areas of the brain, complicated by inflammatory reactions. Different types of dementia exist, of which Alzheimer's disease (AD) is the most prevalent, followed by vascular dementia (Pasinetti 1996; Ritchie & Lovestone 2002). AD is characterized by the presence of neurofibrillary tangles and amyloid plaques that are clearly visible by microscopy in brains of those afflicted with AD (Savya 2009; Welsh-Bohmer 2009). Vascular dementia is characterized by cerebrovascular lesions secondary to vascular disease. There is evidence, however, that pathologies for AD and vascular dementia overlap, and CVD risk factors have consistently been associated also with the occurrence of AD (Breteler 2000; Waldstein 2001; Alonso 2009; Helzner 2009).

Cognitive decline is a strong risk factor for developing dementia (Knopman 2010). The main dimensions of cognitive function are memory and learning, attention and concentration, thinking, language, and visual and spatial skills. The level of cognitive functioning, the time of onset of cognitive decline and the rate of cognitive decline differ between persons of the same age, which indicates that not only age but also other factors are important, which could include diet (Féart 2009; Solfrizzi 2009; Albanese 2009). It has been suggested that n-3 fatty acids from fish, i.e. eicosapentaenoic acid (EPA; C20:5, n-3) and docosahexaenoic acid (DHA; C22:6, n-3), play a beneficial role in brain physiology and could prevent cognitive decline and dementia (Issa 2006; Lim 2006; Kröger 2009; Calderon

2009). DHA is highly concentrated in brain membrane phospholipids and is important in neuronal membrane structure and stability, neuroplasticity, signal transduction, neurotransmission, and gene expression (Calderon 2009; McNamara 2010, Cole 2010). EPA adds less to the total brain fatty acid composition, but together with DHA is important as a precursor of the eicosanoids (i.e., prostaglandins, prostacyclins, thromboxanes and leukotrienes) with anti-thrombotic and anti-inflammatory tendencies (Pasinetti 1996). In humans, EPA can be derived after elongation of alpha-linolenic acid (ALA; C18:3, n-3), the parent n-3 PUFA derived from plant foods, but this is an inefficient process (Brenna 2009).

Cognitive performance has been examined in relation to intake of fish or n-3 fatty acids and blood n-3 fatty acid concentrations in a number of epidemiological studies and randomized controlled trials (Issa 2006; Lim 2006; Cole 2010). In the Zutphen Elderly Study a significant inverse association was observed between the intake of fish and n-3 PUFA with 5-year cognitive decline (Van Gelder 2007). Other prospective studies confirmed this beneficial association of fish and n-3 PUFA in relation to cognitive decline, although negative results have also been reported (Issa 2006; Lim 2006; Devore 2009). Randomized controlled trials of fish or n-3 fatty acids intake and cognitive function are limited (Lim 2006). Two trials were performed in elderly patients with dementia (Terano 1999; Yehuda 1996). Though both trials observed a beneficial effect of n-3 PUFA supplementation on general cognitive outcomes they add little weight to the evidence, because of sample size (n=20) or short duration (4 weeks). In a 6-month trial in 174 subjects with mild to moderate AD no overall effect of EPA and DHA intake on cognitive performance was found, except in a subgroup of 32 patients with only mild cognitive impairment (Freud-Levi 2006). Van de Rest et al performed a 6-month double-blind, placebo-controlled trial in 302 subject aged 65 and over without cognitive impairment. Subjects were randomly assigned to 1,800 mg/d EPA+DHA, 400 mg/d EPA+DHA, or placebo for 26 weeks and cognitive performance was monitored using an extensive neuropsychological test battery. Overall, there were no significant changes in any of the cognitive domains for either low or high doses of fish oil (Van de Rest 2009). The fact that no effect of EPA+DHA supplementation on cognitive function was found in randomized placebo-controlled trials may be due to the relatively short duration of these studies, i.e. 6 months or less.

The fact that no effect of EPA+DHA supplementation on cognitive function was found in randomized placebo-controlled trials may be due to the relatively short duration of these studies, i.e. 6 months or less. It is conceivable that this duration is insufficient for a beneficial effect of n-3 fatty acids to occur since cognitive decline is a slowly developing process. With regard to ALA intake, there is a lack of data in relation to cognitive function. Cognitive impairment is a significant and frequent disability in older CVD patients, particularly in patients with heart failure (Vogels 2006). No randomized

controlled studies of n-3 PUFA supplementation on cognitive decline in CVD patients have yet been performed.

The Alpha Omega Trial provides the unique opportunity to examine the effect of low doses of EPA+DHA and ALA in relation to change in cognitive function, as assessed by the Mini-Mental State Examination (Folstein 1975), and cognitive decline in over 3000 post-MI patients with 40 months of follow-up.

2.3 Objective

The aim of this ancillary analysis to the Alpha Omega Trial is to examine whether low-dose supplementation of (very-)long-chain n-3 PUFA is effective against cognitive decline in subjects with a history of MI.

2.4 Study participants

Eligible for participation in the Alpha Omega Trial are men and women aged 60-80 years who had a clinically diagnosed MI up to 10 years before randomization. Study participants were identified and recruited in 32 Cardiology centers in The Netherlands.

2.5 Design

The Alpha Omega Trial is a multicenter, double-blind, randomized, placebo-controlled clinical trial. Equal numbers of eligible subjects will be randomly allocated according to a 2x2 factorial design to one of the following treatment strategies:

1. EPA + DHA: Experimental

Margarine spread that yields 400 mg of EPA + DHA per day for average margarine use of 20 grams per day;

2. ALA: Experimental

Margarine spread that yields 2 grams of ALA per day for average margarine use of 20 grams per day;

3. EPA + DHA plus ALA: Experimental

Margarine spread that yields 400 mg of EPA + DHA per day plus 2 grams of ALA per day, for average margarine use of 20 grams per day;

4. Placebo: Placebo Comparator

Margarine spread that contains no EPA, DHA or ALA (exchanged for oleic acid).

The total intervention period was set at 40 months. Research staff, participants and couriers for margarine transport were blinded towards the type of margarine that subjects received. Margarines for the trial were produced at regular intervals by Unilever, Vlaardingen, The Netherlands. A frequently consumed margarine available on the Dutch market served as the basis for trial margarines. Enriched margarines and placebo were of identical taste and appearance to ensure double-blind conduct of the trial. Treatment codes could not be derived from margarine tubs. After 4-6 weeks on placebo margarine, trial margarines (8 tubs of 250 g) were delivered free of charge to subjects' homes every 12 weeks by cooled courier transport.

2.6 Data collection

At baseline, data were collected on demographic factors (including educational level), lifestyle (including smoking status and alcohol use), self-perceived health, medical history, and current health status. Dietary data, including intake of different types of fish, were collected by a 203-item food frequency questionnaire that was an extended version of a previously validated food frequency questionnaire. Physical activity was also assessed by a validated questionnaire. Self-reported medication (including psychotropic drugs) of the participants was coded according to the Anatomical Therapeutic Chemical Classification System (ATC). Subjects were physically examined by trained research nurses at home or in the hospital, which included anthropometry, blood pressure, heart rate, and blood sampling.

The Mini-Mental State Examination (Folstein 1975) was performed to test cognitive function by a trained research nurse either at the participant's home, or at the hospital. Subjects with Mini-Mental State Examination scores ≤21 were excluded to avoid problems with trial procedures and dietary recall. Body weight (kg) and height (cm) were measured with the subject wearing light indoor clothing without shoes, and the body mass index (kg/m^2) was computed. Waist circumference (cm) was measured at the midpoint between the bottom rib and the top of the hipbone. Systolic and diastolic blood pressures (corresponding to the 1^{st} and 5^{th} Korotkoff sounds, respectively) were measured with the subject seated, after a 10-minute rest. Measurements were performed twice on the left upper arm with an automatic device (Omron HEM-711), and values were averaged. Finally, venous blood (30 mL) was drawn for the assessment of serum lipids, plasma glucose, and the fatty acid composition of plasma cholesteryl esters. Buffy coats were stored for DNA analysis. We asked subjects to remain fasted if physical examination was scheduled before 10:30 AM or to consume only a light meal when the examination was scheduled after 10:30 AM and to refrain from smoking or drinking coffee 1 hour before blood sampling.

2.7 Study endpoints

2.7.1 Endpoint reporting

A continuous update of the vital status of trial participants was provided by the National Institute for Public Health and the Environment (RIVM), Bilthoven, via a computerized link with the municipal health registries in the Netherlands. A 100% follow-up for vital status was achieved. All subjects provided contact details of a close relative or friend (not a household member) and gave informed consent to contact their general practitioner (GP) and cardiologist for information. For subjects who died, the GP was asked to fill out a standard form on primary and contributing causes of death. This form included two additional questions on whether the deceased patient suffered from dementia or cognitive decline. In addition, for all subjects who died, the death certificate was obtained from the Dutch National Mortality Registry (Statistics Netherlands). Self-reported non-fatal CVD events and cardiac interventions (PCI, CABG) were verified using patient records at the hospital Cardiology department. In case that dementia or cognitive decline was stated in the medical record, this information was also registered in the database of the Alpha Omega Trial.

Subjects were asked to record all hospitalizations and any changes in medication in a structured patient diary. In addition, subjects informed research staff about vacations, change of address, and hospitalizations by means of postage-paid reply cards. Spontaneous notifications of health problems (including cognitive decline and dementia) by the participant or persons affiliated with the participant, either by letter, email, telephone, or noted on trial questionnaires, were recorded in the computer database by trained research staff. Structured telephone interviews on major lifestyle factors, health status and changes in medication were conducted in the total cohort after 1 and 2 years of follow-up using a computerized questionnaire, by trained research staff. At the end of the follow-up period, subjects underwent physical examination and an extensive interview, including the MMSE test, by a research nurse at home or at the hospital. From 1 January 2009 onward, examinations by research nurses were no longer performed because of financial limitations. Thus, no MMSE tests were performed after that date. Subjects who refused participation in the final examination by the research nurse and subjects who left the study in 2009 received questionnaires by regular postal mail. In case of no response or refusal to fill out the questionnaires, a research nurse made a final attempt to interview the participant by telephone, using a condensed version of the questionnaire.

2.7.2 Follow-up

According to the original protocol, the first 4000 trial participants were followed for the incidence of endpoint events for a period of 40 months since their randomization. On 27 September 2005, the Steering Committee decided to enlarge the sample to a maximum of 5000. The Steering Committee also decided to terminate the follow-up on 5 November 2009 for all participants. Consequently, the maximal follow-up of the additional subjects might vary from 30-39 months. Anecdotally, participants from the original cohort might have a follow-up that exceeds 40 months. Analyses of the efficacy endpoints that are described below are based on data that emerged during the maximum available follow-up for each individual participant in the cohort that is eligible for the study of cognitive decline (as described in 3.1).

2.7.3 Endpoint adjudication process

Fatal events were coded according to International Classification of Diseases, 10th revision (ICD-10), by 3 independent members of the Endpoint Adjudication Committee (EAC), who remained blinded for the subjects' identity, treating physicians, and allocated treatment. EAC members coded all events independently, and regular meetings were organized to reach consensus in case of discrepancies. Non-fatal events, including spontaneous reports of dementia or cognitive decline by the participant or person affiliated with the participant, were not coded by the EAC.

2.8 Endpoints of cognitive decline

2.8.1 Change in cognitive function (continuous variable)

Overall cognitive function was assessed by the MMSE (Folstein 1975). Change in cognitive function will be calculated by subtracting pre-treatment (=baseline) from post-treatment (=after 40 months) MMSE scores in all subjects with complete data.

2.8.2 Cognitive decline (dichotomous variable)

Cognitive decline (0=absent, 1=present) is defined as a decrease of 3 points or more in MMSE score during follow-up. When data on change in MMSE score are not available, subjects will be categorized on basis of other information that indicates the presence of dementia or severe cognitive decline during follow-up. Cognitive decline in these subjects will be considered present in case that:

- the subjects died from dementia (ICD-10 codes F00, F01, F02 and F03);
- the General Practitioner stated on "cause of death from" that the patient suffered from dementia or cognitive decline;

- dementia or cognitive decline was stated in the medical record of the participant at the Cardiology center, as described in 2.7.1;
- when treatment with anti-dementia drugs was initiated during follow-up (ATC code N06D);
- dementia or cognitive decline was reported by the participant or persons affiliated with the participant and recorded in the computer database of the Alpha Omega Trial, as described in 2.7.1.

2.8.3 Severe cognitive decline (dichotomous variable)

Severe cognitive decline (0=absent, 1=present) is defined as a decrease of 5 points or more in MMSE score during follow-up. When data on change in MMSE score are not available, subjects will be categorized on basis of other information that indicates the presence of dementia or severe cognitive decline during follow-up, as stated in 2.8.2.

2.9 Power calculation

A post-hoc power calculation for the present analysis was conducted, based on the given numbers of ~1500 experimental subjects and ~1500 control subjects (two-way analysis for EPA+DHA and ALA, respectively, making use of the 2x2 factorial design). Assuming a standard deviation of 2.3 points for change in MMSE score during 40 months of follow-up within the groups and a true difference of 0.3 points in MMSE change between the groups, the power of rejecting the null hypothesis of "no difference in MMSE change between the groups" is 95%. The Type I error probability associated with testing this null hypothesis is 0.05.

3.0 Statistical handling policy

3.1 Analysis conventions

This section details general policies to be used for the statistical analysis. Departures from these general policies may be given in the specific detailed sections of this statistical analysis plan. When this situation occurs, the rules set forth in the specific section take precedence over the general policies.

3.2 Significance testing

All statistical tests will use a significance level of α=0.05. Two-tailed tests will be performed for all analyses that use statistical testing. Confidence intervals will be presented with 95% degree of confidence.

All p-values will be rounded to 3 decimal places. All p-values that round to 0.000 will be presented as '<0.001' and p-values that round to 1.000 will be presented as '>0.999'. Any p-value $\leq$0.05 will be considered statistically significant.

3.3 Multiplicity

No adjustments for multiplicity are planned within any of the analyses performed.

3.4 Data summarization

Summary statistics will consist of the number and percentage of responses in each category for discrete variables, and the mean, median, standard deviation (SD), minimum, and maximum for continuous variables. In addition quartiles will be presented in cases where a non-parametric analysis of continuous data is performed.

All mean and median values will be formatted to one more decimal place than the measured value. Standard deviation values will be formatted to two more decimal places than the measured value. Minimum and maximum values will be presented to the same number of decimal places as the measured value.

All percentages will be rounded to one decimal place. The number and percentage of responses will be presented

All analysis and summary tables will have the population sample size for each treatment group in the column heading.

3.5 Missing data

In case of missing data on the endpoint 'Change in cognitive function' (2.8.1), a decrease of 5.0 points in MMSE score during follow-up will be imputed for patients who have no MMSE score at the end of follow-up, but who meet the criteria for cognitive decline as stated in paragraph 2.8.2. No other imputations for missing data will be performed.

3.6 Data distribution and model checking

For the analysis of dichotomous data chi-square tests (with continuity correction) will be used, presented with the 95% confidence interval for the difference in proportions (normal approximation, large sample theory). If expected cell frequencies are small (less than 5), then Fisher's exact tests will be used instead.

For the analysis of continuous data that were measured at one point in time one-way analysis of variance (ANOVA) and unpaired Student's T-tests will be used. For the analysis of continuous data that were repeatedly measured over time, two-way ANOVA and paired Student's *t* tests will be used.

If the assumptions underlying ANOVA (normal distributions of residuals, homogeneity of variances) or Student's *t* tests (normal population distribution, particularly relevant for small samples) are not satisfied, then data transformation (e.g., logarithmic or square root transformation) will be applied to meet these assumptions. This includes the endpoint of cognitive decline, i.e. change in MMSE score during intervention, should this variable have a skewed distribution. Non-parametric tests will be applied, if the assumptions cannot be met with transformation.

When studying the dichotomous outcomes (described in 2.8.2 and 2.8.3) crude and multivariable logistic regression analysis will be used. Confounders in the multivariable models will include age, gender, educational level, smoking, alcohol use, and psychotropic medication (other than anti-dementia drugs, which forms part of the endpoint of interest).

3.7 Software

Version 9.1, or later of SAS® will be the statistical software package used to produce all summaries, listings, statistical analyses, and graphs.

4.0 Statistical analyses

4.1 Analysis populations

The source population for the present analysis is the cohort of the Alpha Omega Trial that includes men and women aged 60-80 with a history of MI and with baseline MMSE scores of 22 or higher, according to predefined inclusion criteria. Eligible for the analysis of cognitive decline are ~3000 randomized patients (62% of the total cohort) who were enrolled during the first years of the study and who were scheduled to complete the trial before 1 January 2009. This subcohort also includes patients who fulfilled this criterion and died during the trial. The reason that only part of the Alpha Omega Trial cohort can be included in the present analysis is the following: from 1 January 2009 onward, the MMSE test was no longer part of the final examination of the Alpha Omega Trial and changes in MMSE score can therefore not be computed in patients who left the trial after that date. The patient populations to be studied are described below.

4.1.1 Intention-to-treat population

The Intention-to-treat (ITT) population includes ~3000 subjects who were scheduled to complete the trial before 1 January 2009. This includes subjects who fulfilled this criterion and died during the trial.

4.1.2 Population not cognitively impaired at baseline

This population includes subjects not cognitively impaired (NCI) at baseline, i.e. MMSE score of 24 or higher (Siu 1991) or treatment with anti-dementia drugs, and who were scheduled to complete the trial before 1 January 2009. This includes subjects who fulfilled this criterion and died during the trial.

4.1.3 Per protocol

The following per protocol (PP) populations have been defined:

- PP-10: This population includes all subjects in the ITT population who had an average margarine intake of at least 10 g/d (which is equivalent to the target of >200 mg/d extra EPA + DHA and >1 g/d extra ALA).
- PP-20: This population includes all subjects in the ITT population who had an average margarine intake of at least 20 g/d (which is equivalent to the target of >400 mg/d extra EPA + DHA and >2 g/d extra ALA).
- PP-EXP: This population includes all subjects in the ITT population actually exposed to margarine use. The exposed period is defined as the time period between the date of first delivery of trial margarines to the participant's home until the date at which the last trial margarine was consumed.

4.2 Subject characteristics

Subject demographics and pre-treatment characteristics will be summarized for the ITT, NCI and PP populations. The demographics and pre-treatment characteristics will be summarized for each treatment group and for all subjects combined.

4.2.1 Demographics and pre-treatment subject characteristics

The summary of demographics includes:

- Age;
- Gender;
- Race;
- Educational level (4 categories);
- Marital status (2 categories).

The summary of medical history and risk factors includes:

- Height (cm);
- Body weight (kg);
- Body mass index (kg/m^2);
- Physical activity as assessed by the PASE questionnaire;
- Systolic blood pressure (mmHg);
- Diastolic blood pressure (mmHg);
- Serum total cholesterol (mmol/L);
- Serum HDL cholesterol (mmol/L);
- Serum triglycerides (mmol/L);
- Serum glucose (mmol/L);
- Self -perceived health, in categories (%);
- Smoking habits (current / former / never);
- Alcohol use (%);
- Alcohol intake, in drinkers only (g/d);
- Fish consumption;
- Fish intake, in consumers only (g/d);
- Time since last MI (y);
- Self-reported history of stroke (%);
- Presence of diabetes mellitus (%);
- Self-reported history of PCI or CABG (%).

4.2.2 Medication

The summary of prior and concomitant medications will at least include supplements containing n-3 fatty acids and the following list of medication, ordered by ATC code:

- A10 Drugs used in diabetes
- A10A Insulin
- B01 Antithrombotic medication
- C01 Cardiac therapy
- C02 Antihypertensives
- C03 Diuretics
- C04 Peripheral vasodilators
- C05 Vasoprotectives
- C07 Beta blocking agents
- C08 Calcium channel blockers
- C09 Agents acting on the renin-angiotensin system
- C10 Lipid modifying agents
- C10AA HMG CoA reductase inhibitors (statins)
- G03 Sex hormones and modulators of the genital system
- N05A Antipsychotic drugs;
- N06A Antidepressant drugs;
- N06AA Tricyclic antidepressants;
- N06AB Selective serotonin reuptake inhibitors;
- N06D Anti-dementia drugs, including anticholinesterases

In addition, the following groups of medication will be created and analyzed:

- All blood pressure lowering drugs: C02 + C03 + C07 + C08 + C09;
- Aspirin: A01AD05 + B01AC06 + N02BA01;
- Benzodiazepines (N05BA, N05CD, N03AE01, and so called "Z-drugs" zopiclone and zolpidem: N05CF);
- Antipsychotic drugs (N05A) except lithium (N05AN);
- All psychotropic drugs (N05 [Psycholeptics] and N06 [Psychoanaleptics]);
- Antidepressant drugs other than tricyclic antidepressants and selective serotonin reuptake inhibitors (N06A minus N06AA + N06AB);

4.3 Monitoring of compliance, patient characteristics and medication

As a measure of compliance to margarine use, the fatty acid composition of plasma cholesteryl esters (as a biomarker of n-3 fatty acid intake) was assessed in randomly selected subsets of men (n=405) and women (n=405) at baseline, and after 20 and 40 months of follow-up. Patient characteristics (see 4.2.1), the use of n-3 PUFA supplements and changes in psychotropic medication (see 4.2.2) were also assessed in these subsets of patients during follow-up. Descriptive and inferential (differences in relation to randomly allocated treatment) statistics for the abovementioned variables will be determined in the randomly selected subsets at baseline, after 20 months and after 40 months.

4.4 Efficacy analysis

All efficacy analyses will be performed for the ITT, NCI and PP populations. Efficacy parameters will be determined for all 4 randomly allocated treatments using Student's *t* tests for independent samples, and using analysis of covariance (outcome stated in 2.8.1) or multivariable logistic regression (outcomes stated in 2.8.2 and 2.8.3) while adjusting for the main potential confounders (i.e., age, gender, educational level, smoking, alcohol use and psychotropic medication).

4.4.1 Efficacy of EPA + DHA

The subjects who are randomized to EPA + DHA alone or to EPA + DHA plus ALA will be merged in one cohort (i.e. EPA + DHA experimental treatment). Their efficacy parameters will be compared with the cohort of subjects who are randomized to ALA alone or to placebo (i.e. EPA + DHA placebo). Thus an estimate of the efficacy of EPA + DHA treatment will be obtained.

4.4.2 Efficacy of ALA

The subjects who are randomized to ALA alone or to EPA + DHA plus ALA will be merged in one cohort (i.e. ALA experimental treatment). Their efficacy parameters will be compared with the cohort of subjects who are randomized to EPA + DHA alone or to placebo (i.e. ALA placebo). Thus an estimate of the efficacy of ALA treatment will be obtained.

4.4.3 Combined efficacy of EPA + DHA and ALA

The subjects who are randomized to ALA alone, to EPA + DHA plus ALA, to EPA + DHA alone, and to placebo are compared. Thus an estimate of the efficacy of ALA, EPA + DHA, and the combination of ALA plus EPA + DHA treatment will be obtained.

4.5 Subgroup analyses

All analyses will be repeated in strata according to

- age (<70 vs. ≥70 yr);
- gender (men vs. women);
- use of psychotropic medication at baseline (yes vs. no);
- years since the index MI (<median vs. ≥median);
- background average intake of EPA + DHA (<50 mg/d vs. ≥50 mg/d and <100 mg/d vs. ≥100 mg/d);
- background average intake of fish (<5 g/d vs. ≥5 g/d and <10 g/d vs. ≥10 g/d).

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List of abbreviations

AD	Alzheimer's disease
ALA	alpha-linolenic acid
ANOVA	analysis of variance
ATC	Anatomical Therapeutic Chemical Classification System
CABG	coronary artery bypass grafting
CHD	coronary heart disease
CVD	cardiovascular diseases
DHA	docosahexaenoic acid
EAC	Endpoint Adjudication Committee
EPA	eicosapentaenoic acid
GP	general practitioner
ITT	intention-to-treat
MI	myocardial infarction
n-3 PUFA	omega-3 (n-3) polyunsaturated fatty acid(s)
NCI	not cognitively impaired
PCI	percutaneous coronary intervention
PP	per protocol
SAP	statistical analysis plan