# RESEARCH PROTOCOL

# PURPOSE: Placebo-controlled trial in subjects at Ultra-high Risk for Psychosis with Omega-3 fatty acidS in Europe

**Study protocol**

**Version 7, dated December 23rd 2021**

**PROTOCOL TITLE**

'Placebo-controlled trial in subjects at ultra-high risk for psychosis with omega-3 fatty acids in Europe'

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**TABLE OF CONTENTS**

**1. INTRODUCTION AND RATIONALE ..... 14**

**2. OBJECTIVES ..... 15**

**3. STUDY DESIGN ..... 16**

3.1 Visits and study procedures UHR subjects ..... 19

3.2 Visits and study procedures healthy controls ..... 21

**4. STUDY POPULATION ..... 24**

4.1 Population ..... 24

4.2 In- and exclusion criteria UHR subjects ..... 24

4.3 In- and exclusion criteria healthy controls ..... 26

4.4 Sample size calculation ..... 26

**5. TREATMENT OF SUBJECTS ..... 27**

5.1 Investigational product ..... 27

5.2 Concomitant treatment ..... 27

**6. INVESTIGATIONAL PRODUCT ..... 28**

6.1 Name and description of investigational product ..... 28

6.2 Summary of findings from clinical studies ..... 28

6.3 Summary of known and potential risks and benefits ..... 29

6.4 Description of administration and dosage ..... 30

6.5 Preparation and labelling of Investigational Medicinal Product ..... 30

6.6 Drug accountability ..... 30

**7. METHODS ..... 30**

7.1 Study parameters ..... 30

7.2 Randomisation, blinding and treatment allocation ..... 31

7.3 Study procedures ..... 32

7.4 Withdrawal of individual subjects ..... 37

7.5 Replacement of individual subjects after withdrawal ..... 37

7.6 Follow-up of subjects withdrawn from treatment ..... 37

7.7 Termination of participating study centre ..... 37

7.8 Premature termination of the study ..... 38

**8. SAFETY REPORTING ..... 38**

8.1 General aspects ..... 38
8.2 Definitions ..... 38
8.3 Reporting procedures ..... 39
8.4 Follow-up of adverse events ..... 40
8.5 Data Safety Monitoring Board ..... 40
**9. STATISTICAL ANALYSIS ..... 40**
9.1 General statistical considerations ..... 41
9.2 Baseline comparisons ..... 41
9.3 Primary analyses ..... 41
9.4 Secondary analyses ..... 41
9.5 Dropout rates and missing values ..... 42
**10. ETHICAL CONSIDERATIONS ..... 42**
10.1 Regulation statement ..... 42
10.2 Recruitment and consent ..... 42
10.3 Consent procedures in minors ..... 43
10.4 Risk-benefit assessment ..... 44
10.5 Compensation for injury ..... 45
10.6 Incentives ..... 45
**11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION ..... 45**
11.1 Handling and storage of data and documents ..... 45
11.2 Monitoring and Quality Assurance ..... 47
11.3 Amendments ..... 48
11.4 Annual progress report ..... 48
11.5 End of study report ..... 48
11.6 Public disclosure ..... 48
11.7 Publication Policy ..... 48
**12. REFERENCES ..... 51**
**Appendix 1: Protocol signature page Principal Investigator ..... 57**

## LIST OF ABBREVIATIONS

| | |
|---|---|
| **ADNI** | **Alzheimer's Disease Neuroimaging Initiative** |
| **AE** | **Adverse Event** |
| **BDI** | **Beck's Depression Inventory** |
| **BIF** | **Behaviour Identification Form** |
| **CAARMS** | **Comprehensive Assessment of At-Risk Mental State** |
| **CBCL** | **Child Behaviour Checklist** |
| **CGI** | **Clinical Global Impression** |
| **CNB** | **Computerised Neurocognitive Battery** |
| **CPT** | **Continuous Performance Task** |
| **DHA** | **Docosahexaenoic acid** |
| **DSMB** | **Data Safety Monitoring Board** |
| **DTI** | **Diffusion tensor imaging** |
| **eCRF** | **Electronic Case Report Form** |
| **EHI** | **Edinburgh Handedness Inventory** |
| **EPA** | **Eicosapentaenoic acid** |
| **EudraCT** | **European drug regulatory affairs Clinical Trials** |
| **GAF** | **Global Assessment of Functioning** |
| **GCP** | **Good Clinical Practice** |
| **GF:R** | **Global Functioning Role** |
| **GF:S** | **Global Functioning Social** |
| **ICH-GCP** | **International Conference on Harmonisation - Good Clinical Practice** |
| **IL** | **Interleukin** |
| **IMP** | **Investigational Medicinal Product** |
| **IMPD** | **Investigational Medicinal Product Dossier** |
| **K-SADS-PL** | **Kiddie - Schedule for Affective Disorders and Schizophrenia - Present and Lifetime Version** |
| **MRI** | **Magnetic Resonance Imaging** |
| **NAPLS** | **North American Prodrome Longitudinal Study** |
| **PANSS** | **Positive and Negative Syndrome Scale** |
| **SAE** | **Serious Adverse Event** |
| **SOFAS** | **Social and Occupational Functioning Assessment Scale** |
| **SPC** | **Summary of Product Characteristics** |
| **SUSAR** | **Suspected Unexpected Serious Adverse Reaction** |
| **UHR** | **Ultra-high risk** |

| | |
|---|---|
| **WAIS** | **Wechsler's Adult Intelligence Scale** |
| **WISC** | **Wechsler's Intelligence Scale for Children** |
| **WHO** | **World Health Organisation** |
| **YMRS** | **Young Mania Rating Scale** |
| **YSR** | **Youth Self Report** |

**SUMMARY**

**Rationale:** Psychosis is a chronic and severe mental disorder, usually preceded by a prodromal phase of attenuated psychotic symptoms and decline in function. Intervention during this prodromal state could prevent transition to psychosis, and is therefore of high significance. A recent randomised controlled trial with individuals at ultra-high risk (UHR) for psychosis showed a significantly lower transition rate to psychosis and reduced psychotic symptoms in those subjects who were treated daily with omega-3 fatty acids compared to placebo. Here we further evaluate the potential of omega-3 fatty acids in the prevention of psychosis.

**Objective:** Primary objective of this randomised controlled trial is to compare transition rates to psychosis between individuals who are at UHR for developing psychosis and randomised to treatment with omega-3 fatty acids to those randomised to placebo, as determined through the Comprehensive Assessment of At-Risk Mental State (CAARMS). Secondary objectives include a comparison between the two treatment arms with regard to discontinuation rate, tolerability, symptomatology, psychosocial and cognitive function, quality of life, blood levels of bioactive lipids, (epi)genetic markers and immune parameters. Third, the ability of both MRI and blood parameters to serve as potential biomarkers that may predict clinical response of UHR subjects will be elucidated. Finally, both cross-sectional and longitudinal comparisons of study parameters will be conducted between UHR subjects and healthy controls.

**Study design:** A European, multicentre, randomised, double-blind, placebo-controlled clinical trial.

**Study population:** The study aims to recruit a sample of 220 individuals at UHR for psychosis, aged 13 - 20, who subsequently will be treated with omega-3 fatty acids or placebo. A limited number of participating centres will additionally recruit a sample of 110 healthy controls without an intervention.

**Intervention:** UHR subjects are randomised to daily treatment with either 1.2 gram omega-3 polyunsaturated fatty acids or placebo for six months, followed by a 1.5-year clinical follow-up.

**Main study parameters:** Transition to psychosis will be defined using the CAARMS criteria. Symptomatology will be examined using semi-structured interviews and questionnaires including the Positive and Negative Syndrome Scale (PANSS), Global Assessment of Functioning scale (GAF), Clinical Global Impression Scale (CGI) and Beck’s Depression Inventory (BDI). Cognition will be assessed using subscales of the Wechsler's Intelligence

Scale. Blood samples will be drawn to assess levels of bioactive lipids, (epi)genetic markers and immune parameters. Finally, brain structure and function are measured in an MRI session, consisting of structural MRI, resting state functional MRI, Diffusion Tensor Imaging (DTI), and functional MRI during reward processing.

**Nature and extent of the benefit, burden and risks associated with participation:** This study includes eight site visits. The first two visits occur before treatment initiation, and consist of screening for in- and exclusion criteria, a diagnostic interview, and baseline examination of symptomatology, psychosocial and cognitive function, quality of life and drug use. Four blood samples are drawn to perform standard clinical laboratory tests and to assess levels of bioactive lipids, (epi)genetic markers and immune parameters. Subjects will undergo a baseline MRI session of at maximum sixty minutes. Both blood sampling and MRI are safe procedures, and standard procedures will be followed to minimise risks. Subsequent visits take place at 1 month, 3 months, 6 months, 1 year, 1.5 years and 2 years after start of study medication. During these visits, interviews and questionnaires performed at baseline will be repeated. At visits 5 and 8, three blood samples will be drawn to assess levels of bioactive lipids, (epi)genetic markers and immune parameters. Transition to psychosis will be assessed every six months using the CAARMS criteria. UHR individuals younger than 18 years of age will be included in this study, as attenuated symptoms of psychosis typically emerge in adolescence. However, participation in this randomised controlled trial may be of therapeutic benefit to UHR subjects since treatment with omega-3 fatty acids has been associated with prevention of the transition to psychosis. Omega-3 fatty acids are food supplements with important physical health benefits and are minimally associated with adverse effects.

## 1. INTRODUCTION AND RATIONALE

Schizophrenia is a chronic and devastating mental disorder characterised by disturbances of thought, perception, volition, and cognition. Although the incidence of schizophrenia is relatively low (10 - 22 per 100 000), its prevalence is relatively high (0.3 - 0.7 per 100) due to the chronic nature of the illness (McGrath et al., 2008). The first stage of the disorder involves a syndrome of attenuated symptoms of psychosis, social anxiety and a marked decline in function (Häfner, 2000). Once these features are present, there is a 30% risk of developing a psychotic disorder within two years, which is about 400 times greater than in the general population (Fusar-Poli et al., 2012). Therefore, subjects at this stage are regarded to be at Ultra High Risk (UHR) for psychosis. Clinical trials with antipsychotic treatment of UHR individuals do not show a significant reduction in the risk of later progression to psychosis (McGorry et al., 2002; McGlashan et al., 2006; Yung et al., 2011; McGorry et al., 2013). In addition, in practice, most UHR subjects refuse to take antipsychotic medication, as it is associated with the stigma of

psychosis, and with serious metabolic and neurological adverse effects. Therefore, there is a pressing need for interventions that can reduce the risk of progression to psychosis, but that have minimal side effects and are not associated with the treatment of psychosis, and therefore stigmatising.

In this respect, the class of long-chain omega-3 polyunsaturated fatty acids is a highly promising candidate. Omega-3 fatty acids are known to have important physical health benefits and minimal side effects (De Caterina, 2011). Despite inconclusive findings of clinical trials with omega-3 fatty acids in patients with schizophrenia (see for meta-analyses Freeman et al., 2006; Irving et al., 2011; Fusar-Poli and Berger, 2012), the only randomised placebo-controlled study in subjects at UHR for developing psychosis showed encouraging results (Amminger et al., 2010). In this study, 81 UHR individuals aged 13 - 25 were randomised to 12 week treatment with either daily 1.2 gram omega-3 fatty acids or placebo, followed by a 40-week monitoring period. At the end of the study, the transition rate to psychosis was significantly lower in the fatty acid group (4.9%, 2 of 41 subjects) compared to the placebo group (27.5%, 11 of 40 subjects). In addition, treatment with omega-3 fatty acids significantly reduced psychotic symptoms and improved functioning of UHR individuals. No differences in adverse events were demonstrated (Amminger et al., 2010).

To date, the randomised controlled trial performed by Amminger and colleagues is the only study that examined the effectiveness of omega-3 fatty acids in the prevention of psychosis. Notwithstanding the highly promising results, the relatively modest sample size requires that care be taken in generalising findings to other groups and settings. In addition, the 12-month study period entails the possibility that transition to a first episode of psychosis may have been delayed rather than prevented. In order to clarify these important issues and to further evaluate the potential of omega-3 fatty acids in the prevention of psychosis, we aim to perform a large multicentre trial in which individuals who are at ultra-high risk for psychosis are treated daily with either omega-3 fatty acids or placebo for six months, and clinically followed up for 1.5 year.

## 2. OBJECTIVES

The primary objective of this randomised controlled trial is to compare the rate of transition to psychosis between individuals who are at UHR for developing psychosis and randomised to omega-3 fatty acids to those randomised to placebo. Secondary objectives include comparisons between the two treatment arms regarding discontinuation rate, tolerability, symptomatology, psychosocial and cognitive function, quality of life, blood levels of bioactive lipids, (epi)genetic markers and immune parameters. Third, the ability of both MRI and blood parameters to serve as potential biomarkers that may predict clinical response of UHR subjects will be elucidated.

Finally, both cross-sectional and longitudinal comparisons of study parameters will be conducted between UHR subjects and healthy controls.

## 3. STUDY DESIGN

The impact of omega-3 fatty acid treatment of individuals at UHR for developing psychosis will be assessed using a randomised, double-blind, placebo-controlled design. UHR subjects will be treated daily with either 1.2 gram omega-3 polyunsaturated fatty acids or placebo for six months (please see for details regarding medication paragraph 6). After an extensive baseline examination, subsequent assessments will occur at 1 month, 3 months, 6 months, 1 year, 1.5 years and 2 years after treatment initiation. Transition to psychosis will be assessed every six months using the criteria in the Comprehensive Assessment of At-Risk Mental State (CAARMS). Assessment of cognitive function occurs at baseline and at 2 years after start of study medication. Blood draws take place during the screening visit, and 6 months as well as 2 years after treatment initiation. An MRI scan will be obtained at baseline (see table 1).

Assignment to a treatment arm will be performed randomly to eliminate the possible influence of arbitrary allocation of subjects on the study results. Using random assignment, known and currently unknown factors potentially influencing outcomes (e.g. demographic factors, findings at baseline examination) are evenly distributed among the two groups, which increases the validity of statistical analyses. Administration of medication will be double-blind in order to minimise the influence of expectancy regarding the type of medication on data collection and analysis. A placebo arm is included as the effectiveness of omega-3 fatty acids in the prevention of psychosis will be examined, and no beneficial treatments prescribed to UHR subjects are available as a comparison.

This study examines the effectiveness of omega-3 fatty acids in UHR individuals who are between 13 and 20 years of age. Inclusion of minors is essential, as attenuated symptoms of psychosis typically emerge in adolescence and young adulthood, with the incidence of psychosis rapidly increasing from the age of 15 with a peak age of onset around 20 (Figure 1; Jones et al., 2013). Indeed, established at-risk services report an intake of UHR subjects younger than 20 years (Yung et al., 2011). In addition, the only randomised placebo-controlled study with omega-3 fatty acid treatment of UHR individuals to date included subjects with a mean age of 16.8±2.4 years (Amminger et al., 2010). Since we aim to replicate and extend these findings we will include participants with a similar age. Finally, the clinical effect of omega-3 fatty acids is hypothesised to be most significant in young UHR subjects, as fatty acids have been implicated in the process of adolescent brain maturation (McNamara and Carlson, 2006).

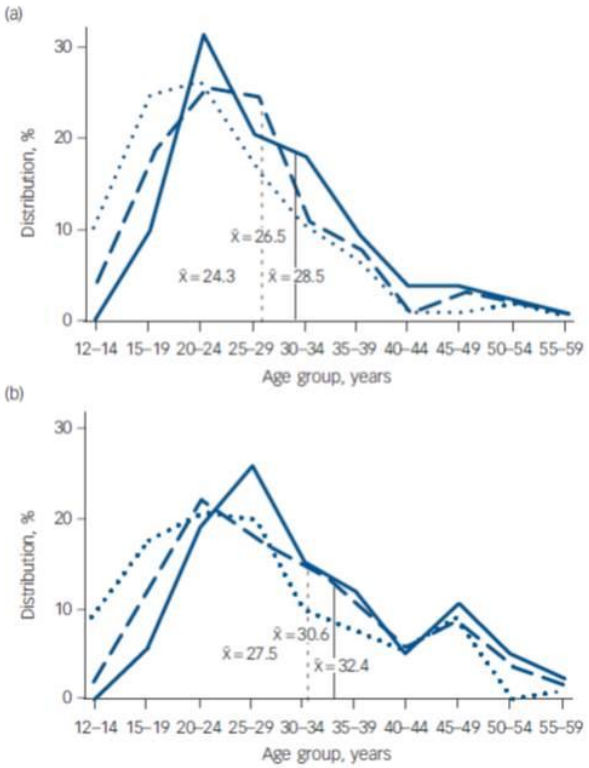



**Figure 1** Distribution of age of onset (as percentage of the whole sample, with being mean age years) of schizophrenia. A) Males: earliest sign of mental disturbance, n=117 (dotted line); first psychotic symptom, n=125 (dashed line); index admission, n=133 (solid line). B) Females: earliest sign of mental disturbance, n=131 (dotted line); first psychotic symptom, n=139 (dashed line); index admission, n=143 (solid line).

**Table 1** Visits and study procedures UHR subjects

| | Visit number | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Duration (mins) |
| **Assessments** | Screening | Baseline | 1 month* (+/- 1 week) | 3 months* (+/- 1 week) | 6 months* (+/- 2 weeks) | 1 year* (+/- 2 weeks) | 1.5 years* (+/- 2 weeks) | 2 years* (+/- 2 weeks) | |
| Informed consent | X | | | | | | | | 5 |
| Inclusion and exclusion criteria | X | | | | | | | | 10 |
| Sociodemographics | X | | | | | | | | 5 |
| Medical history | X | | | | | | | | 5 |
| Family history | X | | | | | | | | 5 |
| Physical health | X | | X | X | X | X | X | X | 5 |
| Current medication use | X | | X | X | X | X | X | X | 5 |
| Recent psychiatric history | X | | X | X | X | X | X | X | 5 |
| Adverse events | X | | X | X | X | X | X | X | 5 |
| **Self-report measures** | | | | | | | | | |
| Edinburgh Handedness Inventory | | X | | | | | | | 2 |
| Tanner Criteria | | X | | | | | | X | 2 |
| YSR / CBCL | | X | | | X | | | X | 15 |
| Beck's Depression Inventory | | X | X | X | X | X | X | X | 5 |
| Connor-Davidson Resilience Scale | | X | X | X | X | X | X | X | 10 |
| Perceived Stress Scale | | X | X | X | X | X | X | X | 2 |
| KIDscreen-27 | | X | | X | X | X | X | X | 10 |
| Medication Adherence Questionnaire | | | X | X | X | | | | 1 |
| **Clinical measures** | | | | | | | | | |
| K-SADS-PL | X | | | | | | | X | 75 |
| CAARMS Positive Symptoms[1] | X | | | | X | X | X | X | 30 |
| PANSS | X | | X | X | X | X | X | X | 45 |
| GAF / SOFAS / GF:R / GF:S | X | | X | X | X | X | X | X | |
| CGI | | X | X | X | X | X | X | X | |
| YMRS | | X | X | X | X | X | X | X | 10 |
| WHO Assist 3.0 Drug Use | | X | | X | X | X | X | X | 10 |
| **Cognitive measures** | | | | | | | | | |
| WISC / WAIS | X | | | | | | | X | 40 |
| **Blood measures** | | | | | | | | | |
| Standard clinical laboratory tests | X | | | | | | | | 15 |
| Blood sampling bioactive lipids | X | | | | X | | | X | |
| (Epi)genetic markers | X | | | | X | | | X | |
| Blood sampling immune markers | X | | | | X | | | X | |
| **Other measures** | | | | | | | | | |
| Urinary drug screening | | X | | X | X | X | | X | 2 |
| MRI | | X | | | | | | | 60 |
| **Drug dispensing** | | X | | X | | | | | |
| **Estimated duration (mins)** | **255** | **82** | **76** | **88** | **118** | **117** | **115** | **187** | |
| **Estimated duration (hrs)** | **4,3** | **1,4** | **1,3** | **1,5** | **2** | **2** | **1,9** | **3,1** | |

X To be completed at home; * Timing is relative to baseline visit; [1] When several interviews are planned at one visit, then interviews will be combined which results in a shorter estimated duration. YSR, Youth Self Report; CBCL, Child Behaviour Checklist; K-SADS-PL, Kiddie - Schedule for Affective Disorders and Schizophrenia - Present and Lifetime Version; CAARMS, Comprehensive Assessment of At-Risk Mental State; PANSS, Positive And Negative Syndrome Scale; GAF, Global Assessment of Functioning scale; SOFAS, Social and Occupational Functioning Assessment Scale; GF:R, Global Functioning Role; GF:S, Global Functioning Social; CGI, Clinical Global Impression scale; YMRS, Young Mania Rating Scale; WHO, World Health Organisation; WISC, Wechsler's Intelligence Scale for Children; WAIS, Wechsler's Adult Intelligence Scale; MRI, Magnetic Resonance Imaging.

In the current study, data of both UHR individuals and healthy controls will be obtained. Healthy controls are not subject to an intervention. The inclusion of a control group of healthy volunteers is required as a possible signature that is associated with the beneficial effect of omega-3 fatty acid treatment and/or the transition to psychosis needs to be validated by understanding how these measures are different in controls. In addition, healthy controls are required for secondary cross-sectional and longitudinal case-control comparisons. However, whereas all participating centres will contribute to the recruitment of a sample of UHR subjects (N=220), only a limited number will additionally recruit a sample consisting of healthy controls (N=110), which thus will be of comparable size to each arm of UHR subjects. Study design and in- and exclusion criteria for healthy controls will be provided in paragraphs 3.2 and 4.3, respectively.

### 3.1 Visits and study procedures UHR subjects

#### 3.1.1 Informed consent and screening (visit 1)

During the first meeting with the investigator, the study will be explained to the subject including possible benefits and disadvantages of participation. For individuals younger than 18 years of age, additional procedures, rules and regulations apply, which are described in section 10.3. When the subject's competence is assured and written informed consent is provided, the subject is screened extensively for in- and exclusion criteria. This includes the withdrawal of a blood sample to perform standard clinical laboratory tests. To minimise the burden for participants, blood to assess levels of bioactive lipids, (epi)genetic markers and immune parameters will be collected simultaneously. Information about sociodemographics, medical history, current medication use, recent psychiatric history, and psychiatric disorders in first-degree relatives will be collected. A physical examination including heart rate, blood pressure, weight, height and abdominal circumference will be conducted. Current and past episodes of psychopathology will be determined using the semi-structured diagnostic interview Schedule for Affective Disorders and Schizophrenia for School Aged Children (K-SADS-PL). The positive symptoms scale of the Comprehensive Assessment of At-Risk Mental State (CAARMS) (Yung et al., 2005) is used to assess UHR criteria. Symptomatology will be examined using the Positive and Negative Syndrome Scale (PANSS), and psychosocial function with the Global Assessment of Functioning scale (GAF), Social and Occupational Functioning Assessment Scale (SOFAS), Global Functioning Role (GF:R) and Global Functioning Social (GF:S). Cognition and IQ will be assessed using subscales of the Wechsler's Intelligence Scale. Due to the elaborate assessment at visit 1, it is allowed to divide the assessments over multiple days. However, all visit 1 procedures will have to be completed within one week after signing informed consent. At the end of the screening visit, subjects will be given questionnaires that can be filled

in at home before the next visit. If the CAARMS/K-SADS-PL has been completed for a participant in the past, these data may be used if the interview has taken place not longer than three months before inclusion in the study. If the CAARMS interview was administered in a period of 1 to 3 months prior to study inclusion, the data should be updated to the current situation.

### 3.1.2 Baseline examination (visit 2)

Visit 2 takes place at maximum two weeks after the last visit 1 procedure has been completed. At baseline, thus before administration of any study medication, current drug use will be assessed with a urinary drug screening, and information about history of drug use is collected with the WHO Assist interview. Mania symptoms will be assessed with the Young Mania Rating Scale (YMRS), and the Clinical Global Impression Scale (CGI) will be filled in by the investigator. Subjects will undergo an MRI session of sixty minutes at maximum which includes structural MRI, resting state functional MRI, diffusion tensor imaging (DTI), and functional MRI during reward processing. The self-report questionnaires which were provided to the subject during the screening visit will be collected by the investigator. Please see table 1 and paragraph 7.3 for details of assessments. When all screening and baseline procedures are completed and the participant is deemed eligible, the subject can be randomised to either omega-3 fatty acid treatment or placebo. At the end of the baseline visit, study medication will be dispensed for the first time. Assessments need to be completed within one day.

### 3.1.3 Follow-up examinations (visits 3 to 8)

Follow-up assessments occur at 1 month, 3 months, 6 months, 1 year, 1.5 years and 2 years after treatment initiation. Before each visit, subjects will be sent questionnaires that can be filled in at home. During all visits, information about current medication use, recent psychiatric history and adverse events will be collected, and a brief physical examination will be conducted. In addition, measures of symptomatology and psychosocial function that are performed at baseline will be repeated. At visit 4, new study medication will be dispensed and used medication will be returned. At visits 3 to 5, the Medication Adherence Questionnaire will be applied, and at visits 4, 5, 6 and 8, current drug use will be assessed with a urinary drug screening. Cognitive function is assessed at visit 8. Blood samples will be drawn at visits 5 and 8 to assess levels of bioactive lipids, (epi)genetic markers and immune parameters. Transition to psychosis will be assessed every six months (visits 5 - 8) using the CAARMS criteria (see table 1). Assessments for all visits may be divided over multiple days. Please note that timing of visits is relative to the baseline visit.

### 3.1.4 Telephone calls to improve drug adherence

During treatment, UHR subjects are contacted at least once every two weeks by telephone to monitor drug adherence. Information on the intake of capsules will be collected.

### 3.1.5 Endpoint

If a subject makes a transition to psychosis, as determined through the CAARMS, or when a subject exceeds the maximally allowed dose of antipsychotics during the study (i.e. equivalent of a total haloperidol use of 10 mg within two months, except for situations as described in section 5.2), all measures as described for visit 8 (2 years after start of study medication) will be conducted as soon as possible after transition. These subjects will be followed up and/or be treated as usual in normal daily practice, but will not be followed up as part of this study. That is, no study medication will be dispensed and no visits are scheduled within the context of this trial. However, if data is available that would be collected within the context of the study, this info can be collected from the subject's medical file and entered into the electronic case report form.

### 3.1.6 Allowed visit windows

All assessments related to the screening visit (visit 1) need to be completed within one week. Please note that signing the informed consent is regarded as a screening visit procedure, meaning that all visit 1 procedures need to be completed within one week after the informed consent is signed. Baseline examination (visit 2) takes place at maximum two weeks after the last visit 1 procedure has been completed. All visit 2 assessments need to be completed within one day. The timing of visits 3 to 8 is *relative to the baseline visit*. This will result in a comparable study completion period between subjects, ranging between 102 and 106 weeks in total. Visit 3 occurs 1 month +/- 1 week after the baseline visit, visit 4 occurs 3 months +/- 1 week after the baseline visit, visit 5 occurs 6 months +/- 2 weeks after the baseline visit, visit 6 occurs 1 year +/- 2 weeks after the baseline visit, visit 7 occurs 1.5 years +/- 2 weeks after the baseline visit, and visit 8 occurs 2 years +/- 2 weeks after the baseline visit.

## 3.2 Visits and study procedures healthy controls

A cohort of 110 healthy volunteers will be used as controls for the cohort of UHR subjects, and will be recruited by a limited number of participating centres in order to reduce any variability introduced by the use of multiple MRI scans. Healthy controls will be assessed at three time points: at baseline, at 1 year and at 2 years after inclusion in the study (see table 2). Study procedures for healthy controls are a subset of those described for UHR subjects, and are

described in more detail in paragraph 7.3. MINI-KID will be used in healthy controls to assess psychiatric history.

### 3.2.1 Allowed visit windows

All assessments related to the baseline visit (visit 1) need to be completed within one week. Please note that signing the informed consent is regarded as a baseline visit procedure, meaning that all visit 1 procedures need to be completed within 1 week after the informed consent is signed. The timing of visits 2 and 3 is *relative to the baseline visit*. This will result in a comparable study completion period between subjects, ranging between 102 and 106 weeks in total. Visit 2 occurs 1 year +/- 2 weeks after the baseline visit, and visit 3 takes place 2 years +/- 2 weeks after the baseline visit.

**Table 2** Visits and study procedures healthy controls

| | **Visit number** | | | |
|---|---|---|---|---|
| **Assessments** | 1<br>Baseline | 2<br>1 year*<br>(+/- 2 weeks) | 3<br>2 years*<br>(+/- 2 weeks) | **Duration (mins)** |
| Informed consent | **X** | | | 5 |
| Inclusion and exclusion criteria | **X** | | | 10 |
| Sociodemographics | **X** | | | 5 |
| Medical history | **X** | | | 5 |
| Family history | **X** | | | 5 |
| Physical health | **X** | **X** | **X** | 5 |
| Current medication use | **X** | **X** | **X** | 5 |
| **Self-report measures** | | | | |
| Edinburgh Handedness Inventory | **X** | | | 2 |
| Tanner Criteria | **X** | | **X** | 2 |
| **Clinical measures** | | | | |
| MINI-KID | **X** | | **X** | 30 |
| CAARMS Positive Symptoms | **X** | | **X** | 10 |
| WHO Assist 3.0 Drug Use | **X** | **X** | **X** | 10 |
| **Cognitive measures** | | | | |
| WISC / WAIS | **X** | | **X** | 40 |
| **Other measures** | | | | |
| Urinary drug screening | **X** | **X** | **X** | 2 |
| MRI | **X** | | | 60 |
| **Estimated duration (mins)** | **196** | **22** | **104** | |
| **Estimated duration (hrs)** | **3,3** | **0,37** | **1,7** | |

* Timing is relative to the baseline visit. MINI-KID, MINI International Neuropsychiatric Interview for Children and Adolescents; CAARMS, Comprehensive Assessment of At-Risk Mental State; WHO, World Health Organisation; WISC, Wechsler's Intelligence Scale for Children; WAIS, Wechsler's Adult Intelligence Scale; MRI, Magnetic Resonance Imaging.

### 3.3 Alternative study procedures

Due to the current world-wide affecting pandemic, in order to maintain the safety of patients, family members and research staff, alternative procedures can be put in place to facilitate remote assessments when preferred. However, visits where lab analyses and other face to face assessments are required, will continue to be carried out by in-person visits. This can be done onsite or by, e.g., home visits, depending on local requirements and regulations. To minimize the time of the visit to the hospital, interviews corresponding to these visits can be done via secured/end to end encrypted videoconference. Interviews will be preferably performed via videoconference facilities, but if video-conference is not possible, these can be done via telephone.

Specifically, for UHR subjects, this means that (at least) the following assessments are done face to face:

- V1: CAARMS, WISC/WAIS and blood tests.
- V2: Urinary drug screening and MRI session. Considering that visit 1 and 2 may be combined and in order to minimize the number of visits to the hospital, the urinary drug screening and MRI corresponding with visit 2 should preferably be carried out on the same day as the face to face assessments corresponding with visit 1.
- V5: CAARMS, blood tests, urinary drug screening.
- V8: CAARMS, WISC/WAIS, blood tests and urinary drug screening.

In HC subjects, at least the following assessments are carried out by in-person visits:

- V1: CAARMS, WISC/WAIS, urinary drug screening and, if possible, MRI.
- V3: CAARMS, WISC/WAIS and urinary drug screening.

Urinary drug screening may be skipped during intermediate visits (i.e. visit 4 and 6 for UHR subjects and visit 2 for healthy controls) but this should be documented as a protocol deviation. Physical examination (i.e. heart rate, blood pressure, weight, height and abdominal circumference) can be reported to the research team on the phone by, for example, using equipment at home or taking the patient to a local pharmacy. Self-report questionnaires can also be completed at home.

Medication can be delivered via courier from trial sites/hospital pharmacies to trial participants, provided that data protection rules are followed and every effort is made to maintain the medication at the adequate temperature during transit. If a temperature deviation is expected, the sponsor should be notified. Documentation of receipt by the trial participants should be filed. If a medication compliance check is due but the study visit is not carried out in person,

participants should do a pill count themselves and report the result to the research team on the phone. Participants should retain unused/empty medication bottles (and, e.g., return them to the investigator when they next have a visit to the investigator site), so that complete drug accountability can be guaranteed.

## 4. STUDY POPULATION

### 4.1 Population

A sample of 220 UHR subjects (110 in each group) will be recruited (refer to paragraph 4.4 for power analysis). Participants will be recruited at institutions situated in Europe and Israel, which are highly experienced in providing healthcare to UHR subjects, and have excellent research facilities. Due to the naturalistic design of this study, it is expected that the majority of the subjects treated by the mental health teams meet the in- and exclusion criteria stated below.

In addition, a limited number of participating centres will recruit a sample consisting of healthy controls (N=110) that is not subject to an intervention. Healthy control subjects will be recruited at the following institutions: University of Tübingen, University Medical Center Utrecht, Hospital Clinic de Barcelona, Hospital General Universitario Gregorio Marañon (Madrid) and ZKJP University Zürich. This will result in a group of healthy controls that is representative for the entire consortium. The following sites will serve as back-up centres for the recruitment of healthy controls: University Hospital Jena and Sant Joan de Deu - Hospital Infantil (Barcelona). These sites will only be called upon by the sponsor if the recruitment rate of healthy controls is not deemed to be satisfactory during the course of the study. Controls will be recruited by advertisement and flyers, and will be matched with UHR subjects for age, gender and socioeconomic status of parents.

Recruitment will last for four years after inclusion of the first subject. The number of sites, duration of recruitment, and estimated site recruitment rates are deemed sufficient to attain the required number of UHR subjects and healthy controls. Moreover, these aspects are expected to be comparable to previous European multicentre studies such as EU-GEI (http://www.eu-gei.eu), which have successfully recruited hundreds of UHR subjects over 2-4 years.

### 4.2 In- and exclusion criteria UHR subjects

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Age 13 - 20 (inclusive)

- Written informed consent of the subject. For individuals below the age of legal capacity the parents / legal representatives need to give consent, and the subject can provide assent (whether the latter is required depends on local laws and regulations).
- UHR diagnosis as made using the Comprehensive Assessment of At-Risk Mental States (CAARMS) (Yung et al., 2005). Subjects have to meet one or more of the following criteria: (a) attenuated psychotic symptoms, (b) brief limited intermittent psychotic symptoms (a history of one or more episodes of frank psychotic symptoms that resolved spontaneously within 1 week in the past year), or (c) either the presence of schizotypal personality disorder or a family history of psychosis in a first-degree relative, all three together with a recent decline in function.

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Any clinically significant medical condition that may influence the results of the trial or affect the ability to take part in a trial.
- Laboratory screening values considered clinically relevant by a medical doctor for transaminases, thyroid hormones or coagulation parameters
- Current or past DSM-IV diagnosis of psychosis, as measured with K-SADS-PL
- Intake of an antipsychotic or mood-stabilising agent in the two weeks prior to study inclusion, except for situations as described in section 5.2
- Intake of an antipsychotic agent equivalent to a total haloperidol use of >50 mg in the six months prior to study inclusion, except for situations as described in section 5.2
- A first-degree relative (i.e. parents, offspring or siblings) participating in this study
- UHR diagnosis on the basis of attenuated psychotic symptoms that are entirely explained by acute intoxication
- Current aggression or dangerous behaviour (PANSS G14 score 5 or above)
- Current suicidality / self-harm (PANSS G6 score 7)
- Current DSM-IV diagnosis of alcohol or substance dependence as measured with K-SADS-PL
- Any current or previous neurological disorder, including epilepsy
- History of head injury resulting in unconsciousness lasting at least 1 hour
- IQ < 70
- More than 4 weeks of regular omega-3 supplementation (>2 daily capsules standard strength providing >600 mg combined EPA/DHA) within the last 6 months.

A potential subject with any contraindications to MRI will be excluded from MRI sessions.

### 4.3 In- and exclusion criteria healthy controls

In order to be eligible to participate in this study, healthy controls must meet all of the following criteria:

- Age 13 - 20 (inclusive)
- Written informed consent of the subject. For individuals below the age of legal capacity the parents / legal representatives need to give consent, and the subject can provide assent (whether the latter is required depends on local laws and regulations).

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Any clinically significant medical condition that may influence the results of the trial or affect the ability to take part in a trial
- Lifetime history of a DSM Axis-I psychiatric disorder, as measured with MINI-KID
- At ultra-high risk for developing psychosis as determined through CAARMS
- First-degree relative with a lifetime history of affective or non-affective psychosis (defined by treatment or diagnosis)
- Previous intake of antipsychotic medication, except for situations as described in section 5.2
- A first-degree relative (i.e. parents, offspring or siblings) participating in this study
- Current intake of psychoactive medication, regardless of the indication for use
- Any previous neurosurgery or neurological disorder, including epilepsy
- History of head injury resulting in unconsciousness lasting at least 1 hour

A potential subject with any contraindications to MRI will be excluded from MRI sessions.

### 4.4 Sample size calculation

In the only randomised, placebo-controlled trial with omega-3 fatty acids so far, 12-week daily treatment of UHR subjects resulted in an 82% reduction in transition rate to psychosis at one year after inclusion in the study (from 27.5% to 4.9%) (Amminger et al., 2010). In addition, a recent meta-analysis shows a mean transition rate to psychosis of 18% of UHR individuals at six months of follow up, 22% at one year, and 29% at two years (Fusar-Poli et al., 2012). Following these results, power analysis indicates that 170 subjects are required in order to detect a relatively conservative 70% reduction in the expected transition rate at one year of follow up, thus corresponding to a transition rate of 22% in the placebo group and 6.6% in the omega-3 group, using a two-sided log-rank test with $\alpha = 0.05$ (probability of type I error) and 80% statistical power ($\beta = 0.2$, probability of type II error) (Dupont and Plummer, 1990). Given

the long duration of both treatment and follow-up we anticipate a drop-out rate of 20 to 30%, which results in the overall required inclusion of 220 subjects in the current study. This sample size will also be largely sufficient to detect a significant improvement in both total PANSS and GAF scores with an effect size of 0.7 as reported by Amminger et al. (2010). Please note that these calculations are based on a treatment of 12 weeks, whereas our study includes six months of treatment. To be able to perform both cross-sectional and longitudinal case-control comparisons, a sample of 110 controls will be recruited to provide a group that will be of comparable size to both arms of UHR subjects.

## 5. TREATMENT OF SUBJECTS

### 5.1 Investigational product

UHR subjects will receive either 1.2 gram/day omega-3 polyunsaturated fatty acids or placebo daily, for six months (see paragraph 6.1).

### 5.2 Concomitant treatment

Use of antipsychotic medication and mood stabilizers (including but not limited to lithium and anti-epileptics) is not permitted at study entry, but is allowed during the study in case of medically required treatment (i.e. significant worsening of symptoms) with as a maximally allowed dose of antipsychotics the equivalent of a total haloperidol use of 10 mg within two months. The use of antipsychotics is also allowed if they are prescribed for sleep problems, but only when used in low doses that are non-effective for the treatment of psychotic symptoms (e.g. a very low daily dose of quetiapine) and after consultation of the sponsor. Use of antidepressants and benzodiazepines is not limited or directed by the protocol. Subjects who start using or continue using antidepressants or benzodiazepines will not be excluded from the study. Existing prescriptions of other psychiatric medication is evaluated at baseline and continued if clinically indicated. Regular omega-3 supplementation (>2 daily capsules standard strength providing >600 mg combined EPA/DHA) is not allowed for more than one week during study treatment, but is not limited or directed by the protocol after completion of study treatment. Concurrent psychological interventions such as cognitive-behavioural therapy is permitted. Both medication use and psychological interventions as well as use of at-risk services will be documented.

#### Tapering off of medication

Some UHR individuals are prescribed a low dose of antipsychotic or mood-stabilising medication. When this is the case with a potential study participant at the moment of signing

informed consent, it is allowed to gradually taper off medication, and to do all other visit 1 assessments except CAARMS at least two weeks after the last medication is taken. CAARMS will be done immediately after signing informed consent to assess if someone meets UHR criteria, which is the main inclusion criterion in this study. This procedure can only be followed if this is agreed upon by both the UHR individual and the treating physician. A potential participant will only be included in the study if none of the exclusion criteria are met two weeks after cessation of medication, including the maximum intake of an antipsychotic agent equivalent to a total haloperidol use of 50 mg in the six months prior to study inclusion (please see paragraph 4.2 'In- and exclusion criteria UHR subjects'). All assessments related to visit 1 other than signing informed consent and CAARMS need to be completed within one week.

## 6. INVESTIGATIONAL PRODUCT

### 6.1 Name and description of investigational product

The active treatment is a supplement of 0.6-g yellow gelatin capsules containing concentrated marine fish oil. The daily dose of 4 capsules provides 720 mg of eicosapentaenoic acid (EPA) (20:5n3), 480 mg of docosahexaenoic acid (DHA) (22:6n3), and 7.6 mg of mixed tocopherol (vitamin E). The daily amount of other omega-3 fatty acids (18:3n3, 18:4n3, 20:4n3, 21:5n3, and 22:5n3) provided with the study medication is 240 mg. Placebo capsules will be matched to the fish oil capsules in size and appearance; 4x 0.6-g matched capsules will be administered orally and daily for 6 months. Placebo capsules will contain a 1:1 combination of coconut oil and medium chain triglycerides because it does not contain polyunsaturated fatty acids and has no impact on omega-3 fatty acid metabolism. Placebo capsules also contain the same amount of vitamin E as the omega-3 capsules and 1% fish oil to mimic flavour and taste. Medication in the current study is consistent with that of the randomised controlled trial conducted in UHR subjects by Amminger and colleagues (2010).

### 6.2 Summary of findings from clinical studies

Omega-3 fatty acids are food supplements with important physical and psychosocial health benefits (De Caterina, 2011; Mozaffarian and Wu, 2011). Positive effects of omega-3 fatty acids were originally investigated in the context of cardiovascular disease. More than twenty-five epidemiological studies and several meta-analyses, involving a total of more than 280,000 participants, indicate that consumption of fish or fish oil (which is very high in omega-3 fatty acids) significantly reduces mortality from coronary heart disease, including fatal myocardial infarction and sudden cardiac death, in populations with and without established cardiovascular disease. Meta-analyses of large-scale randomised clinical trials also demonstrate significant reductions in coronary heart disease mortality associated with omega-3 fatty acid intake

(Mozaffarian and Rimm, 2006; Leon et al., 2008; Marik and Varon, 2009). In the context of psychosis, clinical trials with omega-3 fatty acids in schizophrenia patients show inconclusive results. Several controlled trials in people with schizophrenia have found beneficial effects of omega-3 supplementation in terms of improved psychopathology as measured with PANSS and a reduction in both the need for antipsychotic medication and associated side effects (Peet et al., 2001; Emsley et al., 2002; Berger et al., 2007), while other studies report negative findings (Fenton et al., 2001; Peet et al., 2002). Meta-analyses confirm that it is still too early to draw any conclusions on the efficacy of omega-3 fatty acids in schizophrenia (Freeman et al., 2006; Irving et al., 2011; Fusar-Poli and Berger, 2012). However, the only randomised placebo-controlled study in subjects at UHR for developing psychosis shows encouraging results (Amminger et al., 2010). In this study, 81 UHR individuals aged 13 - 25 were randomised to 12 week treatment with either daily omega-3 fatty acids or placebo, followed by a 40-week monitoring period. At the end of the study, the transition rate to psychosis was significantly lower in the fatty acid group (4.9%, 2 of 41 subjects) compared to the placebo group (27.5%, 11 of 40 subjects). In addition, treatment with omega-3 fatty acids significantly reduced psychotic symptoms (change from baseline to 12-month assessment in total PANSS score -15.7±2.8 and -4.4±2.8 after omega-3 fatty acids and placebo, respectively) and improved functioning of UHR individuals (change from baseline to 12-month assessment in GAF score 17.7±2.3 and 7.2±2.3 after omega-3 fatty acids and placebo, respectively) (Amminger et al., 2010).

### 6.3 Summary of known and potential risks and benefits

Prospective observational studies and adequately powered randomised clinical trials provide strong concordant evidence that consumption of fish or omega-3 fatty acids reduces mortality from coronary heart disease. Efficacy in the context of schizophrenia is less convincing, although treatment with omega-3 fatty acids of subjects at UHR for developing psychosis has been associated with prevention of the transition to psychosis. Omega-3 fatty acids are minimally associated with adverse effects. The most common adverse effect is a fishy aftertaste. Importantly, high omega-3 fatty acid consumption (>3g/day) has been associated with prolonged bleeding time (Kris-Etherton et al., 2002), which warrants caution with the use of supplemental omega-3 fatty acids in those who are at increased risk of excessive bleeding. For this reason, coagulation parameters are examined at screening and subjects with abnormal values are excluded from participation in the study. Altogether, international guidelines have converged on consistent recommendations for the general population to consume at least 250 mg per day of long-chain omega-3 polyunsaturated fatty acids or at least two servings per week of oily fish (Mozaffarian and Wu, 2011). For women who are or might become pregnant as well as for children, additional specific recommendations are available for minimum DHA consumption to optimise children’s brain development (FAO/WHO, 2008).

### 6.4 Description of administration and dosage

1.2 gram/day omega-3 polyunsaturated fatty acids or placebo will be given daily in four 0.6-g gelatin capsules, which includes a daily intake of 720 mg of EPA and 480 mg of DHA. This EPA:DHA ratio of 3:2 has been proven safe and effective in the clinical trial of Amminger and colleagues (2010). Please note that omega-3 fatty acids have been proven safe at much larger doses of up to 3 g/day in a total of more than 100,000 cardiovascular patients (De Caterina, 2011; Mozaffarian and Wu, 2011).

### 6.5 Preparation and labelling of Investigational Medicinal Product (IMP)

The study medication will be provided centrally, carrying appropriate labels that adhere to European as well as local laws and regulations.

### 6.6 Drug accountability

Subjects will be asked to return any unused medication to the site. Accountability and subject adherence will be assessed by maintaining drug dispensing and return records. Adherence to the study medication will also be monitored by self-report, and the measurement of the omega-3 to omega-6 ratio in blood at screening and after six months will provide a measure of drug compliance (see paragraph 7.3.3).

A Drug Accountability Log must be kept current and should contain the following information:

- the identification of the subject to whom the medication was dispensed
- the date[s], quantity of the medication dispensed *to* the subject
- the date[s] and quantity of the medication returned *by* the subject

This inventory must be available for inspection by the monitor.

The study medication will be provided centrally. The shipping, receipt, returning and destruction will be tracked and documented in line with Good Clinical Practice (GCP) guidelines.

## 7. METHODS

### 7.1 Study parameters

#### 7.1.1 Main study parameter

The main study parameter is the rate of transition to psychosis as defined using the criteria in the Comprehensive Assessment of At-Risk Mental State (CAARMS) (Yung et al., 2005).

### 7.1.2 Secondary study parameters

Secondary study parameters include 1) discontinuation rate and tolerability associated with omega-3 fatty acid treatment, 2) symptomatology as examined using semi-structured interviews and questionnaires such as the Positive and Negative Syndrome Scale (PANSS), Clinical Global Impression Scale (CGI) and Beck's Depression Inventory (BDI), 3) psychosocial functioning as assessed using the Global Assessment of Functioning scale (GAF), Social and Occupational Functioning Assessment Scale (SOFAS), Global Functioning Role (GF:R) and Global Functioning Social (GF:S), 4) cognitive function as examined using subscales of the Wechsler's Intelligence Scale, 5) MRI measures including structural MRI, resting state functional MRI, Diffusion Tensor Imaging and functional MRI during reward processing, and 6) blood levels of bioactive lipids, (epi)genetic markers and immune parameters.

### 7.1.3 Other study parameters

Other study parameters include sociodemographics, medical history, physical health, current medication use, recent psychiatric history, psychiatric disorders in first-degree relatives, use of drugs of abuse, number and type of psychological interventions, and use of at-risk services. Handedness (Edinburgh Handedness Inventory), pubertal status (Tanner criteria), behavioural, social and physical functioning (Youth Self Report / Child Behaviour Checklist), resilience (Connor-Davidson Resilience Scale), perceived stress (Perceived Stress Scale) quality of life (KIDScreen-27) and drug adherence (Medication Adherence Questionnaire) will be assessed with self-report questionnaires. Current and past episodes of psychopathology will be determined using the Kiddie - Schedule for Affective Disorders and Schizophrenia - Present and Lifetime Version (K-SADS-PL) in UHR subjects and with the MINI International Neuropsychiatric Interview for Children and Adolescents (MINI-KID) in healthy controls.

## 7.2 Randomisation, blinding and treatment allocation

Randomisation will take place at a 1:1 into two treatment groups, using computer generated randomisation which will be stratified for site and gender. The data manager who performs the blinding holds the key to the randomisation. Researchers and subjects will not be informed until the total clinical trial is completed and the database is locked.

### 7.2.1 Unblinding procedures

Unblinding of study medication before the end of the study may only occur on an individual basis if the information can help treat a (Serious) Adverse Event and for safety reasons. The decision to unblind is at the discretion of the investigator. For this purpose, a set of sealed envelopes comprising the information on the type of medication stored in every medication box used in the trial will be sent to the pharmacy at the UMC Utrecht, where the central study team

is based. This pharmacy will provide a 24/7 backup emergency unblinding service. In the case of a medical emergency situation as described below, the envelope can be opened and the treatment assignment of a subject will be unblinded. This subject will be excluded from subsequent statistical analyses.

Emergency unblinding is indicated in the following situations only:

1. unblinding is necessary for the subjects emergency treatment at the investigator's discretion.
2. unblinding is required by local laws or regulations (in case of Suspected Unexpected Serious Adverse Reaction).
3. the Data Safety Monitoring Board decides that unblinding is necessary for proper study management of the subjects and the overall safety of the other subjects in the study.

After closing of the database every site will be informed about each subject's assigned treatment group.

### 7.3 Study procedures

#### 7.3.1 Interviews and questionnaires

The following interviews, questionnaires and scales will be applied:

- Edinburgh Handedness Inventory (EHI; Oldfield, 1971)
  Four-item questionnaire to determine handedness.
- Tanner Criteria (Marshall and Tanner, 1969; 1970)
  Self-report form on pubertal status.
- Youth Self Report / Child Behaviour Checklist (YSR/CBCL; Achenbach, 1991)
  The YSR is a self-report questionnaire for adolescents with age above twelve years. It consists of 113 items which are rated on a three-point scale, grouped into nine syndrome scales of behavioural, social and physical functioning. Age- and gender-adjusted norm scores are available to determine the clinical range of psychopathology. The CBCL is a similar questionnaire to be filled in by the caregiver of a child.
- Beck's Depression Inventory (BDI; Beck et al., 1996)
  21-question multiple-choice self-report inventory to measure severity of depressive symptoms.
- Connor-Davidson Resilience Scale (Connor and Davidson, 2003)
  25-item self-report questionnaire to assess resilience.
- Perceived Stress Scale (Cohen et al., 1983)
  10-item self-report questionnaire measure the perception of stress.

- KIDscreen-27 (Ravens-Sieberer et al., 2005)
  Self-report questionnaire to measure quality of life in ten dimensions in kids and adolescents.
- Kiddie - Schedule for Affective Disorders and Schizophrenia - Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997)
  An interviewer-oriented diagnostic interview designed to assess current and past DSM-IV symptoms resulting in diagnoses in children and adolescents, by interviewing the parent(s) and child separately.
- Comprehensive Assessment of At-Risk Mental State (CAARMS; Yung et al., 2005)
  Semi-structured interview; only the 4-item rating scale on positive symptoms will be used to assess UHR diagnosis and transition to psychosis.
- Positive and Negative Syndrome Scale (PANSS; Kay et al., 1987)
  Semi-structured interview; a 30-item rating scale designed to measure severity of psychopathology.
- Global Assessment of Functioning (GAF; Hall, 1995)
  Scale used to indicate psychosocial function, defined as a score between 0 and 100.
- Social and Occupational Functioning Assessment Scale (SOFAS; Goldman et al., 1992)
  The SOFAS scale is similar to the GAF but looks at social and occupational functioning rather than symptoms.
- Global Functioning Social (GF:S; Auther et al., 2006) and Global Functioning Role (GF:R; Niendam et al., 2006)
  Scales used to assess age-appropriate social contacts and interactions outside the family (GF:S), and demands of the role, level of independence or support provided to the individual and the individual's overall performance in the role given the level of support (GF:R). Scores for both scales range between 1 and 10, with 10 indicating superior functioning and 1 representing severe dysfunction.
- Clinical Global Impression Scale (CGI; Guy, 1976)
  Three-item observer-rated scale that measures illness severity, global improvement or change and therapeutic response.
- Young Mania Rating Scale (YMRS; Young et al., 1978)
  11-item rating scale to measure severity of manic episodes.
- WHO Assist 3.0 (WHO ASSIST Working Group, 2002)
  Interview about substance and drug abuse.
- Medication Adherence Questionnaire (Morisky et al., 1986)
  4-item rating scale to measure drug adherence.
- MINI-KID (Sheehan et al., 2010)

A short structured diagnostic interview to assess current and past psychiatric disorders in children and adolescents.

### 7.3.2 Cognition

Cognition will be assessed at screening and at 2 years after start of study medication. For adolescents aged fifteen and under, cognitive function will be measured using subscales of the Wechsler's Intelligence Scale for Children (WISC-III; Wechsler, 1991; WISC-IV; Wechsler, 2003; WISC-V; Wechsler, 2014), whereas for adolescents aged sixteen and over Wechsler's Adult Intelligence Scale (WAIS-III; Wechsler, 1997; WAIS-IV; Wechsler, 2008) will be used. These measures have been commonly used and validated for the respective age groups, and will also provide an IQ estimate. Total duration is approximately 40 minutes. If a participant is 15 years old at baseline, then the WISC will be administered both at baseline and at 2-year follow-up. If a participant has completed the WISC/WAIS before, these data may be used if the cognitive assessment has taken place not longer than three months before inclusion in the study.

### 7.3.3 Biological blood markers

Blood sampling procedures are described in more detail in the separate Standard Operating Procedure ‘Blood Sampling’.

Blood samples will be drawn from UHR subjects only at screening, and at 6 months and 2 years after treatment initiation to assess levels of bioactive lipids, (epi)genetic markers and immune parameters. Levels of bioactive lipids that are determined include those of omega-3, 6 and 9 fatty acids. These are of interest as 1) assessment of the omega-3 to omega-6 ratio provides a measure of drug compliance, and 2) changes in membrane fatty acid profiles have been implicated in both the beneficial effect of omega-3 fatty acid treatment in UHR subjects as well as in the transition to psychosis (Smesny et al., 2014). Genetic and epigenetic markers of interest include but are not restricted to genes coding for catechol-O-methyltransferase (COMT), dopamine D2 receptor (DRD2), metabotropic glutamate mGlu3 receptor (mGluR3), glutamic acid decarboxylase67 (GAD1) and reelin (RELN). These genetic loci have been identified in large-scale genome-wide association studies as genetic risk factors for the development of schizophrenia (Ripke et al., 2014), are substantially implicated in therapeutic targets for antipsychotic medication (Davis et al., 1991; Wassef et al., 2003; Moghaddam and Javitt, 2012), and explain a significant part of variation in brain structure and function of schizophrenia patients (Addington et al., 2005; Meyer-Lindenberg et al., 2006; Ohnishi et al.,

2006; Bertolino et al., 2009). Immune parameters that are assessed include but are not restricted to interferon-γ, interleukin (IL)-1α, IL-1RA, IL-6, IL-10, IL-12, IL-15, tumour necrosis factor-α, c-reactive protein, S100B, leukocytes and monocytes. Altered levels of these parameters have been shown in schizophrenia patients, and may predict both the course of the illness and response to antipsychotics (Miller et al., 2011; Schwarz et al., 2012; de Witte et al., 2014). In the current study, it will be examined 1) how levels of bioactive lipids, (epi)genetic markers and immune parameters are related to both the beneficial effect of omega-3 fatty acid treatment and the transition to psychosis, and 2) if these blood parameters are able to serve as potential biomarkers that may predict clinical response of UHR subjects and/or the transition to psychosis.

Blood samples for bioactive lipids will be sent every eighteen months to the lab of Dr Vaz at the Academic Medical Center in Amsterdam, the Netherlands. Blood samples for genetic and immune analyses will be sent to the Rudolf Magnus Brain Center, Department of Psychiatry, University Medical Center Utrecht, the Netherlands. Each site will send the samples for genetics and immune analyses after the last visit of the last patient has been performed locally. These.blood samples will be stored in a -80°C freezer of the Central Biobank of the University Medical Center Utrecht. Since multiple studies are currently investigating biomarkers in the development of schizophrenia, bloods will be stored after the end of the study to be able to measure potential novel biomarkers.

Over the course of the entire study, a total amount of approximately 75 ml blood will be drawn: 1x 3 ml to perform standard clinical laboratory tests at screening (this amount may vary depending on requirements of local labs), 3x 4 ml for bioactive lipids, 3x 10 ml for (epi)genetic markers, and 3x 10 ml for immune markers. Blood sampling must be done for all UHR subjects at screening as this information is required to determine eligibility. The aim is to collect all further blood samples for all UHR subjects, however if subjects do not want to have subsequent blood samples drawn, they can still participate in the study.

### 7.3.4 Magnetic Resonance Imaging (MRI)

MRI procedures are described in more detail in the separate Standard Operating Procedure ‘Magnetic Resonance Imaging (MRI)’.

Subjects will undergo an MRI session of at maximum sixty minutes at baseline. This includes structural MRI, diffusion tensor imaging (DTI), resting state functional MRI, and functional MRI during reward processing. Alterations in these MRI measures have been shown in both UHR

subjects and schizophrenia patients, and appear to be associated with the later onset of psychosis (see for reviews Smieskova et al., 2010; Fusar-Poli et al., 2013). For example, in UHR subjects who later developed a psychotic episode, significant baseline grey matter volume reductions have been shown in the temporal and prefrontal cortex compared to those who did not transition to psychosis (Pantelis et al., 2003; Fusar-Poli et al., 2011; Mechelli et al., 2011). In addition, using DTI, a progressive reduction in white matter integrity in the left frontal cortex was observed in UHR subjects who developed psychosis, which was not evident in UHR subjects who did not make a transition (Carletti et al., 2012). Finally, abnormalities in brain activity patterns underlying reward processing have been shown in UHR subjects (Juckel et al., 2012), schizophrenia patients (Schlagenhauf et al., 2009; Nielsen et al., 2012), unaffected siblings of patients (De Leeuw et al., 2015) and adolescent offspring of patients (Vink et al., under review). In the current study, it will be examined 1) if MRI measures are able to serve as potential biomarkers that may predict clinical response of UHR subjects and/or the transition to psychosis, and 2) how MRI measures differ between UHR subjects and healthy controls. The aim is to obtain MRI scans of all subjects, however if subjects do not want to be scanned, they can still participate in the study.

*Image acquisition and storage*

Data will be collected on 3.0 Tesla MRI scanners using standardised scan sequences that have been previously employed in other European multicentre studies such as OPTiMiSE (http://www.optimisetrial.eu) and EU-GEI (http://www.eu-gei.eu). Parameters of structural images are based on those of the Alzheimer's Disease Neuroimaging Initiative (ADNI), which have been specifically developed for multi-centre imaging studies, and can be run on recent Siemens, Phillips and GE scanners (for full details see http://adni.loni.usc.edu/methods/mri-analysis/mri-acquisition). Data will be archived by each site according to existing local procedures, and will also be transferred (using the DICOM protocol supported by all scanners) to a project-specific computer, where, following basic consistency checks, it will be pseudonymised for transfer to the coordinating site using a central computer system. Data will be stored centrally by Brain Center Rudolf Magnus, University Medical Center Utrecht.

*Standardisation of data quality across sites*

Before the start of the study, sites will be requested to scan both a phantom and a healthy volunteer with the scan protocol, and to send the data to Brain Center Rudolf Magnus, University Medical Center Utrecht, for quality control. Data will be analysed locally (to allow a rapid 'turn round' if any issue is uncovered requiring servicing of the scanner), and also transferred to the coordinating site (UMC Utrecht, the Netherlands).

### 7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences.

Reasons to terminate a subject's participation:

- A subject withdraws her/his consent
- Intolerance to the study drug
- More than one week of regular omega-3 supplementation (>2 daily capsules standard strength providing >600 mg combined EPA/DHA) during study treatment
- The investigator considers a subject's continued participation in the study to be unjustifiable on medical grounds (i.e. because of side effects or unusual risks).

### 7.5 Replacement of individual subjects after withdrawal

As an expected drop-out rate of 20-30% has been taken into account in the sample size calculation, subjects that drop out will not be replaced.

### 7.6 Follow-up of subjects withdrawn from treatment

When an individual subject is discontinued due to one of the reasons mentioned in paragraph 7.4, this subject will be treated as usual in normal daily practice. If a subject drops out, the investigator should make an effort to perform an Early Termination Visit, which includes all measures as described for visit 8 (2 years after start of study medication). An Early Termination Visit will be conducted as soon as possible after dropout, but only if there are no direct or indirect signs of objection from the subject.

### 7.7 Termination of participating study centre

Reasons to terminate participation of a study centre:

- Inability to conduct the study in accordance with protocol at a particular study site, to maintain agreements made with the study sponsor, or to meet guidelines of International Conference on Harmonisation - Good Clinical Practice (ICH-GCP).
- Insufficient inclusions, i.e. <5 subjects included per year.

If a study centre discontinues the study due to one of the above-mentioned reasons, the subjects that have already been included will be followed up, but no new subjects will be included.

### 7.8 Premature termination of the study

The study may be prematurely terminated in case the approval conditions and/or risk profile of the investigational medicinal product change, making a new assessment of the use of the drug in this study necessary. In case the entire study is discontinued, subjects will be followed up and/or be treated as usual in normal daily practice, depending on the severity of the changes in risk profile and the extent to which this increase in risk is applicable for individual subjects.

## 8. SAFETY REPORTING

Safety aspects are described in more detail in the separate Standard Operating Procedure 'Safety Review and Reporting Procedure'.

### 8.1 General aspects

This study will be performed according to the Declaration of Helsinki (2013) and ICH-GCP**.** The definitions of adverse events and serious adverse events described in these guidelines will be used for the present study.

Medication to be administered in this study is a nutritional food supplement with important physical health benefits and is minimally associated with adverse effects. The investigator will inform the subjects and the reviewing accredited Independent Ethics Committee if any new relevant safety information becomes available, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the Independent Ethics Committee, except insofar as suspension would jeopardise the subjects' health. The investigator will ensure that all subjects are kept informed.

### 8.2 Definitions

Adverse Events (AE) are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational drug.

A Serious Adverse Event (SAE) is any untoward medical occurrence or effect that results in:

- death
- is life threatening (at the time of the event)
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect

An event qualifies as a Suspected Unexpected Serious Adverse Reaction (SUSAR) when:

- the criteria for an SAE are met
- it is plausible that the event is caused by the study medication
- the event is unexpected, meaning that the nature, or severity, is not consistent with the applicable product information (i.e. Summary of Product Characteristics).

**8.3 Reporting procedures**

All AEs reported spontaneously by the subject or observed by the investigator or his staff will be recorded in the electronic case record form (eCRF) by the investigator. Any SAE must be reported to the sponsor/coordinator (UMC Utrecht, the Netherlands) regardless of their estimated relatedness to the study drug. SAEs should be reported by filling out the SAE form in the eCRF within 24 hours after the investigator became aware of an SAE. By means of the eCRF, an email alert will be sent immediately to the UMC Utrecht team, enabling the reporting procedure to the regulatory authorities. When the eCRF system does not function, reporting must be performed by emailing the completed SAE form to the trial assistant (purpose@umcutrecht.nl). Should the investigator become aware of an SAE that occurs within 30 days after stopping the study treatment, the SAE must be reported in accordance with procedures specified above. If a reported SAE is fatal or life threatening, all participating centres as well as the regulatory authorities will be notified within 7 days after the investigator at the centre where the event occurred became aware of the event. Otherwise, the SAE will be reported to the centres and authorities within 15 days. SUSARs will be reported using the same procedure and timelines as SAEs described above. In case of a SUSAR, treatment will be unblinded in line with the Clinical Trial Directive. However, the unblinding can be limited to a designated member of the central study team as well as local study team; the extent of communication of the treatment allocation will be determined on a case by case basis.

Any information that is needed to adhere to local laws and regulations of participating countries regarding the reporting of SAEs/SUSARs will be provided by the trial coordinator and/or the project manager to the Country Coordinator and local Principal Investigator. All SAEs and SUSARs will be reported by the central study team to the appropriate Dutch authorities, in accordance with local laws and regulations. SAE/SUSAR reporting to appropriate national authorities is delegated to the Country Coordinator / investigator sites as these investigators are fully aware of their local laws and regulations. SAEs cannot be reported centrally on a European level, so each Country Coordinator / investigator needs to ensure that local laws and regulations are adhered to in case an SAE occurs in their centre, that is: the independent ethics committee or other national authorities may need notification. The central study team is able to report SUSARs on a European level, but nevertheless investigators need to make sure that any

additional local/national law/regulation is adhered to. All information regarding SAEs, SUSARs and the reporting of these events will be filed in the central study file as well as the investigator site files.

### 8.3.1 Annual safety report

A list of all SAEs and SUSARs (composed by the central study team, submitted to national authorities by the local investigators) will be provided to the appropriate authorities. This includes a summary table arranged by organ systems and a review of all new scientific data as well as new safety signals. If possible (if timing of the meeting and Annual Safety and Progress Report coincide), a cost-benefit evaluation performed by the Data Safety Monitoring Board is included, based on a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

## 8.4 Follow-up of adverse events

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

## 8.5 Data Safety Monitoring Board

The safety and integrity of the study will be judged on a regular basis by an independent committee of experts, at a frequency of at least once a year. The members of this board will have access to all SAEs and SUSARs and to the inclusion and drop-out rates. This Data Safety Monitoring Board (DSMB) is authorised to suggest changes to the protocol or to provide an altered judgement of feasibility when information from the annual safety report or new information about the applied study medication has become available. If deemed necessary for their evaluation, the DSMB is allowed to review unblinded data. The central study team members will not be exposed to these data. Composition of the DSMB as well as roles, responsibilities and criteria for premature termination of the trial are described in a separate DSMB Charter.

# 9. STATISTICAL ANALYSIS

The primary intent of this randomised controlled trial is to compare the rate of transition to psychosis between individuals who are at UHR for developing psychosis and randomised to omega-3 fatty acids to those randomised to placebo. Hence the null hypothesis is that the transition rate in the omega-3 fatty acids arm does not differ from the rate in the placebo arm.

### 9.1 General statistical considerations

Descriptive statistics will be provided for all data broken down by medication (omega-3 fatty acids and placebo), group (UHR subjects who made a transition to psychosis, UHR subjects who did not make a transition to psychosis, and healthy controls), visit and country. Mean, median, standard deviation, range and number of observations will describe continuous variables. Frequencies and percentages will describe discrete variables. All statistical tests will be carried out two-tailed; the alpha (level of significance) is 5%. Continuous variables will be tested with one-way analysis of variance (Student's-t-test for pairwise comparisons) in case of a normal distribution, and Kruskal-Wallis test (Mann Whitney-U-test for pairwise comparisons) in case of a skewed distribution. Discrete variables are analysed with Chi-square or Fisher's exact test.

### 9.2 Baseline comparisons

The randomisation procedure will be evaluated by a comparison of the two treatment groups for all relevant variables recorded at baseline.

### 9.3 Primary analyses

The primary objective of this study will be examined with survival analyses, both Kaplan Meier survival analysis and uni- and multivariate Cox proportional-hazards regression analysis, to estimate differences of transition probabilities during 2 years of follow-up between the omega-3 fatty acids arm and the placebo arm. Differences will be expressed in Hazard Ratios (HRs), with corresponding 95% Confidence Intervals (CIs). Starting point is the first administration of medication at the end of visit 2. Endpoint is the moment that a UHR subject makes a transition to psychosis according to the CAARMS criteria.

### 9.4 Secondary analyses

Differences between the two treatment arms in discontinuation rate will be analysed with both Kaplan Meier survival analysis and uni- and multivariate Cox proportional-hazards regression analysis. Continuous safety and tolerability outcomes will be assessed using linear regression; dichotomous safety and tolerability outcome measures will be studied using Poisson regression analyses. Longitudinal effects are statistically analysed using repeated measures ANOVA with time (visits) as within-subjects and group (UHRs-transitioned, UHRs-non-transitioned and/or healthy controls) as between-subjects factor. Cross-sectional differences are examined using an independent sample t test (two groups) or one-way ANOVA (three groups) in case of a normal distribution, and Mann Whitney-U-test (two groups) or Kruskal-Wallis test (three groups) in case of a skew distribution.

### 9.5 Dropout rates and missing values

The expected drop-out rate is 20-30%, which is taken into account in the sample size calculation. In case of missing data on questionnaires or other measures, we will use the multiple imputation method as described by Rubin (1987;1996) using the Software „SOLAS for missing data analysis“ (Version 3.0), or an equivalent method according to the appropriate standards at the time of analyses.

## 10. ETHICAL CONSIDERATIONS

### 10.1 Regulation statement

The study will be conducted in accordance to the Declaration of Helsinki (2013), ICH-GCP, and other applicable (national) laws and regulations. In line with these guidelines, insurance is arranged by the sponsor for all participating sites if this is required by their local laws and regulations and the participating centre does not have a participant insurance which can be used for this purpose. All investigators involved in the present study will receive a copy of the GCP guidelines, and extensive training on the study protocol, procedures and GCP will be provided during the investigators meeting at the beginning of the study and again during site initiation. Every investigator involved in the study is responsible for compliance with protocol, ICH-GCP guidelines, and other existing (local) laws and regulations.

Before study start, the principal investigator of each participating site will submit a complete set of relevant documentation to the respective independent ethics committee and competent authority for approval, in line with the national / local regulations. When multiple sites within one country participate, a Country Coordinator will be appointed, who will be responsible for obtaining regulatory approval on a national level. The study can commence once all documents have been evaluated by the ethics committee / competent authority, and have been approved without reservations. A written confirmation of the unreserved acceptance of the proposal under consideration of the presented documents must be present. Over the study’s course, the principal investigator of each participating site is also responsible for informing the ethics committee, and any other regulatory agency if applicable, on the progress of the study and changes in the protocol or other study-related documentation according to the local regulations.

### 10.2 Recruitment and consent

Recruitment will last for four years after inclusion of the first subject. The number of sites, duration of recruitment, and estimated site recruitment rates are deemed sufficient to attain the required number of UHR subjects and healthy controls. Moreover, these aspects are comparable to previous European multicentre studies such as EU-GEI (http://www.eu-gei.eu),

which have successfully recruited hundreds of UHR subjects over 2-4 years. A previous randomised placebo-controlled study with omega-3 fatty acid treatment that was conducted in one research centre successfully recruited 81 UHR subjects over three years (Amminger et al., 2010).

UHR subjects will be recruited from mental health units. Due to the naturalistic design of this study, it is expected that the majority of the subjects treated by these mental health teams satisfy the inclusion and exclusion criteria. Healthy controls will be recruited by advertisement, and will be matched with UHR subjects for age, gender and socioeconomic status. Participation in the study is preceded by counselling by a doctor or investigator in accordance with ICH-GCP guidelines, during which the subject must be informed about the entire course of the study, potential individual benefits and personal risk. Here it will be re-emphasised that participation is absolutely voluntary. Subjects are given sufficient time to read all the provided information (approved by the appropriate authorities), counsel partners or relatives, and clarify any questions with the investigator. Regarding data privacy, subjects will be informed about pseudonymous recording and sharing of data in accordance with the requirements for documentation and information (discussed in more detail in section 11.1). In case subjects cannot agree to this central collection of data they cannot participate in the study. Participation only becomes possible after the subject as well as the investigator/doctor have signed the informed consent document. This consent can be revoked at any time without citing reasons and without any consequences. No examinations or other activities will take place before the informed consent procedure is completed. A copy of the consent form and the patient information sheet will be given to the participant.

In case the patient information sheet or the consent form change, participants will be informed immediately and relevant information will be passed on to the ethic committees for approval. New patient information and consent will be discussed in detail again, the participant will again be asked for written consent, and a copy of the documents will be given to the subject.

### 10.3 Consent procedures in minors

For minors, informed consent should be provided by their legal representatives/parents, in line with the Clinical Trial Directive, the Declaration of Helsinki and ICH-GCP. Their consent must represent the minor's presumed will and may be revoked at any time, without detriment to the minor. Whenever appropriate, the minor should participate in the informed consent process together with the parents. If the minor is deemed to be able to give assent to decisions about participation in research, the researcher will obtain this assent in addition to the consent of the

legal representatives/parents. If the minor's assent is not obtained, it is recommended that this is documented with justification in the consent form which is signed by the legal representatives/parents and investigator. The minor's assent is not sufficient to allow participation in the study; informed consent of the legal representatives/parents is required. Consent from legal representatives/parents and assent from the minor should be sought at the same time. In any case, the minor will receive information according to its capacity of understanding, from staff with experience in minors, regarding the study, its risks and benefits. The explicit wish of a minor who is capable of forming an opinion and assessing this information to refuse participation will be followed; in such case, the minor can be withdrawn from the study at any time. If a minor subject does not want to participate, the minor will not be included in the study. This is also explicitly stated in the patient information sheet.

In case a minor reaches adulthood (i.e., age of 16 or 18, depending on local laws and regulations) during the study, the researcher is obliged to obtain informed consent from this participant as soon as possible. Informed consent from legal representatives/parent is no longer required, although it is recognised that an adolescent is still vulnerable and may require additional discussions and explanations.

In case the above described procedures are not in line with any applicable local law or regulation, any deviations needs to be discussed and agreed upon with the sponsor, as well as clearly documented. In general, the most strict procedure will be upheld.

**10.4 Risk-benefit assessment**

Participation in this trial implies that subjects contribute to the development of medical knowledge of which they and other individuals could benefit. An advantage of participation could be that subjects will be more thoroughly followed and examined, and that therefore the progress of current attenuated symptoms and/or decrease in function are better measured compared to normal practice, where these subjects are not systematically seen by healthcare professionals in this at-risk stage. Participation in a randomised clinical trial implies that there is a risk of side effects. However, omega-3 fatty acids are food supplements with important physical health benefits and are minimally associated with adverse effects. They have been proven safe at much larger doses than used in the current trial in a total of more than 100,000 cardiovascular patients. In addition, assessments (with the exception of drawing blood) are essentially non-invasive, and standard procedures are followed, including an extensive screening for MRI eligibility. Although there are a substantial number of assessments, it is allowed to complete assessments of each visit in multiple days and participants will be offered frequent breaks. Hence, both risks and burden associated with participation are deemed minimal. Individuals

younger than eighteen years of age (≥13) can be included in this study, as attenuated symptoms of psychosis typically emerge in adolescence and young adulthood.

### 10.5 Compensation for injury

If required by local laws and regulations, and when it is not an option to use available insurance for research subjects from the individual participating centre, the sponsor will arrange subject insurance in line with the local laws and regulations. The participating centres are responsible for liability insurance.

### 10.6 Incentives

In line with the Clinical Trial Directive, there may not be any financial inducement to enrol a child (age <18) into the study; no financial incentive should be offered except compensation and expenses for the legal representatives/parents. For participants of 18 years or older, local study teams can decide whether or not reasonable incentives are to be provided, e.g. a monetary compensation for time spent.

## 11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

### 11.1 Handling and storage of data and documents

#### 11.1.1 Data privacy

Privacy laws and regulations will be adhered to during all procedures related to this study. The collection and processing of participants' personal information will be limited to what is necessary to insure the study's scientific practicability and to assess the research questions. Information collected about participants during this clinical investigation will be treated confidentially. The local investigator or her/his co-workers will collect data and transfer it without recording the subject's name or date of birth, but coded with a subject identification code. Therefore, data is not directly traceable to individual subjects. A subject identification code links the data to the individual subject. The code will be safeguarded by the responsible investigator at the site; the key to this code (subject identification code list) will be kept at the site, with limited access by study team members only. Data will be entered into a central database for processing and analysis. After the last subject has completed the study, (coded) source data will be stored by the participation centre or sponsor for at least 25 years, in line with GCP requirements.

Anonymous (coded) data will be relayed to the central study team for scientific analysis or made available, if necessary, to the responsible federal supervisory authority (in case of audits during the course of the study). Only qualified and authorised collaborators of the study sponsor will enter the pseudonymous data into a computerised database. The acquired data will be used without participants' names for scientific analysis. Participants' names will not be mentioned in any publication of study results. Persons monitoring the data will have access to all information needed to ensure the validity of the study data, and are required to keep information confidential as well as to respect data privacy. Participants have the right to look into their data and can check their data in accordance with the relevant judicial regulations and procedures.

### 11.1.2 Study registration

Before the start of the study all required documents will be submitted to the responsible independent ethics committees, and competent authority if necessary. The study will be registered in both the European Clinical Trials Database (EudraCT) and the Clinicaltrials.gov registry database.

### 11.1.3 Documentation of study results

Local investigators will enter the acquired data and examination results into an eCRF that is accessible via the internet. Investigators will receive personal user names and passwords for this purpose, and data will be encrypted for transfer. For each site, it will be agreed before the start of the study which documents serve as source documents for all data entered into the eCRF. The investigator must (electronically) sign that entries into the eCRF are true and complete.

For the electronic data capture, storage, monitor and export for statistical analyses, the latest version of the study software SecuTrial® will be used. SecuTrial® meets the regulatory requirements according to GCP and FDA 21 CFR Part 11 und includes among others Audit-Trial, electronic signature, programmable tests of plausibility, consistency and value range, a query management system for online monitoring and a customizable system to assign roles and authorizations. Details for the software and its functionalities are described in separate documents which can be provided on request.

### 11.1.4 Quality control of data acquisition

Study sites and associated investigators will be carefully selected and comprehensively informed and trained regarding GCP, all study procedures and the required examinations and documentation before the start of the study. The quality of data acquisition will be ensured by regular monitoring visits and the continuous availability of the monitor and trial-coordinator for consultation. After data have been submitted to the study centre, another thorough inspection of the completeness and plausibility of entries will be conducted. If needed, questions for clarification will be addressed to the sites. Only after all questions regarding data quality have been answered, the database will be locked.

### 11.1.5 Archiving of study documents

In accordance with national laws and guidelines and the specifications of the ICH-GCP guidelines, the investigators from participating sites and the coordinating centre are obligated to archive all documents pertaining to the study for 25 years after the last subject has completed or discontinued from the study.

### 11.2 Monitoring and Quality Assurance

The project manager/monitor, located at the UMC Utrecht, will perform monitoring according to national laws and guidelines and the specifications of the ICH-GCP guidelines. The project manager/monitor will visit study sites at regular intervals to monitor the execution of the study, remotely if needed. They will have access to all documents that are needed to perform their task according to the above mentioned guidelines. The project manager/monitor will check whether requirements to conduct the study are met and study procedures are followed correctly, and will check the study site’s documentation, the participants’ source data, eCRF entries, and the correct maintenance of the Investigator Site File. A separate Monitoring Manual contains an elaborate Monitoring Plan as well as an extensive monitoring checklist. Remote monitoring visits (e.g. remote source data verification or an informed consent check) may be appropriate and will always be conducted in accordance with the latest EMA guidelines. Remote actions should be proportionate based on benefit-risk considerations. The exact procedures will be decided on a case-by-case basis and at the project manager/monitor's discretion. The study sponsor or responsible regulatory authorities can audit, i.e. thoroughly investigate, a study site at any time. In this case, auditors are to be given access to all documents relevant to the study and its correct execution.

### 11.3 Amendments

Changes to the protocol will be documented in a protocol amendment and submitted to the independent ethics committees and, if necessary, national authorities according to the regulations, taking into account the distinction between substantial and non-substantial amendments.

### 11.4 Annual progress report

The sponsor/investigator will submit a summary of the study progress to the appropriate authorities following local laws and regulations. Information will be provided on the date of inclusion of the first subject, the number of subjects included in the study, the number of subjects that have completed the study, serious adverse events, any issues and amendments.

### 11.5 End of study report

The sponsor will notify the appropriate authorities of the end of the study within a period of 90 days. The end of the study is defined as the last subject's last visit. In case the study is terminated prematurely, the sponsor/investigator will notify the appropriate authorities within timelines set in local laws and regulations, including the reasons for the premature termination. Within one year after the end of the study, the sponsor/investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the appropriate authorities if required by local laws and regulations.

### 11.6 Public disclosure

The aim is to publish the results of the current study in scientific journals and to present them at (inter)national scientific conferences. The results can be shared by the local investigators with participating subjects after the last subject has completed the study and analyses have been performed. Individual subjects will never be identified in any report or publication.

### 11.7 Publication policy

This section describes the policy and plan for data analysis and publications, which has been derived from policies and practices utilised in other multi-centre academic studies, and which is in accordance with the principles and standards of scientific research and scholarship within the fields of biomedical research and scientific journalism. The goals of this policy are: 1) to provide for the timely, scholarly and comprehensive reporting of the data in the scientific literature; 2) to provide for the assignment of authorship and data analytic opportunities to study investigators in a manner that is equitable and supports career development; 3) to ensure that the analysis and reporting of data are consistent with regulatory agency requirements.

### 11.7.1 Administrative structure

The data analysis and publication strategy will be determined by the Steering Committee comprising the UMC Utrecht Purpose study team and the Principal Investigators of the participating research centres. All study publications must be submitted for review to the Steering Committee.

### 11.7.2 Data analyses

Data analyses will be conducted in a timely fashion in a manner that ensures the study's scientific integrity. Final analyses will be performed after all subjects have completed all study visits and queries have been solved. No interim analysis is planned. Data will be analysed first for the comprehensive sample. After the first publication of the comprehensive sample has been published, reports of individual sites' data may be developed. Data from the individual sites may be used in the development of grant applications at any time provided it doesn't compromise the study's integrity.

It is expected that there will be at least four levels of analyses and reports from the study. The first contains analyses associated with the primary objective of the protocol. The second consists of those associated with other objectives of the protocol. In the third there are analyses of variables or subvariables that are not associated with the objectives described in the protocol nor are directly derivative of them. The fourth level consists of analyses of individual site(s) data. The primary and secondary analyses will be determined by the Steering Committee.

### 11.7.3 Submission and review procedures for tertiary and quaternary analyses

Proposals for data analyses and manuscript development will be submitted to the Steering Committee for review. The Principal Investigators will coordinate this process. Each proposal will be logged in and distributed to each country coordinator. The format for the proposal will be no more than two pages and include 1) Specific Aims; 2) Hypotheses; 3) Background and Rationale; 4) Variables to be analysed including time period. Preliminarily approved proposals will be assigned for development to the proposing investigator and interested investigators from other sites. Manuscripts will be developed as per the guidelines below.

### 11.7.4 Determination of Authorship

The number and composition of co-authors will be defined based on prevailing standards of scientific scholarship. The following criteria will be utilised in a system for allocation of authorship:

- Expertise
- Source of idea for analysis or report.
- Role in the study's development and implementation.
- Role in data analyses.
- Role in writing the article.
- Study recruitment

Conditions and Limitations on Authorship:

- First overall study publication: Principal Investigators, Project Manager and country coordinators.
- Not every site will necessarily get a first author comprehensive study publication.
- Some form of rotation will be developed for authorship on comprehensive study publications.
- Executive Board will review site-specific proposals for publication.
- A time limit within which lead authorship assignments must be initiated and/or completed will be established (e.g. six months from assignment and availability of data to the production of a first draft of manuscript). After this time has elapsed lead authorship will be reassigned.

To resolve any differences or questions regarding authorship or prioritisation of data analyses the following criteria will be used:

- Scientific merit of the topic.
- Resource requirements in terms of time and effort of the request.
- Number of prior proposals and requests of the investigator.
- Number of prior first authored papers by the investigator.
- Overall contribution to the study.

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**Appendix 1: Protocol signature page Principal Investigator**

**Protocol Title**: 'Placebo-controlled trial in subjects at ultra-high risk for psychosis with omega-3 fatty acids in Europe'

**Version 7, dated December 23rd 2021**

I agree to the conditions relating to this study as set out in the above named protocol. I fully understand that any changes instituted by the Investigator(s) without previous discussion with the appropriate sponsor personnel would constitute a violation of the protocol, including any ancillary studies or procedures performed on study subjects (other than those procedures necessary for the well–being of the subjects).

I acknowledge that I have read the above named protocol and agree to carry out all of its terms in accordance with the applicable regulations and law, to follow ICH GCP guidelines for good clinical practice, to obtain the required regulatory approvals prior to implementation, to allow direct access to source documents, and agree to inspection by auditors from regulatory authorities, as required by ICH GCP.

To be signed by Principal Investigator.

| | |
|---|---|
| Full name of Principal Investigator | |
| Signature | |
| Date | |