Allegato 1

Strategic project promoted

by the Italian Medicines Agency (AIFA)

INTERCEPTOR PROJECT

ON EARLY DIAGNOSIS OF THE PRODROMAL STAGE OF ALZHEIMER DISEASE. THE PROGRESSION FROM MILD COGNITIVE IMPAIRMENT (MCI) TO DEMENTIA: THE ROLE OF BIOMARKERS IN EARLY INTERCEPTION OF PATIENTS CANDIDATE FOR PRESCRIPTION OF FUTURE DISEASE-MODIFYING DRUGS

Study Protocol

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(Version 1.0)

Sponsor: AIFA (Agenzia Italiana del Farmaco) and Ministry of Health

Principal Investigator: Paolo Maria Rossini

Coordinating Committee: Institute of Neurology, Catholic University of The Sacred Heart, Rome, (Responsible: Paolo Maria Rossini); IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia (Responsible: Stefano Cappa); IRCCS Foundation "Carlo Besta" Neurological Institute, Milan (Responsible: Fabrizio Tagliavini); National Institute of Health (Responsible: Nicola Vanacore); Associazione Italiana Malattia Alzheimer - AIMA (Responsible: Patrizia Spadin); Istituto Nazionale Ricovero e Cura Anziani – IRCCS-INRCA (Responsible: Fabrizia Lattanzio).

Study classification: This is an interventional non therapeutic clinical trial. It is a diagnostic trial which is aimed at finding new ways for early detection and diagnosis of medical conditions.

Biomarkers included in the study: MMSE; FCSRT; CSFp-tau and CSF p-tau/ABeta; (^{18}F)FDG-PET SCAN; Volumetric MRI; ApoE4; EEG.

Design: multicenter, interventional cohort study.

Number of patients in the study: 400.

Number of participating Recruiting Centres: 20 centres (identified following a public call by AIFA).

Number of Expert Centres: 4 (Institute of Neurology, Catholic University of The Sacred Heart for EEG; IRCCS Centro San Giovanni di Dio Fatebenefratelli for neuropsychological tests and volumetric MRI; IRCCS Foundation "Carlo Besta" Neurological Institute for liquoral markers and APOE4; IRCCS, Nuclear Medicine, San Raffaele Hospital, Milan for PET).

Funding: AIFA and Ministry of Health

LIST OF ABBREVIATIONS

A-beta42= Beta amyloid peptide of 42 amminoacids

AD= Alzheimer's Disease

AES= Advanced Encryption Standard it is a specification for the encryption of electronic data established by the U.S. National Institute of Standards and Technology

AIFA= Agenzia Italiana del farmaco; Italian Medicines Agency

aMCI= amnesic Mild Cognitive Impairment

ApoE= Apolipoprotein E

AEs= Adverse Events

BA= Brodmann Areas (histologic classification of cortical brain areas)

CDCD= Centro Disturbi Cognitivi e Demenza; Center for Cognitive Disorders and Dementia

CDR= Clinical Dementia Rating scale

CDMS= Clinical Data Management System

CC= Coordinating Committee

CRF= Case Report Form

CSF= Cerebro-SpinalFluid

DFR= Delayed Free Recall (neuropsychological test)

DTI= DIffusor Tension Imaging (method of MRI analysis for fiber tracts)

EC= Expert Centre

EDTA= Etilen DiamminoTetraacetic Acid

EEG= Electroencephalogram

EOAD= Early Onset Alzheimer's Disease

FCSRT= Free & Cued Selective Reminding Test

(^{18}F) FDG= (^{18}F) Fludeoxyglucose (radionuclide for PET)

FTP= Full Time Person

GANNT (diagram)= instrument for project management in memory of Henry L. Gantt

GCP= Good Clinical Practice

HIV= Human Immunodeficiency Virus

HTLM= HyperText Markup Language; it is the language to create web pages and other documents visible on a browser

Hz= Hertz (cycles/sec)

ICA= Independent component Analysis

INRCA= Istituto Nazionale Ricovero e Cura Anziani; National Institute for Hospitalization and Elderly Care

IRB= Institutional Review Board

IRCCS= Istituti di Ricovero e Cura a Carattere Scientifico; Scientific Institutes for Hospitalization and Care

IWG2= International Working Group (on Alzheimer diagnostic criteria)

LORETA= Low Resolution brain Electromagnetic Tomography

NPT= Neuro Psychological Tests

MCI= Mild congnitive Impairment

MMSE= Mini Mental State Examination

MRI= Magnetic Resonance Imaging

NIA-AA= National Institute on Aging - Alzheimer Association

PET= Positron Emission Tomography

RC= Recruiting Centre

RCT= Randomized Controlled Trial

ROI= Region of Interest

SIN-Dem= Società Italiana Neurologia - gruppo di studio Demenze; Italian Society of Neurology - Working Group on dementia

SPSS= Statistical Package for Social Science

STARD= Standards for Reporting Diagnostic Accuracy (statement developed in order to improve the standards for reporting of studies on diagnostic accuracy)

1.5-3T= 1.5-3 Tesla (intensity of the MRI magnetic field)

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SYNOPSIS

In the next 8 years a number of phase 2-3 trials will end up which utilize experimental drugs possibly *disease modifying* for Alzheimer Dementia. They target different disease stages: mild-moderate AD, 'early' AD, MCI prodromic to AD (MCI + for biomarkers heralding progression to AD), pre-symptomatic AD in pathogenetic gene mutation carriers of familiar AD forms. This dense clinical trials activity has triggered a fundamental question both from Patients and Scientific Communities and Health Authorities/Insurances: on which basis will the new drugs –if effective- be distributed to patients or at-risk population? This question mainly deals with the "MCI prodromal to AD"condition since the MCI population actually includesabout 50% of those who will progress to AD (the real "prodromic to AD" MCI form) while the remaining 50% will never convert to AD. Since the new drugs –if effective- will carry both elevated unit costs and not marginal side-effects, they should be administered selectively to those MCI with a severely high risk of conversion (i.e. 90% or more) and particularly to those with a highrisk of rapid conversion (1-2 years from diagnosis of the MCI condition).

The INTERCEPTOR project is focused on the prodromic AD condition (IWG2) or the MCI condition (NIA-AA) which form the neuropsychological point of view and is characterized by means of: cognitive questionnaire, screening test (MMSE), extended neuropsychological evaluation (incl. 2 episodic memory tests, language test, visuo-spatial abilities evaluation, behavioural scales Costa et al, 2017; Cerami et al, 2017), functional scales (Annex), full neurological examination. CDR must be 0.5. Atypical types of cinical presentations must be particularly considered (i.e. non amnesic debut) for a differental diagnosis where the role of biomarkers is pivotal.
In Italy, according to a recent study (Cohort Studies Memory in an International Consortium-COSMIC), an MCI prevalence of 5.9% has been evaluated in over 60 yrs population, with an increase of 4.5% between 60 & 69 yrs, of 5.8% between 70 & 79 yrs, and up to 7.1% in the 80 - 89 yrs range. On this epidemiological basis about 735.000 MCI cases are estimated for 2016in the resident Italian population .

Study design and methods

Multicentric non therapeutic cohort study in subjects fulfilling the MCI "core" criteria as defined by the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease (Albert et al., 2010). The study design reflect a longitudinal cohort study in which the baseline clinical and biomarkers characteristics of the enrolled MCI subjects at baseline will be compared for those classified as "AD converters" after 3.0 years of follow-up with respect to those "non converters". MCI subjects who will convert to other forms of dementia will be examined separately.

Number of MCI to enroll about 480 with a target of 400 full evaluated having about 20% of dropout. Total duration of the study 54 months.
All the medications at baseline are allowed without modifications for the whole protocol except for urgencies.
Neuropsychological follow-up tests will be carried out at 6 months intervals (total 7 from T0= baseline to T6= 42 months).
Within 60 days from T0 biomarkers must be carried out/collected including:

MMSE & DRF – FCSRT)
DNA extraction and ApoE typing
Lumbar Puncture for Beta/Tau metabolites
EEG for brain connectivity with graph theory
MRI + hippocampal volumetry
(^{18}F)FDG-PET

Endpoints

Primary Endpoint
It will be considered the conversion to Alzheimer's disease within 3.0 years after diagnosis of MCI, together with the assessment of those who remain in a stable condition and those who have a reversion to normal cognitive profile. People with MCI who convert to other forms of dementia will be considered separately. The biomarker or a set of biomarkers that can predict the conversion to Alzheimer's disease with higher accuracy will be evaluated.

Secondary Endpoints
Evaluation of cost/benefit ratio of individual and combination of biomarkers compared to their accuracy to predict progression from MCI to AD and their financial sustainability, availability in the country and non-invasiveness for patients.

Inclusion criteria:
a) age between 50 and 85 years;
b) age and education corrected Mini Mental State Examination score equal or superior to 24/30;
c) Clinical Dementia Rating (CDR) global score of 0.5;
d) concerns about cognitive modifications, expressed as subjective complaints by the subject, or by impression by a close acquaintance or an expert clinician;
e) defective performance with reference to age and education matched controls in one cognitive domain (memory, executive function, attention, language, visuospatial function): if repeated assessments are available, evidence of performance decline;
f) preserved functional autonomy: the subject remains fully independent, even if specific performamces may be slower, less efficient than usual level, with occasional errors;
g) no dementia: the cognitive modifications do not significantly hamper social function or work activities.

Exclusion criteria:
a) history of cerebrovascular disease (i.e. stroke episodes), alcohol abuse, severe medical disorders associated with cognitive impairment (organ failures, endocrine disorders, in particular thyroid disease and B12/folates deficiency); neuroimaging evidence of other potential causes of cognitive decline (e.g. subdural haematoma, malignancy etc.); chronic treatment with psychotropic drugs; women in reproductive age;
b) history of malignancy < 5 years;
c) contraindications for Magnetic Resonance Imaging (MRI): pacemaker; spinal stimulators; defibrillator; any other condition incompatible with MRI acquisition;
d) presence of spinal malformations or any other contraindications to lumbar puncture, according to the investigator's judgement;
e) HIV infection;

f) use of drugs potentially affecting cognitive function, according to the investigator's judgement;
g) subjects are not allowed to partecipate in any trial with experimental drug;

Exclusion criteria specific to lumbar puncture:
Patients who refuse to or cannot temporarily interrupt antiplatelet or anticoagulant therapy 14 days prior to sampling visit.

1. INTRODUCTION

1.1 The 3 main scenarios

There is a tremendous impetus in clinical research in the attempt of finding out a disease-modifying therapy for the various forms of dementia, namely of the Alzheimer's type dementia(Alzheimer's disease= AD). Below an outline of the 3 most frequent scenarios in daily clinical practice,which represent the respective targets for experimental therapies aiming to interfere with the natural course of the disease either blocking or slowing down its progression.

Scenario 1 - Asymptomatic subjects at high risk; this definition is presently applicable to overall healthy subjects carrying genetic mutations which are pathogenic for AD or FTD (guidelines for detailed description of high-risk subjects are fully described in SINDEM consensus paper by Bocchetta et al. 2016). The asymptomatic stage must be verified by means of a dedicated questionnaire for cognitive symptoms, of an accurate neuropsychological evaluation and a full neurological examination. The Clinical Dementia Rating scale (CDR) must be 0. In the case of familial history of dementia, a genetic counselling and testing shall be performed together with an accurate analysis of the age of onset of symptoms with the aim of establishing in advance the age for starting therapy. If the family carries patogenetic mutations, the use of biomarkers is considered useful just for follow-up, but not for diagnostic purposes. It is estimated that early onset AD cases (EOAD) represent 35 per 100.000 inhabitants between 45-64 years of age (Stevens JC et al. 2011). With this value in mind, it can be assumed that in Italy there are presently (2016) about 6000 cases with EOAD,about 50% of whichcarries mutations of the patogenic genes.

Scenario 2 – the one to which the present research protocol is targeted- Subjects with a prodromal stage of AD (IWG2) or Mild cognitive impairment (MCI) prodromic to AD (NIA-AA). MCI is a clinical and neuropsychological state in the elderly brain – an intermediate stage between normal cognition and dementia. It is mainly characterized by objective evidence of memory impairment during a neuropsychological examination which does not yet fulfill the definition of dementia. Epidemiological research suggests that amnesic MCI (aMCI) is a precursor to Alzheimer' Disease (AD), based on the high rate of progression from this state to AD. Between 50 and 60% of MCI subjects convert to dementia. The remaining individuals will either stay in the MCI condition or return to a fully normal one and never progress to dementia. In order to plan optimal and early therapeutic, organizational and rehabilitative interventions, aMCI diagnosis should be combined

with the most reliable prognosis on the likelihood and time of progression to dementia. The MCI definition requires the following: cognitive questionnaire, screening tests (MMSE), neuropsychological evaluation (including 2 tests for episodic memory, tests for language, visuo-spatial abilities and behavioural scales with appropriate normative thresholds; Costa et al. 2017; Cerami et al. 2017), functional scales, neurological examination and a CDR score of 0.5. Growing evidence suggests that early diagnosis reduces health and social costs for dementia management. Moreover, and strictly related to this research protocol, MCI prodromal to AD is becoming increasingly frequent and is the preferred target for clinical trials with potential disease-modifying experimental drugs. Early diagnosis of the MCI condition prodromal to AD can presently be achieved with a very high sensitivity and specificity by combining a number of tests (i.e. hippocampal volumetric MRI, (^{18}F)FDG-PET and lumbar puncture for CSF examination). However, due to their elevated costs, low availability and/or invasiveness, these cannot be applied to evaluate a large population sample -for instance on a national scale-. In a recent study by an international consortium (Cohort Studies Memory in an International Consortium-COSMIC - Sachdev PS et al., 2015) it was attempted to define the epidemiological boundaries of the MCI condition through a metanalysis of the published data. A prevalence of 5.9% has been estimated in population with >60 years, with an increment of the stratified age ranges from 4.5% (60 to 69 years), to 5.8% (70 to 79 years) and 7.1% (80 to 89 years). Based on such values, around 735.000 MCI subjects are estimated in the Italian population as of 2016. As aforementioned, the motivation of focusing the present research project on this type of subjects (scenario 2) relies on the growing evidence that the prodromic stage seems the most responsive to experimental disease modifying drugs (including some of those which have recently failed in the early/moderate AD stage).

Scenario 3- Patients with early AD condition which is defined by means of the following: cognitive questionnaire, screening tests (MMSE after correction for age and education, score between 21 and 25/30), neuropsychological evaluation (including 2 tests for episodic memory, tests for language, visuo-spatial abilities and behavioural scales with appropriate normative thresholds; Costa et al. 2017; Cerami et al. 2017), functional scales, neurological examination and a CDR score of 1. Epidemiological evidence indicate that in Italy there are presently about 500.000 AD cases. Although it is not easy to evaluate how many of them are in the early stage, by taking advantage of previous attempts from the CRONOS project, it can be predicted that about 60% of them – nearly 300.000 patients- are currently in an 'early' stage of disease (Vanacore et al. 2004).

1.2 Biomarkers role

The role of biomarkers in the diagnosis of dementia is becoming all the more important as the need for an 'early' diagnosis is now prominent, namely a diagnosis in pauci- or asymptomatic disease stages. Three separate research strings have been launched on PUBMED:

A) (biomarker* OR "blood" OR "serum" OR "plasma" OR "cerebrospinal" OR "cerebro-spinal" OR "csf" OR liquor* OR biops* OR bioptic* OR urine* OR saliva OR neuroimag* OR neuro-imag* OR imaging) AND ("mild neurocognitive disorder" OR "minor neurocognitive disorder" OR "mild cognitive impairment" OR "MCI") AND (predictive* OR prediction*) AND (progression OR conversion).

B) (biomarker* OR "blood" OR "serum" OR "plasma" OR "cerebrospinal" OR "cerebro-spinal" OR "csf" OR liquor* OR biops* OR bioptic* OR urine* OR saliva OR neuroimag* OR neuro-imag* OR imaging) AND ("major neurocognitive disorder" OR "mild neurocognitive disorder" OR "minor neurocognitive disorder" OR "mild cognitive impairment" OR "MCI" OR Alzheimer*) AND (diagnosis OR diagnostic*).

C) (*encephalography* OREEG) AND (alzheimer* OR "mild cognitive impairment") AND ("diagnosis" OR "diagnostic" OR "progression" OR "conversion").

The main criterion for quality selection was focussed on those biomarkers which best predict progression from MCI to AD in longitudinal studies, while cross-sectional studies were excluded. If in the review a 'pooled' estimate was not available, the studywith the larger sample was selected in order to obtain a more careful estimate with a small 95% CI . When considering the best evidence for each biomarker, Cochrane reviews were preferred (whenever available) which identify quality of the studies by means of the checklist QUADAS (Quality Assessment of Diagnostic Accuracy Studies) and STARD (Standards for Reporting Diagnostic Accuracy) . Only for EEG a single study was taken into account.

Below is the best scientific evidence on the *sensitivity* and *specificity* parameters for individual biomarkers identified with a95% CI threshold in case 400, 600 or 800 MCI subjects are enrolled.

<u>Biomarker 1: Mini-Mental State Examination</u>

Cochrane review title: "Mini-Mental State Examination (MMSE) for the detection of Alzheimer's disease and other dementias in people with mild cognitive impairment (MCI) (Arevalo-Rodriguez

et al. 2015)". The authors' conclusions underline a "Limited investigation of heterogeneity due to insufficient number of studies".

The pooled estimateis not present, thereforeit was considered the study of Xu G., et al., 2002 performed on 351 patients with MCI.

	Xu 2002 (n=351)	n=400	n=600	n=800
Sensitivity (CI95%)	0.57 (0.46; 0.68)	0.57 (0.46; 0.68)	0.57 (0.48; 0.65)	0.57 (0.50; 0.64)
Specificity (CI95%)	0.86 (0.81; 0.90)	0.86 (0.81; 0.90)	0.86 (0.82; 0.89)	0.86 (0.83; 0.88)

Biomarker 2: Delayed Free Recall and Free and Cued Selective Reminding Test

Study by Giulia Grande (Grande et al. submitted to J Neurol Neurosurg Psychiatry) on the use of Free and Cued Selective Reminding Test (FCSRT) in the conversion from MCI to dementia.

	Grande n=187	n=400	n=600	n=800
Sensitivity (CI95%)	0.81 (0.70; 0.89)	0.81 (0.74; 0.87)	0.81 (0.75; 0.86)	0.81 (0.76; 0.85)
Specificity (CI95%)	0.71 (0.62; 0.79)	0.71 (0.65; 0.76)	0.71 (0.66; 0.76)	0.71 (0.67; 0.75)

Biomarker 3: Cerebro-Spinal-Fluid (CSF) p-tau e ratio tau/ABeta

Cochrane review title: "CSF tau and the CSF tau/ABeta ratio for the diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI) (Ritchie c., et al., 2017)". The authors' conclusions undeline a "Limited investigation of heterogeneity due to insufficient number of studies. Lack of common thresholds "

The "pooled" estimate is missing; therefore, it was considered the study of Vos SJ.et al. 2013 performed on 214 patients with MCI.

	Vos 2013 (n=214)	n=400	n=600	n=800
Sensitivity	0.71 (0.61; 0.80)	0.71 (0.64; 0.78)	0.71 (0.65; 0.77)	0.71 (0.66; 0.76)

(CI95%)				
Specificity (CI95%)	0.77 (0.69; 0.84)	0.77 (0.71; 0.83)	0.77 (0.72; 0.82)	0.77 (0.73; 0.81)

Biomarker 4: (^{18}F)FDG-PET

Cochrane review title: "^{18}F-FDG PET for the early diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI) (Smailagic N. et al. 2015)". The authors' conclusions underline a "Limited investigation of heterogeneity due to insufficient number of studies. Lack of common thresholds". The pooled estimate is not present, therefore it was consideredthe study of Herholz K. et al, 2011, performed on 94 patients with MCI.

	Herholz2011 (n=94)	n=400	n=600	n=800
Sensitivity (CI95%)	0.57 (0.37; 0.75)	0.57 (0.48; 0.65)	0.57 (0.49; 0.64)	0.57 (0.50; 0.63)
Specificity (CI95%)	0.67 (0.54; 0.78)	0.67 (0.61; 0.73)	0.67 (0.62; 0.72)	0.67 (0.63; 0.71)

Biomarker 5: at least the presence of one allele APOE ε 4

Title meta-analysis of four prospective studies: "Apolipoprotein 4 allele ε4-as a significant risk factor for conversion from mild cognitive impairment to Alzheimer's disease: a meta-analysis of prospective studies (n = 458) (Fei M. et al. 2013)".

	Fei 2013 n=458	n=400	n=600	n=800
Sensitivity (CI95%)	0.52 (0.45; 0.60)	0.52 (0.44; 0.60)	0.52 (0.46; 0.59)	0.52 (0.47; 0.58)
Specificity (CI95%)	0.66 (0.59; 0.71)	0.66 (0.59; 0.71)	0.66 (0.60; 0.70)	0.66 (0.61; 0.70)

Biomarker 6: multiple six electroencephalogram (EEG) biomarkers into a diagnostic classification index

It was identified the following study "Integrative EEG biomarkers predict progression to Alzheimer's disease at the MCI stage." (Poil SS et al., 2013), which shows interesting values of sensitivity and specificity of the EEG via modern methods of analysis.

	Poil n=30	n=400	n=600	n=800
Sensitivity (CI95%)	0.87 (0.47; 1.00)	0.87 (0.80; 0.93)	0.87 (0.81; 0.92)	0.87 (0.82; 0.91)
Specificity (CI95%)	0.64 (0.41; 0.83)	0.64 (0.58; 0.69)	0.64 (0.59; 0.68)	0.64 (0.60; 0.68)

Biomarker 7: volumetric MRI

Title pooled analysis: "Imaging markers for Alzheimer's disease: which vs how. Neurology 2013; 81:487-500, (Frisoni G.B. et al., 2013)".

	Frisoni 2013 (n=416)	n=400	n=600	n=800
Sensitivity (CI95%)	0.60 (0.51; 0.68)	0.60 (0.51; 0.68)	0.60 (0.54; 0.66)	0.60 (0.55; 0.65)
Specificity (CI95%)	0.75 (0.67; 0.82)	0.75 (0.67; 0.82)	0.75 (0.70; 0.79)	0.75 (0.71; 0.79)

The present study protocol is focused on the second scenario and therefore includes the definition of prodromal AD (IWG2) or MCI (NIA-AA).

2. OBJECTIVES OF THE STUDY

Whereas scientific literature is always more abundant in contributions to the profiles of individual sensitivity and specificity biomarkers, much fewer studies have evaluated combinations of multiple markers on the same study population. Furthermore, to date no study has assessed and validated an organization model for screening large part of the population using combinations of biomarkers, in terms of feasibility of costs, distribution and operational feasibility. Indeed these are the key points that this Protocol seeks to address.

The primary aim is to identify a biomarker or a set of biomarkers able to predict with greater accuracy the conversion of MCI to Alzheimer's dementia after 2 or 3 years of follow-up. The secondary aim is to define an optimal organizational model, as regards transferability in clinical practice of the defined diagnostic path of the primary objective as well as sustainability costs, in order to identify patients eligible to prescription of antidementia drugs which are currently under experimentation by RCTs.

3. METHODS-STUDY DESIGN

3.1 Overall design

Multicentric interventional non therapeutic cohort study on a sample of 400 subjects consecutively diagnosed with MCI at the Center for Cognitive Disorders and Dementia (the Italian acronym is CDCD). The subjects shall fulfill the "core" criteria for MCI as defined by the *National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease* (Albert et al., 2011).

The MCI sample shall be recruited in the cohort study, with the aim of comparing the baseline characteristics (clinical and biomarkers) between the group progressing to AD after 2 and 3 years from diagnosis and the group remaining stable or reverting to a normal condition. The recruited MCI subjects progressing to a non-AD dementia shall be considered separately.

3.2 Population

Inclusion and exclusion criteria are reported in the following table 1.

Table 1

Inclusion criteria:

- Age between 50 and 85 years
- Age and education corrected Mini Mental State Examination score equal or higher than 24/30
- Clinical Dementia Rating (CDR) global score of 0.5

a) concerns about cognitive modifications, expressed as subjective complaints by the subject, or an an impression by a close acquaintance or an expert clinician;

b) defective performance with reference to age and education matched controls in one or more cognitive domains (memory, executive function, attention, language, visuospatial function): if repeated assessments are available, evidence of performance decline;

c) preserved functional autonomy: the subject remains fully independent, even if specific performances may be slower, less efficient than the usual level, with occasional errors;

d) no dementia: the cognitive modifications do not significantly hamper social functioning or work.

Exclusion criteria:

- History of cerebrovascular disease (i.e. stroke episodes), alcohol abuse, severe medical disorders associated to cognitive impairment (i.e. organ failures, endocrine disorders, in particular thyroid disease and B12/folates deficiency); neuroimaging evidence of other potential causes of cognitive decline (e.g. subdural haematoma, malignancy etc.); chronic treatment with psychotropic drugs; women in reproductive age

- History of malignancy < 5 years

- Contraindications for Magnetic Resonance Imaging (MRI): pacemaker; spinal stimulators; defibrillator; severe claustrophobia; any other condition incompatible with MRI acquisition

- Presence of spinal malformations or any other contraindications to lumbar puncture, according to the investigator's judgement

- HIV infection

- Use of drugs potentially affecting cognitive function, according to the investigator's judgement

- Participation in trials with experimental drugs

Exclusion criteria specific to lumbar puncture: patients who refuse to or cannot temporarily interrupt antiplatelet or anticoagulant therapy 7-14 days prior to sampling visit depending upon the type of treatment. The a) and b) criteria shall be defined using scales and neuropsychological tests, including:

a) for the assessment of subjective complaints and awareness, the battery of questionnaires developed for the INSIGHT-AD project (Cacciamani et al., 2017), which includes 6 scales administered to subjects and family members;

b) besides the screening test (MMSE), the following neuropsychological battery will be administered:

Memory:

-Free and Cued Selective reminding Italian version (Frasson et al. 2011; Clerici et al. 2017);

-Episodic Memory Score (Marra et al., 2016), a composite measure based on the score on Rey's wordlist learning test (Gainotti et al., 2001) and delayed recall of Rey's figure (Caffarra et al., 2002);

-DSM-48 (Barbeau et al., 2004);

Language:

- Screening for Aphasia in Neurodegeneration (Catricalà et al., 2017), a screening test with normative data

- Phonological and semantic fluency (Novelli et al., 1986);

Visuospatial function:

-copy of Rey's figure (Caffarra et al., 2002);

-Visual Object and Space Perception Battery (Warrington e James, 1991);

Executive function and behaviour :

-Trail Making Test (Giovagnoli et al., 1996);

-Stroop colour-word test (Caffarra et al., 2002);

-Frontal Assessment Battery (Appollonio et al., 2005);

-Neuropsychiatric Inventory (Binetti et al., 1998);

For criterion c) (functional assessment) Amsterdam IADL questionnaire (Koster et al., 2015)

Criterion d) requires a CDR score of 0.5

Every 6 months the subjects will be administered the full neuropsychological assessment and will be submitted to a control neurological examination. The specialist shall diagnose whether the subject has progressed to dementia on the basis of test performances and clinical examination, including the CDR.

3.3 Biomarkers included in the study

Biomarker 1: MMSE

Biomarker 2: DFR (FCSRT)

Biomarker 3: cerebrospinal fluid levels of Aß42, total tau, ph-tau, and tau/Aβ Ratio. Lumbar puncture for determing the levels of Aβ and tau in the CSF (procedure for collection, storage and lab tests):

- lumbar puncture (L4-L5) to be performed in the morning, approximately between 8-11 a.m.;
- collect 6 ml of CSF;
- centrifuge at 2000 x g for 10 minutes within one hour from collection;
- aliquot samples in 12 Sarstedt popypropylene tubes (each tube should contain approximately 0.5 ml of CSF) and freeze them preferably at -80°C (if not possible at least at -20°C).

Shipping to the expert center must be performed in dry ice.

Aß1-42, total tau and ph-tau levels shall be measured using commercially available enzyme-linked immunosorbent assays (Innogenetics and/or Fujirebio) according to a standardized procedure.

Antiplatelet or anticoagulant therapy (including but not limited to: aspirin, clopidogrel, dipyridamole, warfarin, dabigatran, rivaroxaban and apixaban) can be temporarily interrupted within the 7 to 14 days' period prior to sampling visit, depending on the type of therapy and societal guidelines (Engelborghs et al., 2017; https://clinicaltrials.gov/ct2/show/NCT02855476).

Biomarkers 4: (^{18}F)FDG-PET

Acquisition on a 3D PET scanner

- (^{18}F)FDG-PET with voxel-based single subject analysis using the optimized procedure based on SPM, large database of normal controls for comparison and the new FDG atlas for normalization (Della Rosa et al., 2014; Perani et al., 2014).

Biomarker 5: APOE4 Genotype

❖ *Biosample (DNA, plasma and serum) preparation*

Blood samples should be collected before breakfast and after an overnight fast, using three 6 ml EDTA tubes for DNA and plasma, and one 6 ml plain tube for serum.

A. DNA and plasma preparation

- Add 15 ml of Ficoll-Paque in a 50 ml Arnika tube and centrifuge at maximum speed for 5 minutes to force the Ficoll under the filter.
- Add the undiluted whole blood to the Arnika tube and centrifuge at 1400 x g for 15 minutes. A buffy coat will occur over the filter which is clearly distinguishable from the plasma.
- Transfer the plasma to polypropylene tubes (12 aliquots, 0.5 ml each), freeze and store at -80^{o}C (if not possible at least at -20°C).
- Transfer the supernatant containing the buffy coat to a 50 ml tube and centrifuge at 1800 x g in order to pellet the lymphocytes.
- Discard the supernatant, suspend the pellet in 10 ml of PBS. Transfer the solution to a 15 ml tube and centrifuge at 1800 x g for 15 minutes.
- Discard the supernatant and store the pellet at -20^{o}C. For DNA extraction, add (i) 3 ml of lysis buffer *, (ii) 500 µl of PK solution ** and (iii) 200 µl of 10% SDS * to the pellet .
- Incubate at 37°C over night.
- The day after, add 1 ml of NaCl 6M.
- Vortex for 30 seconds.
- Centrifuge at 1100 x g for 15 minutes.
- Transfer the limpid supernatant to a tube containing 3 ml of ice cold 100% ethanol.
- Transfer the DNA (with the least amount of fluid) to a 1.5 ml tube. Centrifuge and discard the supernatant.
- Wash the DNA pellet with 300 µl of 70% ethanol (add the ethanol and discard it after centrifugation).
- Allow the pellet to dry.
- Suspend the pellet in 100 µl of TE 1X. Transfer the sample in two 50 µl Sarstedt polypropylene tubes and report the DNA concentration on every tube. Store DNA at 4^{o} C.

* Lysis Buffer: 10 mM Tris-HCl (pH 8), 400 mM NaCl, 2 mM EDTA.

** PK Solution: dissolve 2 mg of K proteinases in 1 ml of 1% SDS and 2mM EDTA solution.

B. Serum preparation

- Allow the blood to clot at room temperature for 30 minutes.
- Centrifuge at 2000 x g for 15 minutes at room temperature.
- Transfer the serum to Sarstedt polypropylene tubes (4 aliquots, 0.5 ml each).
- Freeze and store at -80^{o}C (if not possible at least at -20°C).

❖ *Apolipoprotein E (APOE) Genotyping*

Polymerase Chain Reaction (PCR) shall be performed as described by Wenham et al. (Wenham PR et al., 1991) and the final products shall be digested with restriction enzyme CfoI for assessing the APOE polymorphic sites.

Biomarker 6: EEG for brain connectivity

Digital EEG for the study of brain connectivity. The dedicated software will be provided via a cloud computing platform. The EEG recording will be performed at rest, with closed eyes and no-task conditions (for at least 5 minutes). The subjects will be seated and relaxed, in a sound-attenuated and dimly lit room. Electroencephalographic signals will be recorded with a standard montage from 19 electrodes (Fp1, Fp2, F7, F8, F3, F4, T3, T4, C3, C4, T5, T6, P3, P4, O1, O2, Fz, Cz and Pz) positioned according to the International 10-20 system. Eye movements will be monitored from two different channels with vertical and horizontal montages. Skin/electrode impedances will be below 5 KΩ.

Data will be analyzed and processed in centralized Expert Centres with Matlab R2011b software (Math Works, Natick, MA) and using scripts based on the EEGLAB 11.0.5.4b toolbox (Swartz Center for Computational Neurosciences, La Jolla, CA; sccn.ucsd.edu/eeglab). The EEG recordings will be band-pass filtered from 0.2 to 47 Hz using a finite impulse response (FIR) filter, and a 256 Hz sampling rate. Ocular, muscular, cardiac and other types of artifacts will be inspected on imported data fragmented in 2 s duration epochs as follows: 1) epochs with aberrant waveforms or with evident artefactual activity will be manually discarded by an expert EEGer; 2) detection and rejection of artifacts will be completed through independent component analysis (ICA) using the

Infomax ICA algorithm, as implemented in the EEGLAB. ICA is a blind source decomposition algorithm that enables the separation of statistically independent sources from multichannel data.

Functional connectivity analysis: EEG functional connectivity analysis will be performed using eLORETA exact low resolution electromagnetic tomography software. The eLORETA algorithm is a linear inverse solution for EEG signals that has no localization error to indicate sources under ideal (noise-free) conditions. According to the scalp-recorded EEG potentials distribution, the exact low resolution brain electromagnetic tomography (eLORETA) software will be used to compute a discrete, three-dimensionally (3D) distributed linear, weighted, minimum norm inverse solution. The particular weights used in eLORETA endow the tomography with the property of exact localization necessary to test point sources, yielding images of the current density with exact localization, albeit with a low spatial resolution (i.e. the neighboring neuronal sources are highly correlated). To obtain a topographic view of the whole brain, brain connectivity will be computed with eLORETA software in 84 regions, positioning the center in the available 42 Brodmann Areas (BAs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47) in the left and right hemispheres. Regions of Interest (ROIs) are needed for the estimation of the electric neuronal activity that is used to analyze brain-functional connectivity. The signal at each cortical ROI will consist of the average electric neuronal activities of all voxels belonging to that ROI, as computed with eLORETA. For each hemisphere, among the eLORETA current density time series of the 84 ROIs, intracortical Lagged Linear Coherence, extracted by the “all nearest voxels” method (34), will be computed between all possible pairs of the 84 ROIs for each of the 7 independent EEG frequency bands of delta (2–4 Hz), theta (4–8 Hz), alpha 1 (8–10.5 Hz), alpha 2 (10.5–13 Hz), beta 1 (13–20 Hz), beta 2 (20–30 Hz) and gamma (30-45 Hz).

Starting with the definition of the complex valued coherence between time series x and y in the frequency band ω—which is based on the cross-spectrum given by the covariance and variances of the signals—the lagged linear coherence in the frequency band ω is reported in accordance with the following equation:

$$LagR^2_{xyw} = \frac{[ImCov(x,y)]^2}{Var(x)*Var(y)-[ReCov(x,y)]^2}$$

Where Var and Cov are variances and the covariance of the signals. This equation was developed as a measure of true physiological connectivity not affected by volume conduction and low spatial resolution. The values of lagged linear connectivity computing between all pairs of ROIs for each frequency band will be used as weights of the networks built in the graph analysis.

Graph analysis: a network is a mathematical representation of a real-world complex system. It is defined by a collection of nodes (vertices) and links (edges) between pairs of nodes. Nodes usually represent brain regions, while links represent anatomical, functional or effective connections, depending on the dataset. Anatomical connections typically correspond to white matter fiber tracts between pairs of grey matter brain regions (cortical areas or subcortical relays). Functional connections correspond to magnitudes of temporal correlations in activity and may occur between pairs of anatomically unconnected regions. A weighted graph is a mathematical representation of a set of elements (vertices) that may be linked through connections of variable weights (edges). Weighted and undirected networks will be built, the vertices of the network being the estimated cortical sources in the BAs, and the edges being weighted by the Lagged Linear value within each pair of vertices. The software instrument for the graph analysis will be the Brain Connectivity Toolbox (BCT, brain-connectivity-toolbox.net), adapted with our own Matlab scripts.

The Small World (SW) parameter will be evaluated on brain networks, since it measures the balance between local connectedness and the global integration of a network, representing brain network organization. Small-world organization is intermediate between that of random networks, the short overall path length is associated with a low level of local clustering, and that of regular networks or lattices, the high-level of clustering of which is accompanied by a long path length. The measure of network small-worldness will be defined as the ratio of the normalized Clustering Coefficient Cw and the normalized Path Length Lw. To obtain individual normalized measures, the values of characteristic path length and of the clustering coefficient will be divided by by the mean values obtained from the average values of each parameter in all frequency bands of each subject.

Biomarker 7: Magnetic Resonance Imaging

Brain MRI at 1.5 or 3T: conventional sequences + volumetric imaging in T1 at high resolution, DTI sequence for high resolution tractography, functional acquisition (EPI) for resting-state analysis. During acquisition the subjects must be motionless, awake with eyes closed.

Data analysis. Voxel based morphometry and FreeSurfer (http://surfer.nmr.mgh.harvard.edu/) for gray matter density and cortical thickness; for DTI: FMRIB software library (FSL, www.fmrib.ox.ac.uk/fsl/), CAMINO (www.camino.org.uk) for fractional anisotropy and mean diffusivity (Pierpaoli and Basser 1996), probabilistic tractography (Parker, et al. 2003).

Statistical parametric mapping (SPM8) and Group ICA, fMRI toolbox, (GIFT, http://icatb.sourceforge.net/) for resting state data analysis; graph-based connectivity analysis. Multivariate analyses for the calculation of predictive indices.

3.4 Biorepository

Anonymized aliquots of biological samples (DNA, plasma, serum, CSF) will be stored in a certified biorepository exclusively dedicated to the purposes of the current protocol that will ensure their optimal preservation. Therefore, if new biomarkers (useful for fulfilling the objective of this study) are identified in the future, our samples will be available for further analysis. Samples will be stored for 10 years. A Scientific Committee including the Coordinating Committee and the Funding Bodies will evaluate all the requests for new biomarkers testing.

3.5 Operational phases

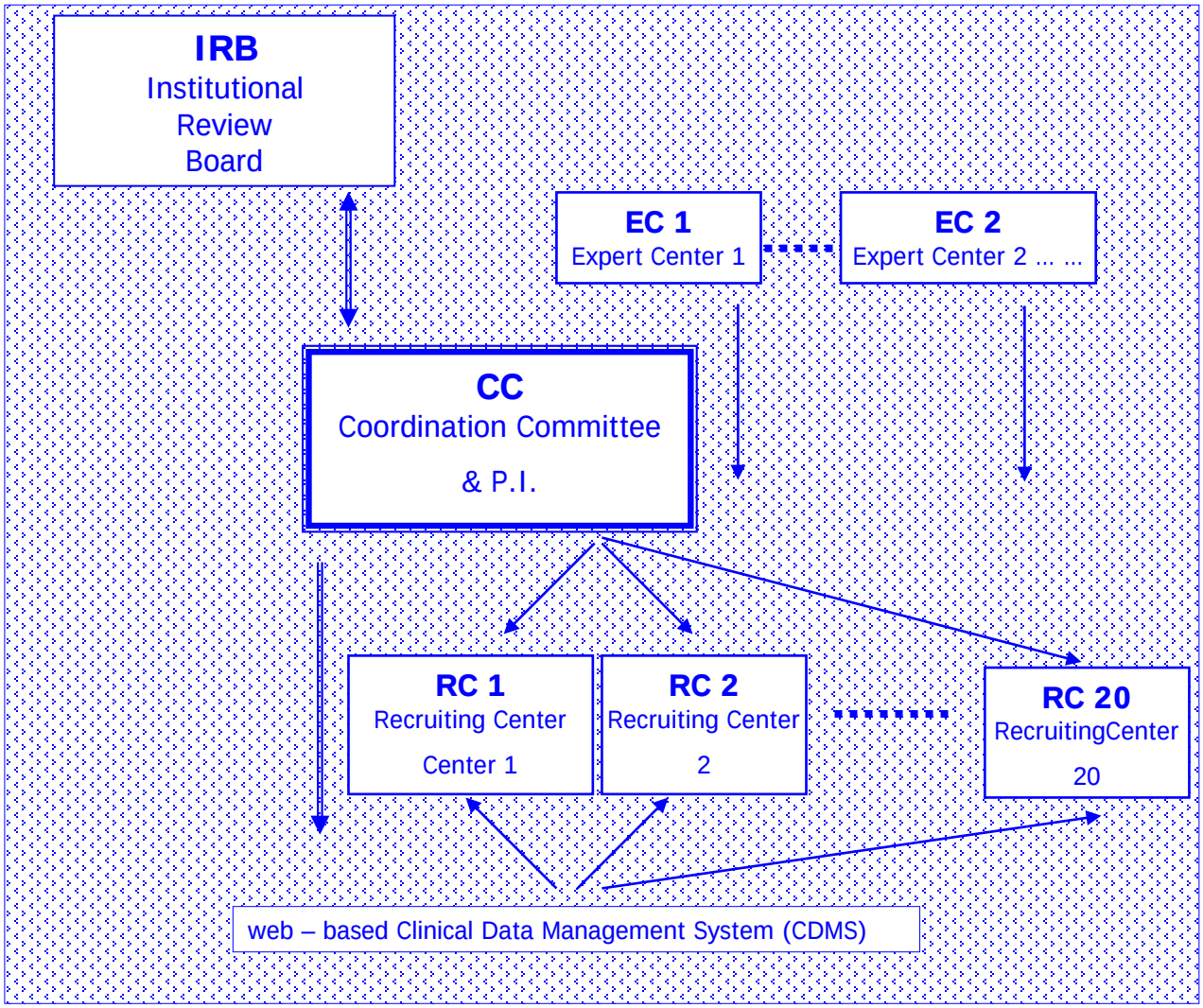
The study will last for 54 months. Six months will be used to prepare the organizational and operational network, 6 months for the recruitment of patients and collecting the bio-markers, 3 years of clinical follow-up, 6 months to analyse the results of all markers and to prepare the final report. The frequency of neuropsychological and clinical follow-up will be every 6 months, therefore 7 ratings from T0 (recruitment) to T6 (42 months) shall be performed.

All drugs taken at the time of diagnosis of MCI -except emergencies subsequent to recruitment to manage- are admissible without changes in the course of the study, unless the physician judges that they interfere with attentional-cognitive processes to the extent that the neuropsychological tests performedare unreliable. No participation in other clinical trials with experimental drugs shall be allowed. Within 60 days from T0 (recruitment on the basis of the NTP battery and acquisition of the 'informed consent') each MCI subject shall perform the markers identified in the study. Specific steps are shown in the Gantt chart (Figure 3).

3.6 Organizational structure of the study

The overall organization of competitive- balanced for geographical distribution and multicenter study(faster recruiting centres will include more subjects than slower centres) is shown in Figure 1. Competitive means that once the threshold of 400 completed subjects is reached, the study will be interrupted, independently of the recruitment rate of the individual centres.

Figure 1. The figure shows the overall organization of competitive- balanced for geographical distribution and multicenter study.



3.6.1 Coordination Committee (CC) & Principal Investigator (P.I.)

The study will be coordinated by a Coordination Committee (CC) (Responsible: Paolo Maria Rossini) at the Institute of Neurology, University Policlinic A. Gemelli Foundation, Catholic University of The Sacred Heart, Rome. The CC shall be formed bythe Institute of Neurology, Catholic University of The Sacred Heart, Rome, (Responsible: Paolo Maria Rossini); IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia (Responsible: Stefano Cappa); IRCCS Foundation

"Carlo Besta" Neurological Institute, Milan (Responsible: Fabrizio Tagliavini); National Institute of Health (Responsible: Nicola Vanacore); IRCCS-INRCA, Ancona (Fabrizia Lattanzio); Associazione Italiana Malattia Alzheimer (AIMA) (Patrizia Spadin). During the conduct of the study the CC will monitor the approval procedures, the conduct of the study and its closure. A Scientific Committee including the Coordinating Committee and the Funding Bodies will evaluate all the requests for new biomarkers testing. However, the final decision on any further request for new biomarkers testing shall be shared with AIFA and the Ministry of Health. P.I. shall interact with the ECs and RCs in order to monitor their activity and refer to the CC. P.I. shall also manage the preparation of the digital platform for signal/images transfer from RCs to ECs. P.I. will arrange the harmonization meetings with the ECs. Coordination meetings of the CC are expected every 3-6 months with all the clinical and expert centres (RC & EC) included in the study.

The CC shall prepare the publication policy and the dissemination plan to govern the relevant activities including -but not restricted to- publications and presentations in agreement with the provisions of the Funding Agencies.

3.6.2 Institutional Review Board (IRB)

The study will be reviewed and monitored by the IRB, made up of researchers not directly involved in the interventional study indicated by the following institutions: AIFA and Ministry of Health. The IRB reviews all research on human subjects to ensure protection of rights, safety and well being of the patients involved in the study. The IRB shall review and approve the study protocol. In particular, the materials and methods used for obtaining and documenting informed consent of the patients.

3.6.3 Recruiting Centers (RC)

MCI subjects shall be recruited by Centers with a documented expertise in diagnosis and treatment of Alzheimer's disease and MCI (Centri per i Disturbi Cognitivi e Demenza - CDCD) distributed on the national territory to reflect a representative reproduction of the Italian Health system organization in this field; they will be selected through a public call by AIFA. Twenty RC will be identified, 4 in each of the country's 5 macroareas (Northwest; North East; Central, South, Islands). The RC will be identified through apublic call organized by AIFA. Each RC will identify a local coordinator with a documented experience and a contact point for families and enrolled individuals. The local coordinator shall take care of the approval by the ethics committees of the

centre, shall be responsible for the conduct of the interventional study and shall ensure that all medical and nursing staff involved in patient care is sufficiently informed on the study, and particularly mindful of patient communication. Each RC shall perform neuropsychological evaluation, a blood sample, lumbar puncture and an EEG, PET scan and volumetric MRI either 'in house' or at facilities associated with the RC and approved by the experts centers (EC) for individual biomarkers.

For the final definition of the implementing rules of all examinations, specific meetings shall be arranged of the Operators participating to the project between CC and RC, with a view topromoting full harmonization procedures. Furthermore, on such meetings, the medical point of contact shall be provided with guidelines for ongoing communication with patients(compliance meeting).

3.6.4 Expert Center (EC)

Evaluation of "biomarkers" will be performed in expert centers previously identified and connected by the technological platform or receiving the samples stored at -80° C and shipped in dry ice except DNA, which must be shipped at room temperature environment (according to the harmonised Protocol developed and adopted within the framework of Network Project titled "Development of operational research diagnostic criteria for diagnosis of Alzheimer's disease in the preclinical/predementia phase and implementation of SOPs for imaging and CSF biomarkers in Memory Clinics. An integrated care pathway for early diagnosis and best management in the National Health Service of five Italian regions", code NET-2011-02346784, funded by the Italian Minister of Health). The following 4 expert centres have been identified through a selection by AIFA and by the Ministry of Health based on their clinical (i.e number of patients followed by the center) and research activities (i.e number of scientific publications and clinical trials performed) and in light of their scientific production in the area of Alzheimer's disease within the network of scientific institutes belonging to the Ministry of Health: Institute of Neurology, Catholic University of The Sacred Heart for EEG connectivity analysis; IRCCS Centro San Giovanni di Dio Fatebenefratelli for neuropsychological tests and volumetric MRI; IRCCS Foundation "Carlo Besta" Neurological Institute for liquoral markers and APOE4; IRCCS, Nuclear Medicine, San Raffaele Hospital, Milan for PET scan. Each EC can relate with other centres throughout the country with specific expertise on biomarkers included in this study. The Institutions where EC are located

might also apply for the RC selection call. However, in this case, none of the participating team of Experts can also be part of the RC team.

3.6.5 Data Management

The activities of the interventional study will be managed and controlled at all times through a clinical data management system (Clinical Data Management System – CDMS) implemented by the coordination centre, according to the following general requirements:

- adherence to Good Clinical Practice (GCP) and the laws and regulations regarding conduct of clinical trials (e.g. confidentiality, security and integrity of clinical data according to the standard ISO 27001);
- adherence to HTML/XHTML standards.

The main functional features of CDMS are:

- access based on user profiles with custom functions: Profile data input CC, Profile EC, Profile Coordinator Group;
- networking (web community area):

  a. communication Area: sending e-mail, teleconferencing via skype, etc.;

  b. document exchange Area : documents can be uploaded for sharing, be discussed and reviewed;

  c. forum: forum participants can ask questions and receive answers on issues related to the conduct of the trial;
- electronic management of case report form (CRF) (enrolment, follow-up, etc.);
- data standardization and classification (for example, classification of adverse events according to MedDRA);
- management of the e-Queries: electronic forms to track changes to data and to request additional information. They can be opened by centers or coordination. Unless an e-query is opened, no change may be made to the variables collected;
- control of the study, analysis of the e-queries and quality monitoring of the centres-: a module which allows the study coordinator to monitor and control all indicators such as: enrolment trends, activities of the centres (progress report), e-queries and data quality. The reports are organized into surfable paths according to the drill down technique, to allow consultation of individual CRF.

3.7 Endpoints

Primary Endpoint

It shall be considered the conversion to Alzheimer's disease within 3.0 years after diagnosis of MCI, together with the assessment of the subjects remaining in a stable condition and of those showing a reversion to the normal cognitive profile. People with MCI who convert to other forms of dementia will be considered separately. The biomarker or a set of biomarkers will be evaluated which can predict the conversion to Alzheimer's disease with higher accuracy.

Secondary Endpoints

Evaluation of cost/benefit ratio of individual biomarker or combination of biomarkers compared to their accuracy to predict progression from MCI to AD and their financial sustainability, availability in the country and non-invasiveness for patients.

3.8 Safety aspects

All subjects included in the interventional non therapeutic clinical study will be evaluated for safety determined through the assessment of adverse events (AES), serious adverse events, evaluation of laboratory tests, physical examination and vital signs. All information on safety will be collected, analyzed and evaluated by the CC in compliance with regulatory obligations provided for protection of patients in the study. Adverse events, whether observed by staff in clinics, or reported spontaneously bythe patient or caregiver, or in response to a request, shall be documented in patients' CRF. Adverse events detected upon performing diagnostic techniques are defined as any adverse event associated with the use of the technique, whether or not it is considered technique-related.

3.9 Sample size

In order to calculate the sample size of this study which aims at identifying with the greatest possible accuracy the biomarker or set of biomarkers which could predict conversion from MCI to Alzheimer's dementia, the biomarkers are used as screening tests and then for their level of the sensitivity and specificity. The following table shows the sensitivity value pairs of seven biomarkers that were proposed in this study on the basis of evaluation of the literature reported in section 1.2 of the introduction.

Table 1. Pairs of sensitivity values of seven biomarkers included in the study. The table shows that one pair of biomarkers (in bold and with an asterisk) is the combination of values of the highest sensitivity, including the use of

DFR (FCSRT) and EEG. These two biomarkers were therefore taken into account for the calculation of sample size. Tables 2 and 3 show the values of sensitivity, specificity andpositive/negative predictive value of the two biomarkers.

	MMSE	CSF p-tau	(^{18}F)FDG-PET	DFR (FCSRT)	APOE4	EEG	Volumetric MRI
MMSE							
CSF p-tau	57/71						
(^{18}F)FDG-PET	57/57	71/57					
DFR (FCSRT)	57/81	71/81	57/81				
APOE4	57/52	71/52	57/52	81/52			
EEG	57/87	71/87	57/87	**81/87***	52/87		
Volumetric MRI	57/60	71/60	57/60	81/60	52/60		

Table 2 Data included in Grande's paper et al. submitted

Giulia		AD					
follow-up 2.5 anni		1	0				
DFR	1	59	33	92		sens	0.81
	0	14	81	95		spec	0.71
		73	114	**187**		vpp	0.64
						vpn	0.85

Table 3 Poil's study et al. 2013 (follow-up of 2 year) (EEG)

Poli 2013		AD					
follow-up 2 anni		1	0				
EEG	1	7	8	15		sens	0.88
	0	1	14	15		spec	0.64
		8	22	**30**		vpp	0.47
						vpn	0.93

In 2 years of the follow-up the convertion rate from MCI to AD is 26.6% (8/30). In summary, after a follow-up of about 2.5 years, the prevalence of AD in a cohort of patients with MCI ranges from 26.6% to 39%. Based on these data, it was decided to adopt 2 approaches, one based on the methodological work of Bujang & Adnan (Requirements for Minimum Sample Size for Sensitivity and Specificity Analysis. J Clin Diagn Res. 2016 Oct; 10 (10): YE01-YE06), the other using the logic of a case control study, in which the cases are represented by patients converting to Alzheimer's disease, while the control is represented by patients with MCI. In the latter case an OR was

calculated between the biomarker and positive AD presence. In detail, the first approach allows us to predict a number of patients with MCI ranging from 78 to 388 in the hypothesis of a prevalence of 40% Alzheimer Dementia after 3 years of follow-up and a sensitivity value (working hypothesis) of 80-90% for an individual biomarker with a potency of about 81% and a p value lower than 5% (see below-from Bujang & Adnan 2016).

n (Sensitivity)						
Prev	H_o	H_a	Power	p-value	N1	N
40%	0.70	0.80	0.818	0.044	155	388
40%	0.70	0.90	0.807	0.048	31	78
40%	0.80	0.90	0.819	0.040	107	268

In detail, the second approach, typical of a case control study, has allowed to estimate with a power of 99% and a p value of 0.001 the following patients with MCI, depending on the type of biomarker. For the purposes of this study, it was decided to use very high values for alpha and beta parameters in order to minimize the statistical error ofthe first and second kind, thus giving giving priority to the use of the data for a decision at an individual level.

OpenEpi software was employed to calculate data in the tables 2 and 3. Tables 4 and 5 show details of sample size for two separate biomarkers by highlighting the value of OR between biomarker positivity and onset of Alzheimer's dementia.

Table 4 Delayed Free Recall (DFR) (Free and Cued Selective Reminding Test -FCSRT)

Sample size dimensions for a case-control unpaired study

For:

Level of bilateral confidence (1-alpha)	99.9
Potency (% possibilità di individuazione)	99
Ratio controls-cases	1
Hypothetical proprtion of controls with exposure	29
Hypothetical proprtion of cases with exposure	81
Minium Odds Ratio to identify:	10.44

	Kelsey	**Fleiss**	**Fleiss con CC**
Sample size – Cases	58	51	55
Sample size - Controls	58	51	55

Sample Size- total sample	116	102	110

Table 5. EEGSample size dimensions for a case-control unpaired study

For:		
	Level of bilateral confidence (1-alpha)	99.9
	Potency (% possibility of identification)	99
	Ratio controls-cases	2
	Hypothetical proprtion of controls with exposure	36.4
	Hypothetical proprtion of cases with exposure:	87.5
	Odds Ratio minimo estremo da rilevare:	12.23

	Kelsey	**Fleiss**	**Fleiss con CC**
Sample size – Cases	46	38	41
Sample size - Controls	91	75	81
Sample Size- total sample:	137	113	122

It is not possible to apply an estimate of sample size to a pair of biomarkers as there are no specific data in the literature for the 2 couples with the highest sensitivity values previously reported (table 1). It should be noted that -as will be reported in the next paragraph- it is necessary tocapture the full data set in order to have the number of cases which convert and do not convert to Alzheimer's dementiafor each of the 4 combinations of a biomarker pair (++; +-;-+;--). On the basis of relevant literature, using two specific approaches for sample size calculation for a single biomarker and estimating an approximate 20% drop-out rate for various reasons (death, severe neurodegenerative disease, dropout for poor compliance) during the follow up, a sample size of 400 patients with MCI is considered sufficient to highlight a biomarker or a pair of biomarkers with such a level of accuracy as to trigger the decision to prescribe a new anti-dementia medicinal product.

3.10 Statistical Analysis

Statistical analysis will be carried out following three areas:

1. Preparation of contingency tables for each of the seven biomarkers (MMSE; FCSRT; CSF CSF tau and p-tau/ABeta; (^{18}F)FDG-PET; ApoE4; EEG) and processing parameters of sensitivity, specificity, positive predictive value and negative predictive value and overall accuracy. We will also calculate the positive and negative likelihood ratios (LR). The related 95% CI for each of these parameters shall also be calculated . The results will be compared with the positive and negative LR values, which are considered the gold standard for the placing on the market of a diagnostic test. (Jaeschke R, Guyatt GH, Sackett DL 1994)(see below).

Table 6. Interpretation of likelihood ratios

Likelihood ratio Positive	Likelihood ratio negative	Changes in probability of the condition	Results
> 10	< 0.1	Large	Conclusive
5–10	0.1–0.2	Moderate	Moderately useful
2–5	0.2–0.5	Small	Sometimes useful
< 2	> 0.5	Tiny	Rarely useful
1	1	No change at all	Not useful

(*)*Modified from: The investigation and management of the small-for-gestational-age fetus Royal College Obstetricians and Gyneacologists - RCOG Guideline No. 31 November 2002*

2. For the two biomarkers with the highest sensitivity value we will proceed with an assessment in parallel with a mode "OR". Please note that in this study we use biomarkers in parallel in order to improve the overall specificity with respect to the use of every single biomarker. This implies that the purpose of the study is to develop an organizational model capable of identifying those subjects with MCI with the highest risk of converting to Alzheimer's disease (low percentage of false negative). Consider this theoretical example (http://www.quadernodiepidemiologia.it/epi/screen/tst_com.htm) which will be replicated with data captured in this study. Contingency tables can be set up separately for each of the 2 tests to calculate their sensitivity and specificity.

Compared to using a single test, in the case of interpretation «OR» it is less likely to miss a person with MCI converting to Alzheimer's dementia. However, there will be a low percentage of false negative, i.e. of subjects with AD at the end of the study but with biomarker negative at baseline. With this mode we might get an increase in sensitivity at the expense of specificity. In the case of interpretation «AND», it is easier to miss subjects with MCI converting to Alzheimer's disease, but there is a lower probability that a person with MCI is classified as a subject that will convert to Alzheimer's disease. Specificity increases at the expense of sensitivity.

3. Cox logistic regression models will be performed that will help to identify the best pattern of variables for a subject with MCI candidate for conversion to Alzheimer's disease. For each patient included in the study, biographic and clinical information will be collected that will be used to construct multivariate models. In this regard, the months-person observation in the cohort will be calculated for each patient from the baseline visit to the end of the study or to his/her last visit or death.

Finally, in order to identify the combination of biomarkers to be proposed for selection of patients eligible for treatment with the new anti-dementia drugs, it shall be taken into account both costs and availability on the national territory of centres able to propose the diagnostic work-up, along with the level of invasiveness of the test. From a perspective of research in public health, the critical issues shall be carefully considered relating to standardization of analytical procedures for the determination of a biomarker, as documented by numerous reviews of the the Cochrane Collaboration. In this context the following solutions have been identified:

1. evaluation of "markers" shall occur in "expert centres" previously identified and connected through technology platform or receiving the samples stored at -80° C;

2. Training meetings shall be arranged for the panel of "expert centres" assessors in order to harmonize procedures, minimize the inter-rater variability and create a panel of "expert assessors" for each type of marker certifying the results for the individual subjects;

3. all information of the 400 subjects shall be blinded within a database provided by a professional biostatistical company approved by and under the supervision of the CC so that the assessors' panel of the individual exams is totally unaware whether MCI is 'Stable' or 'Converted' during follow-up. Only at the end of the Protocol will

the encrypted archive be opened and the diagnosis of all the NPT follow-up be coupled to each person;

4. all markers shall be arranged in 2 different presentations (each sample of CSF divided into 2 tubes, each image split into 2 sets of images, each EEG divided into 2 lengths of EEG tracing) in order to have 2 labels for each subject which are also blinded. This procedure will ultimately also provide an idea of the repeatability of the method of evaluation on the same sample/profile/path. Obviously, this does not apply to ApoE genotyping. Also in this case the assessors will be blinded to the fact that a particular exam is the second of the pair of an individual patient or the first of another patient;

5. each individual marker shall express a single assessment (altered/not altered) taken unanimously by the assessors. In the case of "altered", a severity level of 2 or 3 of the alteration (A1, A2 ...) will be scored to check at a later time whether they correlate with rapidity of MCI→AD progression and with the possible rapid worsening of the disease. In the event of a discrepancy between the evaluators who have assessed that particular marker (called N), a strategy of consensus will be built among the evaluators in order that the exam be considered in the final statistical evaluation. K statistics shall be used to estimate the degree of agreement between 2 raters.

Statistical analyses shall be performed by using the softwares STATA and SPSS (version 25.0).

3.11 Monitoring of the study

Some general aspects of the study will be monitored centrally by the P.I. and the CC through CDMS as, for example, evaluation of the conduct of the study, including periodic evaluation of the quality and timely arrival of data, patient recruitment, evaluation of the centres' activities , etc. The monitors shall use the medical records as central documentation. After every visit at the centres, the monitor shall fill in an *ad hoc* form "Summary of monitoring results". This documentation will be evaluated by the CC. During the study each CC will receive 3 monitoring visits by the CC.

3.12 Ethical aspects

Once signed the 'informed consent', the MCI candidate will be notified by the CC prior to enrollment for approval. Researchers will establish and maintain ongoing communication with patients, since in the MCI prodromal to AD no caregiver is needed, in line with the autonomy of individuals and with medical confidentiality. Each recruiting centre shall identify a "contact point for the study protocol", adequately trained for the enrolled subjects. The study shall be submitted for approval of the Ethics Committee of the coordinators' centre and of the ethics committees of the participating clinical centres.

3.13 Gantt Diagram

The activities of the various phases of the study will be as follows: 6 months for the preparatory activities, recruitment and collection of biomarkers, 42 months for follow-up and data analysis and 6 months for the release of the results. The Gantt chart is shown in figure 2.

Figure 2. The Gantt chart

	M1-6	M7-12 (T0)	M13-18 (T1)	M19-24 (T2)	M25-30 (T3)	M31-36 (T4)	M37-42 (T5)	M42-48 (T6)	M49-54
Identification of the Hubs of the network, harmonization meetings from ECs, preparation of technological platform									
Subjects enrollment									
Neuropsychological testing									
Neurological and Medical exam									
Biomarkers collection (MMSE; FCSRT; CSF p-tau e CSF tau/ABeta ;(^{18}F)FDG-PET;APOE4;EEG, volumetric MRI)									
Neuropsychological tests and functional scales (follow-up)									
Neurological exam (follow-up)									
Results evaluation									

3.14 Costs of the study (Euros)

COSTS	Reserved to the Call For Recruiting Centers	Reserved for Expert Centres	General costs	Total
(^{18}F)FDG-PET 400 x 1.000 Euro each	320.000	80.000	-	400.000
Volumetric MRI 400 x 400 Euro each	128.000	32.000	-	160.000
ApoE 400 x 150 Euro each	30.000	30.000	-	60.000
Lumbar Puncture for Beta/Tau metabolites 400 x 300 Euro each	60.000	60.000	-	120.000
Digital EEG recording and connectivity analysis. 400 50 Euro each	10.000	10.000	-	20.000
Neuropsychological battery (400 pz x 7 follow-up evaluations) 2800 70 Euro each	156.000	40.000	-	196.000
Transfer of Patients	280.000	-	-	280.000
Biologic sample shipping	10.000	-	-	10.000
Web master	-	-	20.000	20.000
Development of technological platform for data transfer	-	-	50.000	50.000
Database & Insurance	-	-	294.000	294.000
Study Coordination	-	-	50.000	50.000
Clinical Centres (n=20) (1 FTP each x 4 years)	1.200.000	-	-	1.200.000
Expert Centres (n=4) (1 FTP x 4 years)	-	400.000	-	400.000
Statistics & database management (1 FTP x 4,5 years)	-	-	90.000	90.000
Biorepository	-	-	-	20.000
Meetings for Harmonization (one for each Marker, 2 full days) +	-	-	63.000	63.000

compliance meeting (3 h)				
Other costs	20.000	20.000	20.000	60.000
Total	**2.214.000**	**672.000**	**587.000**	**3.473.000**

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Annex 1. Consenso al trattamento

CONVERSIONE DAL MILD COGNITIVE IMPAIRMENT (MCI) A DEMENZA: IL CONTRIBUTO DEI BIOMARCATORI NELL'IDENTIFICARE I PAZIENTI CANDIDATI ALLA PRESCRIZIONE DI FARMACI CON POSSIBILE AZIONE DISEASE-MODIFYNG

Centro: _____

Dichiarazione di consenso (paziente)

Io sottoscritto/a (Cognome)_____(Nome)_____,

Sesso____, Data di nascita_____, Luogo di nascita_____,

dichiaro di aver ricevuto dal Dottor _____
spiegazioni esaurienti in merito alla richiesta di partecipazione allo studio di coorte multicentrico in oggetto, secondo quanto riportato nella scheda informativa qui allegata, copia della quale mi è stata consegnata prima d'ora.

Dichiaro inoltre di aver potuto discutere tali informazioni, aver potuto porre tutte le domande che ho ritenute necessarie e di aver ricevuto riposte soddisfacenti, come pure di aver avuto la possibilità di informarmi in merito ai particolari dello studio con persona di mia fiducia.

Sono consapevole che il Dr…….. (disponibile nei giorni……., dalle ore…….alle ore……..) avrà funzione di "collegamento" per ogni esigenza dovesse insorgere in relazione alla mia partecipazione alla sperimentazione prevista dal protocollo di ricerca in oggetto.

Sono consapevole che i referti degli esami a cui sarò sottoposto NON mi saranno forniti sino a che la mia situazione clinica rimarrà stabile come alla data d'ingresso nello studio. Solo in caso ci dovesse essere una progressione verso uno stato di "Demenza Iniziale" tutti i risultati degli esami e delle valutazioni neuropsicologiche effettuate mi verranno resi noti per gli usi clinici connessi.

Sono consapevole che per tutta la durata della mia partecipazione allo studio NON potrò entrare in *trial* clinici con farmaci sperimentali.

Accetto dunque liberamente di partecipare alla sperimentazione, avendo avuto la possibilità di capire completamente il significato della richiesta e avendo compreso i rischi e i benefici che sono implicati.

Accetto il trattamento delle informazioni da parte di persone tenute al segreto medico.

In nessun modo il mio nome figurerà nei documenti dello studio e nella pubblicazione di dati.

La mia pratica potrà essere consultata da un medico di mia scelta.

Acconsento all'archiviazione, in forma anonima, dei miei dati e dei risultati degli esami eseguiti, su una piattaforma informatica centralizzata sicura. Acconsento alla conservazione dei miei campioni biologici, anonimizzati, in un *biorepository* (banca per la conservazione a lungo termine dei campioni biologici) dedicato allo studio. Acconsento

all'utilizzo dei miei campioni biologici, anche oltre il termine dello studio, per ulteriori ricerche o sperimentazioni approvate dal Comitato Etico nell'ambito scientifico di indagine dello studio descritto nel foglio informativo, anche nel caso in cui decidessi di interrompere anticipatamente la mia partecipazione allo studio anticipatamente. Acconsento alla pubblicazione dei risultati dello studio su riviste scientifiche e alla presentazione degli stessi a convegni scientifici. Acconsento alla comunicazione della mia partecipazione a questo studio al mio medico di medicina generale ed allo scambio delle mie informazioni cliniche tra il medico dello studio e il medico di medicina generale e/o gli altri miei medici curanti, se necessario.

Sono a conoscenza di poter, in ogni momento e per qualsiasi motivo, chiedere di uscire dalla sperimentazione.

Data_____Firma_____

(Medico che ha informato il paziente)

Data_____Firma_____

(Paziente)

Foglio informativo per il paziente

INVITO A PARTECIPARE AD UNA SPERIMENTAZIONE CLINICA PER IL SEGUENTE STUDIO: CONVERSIONE DAL MILD COGNITIVE IMPAIRMENT (MCI) A DEMENZA: IL CONTRIBUTO DEI BIOMARCATORI NELL'IDENTIFICARE I PAZIENTI CANDIDATI ALLA PRESCRIZIONE DI FARMACI CON POSSIBILE AZIONE DISEASE-MODIFYING

Gentile Signora/e,

- Le è stata diagnosticata una condizione neuropsicologica di MCI (Mild cognitive impairment), nel giro di 2 o 3 anni potrebbe evolvere in demenza di Alzheimer o rimanere stabile o tornare ad un profilo cognitivo normale. La malattia di Alzheimer causa un danno a carico delle cellule nervose e una progressiva perdita di memoria.

- Frequentemente nel corso della malattia di Alzheimer si possono presentare disturbi comportamentali e psicologici (tra cui agitazione, aggressività, disturbi del sonno, allucinazioni) che rendono più difficile la gestione del malato.

- Tra qualche anno potrebbero essere in commercio farmaci in grado di modificare il decorso della malattia di Alzheimer (*disease modifyng*).

- L'obiettivo primario è quello di identificare il "biomarcatore" o "l'insieme di biomarcatori" (esami strumentali che permettono di completare la diagnosi) in grado di predire con maggiore accuratezza l'evolvere in 2 o 3 anni dalla diagnosi di MCI a quella di demenza di Alzheimer.

- L'obiettivo secondario è quello di definire lo scenario maggiormente sostenibile, sia dal punto di vista della applicazione pratica che economico, per l'uso di biomarcatori nell'identificazione dei pazienti candidati alla prescrizione dei farmaci antidemenza in corso di sperimentazione.

- La Sua partecipazione allo studio durerà al massimo 48 mesi.

- Le stiamo proponendo di partecipare a questo studio per aiutarci a capire quale biomarcatore o l'insieme di biomarcatori siano utili per identificare quali soggetti con MCI evolveranno ad una condizione di Alzheimer. Lei è libero di decidere se partecipare o meno allo studio. Prima di decidere è importante che comprenda perché lo studio viene effettuato e cosa comporterà la partecipazione. La preghiamo di leggere con attenzione le pagine che seguono e di discuterne con altri se desidererete farlo.

- Nella Parte 1 troverà le spiegazioni sul perché viene effettuato questo studio.

- Nella Parte 2 troverà informazioni più dettagliate su come verrà condotto lo studio e su cosa avverrà se deciderà di partecipare.

Studio

Conversione dal Mild cognitive impairment (MCI) a demenza: il contributo dei biomarcatori nell'identificare i pazienti candidati alla prescrizione di farmaci capaci di modificare il naturale decorso della malattia. I biomarcatori in studio sono esami strumentali che permettono di completare la dignosi di MCI inizialmente definita solo sulla base di test neuropsicologici. I biomarcatori in studio sono MMSE; FCSRT; CSF p-tau e CSF tau/ABeta ; (^{18}F)FDG-PET; APOE4; EEG; RM volumetrica. A questi potrebbero aggiungersene altri sulla base di nuove evidenze scientifiche che nel suo caso saranno esaminati facendo ricorso ai campioni già presenti nella banca dei campioni biologici, alla cui raccolta Le verrà chiesto di dare il consenso.

Codice identificativo dello studio:

Parte 1:

Cosa è la condizione di MCI (mild cognitive impairment)?

È una condizione clinica caratterizzata da una sfumata difficoltà in uno o più domini cognitivi (quali, ad esempio, memoria, attenzione o linguaggio), oggettivata attraverso i test neuropsicologici, tale però da non compromette le normali e quotidiane attività di una persona.

Le persone con il decadimento cognitivo lieve di solito incontrano qualche difficoltà ad ultimare alcuni compiti complessi, che prima avevano sempre eseguito senza difficoltà, come occuparsi dei propri affari finanziari, prepararsi un pasto oppure fare la spesa. Potrebbero necessitare di tempi più lunghi, oppure essere meno efficienti o fare più errori rispetto al passato nelle medesime attività, ma ciononostante mantenere la loro autonomia e indipendenza. Un gran numero di soggetti con MCI non andrà mai incontro a demenza di Alzheimer e una parte di loro potrà anche ritornare a un profilo cognitivo normale.

Lo studio si propone principalmente di identificare il "biomarcatore" o "l'insieme di biomarcatori" in grado di predire con maggiore accuratezza l'evolvere in 2 o 3 anni dalla diagnosi di MCI quella di demenza di Alzheimer.

Inoltre si propone di definire lo scenario maggiormente sostenibile, sia dal punto di vista della applicazione pratica che economico, per l'uso di biomarcatori nell'identificazione dei pazienti candidati alla prescrizione dei farmaci antidemenza in corso di sperimentazione.

Parte 2:

In cosa consiste lo studio Conversione dal Mild cognitive impairment (mci) a demenza: il contributo dei biomarcatori nell'identificare i pazienti candidati alla prescrizione di farmaci con possibile effetto positivo sul decorso della malattia?

Lo studio è una sperimentazione clinica coordinata dal Policlinico Gemelli di Roma e sponsorizzata dall'AIFA e dal Ministero della Salute che verrà condotta su circa 400 pazienti assistiti da 20 centri in tutta Italia.

Questo studio si propone di arruolare soggetti con MCI e di confrontarne le caratteristiche cliniche all'inizio dello studio e le risposte ai biomarcatori e di seguirne l'evoluzione per tre anni.

- La sua partecipazione allo studio durerà al massimo 48 mesi.

Durante questo periodo lei dovrà presentarsi ad alcune visite presso questo Centro, dopo la prima visita, è necessario che sia valutato una volta ogni sei mesi. Sarà inoltre possibile contattare il medico anche al di fuori delle visite programmate, qualora se ne dovesse verificare la necessità. Per tutta la durata della Sua partecipazione allo studio Lei NON potrò entrare in *trial* clinici con farmaci sperimentali.

All'inizio dello studio Le saranno effettuati i seguenti esami:

- test neuropsicologici (MMSE Mini mental state examination, e DRF delayed-free recall – FCSRT Free And Cued Selective Reminding Test);
- neuroimmagini funzionali con (^{18}F)FDG-PET;
- prelievo liquor (tramite la puntura lombare) per metaboliti Beta amiloide e Tau;
- prelievo di sangue per test genetico APOE4;
- l'elettroencefalografia per lo studio della connettività cerebrale.

Verranno inoltre effettuate una visita neuropsicologica e una visita clinica e una misurazione della pressione arteriosa. Inoltre, verrà sottoposto (all'inizio dello studio e a tempi prestabiliti nel corso dello studio) a una serie di test neuropsicologici nel corso dei quali le verranno poste domande sui sintomi della malattia e Le verrà chiesto di eseguire alcune semplici attività.

I risultati di questi test ci aiuteranno a valutare lo stato iniziale e la progressione della malattia. Lei non riceverà i risultati dei test eseguiti sino al termine dello studio o sino all'eventuale progressione verso uno stato di "Demenza Iniziale". Se tale progressione dovesse avvenire prima del termine dello studio, Lei riceverà i risultati di tutti gli accertamenti effettuati sino a quel momento per gli usi clinici correlati.

Come verranno gestiti i dati raccolti durante lo studio e gli esiti delle mie valutazioni?

Il trattamento delle Sue informazioni personali avverrà nel rispetto della vigente normativa sulla privacy.

Tutti i Suoi dati digitalizzati saranno anonimizzati ed archiviati su una piattaforma informatica centralizzata e sicura. Nello specifico, i dati clinici/neuropsicologici, biochimici/molecolari e di neuroimmagine che La riguardano saranno contrassegnati con un codice numerico e non riporteranno il Suo nome o altre informazioni in grado di identificarLa direttamente.

I Suoi campioni biologici, anonimizzati, saranno conservati, insieme a quelli delle altre persone partecipanti allo studio, in un *Biorepository* dedicato allo studio.

Firmando questo consenso ci consentirà di utilizzare i Suoi campioni biologici in forma totalmente anonima anche oltre il termine dello studio, per scopi di ricerca strettamente connessi all'ambito scientifico di indagine indicato in questo modulo informativo ed approvati da un Comitato Tecnico Scientifico in cui sono rappresentati gli enti e le istituzioni che promuovono il presente protocollo, al solo fine di individuare quale biomarcatore o insieme di biomarcatori siano utili per identificare i soggetti affetti da declino cognitivo lieve che hanno un rischio elevato di evolvere verso una malattia di Alzheimer, in modo da consentire interventi farmacologici e non farmacologici in una fase precoce.

I risultati dello studio verranno pubblicati su riviste mediche e presentati a convegni.

Quali sono i rischi legati alle metodiche di analisi ?

Le tecniche diagnostiche utilizzate possono causare eventi evversi che sono, comunque, molto rari.

Tutti gli eventi avversi devono essere comunicati tempestivamente al Centro che la segue e saranno prese le necessarie misure per gli eventuali disturbi.

Se Lei ha un'assicurazione medica privata potrebbe voler parlare con la sua assicurazione prima di decidere se partecipare a questo studio, in modo da verificare che la partecipazione alla sperimentazione non influenzi la Sua assicurazione medica.

Quali sono i possibili benefici derivanti dalla partecipazione a questo studio?

Se deciderà di partecipare a questo studio contribuirà a migliorare le nostre conoscenze su quali biomarcatori saranno utili per prevedere la conversione ad Alzheimer nei soggetti con MCI. In questo modo sarà possibile utilizzare i nuovi farmaci per quei soggetti con MCI che progrediranno ad Alzheimer.

Come dovrò comportarmi se deciderò di partecipare allo studio?

Se deciderà di partecipare a questo studio dovrà riferire al medico che La seguirà nel corso della sperimentazione tutte le medicine che sta assumendo. Il Suo medico stabilirà se ci sono farmaci che non devono essere assunti durante il periodo dello studio. Se Lei deciderà di partecipare a questo studio non dovrebbe partecipare contemporaneamente ad altre sperimentazioni farmacologiche.

Sono tenuto a partecipare a questo studio?

Non deve necessariamente partecipare a questo studio, né deve fornire spiegazioni nel caso in cui decida di non partecipare.

Prima di decidere se partecipare o meno è opportuno che legga questo foglio informativo con attenzione e faccia domande al medico che la segue se ci sono cose che risultano poco chiare.

Se deciderà di entrare nello studio Le chiederemo di firmare un consenso informato in cui è scritto che ha compreso cosa lo studio comporta.

La mia partecipazione allo studio modificherà i suoi diritti legali?

Sia che partecipi o no alla sperimentazione manterrà gli stessi diritti legali di qualsiasi altro paziente assistito dal Sistema Sanitario Nazionale.

Potrò decidere di ritirarmi dallo studio?

In qualsiasi momento e per qualsiasi motivo potrà decidere di non partecipare più alla sperimentazione e le sarà comunque fornita la migliore assistenza possibile. Firmare il modulo del consenso informato non La obbliga a partecipare all'intera durata dello studio. Se dovesse cambiare idea in un secondo momento non sarà necessario fornire una spiegazione, ma sarebbe di aiuto alla nostra ricerca se accettasse di completare comunque i questionari per farci sapere l'andamento delle sue condizioni cliniche. Firmando questo consenso, Lei ci darà il permesso di utilizzare le informazioni che ci ha fornito prima di lasciare lo studio per scopi di ricerca strettamente connessi all'ambito scientifico di indagine indicato in questo foglio informativo. Tali informazioni saranno conservate anche dopo il termine dello studio. Qualora ne faccia esplicita richiesta, provvederemo alla distruzione dei campioni biologici e alla rimozione di tutte le informazioni che La riguardano dalla piattaforma centralizzata.

Da chi è organizzato e sponsorizzato questo studio?

Questo studio è coordinato dal Policlinico Gemelli ed è sponsorizzato dall'Agenzia Italiana del Farmaco AIFA e dal Ministero della Salute.

Ci sono altre domande che vorrebbe porre?

Dopo aver letto questo foglio informativo noi ci auguriamo che Lei deciderà di partecipare a questo studio. Se ci sono altre domande che vorrebbe porre al Suo medico relativamente allo studio ora o in un secondo momento si senta libero di farlo.

Troverà alla fine di questo foglio il nome del medico a cui potrà rivolgersi e il numero telefonico. Se preferisce rinviare la Sua decisione, ad esempio per poterne discutere con amici o parenti, può prendere appuntamento per tornare in un secondo momento. La preghiamo però di ricordarsi di conservare questi fogli in un posto sicuro e di appuntarsi i nomi e numeri di telefono nella sua agenda. Grazie.

Per ulteriori informazioni la preghiamo di contattare:

Dr. ……. Numero di telefono: ……, che nei giorni ……, dalle ore……alle ore…… sarà a Sua disposizione per ulteriori chiarimenti e informazioni. A tal fine può contattare anche il numero verde di AIMA 800 679679, a Sua disposizione dal lunedì al venerdi dalle 9,30 alle 17,30.

CONVERSIONE DAL MILD COGNITIVE IMPAIRMENT (MCI) A DEMENZA: IL CONTRIBUTO DEI BIOMARCATORI NELL'IDENTIFICARE I PAZIENTI CANDIDATI ALLA PRESCRIZIONE DI FARMACI CON POSSIBILE AZIONE DISEASE-MODIFYNG

Centro: _____

Dichiarazione di acquisita informazione (familiare)

Questa richiesta viene avanzata solo previa approvazione da parte del Paziente

Io sottoscritto/a (Cognome)_____(Nome)_____, Sesso____Data di nascita_____,Luogo di nascita_____, Familiare (specificare grado di parentela) di _____, dichiaro di aver ricevuto dal Dottor _____esaurienti spiegazioni in merito alla richiesta di partecipazione del mio familiare allo studio di coorte multicentrico in oggetto, secondo quanto riportato nella scheda informativa qui allegata, copia della quale mi è stata prima d'ora consegnata.

Dichiaro altresì di aver potuto discutere tali informazioni, aver potuto porre tutte le domande che ho ritenute necessarie e di aver ricevuto riposte soddisfacenti, come pure di aver avuto la possibilità di informarmi in merito ai particolari dello studio con persona di mia fiducia.

Sono consapevole che il Dr…… avrà funzione di "collegamento" per ogni esigenza dovesse insorgere in relazione al protocollo di ricerca in oggetto.

Sono consapevole che i referti degli esami a cui sarà sottoposto NON gli saranno forniti sino a che la situazione clinica rimarrà stabile come alla data d'ingresso nello studio. Solo in caso ci dovesse essere una progressione verso uno stato di "Demenza Iniziale" tutti i risultati degli esami e delle valutazioni neuropsicologiche effettuate verranno resi noti al Paziente per gli usi clinici connessi.

Sono consapevole che per tutta la durata della sua partecipazione allo studio il mio parente NON potrà entrare in *trial* clinici con farmaci sperimentali.

Accetto dunque liberamente di far partecipare il mio familiare allo studio osseravazionale, avendo potuto capire completamente il significato della richiesta e avendo compreso i rischi e i benefici che sono implicati.

Accetto il trattamento delle informazioni da persone autorizzate al segreto medico.

In nessun modo il nome del mio familiare figurerà nei documenti dello studio e nella pubblicazione di dati. La pratica potrà essere consultata da un medico di mia scelta.

Sono a conoscenza del fatto che il mio familiare possa, in ogni momento e per qualsiasi motivo, chiedere di uscire dalla sperimentazione.

Data_____Firma_____

(Medico che ha informato il familiare)

Data_____Firma_____

(Familiare)

Foglio informativo per il familiare

Tale informazione viene rivolta solo previa approvazione da parte del Paziente

INVITO A PARTECIPARE AD UNA SPERIMENTAZIONE CLINICA PER IL SEGUENTE STUDIO: CONVERSIONE DAL MILD COGNITIVE IMPAIRMENT (MCI) A DEMENZA: IL CONTRIBUTO DEI BIOMARCATORI NELL'IDENTIFICARE I PAZIENTI CANDIDATI ALLA PRESCRIZIONE DI FARMACI CON POSSIBILE AZIONE DISEASE-MODIFYING

Gentile Signora/e,

- Al Suo familiare è stata diagnosticata una condizione neuropsicologica di MCI (Mild Cognitive Impairment – Lieve Deficit Cognitivo), nel giro di 2 o 3 anni potrebbe progredire a demenza di Alzheimer o rimanere stabile o tornare ad un profilo cognitivo normale. La malattia di Alzheimer causa un danno a carico delle cellule nervose e una progressiva perdita di memoria e dell'autonomia personale del soggetto colpito.

- Frequentemente nel corso della malattia di Alzheimer si possono presentare disturbi comportamentali e psicologici (tra cui agitazione, aggressività, disturbi del sonno, allucinazioni) che rendono più difficile la gestione del paziente.

- In un futuro prossimo potrebbero essere in commercio farmaci in grado di modificare il decorso della malattia di Alzheimer (disease modifyng).

- L'obiettivo primario è quello di identificare il "biomarcatore" o "l'insieme di biomarcatori" in grado di predire con la maggiore accuratezza la progressione, nell'arco temporale di 2 - 3 anni, da un declino cognitivo lieve alla demenza di Alzheimer.

- L'obiettivo secondario è quello di definire lo scenario maggiormente sostenibile, sia dal punto di vista della trasferibilità che economico, per l'uso di biomarcatori nell'identificazione dei pazienti candidati alla prescrizione dei farmaci antidemenza attualmente in corso di sperimentazione.

- La sua partecipazione allo studio durerà al massimo 48 mesi.

- Stiamo proponendo al Suo familiare di partecipare a questo studio per aiutarci a capire quale biomarcatore o l'insieme di biomarcatori siano utili per identificare quali soggetti con diagnosi di MCI evolveranno ad una condizione di demenza di Alzheimer. Il Suo familiare è libero di decidere se partecipare o meno allo studio. Prima di decidere è importante che comprenda perché lo studio viene effettuato e cosa comporterà la partecipazione. La preghiamo di leggere con attenzione le pagine che seguono e di discuterne con altri se desidererete farlo.

- Nella Parte 1 troverete le spiegazioni sul perché viene effettuato questo studio.

- Nella Parte 2 troverete informazioni più dettagliate su come verrà condotto lo studio e su cosa avverrà se il suo familiare deciderà di partecipare.

Studio

Conversione dal Mild Cognitive Impairment (MCI) a demenza: il contributo dei biomarcatori nell'identificare i pazienti candidati alla prescrizione di farmaci con possibile azione disease-modifying.

Biomarcatori in studio (MMSE; FCSRT; CSF p-tau e CSF tau/ABeta ;(^{18}F)FDG-PET; APOE4; EEG; RM volumetrica)

Codice identificativo dello studio:

Parte1:

Cosa è la condizione di MCI (Mild Cognitive Impairment)?

Al Suo familiare è stata diagnosticata una condizione clinica caratterizzata da una sfumata difficoltà in uno o più domini cognitivi (quali, ad esempio, memoria, attenzione o linguaggio), oggettivata attraverso test neuropsicologici specifici, tale però da non compromettere le normali e quotidiane attività di una persona.

Le persone con un decadimento cognitivo lieve di solito incontrano qualche difficoltà ad ultimare alcuni compiti complessi, che prima avevano sempre eseguito senza difficoltà, come occuparsi dei propri affari finanziari, prepararsi un pasto oppure fare la spesa. Potrebbero necessitare di tempi più lunghi, oppure essere meno efficienti o fare più errori rispetto al passato nelle medesime attività, ma ciononostante mantenere la loro autonomia e indipendenza. Un gran numero di soggetti con MCI non andrà mai incontro a demenza di Alzheimer e una quota di loro può anche ritornare a un profilo cognitivo normale.

Lo studio si propone principalmente di identificare il "biomarcatore" o "l'insieme di biomarcatori" in grado di predire con la maggiore accuratezza la progressione, nell'arco temporale di 2 - 3 anni, dalla diagnosi di MCI alla demenza di Alzheimer.

Inoltre si propone di definire lo scenario maggiormente sostenibile, sia dal punto di vista della trasferibilità che economico, per l'uso di biomarcatori nell'identificazione dei pazienti candidati alla prescrizione dei farmaci antidemenza attualmente in corso di sperimentazione.

Parte 2:

In cosa consiste lo studio "Conversione dal Mild Cognitive Impairment (MCI) a demenza: il contributo dei biomarcatori nell'identificare i pazienti candidati alla prescrizione di farmaci con possibile azione disease-modifying"?

Lo studio è una sperimentazione clinica coordinata dal Policlinico Gemelli di Roma e sponsorizzata dall'AIFA (Agenzia Italiana del Farmaco) e dal Minsitero della Salute che verrà condotta su circa 400 pazienti assistiti da 20 Centri specializzati in tutta Italia.

Questo studio si propone di arruolare soggetti con MCI e di confrontarne le caratteristiche al momento della diagnosi e le risposte ai biomarcatori tra coloro che progrediranno nell'arco temporale di 2 – 3 anni a demenza di Alzheimer, quelli che rimarranno in una condizione di stabilità e coloro che torneranno ad un profilo cognitivo normale..

- La partecipazione allo studio del suo familiare durerà al massimo 48 mesi.

Durante questo periodo il suo familiare dovrà presentarsi ad alcune visite presso questo Centro; dopo la prima visita è necessario che torni una volta ogni sei mesi per i necessari controlli clinici. Sarà inoltre possibile contattare il medico anche al di fuori delle visite programmate, qualora se ne dovesse verificare la necessità. Per tutta la durata della sua partecipazione allo studio, il suo Familiare NON potrà entrare in trial clinici con farmaci sperimentali.

All'inizio dello studio al suo familiare saranno effettuati i seguenti esami:

- Test neuropsicologici (MMSE Mini mental state examination, e DRF delayed-free recall – FCSRT Free And Cued Selective Reminding Test);
- Neuroimmagini funzionali con (^{18}F)FDG-PET;
- Prelievo liquor per metaboliti Beta amiloide e Tau;
- Prelievo ematico per test genetico APOE4;
- Elettroencefalografia per lo studio della connettività cerebrale.

Verrà effettuata una visita neuropsicologica, una visita medica generale e verranno raccolti tutti i dati anamnestici. Inoltre saranno somministrati (all'inizio dello studio e a tempi prestabiliti nel corso dello studio) una serie di test neuropsicologici nel corso dei quali verranno poste domande sui sintomi della malattia e verrà chiesto di eseguire alcune semplici attività.

Anche la persona che assiste il/la paziente parteciperà alla valutazione della memoria, delle capacità relative alle attività della vita quotidiana e del comportamento.

I risultati di questi test ci aiuteranno a valutare lo stato iniziale e l'eventuale progressione della malattia.

- Tutte le informazioni raccolte in questo studio rimarranno strettamente confidenziali, analogamente a quanto avviene per tutte le altre documentazioni cliniche. Le informazioni raccolte verranno inserite in un computer e analizzate, ma né il suo familiare, né alcun altro paziente dello studio potrà essere identificato quando verranno resi noti i risultati dello studio. La documentazione clinica del Suo familiare potrà essere consultata da un medico di Sua fiducia.

Quali sono i rischi legati alle metodiche di analisi ?

Le tecniche diagnostiche utilizzate possono causare eventi evversi che sono, comunque, molto rari.

Tutti gli eventi avversi devono essere comunicati tempestivamente al Centro che segue il Suo familiare e saranno prese le necessarie misure per alleviare i disturbi.

Se il Suo familiare ha una polizza assicurativa medica privata potreste voler verificare, prima di decidere se partecipare a questo studio, se l'arruolamento non interferisca con le clausule contrattuali dell'assicurazione stessa.

Quali sono i possibili benefici derivanti dalla partecipazione a questo studio?

Se il Suo familiare deciderà di partecipare a questo studio contribuirà a migliorare le nostre conoscenze su quali biomarcatori saranno utili per prevedere la progressione a demenza di Alzheimer nei soggetti con MCI. In questo modo sarà possibile utilizzare i nuovi farmaci per quei soggetti con MCI che progrediranno ad Alzheimer .

Come dovrà comportarsi il Suo familiare se deciderà di partecipare allo studio?

Se il Suo familiare deciderà di partecipare a questo studio dovrà riferire al medico che lo ha in cura tutte le medicine che sta assumendo. Il medico stabilirà se ci sono farmaci che non devono essere assunti durante il periodo dello studio. Se il Suo familiare deciderà di partecipare a questo studio non dovrebbe partecipare contemporaneamente ad altre sperimentazioni farmacologiche.

Il mio familiare è tenuto a partecipare a questo studio?

Il Suo familiare non deve necessariamente partecipare a questo studio, né deve fornire spiegazioni nel caso in cui decida di non partecipare.

Prima di decidere è opportuno che legga questo modulo informativo con attenzione e faccia domande al medico che segue il Suo familiare nel caso in cui ci fossero cose che risultano poco chiare.

Se il Suo familiare deciderà di partecipare allo studio chiederemo sia a lei che al Suo familiare di firmare un consenso informato in cui è scritto che avete compreso ogni singolo aspetto dello studio stesso.

La partecipazione del mio familiare allo studio modificherà i suoi diritti legali?

Sia che il Suo familiare partecipi o non partecipi alla sperimentazione manterrà gli stessi diritti legali di qualsiasi altro paziente assistito dal Sistema Sanitario Nazionale.

Il mio familiare potrà decidere di ritirarsi dallo studio?

In qualsiasi momento e per qualsiasi motivo il Suo familiare potrà decidere di non partecipare più alla sperimentazione e gli sarà comunque fornita la migliore assistenza possibile. Firmare il modulo del consenso informato non obbliga il Suo familiare a partecipare all'intera durata dello studio. Se dovesse cambiare idea in un secondo momento non sarà necessario fornire una spiegazione, ma sarebbe di aiuto alla nostra ricerca se accettasse di completare comunque i questionari per farci conoscere l'andamento delle sue condizioni cliniche.

Da chi è organizzato e sponsorizzato questo studio?

Questo studio è coordinato dal Policlinico A. Gemelli di Roma ed è sponsorizzato dall'AIFA e dal Ministero della Salute.

Ci sono altre domande che vorrebbe porre?

Dopo aver letto questo modulo informativo noi ci auguriamo che il Suo familiare deciderà di partecipare a questo studio. Se ci sono altre domande che vorrebbe porre al medico del Suo familiare relativamente allo studio ora o in un secondo momento si senta libero di farlo.

Troverà in calce a questo modulo il nome del medico a cui potrà rivolgersi e il numero telefonico. Se preferisce rinviare la sua decisione, ad esempio per poterne discutere con amici o parenti, può prendere appuntamento per tornare in un secondo momento. La preghiamo però di ricordarsi di conservare questi moduli in un posto sicuro e di appuntarsi i nomi e numeri di telefono nella sua agenda. Grazie.

Per ulteriori informazioni la preghiamo di contattare:

Dr. …….. Numero di telefono: ……..

Annex 2. Neuropsychological tests and rating scales

The neuropsychological assessment includes: a) an assessment of subjective complaints and of the awareness of cognitive modifications by the subject and his/her relatives/close acquaintances; b) a formal assessment of the main cognitive domains; c) a quantitative functional evaluation; d) a global clinical judgement of severity. These four aspects are jointly required to diagnose a MCI condition, which is the entry criteria for the study (subjective complaints, objective cognitive impairment, no relevant functional impairment, no dementia).

a) The importance of a quantitative assessment of subjective complaints in the AD continuum has emerged only recently (see for example the INSIGHT-AD study- Cacciamani et al., 2017). There is a limited choice of tools standardized for the Italian language. We will adapt 2 instruments validated in English:

-Cognitive Function Instrument (CFI; Walsh et al., 2006; an Italian adaptation is under way): 14 questions, given to the subject and to an informant. In a longitudinal study with a 4-year follow-up (Amariglio et al., 2015), the combination of CFI and CDR was highly predictive of cognitive impairment progression .

-Healthy Aging Brain Care Monitor (HABC-M; Monahan et al., 2012; 2014), assessing how frequently the subject has met difficulties in daily life during the last 2 weeks, as judged by the subject and by an informant. The difficulties may involve 3 macro-domains: cognitive (6 questions), functional (11 questions) and psycho-behavioural (10 questions).

b) Besides the screening test (MMSE), the neuropsychological battery includes the following tests:

<u>Memory:</u>

The assessment of episodic memory is required as a core criterion for the diagnosis of a typical presentation of AD:

-Free and Cued Selective reminding according to the Italian version (Frasson et al. 2011; Clerici et al. 2017): it is an episodic memory test controlling for encoding of information and for the effectiveness of cueing during retrieval, following the recommendations of the IWG.

-Episodic Memory Score (Marra et al., 2016), a composite measure based on the score of Rey's wordlist learning test (Gainotti et al., 2001) and the delayed recall of Rey's figure (Caffarra et al., 2002).

<u>Language:</u>

-The SAND battery (Catricalà et al., 2017) is a brief but comprehensive language test, which has been standardized and fully validated in the Italian population.

-Semantic and phonological fluency (Novelli et al., 1986) are tests of language and executive function with a high sensitivity for both typical and language-based presentations of AD.

Visuospatial function:

An in-depth assessment is required, given the possible posterior atypical presentations of AD (posterior cortical atropy, PCA):

-copy of Rey's figure (Caffarra et al., 2002);

-visual Object and Space Perception Battery (Warrington and James, 1991): it is a brief but comprehensive evaluation of the visuo-perceptual function, which is sensitive to both ventral and dorsal presentations of PCA. Normative data are not available for the Italian population, but this is not a major limitation, given the non verbal nature of the test.

Executive function and behaviour:

The assessment of the executive function and behaviour is required for the diagnosis of atypical frontal presentations of AD, and is based on tests and rating scales:

-Trail Making Test (Giovagnoli et al., 1996): a classical brief test of psychomotor efficiency.

-Stroop Coulour Word test (Caffarra et al., 2002): a classical test of selective attention and resistence to interference.

-Frontal Assessment Battery (Appollonio et al., 2005): a brief scale for behavioural assessment.

-Neuropsychiatric Inventory (Binetti et al., 1998): the neuropsychiatric scale most widely used in neurodegenerative disorders.

c) Functional assessment shall be based on the Amsterdam IADL (Koster et al., 2015). It is an informant-based evaluation of the level of functioning in complex activities of daily life, which has been demonstrated to be sensitive to change.

d) The inclusion requires a score of 0,5 at Clinical Dementia Rating (CDR; Hughes et al., 1982; Hayman et al., 1987), a widely diffused clinical scale aiming at the quantification of symptom severity and disease stage.