A Strategic Research Agenda to the Biomarker-Based Diagnosis of Prodromal Alzheimer's Disease

Giovanni B. FRISONI, Departments of Psychiatry and Internal Medicine, University Hospitals and University of Geneva, Switzerland

Marina BOCCARDI, LANVIE – Laboratory of Neuroimaging of Aging of Aging, Department of Psychiatry, University of Geneva, Switzerland; LANE - Laboratory of Alzheimer Neuroimaging & Epidemiology, IRCCS S. Giovanni di Dio - Fatebenefratelli, Brescia, Italy

Frederik BARKHOF, VUMC, Amsterdam, The Netherlands, and Institutes of Neurology & Healthcare Engineering, UCL, London, UK and ESNR – European Society of Neuroradiology

Kaj BLENNOW, Inst. of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at University of Gothenburg, and Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden; International Federation of Clinical Chemistry and Laboratory Medicine Working Group for CSF proteins (IFCC WG-CSF)

Stefano CAPPA, IRCCS Fatebenefratelli, Brescia, Italy, and FENS – Federation of European Neuropsychological Societies

Konstantinos CHIOTIS, Department of Neurobiology, Care Sciences and Society, Center for Alzheimer Research, Translational Alzheimer Neurobiology, Karolinska Institutet, Stockholm, Sweden

Jean-Francois DÉMONET, Leenards Memory Center, Department of Clinical Neuroscience, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Valentina GARIBOTTO, Nuclear Medicine and Molecular Imaging Division Geneva University and Geneva University Hospitals

Panteleimon GIANNAKOPOULOS, Department of Mental Health and Psychiatry, University Hospitals of Geneva, Geneva, Switzerland.

Anton GIETL, University of Zurich, Institute for Regenerative Medicine-IREM, Switzerland

Oskar HANSSON, Memory Clinic, Skåne University Hospital and the Clinical Memory Research unit, Lund University, Sweden

Karl HERHOLZ, The University of Manchester Wolfson Molecular Imaging Centre, Manchester, UK

Clifford R JACK Jr, Department of Radiology, Mayo Clinic, Rochester, MN, USA

Flavio NOBILI, Department of Neuroscience (DINOGMI), University Genoa and IRCCS AOU San Martino-IST Genoa, Italy, and EANM – European Association of Nuclear Medicine

Agneta NORDBERG, Department of Neurobiology, Care Sciences and Society, Center for Alzheimer Research, Translational Alzheimer Neurobiology, Karolinska Institutet; Department of Geriatric Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden

Heather M. SNYDER, Alzheimer's Association, Medical & Scientific Relations, Chicago, USA

Mara TEN KATE, VU University Medical Center, Amsterdam, the Netherlands

Andrea VARRONE, Karolinska Institutet, Department of Clinical Neuroscience, Centre for Psychiatry Research, Stockholm, Sweden

Emiliano ALBANESE, Department Psychiatry, University Hospitals and University of Geneva, Switzerland

Patrick BOSSUYT, Clinical Epidemiology at the University of Amsterdam, The Netherlands, and EFLM – European Federation of Laboratory Medicine

Maria CARRILLO, Alzheimer's Association, Chicago, USA

Chiara CERAMI, Vita-Salute San Raffaele University and San Raffaele Scientific Institute, Milan, Italy

Bruno DUBOIS, Institut de la Mémoire et de la Maladie d'Alzheimer, Hôpital Pitié Salpêtrière, UPMC University Paris 6, France

Valentina GALLO, Queen Mary, University of London

Ezio GIACOBINI, Department of Internal Medicine, Rehabilitation and Geriatrics, University Hospitals and University of Geneva, Switzerland

Gabriel GOLD, Service of Geriatrics, Department of Internal Medicine Rehabilitation and Geriatrics, University Hospitals of Geneva, Geneva, Switzerland

Samia HURST, Institute for Ethics History and the Humanities, University of Geneva Medical School, Geneva, Switzerland

Anders LÖNNEBORG, Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University

Karl-Olof LOVBLAD, Head of Diagnostic and Interventional Neuroradiology, University Hospital of Geneva, Switzerland

Niklas MATTSSON, Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University; Memory Clinic, and Department of Neurology, Skåne University Hospital, Sweden

José-Luis MOLINUEVO, BarcelonaBeta Brain Research Center, Pasqual Maragall Foundation, Barcelona, Spain

Andreas MONSCH, Association of Swiss Memory Clinics, University Center for Medicine of Aging, Basel, Switzerland; University of Basel, Switzerland

Urs MOSIMANN, Department of Old Age Psychiatry, University of Bern, Switzerland

Alessandro PADOVANI, Department of Clinical Neurosciences; Dean, Faculty of Medicine; University of Brescia, Italy

Agnese PICCO, University of Genoa, Department of Neuroscience (DINOGMI), Clinical Neurology, Genoa, Italy; Memory Clinic and LANVIE – Laboratory of Neuroimaging of Aging, University Hospitals and University of Geneva, Switzerland

Corinna PORTERI, Bioethics Unit, IRCCS S. Giovanni di Dio - Fatebenefratelli, Brescia, Italy

Osman RATIB, Department of Radiology and Head of the Division of Nuclear Medicine at the University Hospital of Geneva, Switzerland

Laure SAINT-AUBERT, Department of Neurobiology, Care Sciences and Society, Center for Alzheimer Research, Translational Alzheimer Neurobiology, Karolinska Institutet, Stockholm, Sweden

Charles SCERRI, Department of Pathology, Faculty of Medicine & Surgery, University of Malta, Malta; Alzheimer's Europe, Luxembourg

Philip SCHELTENS Alzheimer Center, VU University Medical Center, Amsterdam, The Netherlands

Jonathan SCHOTT, Dementia Research Centre, Department of Neurodegeneration, UCL Institute of Neurology, London, UK

Ida SONNI, Division of Nuclear Medicine and Molecular Imaging, Stanford University, CA, USA, former visiting researcher in the PET Center of the Department of Clinical Neurosciences, Karolinska Institutet, Stockholm, Sweden

Stefan TEIPEL, German Center for Neurodegenerative Diseases (DZNE) - Rostock/Greifswald, Rostock, Germany; Department of Psychosomatic Medicine, University of Rostock, Rostock, Germany

Paolo VINEIS, Faculty of Medicine, Imperial College London, UK

Pieter-Jelle VISSER, Alzheimer Center & Department of Neurology, Neuroscience Campus Amsterdam, VU University, Medical Center, Amsterdam, Netherlands; Department of Psychiatry & Neuropsychology, School for Mental Health and Neuroscience, Maastricht University, Maastricht, the Netherlands

Yutaka YASUI, University of Alberta, Edmonton, Canada

Bengt WINBLAD, European Alzheimer's Disease Consortium; Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet Huddinge, Sweden

Correspondence to:

Giovanni B Frisoni, University Hospitals and University of Geneva, Geneva, Switzerland, giovanni.frisoni@gmail.com

Abstract

Biomarkers sensitive to functional impairment, neuronal loss, tau, and amyloid pathology based on MR, PET, and CSF studies are increasingly used to diagnose Alzheimer's disease (AD), but clinical validation is incomplete, hampering reimbursement by payers, widespread clinical implementation, and impacting on health care quality. An expert group convened to develop a strategic research agenda to foster the clinical validation of AD biomarkers. These demonstrated sufficient evidence of analytical validity (phase I of a structured framework adapted from oncology). Research priorities were identified based on incomplete clinical validity (phases II and III), and clinical utility (phases IV and V). Priorities included: definition of the assays; reading procedures and thresholds for normality; performance in detecting early disease; accounting for the effect of covariates; diagnostic algorithms comprising combinations of biomarkers; and developing best practice guidelines for the use of biomarkers in qualified memory clinics in the context of phase IV studies.

Glossary

Biomarker. An objective measure of a biological or pathogenic process with the purpose of evaluating disease risk or prognosis, guiding clinical diagnosis or monitoring therapeutic interventions. While the term originally referred to traceable substances produced by or introduced into an organism, it later evolved to any measurable parameter, including those obtained via imaging procedures.

Roadmap. Objective-oriented, structured, and efficient action plan. In science and technology also called "strategic research agenda".

*Alzheimer's disease (AD) dementia***.** Traditionally and according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria, Alzheimer's disease was defined as a syndrome with progressive cognitive impairment severe enough to impact on daily activities. A diagnosis of Alzheimer's disease could only be made after exclusion of other possible causes.[1] Sixty-five to 80% of cases of patients fulfilling these criteria have Alzheimer's pathology (plaques and tangles), the remainder having a range of other pathologies. In order to increase diagnostic certainty, contemporary criteria for AD dementia incorporate biomarker evidence for different aspects of Alzheimer's pathology, including imaging (magnetic resonance imaging - MRI - measures of atrophy; ^{18}F-fluorodeoxyglucose-positron emission tomography - FDG-PET - measures of cerebral hypometabolism; amyloid PET measures of fibrillar β-amyloid - Aβ - deposition) and cerebrospinal fluid - CSF (decreased levels of Aβ42, increased levels of tau and phospho-tau).[2,3]

*Alzheimer's disease process***.** Recognizing that AD pathology is present many years before symptoms emerge, new criteria classify the disease process on a continuum from asymptomatic to prodromal and finally to dementia stage.[4] Individuals at the asymptomatic stage can only be identified by biomarkers of Alzheimer's pathology. Nonetheless, it is still unclear to what extent biomarker positivity in the asymptomatic stage predicts clinical symptoms in the future.

Prodromal Alzheimer's disease. Alzheimer's pathology at a symptomatic stage but with yet no disability (MCI).

Alzheimer's pathology. The major hallmarks are extracellular plaques and intraneuronal neurofibrillary tangles consisting mainly of β-amyloid and hyperphosphorylated tau, respectively. Neuronal and synaptic loss usually but not invariably co-localize with tangles. Three pathological variants are recognized based on the distribution of tangles: typical (limbic and neocortical), limbic predominant, and hippocampal sparing.[5]

Biomarker development and validation. The process of discovery, analytical validation, clinical validation, and demonstration of clinical utility. A structured framework has been developed using the paradigm of oncology biomarker development and validation comprising 5 phases: (i) preclinical exploratory studies; (ii) clinical assay development for overt disease; (iii) retrospective longitudinal repository studies; (iv) prospective diagnostic studies, and (v) disease control studies.[6]

Mild Cognitive Impairment (MCI). A syndrome of acquired cognitive impairment without functional limitation. As with dementia, MCI is a heterogeneous entity with different underlying pathologies.

In memory clinics, depending on the threshold for cognitive impairment (performance on cognitive testing 1 to 1.5 age- sex- and education-adjusted standard deviations below the mean), up to two thirds of patients with amnestic MCI have underlying Alzheimer's pathology (prodromal AD), 15-25% have neurodegenerative disease other than Alzheimer's (hippocampal sclerosis, tangle-only dementia, primary age-related tauopathy, frontotemporal degeneration, pure cortical Lewy body disease, etc.), and the remaining represent variations of normal ageing.[7–9] MCI is the condition of interest of the effort described in this paper.

Introduction

In the last decade, the research definition of Alzheimer's disease (AD) and associated forms of dementia has been moving from a clinico-pathological paradigm to a more biological approach, integrating emerging evidence for the specific underlying pathophysiology associated with the disease. Biomarkers including hippocampal atrophy on magnetic resonance imaging (MRI), cortical hypo-metabolism on ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET), decreased Aβ42 and increased tau and phospho-tau in the cerebrospinal fluid (CSF), and increased retention of amyloid tracers on PET are now incorporated in contemporary research diagnostic criteria.[10,11] Other biomarkers can be exclusionary, providing information about systems that should be typically preserved in AD, namely the dopaminergic system.

Despite research criteria are not supposed to be used in clinical settings, many academic memory clinics use the latest research criteria for AD in their routine practice. However, the lack of a consistent framework to assess the validity of biomarkers for AD has led to high heterogeneity of their operationalisation and inconsistent reimbursement by payers – all factors negatively affecting the provision of high quality care to patients. This paper summarizes an international and interdisciplinary effort to develop a strategic research agenda (or "roadmap") to accelerate the evaluation and adoption of biomarkers for AD for use in clinical practice.

The following sections outline the clinical context and need for AD biomarkers, discuss issues related to biomarker development and validation with specific reference to AD, outline the gaps in our knowledge on AD biomarkers, present a structured framework for biomarker originally developed for cancer, and propose a list of research priorities for AD biomarker validation. This paper focuses on detecting the AD process, as defined in the Glossary, at the MCI stage, as the clinical condition of interest, having the highest diagnostic uncertainty and stakes.

1. The clinical context: the diagnosis of prodromal AD requires biomarkers

In 1906, Alois Alzheimer's defined the disease that was later to carry his name, as a clinico-pathological condition consisting of progressive cognitive impairment and behavioural change underpinned by senile plaques and neurofibrillary tangles in brain grey matter.[12] Decades later, plaques and neurofibrillary tangles were found to be composed of β-amyloid and hyper-phosphorylated tau protein respectively,[13,14] and the clinical symptoms were shown to correlate with synaptic and neuronal loss.[15,16] The first NINCDS-ADRDA criteria for AD were developed in 1984 at a time when biomarker development for AD was still in its infancy; accordingly, at this stage a diagnosis of AD was based on clinical findings alone, with biomarkers, especially brain imaging, used only to exclude other causes for cognitive decline. [1]

Panel 1. Consolidated biomarkers for the diagnosis of prodromal Alzheimer's disease (AD). Tau-PET is still under development and not reported.				
Exam	**Marker**	**Abnormality**	**Pathology**	**Target**
MRI	Regional anatomy	↓ volume of hippocampus and other temporal lobe structures	Tissue loss, neurodegeneration	AD
PET	^{18}F-fluorodeoxy-glucose (FDG)	↓ uptake in posterior cingulate-precuneus and temporo-parietal cortex	Glucose hypometabolism, neurodegeneration	AD
PET	^{11}C-PIB, ^{18}F tracers (florbetapir, flutemetamol, florbetaben)	↑ cortical retention	Deposition of cortical β-amyloid	AD
CSF	Aβ42 or Aβ42/ Aβ40 ratio	↓ concentration, ↓ ratio	Abnormal metabolism of β-amyloid	AD
CSF	Total tau, phospho-tau	↑ concentration	Neuronal damage, and accumulation of tau pathology. P-tau is more specific for AD neurodegeneration	AD

Over subsequent decades, considerable technological advances in imaging (PET and MRI) and CSF analysis, allowed the development of biomarkers related to neurodegeneration, cerebral β-amyloid deposition and tau-pathology (Panel 1). Based on an extensive body of literature demonstrating their analytical and early clinical validity, diagnostic criteria integrating these biomarkers were proposed with the aim of moving from an exclusionary approach to a positive diagnosis, re-conceptualizing AD from the clinico-pathological condition originally described by Alois Alzheimer.[2,3,10,17] Noting that changes in these biomarkers can already be identified when cognitive impairment is mild and in the absence of functional disability (so-called prodromal, or mild cognitive impairment – MCI – stage)[11] new diagnostic criteria incorporating biomarkers now allow for a diagnosis of AD before the development of full-blown dementia (Panel 2).[2,10,17,18] Biomarkers can also be used in the full-blown dementia stage for a more accurate aetiologic diagnosis[19,20], and, potentially, to screen the general population for persons at high risk of entering the symptomatic stages.[18,21]

Panel 2. Biomarkers in the clinical context of the diagnosis of prodromal Alzheimer's disease (AD) in patients with mild cognitive impairment.

Individual with cognitive impairment satisfying MCI criteria [22–24]

- cognitive complaints reported by the patient, relatives, or physician
- new onset of cognitive complaints for at least 6 months
- complaints of episodic memory but occasionally of language, visuospatial tasks, or topographic disorientation)
- independent for daily activities although some may be performed at a lower level than previously
- major behavioral disturbances mostly absent or mild (sleep disorders, apathy, depression); if dominant, other diagnoses, e.g. frontotemporal lobar degeneration (FTLD) or dementia with Lewy-bodies (DLB) should be considered
- neurological exam is normal; if parkinsonism is present, differential diagnosis with DLB or rare genetic forms of AD or FTLD should be considered
- mini-mental state examination score between 24 and 30 inclusive
- consistent abnormal performance, compared to age- and education-specific mean, on memory test
- symptoms not explained by psychiatric history and assessment
- structural imaging and lab exams exclude a non-degenerative or metabolic cause

Biomarker assessment

- Absence of medial temporal (mainly hippocampal) atrophy on MRI in typical (memory) presentations makes AD less likely. Atypical (neocortical) presentations of AD may spare the medial temporal regions especially in younger patients. The presence of medial temporal atrophy supports a neurodegenerative process, including but not limited to AD (e.g. DLB, FTLD).
- Reduced cortical metabolism on fluorodeoxyglucose PET in posterior cingulate-precuneus and temporo-parietal cortex increases the likelihood that AD is the cause of the cognitive impairment. In this context, complete normality of FDG-PET militates against a neurodegenerative disease.
- Abnormal CSF protein profile indicating abnormal amyloid metabolism (low Aβ42, a low Aβ42/Aβ40 ratio) and neuronal damage (high tau and phospho-tau) increases the likelihood that AD is the cause of the cognitive impairment. The combination of a normal CSF Aβ42 and a normal CSF Aβ42/Aβ40 ratio makes AD very unlikely.
- Absence of brain amyloidosis on amyloid PET makes AD a very unlikely cause of the cognitive impairment. Positive amyloid PET supports cognitive impairment to be due to AD in younger patients, where the *a priori* risk of being amyloid positive is lower than in older persons, where a significant proportion of cognitively intact individuals are amyloid positive.
- Positivity to one biomarker of amyloidosis and one biomarker of neurodegeneration is strongly associated with progression over time and development of disability and dementia within 5-7 years.

Diagnosis

- MCI patients with positive AD biomarkers are diagnosed as *MCI due to AD* or *prodromal AD*[2,11]

2. Peculiar challenges of AD biomarker development, validation and use in the clinic

Clinical needs, scientific knowledge, technical developments, the regulatory milieu, and market opportunities are the determinants of the current and future use of diagnostic AD biomarkers in the clinic. This section illustrates key challenges that should be taken into account to foster their clinical use.

2.1 Diagnostic pressure leads to the development of local diagnostic tests for AD. Patients' expectations of increasingly accurate diagnosis and little incentive for biotech or pharma companies to engage in the effortful procedures for biomarker development, validation, market approval and reimbursement stimulate the local development of tests and cut-off values in individual laboratories which are used to guide diagnosis and treatment.[25] In the AD field, examples are hippocampal volumetry[26] and metrics of cortical hypometabolism[27,28] for both of which numerous methods are used. These tests have undergone variable standardization and, with few notable exceptions,[29,30] fall short of approval for clinical use by regulatory agencies. They are nevertheless often used to guide diagnosis and symptomatic treatment in the clinical setting under local responsibility [31,32].

2.2 Local diagnostic tests for AD tend to enter clinical use before regulatory approval. The promise of AD biomarkers to allow earlier and more precise diagnosis leads clinicians to use them even in the absence of regulatory approval. Since at stake is diagnosis rather than drug treatment, the underlying assumption may implicitly be backed by physicians that the margin of error potentially intrinsic in a not approved biomarker would not cause direct harm to patients. However, the use of a diagnostic test with poor performance or insufficient validity can have important implications: poor sensitivity (leading to false negatives) may result in a patient being given false reassurance as well as being excluded from appropriate treatments or access to trials. Poor specificity may result in over-diagnosis and cause unnecessary anxiety, over-treatment and inappropriate inclusion in clinical trials, exposing patients to unnecessary side-effects as well as diluting potential treatment effects.[33] Physicians should be fully aware of the clinical and ethical implications of the pre-regulatory use of biomarkers.

2.3 The validation of some diagnostic tests for AD is incomplete. Standardization and validation efforts are heterogeneous across biomarkers. Whilst some biomarkers are the subject of one or more initiatives aimed at standardization or utility analysis, others are relatively less investigated. For example, despite being widely regarded as a useful biomarker for the early detection of AD,[34,35] there is currently no structured program to standardize the readout of cortical hypometabolism as measured using FDG-PET.

The assessment of the diagnostic performance of AD biomarkers in representative and real-world populations (phase IV of Panel 3) is of particular relevance when it comes to implementing biomarkers in the clinical routine. Indeed, the highly selected samples that are used for the biomarker evaluation in phases II and III may not be representative of clinical routine populations with regard to co-morbidity, socio-economic status, and education – such that biomarkers may perform differently in phase III and phase IV samples.[36] To date, there are no data on changes in health outcomes (disability, mortality, morbidity, quality of life) attributable to the use of AD biomarkers. This is in part due to the current lack of disease modifying drugs, access to which is

recursively likely to depend on fulfilment of biomarker supported criteria. Developing phase IV studies is further hampered by the lack of a consistent methodological framework for biomarker validation.

2.4 Synergies among research initiatives addressing biomarker standardization. To address many of the issues listed above, a number of AD biomarker validation programs have been launched. The Alzheimer's Association leads the Global Biomarker Standardization initiatives, including both the Association's *External Quality Control Program for CSF Biomarkers*[37] and sponsored the EADC-ADNI *Harmonized Protocol for Manual Hippocampal Segmentation on Magnetic Resonance* (HarP).[38] More recently, the Joint Programming Neurodegenerative Diseases (JPND) of the European Commission has funded *BiomarkAPD*[39], the aims of which are similar to those of the *External Quality Control Program for CSF Biomarkers*. The Radiological Society of North America has launched the Quantitative Imaging Biomarkers Alliance (QIBA) to unite researchers, healthcare professionals, and the industry to advance quantitative imaging and the use of imaging biomarkers in clinical trials and clinical practice (https://www.rsna.org/qiba/, 2016). For amyloid imaging, the Alzheimer's Association leads the *US Imaging Dementia—Evidence for Amyloid Scanning (*IDEAS) study, with management by the American College of Radiology Imaging Network (https://clinicaltrials.gov/ct2/show/NCT02420756). In Europe, the European Commission and EFPIA have recently funded the *Amyloid imaging to Prevent Alzheimer's Disease* (AMYPAD, http://www.amypad.org/, 2016).

Whilst these initiatives address important issues related to the standardisation and/or clinical utility of biomarkers, it is notable that each focuses on one biomarker or class of biomarkers. In the absence of a common frame of reference it is not straightforward for synergies to be exploited, and collaborative efforts to be harnessed.

2.5 The impact of AD biomarker research on clinical practice should be improved. Despite a very extensive literature which has amassed a huge amount of evidence both in the form of single centre, multi-centre and in many cases meta-analytical data supporting the use of biomarkers for the diagnosis of AD, practices are very diverse in how biomarkers are used and reimbursed for across the world (Panel 4). *FDG-PET* is currently reimbursed today by US Centers for Medicare and Medicaid Services (CMS, https://www.cms.gov/Regulations-and-Guidance/Guidance/Manuals/downloads/ncd103c1_part4.pdf) with indication to exclude AD in patients who meet diagnostic criteria for both AD and fronto-temporal dementia. Most national health systems in Europe authorize reimbursement of FDG-PET for the differential diagnosis between AD and frontotemporal dementia. It should be noted that what is being reimbursed are image acquisition and the traditional visual subjective readout,[40] which is operator-dependent.[41]

MRI scanning is reimbursed widely, but often the indication is the exclusion of alternative causes (non-degenerative or surgical) of cognitive impairment rather than the positive diagnosis of AD through assessment of, e.g., medial temporal atrophy. When surveyed in 2009, CSF biomarkers were reimbursed in only about half of European countries.[42] In a recent survey in Italy, besides heterogeneous use, CSF biomarkers turned out to be reimbursed by the national health system only in one (Umbria) out of the 20 Italian regions.[43]). At the current time, amyloid PET is available in the clinic in some European countries, albeit with significant limitations (Panel 4).

2.6 The impact of non-evidence-based factors on the use of diagnostic AD biomarkers in the clinic. The inconsistent evidence on biomarker utility has led to most biomarkers largely being excluded from available evidence-based guidelines. Importantly, the current lengthy guideline development procedures do not provide ready means for such criteria to be easily updated as more evidence accumulates. The American Academy of Neurology's practice parameters for the diagnosis of dementia, now over a decade old, state that there is not enough evidence to support or refute the use of PET and CSF or other biomarkers for AD.[44] The more recent European guidelines state that in clinical practice, CT and MRI should be used to exclude (usually non-degenerative) causes of dementia (class I evidence), and that diagnostic biomarkers are only needed in a proportion of cases. Here, FDG-PET and CSF biomarkers are rated as class II and class III evidence for discriminating AD, FTLD and DLB.[45] The only recommendations emphasizing the use of a biomarker to make a specific diagnosis of AD are those for amyloid PET, the biomarker with the greatest financial implications.[46] Whilst a number of local (either national, regional or individual centre) guidelines recommend the use of biomarkers, this is usually driven by active scientific groups or societies, again reflecting the lack of a common framework to ensure consistency.[47,48]

The lack of consistent guidelines and recommendations may lead clinicians to use biomarkers based on practical considerations, reflective of resources and experience, rather than clinical and evidence-based considerations. A few years ago, an Italian study on imaging biomarkers showed that the choice of the imaging technique (among CT, MRI and FDG-PET) in the workup of dementia was driven as much by the exam availability, by the physicians' familiarity with that technology, and by the length of patients waiting list for that exam, as by the proper clinically relevant parameters, such as the patient's age, severity of cognitive impairment, and diagnostic question (e.g. clinical suspicion of cerebrovascular disease).[49] Where there are alternative means of determining the same pathology, financial considerations may take precedence over other factors. In the case of brain amyloid detection, there are pros and cons of using amyloid PET or CSF in terms of availability, reproducibility, cost, and doctors' and patients' acceptance and attitude towards the methods.[50,51] In France however, CSF examination is preferred over amyloid PET which is not reimbursed although it has been authorized.[52]

Despite these limitations, the increasing availability of biomarkers and pressure by better informed patients makes urgent the development of recommendations for the use of biomarkers for diagnosis in the MCI population.

2.7 The use of diagnostic AD biomarkers in a time devoid of disease modifying drugs. The net result of all of the issues discussed above is a delayed and uncoordinated penetration of biomarker use in the clinic. As disease-modifying drugs for AD, with potentially significant benefits but possible side-effects,[53,54] become available, determining the diagnosis of AD as certainly as possible in prodromal stages will be vital; and despite some drawbacks with several such trials still due to report imminently we cannot wait until licensing to begin to alter clinical practice. Moreover, even in the absence of disease modifying drugs, the advantages of an accurate diagnosis justify the use of (relatively) advanced diagnostic technology.[55] Knowing the aetiology of an individual's cognitive impairment before the dementia stage already allows for the delivery of timely and appropriate personalized care, including appropriate counselling and future planning, preventing the use of inappropriate medications and ancillary investigations, taking eventually

appropriate steps to prevent unsafe behaviours (e.g., driving), allowing access to currently available symptomatic drugs,[55] and recruitment to research and clinical trials. In Europe and the US less than 50% of people with clinical dementia receive a formal diagnosis of dementia in primary care.[56] With limited access to specialized care for the majority of cases and countries the penetration of validated biomarkers into routine care may leverage specialized care resources to provide broader access to diagnosis and treatment options for the entire population of people with manifest symptoms of dementing neurodegenerative disorders.

Panel 3. The 5-phase framework for the development of cancer screening biomarkers in the population, adapted to AD biomarkers for the early diagnosis of AD in clinical settings. From Boccardi et al., 2016.[20]

Phase	Primary and secondary aims (PA, SA)	Adaptations from cancer to dementia
I — Preclinical Exploratory Studies	PA—To identify leads for potentially useful biomarkers and (ii) prioritize identified leads.	No significant change. Gold standard is pathology.
II — Clinical Assay Development for Overt Dementia	PA—To estimate the true and false positive rate or ROC curve and assess ability to distinguish subjects with and without AD dementia. SA 1—To optimize procedures for performing the assay and to assess the reproducibility of the assay within and between laboratories. SA 2—To determine the relationship between biomarker measurements made on tissue (phase I) and the biomarker measurements made on the noninvasive clinical specimen (phase II). SA 3—To assess factors (e.g. gender, age, etc.), associated with biomarker status or level in control subjects. If such factors affect the biomarker, thresholds for test positivity may need to be defined separately for target subpopulations. SA 4—To assess factors associated with biomarker status or level in diseased subjects—in particular, disease characteristics.	"Established disease" in cancer is believed to correspond to "overt dementia" in AD. The preferable standard of reference is Alzheimer's pathology. Alzheimer's dementia is acceptable where there is reason to believe that the majority have Alzheimer's pathology (e.g. NINCDS-ADRDA probable Alzheimer's dementia.[3]
III — Retrospective Longitudinal Repository Studies	PA 1—To evaluate the capacity of the biomarker to detect AD at the MCI/prodromal stage. PA 2—To define criteria for a positive screening test in preparation for phase IV. SA 1—To explore the impact of covariates on the discriminatory abilities of the biomarker before clinical diagnosis. SA 2—To compare markers with a view to selecting those that are most promising. SA 3—To develop algorithms for positivity based on combinations of markers. SA 4—To determine a biomarker testing interval for phase IV if repeated testing is of interest	In oncology, phase III is retrospective nested case-control studies of longitudinal cohorts. This is not feasible in AD for the scarcity of studies where biomarkers have been collected in the past and current presence of early Alzheimer's disease (MCI due to AD/prodromal AD) is known. This design is replaced by prospective longitudinal repository studies, where the biomarker is measured at baseline in MCI patients, and Alzheimer's disease status ascertained at follow-up. The preferable standard of reference is Alzheimer's pathology, but incident Alzheimer's dementia or cognitive progression are also acceptable. As in phase III cancer studies, biomarker results are not used for diagnosis and treatment.
IV — Prospective Diagnostic Studies	PA—To determine the operating characteristics of core AD biomarkers in MCI patients in a memory clinic setting by determining the detection rate and the false positive rate. SA 1—To describe the characteristics of disease detected by the biomarker test—in particular, with regard to the potential benefit incurred by early detection. SA 2—To assess the practical feasibility of implementing the case finding program and compliance of test-positive subjects with work-up and treatment recommendations. SA 3—To make preliminary assessments of the effects of biomarker testing on costs and mortality associated with the disease. SA 4—To monitor disease occurring clinically but not detected by the biomarker testing protocol.	The major difference with phase IV in oncology is that studies will not involve clinically asymptomatic, but rather symptomatic and non-demented (MCI) patients. Specific to AD is also the need of highly specialized clinics and clinical guidelines for the collection, measurement, and interpretation of the biomarkers. As in oncology, biomarker results will be used for diagnosis and treatment.
V — Disease Control Studies	PA—To estimate the reductions in AD-associated mortality, morbidity, and disability afforded by the diagnostic test. SA 1—To obtain information about the costs of biomarker testing and treatment and the cost per life saved or per quality-adjusted life year. SA 2—To evaluate compliance with testing and work-up in a diverse range of settings. SA 3—To compare different biomarker testing protocols and/or to compare different approaches to treating test positive subjects in regard to effects on mortality and costs.	No adaptation is currently required. However, the current lack of AD modifiers make the achievement of phase V outcome unlikely.

Panel 4. Indication for prescription and reimbursement of biomarkers examination in the work-up for the diagnosis of dementing neurodegenerative disorders. AD: Alzheimer's disease. FTLD: frontotemporal lobar degeneration. PD: Parkinson's disease. PDD: dementia of Parkinson's disease. VD: vascular dementia.				
Country	MR	FDG-PET	Amyloid-PET	CSF
France	INDICATION: Not restricted. REIMBURSEMENT: social security and private insurance.	INDICATION: early diagnosis of AD / atypical presentation / diagnostic doubt of FTLD. Useful in the diagnosis of probable AD. REIMBURSEMENT: social security and private insurance.	INDICATION: to estimate β-amyloid neuritic plaque density, in combination with clinical evaluation, for the diagnosis of AD in patients with cognitive decline. REIMBURSEMENT: no	INDICATION: not restricted REIMBURSEMENT: social security and private insurance.
Germany	INDICATION: Not restricted. REIMBURSEMENT: yes, health care insurance. [GE]	INDICATION: criteria not defined. REIMBURSEMENT: no. Individual exceptions should be negotiated with health care insurance, but criteria for reimbursement are not clearly defined.	INDICATION: see above. REIMBURSEMENT: No	INDICATION: ordinary memory assessment in tertiary clinic. Sometimes in specialized physician practices. REIMBURSEMENT: yes, health care insurance. Reimbursement in outpatient care does not cover costs and lumbar puncture is often performed in inpatient or day clinic settings.
Italy	INDICATION: routine use of imaging techniques for the differential diagnosis of disorders causing dementia is not recommended. Indicated for the differential diagnosis between AD and VD and between AD and FTLD. REIMBURSEMENT: in practice, acquisition without contrast is reimbursed once during the disease by the National Health Service at €250-300.	INDICATION: recommended for the differential diagnosis between AD and VD, and between AD and FTLD.[I] REIMBURSEMENT: by the National Health Service	INDICATION: see above. REIMBURSEMENT: The procedure is reimbursed at the same level than brain FDG-PET. Additional cost should be covered by the hospital budget.	INDICATION: no formal indication. REIMBURSEMENT: no. Reimbursed by the National Health Service only in one region – Umbria[43]).
Netherlands	INDICATION: evaluation of dementia. REIMBURSEMENT: Healthcare insurance.	INDICATION: FTLD; unexplained dementia. REIMBURSEMENT: Healthcare insurance	INDICATION: see above. Only selected cases. REIMBURSEMENT: under discussion.	INDICATION: evaluation of dementia, especially in younger cases. REIMBURSEMENT: Healthcare insurance
Spain	INDICATION: no formal indication. REIMBURSEMENT: social security.	INDICATION: no formal indication. In most guidelines: for differential diagnosis of AD with other dementias REIMBURSEMENT: social security.	INDICATION: see above.[SP] REIMBURSEMENT: social security	INDICATION: no formal indication. In practice, frequently used in the same population as amyloid PET. REIMBURSEMENT: no.
Sweden	INDICATION: used in ordinary memory assessment in tertiary clinic. REIMBURSEMENT: by the clinic.	INDICATION: used in tertiary clinics when diagnosis is still unclear after ordinary memory assessment. REIMBURSEMENT: by the clinic.	INDICATION: used when some special cases when diagnosis is still unclear after ordinary memory assessment at tertiary clinic REIMBURSEMENT: by the clinic	INDICATION: used in ordinary memory assessment in tertiary clinic. REIMBURSEMENT: by the clinic.

Switzerland	INDICATION: no restriction for use. REIMBURSEMENT: health insurance.	INDICATION: second level investigation in unclear cases, after a visit by a neurologist, psychiatrist or geriatrician, below 80 years of age, MMSE of 10 or higher, max. disease duration of 5 y, no previous brain PET or SPECT.[CH1] REIMBURSEMENT: health insurance.	INDICATION: see above. [CH2] REIMBURSEMENT: no.	INDICATION: no formal indication. Indication currently under discussion. REIMBURSEMENT: partial reimbursement by health insurance.
UK	INDICATION: recommended for all patients being investigated for dementia.[UK1] REIMBURSEMENT: yes, via the National Health Service.	INDICATION: to help differentiate between Alzheimer's disease, vascular dementia, and frontotemporal dementia if the diagnosis is in doubt.[UK1] REIMBURSMENT: yes, via the National Health Service	INDICATION: highly selected patients with cognitive impairment where i) AD is a possible diagnosis but remains uncertain after comprehensive evaluation by a dementia expert and conventional imaging work-up and ii) where knowledge of the presence or absence of amyloid is expected to increase diagnostic certainty and influence patient management and: (iii) Persistent or progressive unexplained memory impairment confirmed by standard medical tests and/or (iv) An unusual clinical presentation and/or an atypically early age of onset (<65)[UK2] REIMBURSEMENT: no.[UK3] Available in some centres via local arrangements.	INDICATION: if Creutzfeldt–Jakob disease or other forms of rapidly progressive dementia are suspected.[UK1] Used in certain tertiary clinics especially for young onset cases. REIMBURSMENT: yes, via the National Health Service.

[UK1] National Institute for Health and Care Excellence guideline - CG42 (https://www.nice.org.uk/guidance/CG42/chapter/1-Guidance#diagnosis-and-assessment-of-dementia)

[UK2] https://www.rcr.ac.uk/system/files/publication/field_publication_files/bfcr163_pet-ct.pdf

[UK3] https://www.engage.england.nhs.uk/consultation/specialised-services-consultation/user_uploads/pet-ct-policy-statemnt.pdf

[CH1] Krankenpflege-Leistungsverordnung. Verordnung des EDI über Leistungen in der obligatorischen Krankenpflegeversicherung. https://www.admin.ch/opc/de/official-compilation/2016/4639.pdf

[CH2] https://compendium.ch/mpro/mnr/24891/html/fr

[GE] Gemeinsamer Bundesausschuss http://www.english.g-ba.de/.

[I] *Impiego delle tecniche di imaging nelle demenze. Sistema nazionale per le linee guida (SNLG). A cura del Istituto Superiore di Sanità e del Ministero della Salute. Linea Guida 19, edizione settembre 2010, aggiornamento del settembre 2013.*

[SP] https://www.aemps.gob.es/informa/boletines-AEMPS/boletinMensual/2014/junio/docs/boletin-mensual_junio-2014.pdf

3. A structured 5-phase framework for AD biomarker development and validation

Oncology is significantly more advanced than the AD field in the development and use of biomarkers for screening and delivery of precision medicine treatments. In 2001, Pepe and colleagues devised a 5-phase framework for the development of screening biomarkers in the general population (Panel 3).[6] Each phase consists of one or two main aims, pertinent primary outcome measures, and secondary aims.

Allowing for the greater taxonomic maturity of oncology, the major differences with cancer biomarker validation consist of (i) the use of AD biomarkers for *diagnosis* rather than *screening*, (ii) the difficulty of accessing neuropathological data, the gold standard for AD diagnosis, and (iii) the current lack of interventions able to positively alter the course of AD and thus impact significantly on phase V outcomes (AD-associated mortality, morbidity, and disability afforded by the diagnostic test) (Panel 3). Taking into consideration these basic differences, the oncology framework was adapted to the specifics of AD as it is understood today[57].

4. Research priorities for AD biomarker validation to launch phase IV studies

In the context of the 5-phase framework, we reviewed the available evidence on the validity of AD biomarkers based on the framework adapted from oncology. The clinical context of the exercise was confined to the diagnosis of prodromal AD. This is where prognosis is most uncertain (up to 50% of MCI patients do not progress to develop dementia) and the stakes of an accurate diagnosis are higher. The initiative included the core AD biomarkers for their greater evidence of validity. Tau PET was not included as it is an emerging technology currently used only in research (phase I/phase II development stages), as briefly outlined further on.[58] The roadmap we propose provides a general framework that can benefit other technologies or techniques, including tau PET.

The methods and results of this exercise are reported in detail elsewhere.[55,57,59–64] Briefly, literature reviews were performed using harmonized strings for all individual aims of the 5 phases (Supplementary Table). The core data are reported in Panel 5 and briefly summarized below. Overall, each of the biomarkers in question has been validated to varying degrees. Phase V studies are not available for any of the biomarkers, and only preliminary evidence for individual phase IV aims could be found for CSF, FDG-PET and visual MRI measures of medial temporal atrophy [61]. Unsurprisingly, given that individual biomarkers have been assessed so variably, evidence from studies comparing combinations of biomarkers is very limited (phase III, secondary aims 2 and 3). The following paragraphs summarize the conclusions of the individual reviews and highlight research priorities.

4.1 Neuropsychology.[59] Most task force members agreed that neuropsychological tests do not qualify as biomarkers, but they should rather be considered as the “gatekeeper” to the use of biomarkers. In particular, neuropsychological tests are used to demonstrate objective impairment allowing for MCI to be distinguished from subjective cognitive complaints. Indeed, the positive and negative predictive values of biomarkers is closely dependent on the psychometric properties of neuropsychological tests and the case mix of the population undergoing biomarker assessment.

Cerami and colleagues focused on delayed free and cued recall tasks as they represent the most sensitive measures of memory decline of hippocampal type and are relatively specific to the medial temporal lobe dysfunction typical of AD[2]. They concluded on the existence of a large number of valid tests tapping the same function, but lacking standardization of administration, scoring, and normative values.

Research priorities for neuropsychological tests
Comparing different neuropsychological tests assessing memory function for sensitivity, specificity, positive and negative predictive values
Defining a consensus delayed recall test with multilingual versions and the relative normative populations
Define a consensus algorithm based on neuropsychological tests to access biomarker assessment
Define a consensus neuropsychological test battery required to support a diagnosis of "atypical" (non-memory) AD presentations

4.2 Medial temporal atrophy.[61] Ten Kate and colleagues reviewed the evidence base for visual rating and volumetric measures of medial temporal atrophy. Medial temporal atrophy is the only biomarker where not only phase I, but also phase II is almost completely achieved and where the validation process is most complete – i.e. validation from upstream to downstream phases has been achieved. However, this is more likely due to its being one of the oldest, more accessible, and most studied biomarkers, rather than to coordination of activities and projects within the scientific community. Similar to most biomarkers, phases IV and V have not yet started except for sparse preliminary data on the practical feasibility of the visual assessment of MTA. However, the limited specificity of MTA when used as a stand-alone, a feature shared to different extents by the other biomarkers, requires that phases IV and V examine its performance in combination with other biomarkers.

The strength of this biomarker consists in the feasibility of visual assessment with a well-consolidated technique, and the reliability of a protocol for manual hippocampal segmentation with worldwide harmonization (HarP) against which new automated algorithms can be validated. Visual assessment has the potential to be rapidly implemented in current clinical practice, but in the long term automated volumetry might be the standard tool for its greater stability.

The HarP is now being implemented into algorithms and should help calibrate the various volumetric approaches. Heterogeneous normative reference populations, differences in measurements across algorithms, and short follow-ups have so far not allowed validating a single threshold for positivity for volumetry, weakening their clinical usability. The major weakness is the poor specificity against non-AD causes of cognitive impairment.

Research priorities for hippocampal atrophy on MRI		
Phase II	Clinical Assay Development for Clinical Disease	
	SA1	Define a standard validation procedure for automated segmentation algorithms based on the harmonized manual segmentation protocol Assess reproducibility between different algorithms
Phase III	Prospective Longitudinal Repository Studies	
	PA1	Assess accuracy of prediction of MCI progression to AD in clinical samples with adequate follow-up
	PA2	Define the threshold for hippocampal atrophy taking into account the effect of covariates
	SA1	Explore the impact of covariates on the discriminatory abilities of hippocampal volumetry in detecting MCI due to AD

4.3 FDG-PET.[60]. FDG-PET is also at a relatively advanced stage of validation. The greater availability of phase IV preliminary data on the impact and cost-effectiveness of FDG-PET in atypical and early onset patients is weakened by the incomplete achievement of upstream studies. In particular, the lack of a sufficient amount of evidence about the effect of covariates, such as apoE genotype, disease duration, or amount of cortical atrophy, on hypometabolism in AD patients (Phase II, Secondary Aim 4) implies that the capacity of FDG-PET to detect prodromal AD (i.e. Primary Aim 1 of phase III, regarded as "achieved" based on the substantial availability of studies) probably contains variability that might be reduced after achievement of Phase II, Secondary Aim 4. This problem, in addition to the availability of different readout procedures, each with its threshold for positivity (Phase III, Primary Aim 2) may contribute to the highly variable accuracy values in detecting prodromal AD.[65]

Research priorities for ^{18}F-fluorodeoxy-glucose PET		
Phase II	Clinical Assay Development for Clinical Disease	
	SA4	Assess the effect of covariates and disease characteristics (stage, onset of disease, clinical presentation, reserve capacity, comorbidities, genotype) on levels and distribution of cerebral glucose hypometabolism and on normality thresholds.
Phase III	Prospective Longitudinal Repository Studies	
	PA1	Assessment of the accuracy of FDG-PET in prodromal AD detection may need to be re-assessed after completion of SA4, to investigate possibly better performance
	PA2	Harmonize reading criteria and determine a standard threshold for hypometabolism; validate the reading procedures for reproducibility

4.4 CSF Aβ42, tau, p-tau.[63] CSF biomarkers are at an advanced stage of development. However, the currently used manual immunoassays are sufficiently stable only in expert laboratories implementing specific quality control procedures (secondary aim 1 of phase II). During the course of the present initiative, a potentially significant advancement took place consisting in the

development of novel fully automated assays based on electrochemiluminescence, featuring coefficients of variability almost one order of magnitude lower than the traditional assays. Further, fully-automated assays from other vendors are under development. However, an optimized protocol for standardized pre-analytical handling of CSF samples needs to be developed and implemented in worldwide practice. We do not anticipate the need of re-running all phase II and III studies on the newer or other future assays, but the normality cut-off will need to be defined for both the new pre-analytical protocol and new immunoassays using a suitable reference (preferably neuropathology) (phase III, primary aim 2). Research for several priorities for CSF biomarkers identified in this roadmap are already underway, such as the development of international certified reference materials to better bridge results between different assays.

Research priorities for CSF Aβ42, tau, p-tau		
Phase II	Clinical Assay Development for Clinical Disease	
	SA1	Develop and implement an optimized protocol for standardized pre-analytical handling of CSF samples. Validate novel fully automated immunoassays, using Certified Reference Materials (CRM).
	SA3	Determine in greater detail the effects of non-AD brain pathologies on the CSF levels of different variants of Aβ and tau.
	SA4	Assess in greater detail the effects of disease characteristics (stage, genotype, disease onset, clinical manifestation) and other covariates on the levels of CSF biomarkers.
Phase III	Prospective Longitudinal Repository Studies	
	PA2	Define cut off values for all CSF biomarkers (or CSF biomarker ratios) using the optimized protocol for standardized pre-analytical handling of CSF samples. This needs to be done for each new fully automated immunoassay using a suitable reference (e.g. pathology).
	SA2	Determine the optimal combination of different CSF biomarkers for detection of MCI due to AD when using the optimized protocol for standardized pre-analytical handling of CSF samples in combination with novel fully automated immunoassays and Certified Reference Materials (CRM).
	SA3	Develop optimal algorithms combining CSF biomarkers with other measures, including MRI and cognitive tests.
	SA4	Determine the intra-individual changes of CSF biomarkers over time during prodromal stages of AD when using the optimized protocol for standardized pre-analytical handling of CSF samples in combination with novel fully automated immunoassays and Certified Reference Materials (CRM).

4.5 Amyloid PET.[64]. In the field of amyloid PET, despite general consensus on the equivalence of the three regulatory approved fluorinated tracers, the interpretation of phase III studies is complicated by imperfect standardization of comparative reading or quantification procedures (Phase II, Secondary Aim 1) and thresholds for positivity (Phase III, Primary Aim 2). The effect of covariates on cases (Phase II, Secondary Aim 4) and controls (Phase II, Secondary Aim 3) has been assessed quite extensively, but may provide more stable results when a procedure harmonized across tracers (e.g. the centiloid project) will be more widely implemented. Similarly, a harmonized procedure may impact the ability of this biomarker to detect prodromal AD (Phase III, Primary Aim 1). This objective was considered fully achieved based on studies published so far; however, problems are also evident in this procedure,[66] and, moreover, it relies on the

assumption that the different tracers have similar discriminative abilities, for which there is only preliminary evidence.

Phase III studies will also need to address the impact of clinical covariates on the detection of AD in MCI patients (Phase III, Secondary Aim 2). Recent evidence indicates that subjects with borderline retention levels, but still in the range currently believed to be normal, could be "accumulators" who will become amyloid positive shortly. This is relevant as it potentially impacts on the definition of the threshold for positivity (Phase III, Secondary Aim 4).

These uncertainties notwithstanding, some small scale phase IV studies are already available, and collaborative efforts between researchers and tracer developers have led to ongoing large scale phase IV studies (IDEAS, http://www.ideas-study.org/; AMYPAD http://www.amypad.org).

Research priorities for ^{18}F amyloid PET		
Phase II	Clinical Assay Development for Clinical Disease	
	SA1	Assess on the same population comparability and reproducibility of tracers, operating procedures, and readout methods.
	SA3	Assess the impact of covariates (gender, education, levels of cognitive activity) on tracer retention and define whether and how they should affect the definition of positivity.
	SA4	Assess the effect of disease characteristics (stage, genotype, disease onset, clinical manifestation) and other covariates in patients on levels of retention, to quantify the informative value of amyloid imaging in patients
Phase III	Prospective Longitudinal Repository Studies	
	PA1	Discrimination ability of MCI due to AD may provide more stable results if re-run after definition of one standard procedure
	PA2	Progress the definition of positivity mainly by standardizing the reading criteria
	SA1	Collect evidence on the impact of covariates on the discriminatory abilities of the biomarker
	SA2	Compare the predictive performance of amyloid imaging versus other biomarkers (particularly CSF Aβ42, assessed with the new standard)
	SA3	Develop sensitive algorithms for positivity based on combinations of amyloid imaging and other markers
	SA4	Investigate the meaning of intermediate levels of retention (quantitative assessment) or dubious cases (visual assessment) and define whether repeated testing may be useful, at which time interval, and for which patients

4.6 Tau PET. In recent years, the emergence of PET tracers targeting deposits of abnormally hyperphosphorylated tau protein, a key pathological hallmark of AD, is opening up the possibility of using PET to measure the prevalence of different forms of tau deposits in the brain of patients with both AD and other tauopathies. These tracers showed in vitro high specificity to tau pathology (specifically to 3r/4r paired helical filamentous tau aggregates that are characteristic of AD), although the agreement between tracer binding and tau immunohistochemistry appears more complex.[67–71] The favourable pharmacokinetics of those tracers [72–74] completed the requirements for Phase I. A number of rather small and non-consecutive Phase II studies have

already reported good discrimination between healthy volunteers and AD patients,[67,75–78] with preliminary evidence, available for only one of the tracers (AV-1451), supporting the agreement between antemortem PET quantification of the tracer retention and postmortem evidence of tau pathology in the same non-AD patients, a carrier of a MAPT mutation (p.R406W), and two patients with corticobasal degeneration, although questions remain about the specific target of the tracer[58,79,80] (Phase II, SA 2). Further research on tau PET imaging is required to understand the binding characteristics of the different tracers, before exploring further the clinical validity of this novel biomarker.

4.7 Combination of biomarkers. The most widely accepted diagnostic criteria predicate that the highest accuracy can be achieved with a combination of markers of amyloidosis (either amyloid PET or CSF Aβ42) and neurodegeneration (medial temporal atrophy, ^{18}F-fluorodeoxy-glucose PET, and CSF tau and p-tau) [11] or amyloidosis and tauopathy (amyloid PET and CSF Aβ42, tau and p-tau).[4] However, as reported in greater detail elsewhere,[59–64] the findings on the performance of combinations of biomarkers are inconsistent, the only conclusion being that more biomarkers provide better accuracy.

Proceeding effectively in the definition of an efficient combination of biomarkers requires that the discriminant ability of biomarkers is assessed (i) based on operative procedures that have successfully passed upstream phases, and (ii) on a patient population where all the tested biomarkers are simultaneously measured. Such a design is feasible for phase III studies of MTA, FDG-PET, and CSF or amyloid PET.

5. Additional conditions to launch phase IV AD biomarker validation studies

The research priorities listed in the previous section address the gaps of knowledge of phases II and III. Indeed, Panel 5 shows that phase IV is largely unattained whatever AD biomarker is considered and completion of phases II and III is a required step to design phase IV studies. However, completion of phases II and III is a necessary but not sufficient condition to the setup of methodologically sound phase IV studies. We believe that at least three additional conditions should be satisfied.

5.1 Biomarker-qualified memory clinics. Biomarkers demonstrating valid after completion of phases II and III will be sufficiently robust to be used in phase IV studies, i.e. in realistic setting for diagnosis and treatment. However, the biomarker may not necessarily be a commercial ready-to-use product. For instance, ^{18}F–amyloid tracers have not yet completed phase III and are already commercial products, while it is not clear whether hippocampal volumetry will ever become one, even after phase III will be completed. For this reason, it may be necessary to carry out phase IV studies in memory clinics where the biomarker can be collected and measured in a standardized fashion. This is the approach taken by a national project which is being carried out in Italy where 6 memory clinics are qualified to collect CSF, structural MRI following the ADNI protocol, and extract hippocampal volume and FDG-PET metrics of temporo-parietal hypometabolism with a number of automated segmentation tools and amyloid load by PET imaging. The results of biomarker measurement will be used to direct diagnosis and treatment of patients with MCI (Italian Health Ministry grant: NET-2011-02346784).

5.2 Clinical guidelines. Biomarkers cannot be used in a phase IV study without clinical guidelines providing indications on their appropriate use and how to integrate the results as part of a patient's diagnostic workup. Clinical and imaging guidelines specific to amyloid PET have been developed by US, European, UK, Italian, and Canadian task forces;[81–85]. These, however, have addressed amyloid PET as a stand-alone biomarker, while in practice it will be used and hence interpreted in association with other biomarkers, and on occasions with other measures of amyloid deposition (e.g. CSF biomarkers).[86] The US clinical guidelines are being used in a large amyloid PET phase IV study where over 18.000 persons will be scanned (http://www.ideas-study.org). Other guidelines focusing on neuroimaging[87] take a traditional approach to imaging biomarkers, where qualitative readings are privileged, and that do not provide guidance on quantitative approaches recommended by this roadmap. Biomarker-specific guidelines will need to be developed for appropriately designed phase IV studies that inform use and interpretation of biomarkers in the clinical setting where they are available.

5.3 Ethics. Phase IV studies imply that biomarkers not approved for clinical use will guide diagnosis and treatment of real-life patients. When such studies become research projects rather than audit studies or clinical evaluations, Good Clinical Practice research frameworks will need to be followed including ascertainment of relevant ethical approvals, and informed consents for anonymized data storage and sharing. Of particular relevance to implementation of "real-life" diagnostic studies using biomarkers is that patients are informed about the degree of uncertainty and the reading limitations of biomarkers in the current stage of development, and how biomarkers will be used to guide diagnosis and treatment. Guidelines for the disclosure of the diagnosis in the pre-dementia stage of AD that include patients' views will need to be developed. Structured training for medical doctors on how to communicate the diagnosis to the individual should also be promoted.

Against these guidelines, already today MRI based volumetry and (amyloid) PET are being used as a business case for screening for Alzheimer's disease in asymptomatic people without evidence for clinical usefulness. This development outside of academia underscores the urgent need for the evaluation and adoption of stricter rules for biomarker use in persons with cognitive concerns.

Panel 5. Key points of the strategic research agenda to the biomarker-based diagnosis of prodromal Alzheimer's disease.	
Problem	Biomarkers for AD have incomplete evidence of clinical validity, adversely affecting clinical use and reimbursement on a large scale.
Aim	Define a strategic research agenda aimed to synchronize research efforts and complete validation effectively, to have biomarkers approved for proper clinical use.
Action	We interpreted available evidence on validity of AD biomarkers in the context of a 5-phase framework for structured validation adapted from oncology (see Panel 3) where phase I is preclinical exploratory studies, phase II clinical assay development for overt disease, phase III prospective longitudinal repository studies, phase IV prospective diagnostic studies, and phase V disease control studies.
Results	The validation of all biomarkers is completed only for phase I. Research priorities were identified for phases II and III including definition of the assays, reading procedures and thresholds for normality, performance in detecting the disease, accounting for effect of covariates, and diagnostic algorithms comprising combinations of biomarkers.
Future actions	Define guidelines for best practice use of biomarkers and of combinations thereof. Complete phases II and III with priority to filling upstream gaps; set up phase IV studies.
Recommendations	Set up the validation of new biomarkers according to the 5-phase framework. Partial validation of available biomarkers, lacking upstream evidence, may need to re-run from earlier phases. Biomarkers should be validated in phase IV studies run in qualified memory clinics and with appropriate ethics approval and informed consent.

6. Conclusions and future directions

We have identified gaps of evidence that prevent AD biomarkers from being used rationally and cost-effectively in clinical practice, and formulated research priorities to fill these gaps. The effort aims to influence funding agencies of healthcare research, pharma companies, scientists and scientific societies, and policy makers.

The agreement on the need for further efforts of biomarker validation is not universal in the AD scientific community. In a recent debate on CSF, some researchers acknowledged the need for more systematic validation[88,89]), while others regarded the currently available evidence as

sufficient to support their use in the clinic[90]. If experts do not agree, it is not surprising that health care payers are reluctant to reimburse. The adoption of the 5-phase framework that we propose might contribute to harmonize biomarker validation and ultimately reduce heterogeneity of prescription and reimbursement.

Our effort is not the first of this kind: an earlier attempt to develop a validation framework was proposed for quantitative imaging biomarkers for AD [91]; however, maybe due to lack of general consensus, it failed to significantly impact on the field.

The 5-phase framework requires that a given phase is addressed only after the previous ones are completed. In practice, especially for biomarkers where validation studies have already been undertaken, the process will be less orderly due to factors including, but not limited to market pressure, funding opportunities, co-operation between researchers, responses by regulators, and patient and societal pressures. On the other hand, in the absence of disease-modifying therapies, Phase V studies, aiming to estimate the reductions in AD-associated mortality, morbidity, and disability as allowed by the use of biomarkers, are necessarily limited. A number of pharmacologic phase III trials are currently ongoing[92] where the drug is offered only to patients positive to a diagnostic biomarker. Most of the currently investigated drugs are amyloid-lowering agents and amyloid biomarker abnormality may be required (either PET or CSF). Should one of these drugs be effective, the associated biomarker will enter the market as a "theranostic" (test employed to select focused therapy). Phase V studies of the theranostic biomarker will, at this point, correspond to phase III studies of the drug.

We believe that the success of a disease modifying drug in AD (e.g. results on Aducanumab[54]), at the MCI/prodromal stage will not make our effort obsolete. Only 20% of patients with AD are estimated to be treated with the approved, reimbursed, orally administered, and relatively low cost cholinesterase inhibitors.[93] It can be predicted that the proportion of patients treated with disease modifiers, some of which will be significantly more expensive and of less practical administration route (e.g., intravenous), may be even lower. Disease modifiers will increase the relevance and urgency for accurate diagnostic tools to select the population with the target condition (i.e., presence of amyloid or tau aggregates), properly stage the disease (treatment may be effective or authorized only at specific stages), and monitor response to treatment. Last, even in a world with effective disease modifying therapies, the many patients not qualifying for treatment will always need to receive an accurate diagnosis and the best possible treatment. Indeed, the high cost and low practical routine administration of disease modifiers will make these drugs indicated in a portion of all AD patients even lower than the 20% currently treated with anticholinesterase inhibitors. Finally, the new diagnostic or theranostic biomarkers that the very active research in the field is likely to bring out in the next years[94] will also need to undergo the same 5-phase process described in this paper. The availability of the virtuous example of the core biomarkers will represent a significant facilitator.

7. Acknowledgements

The Geneva Task Force for the Roadmap of Alzheimer's Biomarkers includes the participants to a workshop held in Geneva on December 8-9, 2014, aimed to accelerate the evaluation and adoption of biomarkers for AD for use in research and clinical practice, by adapting a methodological framework for validation of oncology biomarkers to the specifics of AD, as it is understood today. Participants included some of the scientists who contributed to developing the oncology framework (authors YY and PV), and other European acknowledged experts on clinical use of AD biomarkers. The P.I. of the initiative is Giovanni B Frisoni, with Bengt Winblad and Clifford R Jack Jr as co-PIs. The task force includes experts in biomarkers for oncology; experts on diagnostic AD biomarkers from Switzerland and Europe; representatives of pertinent scientific societies (Federation of European Societies of Neuropsychology - FENS, European Neurological Society of Neuroradiology - ENSNR, International Foundation of Clinical Chemistry and Laboratory Medicine - IFCCLM, European Association of Nuclear Medicine - EANM, and Swiss Federation of Clinical Neuro Societies - SFCNS); representatives of patient advocates, bioethicists and regulatory agencies, and early career researchers. The Geneva Task Force has been endorsed by the EADC – European Alzheimer's Disease Consortium.

The workshop was funded thanks to a competitive grant by the Swiss National Science Foundation and unrestricted grants from: Alzheimer Forum Switzerland, Association pour la Recherche sur Alzheimer, Genève; Piramal, Eli Lilly & Company, General Electric, Guerbet, TEVA Pharma; Academie Suisse de Sciences Médicales, Vifor Pharma Switzerland., Novartis, Siemens, and IXICO. We acknowledge the help from Margherita Mauri and Daria Gennaro (IRCCS Fatebenefratelli, Brescia, Italy) and Agnese Picco (Università di Genova, Genova, Italy) who took care of the logistics of the workshop.

The Alzheimer's Association hosted the first follow-up meeting of the initiative at the 2015 AAIC congress in Washington.

The following authors took part to the Geneva Workshop for the Roadmap of Alzheimer's Biomarkers on December 8-9 2014 as representatives of scientific societies, patient advocate associations, and regulators. Flavio Nobili was delegate from the European Association of Nuclear Medicine (EANM) Neuroimaging Committee. Kaj Blennow was delegate and Chair of the International Federation of Clinical Chemistry and Laboratory Medicine Working Group for CSF proteins (IFCC WG-CSF). Frederik Barkhof was delegate from the European Society of Neuroradiology (ESNR). Stefano Cappa was delegate and Chair of the Federation of European Societies of Neuropsychology (FENS). Urs Mosimann was delegate from the Swiss Federation of Clinical Neuro Societies (SFCNS). Mark Baker was delegate from the National Institute for Health and Care Excellence, UK. Heather Snyder was delegate and Director of Medical Affairs of the Alzheimer's Association, Chicago, Ill. Charles Scerri was delegate of Alzheimer's Europe, Luxembourg. The content of this paper represents the opinion of the individual authors and is not necessarily endorsed by the entities authors are affiliated to.

The Alzheimer's Association, Chicago, hosted two coordinating meetings at AAIC in Washington, (2015) and Toronto (2016).

This work has received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking (EMIF grant n°115372).

Frederik Barkhof was supported by the National Institute for Health Research Biomedical Research Centre at University College London Hospitals.

Funders' contribution

The funders had no role in study design, nor in the collection, analysis, interpretation of data, nor in the writing of the report, nor in the decision to submit the paper for publication.

Corresponding author

Giovanni Frisoni confirms that he had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Authors' contribution

All authors have finally approved the manuscript and are fully accountable for the work. In addition:

STUDY CONCEPTION, MANUSCRIPT DRAFT: Giovanni B. Frisoni

MANUSCRIPT DRAFT, ANALYSIS AND INTERPRETATION OF DATA FOR THE WORK: Bengt Winblad, Marina Boccardi

ACQUISITION, ANALYSIS, OR INTERPRETATION OF DATA FOR THE WORK AND CRITICAL REVISION OF THE WORK FOR IMPORTANT INTELLECTUAL CONTENT:

Frederik Barkhof, Kaj Blennow, Stefano Cappa, Konstantinos Chiotis, Jean-Francois Démonet, Valentina Garibotto, Panteleimon Giannakopoulos, Anton Gietl, Oskar Hansson, Karl Herholz, Clifford R Jack Jr, Flavio Nobili, Agneta Nordberg, Heather M. Snyder, Mara ten Kate, Andrea Varrone, Emiliano Albanese, Mark Baker, Stefanie Becker, Patrick Bossuyt, Maria Carrillo, Chiara Cerami, Bruno Dubois, Valentina Gallo, Ezio Giacobini, Gabriel Gold, Samia Hurst, Anders Lönneborg, Karl-Olof Lovblad, Niklas Mattsson, José-Luis Molinuevo, Andreas Monsch, Urs Mosimann, Alessandro Padovani, Agnese Picco, Corinna Porteri, Osman Ratib, Laure Saint-Aubert, Charles Scerri, Philip Scheltens, Jonathan Schott, Ida Sonni, Stefan Teipel, Paolo Vineis, Pieter-Jelle Visser, Yutaka Yasui.

Panel 5. Current state of development and validation of biomarkers for the diagnosis of prodromal Alzheimer's disease. PA: primary aim. SA: secondary aim. Green: fully achieved; yellow: partly achieved; orange: preliminary evidence; red: not achieved; white: not applicable.
* colours represent the least developed level between visual and volumetric medial temporal atrophy.

Biomarker	Phase I	Phase II					Phase III						Phase IV					Phase V
	Pilot Studies	Clinical Assay Development for Clinical Disease					Retrospective Longitudinal Repository Studies						Prospective Diagnostic Studies					Disease Control Studies
	PA	**PA**	**SA1**	**SA2**	**SA3**	**SA4**	**PA1**	**PA2**	**SA1**	**SA2**	**SA3**	**SA4**	**PA**	**SA1**	**SA2**	**SA3**	**SA4**	
MRI medial temporal atrophy*																		
^{18}F-fluorodeoxy-glucose PET																		
^{11}C-PIB, ^{18}F amyloid tracers PET																		
CSF (Aβ42, tau, p-tau)																		

References

1 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984; **34**: 939-44.

2 Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol* 2007; **6**: 734-46.

3 McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; **7**: 263-9.

4 Dubois B, Hampel H, Feldman HH, et al. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement* 2016; **12**: 292-323.

5 Murray ME, Graff-Radford NR, Ross OA, Petersen RC, Duara R, Dickson DW. Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. *Lancet Neurol* 2011; **10**: 785-96.

6 Pepe MS, Etzioni R, Feng Z, et al. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001; **93**: 1054-61.

7 Bennett DA, Wilson RS, Boyle PA, Buchman AS, Schneider JA. Relation of neuropathology to cognition in persons without cognitive impairment. *Ann Neurol* 2012; **72**: 599-609.

8 Rowe CC, Ellis KA, Rimajova M, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. *Neurobiol Aging* 2010; **31**: 1275-83.

9 Jack CR,Jr, Lowe VJ, Senjem ML, et al. 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. *Brain* 2008; **131**: 665-80.

10 Dubois B, Feldman HH, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol* 2014; **13**: 614-29.

11 Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; **7**: 270-9.

12 Alzheimer A. Über einen eigenartigen schweren Erkrankungsprozeß der Hirnrinde. *Neurologisches Centralblatt* 1906; **23**: 1129-36.

13 Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A* 1985; **82**: 4245-9.

14 Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci U S A* 1986; **83**: 4913-7.

15 Terry RD, DeTeresa R, Hansen LA. Neocortical cell counts in normal human adult aging. *Ann Neurol* 1987; **21**: 530-9.

16 Giannakopoulos P, Herrmann FR, Bussiere T, et al. Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer's disease. *Neurology* 2003; **60**: 1495-500.

17 Jack CR,Jr, Albert MS, Knopman DS, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; **7**: 257-62.

18 Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011; **7**: 280-92.

19 Zwan MD, Bouwman FH, Konijnenberg E, et al. Diagnostic impact of [18F]flutemetamol PET in early-onset dementia. *Alzheimers Res Ther* 2017; **9**: 2,016-0228-4.

20 Boccardi M, Altomare D, Ferrari C, et al. Assessment of the Incremental Diagnostic Value of Florbetapir F 18 Imaging in Patients With Cognitive Impairment: The Incremental Diagnostic Value of Amyloid PET With [18F]-Florbetapir (INDIA-FBP) Study. *JAMA Neurol* 2016; **73**: 1417.

21 Jessen F, Amariglio RE, van Boxtel M, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimers Dement* 2014; **10**: 844-52.

22 Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol* 1999; **56**: 303-8.

23 Petersen RC, Doody R, Kurz A, et al. Current concepts in mild cognitive impairment. *Arch Neurol* 2001; **58**: 1985-92.

24 Aisen PS, Petersen RC, Donohue MC, et al. Clinical Core of the Alzheimer's Disease Neuroimaging Initiative: progress and plans. *Alzheimers Dement* 2010; **6**: 239-46.

25 Home-brew tests need regulation. *Nature* 2014; **512**: 5.

26 Geuze E, Vermetten E, Bremner JD. MR-based in vivo hippocampal volumetrics: 1. Review of methodologies currently employed. *Mol Psychiatry* 2005; **10**: 147-59.

27 Chen K, Ayutyanont N, Langbaum JB, et al. Characterizing Alzheimer's disease using a hypometabolic convergence index. *Neuroimage* 2011; **56**: 52-60.

28 Landau SM, Harvey D, Madison CM, et al. Associations between cognitive, functional, and FDG-PET measures of decline in AD and MCI. *Neurobiol Aging* 2011; **32**: 1207-18.

29 Brewer JB, Magda S, Airriess C, Smith ME. Fully-automated quantification of regional brain volumes for improved detection of focal atrophy in Alzheimer disease. *AJNR Am J Neuroradiol* 2009; **30**: 578-80.

30 Herholz K, Salmon E, Perani D, et al. Discrimination between Alzheimer dementia and controls by automated analysis of multicenter FDG PET. *Neuroimage* 2002; **17**: 302-16.

31 Vos S, van Rossum I, Burns L, et al. Test sequence of CSF and MRI biomarkers for prediction of AD in subjects with MCI. *Neurobiol Aging* 2012; **33**: 2272-81.

32 Galluzzi S, Geroldi C, Amicucci G, et al. Supporting evidence for using biomarkers in the diagnosis of MCI due to AD. *J Neurol* 2013; **260**: 640-50.

33 Hayes DF. Biomarker validation and testing. *Mol Oncol* 2015; **9**: 960-6.

34 Frisoni GB, Bocchetta M, Chetelat G, et al. Imaging markers for Alzheimer disease: which vs how. *Neurology* 2013; **81**: 487-500.

35 Insel PS, Mattsson N, Mackin RS, et al. Accelerating rates of cognitive decline and imaging markers associated with beta-amyloid pathology. *Neurology* 2016; **86**: 1887-96.

36 Teipel S, Kilimann I, Thyrian J, Klöppel S, Hoffmann W. Potential role of neuroimaging markers for early diagnosis of dementia in primary care. *Curr Alzheimer Res* In Press 2016; .

37 Mattsson N, Andreasson U, Persson S, et al. The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimers Dement* 2011; **7**: 386,395.e6.

38 Frisoni GB, Jack CR,Jr, Bocchetta M, et al. The EADC-ADNI Harmonized Protocol for manual hippocampal segmentation on magnetic resonance: evidence of validity. *Alzheimers Dement* 2015; **11**: 111-25.

39 Winblad B. BIOMARKAP: Biomarkers for Alzheimer's disease and Parkinson's disease. 2012; .

40 Varrone A, Asenbaum S, Vander Borght T, et al. EANM procedure guidelines for PET brain imaging using [18F]FDG, version 2. *Eur J Nucl Med Mol Imaging* 2009; **36**: 2103-10.

41 Morbelli S, Brugnolo A, Bossert I, et al. Visual versus semi-quantitative analysis of 18F-FDG-PET in amnestic MCI: an European Alzheimer's Disease Consortium (EADC) project. *J Alzheimers Dis* 2015; **44**: 815-26.

42 Hort J, Bartos A, Pirttila T, Scheltens P. Use of cerebrospinal fluid biomarkers in diagnosis of dementia across Europe. *Eur J Neurol* 2010; **17**: 90-6.

43 Sancesario GM, Toniolo S, Chiasserini D, et al. The Clinical Use of Cerebrospinal Fluid Biomarkers for Alzheimer's Disease Diagnosis: The Italian Selfie. *J Alzheimers Dis* 2017; **55**: 1659-66.

44 Petersen RC, Stevens JC, Ganguli M, Tangalos EG, Cummings JL, DeKosky ST. Practice parameter: early detection of dementia: mild cognitive impairment (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology* 2001; **56**: 1133-42.

45 Sorbi S, Hort J, Erkinjuntti T, et al. EFNS-ENS Guidelines on the diagnosis and management of disorders associated with dementia. *Eur J Neurol* 2012; **19**: 1159-79.

46 Johnson KA, Minoshima S, Bohnen NI, et al. Appropriate use criteria for amyloid PET: a report of the Amyloid Imaging Task Force, the Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer's Association. *J Nucl Med* 2013; **54**: 476-90.

47 Nationell utvärdering – Vård och omsorg vid demenssjukdom 2014 – Rekommendationer, bedömningar och sammanfattning. Socialstyrelsen; 2014.

48 Deuschl G. and Maier W. Demenzen. Deutsche Gesellschaft fur Neurologie. patent AWMF register Nummer: 038-013. 2016 .

49 Frisoni GB, Galluzzi S, Riello R. Prescription and choice of diagnostic imaging by physician specialty in Alzheimer's Centers (Unita di Valutazione Alzheimer - UVA) in Northern Italy. *Aging Clin Exp Res* 2005; **17**: 14-9.

50 Blennow K, Mattsson N, Scholl M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. *Trends Pharmacol Sci* 2015; **36**: 297-309.

51 Palmqvist S, Mattsson N, Hansson O. Reply: Do we still need positron emission tomography for early Alzheimer's disease diagnosis? *Brain* 2016; **139**: 1.

52 Haute Autorité de Santé. Maladie d'Alzheimer et maladies apparentées: diagnostic et prise en charge. *HAS / Service des bonnes pratiques professionnelles* 2001; .

53 Carlson ,Christopher, Siemers ,Eric, Hake ,Ann, et al. Amyloid-related imaging abnormalities from trials of solanezumab for Alzheimer's disease. *Alzheimers Dement (Amst)* 2016; **2**: 75.

54 Sevigny J, Chiao P, Bussiere T, et al. The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. *Nature* 2016; **537**: 50-6.

55 Porteri C, Albanese E, Scerri C, et al. The Biomarker-based Diagnosis of Alzheimer's Disease. 1 – Ethical and Societal Issues. *Neurobiol Aging* 2017; **52**: 132-40.

56 Eichler T, Thyrian JR, Hertel J, et al. Rates of formal diagnosis in people screened positive for dementia in primary care: results of the DelpHi-Trial. *J Alzheimers Dis* 2014; **42**: 451-8.

57 Boccardi M, Gallo V, Yasui Y, et al. The Biomarker-based Diagnosis of Alzheimer's Disease. 2 – Lessons From Oncology. *Neurobiol Aging* 2017; **52**: 141-52.

58 Josephs KA, Whitwell JL, Tacik P, et al. 18F]AV-1451 tau-PET uptake does correlate with quantitatively measured 4R-tau burden in autopsy-confirmed corticobasal degeneration. *Acta Neuropathol* 2016; **132**: 931.

59 Cerami C, Dubois B, Boccardi M, Monsch A, Demonet J, Cappa S, The Geneva Task Force for the Roadmap of Alzheimer's Biomarkers. Clinical validity of free and cued wordlist recall as a gateway-biomarker for Alzheimer's disease in the context of a structured 5-phase development framework. *Neurobiol Aging* 2017; **52**: 153-66.

60 Garibotto V, Herholtz K, Boccardi M, et al. Maturity of FDG-PET as a biomarker for Alzheimer's diseas in the context of a structured 5 -phase development framework. *Neurobiol Aging* 2017; **52**: 183-95.

61 Ten Kate M, Barkhof F, Boccardi M, et al. Clinical validity of medial temporal atrophy as a biomarker for Alzheimer's disease in the context of a structured 5–phase development framework. *Neurobiol Aging* 2017; **52**: 167-82.

62 Sonni I, Ratib O, Boccardi M, et al. Clinical validity of presynaptic dopaminergic imaging with ^{123}I-ioflupane and noradrenergic imaging with ^{123}I-MIBG SPECT in the differential diagnosis between Alzheimer's disease and Dementia with Lewy bodies in the context of a structured 5–phase development framework. *Neurobiol Aging* 2017; **52**: 228-42.

63 Mattsson N, Lönnborg A, Boccardi M, Blennow K, Hansson O, The Geneva Task Force for the Roadmap of Alzheimer's Biomarkers. Maturity of Aβ42, tau, and phospho-tau in the cerebrospinal fluid as biomarkers for Alzheimer's disease in the context of a structured 5-phase development framework. *Neurobiol Aging* 2017; **52**: 196-213.

64 Chiotis K, Saint-Aubert L, Boccardi M, et al. Clinical validity of increased cortical uptake of amyloid ligands on PET as a biomarker for Alzheimer's disease in the context of a structured 5-phase development framework. *Neurobiol Aging* 2017; **52**: 214-27.

65 Smailagic N, Vacante M, Hyde C, Martin S, Ukoumunne O, Sachpekidis C. (1)(8)F-FDG PET for the early diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). *Cochrane Database Syst Rev* 2015; **1**: CD010632.

66 Jagust WJ, Landau SM, Koeppe RA, et al. The Alzheimer's Disease Neuroimaging Initiative 2 PET Core: 2015. *Alzheimers Dement* 2015; **11**: 757-71.

67 Maruyama M, Shimada H, Suhara T, et al. Imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared to normal controls. *Neuron* 2013; **79**: 1094-108.

68 Lemoine L, Saint-Aubert L, Marutle A, et al. Visualization of regional tau deposits using (3)H-THK5117 in Alzheimer brain tissue. *Acta Neuropathol Commun* 2015; **3**: 40,015-0220-4.

69 Marquie M, Normandin MD, Vanderburg CR, et al. Validating novel tau positron emission tomography tracer [F-18]-AV-1451 (T807) on postmortem brain tissue. *Ann Neurol* 2015; **78**: 787-800.

70 Lowe VJ, Curran G, Fang P, et al. An autoradiographic evaluation of AV-1451 Tau PET in dementia. *Acta Neuropathol Commun* 2016; **4**: 58,016-0315-6.

71 Sander K, Lashley T, Gami P, et al. Characterization of tau positron emission tomography tracer [18F]AV-1451 binding to postmortem tissue in Alzheimer's disease, primary tauopathies, and other dementias. *Alzheimers Dement* 2016; **12**: 1116.

72 Kimura Y, Ichise M, Ito H, et al. PET Quantification of Tau Pathology in Human Brain with 11C-PBB3. *J Nucl Med* 2015; **56**: 1359-65.

73 Jonasson M, Wall A, Chiotis K, et al. Tracer Kinetic Analysis of (S)-(1)(8)F-THK5117 as a PET Tracer for Assessing Tau Pathology. *J Nucl Med* 2016; **57**: 574-81.

74 Wooten D, Guehl NJ, Verwer EE, et al. Pharmacokinetic evaluation of the tau PET radiotracer [18F]T807 ([18F]AV-1451) in human subjects. *J Nucl Med* 2016; .

75 Chiotis K, Saint-Aubert L, Savitcheva I, et al. Imaging in-vivo tau pathology in Alzheimer's disease with THK5317 PET in a multimodal paradigm. *Eur J Nucl Med Mol Imaging* 2016; **43**: 1686-99.

76 Cho H, Choi JY, Hwang MS, et al. Tau PET in Alzheimer disease and mild cognitive impairment. *Neurology* 2016; **87**: 375-83.

77 Johnson KA, Schultz A, Betensky RA, et al. Tau positron emission tomographic imaging in aging and early Alzheimer disease. *Ann Neurol* 2016; **79**: 110-9.

78 Ossenkoppele R, Schonhaut DR, Scholl M, et al. Tau PET patterns mirror clinical and neuroanatomical variability in Alzheimer's disease. *Brain* 2016; **139**: 1551-67.

79 Smith R, Puschmann A, Scholl M, et al. 18F-AV-1451 tau PET imaging correlates strongly with tau neuropathology in MAPT mutation carriers. *Brain* 2016; **139**: 2372-9.

80 McMillan CT, Irwin DJ, Nasrallah I, et al. Multimodal evaluation demonstrates in vivo 18F-AV-1451 uptake in autopsy-confirmed corticobasal degeneration. *Acta Neuropathol* 2016; **132**: 935-7.

81 Johnson KA, Minoshima S, Bohnen NI, et al. Appropriate use criteria for amyloid PET: a report of the Amyloid Imaging Task Force, the Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer's Association. *Alzheimers Dement* 2013; **9**: e,1-16.

82 Guerra UP, Nobili FM, Padovani A, Perani D, Pupi A, Sorbi S, Trabucchi M. Recommendations from the Italian Interdisciplinary Working Group (AIMN, AIP, SINDEM) for the utilization of amyloid imaging in clinical practice. *Neurol Sci* 2015; **36**: 1075-81.

83 Laforce R, Rosa-Neto P, Soucy JP, Rabinovici GD, Dubois B, Gauthier S. Canadian Consensus Guidelines on Use of Amyloid Imaging in Canada: Update and Future Directions from the Specialized Task Force on Amyloid imaging in Canada. *Can J Neurol Sci* 2016; **43**: 503-12.

84 Minoshima S., Drzezga A.E., Djekidel M., et al. SNM MI Procedure Standard - EANM Practice Guideline for Amyloid PET Imaging of the Brain. *J Nucl Med* 2016; **57**: 1316.

85 The Royal College Of Radiologists, Royal College Of Physicians Of London, Royal College Of Physicians And Surgeons Of Glasgow, Royal College Of Physicians Of Edinburgh, British Nuclear Medicine Society, Administration Of Radioactive Substances Advisory Committee. Evidence-based indications for the use of PET-CT in the United Kingdom 2016. *Clin Radiol* 2016; **71**: e171-88.

86 Weston PS, Paterson RW, Dickson J, et al. Diagnosing Dementia in the Clinical Setting: Can Amyloid PET Provide Additional Value Over Cerebrospinal Fluid? *J Alzheimers Dis* 2016; **54**: 1297-302.

87 Filippi M, Agosta F, Barkhof F, et al. EFNS task force: the use of neuroimaging in the diagnosis of dementia. *Eur J Neurol* 2012; **19**: e131,40, 1487-501.

88 Dani M, Brooks DJ, Edison P. Tau imaging in neurodegenerative diseases. *Eur J Nucl Med Mol Imaging* 2016; **43**: 1139-50.

89 Olsson B, Blennow K, Zetterberg H. The clinical value of fluid biomarkers for dementia diagnosis - Authors' reply. *Lancet Neurol* 2016; **15**: 1204-5.

90 Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol* 2016; **15**: 673-84.

91 Prescott JW. Quantitative imaging biomarkers: the application of advanced image processing and analysis to clinical and preclinical decision making. *J Digit Imaging* 2013; **26**: 97-108.

92 Winblad B, Amouyel P, Andrieu S, et al. Defeating Alzheimer's disease and other dementias: a priority for European science and society. *Lancet Neurol* 2016; **15**: 455-532.

93 Franchi C, Lucca U, Tettamanti M, et al. Cholinesterase inhibitor use in Alzheimer's disease: the EPIFARM-Elderly Project. *Pharmacoepidemiol Drug Saf* 2011; **20**: 497-505.

94 Iturria-Medina Y, Sotero RC, Toussaint PJ, Mateos-Perez JM, Evans AC, Alzheimer's Disease Neuroimaging Initiative. Early role of vascular dysregulation on late-onset Alzheimer's disease based on multifactorial data-driven analysis. *Nat Commun* 2016; **7**: 11934.