Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic

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Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic

Harald Hampel[1,2,3,4], Sid E. O'Bryant[5], José L. Molinuevo[6], Henrik Zetterberg[7,8,9,10], Colin L. Masters[11], Simone Lista[1,2,3,4], Steven J. Kiddle[12,13], Richard Batrla[14] and Kaj Blennow[7,8]**

Abstract | Biomarker discovery and development for clinical research, diagnostics and therapy monitoring in clinical trials have advanced rapidly in key areas of medicine — most notably, oncology and cardiovascular diseases — allowing rapid early detection and supporting the evolution of biomarker-guided, precision-medicine-based targeted therapies. In Alzheimer disease (AD), breakthroughs in biomarker identification and validation include cerebrospinal fluid and PET markers of amyloid-β and tau proteins, which are highly accurate in detecting the presence of AD-associated pathophysiological and neuropathological changes. However, the high cost, insufficient accessibility and/or invasiveness of these assays limit their use as viable first-line tools for detecting patterns of pathophysiology. Therefore, a multistage, tiered approach is needed, prioritizing development of an initial screen to exclude from these tests the high numbers of people with cognitive deficits who do not demonstrate evidence of underlying AD pathophysiology. This Review summarizes the efforts of an international working group that aimed to survey the current landscape of blood-based AD biomarkers and outlines operational steps for an effective academic–industry co-development pathway from identification and assay development to validation for clinical use.

Alzheimer disease (AD) is a clinically and pathophysiologically heterogeneous, complex neurodegenerative disease and is the most common cause of age-related neurodegenerative disease, affecting millions of individuals worldwide. Currently, one in nine people over the age of 65 years are living with AD, and its prevalence is expected to grow exponentially over the next few decades[1].

The pathogenesis of AD involves interacting pathophysiological cascades, the core events of which include accumulation of the 42-amino-acid amyloid-β peptide ($A\beta_{1-42}$) into amyloid plaques in the brain parenchyma and the formation of intraneuronal neurofibrillary tangles composed of hyperphosphorylated tau protein[2]. Emerging evidence stresses the existence of additional molecular pathophysiological pathways, such as axonal disintegration[3], synaptic dysfunction and degeneration[4], innate immune responses and neuroinflammation[5,6], vascular and cell membrane dysregulation[7], and brain metabolic dysfunction[8], across the different stages of AD. Moreover, other proteinopathies and pathologies frequently coexist in the ageing brain, including TAR DNA-binding protein 43 (TDP43) or α-synuclein proteinopathies, non-AD tauopathies, vascular pathology and hippocampal sclerosis[9–12]. Consequently, establishing a definitive diagnosis and developing effective treatments for AD is complicated.

The aetiology and pathogenesis of AD are subjects of ongoing research and debate. The amyloid cascade hypothesis proposes that accumulation of aggregated forms of Aβ in the brain is the trigger and/or driver of the disease process[13]. However, recent studies have raised questions about the amyloid cascade as the exclusive cause and/or intervening link between the pathophysiology of AD and its clinical phenotype. The notion that biochemical and cellular mechanisms generate complex cognitive alterations has revitalized AD research, superseding the early descriptive studies with a molecular, mechanistic view.

The exponential increase in knowledge on interacting pathogenic mechanisms in AD holds promise for the development of new targeted therapies and prevention strategies, guided by biomarkers[14–17]. Substantial progress has been made in identifying PET and cerebrospinal fluid (CSF) biomarkers for AD, but financial and logistical issues limit the use of these markers as front-line diagnostic tools. Therefore, attention has turned towards the development of blood-based biomarkers. This Review summarizes the efforts of an international, interdisciplinary working group (BOX 1) that was convened

*e-mail: harald.hampel@med.uni-muenchen.de; kaj.blennow@neuro.gu.se

Key points

- Cerebrospinal fluid (CSF) and PET markers of amyloid-β and tau proteins are accurate in detecting the neuropathological changes of Alzheimer disease (AD).
- The use of CSF and PET biomarkers is limited by invasiveness or high costs; to address these issues, blood-based AD biomarkers are eagerly awaited.
- An international, interdisciplinary expert working group was convened by the Alzheimer's Precision Medicine Initiative to discuss the ideal development process for blood-based biomarkers.
- Nineteen blood-based biomarker assays were selected by the working group for further consideration.
- The working group outlined the pathway from biomarker identification and assay development to validation for clinical use and proposed clear steps for effective academic–industry co-development of blood-based AD biomarkers.
- The development, standardization and validation of blood-based biomarkers will be paramount to the implementation of precision medicine in AD.

to assess the current landscape of blood-based AD biomarkers and devise a roadmap for future biomarker research, from biomarker identification and assay development to clinical validation.

Applications of AD biomarkers

Primary and specialty care

Given the complex clinical phenomenology of AD and other neurodegenerative diseases, clinical, neurological and neuropsychological examinations are still an integral component of accurate late-stage detection of clinically symptomatic individuals. However, waiting times for appointments with specialists in the USA, UK and Ireland (and other countries) can be very long, resulting in substantial and often critical delays for patients and providers[18]. Memory clinics or general neurology clinics in many countries receive a broad range of referrals covering many conditions and diseases; therefore, streamlining of referrals to specialty clinics could have a substantial impact on health-care utilization and costs[18].

US-based legislation requires that elderly people aged ≥65 years receive annual cognitive examinations as part of the Annual Wellness Visit. However, older adults continue to be inadequately assessed for cognitive decline during primary care visits[19] — perhaps not surprising given that the average duration of primary care visits for geriatric patients is 21 min[20]. In addition, cognitive examinations are frequently administered, scored and interpreted incorrectly in primary care owing to a lack of training and expertise[21,22], and considerable differences exist between primary care and specialty care approaches to diagnosis, treatment and social support[23]. Therefore, a process that aids primary care practitioners in deciding which patients should receive a referral to a memory clinic would be of substantial benefit to both specialists and general practitioners. Such a system would reduce the overall clinic and medical system burden by decreasing the numbers of unnecessary referrals and diagnostic procedures[24,25]. To this end, biomarker-based diagnostics can greatly aid multistage selection of patients for appropriate centres.

To date, the literature has focused on diagnostic biomarkers[26,27] for specialty clinic settings, with little attention being paid to the screening instruments that are required for broad-based implementation in primary care settings. In addition, diagnostic paradigms continue to rely substantially on clinical symptoms, with only fairly recent research guidelines taking biomarkers of AD pathophysiology into account[28,29]. Until the mid-2000s, the diagnosis of AD had a clinicopathological basis, with no definitive positive diagnosis being possible until post-mortem confirmation of the presence of Aβ plaques and neurofibrillary tangles, and clinical diagnosis being assigned through exclusion of other dementia aetiologies once a patient had reached the late-stage syndromal dementia threshold[28]. This approach had profound limitations, given that the clinical symptoms of AD are by nature heterogeneous (including an increasing number of atypical clinical phenotypes) and vary regarding onset, progression and sequence of events. The symptoms also show considerable overlap with other CNS proteinopathies that cause dementia and with dementia due to cerebrovascular disease[30].

With the advent of reliable radiotracers with affinity for cerebral amyloid deposits[31] and the availability of clinically well-validated assays for detection of Aβ peptides and tau proteins in the CSF[3], progress has been made towards a clinicobiological diagnostic approach, in which pathophysiological and topographic biomarkers are integrated into the diagnostic paradigm[28] as adjuncts to core clinical criteria[29]. These biomarkers are available in specialty clinic settings in some countries, providing the means for dementia specialists to confirm the aetiological diagnosis with much higher certainty through the presence of characteristic features of AD pathophysiology[14,32,33]. In common with more mature translational research areas of biomedicine, such as oncology and cardiovascular medicine, the AD field is in need of biomarker-based diagnostics that can accurately and reliably identify at-risk individuals as early as possible in the disease process[34–38], preferably in primary care settings.

Precision medicine

Biomarker-based diagnostics are also expected to open up new opportunities for precision medicine, allowing a shift away from the traditional 'one-size-fits-all'

Author addresses

[1]AXA Research Fund and Sorbonne University Chair, Paris, France.
[2]Sorbonne University, GRC n° 21, Alzheimer Precision Medicine (APM), AP-HP, Pitié-Salpêtrière Hospital, Paris, France.
[3]Brain & Spine Institute (ICM), INSERM U 1127, Paris, France.
[4]Institute of Memory and Alzheimer's Disease (IM2A), Department of Neurology, AP-HP, Pitié-Salpêtrière Hospital, Paris, France.
[5]University of North Texas Health Science Center, Fort Worth, TX, USA.
[6]Barcelonaβeta Brain Research Center, Pasqual Maragall Foundation, Barcelona, Spain.
[7]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.
[8]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden.
[9]Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, UK.
[10]UK Dementia Research Institute at UCL, London, UK.
[11]The Florey Institute, The University of Melbourne, Melbourne, Australia.
[12]Department of Biostatistics and Health Informatics, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK.
[13]MRC Biostatistics Unit, Cambridge Institute of Public Health, University of Cambridge, Cambridge, UK.
[14]Roche Diagnostics International, Rotkreuz, Switzerland.

Box 1 | **The APMI Working Group**

The contributors to the Alzheimer's Precision Medicine Initiative (APMI) Working Group are listed below.

Lisi Flores Aguilar (Montréal, Quebec, Canada), Claudio Babiloni (Rome, Italy), Filippo Baldacci (Pisa, Italy), Norbert Benda (Bonn, Germany), Keith L. Black (Los Angeles, CA, USA), Arun L. W. Bokde (Dublin, Ireland), Ubaldo Bonuccelli (Pisa, Italy), Karl Broich (Bonn, Germany), René S. Bun (Paris, France), Francesco Cacciola (Siena, Italy), Juan Castrillo (Derio, Spain), Enrica Cavedo (Paris, France), Roberto Ceravolo (Pisa, Italy), Patrizia A. Chiesa (Paris, France), Olivier Colliot (Paris, France), Cristina-Maria Coman (Paris, France), Jean-Christophe Corvol (Paris, France), Augusto Claudio Cuello (Montréal, Quebec, Canada), Jeffrey L. Cummings (Las Vegas, NV, USA), Herman Depypere (Ghent, Belgium), Bruno Dubois (Paris, France), Andrea Duggento (Rome, Italy), Stanley Durrleman (Paris, France), Valentina Escott-Price (Cardiff, UK), Howard Federoff (Irvine, CA, USA), Maria Teresa Ferretti (Zurich, Switzerland), Massimo Fiandaca (Irvine, CA, USA), Richard A. Frank (Malvern, PA, USA), Francesco Garaci (Rome, Italy), Hugo Geerts (Berwyn, PA, USA), Remy Genthon (Paris, France), Nathalie George (Paris, France), Filippo S. Giorgi (Pisa, Italy), Manuela Graziani (Rome, Italy), Marion Haberkamp (Bonn, Germany), Marie-Odile Habert (Paris, France), Harald Hampel (Paris, France), Karl Herholz (Manchester, UK), Eric Karran (Cambridge, MA, USA), Seung H. Kim (Seoul, South Korea), Yosef Koronyo (Los Angeles, CA, USA), Maya Koronyo-Hamaoui (Los Angeles, CA, USA), Foudil Lamari (Paris, France), Todd Langevin (Minneapolis–Saint Paul, MN, USA), Stéphane Lehéricy (Paris, France), Simone Lista (Paris, France), Jean Lorenceau (Paris, France), Dalila Mango (Rome, Italy), Mark Mapstone (Irvine, CA, USA), Christian Neri (Paris, France), Robert Nisticò (Rome, Italy), Francis Nyasse-Messene (Paris, France), Sid E. O'Bryant (Fort Worth, TX, USA), George Perry (San Antonio, TX, USA), Craig Ritchie (Edinburgh, UK), Katrine Rojkova (Paris, France), Simone Rossi (Siena, Italy), Amira Saidi (Rome, Italy), Emiliano Santarnecchi (Siena, Italy), Lon S. Schneider (Los Angeles, CA, USA), Olaf Sporns (Bloomington, IN, USA), Nicola Toschi (Rome, Italy), Steven R. Verdooner (Sacramento, CA, USA), Andrea Vergallo (Paris, France), Nicolas Villain (Paris, France), Lindsay A. Welikovitch (Montréal, Quebec, Canada), Janet Woodcock (Silver Spring, MD, USA) and Erfan Younesi (Esch-Sur-Alzette, Luxembourg).

approach[34,39–42]. One of the key objectives of precision medicine is to provide new models for prevention, early detection, differential diagnosis and treatment of AD and other neurodegenerative diseases, according to individual biological differences reflected by multimodal biomarkers, with the ultimate goal of improving clinical outcomes and quality of patient care[32,34,36,43]. Following the lead of advanced models of oncology and cardiovascular medicine, innovative biomarker studies are expected to detect specific diagnostic, prognostic and predictive biomarker signatures to enable therapies to be adapted to individual patients[39].

Implementation of the precision medicine paradigm in neurodegenerative diseases such as AD requires an array of converging advanced analytical methods. Systems biology and systems neurophysiology[41] can provide innovative, hypothesis-free models to explain the complex and heterogeneous origin and time course of AD pathophysiology[34]. Genotype–phenotype relationships and disease mechanisms can be probed at multiple levels, including the genome, epigenome, transcriptome, microRNome, proteome, peptidome, metabolome, lipidome and microbiome[34,44,45]. Longitudinal investigations using systems biology-based strategies can help to characterize the complex molecular pathophysiology of the subforms of AD, all of which evolve through the convergence of alterations of homeostasis and/or failures in many systems, networks, signalling pathways and pathophysiological processes[44].

Precision medicine is being facilitated by the development of large-scale biological databases and the evolution of advanced high-throughput 'omics' sciences. Novel methods developed within the omics disciplines have been successfully applied in the cancer field and are expected to transfertilize to AD and other neurodegenerative diseases. For example, validated microarray expression profiling and RNA-seq technologies allow differential gene expression to be analysed at the whole-genome level in any specific sample at any given time point. Numerous studies have exploited these transcriptomics methods to recognize biomarkers in specific cancer subtypes[46]. Cancer-specific microRNAs are useful dynamic indicators of tumour growth, as they can be detected in the blood from the earlier stages of tumour development and their concentration increases as the tumour advances[47]. Innovative proteomics methods, such as immuno-PCR, are improving the sensitivity of protein biomarker detection[48,49]. Powerful computational and integrative network biology tools are needed to assimilate the large volumes of multimodal information generated by the omics exploratory analyses[50].

In AD, many of the underlying pathologies are well known. Therefore, hypothesis-free systems theory approaches can be advanced in synergy with the refinement of methods that directly reflect core pathologies. Such an approach is already being applied to the study of blood-based biomarkers for Aβ pathology[51–53] and axonal degeneration[54,55].

Moving towards blood-based biomarkers

Why are blood-based biomarkers needed?

Advances in PET and CSF biomarker analyses have the potential to improve the accuracy of the diagnostic process for AD. However, these methods have limitations that preclude their use as first-line diagnostic tools. These limitations could be countered by the use of blood-based biomarkers[27].

An accurate and standardized blood test is likely to be more cost-effective than PET scans, the expense of which limits accessibility, generalizability and availability[56,57], particularly outside the USA. Because of this high cost, PET testing in AD is likely to be reimbursed only as an adjunct to other less expensive tests, as was previously the case with PET scans for cancer. In addition, blood testing is less invasive than CSF testing, which requires lumbar puncture[58,59]. Furthermore, blood testing is already an established feature of clinical routines globally, requiring no further introduction and training for health-care professionals, and can be easily performed in a variety of relevant settings — including primary care, community-based medicine centres or even in a patient's home — and at repeated intervals[60]. Global blood sample-handling infrastructures are also well established, allowing samples to be collected and processed using existing systems. Therefore, blood-based screening biomarkers for AD can meet the scalability requirements for primary care settings and even for the broad population-based screening approach that is likely to evolve with the advancing innovative precision medicine framework. Finally, the use of blood-based biomarkers offers the potential to test a large range of comprehensive exploratory and candidate pathophysiological

Sensitivity
Diagnostic sensitivity is the probability that a test result is positive when the disease is present.

biomarkers, reflecting the full spectrum of molecular mechanisms underlying polygenic AD, beyond the conventional Aβ-based and tau-based tests.

Blood-based biomarkers are an ideal choice as the first step of the multistage diagnostic process that begins in primary care settings. These biomarkers provide the means to determine which individuals should be referred for assessment by specialists, including diagnostic CSF analysis, MRI and/or amyloid PET diagnostics[25,61]. In addition to meeting an important clinical need, the availability of these tools would provide a viable path towards regulatory[62] and reimbursement approval, using the cancer paradigm as a model.

Challenges in biomarker development

Identification of potential blood-based biomarkers for CNS diseases presents several challenges (FIG. 1). Blood is highly complex and includes a range of different molecules, including proteins, peptides, nucleic acids, lipids and metabolites, which can be detected in plasma, exosomes and cellular components. Erythrocytes, leukocytes and platelets can be isolated into distinct cell subsets via flow cytometry or from the buffy coat after density gradient centrifugation. Each of the different cellular compartments is a potential source of biomarkers and can introduce variability into analyses[60,63].

The diverse candidate biomarkers in blood include protein concentrations, activity, isoforms and post-translational modifications; metabolic products, such as amino acids, carbohydrates, lipids and organic acids; and nucleic acids. Single-nucleotide polymorphisms (SNPs) in the DNA can act as biological markers, helping to localize genes associated with the disease[64]. When SNPs occur within a gene or in a regulatory region adjacent to a gene, they can affect the gene's function, thereby playing a key part in disease development. High-throughput next-generation DNA sequencing technologies are enabling whole genomes to be screened to identify novel genetic variants that influence the risk of AD[65,66]. The 1000 Genomes Project[67,68], supported by the US National Human Genome Research Institute consortium, has made substantial progress towards this goal.

The CNS is effectively a contained environment, and potential biomarkers might be present at very low concentrations in blood once they have crossed the blood–brain barrier (if they cross it at all as intact molecules)[57,69]. Evidence for peripheral manifestations of AD is limited[70], although there are some indications that the blood–brain barrier becomes increasingly compromised with normal ageing and AD progression[71,72]. In addition, physiological mechanisms occurring in the periphery might restrict the clinical utility of blood-based AD biomarkers. For example, acute-phase or inflammatory proteins, small molecules and metabolites that are associated with AD might also be present in peripheral organs[57]. Specific confounders of blood biomarker assays include biomarker dilution as a consequence of the modest volume of the CSF compared with the blood and extracellular fluid[73]; degradation in the liver or directly in the blood by proteases; matrix effects caused by adherence to plasma proteins or even blood cells; and excretion from the kidneys. These factors might substantially lower the concentrations of the biomarker and decrease the time window for testing.

A further major issue is the frequent existence of overlapping neurodegenerative diseases and comorbidities, including cardiovascular, respiratory, hepatic, renal and rheumatic disease, in patients with AD, all of which might affect the protein profiles in plasma[57]. In addition, as AD is a complex polygenic disease, amyloid and/or tau accumulation is always accompanied by other relevant molecular and cellular pathophysiological mechanisms and brain system failures. Elucidating this true complexity and heterogeneity, including through blood-based biomarker analyses, might substantially improve our understanding of the disease to enable implementation of the precision medicine paradigm[24,39,44].

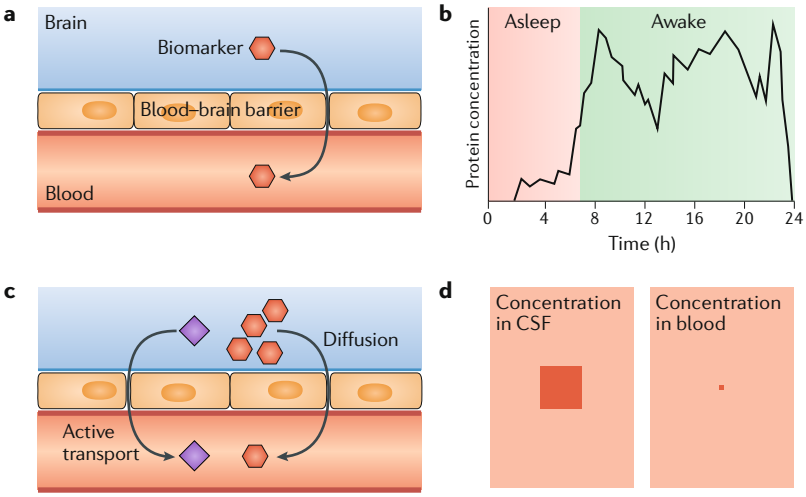

Fig. 1 | **Challenges in developing a blood-based biomarker for CNS disease.** Several factors must be considered when searching for a biomarker for a CNS disease, particularly if the biomarker is to be blood-based. **a** | Blood–brain barrier. To allow detection in the blood, the biomarker must be able to cross the blood–brain barrier. **b** | Diurnal fluctuations in protein levels. Both cerebrospinal fluid (CSF) and blood exhibit diurnal variations in protein concentrations. Biomarker levels might not peak at the same time in CSF as in blood, and a biomarker assay will require sampling at the peak concentration and/or sufficient sensitivity to detect the biomarker throughout the day. **c** | Active versus passive transport. Biomarkers might originate in either CSF or blood. Those originating in CSF could enter the blood via active or passive transport, and understanding the exact nature of the derivation from CSF will be essential to develop an assay. **d** | Differences in concentrations. Biomarker levels are not always similar in CSF and blood; for example, amyloid-β concentrations are tenfold lower in plasma than in CSF (35 pg/ml versus 350 pg/ml).

Gathering a consensus

In view of the strong rationale for the development of a blood-based biomarker for AD, and the associated challenges, an international, interdisciplinary expert working group (BOX 1) was convened by the Alzheimer's Precision Medicine Initiative (APMI) in March 2016 to discuss the ideal development process. The working group was selected through profiling of global experts and other working groups, which was conducted as part of the extensive biomarker literature search. Experts were selected on the basis of their publications and involvement in research in the field of neurodegenerative biomarkers in CSF and blood.

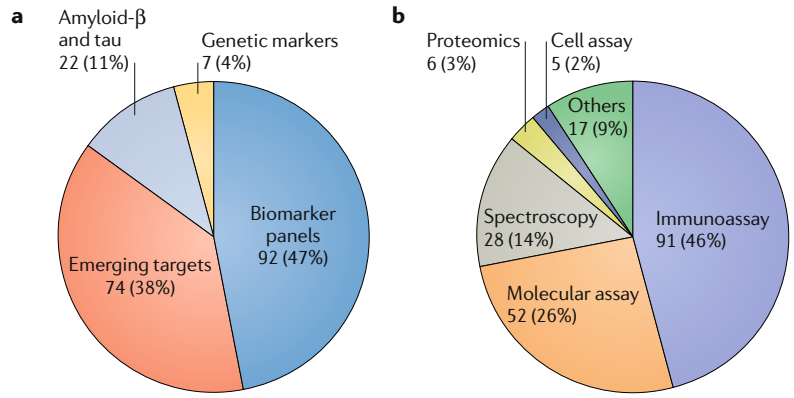

Fig. 2 | **Candidate blood-based biomarkers identified in the landscape analysis.** Technology (part **a**) and platform (part **b**) categories for the 196 high-quality studies identified in the landscape analysis.

The meeting was supported by Roche Diagnostics International, who assisted with the organization of scientific meetings, video and teleconferences according to guidance from the scientific chair and with the support of the scientific writing agency. Roche Diagnostics International also provided the meeting room and refreshments according to the guidance of the compliance guidelines.

In advance of the working group meeting, an in-depth, comprehensive review of the literature was conducted (including the 'grey literature' — that is, patents, press releases and proprietary databases) to identify candidate biomarkers (see next section for further details). A structured and detailed pre-work survey of the meeting expert attendees provided additional material for analysis of the current blood-based biomarker landscape in AD and the most pressing challenges for the successful development of further markers. The aim of the survey was to identify and rank candidate blood-based biomarkers, which were selected from the landscape analysis, in order of priority for further development.

The working group itself provided a forum for definition of an ideal target product profile for a blood-based biomarker in AD. A critical appraisal of the selected biomarkers was then performed to determine whether they met this ideal profile and to assess the general quality of research findings and their suitability for further development. Further considerations were the optimal validation process for a biomarker, the best route to determine the efficacy of a biomarker (predictive values versus accuracy) and the ideal cohorts in which biomarkers could be evaluated. In-depth discussions were also held on several candidate biomarkers, with the aim of defining key questions for evaluating any current or future candidates.

The current biomarker landscape

The working group conducted a landscape analysis to review all blood-based biomarkers in development for AD globally and assessed these biomarkers on the basis of pre-defined selection criteria to prioritize those with the greatest likelihood of successful implementation. The data were sourced from publications (MEDLINE database 2011–2015), patents (worldwide patents, including Patent Cooperation Treaty, USA and China, from 2008 to 2015), press releases and conference abstracts from eight major neuroscience or AD conferences as well as ChinaBio proprietary databases.

After screening of >28,000 reports, 1,404 studies of blood-based biomarkers for AD were identified, 1,039 of which were from publications and conference abstracts. The 1,039 studies were categorized according to biomarker type: 18% were on the conventional AD biomarkers Aβ and tau (or their associated peptides, proteins or variants), 19% were on genetic markers, 29% were on biomarker panels and 34% were on markers related to emerging mechanisms such as inflammation, immune response, oxidative stress, DNA damage, mitochondrial dysfunction and neuronal or microvascular injury. The identified biomarkers were then screened with regard to intended use, biomarker type, technology platform, test type and analyte and the availability of human and/or clinical data. A final screen was performed to focus only on high-quality candidates according to novelty, cohort quality, presence and quality of validation study, diagnostic performance and perceived quality of research. The final list contained 196 candidate biomarkers, with biomarker panels and emerging targets being the most common categories (FIG. 2a). Immunoassays and molecular assays were the most popular technology platforms that were utilized for high-quality AD blood-based biomarkers, accounting for >70% of the studies (FIG. 2b). A potential limitation of the search was the fairly narrow date range, which might have resulted in the exclusion of some potentially useful biomarkers that were published outside this range. The most promising biomarker candidates and the key studies, including those published since the original analysis, are summarized below.

Among the conventional AD biomarkers, the $A\beta_{1-42}$:$A\beta_{1-40}$ ratio has shown potential as a screening or diagnostic marker in several studies. Most early studies on plasma $A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-42}$:$A\beta_{1-40}$ ratio, using enzyme-linked immunosorbent assay (ELISA) methods, found no differences or only minor differences between AD and control groups[74–76]. More recently, a large cohort study using a novel ultrasensitive immunoassay technique (single-molecule array, or Simoa), showed weak but significant correlations between plasma and CSF $A\beta_{1-42}$ and $A\beta_{1-40}$ levels and $A\beta_{1-42}$:$A\beta_{1-40}$ ratio[53]. In addition, plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels and $A\beta_{1-42}$:$A\beta_{1-40}$ ratio were lower in patients with AD than in cognitively healthy individuals and patients with mild cognitive impairment (MCI) or subjective cognitive decline[53].

An alternative technique for measuring Aβ peptides in plasma involves immunoprecipitation and mass spectrometry[52,77]. A pilot study found a trend for a reduction in plasma $A\beta_{1-42}$ levels and $A\beta_{1-42}$:$A\beta_{1-40}$ ratio in patients with AD compared with controls[77]. A separate study, using a modified method involving proteolytic digestion of Aβ peptides before mass spectrometry, found that plasma $A\beta_{1-42}$ levels and $A\beta_{1-42}$:$A\beta_{1-40}$ ratios were reduced in amyloid PET-positive individuals[52]. Furthermore, the plasma $A\beta_{1-42}$:$A\beta_{1-40}$ ratio had good diagnostic accuracy, as indicated by a receiver operating characteristic (ROC)

Subjective cognitive decline
A self-reported decline in cognition, undetected by standard neuropsychological tests.

Receiver operating characteristic
(ROC). The ROC curve is a plot of sensitivity versus 1 – specificity for the different possible cut-off points of a diagnostic test. Accuracy of the diagnostic test is based on the area under the ROC curve; the closer the area under the ROC curve is to 1, the better the test.

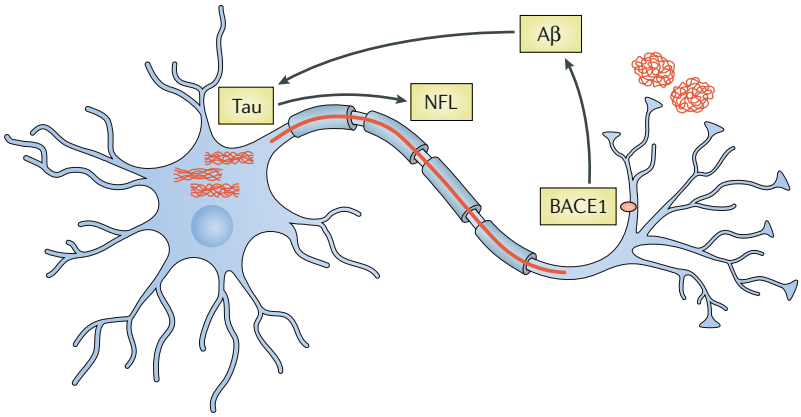

Fig. 3 | **Promising blood-based biomarker candidates.** The figure shows the intracellular and extracellular locations of four promising blood-based biomarker candidates. Arrows indicate the sequence of pathology progression. Amyloid-β (Aβ) peptides, in particular $Aβ_{1–42}$, are implicated in Alzheimer disease (AD) pathogenesis. The $Aβ_{1–42}$:$Aβ_{1–40}$ ratio seems to be the most promising Aβ-related biomarker in the blood. The first step in the generation of Aβ peptides is the cleavage of amyloid precursor protein by β-secretase 1 (BACE1). Measurement of BACE1 activity in the blood might be useful for predicting progression from mild cognitive impairment to AD dementia. Phosphorylated tau protein is a major component of intraneuronal neurofibrillary tangles, which are often present in AD. The abnormal phosphorylation of tau is thought to be driven by Aβ peptides. Tau levels in the blood might be useful as a predictor of future cognitive decline. Neurofilament light (NFL) is an axonal protein that is released into the brain interstitial fluid following neuronal or axonal injury. NFL levels in the blood are elevated in AD and other neurodegenerative diseases; therefore, blood-based NFL could be useful as a biomarker of neurodegeneration.

area under the curve (AUC) value of 0.8865. A study published in 2018 further supports the use of immunoprecipitation–mass spectrometry methods to measure plasma Aβ peptides[51]. Nakamura and colleagues reported high performance of plasma $Aβ_{1–40}$:$Aβ_{1–42}$ ratio and amyloid precursor protein $(APP)_{669–711}$:$Aβ_{1–42}$ ratios, and their composite, for the prediction of amyloid PET burden[51].

Results in the field have been mixed; for example, a multicentre study did not find the plasma $Aβ_{1–40}$:$Aβ_{1–42}$ ratio to be a useful diagnostic[78]. Research also suggests that plasma amyloid markers are modified by cardiovascular and cerebrovascular factors[79,80], thereby limiting the diagnostic and predictive value of these markers[81]. Therefore, though promising, additional work is needed to determine the utility of plasma Aβ as a screening tool for brain amyloidosis and AD, including large clinical and prospective cohorts as well as direct comparisons of different pre-analytical and analytical methodologies, as immunoprecipitation–mass spectrometry could be difficult to generalize for clinical use[82].

Plasma levels of tau protein have also been successfully quantified using novel, highly sensitive immunoassay techniques, and various technologies have shown plasma tau levels to be increased in patients with AD compared with controls[83,84]. Plasma tau levels showed a strong association with AD in a meta-analysis (average ratio of levels in AD versus controls: 1.95, 95% CI 1.12–3.38, $P = 0.02$)[3]. A study based on the large Alzheimer's Disease Neuroimaging Initiative (ADNI) and Biomarkers For Identifying Neurodegenerative Disorders Early and Reliably (BioFINDER) cohorts confirmed an increase in plasma tau in AD dementia, although the levels overlapped substantially with those in elderly individuals without cognitive impairment[85]. Interestingly, longitudinal evaluations showed correlations between high baseline levels of plasma tau and future cognitive decline, increased atrophy rates (measured by MRI) and hypometabolism (measured by ^{18}F-FDG–PET)[85]. Thus, current data suggest a minor increase in plasma tau in AD, although the overlap with control values is too large for this marker to be diagnostically useful. In a 2017 study, elevated plasma tau levels were associated with cognitive decline, independent of elevated brain Aβ (REF.[86]), suggesting a use for plasma tau as a non-disease-specific screening tool.

The 'emerging targets' category includes the axonal protein neurofilament light (NFL), which can be quantified using the ultrasensitive Simoa technique[87]. Serum NFL levels correlate closely with CSF levels, suggesting that blood measurements reflect brain pathophysiology[88]. A recent study on the ADNI cohort found a marked increase in plasma NFL in patients with AD, with an ROC AUC value of 0.87 (REF.[55]), which is comparable to the plasma $Aβ_{1–42}$:$Aβ_{1–40}$ ratio results reviewed above. Plasma NFL levels were highest in MCI patients with positive amyloid PET scans and predicted faster cognitive deterioration and a faster rate of both future brain atrophy (measured by MRI) and hypometabolism (measured by ^{18}F-FDG–PET)[55]. Interestingly, a study on familial AD (FAD) showed that blood NFL levels were increased not only in patients with symptomatic FAD but also in pre-symptomatic mutation carriers[54], and the levels correlated with the estimated time to symptom onset as well as cognitive and MRI measures of disease stage. These findings suggest that plasma NFL reflects neurodegeneration in the preclinical phase of AD. However, high plasma (or CSF) NFL is not specific for AD but is found in several neurodegenerative disorders, including frontotemporal dementia, progressive supranuclear palsy and corticobasal syndrome[89–91]. Thus, plasma NFL might have value as a screening test for neurodegeneration in the initial primary care evaluation of patients with cognitive disturbances.

Also in the emerging targets category was β-secretase 1 (BACE1), the enzyme that is responsible for the first cleavage step in the generation of Aβ peptides from APP[92]. Studies using ELISA-based methods have shown increased BACE1 activity in the plasma of patients with MCI or AD compared with healthy controls[93,94]. One of these studies found that plasma BACE1 activity was significantly higher in individuals with MCI who progressed to AD over a 3-year follow-up period than in those who remained cognitively stable over a 3-year follow-up period[94]. These results suggest that plasma BACE1 activity has potential as a biomarker to predict progression from MCI to AD dementia, which could be valuable in primary care and clinical trial settings; however, further studies are needed to validate these findings. The four candidate blood-based biomarkers — $Aβ_{1–42}$:$Aβ_{1–40}$ ratio, tau, NFL and BACE1 — and their roles in AD pathogenesis are depicted in FIG. 3.

Blood-based biomarker panels are another area of interest for AD, as a combination of markers might show better separation between groups than single

biomarkers. In recent years, several protein panels have shown diagnostic or prognostic potential[61,95–97], including a 21-protein panel for AD screening that has demonstrated a positive predictive value (PPV) of 0.85 and a negative predictive value (NPV) of 0.94 in a preliminary validation[61]. Panels for non-protein analytes, such as amino acids[98], microRNAs[99,100] and lipids[101], have also shown promise, but independent, large-scale validation studies are needed.

The road to the clinic

Historical difficulties with development

Assay reliability and robust replication and validation of initial results are key issues for blood-based biomarker development[25,69,102,103]. Historically, the analytical sensitivity of available technologies has been an important limitation: standard immunochemical methods have often been insufficiently sensitive to allow the reliable quantification of CNS-derived molecules in the blood, although this situation is changing with the advent of ultrasensitive assays[104]. Studies have shown considerable variability owing to inconsistencies in clinical cohorts (for example, discrepancies in diagnostic evaluations and/or disease stages), problems with availability and standardization of the samples, and pre-analytical and analytical differences. As has been observed for CSF assays, consistent use of automated assays across studies might ameliorate analytical differences. However, the lack of standardization of pre-analytical protocols is also a major issue; for example, the majority of errors in proteomic analysis are known to arise in the pre-analytical phase[63,105]. Pre-analytical variables can be divided into several main categories[69,105] (BOX 2). Initial steps towards standardization have already been made[105], with the formation of BBB-PIA, a professional interest area focused on biofluid-based biomarkers, in association with the Alzheimer's Association's International Society to Advance Alzheimer's Research and Treatment (ISTAART). This group will drive towards a consensus on pre-analytical and analytical protocols and will lead the development of a repository of clinical reference samples to enable assay harmonization and clinical performance assessment. However, further action is needed, and communication of these issues to wider audiences is also warranted.

The experimental design of a biomarker evaluation study — including obtaining relevant cohorts with appropriate sample availability — will be important. Although many cohorts exist and potentially have samples suitable for use in the development of a blood-based biomarker, these cohorts might not match the patient profile encountered in the target setting. In addition, any candidate biomarker will need to offer excellent scalability to allow large-scale use.

Ideal biomarker characteristics

The first steps in the development of a blood-based biomarker for AD diagnosis should be to finalize the specific context of use (COU) for regulatory purposes and to determine the target product profile, enabling targeted development of the biomarker to match specific requirements for the intended COU. This prospective approach is likely to improve success compared with current approaches based on a discovery model, whereby biomarkers are first identified in case–control studies before being retrospectively adapted to an intended COU.

The working group agreed that a blood-based biomarker in AD should provide a tool to assess patients reporting cognitive deficits in the primary care setting, allowing identification of the subset of patients who demonstrate biological signs consistent with AD and require further specialist diagnostic testing. Ideally, the biomarker could also be used to rule out other neurodegenerative diseases with symptoms resembling those of AD. Validation against current clinical and biomarker-based diagnostic paradigms would also be required. Ideally, the International Working Group-2 (IWG-2) diagnostic criteria[28] — that is, evidence of cognitive impairment in conjunction with amyloid PET or the CSF 'AD signature' (reduced Aβ and elevated tau CSF concentrations) — should be used. Alternatively, it might be possible to employ the recently proposed biomarker-guided A/T/N classification system[106] in the validation stage.

The predictive accuracy requirements of a screening tool (PPV and NPV), as well as the follow-up diagnostic resources, treatments and associated costs, are dependent

Box 2 | Pre-analytical factors

The results of blood-based biomarker measurements can be influenced by numerous pre-analytical factors, as outlined below.

Patient-related factors
- Demographics (age, sex, weight and ethnicity)
- Diet, including supplements
- Health status
- Medication
- Drug and alcohol use
- Medical conditions
- Exercise
- Posture and bed rest

Blood collection
- Needle gauge and composition
- Withdrawal site
- Collection tube characteristics
- Anticoagulant use
- Time of collection

Blood processing
- Time to storage
- Centrifugation (for example, presence and type of separator, temperature and number of rounds (single or double))
- Addition of protease inhibitor
- Use of denaturation step or protein extraction
- Use of plasma or serum

Sample storage
- Storage temperature
- Storage volume
- Number of freeze–thaw cycles
- Duration

Positive predictive value (PPV). The probability that a patient has the disease when the test result is positive.

Negative predictive value (NPV). The probability that a patient does not have the disease when the test result is negative.

Context of use (COU). A statement that describes the manner and purpose of use for the biomarker in drug development. The supporting data and analyses submitted with the biomarker qualification determine the acceptability of the qualified COU.

A/T/N classification system A classification system that uses three binary biomarker categories reflecting Alzheimer disease pathophysiology. 'A' refers to biomarkers of amyloid-β (Aβ) pathology (cerebrospinal fluid (CSF) $A\beta_{1-42}$ or amyloid PET), 'T' refers to biomarkers of tau pathology (CSF hyperphosphorylated tau or tau PET) and 'N' refers to biomarkers of neurodegeneration or neuronal injury (CSF total tau ^{18}F-FDG–PET or structural MRI).

Specificity
Diagnostic specificity is the probability that a test result is negative when the disease is absent.

on the COU. A primary care-based screening tool would not be intended as a diagnostic but would serve as a gatekeeper to the confirmatory diagnostic procedures utilized by specialty physicians. As with all primary care screening tools, the primary goal is to attain a high NPV. If the base rate of a condition in the general population is very low, the screening tool serves to exclude the vast majority of patients who do not require more invasive and expensive diagnostic procedures. For example, in mammography screening for breast cancer and in depression screening in primary care, PPVs are <30%[61] but are counteracted by excellent NPVs (>90%). Tools with PPVs <40% (or even <20%) but NPVs >90% (or even >95%) are commonplace in primary care settings[61]. For confirmatory diagnostic purposes, the overall diagnostic accuracy of the primary care screening tool must be taken into account, alongside the full diagnostic procedures in specialty clinics, including CSF and/or PET biomarkers (which have excellent PPV). As disease-modifying therapies (DMTs) for AD become available[107], the primary care screen will be increasingly important to constrain costs while broadly opening up access to specialty diagnostics.

The working group consensus was that a candidate biomarker for primary care screening should have as high a PPV as possible — ideally >50%. However, given the ethical implications of false negatives, an NPV of ≥90% would be more important, with 95–98% being an ideal target[61]. On the other hand, a high PPV and an acceptable NPV might be more desirable in a pharmaceutical setting that focuses on identifying as many amyloid-positive patients as possible for inclusion in clinical trials or possible future treatments.

In terms of assay technology, the consensus indicated a strong preference for panel-based assays (that is, a combination of biomarkers, developed together or individually) over single biomarkers. Panels might offer wider applications beyond AD diagnosis, allowing more generalized testing for other neurodegenerative diseases, with each pathological condition having a unique protein signature. Several studies have established panels of biomarkers to discriminate between cognitively healthy controls and individuals with AD, and large arrays of differently combined proteins were assessed to yield high specificity and sensitivity. In view of the need for a holistic approach to standardize blood biomarkers for AD diagnosis, it is crucial to understand the links between various individual biomarkers and overcome the outdated approach of examining a single candidate biomarker at a time[108]. Various methods are available to detect biomarkers in blood[27,108–110], so it is crucial to standardize the technologies and workflows to generate and analyse complex multidimensional data. A general consensus on protocols and ultrasensitive analytical methods is needed across multicentre studies to establish a standardized panel of biomarkers for AD diagnosis[108,109]. Novel ultrasensitive immunoassay methods allow detection and quantification of biomarkers at concentrations several-fold lower than those accessible by existing immunoassays, raising the prospect of a new array of blood biomarkers covering the entire spectrum of molecular pathophysiological mechanisms in AD. Moreover, these technologies may serve as a foundation to enable AD and other neurodegenerative diseases to be diagnosed earlier than ever before[111].

Unfortunately, in the various types of panels that have been developed so far, the numbers of biomarkers have increased the complexity of manufacture and commercial development, complicated validation and standardization processes and led to increased costs — substantial concerns when considering scalability to meet global primary care needs. In addition, analysis of large numbers of candidate molecules in small numbers of patients via panel-based assays can result in data overfitting and misleading results, thereby giving the false impression that the panel performs much better than individual biomarkers[112]. This problem can be partly offset by the use of a training set to initially reduce the dimensionality before validation in an independent cohort[113]. Therefore, although the working group agreed that a multi-marker panel is likely to be needed, they also propose that the number of biomarkers should be restricted as much as possible within the NPV and PPV requirements to help overcome the validation and regulatory hurdles.

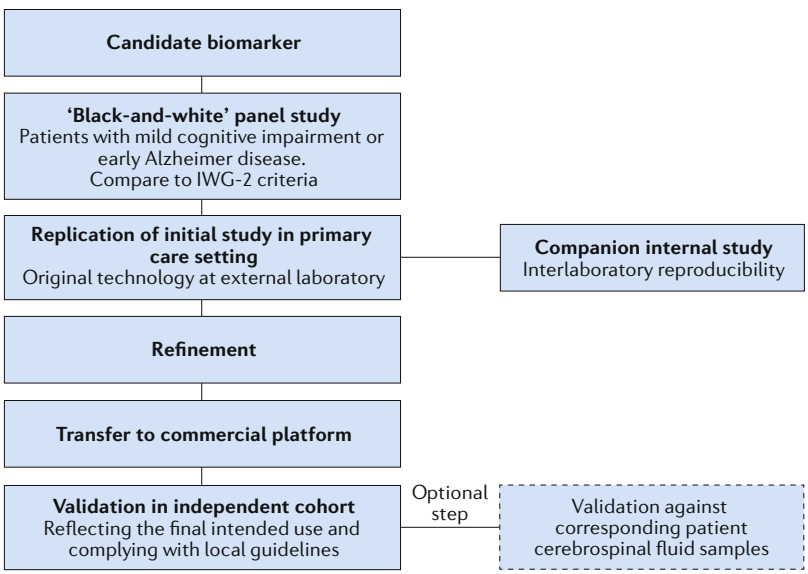

Fig. 4 | **Idealized validation process for blood-based biomarkers.** IWG-2, International Working Group-2.

Refinement and validation

Following identification of a prioritized candidate biomarker, the biomarker will require refinement and validation. Refinement involves agreeing on a best-practice protocol, including blood collection procedures, pre-analytical sample handling and procedures for harmonization between different laboratories. The validation steps are outlined below.

Considerations and recommendations. The consensus process for a pathway towards biomarker validation is shown in FIG. 4. Before validation for widespread use, a biomarker should already have undergone initial clinical validation after the discovery analyses. Validation of target biomarkers should begin with assessment in

Box 3 | **Criteria for evaluation of identified biomarkers**

Tier 1: validated biomarkers
Markers with strong performance data that have successfully completed initial validation studies in subsequent reports or in a multicentre setting with >120 patients

Tier 2: high-performing biomarkers
Markers with good performance data that have not completed validation studies. Markers in this category are further divided into subgroups depending on the intended use and the number of patients tested.

- 2A: Specificity and sensitivity >80%, or specificity or sensitivity >85%, with intended use in early detection, prediction or differential diagnosis; tested in >120 patients
- 2B: Specificity and sensitivity >80%, or specificity or sensitivity >85%, with intended use in early detection, prediction or differential diagnosis; tested in <120 patients
- 2C: Specificity and sensitivity >80%, or specificity or sensitivity >85%, with intended use in diagnosis
- 2D: Same criteria as Tier 2A–C, with markers having applications in both blood and cerebrospinal fluid

Tier 3: promising candidate biomarkers
Innovative markers with promising performance data but yet to produce higher-performance results in clinical settings

a 'black-and-white' panel study, in which samples are obtained from patients with AD who have been diagnosed according to the IWG-2 criteria (amyloid PET or CSF AD signature), and from a comparable number of cognitively healthy controls. This initial study would aim to establish concordance between the novel biomarker and gold standard methods and would be performed using the technology that was employed in the initial development of the biomarker test. Ideally, the study would be conducted by an external investigator blinded to the patient characteristics associated with each sample. Analysis of a black-and-white panel would not test the biomarker within the intended COU but would instead attempt to independently validate the overall accuracy of the biomarker in a known group design. In addition, a single screening test may be limited[61], so multiple tests might be required if the target population is to include both early AD and MCI.

Following the black-and-white panel, the next step would be to attempt to replicate the initial study results in a sample set that more accurately reflects primary care. This second study would again employ the original technology from the developing laboratory but would begin to transfer the technology to an existing diagnostic assay platform that is globally available, capable of meeting the scalability needs for the primary care setting, and developed by an entity with prior experience in undergoing regulatory approval for diagnostics. Technology transfers will probably be mandatory, as discovery platforms are unlikely to meet the stringent requirements for diagnostic assays outlined by the Clinical Laboratory and Standards Institute. Collection and assay of samples across both the original platform and the intended platform for use downstream in the regulatory pathway creates a built-in 'bridge' cohort from the initial trial to test the technology within the intended COU. If these studies show strong concordance of results between internal and external laboratories, data acquired across the original training cohort and the new COU-specific test cohort (using the new technological platform) would be applicable for use in further refinement of the diagnostic algorithm, enabling a case for regulatory approval to be built. Finally, samples would be assayed on the intended platform for regulatory approval across multiple laboratories to establish interlaboratory reproducibility.

Next, the full regulatory pathway, including all additional trials that might be required, would be established in conjunction with regulatory authorities[114–116]. An additional validation study within an independent primary care cohort would probably be required for regulatory approval. For US approval, this study would need to comply with FDA requirements for registrational studies (that is, prospective data or robustly collected retrospective data). An optional further step would be validation using CSF samples collected from the same patients who were included in the original study. Ideally, CSF samples would be collected from both blood-test-negative and blood-test-positive individuals in the primary care setting to set the stage for studies examining the utility of the biomarker (or new biomarkers) in detecting amyloid positivity among cognitively healthy older individuals. However, this latter use would require the entire process to begin anew as it would represent an entirely different COU.

Is a new cohort needed?. For the agreed intended use of the putative assay as a primary care screening tool, the ideal cohort to include in pivotal studies would align closely with the actual epidemiology of AD in primary care. The incidence of AD in this setting is ~10%[1] — a figure that includes patients with either subjective or objective cognitive deficits. The ideal cohort should also provide access to PET and/or CSF data for all patients and should also include information on age, sex, apolipoprotein E ε4 status and education to ensure that these factors are taken into account.

Current cohorts with both CSF and blood samples available are largely based around dementia specialty clinics and, as such, do not meet the needs of the target product profile. Current cohorts are also limited by referral bias: they are enriched for patients with cognitive impairment and, therefore, do not echo the disease prevalence in primary care. An alternative to specifically examining primary care cohorts could be to use a yoked design, whereby the setting is a specialist centre with referrals from primary care where, in addition to referred patients, additional patients not meeting the referral criteria could be included to make the cohort more representative. In addition, collaboration with primary care research networks could also be possible.

In summary, it might be necessary to recruit new cohorts for the purposes of blood-based biomarker assay development. Alternatively, new large-scale cohorts, such as that created as part of the US All of Us Research Program (which evolved from the US Precision Medicine Initiative[117] Cohort Program) or the UK Biobank, could prove extremely valuable in the development of blood-based biomarkers as these cohorts will represent real-world populations with a wide range of genetic and biomarker data available.

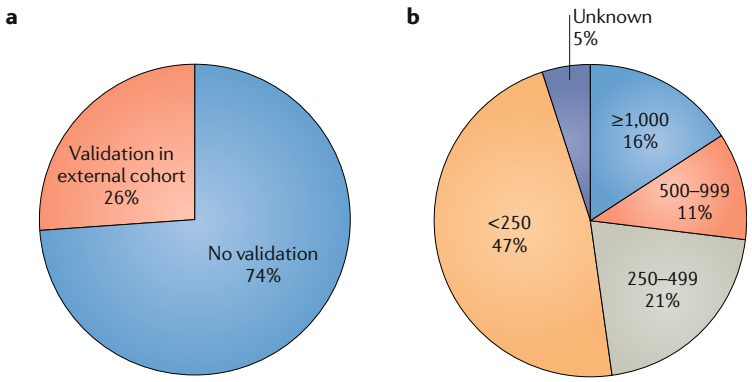

Fig. 5 | **Validation of blood-based biomarkers. a** | Validation status of the 19 prioritized biomarkers. Few of the current blood-based biomarker assays in development have undergone validation in an external cohort. Of the 26% of assays that had some degree of external validation, only two (10.5%) were in large cohorts such as the Alzheimer's Disease Neuroimaging Initiative (ADNI) or Australian Imaging, Biomarker and Lifestyle Study of Ageing (AIBL) cohorts. **b** | Numbers of patients tested. Studies were often underpowered, with small patient numbers.

Recently, to advance the development of the precision medicine paradigm in AD, APMI and its planned pilot-cohort programme (APMI-CP[24,39–42]) were launched and thematically associated with the All of Us Research Program. The mono-centre pilot APMI cohorts, ranging from early asymptomatic preclinical populations through prodromal to late-stage dementia populations, allow standardized recruitment of both cognitively intact individuals at risk of AD and patients with a full spectrum of neurodegenerative diseases to provide an assortment of unique heterogeneous and multidimensional data. The Investigation of Alzheimer's Predictors in Subjective Memory Complainers (INSIGHT-preAD) study[118], a French mono-centre, academic, university-based cohort established at the Institute of Memory and Alzheimer's Disease at the Pitié-Salpêtrière University Hospital in Paris, is the key cohort of the APMI-CP[24,39,40,42]. The main goal of the INSIGHT-preAD study is to investigate the earliest preclinical stages of AD and the subsequent development of the disease, including influencing factors and markers of progression. The cohort consists of cognitively healthy white individuals aged 70–85 years with subjective complaint of memory dysfunction, recruited from the community in the wider Paris area.

Is the landscape fit for purpose?

Following the landscape analysis, the 196 candidate biomarkers (FIG. 2) were further divided into tiers according to additional criteria (BOX 3). Of these 196 candidates, 19 were prioritized for further consideration by the working group (Supplementary Table 1); however, none were deemed to have met the agreed target product profile.

Validation is regarded as a crucial step in biomarker development, yet only five of the biomarker studies identified in the search were followed up with validation studies in external cohorts[95,96,119–121] (FIG. 5). Furthermore, validation was not always conducted by an independent group or based on a model published prior to the study[103]. This scenario results in a risk of hypothesizing after results are known (HARKing), which can lead to selective reporting and inflated predictive accuracy[122]. Validation studies might require additional cohorts or could potentially be conducted within subgroups of the original cohort. However, appropriate cohorts for the ideal intended COU do not currently exist, and the need for cross-cohort validation will therefore be a further hurdle in the development of assays. As previously discussed, the appropriate cohorts, stemming from patients in primary care with CSF and blood samples, as well as PET data, are not available, so the target product profile could not be met by any study.

Additional issues with some biomarkers include PPV and NPV values lower than those specified in the target product profile, as well as small sample sizes (FIG. 5). Studies using large and well-established cohorts such as ADNI and the Australian Imaging, Biomarker and Lifestyle Study of Ageing (AIBL) are more likely to meet the initial requirements. As discussed above, biomarker panels are thought to offer the greatest chance of success, although panels featuring high numbers of markers will be difficult and expensive to develop and validate. In addition, most available technology platforms have a low ceiling on the number of biomarkers that can be contained within the assay, which is usually around five biomarkers. Many emerging areas of interest, such as exosomes, offer promise, although they are insufficiently developed to be considered at this time. In addition, blood-based assays for well-known biomarkers of AD, such as $A\beta_{1–42}$:$A\beta_{1–40}$ ratio, tau, NFL and BACE1, might have potential and should be monitored closely as more information becomes available and novel technologies emerge to address the hurdles associated with the use of these biomarkers.

Blood-based biomarker diagnostics

Blood-based biomarkers could conceivably improve detection and diagnosis of AD by increasing convenience, acceptability and ease of testing and by reducing costs. These biomarkers are likely to provide the most benefit in a number of key areas. The first and most important area is testing for AD in the primary care setting, which will potentially allow identification of patients at the earliest stages of the disease. Ideally, individuals should be tested before any noticeable cognitive deficits develop. The advent of testing in primary care would lead to substantial changes in the current treatment paradigm by allowing patients to be guided towards specialist diagnostics (for example, CSF or PET scans) and access to care earlier in the course of the disease. This facility will be become increasingly important with the emergence of DMTs for AD, but it could provide benefit to patients and caregivers even at the present time[123]. BOX 4, along with previous work by an international working group[25], provides specific considerations and recommendations for the process of developing blood-based biomarkers for use in AD.

Once DMTs become available, we expect to see a tremendous surge in the numbers of patients in primary care settings seeking potential prescriptions. However, without a convenient primary care screen, confirmatory diagnostics, such CSF analysis, MRI or PET, will still be required, which will create a bottleneck in the diagnosis and treatment process and present a considerable

Box 4 | **Checklist for developing a blood-based biomarker**

- Define context of use and setting
- Define attributes of biomarker (how it enters the blood and concentrations and diurnal changes)
- Develop detection method
- Validate against gold standard (International Working Group-2 criteria)
- Replicate in the target setting
- Refine to reach target predictive values
- Demonstrate technical performance
- Validate against large, independent cohorts. Validated biomarkers should be supported by a study showing a difference between individuals with Alzheimer disease and healthy controls and diagnostic utility in individuals with mild cognitive impairment

burden in terms of costs. Availability of a multi-tiered diagnostic process commencing with a blood test in primary care would dramatically increase access to DMTs while conveniently excluding from further diagnosis and treatment the vast majority of patients who would not need to undergo expensive and invasive procedures. An additional potential use of blood-based AD biomarkers is to provide a cost-effective and rapid test for AD pathology in order to determine the eligibility of patients for recruitment into clinical trials of DMTs[124–126]. These tests might also have applicability in the monitoring of disease progression and drug effects on AD pathophysiology during a trial[127]. As a consequence, blood-based biomarker diagnostics might accelerate the development of DMTs.

The development of blood-based biomarkers often stalls in the early stages owing to a disconnect between academia, where biomarkers are identified, and industry, who have the resources to take biomarkers further and develop them commercially (FIG. 6). This disconnect arises from the different needs of these different sectors and from mutual mistrust developed following a history of poor interactions. The current initiative by the APMI Working Group and Roche Diagnostics International represents a step towards improving this process, both efficiently and economically.

Conclusions

Over the past decade, we have witnessed substantial progress in blood-based biomarker research in AD and other neurodegenerative diseases. Human blood — in particular, plasma — holds the largest source of the proteome, and technologies to measure even minor alterations of proteins and peptides in the blood are crucial tools. Mass spectrometry can detect slight alterations in protein concentrations, and immunohistochemistry can recognize specific proteins with high accuracy in the living system. Compared with CSF markers, a validated blood-based AD biomarker would provide a fast, non-invasive and cost-effective method of early detection and diagnosis of the most common age-related neurodegenerative disease worldwide. In addition, venipuncture is a routine, safe procedure that does not pose any harm to the patient. Therefore, in contrast to CSF sampling, examination of blood biomarkers is accepted and easily introduced in the clinical environment[25]. Development of biomarkers reflecting all existing molecular pathophysiological mechanisms in the brain at distinct stages of AD progression will represent the foundation for personalized, tailored, biomarker-guided targeted therapies and constitute a critical step towards the dissemination of precision medicine in AD[24,39]. Furthermore, the development and implementation of a multistage diagnostic approach, beginning with a blood test in primary care, will increase early access to confirmatory diagnostic modalities (such as PET imaging and/or CSF sampling) and provide a clear path for regulatory approval. This tiered approach is expected to increase access to DMTs once they are available.

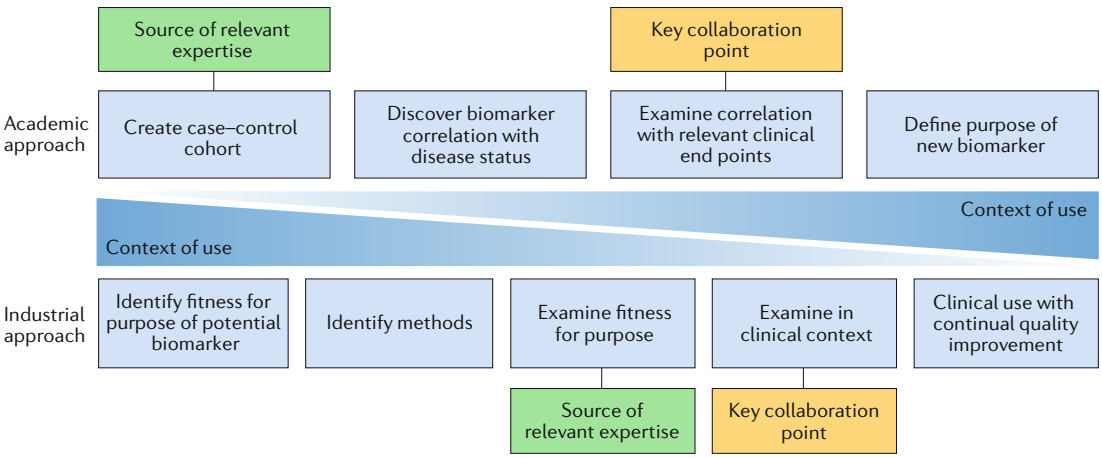

Fig. 6 | **Potential collaboration points between academia and industry.** Academic and industrial approaches to biomarker development are inherently different, but combining these approaches could be extremely useful. Close collaboration between industry and academia would allow sharing of expertise in product testing, access to cohorts and clinical data, and sharing of ideas and theories with regard to clinical end points and context. By merging the two approaches, a method whereby the context of use is the primary focus throughout the process can be established. This model enabled synergistic development of a new biomarker between academics and industrial partners, sharing a wealth of experience.

Big data
A repository of many data sets generated by data-mining tools, including information obtained through systems-theory-based and knowledge-based approaches, and clinical records.

Integrative disease modelling
A multidisciplinary approach to standardize, manage, integrate and interpret multiple sources of structured and unstructured quantitative and qualitative data across biological scales using computational models that assist decision-making for translation of patient-specific molecular mechanisms into tailored clinical applications.

Despite much research, many of the candidate blood-based biomarkers have limitations that currently preclude their use. Close cooperation and coordination are needed among academic institutions, industry partners and regulatory bodies to accelerate the development of a blood-based biomarker assay that is suitable for clinical use. Novel patient cohorts might need to be assembled to allow the development of a new assay to enable population-based screening for AD in primary care.

Omics sciences enable complex biological systems to be visualized in a holistic and integrative manner. Application of systems biology to inspect large multidimensional blood-based omics data will enable the stratification of patient populations into well-defined subsets sharing a common pathophysiology, which can be further explored for targeted interventions. The use of systems biology methods to discover and validate diagnostic biomarkers in specific patient subsets should considerably accelerate the progress of AD precision medicine towards the clinic[25].

Omics techniques generate large and heterogeneous biomedical data sets, leading to the concept of 'big data' in biology and medicine[24,39,41]. Integrated analysis of molecular, cellular, imaging, clinical, demographic and environmental data, produced by academic institutions, clinics and, most recently, mobile devices[128,129], depends on access to appropriate tools for data storage and management and disease modelling. Therefore, effective and sophisticated methods will be essential to systematically screen for novel blood-based biomarkers associated with AD and gain insights into their spatiotemporal interactions with other biomarker categories, as well as to provide complementary information on disease pathophysiology. The Big Data Research and Development Initiative, announced by the Obama Administration in the USA, is a crucial promoter of the implementation of precision medicine through the integration of big and deep biomedical data. The ability to deal with 'big data science', accompanied by the implementation of integrative disease modelling[130], is an essential aspect of APMI and other worldwide initiatives, including ISTAART BBB-PIA[69] and the recently established Cholinergic System Working Group (CSWG)[131].

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