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Precision pharmacology for Alzheimer's disease

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Abstract

The complex multifactorial nature of polygenic Alzheimer's disease (AD) presents significant challenges for drug development. AD pathophysiology is progressing in a non-linear dynamic fashion across multiple systems levels – from molecules to organ systems – and through adaptation, to compensation, and decompensation to systems failure. Adaptation and compensation maintain homeostasis: a dynamic *equilibrium* resulting from the dynamic non-linear interaction between genome, epigenome, and environment. An individual vulnerability to stressors exists on the basis of individual triggers, drivers, and thresholds accounting for the initiation and failure of adaptive and compensatory responses. Consequently, the distinct pattern of AD pathophysiology in space and time must be investigated on the basis of the individual biological makeup. This requires the implementation of systems biology and neurophysiology to facilitate Precision Medicine (PM) and Precision Pharmacology (PP).

The regulation of several processes at multiple levels of complexity from gene expression to cellular cycle to tissue repair and system-wide network activation has different time delays (temporal scale) according to the affected systems (spatial scale). The initial failure might originate and occur at every level potentially affecting the whole dynamic interrelated systems within an organism. Unraveling the spatial and temporal dynamics of non-linear pathophysiological mechanisms across the *continuum* of hierarchical self-organized systems levels and from systems homeostasis to systems failure is key to understand AD. Measuring and, possibly, controlling space- and time-scaled adaptive and compensatory responses occurring during AD will represent a crucial step to achieve the capacity to substantially modify the disease course and progression at the best suitable timepoints, thus counteracting disrupting

critical pathophysiological inputs. This approach will provide the conceptual basis for effective disease-modifying pathway-based targeted therapies.

PP is based on an exploratory and integrative strategy to complex diseases such as brain proteinopathies including AD, aimed at identifying simultaneous aberrant molecular pathways and predicting their temporal impact on the systems levels. The depiction of pathway-based molecular signatures of complex diseases contributes to the accurate and mechanistic stratification of distinct subcohorts of individuals at the earliest compensatory stage when treatment intervention may reverse, stop, or delay the disease. In addition, individualized drug selection may optimize treatment safety by decreasing risk and amplitude of side effects and adverse reactions.

From a methodological point of view, comprehensive “omics”-based biomarkers will guide the exploration of spatio-temporal systems-wide morpho-functional shifts along the *continuum* of AD pathophysiology, from adaptation to irreversible failure.

The Alzheimer Precision Medicine Initiative (APMI) and the APMI cohort program (APMI-CP) have commenced to facilitate a paradigm shift towards effective drug discovery and development in AD.

Keywords

Alzheimer’s disease; Precision pharmacology; Precision medicine; Pathway-based therapy; Pathophysiology; Clinical trials

1. Introduction: precision pharmacology in the context of precision medicine

Complex chronic diseases with global unmet needs such as cancer, diabetes, immune diseases, and brain proteinopathies – including Alzheimer’s disease (AD) – primarily exhibit: I) a multifactorial nature, due to the coexistence of polygenetic/genomic/epigenomic, interactomic, and environmental susceptibility and II) altered networks, affecting relevant modules and interactomes [1,2]. The continuous failure of late stage clinical drug trials, largely developed following the traditional drug development paradigm in AD, demonstrates that a conceptual shift in Drug Discovery & Development programs is required to attain successful breakthrough developments of novel therapies [3,4]. Notably, a critical step for developing effective drugs is to explore and predict the comprehensive effect of a compound on four fundamental levels, such as I) hitting the intended target, II) altering the intended mechanism, III) altering the relevant pathophysiology, and IV) impacting clinical outcome [1].

Precision pharmacology (PP) is a novel conceptual paradigm that aims at exploring and predicting the whole effect of a molecular mechanism of action, i.e. the pharmacodynamic (PD) [5]. As a result, PP is crucial to operate from the perspective of an innovative exploratory, integrative, holistic multi-paradigm or systems level concept, at both experimental and computational level. In order to achieve the full understanding of drug action at the systems level, it is necessary to combine disease mechanism, PD and pharmacokinetic (PK) data into a single model, following the systems pharmacology

paradigm. According to the American Association of Pharmaceutical Scientists (AAPS; https://www.aaps.org/), systems pharmacology is defined as “the science of advancing knowledge about drug action at the molecular, cellular, tissue, organ, organism, and population levels” (available at http://www.aaps.org/Systems_Pharmacology/). Systems pharmacology is an integrative interdisciplinary model providing the potential to investigate drug action though networks of biological pathways, thus allowing the development of predictive models of PD and PK features for a certain molecule [6,7]. Therefore, traditional PK/PD procedures are integrated into the systems biology paradigm to establish predictive models of the whole effect (up-downstream regulated processes, feedback loops) of a given drug, from cell pathways signals to systems outputs [5–7]. This paradigm can lead to the characterization of pathway-based molecular signatures that will allow a mechanistic stratification of individuals and patients for a “stratified pathway-based therapy” [6–8].

The existence of high interindividual variability underlying a genetic/epigenetic different background primarily affects the mechanism of action of the drug under study (Table 1A–H).This categorization process inside drug development relies on the “omic” sciences and aims at achieving personalized predictive models of therapeutic effects, side effects, and adverse [3,9]. Applying PP is assumed to accomplish the following long-term goals: I) developing multi-target therapeutic approaches for multifactorial polygenic diseases, such as AD; and II) providing predictive models/quantitative frameworks of therapeutic efficacy and risk of adverse events for individuals, in the context of Precision Medicine (PM) [3,6]. The implementation of PP in AD is anticipated to result into an innovative and original scientific taxonomy as well as to a distinguished working lexicon and terminology (Table 2). In order to accelerate the development of the PM paradigm in AD, the international Alzheimer PM Initiative (APMI) and its related Cohort Program (APMI-CP) have been established by our consortium and conceptually associated to the U.S. Precision Medicine Initiative (PMI) (available at https://www.whitehouse.gov/precision-medicine) and the U.S. “All of Us Research Program” – evolved from the U.S. PMI Cohort Program (available at https://www.nih.gov/research-training/allofus-research-program). The research using the AMPI cohorts has recently commenced to be facilitated under the structural framework of the newly established French Sorbonne University – “Clinical Research Group in Alzheimer Precision Medicine” (*Sorbonne Université* – “*Groupe de Recherche Clinique – Alzheimer Precision Medicine*”, [*GRC n° 21*]).

Combined downstream and upstream effects on different homeostatic key molecules and pathways are commonly shared on several biological networks which, in turn, underlie apparently unrelated diseases[10]. Pathway-based therapies are anticipated to support the development of novel interventions to treat several diseases which can show misleading clinical divergence. Given the complexity and heterogeneity of many diseases, such as AD, a multi-target approach needs to be performed; in particular, the main “orchestrator” of each pathway – ultimately called target – will be identified by an integrative analysis of comprehensive multi-domain “omic” [2,3,11]. In addition, this advanced holistic systems-level approach is assumed to facilitate the drug repositioning process – also known as drug re-profiling or drug repurposing process – indicating that a drug with a recognized biological effect could be utilized to treat a disease for which it has not been registered [12].

1.1. The road to precision pharmacology: role and contribution of time and space in systems biology for research & development programs

The application of systems biology to investigate multifactorial diseases starts from the elucidation of all gene-interaction networks since complex gene-gene and gene-environment interactions upstream affect the biochemical pathways underpinning the disease with high extent of variability across a patient [3,12]. Therefore, the development of advanced computational/bioinformatic tools made the detection of statistical interactions between genetic loci possible, when examining the data *via* genome-wide association studies (GWAS) [3,9]. Exploratory computational platforms will allow quantitative and dynamic modeling of interacting biological systems active at multiple scales of organization within a *continuum*, i.e. from homeostasis to system failure. Currently available biostatistical approaches facilitate researchers in providing the profile of gene clusters related to several biological processes. There is a growing number of technologies allowing the optimization of data collection from a single biofluid or tissue sample by providing a multimodal profiling, such as genomic/epigenomic, transcriptomic, miRNAomic, proteomic, and metabolomic/lipidomic [13–16].

Charting the molecular dysregulated pathways should be accomplished using pathway-based panels that contain multiple combinations of arrays encompassing several genes, in order to track their direct expression products and the most relevant gene-gene interactions [2,9,17]. This is supposed to substantially transform the Research and Development (R&D) programs, thus paving the way for developing “molecularly” biomarker-guided targeted therapies[18] – i.e., treatments specifically adapted (“tailored”) to the individual – within a short time frame [3,12].

1.1.1. Role of time—The addition of a fourth dimension – time – to the field of structural biology will allow following-up compensatory mechanisms responsible for preserving homeostasis and its dynamic changes over time. In this regard, the identification of transcriptionally active genes and their respective products is a key signature of either active “stress responses” or dynamic loss of homeostasis. Nowadays, the role of advanced nanotechnologies able to dynamically track the time/space coordinates of molecules associated with different pathways is gaining substantial relevance. Expression profiles of genes and proteins are supposed to provide clear outcome measures, i.e., biomarkers, for target engagement as well as for predicting the response to treatment. Simultaneous gene expression and extracellular protein expression profiles can allow exploring a whole cellular species, for instance, to longitudinally investigate immune responses and cell ultrastructural alterations over time. In particular, both overactivation and changes in immune cell surface antigens occur in parallel with the progression of a wide variety of pathophysiological conditions such as in AD and [13,16,17]. As a result, biomarker-guided pathway-based therapies shaped on the comprehensive biological profile of a given subject at a given time point of the disease progression will change according to the evolving biological pattern of the individual.

1.1.2. Role of space—There is a heterogeneous cross-talk between periphery and central nervous system (CNS) pathways, based, for instance, on innate-adaptive

immune system and proteostasis networks. Interestingly, several peripheral and systemic abnormalities have been found to be associated with impaired amyloid beta (Aβ) peptides removal at the level of the CNS. This suggests a crucial role for brain-periphery interaction in the development and progression of brain proteinopathies, including AD [19,20]. Recently, and even more related to AD, an association between peripherally-derived neutrophils, T-regulatory lymphocytes, as well as peripheral immunity loss of function and microglial dysfunction that resulted in protein misfolding has been reported in brain or other tissues [21,22].

In summary, understanding the dynamic regulation of transcellular signals at a system level as well as the mechanisms underlying their bi-directional cross-talks is expected to restore aberrant pathways in pathophysiologically altered tissues/organs by targeting, in turn, other tissues/organs. These insights will promote the identification of remote (i.e., peripheral) key modulators of several cerebral functions, thus providing a reliable open-access to the brain. This step is essential to overcome the high degree of inaccessibility of the brain to pharmacological therapies.

2. Homeostasis and pathway-based therapy

Loss of homeostasis, ultimately leading to a dynamic pathophysiological state, consists of the breakdown of one or more homeodynamic pathways – namely the “stress responses” – originating first of all from maladaptive responses and then from failure of compensatory mechanisms (i.e. decompensation). Compensation is a self-regulatory dynamic counterbalance between regulatory defense mechanisms and disrupting stress-induced signals [23–25]. Compensation occurs through both structural and functional changes and is hierarchically organized from subcellular to cellular level, organs, and, eventually, systems. Compensatory mechanisms aim primarily at protecting the core biosynthetic processes necessary to survival. There is a *continuum* between homeostasis, metastability that precedes adaptation – compensation with an higher risk of failure of compensatory mechanisms over time – finally leading to loss of homeostasis [25–27]. In this scenario, disease is designated as a theoretical construct exhibiting successive and progressive failures (decompensation) in complex interconnected systems or brain networks, according to the notion of “systems failure” [3,9,11]. The primary descriptive concept of this model is that this construct is mostly not the linear result of a unitary etiologic factor; rather, it evolves in time in a non-linear dynamic progressive fashion across physiological and, then, pathophysiological stages – from initial adaptation to compensation and after thresholds to decompensation (leading to failure of homeostatic mechanisms) – and the convergence of failures in several networks/systems, or pathophysiological processes along a *continuum* (Fig. 1).

All living organisms, from nematodes to human beings, are continuously exposed to stress-associated signals triggered by a wide range of endogenous and external stimuli, including physical activity, temperature, UV rays, cosmic radiation, oxidants, bioenergetic restrictions, chronic cellular exposure to impaired metastable proteome and/or conformational [28,29]. Interestingly, the degree of the cellular homeodynamic “stress response” differs in terms of

amplitude and time (short-term/long-term) according to the extent of the stressful stimuli [28,29].

Cellular homeostasis represents the critical point of the individual's health span and refers to all molecular machineries needed at multiple cellular-subcellular compartments to compensate for stress-induced damage, thus finally preserving the cellular functional and metabolic stability. The existence of cellular homeostasis is ensured by "stress responses", including: I) proteostasis networks (exerting mechanisms quality control, from protein synthesis to protein degradation [30,31], II) highly conserved pro-survival and pro-apoptotic gene expression pathways (responsible for multiple level regulation, i.e., from pre-transcriptional to cell trafficking level [23,24,32].

Several studies have shown that preserving cellular homeostasis generally affects the individual's life span while its deterioration over time underlies aging in a bidirectional way [33]. Age-related alterations affecting "stress responses" mainly occur at molecular level: glucose transport, DNA surveillance mechanisms (ensuring repair of DNA lesions), and mitochondrial electron transport play a crucial role to support pro-survival signaling as well as cell development/differentiation, apoptosis, endocytosis, microtubule stability, lipid membrane dynamics, and other key molecular processes [34–36]. As a result, DNA damage, overexpressed oxidative stress, and telomere shortening are typical patterns of aged cells displaying functional decline. This, in turn, has a significant impact on proteostasis leading to a fatal accumulation of misfolded proteins over nucleic acids, lipids, and other molecules. Notably, it is fully acknowledged – also in humans – that the uncontrolled activation of "stress response" pathways is expected to determine loss of homeostasis *via* several mechanisms, in particular through down-regulation performed by negative auto-feedback and bioenergetic depletion due to hyperactivated pathways [29,37,38].

At present, the homeostatic mechanisms have not been completely elucidated. However, it is clear that there is a complex bi-directional crosstalk among numerous anti-stress outputs (Natarajan M et al., 2006) intensifying the presence of intricate networks, where different pathways constitute central hubs coordinating various modules. As a result, the dysfunction of a single component of the network may appear as both the cause and the consequence of the dysfunction of other components, hence substantially and dynamically impacting the whole network [28,38] (Fig. 1).

The comprehensive assessment of the dynamic and mutual interplay among the various cellular "stress response" pathways modulating the individual's life span and aging, will allow disclosing novel insights on aberrant biological conditions. This, in turn, will represent a critical step for developing drugs with efficacy for unresolved medical challenges such as cancer, immune diseases, diabetes, AD (and other brain proteinopathies).

Therefore, a systems biology-based biomarker-guided multi-target therapy relies on a multi-pathway- or multi-network-based approach, which, in the case of AD, should engage selected molecular targets concerning: proteostasis network, immune response (both innate and adaptive) and endothelial dysfunction. In the perspective of a pathway-/network-based drug development strategy, similar systems failures sharing common pathophysiological

pathways appearing as “different diseases” are potentially supposed to be treated with the same molecule [38–41].

Big “omic” data need to be generated from multiple systems levels and integrated to achieve reliable information about the dynamic failures to compensate for complex disruptive signaling that can lead to the disease. Given the partly undiscovered substantial cross-talk between CNS and peripheral systems, it is acknowledged that longitudinal trajectories of blood biomarkers reflect the changes over-time in the interaction between aberrant cerebral networks and peripheral networks. For instance, blood-based inflammatory and metabolomic markers allow to *in vivo* track crucial mechanisms accounting for the pathophysiological evolution of AD along the *continuum*, from adaptation, compensation to decompensation and systems failure and from the earliest preclinical stages to late stage clinical dementia[42].

3. Current status of blood-based biomarkers – inflammatory and metabolomic – for preclinical Alzheimer’s disease

Detailed pathological analyses at autopsy, with the addition of surgical pathology and biochemical studies, have evolved to provide a basis for detecting many human diseases, especially when combined with precise clinical assessments, as in the traditional clinicopathological correlations (CPC) [43]. The traditional conception of modern AD began with such a CPC, provided in the early 20th century, by the German neuroscientist Alois Alzheimer [44]. Since his initial descriptions, the medical and research fields have primarily focused on two of his seminal neuropathological findings, the senile plaques, primarily composed of extracellular Aβ protein fibrils, and the intracellular neurofibrillary tangles, made up of phosphorylated tau protein species [45–47] Not until recently, a key pathological hallmark has gained attention, i.e. the proposed “adipose inclusions” or “lipoid granules” that suggested the existence of dysregulated lipid metabolism [48,49]

Early biomarker investigations related to preclinical AD individuals featured those presenting with an autosomal dominant or familial condition (familial AD, fAD), confirmed via genetic testing and allowed the definition of associated cerebrospinal fluid (CSF), blood, and/or brain (*via* neuroimaging) abnormalities [50]. In such presymptomatic (preclinical) fAD gene mutation carriers, abnormalities in Aβ and tau species concentrations were confirmed compared to controls in each of the matrices, providing a time-dependent course for each protein [50,51], and suggesting different phenoconversion predictive capacity for each protein and combination analyzed. Although these preliminary biomarker investigations correlated between human fAD and certain transgenic rodent models, similar investigations of the vastly more common, late-onset polygenic form of AD (LOAD), remained incomplete. The major limitation in studying LOAD individuals was the absence of an easily attainable preclinical molecular signature that would allow accurate selection and monitoring of disease progression during the preclinical stages. Without such molecular signature for LOAD, and given the shared late neuropathological stage with fAD, a conventional partly reductionistic assumption was generated [52], hypothesizing close links between the pathophysiology of fAD and LOAD [53]. Given the lack of

a holistic understanding for the true basis and unique evolution of LOAD, therapeutic interventions based on fAD and transgenic animal model findings provided no significant evidence of clinical efficacy when tested in LOAD individuals [54]. As a result, in light of the continuous failures of late-stage clinical AD drug trials, there has been a more exploratory, integrative, and holistic reevaluation of additional factors contributing I) to AD pathophysiology, especially related to membrane damage [55], and II) in moving therapeutic interventions into the preclinical stages [56]. Both of the latter require the development of relevant biomarkers for the preclinical stages of AD, particularly targeting other pathophysiological pathways apart from the amyloidogenic one.

Neuroinflammation is such a broad pathophysiological field and has evolved by providing an etiologic explanation for brain membrane injury in AD and in a variety of neurological diseases [57–64]. As a result, the depiction of currently developed blood-based approaches needed to explore the preclinical manifestations of neuroinflammation using both direct – *via* inflammatory biomarkers – and indirect – *via* metabolomic biomarkers – measures seems to be crucial.

3.1. Inflammatory biomarkers

Even though inflammation might not classically considered an initiating factor in ND, there is emerging evidence in animal models that sustained inflammatory responses – involving microglia, the major resident immune cells in the brain, and astrocytes, glial cells with support functions – contribute to disease progression. Sustained inflammation leading to tissue pathology involves the persistence of an inflammatory stimulus or a failure in normal resolution mechanisms. A persistent stimulus may be the result of I) the presence of environmental factors and II) the formation of endogenous factors (for instance, protein aggregates) that are interpreted by the immune system as “unfamiliar” or even dangerous signals. Although some inflammatory stimuli generate positive effects for the organism, such as phagocytosis of debris and apoptotic cells, and inflammation is associated with mechanisms of tissue repair, uncontrolled/uninhibited inflammation may result in production and release of neurotoxic factors intensifying the disease states [65].

At present, the primary role of neuroinflammation in AD is unquestionable. In particular, inflammation occurs in pathologically vulnerable regions of the AD brain and it acts in this way using a plethora of local peripheral inflammatory responses. At the peripheral level, the deposition of highly insoluble abnormal materials, together with degenerating tissue, is a critical factor inducing inflammation. Similarly, at the level of the AD brain, damaged neurons and neurites as well as highly insoluble deposits of Aβ peptide and neurofibrillary tangles provide evident stimuli to trigger inflammation [66]. In this regard, the analysis of *post-mortem* AD brains has provided evidence for inflammatory factors and activated cell types in association with common end-stage pathophysiological features, including amyloid plaques and neurofibrillary tangles [67–69]. The primary cellular sources in the brain responsible for cytokine production are perivascular and meningeal macrophages and microglia [70] (26). Considered uniquely important to AD pathophysiology – especially in context of genetic variants of *TREM2* (encoding the triggering receptor expressed on

myeloid cells 2) gene[71,72] – microglia are known to release various soluble factors and assist in extracellular Aβ clearance.

Genetic factors are fully acknowledged to play a key role in AD. Notably, the search for genes involved in AD has been revolutionized by the application of GWAS, the most common approach to assess genetic variants in the genome using arrays of single nucleotide polymorphism (SNPs) to investigate the potential association with AD[73]. Interestingly, several genetic variants are involved in immune and inflammatory processes, as deeply reviewed [71,74,75]. In particular, two groups of investigators [76,77] independently identified and characterized a rare variant in the *TREM2* gene – a major microglia-specific gene in the CNS – that causes an increased susceptibility to LOAD [78].

Despite the description of the CNS components of neuroinflammation, their putative peripheral manifestations in blood have sometimes provided inconsistent results [79–84], even when comparisons between control subjects and AD patients have been performed. In particular, it has been challenging to develop informative peripheral inflammatory molecular signatures for preclinical AD. Various studies have explored biomarkers potentially associated with inflammatory processes. Cytokines – including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), transforming growth factor-beta (TGF-β), and interleukin-1-beta (IL-1β) – have been measured in CSF of AD patients, but in one *meta*-analysis the only consistent finding was the increased CSF concentrations of TGF-β in AD patients *versus* control groups [85]. TNF-α, expressed by neurons and glia, stimulates the inflammatory responses by recruiting microglia or astrocytes to lesion sites, thus leading to glial cell activation. The TNF receptor complex and its related functional proteins are supposed to be actively involved in AD pathophysiology, thus strictly associating inflammation signaling pathways with the amyloid deposition cycle in a self-propagating and destructive dynamic [86]. TNF-α binds to specific membrane glycoprotein receptors – TNF receptors (TNFRs [TNFR1 and TNFR2]) – that activate signal transduction pathways converging to a common mechanism of neuronal death. The definite function of TNFR1 as crucial mediator of inflammation, apoptosis, and amyloidogenic pathology has been scrutinized [87,88]. Remarkably, since TNFR1 and TNFR2 are both expressed and triggered differentially in AD brains *versus* non-demented brains, distinct pathophysiological mechanisms of neurodegeneration in AD brains have been proposed [89]. In addition, expression levels of TNFR1 and TNFR2 have been documented to be altered in the brains and CSF of subjects with mild cognitive impairment (MCI) and AD patients. The activity of the TNF-α converting enzyme (TACE), cleaving both pro-TNF-α and TNF receptors, is substantially increased in the CSF of AD patients and MCI subjects *versus* healthy controls. Moreover, CSF concentrations of TACE-cleaved soluble forms of TNFR1 (sTNFR1) and TNFR2 (sTNFR2) appear more elevated in AD patients *versus* healthy controls and correlated with TACE activity. Finally, greater levels of TACE activity and soluble TNFRs are present in MCI subjects *versus* AD patients, thus emphasizing an early role of TACE activity and soluble TNFRs during AD pathophysiology and a their potential usefulness as diagnostic markers in MCI and AD dementia stages [90]. In addition, the integrated CSF examination of tau protein with the constituents of the soluble IL-6 receptor complex (sIL-6RC), assumed to be a marker of neuromodulatory and brain inflammatory processes, is assumed to increase the certainty of AD detection/diagnosis [91,92]. Interestingly, peripheral blood

mononuclear cells may provide actionable longitudinal risk information [93] through increased spontaneous production of IL-1 and TNF-α associated with cognitively normal individuals with an increased risk of phenoconversion to AD. Finally, CSF YKL-40–an indicator of microglial activation – has been designated as a pathophysiological biomarker indicating immune/inflammatory mechanisms in AD and other ND, associated with tau protein pathology [64,94].

3.2. Metabolomic biomarkers

The human blood metabolome consists of thousands of small molecular species, typically less than 1500 Da (Daltons; 1.7×10^{-27} kg) in molecular weight and primarily featuring monosaccharides, acylcarnitines, biogenic amines, amino acids, fatty acids, and complex lipids. By far, lipid species make up the largest fraction (45%) of the ~50,000 metabolites currently detectable [95]. Identifiable metabolomic species, including human, pharmacologic, animal, plant, or bacterial, are currently curated in one or more of the following databases: the Human Metabolome Database (HMDB) (available at http://www.hmdb.ca), the METLIN database (available at http://metlin.scripps.edu), and the LIPID MAPS Lipidomics Gateway (available at http://www.lipidmaps.org). A significant number of metabolic species are yet to be annotated, with recent estimates of the total approaching 1 million [95]. Using standard reductionistic approaches, the metabolome is considered a downstream linear reflection of the genome/epigenome, transcriptome, and proteome, sequentially, and in close proximity to the clinical phenotype. Using a systems biology-based perspective, although the aforementioned might be true, complex interrelationships exist between the various “omic” layers [96], that, if properly integrated, are expected to provide an improved understanding of a complex disease state or human health.

Early metabolomic approaches to biomarker development in blood and CSF have featured either nuclear magnetic resonance-based analyses [97,98] or those utilizing mass spectrometry (MS)-based technologies[99,100]. More recent reports of metabolomic biomarkers for AD have been developed using specimens from cross-sectional investigations analyzed with MS platforms, typically comparing metabolite abundances between control subjects and individuals with either prodromal or manifest AD[101–105]. Although consensus is lacking regarding specific metabolites discovered between studies, there is a substantial preponderance for alterations in certain lipid species in blood. Analyses[106,107] from a longitudinal observational study – specifically evaluating preclinical subjects observed to phenoconvert from cognitive normality to either prodromal or manifest AD – reported significant reductions in certain plasma lipid species. Notably, reductions of some of the same species were observed in early AD subjects in an independent therapeutic trial [108].

3.3. Biomarker perspectives

3.3.1. Biomarkers as diagnostics—While there is no unanimously established consensus regarding the selection(s) of molecular biomarker panels that are most informative regarding the preclinical stages of AD, there is growing support for the use of blood-based biomarkers in helping define this crucial therapeutic window. Thanks to their classification sensitivity and specificity comparable to that provided by CSF

and neuroimaging markers, their decreased associated risk, increased patient comfort, and reduced associated cost, blood-based biomarkers are gaining *momentum* as potential screening and prediction tools and for enhanced selection, subject enrichment, and stratification of disease subsets in AD disease-modifying trials [109].

3.3.2. Biomarkers as guides to therapeutics—The ultimate objective is to develop biomarker-guided targeted therapy in AD. The potential utility of certain biomarkers as outcomes and surrogate outcomes during the preclinical stages of AD should will be a development focus. In this specific circumstance, biomarkers indicating the existence of neuroinflammatory and membrane lipid dysregulation processes may be substantially informative.

4. Cns inflammation in Alzheimer's disease stages biomarkers and therapeutic targets

The role of CNS inflammation in the development and progression of AD has been a controversial issue, more specifically whether plaque-related inflammatory and immune processes are disease-aggravating or neuroprotective [64,110–112]. The most significant advance in understanding the role of inflammation in the evolution of the AD pathophysiology is based on the observation that long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) in patients with rheumatoid arthritis reduces the risk of developing AD when compared to the general population [113,114]. These findings originally supported the idea that neuroinflammation represents a key pathophysiological feature in the AD cascade, prompting the pharmaceutical industry to launch several large clinical trials on the use of classic NSAIDs, such as ibuprofen, rofecoxib, celecoxib, and R-flurbiprofen, and other anti-inflammatories, including pioglitazone, steroids, and aspirin, in symptomatic patients diagnosed with AD. Published results from these trials have been the subject of several meta-analyses, all of which have concluded that treatment with anti-inflammatories lacks efficacy in symptomatic, already clinically diagnosed AD dementia patients [115–117]. Only one trial, the Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT), has reported a beneficial clinical outcome when naproxen is administered before the onset of subjective cognitive impairment [118]. These findings indicate that the critical therapeutic window to target neuroinflammation is likely at preclinical AD stages [112].

Although these clinical trials have not yet produced a viable therapeutic option for the treatment or prevention of AD, they have provided insight into the dichotomous function of neuroinflammation in the progression of AD: early inflammation is likely pathogenic and disease-aggravating, whereas late inflammation appears to be dominated by tissue-resolution and phagocytic processes [112]. The idea that inflammation adopts a protective role as the disease progresses through the AD *continuum* is supported by GWAS-based analyses that have identified SNPs in the *CD33* and *TREM2* genes that are associated with an increased risk of developing AD [76,77,119–121]. Disease-relevant variants in both genes, which are primarily expressed by immune cells, result in blunted phagocytic capacity by brain macrophages, thus suggesting that clearance mechanisms likely serve to counteract

late neurodegenerative mechanisms. Inhibiting these protective inflammatory mechanisms, would theoretically exacerbate or, in the least, fail to decelerate the neurodegenerative cascade. As a result, anti-inflammatory therapy is unlikely to be disease-modifying if administered during late symptomatic stages, when the fundamental neuronal networks responsible for higher CNS functions have already been destroyed. Applying an anti-inflammatory therapy earlier in the disease process is the most promising strategy to mitigate the development of the underlying AD pathophysiology.

Recent studies in transgenic animal models of AD have revealed the presence of an early pro-inflammatory process before the development of Aβ plaques. For example, pre-plaque 3xTg mice exhibit increased levels of TNF-α associated with intraneuronal-Aβ pathophysiology in the entorhinal cortex [122]. Inhibiting TNF-α signaling prevents intraneuronal-Aβ accumulation and corrects pre-plaque synaptic deficits and cognitive function in the TGCRND8 and 3xTg mouse models, respectively [123,124]. The McGill-R-Thy1-APP transgenic rat model also exhibits an upregulation of pro-inflammatory molecules at the pre-plaque stage of the amyloid pathology, predominantly in neuronal cells [125,126]. Importantly, treatment with minocycline at the pre-plaque stage restores the balance of inflammatory factors and rescues cognitive deficits in a mouse model of the amyloid pathology [127,128]. Taken together, evidence in animal models suggests that early, plaque-independent inflammation contributes to the progression of the early AD-like amyloid pathology and associated cognitive deficits.

Translating these observations to the human AD pathophysiology has proven to be a major challenge. It is now understood that the underlying AD pathophysiology begins 20–30 years before the first clinical symptoms [129,130]. However, given that current technologies are not sensitive enough to detect the earliest subtle AD pathophysiological features and accompanying CNS inflammation, identifying the initial disease trajectory remains elusive. Positron emission tomography (PET)-scan technology used to measure Aβ plaques, tau pathology, and microglial-TSPO signaling, as well as currently-available CSF and blood biomarkers, only detect advanced AD pathophysiology with a reasonable level of certitude. The critical mass of inflammatory molecules present within the CNS during the long pre-symptomatic phase likely falls below the detection-threshold of current brain imaging techniques. In the absence of reliable early biomarkers, it is virtually unrealistic to unequivocally identify the patient population within the preclinical-AD phase that may be most amenable to anti-inflammatory therapy. It is encouraging that increased astroglial activation was observed by PET imaging 20-years before expected disease onset in patients with autosomal dominant mutations leading to fAD, suggesting that initial detection of astrogliosis may allow clinicians to flag the emergence of the asymptomatic disease phase [131]. Furthermore, a recent report indicates an association between midlife peripheral inflammation and reduction in late-life brain volume in individuals without dementia [132]. These findings suggest that early inflammatory processes could have a detrimental effect in the CNS and this might contribute to the development and progress of the pathophysiology.

It is expected that in the coming years, considerable research efforts will be focused on developing diagnostic methods able of detecting AD progression during preclinical stages, or at least early enough to substantially impact the disease with anti-inflammatory

agents, either as a single or combined therapy. Biologicals, specifically cytokine-directed monoclonal antibodies, are a particularly attractive therapeutic option given that targeting just one cytokine is often sufficient to disrupt the broader molecular cascade that culminates in chronic inflammation [133]. Several TNF-α inhibitors, including TNF-α-directed monoclonal antibodies and recombinant fusion proteins, have already been approved by the Food and Drug Administration (FDA) for the treatment of several inflammatory and auto-immune diseases, including Crohn's disease, ulcerative colitis, and rheumatoid arthritis. In one pilot study, 6-month perispinal extrathecal administration of etanercept (a decoy receptor for TNF-α) in AD patients resulted in an improvement in a variety of cognitive measures [134]; however, these results have yet to be replicated. In another trial, 6-month subcutaneous administration of etanercept in patients with mild-to-moderate AD dementia did not improve cognitive outcomes [135]. In a recent case report, infliximab, a TNF-α-directed monoclonal antibody, administered to a patient with AD led to cognitive improvement along with a decrease in AD pathophysiological biomarkers [136]. Despite these results, exploring the effects of anti-TNF-α therapy in patients with early preclinical AD are still lacking.

Besides its role as lipid sensor and involvement in metabolic pathways, peroxisome proliferator-activated receptor-γ (PPAR-γ) activation leads to the blockage of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)-dependent gene expression, thus inhibiting multiple inflammatory pathways [137]. PPAR-γ agonists have been shown to be beneficial when administered to AD mouse models by decreasing inflammation and AD-related pathophysiological markers [138]. Moreover, clinical trials with rosiglitazone had positive outcomes in mild-to-moderate AD patients [139,140].The PPAR-γ agonist, pioglitazone, an approved treatment for type II diabetes, is currently under a phase 3 clinical trial being conducted in MCI individuals and AD patients (NCT02284906). Further exploration of the therapeutic effects of PPAR-γ activation is needed in early AD stages.

Inhibition of IL-1β signaling represents another promising therapeutic option in treating preclinical AD. Currently-available IL-1β-targeted anti-inflammatory therapies include canakinumab, an IL-1β-directed monoclonal antibody, and anakinra, an IL-1 receptor antagonist [133,141,142]. The potential of anti-IL-1β therapy has yet to be investigated for the treatment of early preclinical AD, either in humans or animal models. Given that IL-β processing and maturation is largely controlled by the multiprotein inflammasome complex [143–146], treatments targeting the assembly and function of the inflammasome may also lead to a reduction in IL-1β signaling in early AD stages. Inflammasome complex activation has been reported in *post-mortem* brains of MCI individuals and AD patients as well as in AD mouse models [147–149]. Moreover, inhibiting the inflammasome in transgenic rodent models of AD leads to a reduction in AD-related pathophysiology and associated cognitive deficits [150]. Proper characterization of inflammasome activation and potential therapeutics at early stages of AD has yet to be explored.

In the absence of early biomarkers and effective therapies to diagnose and treat preclinical AD, the development of compounds targeting CD33 and TREM2 may prove effective in slowing disease progression and symptom severity in already-diagnosed patients with mild-to-moderate AD. Recent studies indicate that disease-relevant variants in *CD33* lead

to increased CD33 expression and impaired phagocytic activity of brain macrophages [151,152], whereas variants in *TREM2* leads to decreased surface cell expression or impaired functioning, also resulting in reduced macrophage phagocytosis [153,154]. The development of small compounds that either inhibit CD33 or promote TREM2 activity may represent a promising therapeutic option to promote phagocytic and clearance mechanisms within the CNS in intermediate-late AD stages. Several monoclonal antibodies targeting CD33 do in fact exist and are in development for the treatment of myeloid leukemia; however, they are currently being evaluated in clinical trials and have not been tested for the treatment of AD, either in humans or animal models [155]. Similarly, the development of a small-compound modulator enhancing TREM2 activity or prolonging its cell-surface expression may promote clearance mechanisms that are likely to be effective in decelerating late neurodegenerative mechanisms.

5. Anti-amyloid beta and anti-tau therapeutic strategies

The clearance of the Aβ peptide, in particular the extracellular overproduction and deposition of the 42-amino acid-long Aβ peptide ($A\beta_{1\text{-}42}$), and the intracellular expression of tau protein, characterized by post-transcriptional phosphorylation, are recognized as critical pathophysiological mechanisms leading to AD. As a result, positivity to amyloid and tau biomarkers is mandatory to establish an effective *in vivo* diagnosis of AD [156]; for this reason, most of the currently developed disease-modifying therapies for AD are targeted on the amyloidogenic and tau pathways.

The conventional hypothesis on AD pathophysiology states that the initial neurodegenerative processes are associated with the imbalance between production and clearance of $A\beta_{1\text{-}42}$ peptides resulting in cerebral accumulation of insoluble and toxic forms of aggregates of misfolded proteins [157]. In this regard, an early, fast, and efficient biomarker-guided screening of individuals during the pre-symptomatic phase might support the development of effective disease-modifying trials before the amyloid-related neurodegenerative processes become irreversible. Indeed, several longitudinal studies clearly indicated that reduced CSF $A\beta_{1\text{-}42}$ concentrations combined with increased cerebral amyloid PET signal currently represent the earliest asymptomatic indicators of AD onset [129,158]. Interestingly, the early pre-symptomatic decrease of CSF $A\beta_{1\text{-}42}$ concentrations or the increase of amyloid PET signal are followed by a “plateau” phase before the individuals become symptomatic (i.e., MCI and dementia stages of AD). Hence, starting an anti-amyloid trial during the dementia or even the MCI stages is predestined to fail. This is supposed to be the key reason accounting for the failure of over 100 anti-amyloid monotherapeutic trials conducted to demonstrate a benefit in slowing the progression of cognitive impairment [159]. Pharmacological anti-amyloid strategy essentially relies on modifying the dynamic balance among Aβ monomers, Aβ oligomers, and fibrils, being Aβ oligomers the most toxic species [160].

Modulation of Aβ secretase enzymes aims at I) increasing α-secretase activity by converting APP into harmless sub-metabolites as well as II) inhibiting β- and γ-secretases activity, to halt the amyloidogenic pathophysiological pathway[161,162]. However, caution should be taken when drug interventions target γ-secretase activity since this enzyme is involved

in various key physiological signaling pathways of proteins modulating cellular trafficking, apoptosis, cholesterol homeostasis, neurogenesis, and angiogenesis [163]. In particular, the amyloid precursor protein (APP) proteolytic processing generates several truncated forms of Aβ which have intrinsic properties providing an essential role for physiological cellular signaling mostly involved in synaptic activity-dependent modulation. Endogenous Aβ monomers show a potential role in the regulation of synaptic vesicles trafficking, thus finally acting as a key electrophysiological modulators of the synaptic firing [164]. Moreover, it has been reported that some Aβ fragments can initiate CREB-mediated cytoprotective pathways [165]. Therefore, an excessive removal of some Aβ monomers by a poorly calibrated pharmacological intervention may prevent hippocampal neurons from surviving aberrant pathways upstream to Aβ deposition.

Interestingly, an alternative option is represented by interfering with APP expression, as previously suggested in AD trials reporting the use of antidiabetic PPAR-γ agonists, including the thiazolidinediones [166]. Another potentially relevant anti-amyloid strategy is stimulating the clearance of amyloid species in the brain by increasing the activity of different proteases including angiotensin and endothelin converting enzymes, insulin degrading enzyme, metalloprotease-9, neprilysin, and plasmin [167]. The aim is to degrade amyloid metabolism byproducts by hindering their oligomerization and aggregation. Interestingly, the concentrations of amyloid degrading enzymes decrease in AD, thus possibly facilitating the deposition of toxic Aβ peptides. However, the modulation of amyloid proteases activity needs further assessment since it may appear as a non-specific and detrimental strategy [168]. In addition, acting on the amyloid transport modulation represents another potential approach. In particular, the multi-ligand receptor for advanced glycation end products (RAGE) efficiently binds Aβ in the blood and promotes its entry into the CNS through the blood brain barrier [2,161]. Finally, the apolipoprotein E, binding the Aβ peptides, allows their entry in the CSF circulation by the lipoprotein receptor related protein-1 and the very-low density lipoprotein receptor mediated transport [169–171].

Currently, anti-amyloid immunotherapies represent the most precisely targeted anti-amyloid treatments. The suggested therapeutic mechanism is that anti-amyloid antibodies may promote the removal of Aβ peptides and Aβ aggregates from the CNS to blood *via* a sort of “peripheral sink” [172]. Passive immunotherapy – based on the intravenous injection of anti-amyloid targeted antibodies – may induce a dosage-dependent increase of both blood and CSF Aβ concentrations. The use of anti-amyloid active immunotherapies has been recently proposed to design next-generation vaccines against small epitopes, instead of developing full length peptides that may generate harmful non-specific immune responses [173]. Unfortunately, the most recently published phase III trials using intravenous immunoglobulin in AD patients did not provide any clinically relevant benefit, in spite of the promising results obtained in preliminary studies [166,174]. Actually, the exact mechanisms of action of these therapies and the origin of their most common side effects, such as cerebral microbleeds, is still unexplored [175]. The ultimate response of an existing proof-of-concept in the anti-amyloid treatment strategies might come from the results of ongoing trials recruiting exclusively AD patients carrying presenilin mutations [176].

Although there is a general consensus that Aβ accumulation represents the initial trigger of AD pathophysiology, the continuous failures of disease-modifying anti-amyloid phase III trials encouraged the design of anti-tau therapeutic strategies. Notably, a robust correlation of tau brain pathology with the severity of the cognitive impairment in AD was reported in several longitudinal studies, thus supporting the interest on anti-tau therapies [129,177,178].

Tau is a microtubule-associated protein involved in axonal stability. It is hyperphosphorylated, separated from microtubules, and then accumulated as a misfolded protein within neurons, in tau-associated neurodegenerative diseases, including AD [179]. Tau targeted treatments can be specifically directed to the phosphorylation process, resulting in the disassembly of microtubules and, consequently, into reduced microtubule stability [180]. One strategy involves acting on the various post-transcriptional modifications monitoring tau intracellular physiological activity [179]. For instance, the up-regulation of tau O-linked glycosylation seems to decrease tau oligomerization process and leads to the deposition of toxic insoluble fibrils. Moreover, the inhibition of tau acetylation may promote tau clearance *via* the ubiquitin/proteasome system (UPS) [181]. Notably, the stimulation of the intracellular autophagy/lysosomal system may represent a way to eliminate the deposition of misfolded tau proteins in the advanced AD stages. Another possible approach includes the down-regulation of tau proteolysis mediated by distinct subtypes of cysteine proteases, namely caspases, calpains, and cathepsins [179,182]. The potential development of active or passive tau immunotherapies appears controversial, given that tau and its toxic brain inclusions have an intracellular position obstructing the tau sinking process mediated by specific anti-tau antibodies [183]. In summary, the development of tau targeted therapies is still in its infancy and, therefore, a further assessment of tau-associated pathophysiological mechanisms (also linked to other neurodegenerative diseases) is mandatory.

Notably, a novel unexplored field in the development of AD therapeutics is to investigate the relationship between the CNS – including the macromolecule circulation and removal within the glymphatyc system – and the periphery [21]. The latter is involved in the clearance of potentially harmful protein byproducts, produced in the brain, that are involved in the pathogenesis of AD and other neurodegenerative diseases. In this regard, emerging data revealed that brain pathophysiological processes are reflected into the periphery; moreover, some CSF biomarkers such as neurofilament light chain [184,185], tau [185], and β-site amyloid precursor protein cleaving enzyme (BACE1) enzyme [86] are reliable blood surrogate proxies of underlying cerebral neurodegenerative mechanisms [86,186–188]. In addition, the Aβ species generated in the CNS can cross the brain blood barrier and be removed by peripheral organs. Actually, systemic diseases may interfere with amyloid clearance, thus contributing to AD development and progression [157]. In this regard, kidney dysfunction might be associated with the amyloidogenic pathophysiological mechanisms leading to AD and is responsible for an increased risk of cognitive/psychiatric alterations and dementia [189]. Interestingly, renal transplantation is assumed to decrease plasma Aβ concentrations and hemodialytic procedures reduce brain Aβ accumulation in subjects suffering from chronic kidney disease [190]. Another interesting observation is that Aβ load in liver tissue is decreased in AD *versus* healthy subjects, hence suggesting the existence of a hepatic Aβ-clearance dysfunction in AD [191]. Notably, epidemiological bidirectional association is evident between diabetes, pure cerebrovascular

cognitive impairment, neurodegenerative diseases such as AD, and mixed forms of dementia [192–198]. In particular, recent studies indicate that Aβ may have detrimental peripheral effects resulting in its atypical accumulation in pancreatic cells [191,199]. In addition, attention should be given to the impaired N-terminal processing of amylin precursor, also called islet amyloid polypeptide (IAPP), an early factor inducing the toxic accumulation and deposition of amyloid in pancreatic β-cells [200].

In conclusion, there is emerging evidence for a bidirectional interplay between brain and peripheral organs in regulating Aβ metabolism and other protein byproducts associated with neurodegenerative pathways. This emphasizes the need for a comprehensive and precise strategy – directed on both CNS and peripheral dysfunctions – based on the systems biology and systems neurophysiology paradigms [157,201]. The traditional “one-drug-fits-all” concept seems to be obsolete and does not reflect the heterogeneity and complexity of neurodegenerative diseases, including potential therapeutic interventions combining precise multi-target drug administrations with lifestyle changes such as diet modifications [202,203] as well as specific and “tailored” cognitive training [204]. These belong to a *spectrum* of diseases caused by the deposition of multiple misfolded proteins and cerebrovascular damage, and are unavoidably affected by systemic diseases [205]. A more flexible and adaptive multi-target strategy, taking into account the complexity of AD pathophysiology, is needed in the upcoming drug development programs.

6. Rethinking and optimizing the design of clinical trials from the precision medicine perspective

PM demands precision drug development. One cannot apply PM concepts of the right drug, in the right dose, for the right patient, without these aspects of drug treatment having been thoroughly tested in clinical trials. Although daunting, the PM approach may be precisely what is needed to resolve the current challenges facing AD drug development. No new treatments have been approved for AD since 2003 and the field has a drug development failure rate in excess of 99% [54]. All drug development programs aimed at developing disease-modifying treatments for AD or any other neurodegenerative disorder have failed [206]. PM offers a means of conceptualizing a resolution to this crisis.

The basic tenet of PM is that humans are biologically heterogeneous and that these differences express themselves in differences in the characteristics of the disease they develop, the stage and rate of progression of the disorder, and the dose needed to abrogate progression or restore function [3,4]. Conduct of clinical trials to meet the demands of PM will require different recruitment approaches, biomarker characterization of participants, dosing strategies, and data analytic approaches. A fundamental need is to better comprehend the basic biology of AD, the druggable aspects of the pathology, the heterogeneity of the disease, and the biomarkers that reveal this heterogeneity to the clinician. These are the building blocks on which precision trials and PM will be built.

6.1. The right drug

The right drug in the PM schema addresses the basic biology of AD. This requires an understanding of the heterogeneous pathology of AD and how it can be addressed pharmacologically. In a recent autopsy study of patients clinically diagnosed with AD and meeting pathologic criteria for AD, 32% of patients had AD pathology only while 68% had combinations of AD pathology, alpha-synuclein/Lewy pathology, and ischemic injury secondary to cerebrovascular disease [207]. In addition to the vascular and degenerative changes, the brains of AD patients exhibit inflammatory, oxidative, mitochondrial, transactive response DNA-binding protein 43 (TDP-43), heavy metal, and epigenetic changes that may contribute to the disease pathophysiological processes and offer opportunities of intervention [208]. The “right drug” for AD will likely be a combination regimen of agents addressing multiple types of pathology. The “right drug” may also evolve over time as the process evolves, changes become more advanced, and new elements participate in the pathophysiological cascade of AD. Clinical trials constructed around PM approach will use biomarkers (discussed below) to link the right drug/combination to the right patient.

6.2. The right dose

Dose exploration is a critical aspect of drug development and clinical trials for AD. Phase 1 should include identification of a maximum tolerated dose (MTD). Without knowledge of the MTD, later efficacy failures will inevitably raise the question of dose adequacy. In some cases, a MTD can be defined by PET occupancy studies showing target saturation thus implying that increased doses will not produce greater effects. Physical limitations, including solubility and acceptable rate of infusion, impose a maximum plausible does for some agents. In all other cases, a MTD should be established and formulation issues should be solved prior to the Phase 1 trial if they may artificially limit the ability to define a MTD.

Dose-response characteristics will also be established in precision clinical trials. Doses that are too low to produce benefit, near the upper limit of tolerability and in the optimal range (minimal 3 doses), should be studied in early phase trials. Adaptive designs may facilitate the elimination of ineffective or toxic doses [209]. Individualization of doses, as required for PM, can be advanced through knowledge of the drug metabolism patterns of the individual, including fast and slow metabolizers and toxic response. In this regard, pharmacogenomics will play a critical role in precision trials and in the PM paradigm [210]. Pharmacogenomics can be broadly defined as the use of genomic and other “omic” information to individualize drug selection, optimize drug efficacy, and reduce adverse drug reactions. In this context, pharmacogenomic information relies on biological markers that label individuals as more or less responsive to specific medications and/or more or less susceptible to experience adverse effects. Moreover, pharmacogenomics can determine treatment response based on disease-causing variants of heterogeneous clinical conditions. Ultimately, pharmacogenomics is expected to remove the traditional “one-size-fits-all” clinical trial methodology in developing and prescribing therapeutic drugs. Hopefully, PM research and interventions will avoid this “trial and error” approach and predict who will respond to a medication and who – in turn – should avoid the same medication. Research in pharmacogenomics is also expected to provide critical information about the genomic

variations that affect response to currently recommended pharmacological treatments and future interventions. Understanding the individual variation and the implications for drug response, metabolism, and drug elimination will allow PM physicians to implement healthcare based on the individual's "omic" biomarker data.

6.3. The right patient

Different therapeutic regimens will likely be required for individuals in different phases of AD. Individuals in preclinical and prodromal AD as well as in mild, moderate, and severe forms of AD exhibit different phenotypes and different underlying "pathologies" that need to be addressed using different drugs/combinations of drugs. Cognitive enhancers are indicated in individuals with cognitive symptoms and psychotropics are indicated in those with neuropsychiatric symptoms. Different drugs/combinations of drugs will be required for those with simple *versus* complex pathology (Fig. 2) and this may evolve as the disease progresses.

The right drug will require use of biomarkers in clinical trials. Biomarkers will define the patient population for which a given therapy or combination is indicated and will link the basic pathophysiology of AD to the proper therapy. Biomarkers of alpha-synuclein, TDP-43, vascular pathology inflammation, and other CNS changes are needed to allow both the trialist and the clinician to construct treatment regimens reflective of the pathology of the individual patient. Experience in the trial will anticipate the needs of the clinician and biomarkers used in trials may evolve to companion biomarkers approved in concert with new therapies and informing their use.

Precision prevention is required for primary prevention of AD in individuals without state or trait biomarkers of the disease or for secondary prevention of those with genetic factors (presenilin-1 [*PSEN-1*], presenilin-2 [*PSEN-2*], amyloid precursor protein [*APP*] mutations or apolipoprotein E [*APOE*] homozygous state) or state biomarkers (positive amyloid PET or CSF signature of AD) indicative of impending AD. Primary prevention trials will focus on life style interventions constructed to match the genomic profile of the individual including risk for diabetes or hypercholesterolemia and other AD risk factors. Primary prevention will involve amyloid prevention agents such as BACE inhibitors. Secondary prevention trials will include lifestyle factors in combination with agents related to tau progression, inflammation, mitochondrial function, and other biological factors. Thus, precision prevention will lead eventually to "precision health".

6.4. Conduct of precision medicine trials for Alzheimer's disease

Precision trials will be structured differently from those currently conducted [211]: substantially extended biological characterization of the population using biomarkers will be required. Biological profiles will be matched to treatment/treatment combinations. Severity may represent an important parameter in selecting the right drug for the right patient. Precise matching of some AD populations to evolving therapies may allow these agents to be developed as orphan drugs for rare diseases [212]. PM trials will be more patient-centric and biomarker-guided than currently conducted trials. Large populations of well-characterized individuals will be required to allow precision trials to be performed. This will require novel

innovative strategies such as mass advertising, enlistment of large populations (e.g., those applying for retirement benefits), on-line testing, mass biomarker collection at convenient locations (such as shopping malls), development of large databases of trial-ready persons, and testing using remote assessments and virtual visits in tele-trials.

Trial analytic strategies will need to evolve to accommodate to PM requirements. PM and precision clinical trial outcomes will address individual responses in more detail. Current analytic approaches provide group data, however, little information about individual responders or non-responders is delivered. Robust analytic techniques applicable to individuals/small groups of individuals will be necessary. This might involve more dependence on well validated biomarkers than previously. Table 3 summarizes how clinical trials will be constructed in the age of PM. The time is now: PM requires precision trials and we should be pursuing these trials now. The trends are already evident with definition of different disease phases and evolution of new biomarkers. These trends need to be validated and accelerated as well as married to aggressive trial methodologies.

7. How can drug discovery programs in Alzheimer's disease accomplish a good level of translational quality to reduce the rate of failures?

7.1. Drug discovery translational for Alzheimer's disease therapeutics

In terms of drug discovery, translation is the process by which non-clinical research is performed that will give insights into the likely behavior of a therapeutic intervention in the individuals. The lack of success in demonstrating efficacy in AD patients of a very wide range of approaches may indicate that translational science is woefully inadequate in the field [54,213]. However, a more considered appraisal suggests a range of reasons for failure that can be grouped into four main – and sometimes overlapping – categories: I) inadequate drug discovery process; II) inadequate target engagement to test the therapeutic hypothesis; III) changing the therapeutic hypothesis to accommodate the compound properties; IV) acceptance of the "null hypothesis."

7.2. Inadequate drug discovery process

In many, but not all, drug discovery programs, work is conducted as reported in Fig. 3. While this is shown as a linear process for simplicity, often steps may be omitted, feedback loops are common, and ideally human data on PK and PD properties of the therapeutic can be fed back to the non-clinical phase to inform the drug discovery program. Moving from *in vitro* to *in vivo* assays, the complexity of the assays increases, as does their relevance to human disease. While an element of this stepwise approach is to filter the large number of test compounds that may need to be synthesized, screened, and assayed in order to find those showing the appropriate potency and selectivity, there is also significant translational value in each step. Notably, biomarkers qualified for use in clinical trials to facilitate marketing authorization and regulatory decision-making should also be available as diagnostic agents. Thus, each biomarker will be useful at one stage or another stage of medical product development, i.e., from discovery to adoption in clinical practice (Fig. 4).

It is a mistake to assume that the *in vivo* model to human transition is the only important translational step: confidence in the therapeutic approach is built throughout the drug discovery process. For instance, if the potency of the potential therapeutic on the isolated target is very significantly reduced (e.g., by >30 fold) when the therapeutic is tested *versus* intact cells, the finding should be investigated and resolved. This may mean that the compound fails to enter cells if the target is intracellular, or that the cell response measured does not solely reflect target activity, and so on. Normally, the pattern of activity of multiple compounds enables trends to be delineated and understood: this is referred to as the structure-activity relationship. If the free drug level (unbound to matrix and, therefore, available to interact with the target) required for a therapeutic response in the target tissue – in this case the brain – is very different from that required for activity in cell culture assays, then this discrepancy needs to be investigated before further advance is considered.

The development and subsequent failure of tarenflurbil reveals several opportunities for significant improvement in the drug discovery process and translation into clinical studies. Tarenflurbil is the R-diasterioisomer of the racemate flurbiprofen, a non-steroidal anti-inflammatory drug approved for human use. The original preclinical data on tarenflurbil showed a dose related decrease in $A\beta_{1\text{-}42}$ production from human embryonic kidney 293 (HEK293) cell line stably transfected with human Swedish mutant APP but with an incomplete dose response curve: the half maximal effective concentration (EC50) being in excess of 250 μM [214]. Additional studies also demonstrated a reduction in $A\beta_{1\text{-}42}$ production from H4 neuroglioma cells expressing Swedish mutant APP695NL but, again, the dose response was incomplete with an EC50 in excess of 250 μM [215]. In the same study, tarenflurbil was administered to Tg2576 Swedish APP transgenic mice, for three days, at three doses: 50, 25, and 10 mg/kg od. All doses reduced $A\beta_{1\text{-}42}$ in brain by a maximum of ~60% but in a non-dose related manner. The group sizes used were small (N = 4-7) and without evidence of a power calculation being employed to guide robust statistical analysis. Crucially, at the top dose of 50 mg/kg, the brain concentration of tarenflurbil was 2.5 μM, a dose more than 100-fold lower than the EC50 in cell culture studies. This significant discrepancy should have been investigated further. In fact, subsequent studies failed to replicate the *in vivo* $A\beta_{1\text{-}42}$ lowering effects of tarenflurbil.

In phase 1 human studies, the highest dose of tarenflurbil administered, 800 mg bid, produced a CSF concentration of approximately 1.2 μM: some 200-fold lower than the EC50 concentration and without any effect on CSF $A\beta_{1\text{-}42}$ concentrations [216]. After the phase 2 study [217] in which target engagement was not assessed and CSF Aβ metabolites were likewise not measured, a phase 3 trial enrolled 1646 mild AD patients in a randomized, double-blind, multisite, placebo controlled trial. Tarenflurbil was administered at 800 mg/kg bid in the active treatment arm for 18 months. The trial failed to meet its coprimary outcome measures of Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) and Alzheimer's Disease Cooperative Study Activities of Daily Living (ADCS-ADL) [218], as did a companion phase 3 trial that was discontinued early. The development of tarenflurbil clearly demonstrates an inadequate translational process during the *in vitro* to *in vivo* phase, coupled then to a likely Type 1 error in *in vivo* efficacy studies that was incorrectly used to support the clinical program.

7.3. Inadequate target engagement to test the therapeutic hypothesis

Aβ is released from the APP holoprotein by the sequential action of BACE and γ-secretase: inhibition of either enzyme is able to reduce the production of the Aβ peptide. Inhibitors of both enzymes have been tested in phase 3 clinical trials in AD patients. Semagacestat is a γ-secretase non-competitive inhibitor, binding at an allosteric site and with a complex mechanism of action [219–221].

Semagacestat inhibited Aβ peptide production in HEK293 cells stably expressing Swedish APPNL with an EC50 of 15 nM [222]. In PDAPP transgenic mice that overexpress the hAPP717 mutant protein, dose related inhibition of brain Aβ production was demonstrated both acutely, and after 7 days' dosing [223]. In a 5-month chronic study, semagacestat was able to lower insoluble Aβ concentrations in a dose-related manner at 3, 10, and 30 mg/kg od [224]. Since this study did not incorporate a baseline group (analyzed at the commencement of dosing), it was not possible to determine whether semagacestat delayed the onset of amyloid deposition or reduced the rate of amyloid deposition, which is critically important with respect to the compound's use in AD patients [213]. In this mouse study, the 30 mg/kg dose reduced plasma Aβ concentrations by approximately 60%.

In a phase 1 study in humans, doses of 60, 100, and 140 mg semagacestat were administered with the peak plasma reduction in Aβ being ~50% at the 60 mg dose and 73% at the 140 mg dose. In this sense, there was evidence for an acceptable nonclinical to clinical translation. However, CSF samples taken 4 h after dosing in humans did not reveal a reduction of Aβ peptide [225]. To investigate this further, the effects of semagacestat on Aβ production were assessed in humans using the stable isotope labelling kinetics (SILK) protocol, which measures the production and clearance of newly synthesized proteins [226]. The oral administration of semagacestat at a single 100, 140, and 280 mg dose was able to inhibit brain Aβ production by 47%, 52%, and 84%, respectively, over a 12 h period, thus confirming semagacestat target engagement [227].

Subsequently, semagacestat was tested at 100 mg and 140 mg in two, Phase 3 trials – Identity 1 and Identity 2–enrolling 2600 mild-to-moderate AD patients in 76 week, placebo-controlled, double blind, randomized, multi-site trials with ADAS-Cog and ADCS-ADL as co-primary outcome measures. Both trials, however, were discontinued following an interim analysis of Identity 1 that revealed a significant worsening of the Clinical Dementia Rating Scale Sum of Boxes (CDR-SB) and the Mini-Mental State Examination (MMSE) scores, together with an increased incidence of skin cancer as well as other adverse events [228].

There has been much discussion about the extent and time duration of Aβ production inhibition at the top 140 mg semagacestat dose [229]. While the plasma half-life of semagacestat is only 2.5 h, there was evidence that the PD effect of the compound exceeded this value in the brain [230]. It is likely that the adverse events, and most probably the worsening of cognition, were caused by an inhibition of γ-secretase mediated notch cleavage and, potentially, other substrates as well. It is very clear, however, that – irrespective of the unfavorable side effect profile of semagacestat – the extent of Aβ inhibition at the top dose of 140 mg was unlikely to have produced an inhibition higher than 25% over a 24 h period, constrained as it was by a combination of short compound half-life

and dose limitation due to preclinical toxicology findings. Thus, the potential efficacy to be derived by robust suppression of Aβ production was not tested because of inadequate target engagement.

7.4. Therapeutic hypothesis is changed to accommodate the compound properties

Solanezumab is a humanized IgG1 antibody derived from m266, a mouse monoclonal antibody that recognizes the mid-domain region (aa16-22) of the Aβ peptide with picomolar (10^{-12}) affinity [231,232]. Nonclinical *in vitro* and *in vivo* studies demonstrated that m266 was able to complex with Aβ so as to deplete Aβ from plasma. Experiments in the PDAPP transgenic mouse model demonstrated that the peripheral Aβ compartment was in communication with Aβ deposited in the brain when m266 was administered, in such a way that the amount of Aβ complexed by m266 in the plasma correlated with the amount of Aβ deposited in the hippocampus [233]. This finding gave rise to the therapeutic rationale for solanezumab for AD – a "peripheral sink hypothesis" – where capturing Aβ in the periphery would alter the soluble to insoluble Aβ equilibrium in the brain thus leading to the dissolution of amyloid plaque [232]. Nonclinical evidence for this hypothesis was, however, rather weak: in fact, it has was not demonstrated that m266 actually cleared amyloid plaque if administered after the beginning of plaque deposition [234]. In addition, reducing peripheral Aβ peptide to undetectable concentrations in plasma of mice using a neprilysin Fc fusion protein showed no effect on brain Aβ levels in wild-type mice. Performing a similar experiment in APP23 transgenic mice with existing plaque likewise was unable to reduce deposited insoluble Aβ levels in the brain of soluble Aβ concentrations in the CSF [235].

During the development of solanezumab, phase 1 clinical studies established that peripheral plasma Aβ increased with dose, as expected if Aβ was being complexed by the antibody and thereby assuming the half-life of the antibody, approximately 30 days [236]. In phase 2 studies, CSF concentrations of total $A\beta_{1\text{-}40}$ peptide (i.e., antibody-bound plus unbound) increased, driven by the very small percentage of solanezumab that entered the central compartment: unbound concentrations of $A\beta_{1\text{-}40}$ decreased. Total concentrations of $A\beta_{1\text{-}42}$ peptide (antibody-bound plus unbound) also increased in the CSF, although unbound concentrations increased: this was taken as evidence of some mobilization of plaque $A\beta_{1\text{-}42}$ [237]. In two, randomized, multisite, blinded, placebo-controlled phase 3 trials – Expedition and Expedition 2 – mild-to-moderate AD patients were administered 400 mg solanezumab by i.v. infusion every 4 weeks for 80 weeks in the active treatment arm. Expedition failed its primary outcome measures of change in ADAS-cog11 and the ADCS-ADL scale from baseline to week 80. On the basis of secondary analyses performed on Expedition, the primary outcome measure for Expedition 2 was changed to ADAS-Cog14 in mild AD patients: Expedition 2 failed this single outcome measure [238]. In secondary analyses of the two trials combined, solanezumab treatment had no effect on the concentrations of unbound CSF $A\beta_{1\text{-}42}$ compared with placebo, unlike that observed in phase 2 studies [239]. More importantly, the treatment had no effect on brain amyloid as measured in a subset of patients using florbetapir PET imaging. A pooled analysis of data from Expedition and Expedition 2 including only the mild AD subset and the ADAS-cog14 as the main outcome showed some evidence for a therapeutic effect of solanezumab. This prompted a previously unplanned,

very large, third phase 3 trial, Expedition 3, in mild AD patients and with ADAS-Cog14 as the single primary outcome measure.

The dosing for Expedition 3 was the same as in Expedition and Expedition 2, i.v. infusion at 400 mg, every 4 weeks, in the active treatment arm in an 80 week trial. Thus, at some point during the clinical development phase, the concept of the "peripheral sink hypothesis" driving plaque resolution was replaced, presumably, by the hypothesis that therapeutic benefit would be mediated, in some way, by penetration of solanezumab into the central compartment and complexing free Aβ. Expedition 3 failed to meet its primary and secondary outcome measures: solanezumab was also shown to fail to reduce brain amyloid in a subset of patients who were assessed using florbetapir PET imaging.

The clinical development of solanezumab continues, however, as the Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease (A4) trial (ClinicalTrials.gov Identifier: NCT02008357) will now test solanezumab at a dose of 1600 mg, every 4 weeks, for 240 weeks in cognitively normal individuals with evidence of brain amyloid pathology measured using florbetapir PET imaging. The primary outcome measure is the change from baseline of the Alzheimer's Disease Cooperative Study-Preclinical Alzheimer Cognitive Composite (ADCS-PACC) to week 240. The rationale for quadrupling the dose of solanezumab from that used in the Expedition trials and lengthening the trial is, presumably, that the trend for an amelioration of cognitive impairment observed in previous phase 3 trials will reach a clinically meaningful level in a treated population that is at the earliest stages of the evolution AD pathophysiology. There are no preclinical or clinical data to support this rationale with respect to solanezumab. Thus, solanezumab's "peripheral sink hypothesis" has clearly been disproven and the current therapeutic hypothesis for solanezumab remains unclear. One can argue that if a therapeutic benefit is ultimately shown for a drug, then the absence of a therapeutic hypothesis is somewhat irrelevant (although, in this eventuality, subsequent therapeutic approaches based on the clinical success would be difficult to enact). The clear danger of this strategy, however, is the risk of chasing a "chimera", coupled to an uninformative clinical experiment should the trial fail.

7.5. What can we do better?

The issues to be surmounted in order to discover and develop a disease-modifying therapy for AD are clearly challenging. There are many lessons to be learned from prior studies:

1. Ensure that the nonclinical efficacy experiments mirror as far as is possible the clinical situation.
    a. In this regard, according to the British statistician George Box, "the most that can be expected from any model is that it can supply a useful approximation to reality: all models are wrong; some models are useful." Therefore, it is important to be aware of the differences between the *in vivo* model utilized to demonstrate therapeutic efficacy and the human disease. In particular, models can be assessed in terms of their "face", "construct", and "predictive" validity:

a. **“Face validity”:** are there elements of the model that resemble the gross appearance/presentation of the human disease?

b. **“Construct validity”:** are there fundamental elements of model construction that are shared between the animal and human disease?

c. **“Predictive validity”:** do results that are derived from the model replicate in human disease?

b. It is crucial to be rigorous in avoiding inappropriate validity assignment. Hence, an APP transgenic mouse model that over-expresses a mutant form of hAPP may well have Aβ amyloid plaque deposition that bears very great similarity to amyloid plaques in AD and also demonstrates learning and memory impairment. While the face and construct validity for plaque deposition is quite robust, it is absent for cognitive impairment, as such mice very often do not have neuronal loss or tau pathology that well correlate with cognitive impairment in AD, when amyloid deposition does not.

c. All AD therapeutics that have completed their clinical testing have been administered to patients with existing disease pathophysiology (notwithstanding patients misdiagnosed with AD). Thus, for therapeutics that might, for example, aim to slow or limit the progression of tau pathology, nonclinical experiments should be conducted in *in vivo* model systems following a therapeutic – dosing commenced after tau pathology onset – rather than preventative – dosing commenced before tau pathology onset – protocol.

d. Concentrations of the therapeutic required for efficacy and or evidence of pharmacological action should not significantly differ from nonclinical assay systems through to clinical testing.

e. c. If possible, to discover translational biomarkers, i.e., to identify changes that can be measured as a consequence of target engagement in the nonclinical efficacy or pharmacology model, that can be measured in humans that will provide confidence that the therapeutic hypothesis will be adequately tested.

2. Be clear on the therapeutic hypothesis and ensure that the clinical phase 3 trial will be sufficiently informative to accept the “null hypothesis”.

a. For instance, if the therapeutic hypothesis is that lowering Aβ production will provide clinical benefit, then robustly interrogate, plan for, and provide evidence for I) the extent of Aβ suppression will be required and II) why.

b. If it is impossible to replicate the conditions of the nonclinical efficacy data in man, e.g. because of adverse reactions to the therapeutic, then

there is a significant risk of conducting a clinical trial where it will be impossible to accept the “null hypothesis” in the event of failure.

c. Ensure that target engagement, or a robust surrogate, has been demonstrated prior to phase 3 clinical testing and appropriate dose ranging studies have been completed.

3. Make sure that the patient/subject population is selected as appropriate to the therapeutic mechanism of action.

a. In this regard, the advent of genetic, imaging, and fluid biomarkers enables a more accurate selection of the population of individuals. Most biomarkers are disease state markers, rather than disease progression markers (e.g., amyloid PET imaging and CSF $A\beta_{1\text{-}42}$ concentrations). Sensitive fluid biomarkers of disease progression might accelerate decision making in otherwise long phase 3 clinical trials.

4. There is a huge unmet clinical need for effective disease-modifying therapeutics for the treatment of AD patients and everyone in the field hopes for a breakthrough. However, “hope” is not a strategy.

8. Perspectives

The development of specific treatment options in biomarker-defined subgroups of patients offers a promising way to treat different diseases more effectively and the use of stratified medicine has gained considerable attention in recent years. Assuming that some drugs act differently in different patients, biomarkers are investigated that are capable to identify patients in which a specific treatment shows a larger effect size or a better tolerability than in the complementary group. Referring to an improved superiority to a control group in the biomarker defined subgroup, these biomarkers are referred to as predictive in contrast to purely prognostic biomarkers that only forecast the course of the disease. Differential treatment effect sizes with respect to different groups of patients – i.e. interactions between subgroup and treatment – are, however, difficult to detect with respect to clinically relevant endpoints due to limited sample size, absence of between-patient comparisons, and blurring effect of additional sources of variability. If the resulting groups of patients are small or if treatment is started in preclinical disease stages, then the development of a confirmatory proof-of-efficacy trial may become extremely difficult to accomplish. On the other hand, pathway-based drugs may work for different diseases and, therefore, proposals were made to investigate these drugs in different diseases simultaneously referring to recently proposed basket trials.

Resulting from these settings and proposals regulatory challenges are to be discussed. In case of an unclear differential treatment benefit, the lack of evidence in the non-selected groups may be an issue and challenge the usefulness of the biomarker-related selection. Data in both biomarker positive and negative patients are necessary. Much effort is still required to explore and confirm reasonable predictive biomarkers. Especially in preclinical AD, early surrogate endpoints able to predict the treatment effect in clinically relevant

endpoints are needed to determine a successful combination of drug and population and to reliably confirm truly predictive biomarkers.

Notably, the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA) (CDER/FDA) will soon release a draft guidance on clinical AD development, encouraging studies in the pre-symptomatic phase [J. Woodcock, personal communication].[1]

Several biomarkers are used in AD for enrichment in clinical studies to define a restricted subpopulation that is expected to profit from treatment. For instance, amyloid PET and CSF $A\beta_{1-42}$ are expected to be useful as predictive biomarkers. Although strongly correlated, both are measuring different aspects of amyloid pathology, fibrillar aggregates of Aβ for PET and soluble $A\beta_{1-42}$ monomer concentrations for CSF $A\beta_{1-42}$. Whereas both are considered acceptable for enrichment, the type of assay and a cut-off needs to be defined and justified [240]. In addition to CSF $A\beta_{1-42}$, total tau (t-tau) or phospho tau (p-tau) concentrations are considered useful, since the $A\beta_{1-42}$/tau ratio was found to have a higher positive predictive value than $A\beta_{1-42}$ alone [158,241].

Downstream topographical markers of brain regional structural and metabolic changes – e.g. hippocampal atrophy assessed by magnetic resonance imaging (MRI) and cortical hypometabolism assessed by ^{18}F-2-fluoro-2-deoxy-D-glucose PET (^{18}F-FDG-PET) – while having insufficient pathological specificity were found to be better related to cognitive decline than Aβ itself and may be particularly valuable for detection and quantification of disease progression. Consequently, the combined use of amyloid and more downstream topographical biomarkers is expected to be more informative [242,243].

Novel biomarkers are currently investigated and may increase the utility of further stratification, e.g. tau PET imaging, biomarkers for neuroinflammation, blood or metabolic signatures [10,106,107,244,245]. Apart from that, epigenomics play an important role: for instance, *APOE ε4* status may be used as one of the means of enrichment. Indeed, *APOE ε4* homozygotes constitute 2–3% of the general Caucasian population and have a particularly high risk of developing symptoms of LOAD (although there seem to be substantial sex-risk differences and the presence of protected *APOE ε4* homozygotes indicates complex individual genetic risk and protection patterns), especially in the presence of AD pathophysiology.

Pathway-related biomarkers should be identified in early development to reliably identify patients groups eligible for specific treatments. Whereas the predictivity of biomarkers expressed in terms of treatment-by-subgroup or treatment-by-biomarker interaction is usually suggested by drug action, further investigations in early phase clinical studies (possibly in surrogate endpoints) would be required to confirm the utility of the biomarker-related selection, but studies to investigate these interactions in hard clinical outcomes appear unrealistic with respect to size and duration.

[1]This reflects the opinion of the author and does not necessarily reflect the position of the Food and Drug Administration.

In case of simultaneously studied diseases for the same drug, questions of how to deal with the multiplicity issue in confirmatory trials and whether and how information can be borrowed from one sub-study to the other arise. The corresponding statistical modeling usually requires additional assumptions that have to be agreed upon. However, even though these studies are considered to be explorative, they should certainly be efficient and informative enough to be justified, especially if long-term outcome is to be measured.

The precision of stratification has greatly improved in recent years, and patient treatment has significantly changed wherever the stratified medicine model has been introduced. This is due to substantial progresses in understanding the molecular basis of the disease, aided particularly by the advent of the genomic era and by the development of targeted therapies to address these new molecular targets. The introduction and refinement of key technologies has allowed these advances, through the increasingly detailed examination of the role of genes, RNAs/miRNAs, proteins/peptides, and metabolites/lipids in disease. These relevant technologies, which are set to further progress, include genomics/epigenomics, transcriptomics, proteomics/peptidomics, metabolomics/lipidomics investigations [201,246] and digital pathology analyses on clinical samples, clinical imaging studies, as well as biomedical and health informatics [247–249]. Standardized protocols for collecting and recording both types of data will be needed to allow comparing and combining samples and datasets, which is required to perform the large-sample-size research that will advance the molecular understanding of the disease. Moreover, recommendations have been recently released by the Academy of Medical Sciences (AMS) (available at https://acmedsci.ac.uk/viewFile/51e915f9f09fb.pdf) to safeguard the continuous development and adoption of stratified medicine products.

In essence, both the exploration and the confirmation of stratified medicine to be used in biomarker-defined subgroups requires a precise understanding of the underlying pathways, considerable amount of comparative data, efficient designs, and challenging integrative statistical modeling (integrative disease modeling, IDM), but also a well-founded appreciation of the remaining uncertainties and the likelihood of false decisions.

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- *In Vitro* Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Patent Number: 8916388
- *In Vitro* Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Patent Number: 8298784
- Neurodegenerative Markers for Psychiatric Conditions Publication Number: 20120196300
- *In Vitro* Multiparameter Determination Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100062463
- *In Vitro* Method for The Diagnosis and Early Diagnosis of Neurodegenerative Disorders Publication Number: 20100035286
- *In Vitro* Procedure for Diagnosis and Early Diagnosis of Neurodegenerative Diseases Publication Number: 20090263822
- *In Vitro* Method for The Diagnosis of Neurodegenerative Diseases Patent Number: 7547553
- CSF Diagnostic in Vitro Method for Diagnosis of Dementias and Neuroinflammatory Diseases Publication Number: 20080206797
- *In Vitro* Method for The Diagnosis of Neurodegenerative Diseases Publication Number: 20080199966
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Abbreviations:

$A\beta_{1-42}$	42-amino acid-long Aβ peptide
AD	Alzheimer's disease
ADAPT	Alzheimer's Disease Anti-Inflammatory Prevention Trial
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive Subscale
ADCS-ADL	Alzheimer's Disease Cooperative Study Activities of Daily Living
ADCS-PACC	Alzheimer's Disease Cooperative Study-Preclinical Alzheimer Cognitive Composite
AMS	Academy of Medical Sciences
APMI	Alzheimer Precision Medicine Initiative

APMI-CP	Alzheimer Precision Medicine Initiative Cohort Program
APOE	apolipoprotein E
APP	amyloid precursor protein
BACE1	β-site amyloid precursor protein cleaving enzyme
CD33	cluster of differentiation 33
CDER	Center for Drug Evaluation and Research
CDER/FDA	Center for Drug Evaluation and Research at the Food and Drug Administration
CDR-SB	Clinical Dementia Rating Scale Sum of Boxes
CNS	central nervous system
CPC	clinicopathological correlations
CSF	cerebrospinal fluid
EC50	half maximal effective concentration
fAD	familial AD
FDA	Food and Drug Administration
^{18}F-FDG-PET	^{18}F-2-fluoro-2-deoxy-D-glucose
GWAS	genome-wide association studies
HEK293	human embryonic kidney 293
HMDB	Human Metabolome Database
IAPP	islet amyloid polypeptide
IL-1β	interleukin-1-beta
IL-6	interleukin-6
LOAD	late-onset AD
MMSE	Mini-Mental State Examination
MRI	magnetic resonance imaging
MS	mass spectrometry
MTD	maximum tolerated dose
NF-κβ	nuclear factor kappa-light-chain-enhancer of activated B cells
NSAIDs	non-steroidal anti-inflammatory drugs

PD	pharmacodynamics
PET	Positron emission tomography
PK	pharmacokinetic
PM	Precision Medicine
PMI	U.S. Precision Medicine Initiative
PP	Precision Pharmacology
PPAR-γ	peroxisome proliferator-activated receptor-γ
PSEN-1	presenilin-1
PSEN-2	presenilin-2
sIL-6RC	IL-6 receptor complex
SILK	stable isotope labelling kinetics
SNPs	single nucleotide polymorphism
TACE	TNFα converting enzyme
TDP-43	transactive response DNA-binding protein 43
TGF-β	transforming growth factor-beta
TNF-α	tumor necrosis factor-alpha
TNFRs	TNF receptors
TREM2	triggering receptor expressed on myeloid cells 2
UPS	ubiquitin/proteasome system

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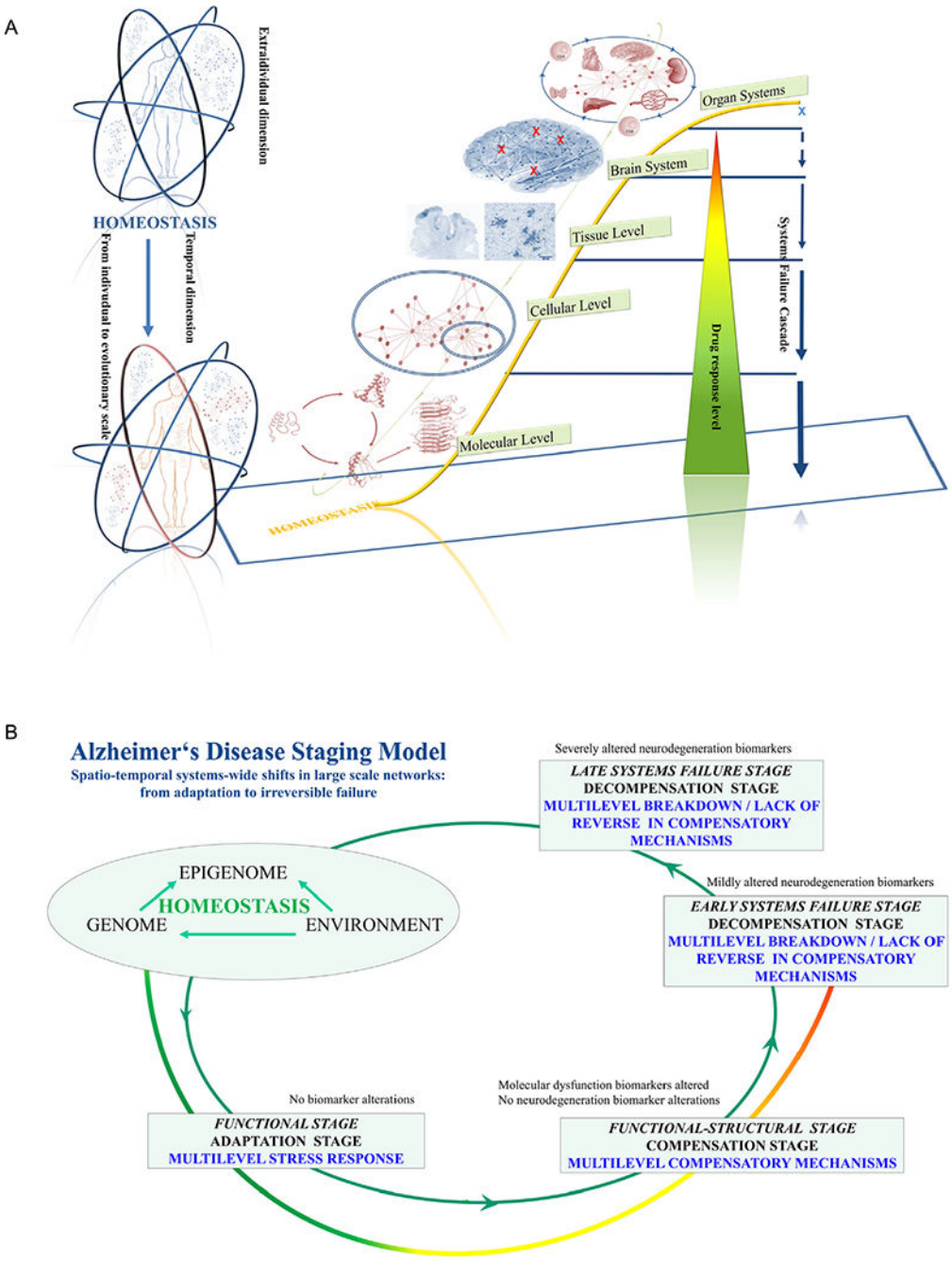

Fig. 1.

(A) Trajectory of pathophysiological mechanisms across the continuum of systems multi-scale hierarchical self-organization, from systems homeostasis to systems failure: conceptual basis for molecular pathway-based therapies.

The preservation of human organism homeostasis is strictly related to the interactions between human systems factors, i.e. genome/epigenome and ecosystem factors, i.e. environment (the circles). Such interactions shows a non-linear fashion with complex dynamic changes over time that are essential at the individual level for adaptation and

survival of the single organism to a certain ecosystem and at extra-individual level for adaptive (genetic) evolutionary transitions finally resulting in the trans-generational process of natural selection. For instance, the impact of a genetic mutation on a single organism may lead to wide-ranging severe maladaptive effects even though from an evolutionary trans-generational perspective this may represent a primary driver for optimized survival and reproduction. Therefore, adaptive responses are differently distributed in space and time scales, aimed at different key roles consistently with the individual, extra-individual and the trans-generational level.

Unrevealing the spatial-temporal coordinates of multilevel adaptive events across human systems (from molecular level to system level) and between these and ecosystem will uncover key notions essential for the comprehensive understanding of complex disease and at an higher level of complexity to achieve a unified theory of genetic adaptation leading to evolution. Thus, an individual vulnerability to stressors exists with an individual threshold of anti-stress response activation and failure.

The non-linear orange-shaped line represents the entire *spectrum* of pathophysiological mechanisms across all systems levels, during the course and progression of disease. Such alterations originate from initial adaptation processes leading through triggers, drivers, thresholds to a point of decompensation at both structural and functional level. The green circle surrounding the five levels represents the marked interplay among the different hierarchical self-organized systems levels. Such interactions support the hypothesis that the initial loss of homeostasis might originate and occur at every level taking into account that a single level potentially affects the whole dynamic interrelated system and, therefore, initially or ultimately the entire affected organism.

The ***molecular level*** shows aberrant conformational states of proteins and dysregulated molecular pathways, including: post-translational modifications, inefficient autophagic mechanisms, dysfunction of membrane dynamics. The **cellular level** originates from the sum of a number of distinct and/or interrelated aberrant molecular pathways. This has a negative impact on anti-stress responses with a subsequent overall impairment of cytoprotective and homeostatic mechanisms. The ***tissue level*** presents a substantial loss of structural and functional organization induced by certain categories of cells. At ***brain system level***, aberrant neural oscillatory, altered metabolic, blood-flow and oxygenation activities might successively or simultaneously occur across different brain system networks, thus affecting different network integration processes and the whole functioning of the system. Therefore, brain-wide shifts in large scale network functioning allow a spatial and temporal processing resources redistribution to cope with stressors. Such hypothetical model can explain how pathophysiological alterations at the brain system level may precede, support and impact abnormal upstream to downstream molecular and cellular pathways. The *organ systems level* represents an enormous and most complex interplay among several networks of different body systems including brain. The existence of many cross-links-talks between CNS and the periphery might account for the hypothesis that brain diseases can originate or be substantially related to peripheral failure. The idea of an isolated brain disease has to be critically assessed in view of the organ systems level.

The colored pyramid represents potential outcome of effective treatment, the potential drug response at each level (from green to red and from the base to the peak there is a decreasing amplitude of effect). The arrows explain the likelihood to restore compensatory mechanisms

(i.e. disease-modifying effect) at the single level; the thicker the arrow is, the higher is the chance that the treatment is effective. The "x" positioned in correspondence of the organ systems failure indicates a hypothetical "point of no return" (pathophysiological irreversibility threshold) without any significant possibility for the drug to reverse, stop or modify the disease dynamic and progression. *Abbreviations:* CNS, central nervous system. (B) Hypothetical model of spatio-temporal systems-wide shifts in large scale networks along the continuum of AD pathophysiological processes: from adaptation to irreversible failure. Organisms are made of systems which are entities consisting in hierarchically self-organized levels with increasing structural complexity resulting in different emerging properties. Multilevel systems are strictly and dynamically interconnected through feedback and cross-talking mechanisms. As a consequence, spatial selective network activation from molecular pathways to systems large scale networks as well as time-dependent cascade of activation can allow to achieve the most effective output to copy with stressors. This, in turn is aimed to maintain homeostasis a dynamic *equilibrium* resulting from the dynamic interaction between genome, epigenome and environment. The regulation of several processes at multilevel of complexity from gene expression to cellular cycle to tissue repair and system-wide network activation has different time delays (time scale) according to the system (space scale). Thus, spatio-temporal systems-wide shifts in large scale network functioning are essential to reallocate processing resources fundamental for adaptation. The understanding of how to measure and possibly control space and time scaled adaptive and compensatory responses occurring during complex polygenic diseases with non-linear pathophysiology, as AD, will represent a crucial step for achieving the capability to effectively modify disease. Biomarkers will guide in exploring how the space and time dimensions are mechanistic involved in complex disease as AD.

***Functional Stage – Adaptation Stage – Multilevel Stress Response*:** from metabolic reconfiguration to functional switch in cellular/tissue/systems network activity aimed to copy with different stressors/pathophysiological processes.

***Functional-Structural Stage – Compensation Stage – Multilevel Compensatory Mechanism*:** structural and functional dynamically balancing one another in order to copy with different pathophysiological processes.

Early Systems Failure Stage – Decompensation Stage – Multilevel Breakdown/Lack of Reverse in Compensatory Mechanisms: initial and progressive loss of physiological interactions and pathophysiological compensations across multilevel systems network.

Late Systems Failure Stage – Decompensation Stage – Multilevel Breakdown/Lack of Reverse in Compensatory Mechanisms: progressed loss of physiological and pathophysiological simultaneous interactions between multilevel systems network

From the first stage to the third stage there is a decreasing chance to restore homeostatic condition (as highlighted by the colors from green to red). No option to recover homeostasis at the last stage.

Abbreviations: AD, Alzheimer's disease.

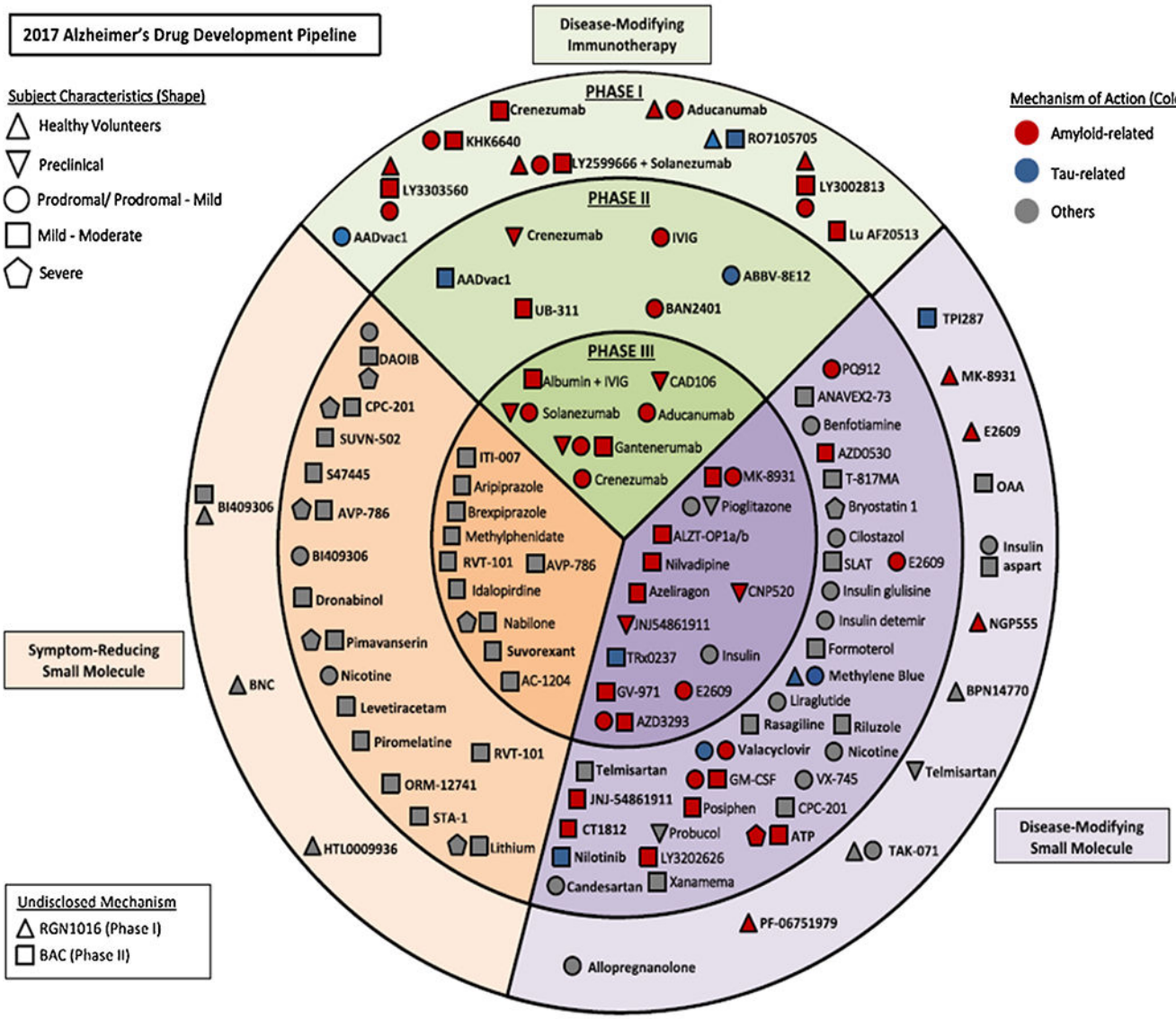

Fig. 2.

Agents in clinical trials for the treatment of Alzheimer's disease in 2017 (from clinicaltrials.gov accessed on 1/5/2017).

Abbreviations: ATP, adenosine triphosphate; BNC, bisnorcymserine; GM-CSF, granulocyte-macrophage colony-stimulating factor; OAA, oxaloacetate; IVIG, intravenous immunoglobulin; SLAT, simvastatin 1L-arginine 1 tetrahydrobiopterin.

From Cummings J et al. "Alzheimer's disease drug development pipeline: 2017." Alzheimers Dement (N Y). 2017 May 24;3(3):367-384. doi: 10.1016/j.trci.2017.05.002.

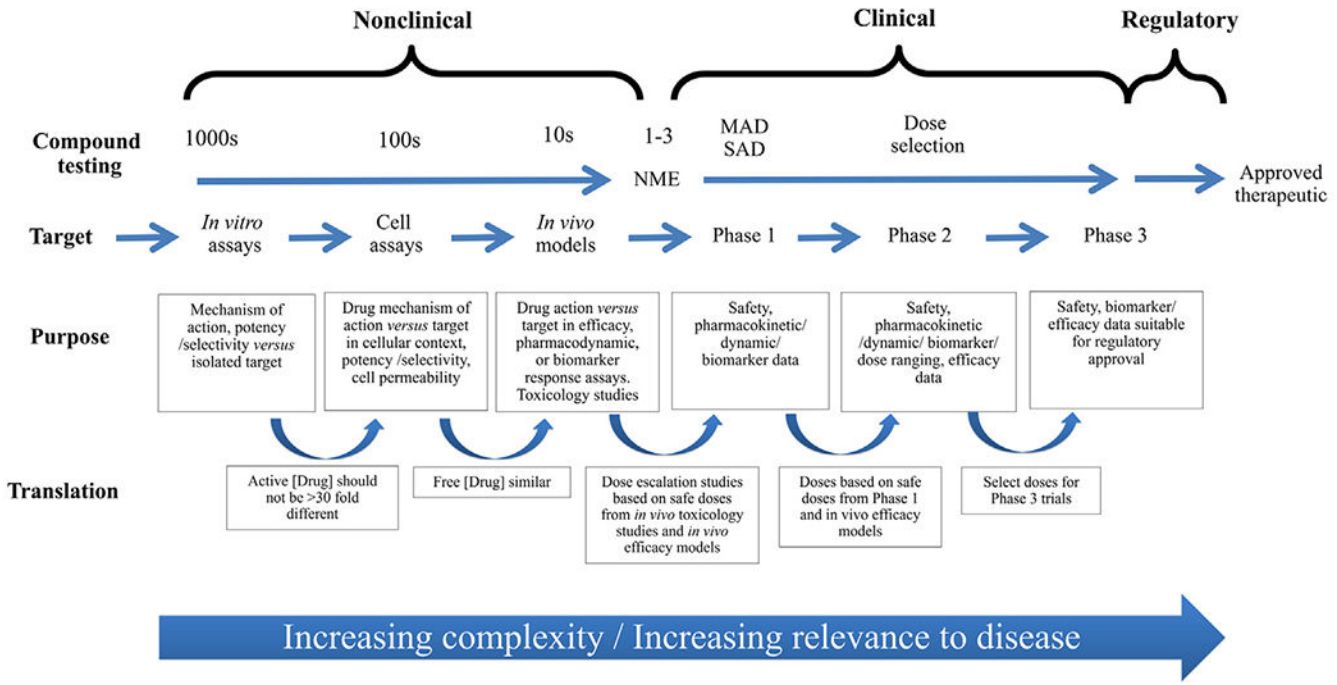

Fig. 3.

Drug discovery programs workflow. Many drug discovery programs progress through a logical sequence where the findings from one type of experiment inform the next step. Significant confidence is generated in programs where the data generated within each phase are concordant with subsequent phases. Programs that lack this translational quality are subject to increasing risk of failure.

Abbreviations: MAD, Multiple Ascending Dose; NME, New Molecular Entity; SAD, Single Ascending Dose.

Adapted from Karran E, Hardy J. "A critique of the drug discovery and phase 3 clinical programs targeting the amyloid hypothesis for Alzheimer disease." Ann Neurol. 2014 Aug;76(2):185-205. doi: 10.1002/ana.24188. Copyright © 2014 Wiley. Reprinted with permission from Wiley.

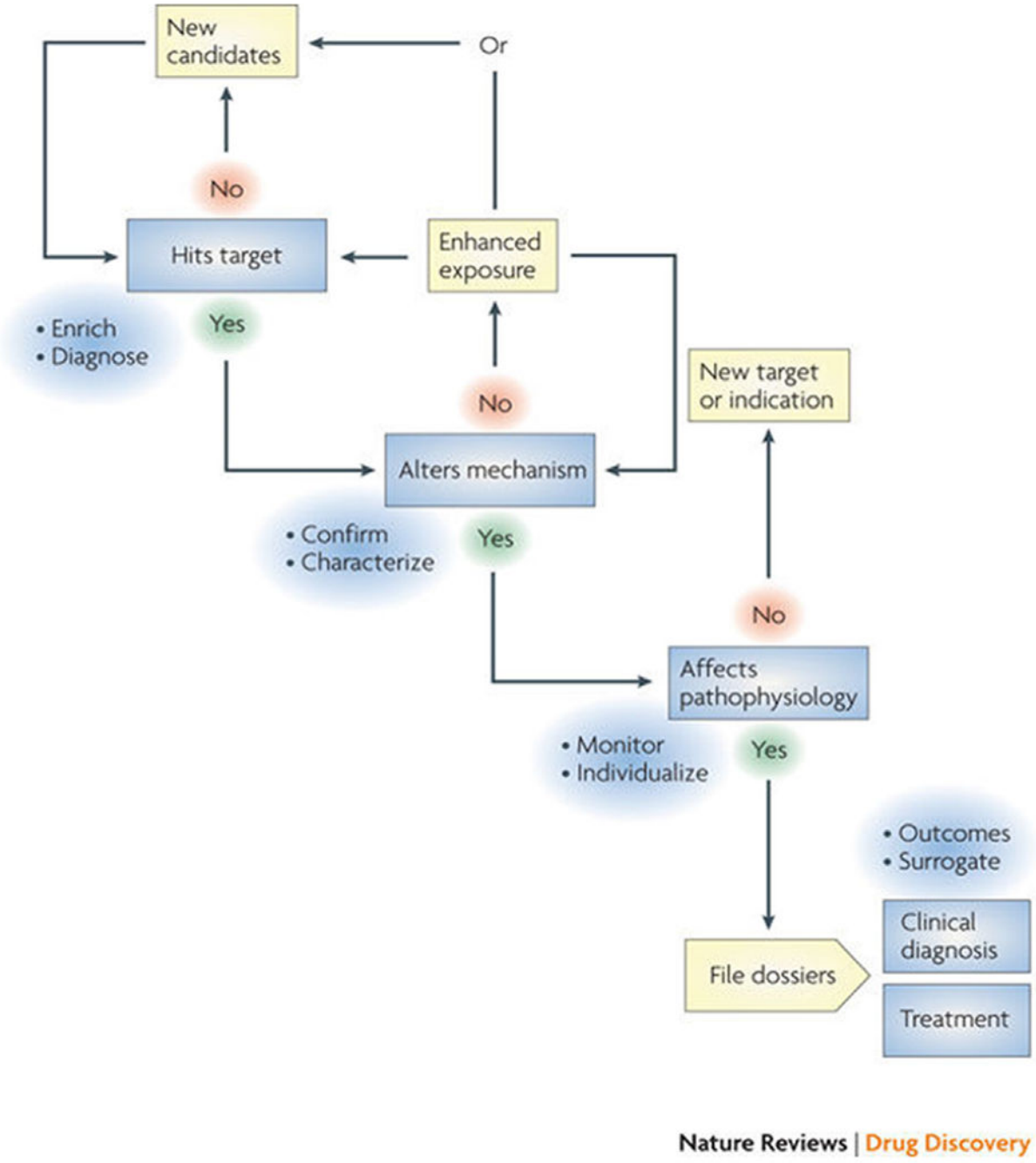

Fig. 4.

The four categories of biomarker: target, mechanism, pathophysiological, and diagnostic. Biomarkers can be categorized into four groups on the basis of their contribution to business, regulatory and clinical decision-making. Clinical decision-making can be further divided into clinical research and patient care diagnostic subcategories. The objective is to use biomarkers as early as possible in the drug development process.

– The initial step is to confirm that **a test compound hits the target** and to quantify the extent to which it does so. Next is to test three concepts in logical sequence.

– First, that hitting this target **alters the pathophysiological mechanism**.
– Second, that altering this mechanism **affects the pathophysiology**.
– Third, that **affecting pathophysiology** predictably improves the clinical status of the patients.
– Biomarkers qualified to confirm the presence of the target and or extent to which the drug candidate hits the target may be validated later as diagnostic tests for early detection or diagnosis of Alzheimer's disease (when that target is expressed differentially between healthy and diseased states).
– Biomarkers qualified for confirming and quantifying mechanistic effects may be validated later as diagnostic tests to inform choice of therapeutic regimen, either in choice of drug or initial dosing regimen.
– Biomarkers qualified for longitudinal quantification of patient response in terms of clinically relevant pathophysiology, may be validated later as diagnostic tests for monitoring and individualization of a therapeutic regimen.
– Biomarkers qualified for either monitoring or individualization of therapy on clinically relevant pathophysiology may also serve as surrogate end points to support regulatory decision-making. In addition, they can be used to ensure appropriateness of use, and as quantifiers of clinical outcomes to support reimbursement decisions.

From Hampel H et al. "Biomarkers for Alzheimer's disease: academic, industry and regulatory perspectives." Nat Rev Drug Discov. 2010 Jul;9(7):560-574. doi: 10.1038/nrd3115.

Table 1

Ongoing and completed clinical trials categorized by molecular targets and mechanisms of action.

A) BACE1 inhibitors

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
BACE1 inhibitors [250–253]	AZD3293 (LY3314814, Lanabecestat)	AstraZeneca, Eli Lilly & Co.	Amyloid (SmallM)		MCI/MD aPET/CSF II-III (Recruting)
	Verubecestat (MK-8931-009)	Merck	Amyloid (SmallM)	Selective inhibitor of BACE1 – It cleaves APP in its ectodomain, generating the amino-(N) terminus of Aβ and the membrane bound carboxyl-terminal fragment C99 from which γ-secretase will generate neurotoxic Aβ oligomers	MCI aPET/CSF III (Recruting)
	CNP520	Amgen, Inc., Novartis Pharmaceuticals Corporation	Amyloid (SmallM)	(Binding of aspartyl protease BACE1 through a competitive and reversible non-covalent interactions with the active site preserving lipophilic profile useful to cross the BBB) [Oral]	AaR $\varepsilon 4^{*}$ II-III (Recruting)
	JNJ-54861911	Janssen, Shionogi Pharma	Amyloid (SmallM)		AaR aPET/CSF II-III (Recruting)
	Elenbecestat (E2609)	Biogen, Eisai Co., Ltd.	Amyloid (SmallM)		MCI/MD aPET II (Recruting)

B) α-secretase modulators

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
α-secretase modulators	Acitretin (Soriatane, Neotigason, RO 101670) [254]	Actavis, Allergan plc	Amyloid (SmallM)	It stimulates the "non-amyloidogenic pathway" of α-secretase ADAM-10, that catalyzes the shedding of the ectodomain of APP in preventing neurotoxic Aβ oligomers formation and producing a neuroprotective-neurotrophic APPs-α It is a synthetic retinoic acid receptor agonist that binds RAR/RXR heterodimers which is a ligand-controlled transcription factors. Thus, it increases gene expression of α-secretase ADAM-10 [Oral]	MD n.a. II-III (Unknown)
	EHT 0202 (Etazolate)[255,256]	ExonHit Therapeutics	Amyloid/ Inflammation/ Transmission (SmallM)	Selective GABA-A receptor modulator and PDE4 inhibitor by which it increases brain APPs-α levels PDE4 hydrolyzes cAMP, inhibiting the cAMP/pCREB/BDNF signaling pathway substantially involved in several pro-inflammatory responses (cytokines release, microglia activation) cAMP functionally modulates the ionotropic GABA-A receptors that potentiate the chloride efflux resulting in the depolarization of the plasma membrane followed by a rise in intracellular calcium. This, in turn, leads to the restoring of calcium homeostasis and preservation of the mitochondrial function with reduction of oxidative stress and a neuroprotective effect. In addition, GABA-A receptors have been linked to sAPPα increased production *via* ADAM10 increased expression [Oral]	MD n.a. II (Completed)
γ-secretase inhibitors or modulators	EVP-0962 (EVP 0015962)	FORUM Pharmaceuticals Inc.	Amyloid (SmallM)	MoA not fully clear – Selective decrease of Aβ1-42 production with an overall shift in the Aβ1-42/Aβ1-40 ratio, increasing Aβ1-38 (non-toxic fragment) without affecting the total Aβ load as well as Notch	MCI/MD n.a. II (Completed)

A) BACE1 inhibitors

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
				receptor processing (whose suppression is associated to systemic toxicity) [257,258]	
	NIC5-15	VA Office of Research and Development		γ-secretase modulators may induce subtle conformational changes of PSEN, the core catalytic site of the γ-secretase complex NIC5-15 is pinitol, a natural cyclic sugar alcohol, that may even have a potential effect as an insulin-sensitizer [Oral]	MD n.a. II (Completed)

D) Aβ and or Tau aggregation/accumulation inhibitors

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
Aβ and or Tau aggregation/ accumulation inhibitors	PBT2	Prana Biotechnology Limited	Amyloid (SmallM)	New generation metal chelator compound promotes cellular Zn and Cu uptake, restoring ions cellular homeostasis and promoting antioxidants cytoprotective signaling. Since cerebral Aβ plaques contain *Cu* and *Zn* ions which are involved in the Aβ oligomer aggregation, and chemical stabilization of plaque itself. Thus, the drug contributes to degrade and prevent ions-facilitated Aβ oligomers aggregation and toxicity [259] [Oral]	MD n.a. II (Completed)
	NPT088	Proclara Biosciences, Inc.	Amyloid/Tau (?) (SmallM)	Ig-GAIM fusion protein: active fragment of g3p and human IgG1-Fc reducing the aggregation of misfolding Aβ and its precipitation in plaques. Possible MoA similar to that of Tau [260]. [IV]	MD aPET I (Recruiting)
	Posiphen (R-phenserine, (−)-Phenserine)	QR Pharma Inc.	Amyloid/α-sin/ Transmission (SmallM)	It potentiates the binding of IRE to IRP1 and, in turn, the activity of the latter. IRP1 controls *Fe*- dependent translational processes of APP synthesis It reduces the production of Aβ, C31 and N-APP that are neurotoxic APP-derived fragments. It blocks neural SNCA mRNA translation Potential anti-neuroinflammatory and anti BACE1 mechanisms. It acts also as a selective, non-competitive AChEi enhancing cholinergic synapse [Oral]	MCI/MD CSF I-II (Recruiting) [261]
	ISIS 814907 [262]	Ionis Pharmaceuticals, Inc.	Tau (SmallM)	Antisense oligo-nucleotides (15–25 nucleotides) that reduces brain levels of neurotoxic aggregating tau proteins by knocking-down Tau mRNA transcription. by binding it to a specific target and causing its destruction by activating the nuclear enzyme RNA hydrolases [IC]	MD CSFI-II (Recruiting)
	PQ912 [263]	Probiodrug AG	Amyloid/Enzyme (SmallM)	Inhibitor of glutaminyl cyclase (metalloenzyme upregulated in AD) that generates pyroglutamil-Aβ, a hydrophobic peptidases-resistant Aβ fragment, with high intrinsic toxicity and also a major component of Aβ plaques [Oral]	MCI/MD CSF II (Recruiting)
	ALZT-OP1 (Cromolyn sodium, Intal) [264]	AZTherapies, Inc.	Amyloid (SmallM)	Cromolyn is a flavonoid derivate that inhibits Aβ aggregation into fibrils by binding Aβ monomers with hydroxyl substituents at specific sites. It may potentially act as γ-secretase modulator [Oral]	MCI/MD CSF III (Recruiting)
	LMTM(TRx0237, LMT-X, Methylene Blue)	TauRx Therapeutics Ltd	Tau (SmallM)	It promotes the oxidation of the two cysteine residues within 4-R tau by a redox cycling mechanism, thus preventing tau aggregation or dissolving its existing aggregates [Oral]	MD – III (Completed)

A) BACE1 inhibitors

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
	TPI 287[265]	University of California, San Francisco	Tau (SmallM)	A tubulin-binding (at beta-tubulin site) acting as microtubule-stabilizer, thus reducing synaptic dysfunction and neuronal loss. [IV]	MD CSF. I (Not recruiting)

E) Immunotherapy (active/passive)

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
Immunotherapy (active)	Affitope AD02 [173,266,267]	AFFiRiS AG	Amyloid (SmallM)	Six amino acids peptides that mimic the N-terminus of Aβ, inducing Th2-type immune response with release of anti-Tau Ab (without prominent Th1 cell activity), with subsequent Fc receptor-mediated phagocytosis and intracellular degradation [IV]	MD n.a. II (Completed)
	AADvac-1	Axon Neuroscience SE	Tau (SmallM)	An Axon peptide 108 conjugated to a KLH and aluminum hydroxide adjuvant. It is a synthetic peptide derived from amino acids 294–305 of the truncated Tau sequence inducing Th2-type immune response with release of anti-Tau Ab (without prominent Th1 cell activity), with subsequent Fc receptor-mediated phagocytosis and intracellular degradation [268] [SC]	MD n.a. I (Completed)
	CAD106 [173]	Novartis Pharmaceuticals Corporation	Amyloid (SmallM)	Multiple copies of the short Aβ1–6 peptide derived from the N-terminal (B cell epitope) of Aβ, inducing Th2-type immune response with release of anti- Aβ-Ab (without prominent Th1 cell activity) binding to Aβ oligomers or fibrils with subsequent Fc receptor-mediated phagocytosis and intracellular degradation [IM]	AaR $\varepsilon 4$: homozygous II-III (Recruiting) MD n.a. II (Completed)
	ACI-24 (Pal1-15 acetate salt)	AC Immune SA	Amyloid (SmallM)	Aβ-liposomal preparation: Antigen Aβ 1–15 anchored on the liposomal surface, by a palmitoylated lysine tandem at each end of the peptide. It elicits Aβ-IgG (without prominent Th1 cell activity) by binding to Aβ oligomers or fibrils with following Fc receptor-mediated phagocytosis and intracellular degradation [269], [SC]	MD n.a. I-II (Recruiting)
	UB-311	United Biomedical	Amyloid (SmallM)	Equimolar mixture of two synthetic peptides, consisting of highly active UBITh® helper T-cell epitopes, coupled to the Aβ1–14 peptide. It elicits Aβ-Ab (without prominent Th1 cell activity) by binding to Aβ oligomers or fibrils with subsequent Fc receptor mediated phagocytosis and intracellular degradation It is a proprietary vaccine delivery system (CpG oligonucleotide [159] [SC]	MD n.a. I (Completed)
	ACI-35	AC Immune SA, Janssen	Tau (SmallM)	Tau-liposomal preparation: a synthetic peptide (16 amino acids) corresponding to human protein tau sequence 393–408, with phosphorylated residues S396 and S404 derivatized with two palmitic acid chains at each terminus to enable integration into liposomes It elicits Tau-Ab without prominent Th1 cell activity [270] [SC]	MD n.a. I-II (Recruiting)
Immunotherapy (passive)[206,271–273]	Aducanumab (BIIB037)	Biogen	Amyloid (monoclonal antibody)	Human monoclonal IgG1; it binds aggregated forms of Aβ at the N-terminus epitope (residues 3–6) (soluble oligomers and insoluble fibrils only) and It promotes Fc-mediated microglial phagocytosis [IV]	MCI/MD aPET/CSF III (Recruiting)

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A) BACE1 inhibitors

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
	Crenezumab (MABT5102A, RG7412)	Hoffmann-La Roche	Amyloid (monoclonal antibody)	Human monoclonal IgG4; it binds oligomeric aggregated forms of Aβ and plaques as well with high affinity, and monomeric Aβ with low affinity, It promotes Fc-mediated microglial phagocytosis [IV]	MCI/MD aPET/CSF III (Recruiting)
	Gantenerumab (RO4909832, RG1450)	Hoffmann-La Roche	Amyloid (monoclonal antibody)	Human conformational monoclonal IgG1; it binds Aβ fibrils encompassing both N-terminal and central amino acids of Aβ and promotes Fc-mediated microglial phagocytosis [SC]	MCI/MD aPET/CSF III (Active, not Recruiting)
	Solanezumab (LY2062430)	Eli Lilly & Co.	Amyloid (monoclonal antibody)	Human monoclonal IgG1; it binds only soluble monomeric Aβ, sequestering and promoting Fc-mediated microglial phagocytosis [IV]	AaR aPET III (Recruiting)
	BAN2401	Biogen, Eisai Co., Ltd.	Amyloid (monoclonal antibody)	Human monoclonal IgG1; it binds selectively to large, soluble Aβ protofibrils and enhances their clearance promoting Fc-mediated microglial phagocytosis [IV]	MCI/MD aPET/CSF II (Active, not recruiting)
	Gamunex	Grifols Biologicals Inc.	Amyloid (monoclonal antibody)	Off-label used human Ig. It binds to several forms of Aβ and promotes Fc-mediated microglial phagocytosis [IV]	MD – II-III (Recruiting)
	AAB-003 (PF-05236812)	Pfizer, Janssen Alzheimer Immunotherapy Research & Development, LLC	Amyloid (monoclonal antibody)	Humanized monoclonal IgG1; it binds to fibrillar and soluble Aβ and promotes Fc-mediated microglial phagocytosis (a derivative of Bapineuzumab) [IV]	MD – I (Completed)
	LY3002813 (N3pG-Aβ mAb)	Eli Lilly & Co.	Amyloid (monoclonal antibody)	Humanized mE8-IgG2a Ab that binds to Aβ (p3-42), a pyroglutamate form of Aβ that is aggregated in plaques. It finally promotes Fc-mediated microglial phagocytosis [IV]	MCI aPET I (Not yet recruiting)
	SAR228810	Sanofi	Amyloid (monoclonal antibody)	Humanized monoclonal IgG1 binds to soluble protofibrillar and fibrillar Aβ and promotes Fc-mediated microglial phagocytosis [IV]	MD – I (Completed)
	MEDI1814	Eli Lilly & Co.	Amyloid (monoclonal antibody)	Human monoclonal IgG1; it binds only to soluble monomeric Aβ, sequestering and removing them [IV]	MD – I (Completed)
	LY3303560	Eli Lilly & Co.	Tau (monoclonal antibody)	Monoclonal Ab inhibiting Tau protein (MoA not disclosed by Sponsor) [SC]	MCI/MD aPET I (Recruiting)

F) Neuroinflammation modulators

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
Neuroinflammation modulators	Sargramostim (Leukine®, GM-CSF)	University of Colorado, Denver	Microglia (SmallM)	Synthetic form of GM-CSF; it might increase phagocytosis of pathogenic protein deposits, activates microglia without increasing microglial release of pro-inflammatory cytokines [SC]	MD aPET II (Active, not recruiting)

A) BACE1 inhibitors

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
	Minocycline	Huntington Medical Research Institutes	Microglia/neurons (SmallM)	Tetracycline derivative capable of crossing the BBB; it modulates the up-regulation of iNOS and COX2 reducing pro-inflammatory response and astrocytes overactivity [274] [Oral]	MCI/MD – II (Completed)
	Neflamapimod (VX-745)	EIP Pharma, LLC	Microglia/neurons (SmallM)	It inhibits p38MAPKα expressed in microglia and neurons. It promotes the shift of microglial activation from a pro-inflammatory to a phagocytic state, thus improving mitochondrial function, reduction of tau hyperphosphorylation, and as a consequence synaptic transmission[275]. [Oral]	MCI/MD – II (Completed)
	Rilapladib (SB-659032)	GlaxoSmithKline (GSK)	Microglia/ macrophages (SmallM)	Potent and selective inhibitor of Lp-PLA2 actively secreted by monocyte-derived macrophages, T lymphocytes, and mast cells. It produces pro-inflammatory factors affecting brain microvascular endothelial cells [276] [Oral]	MCI/MD – II (Completed)
	GC 021109	GliaCure	Microglia (SmallM)	It binds the microglial P2Y6 receptor which activation leads to the synthesis and release of pro-inflammatory cytokines. In addition, purinergic signals are thought to be fundamental in shift of in microglia to an activated phenotype [277]. [Oral]	MD – I (Completed)
	Pioglitazone (AD4833)	Takeda Pharmaceutical Company, Zinfandel Pharmaceuticals Inc.	Microglia/neurons (SmallM)	PPARγ agonists modulate, at a transcriptional level, the microglial response to Aβ plaques deposition increasing Aβ phagocytosis and decreasing cytokine release It blocks the NF-kB-dependent gene expression, thus inhibiting multiple inflammatory pathways Effect on insulin-resistance potentially underling AD PPARγ regulate gene expression by forming heterodimers with RXRs and by binding to a promoterthus reducing BACE1 gene expression [278–280] [Oral]	MCI – III (Recruiting)
	Azeliragon (PF-04494700, TTP488)	Pfizer, TransTech Pharma, Inc., vTv Therapeutics LLC	Microglia/ astrocytes (SmallM)	Inhibitor of RAGE (upregulated in AD astrocytes and microglial cells) that binds to AGEs, Aβ, S100b leading to sustained pro-inflammatory state and contributing to Aβ accumulation and toxicity [281,282] [Oral]	MD – III (Recruiting)
	Masitinib (AB1010)	AB Science	Mast cells/neurons (SmallM)	Inhibition of the Src family Fyn kinase involved in the pathway of tau hyperphosphorylation and tau/Aβ induced toxicity Inhibitor of the c-kit, the proto-oncogene receptor tyrosine kinase. This is a type 3 transmembrane receptor of the mast cell growth factor that contributes to mast cells activation. Thus, the drug preserves BBB and reduces accumulation of pro-inflammatory factors [283] [Oral]	MD – III (Completed)
	Dexpramipexole (R-pramipexole, KNS-760704)	Virginia Commonwealth University	Neurons (SmallM)	The pure R(+) optical enantiomer of the non-ergot dopamine agonist (pramipexole) with low affinity to dopaminergic receptors. It may reduce cell death induced by H2O2 and block mitochondrial permeability transition, thus reducing ROS generation, imbalance of calcium cellular homeostasis. Finally, it can downregulate mitochondrial pathway of the apoptosis cascade [284] [Oral]	MD – II (Completed)
	CHF 5074 [285,286]	CereSpir Incorporated	Microglia/ Neurons (SmallM)	A non-steroidal anti-inflammatory drug that prevents the binding of astrocytic-signaling molecule soluble CD40 ligand to microglia cell surface receptor This in turn, reduce the synthesis and release of TNF-α, IL-1β and iNOS promoting the expression of microglia M2 markers. In addition, it prevents apoptotic pathways by reducing cytochrome c release, NF-κB pathways and caspase-3 activation (neuroprotective	MCI – II (Completed)

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A) BACE1 inhibitors

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
				effect). Previously investigated as γ-secretase modulator [Oral]	

G) Cellular pathway signaling modulators

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
Cellular pathway signaling modulators	Exendin-4 (Exenatide)	National Institute of Aging	Insulin signaling (SmallM)	A protease-resistant GLP-1 analogue that exerts a long acting GLP1 receptor agonism. It induces the sequential activation of an insulin signaling pathway with increased activation of AKT/PI3K and downregulation of GSK-3β activity, thus preventing hyperphosphorylation of tau In addition, the activation of the GLP1 receptor stimulates an adenylyl cyclase and increases cAMP levels, thus enhancing downstream kinases, as PKA, that are related to growth factor signaling [287–289] [Oral]	MCI/MD CSF II (Completed)
	Mitoglitazone (MSDC-0160)	Metabolic Solutions Development Company	Mitochondrial pathways (SmallM)	It modulates mTOT acting as insulin sensitizer improving brain glucose metabolism without PPARγ activation (thus reducing side-effects). In particular, the potential effect is mainly directed to Mpc2 and Mpc1, the mitochondrial pyruvate carrier complex of the mTOT, thus regulating pyruvate entry into the mitochondria [290,291] [Oral]	MD – II (Completed)
	T3D-959 (DB959)	T3D Therapeutics, Inc.	Insulin signaling (SmallM)	Dual PPARδ/γ nuclear receptor agonist, regulating, in neurons, dysfunctional brain glucose, lipid metabolism, gene expression of several molecules involved in Aβ production as BACE1 and pro-survival pathways, thus leading to neuroprotection. It also reduces pro-inflammatory response and microglia activation [292] [Oral]	MD – II (Active, not recruiting)
	Anavex 2–73	Anavex Life Science Corp.	Chaperonine pathways (SmallM)	A mixed ligand for σ_1/muscarinic receptors acting as agonist and enhancing most of their cytoprotective pathways: calcium homeostasis, mitochondrial function and ER stress response. In addition, it modulates the axis AKT/PI3/GSK-3β Downregulation of GSK3 β leads to block tau hyperphosphorylation, thus enhancing ADAM17 expression and reducing BACE1 expression with reduced cellular Aβ toxic monomers load Potential effect of downstream transcription factors activated by GSK3 that are involved in pro-inflammatory response (STAT, NFkB) [293,294] [IV]	MD – II (Active, not recruiting)
	Saracatinib (AZD0530)	AstraZeneca	Src/abl and Fyn kinases pathways (SmallM)	Inhibitor of both the Src family Fyn kinase and Bcr-Abl tyrosine-kinase. The latter participates to several cell signaling most of them converging on transcription factors fundamental for several cytoprotective and cell-growth pathways (as STAT5 and 3). In addition, it is involved in several inflammatory pathways (inducing microglia and pro-inflammatory release) Inhibitor of the Src family Fyn kinase involved in the pathway of tau hyperphosphorylation and tau/Aβ induced toxicity [295,296] Inhibitor of the c-kit, the proto-oncogene receptor tyrosine kinase. This is a type 3 transmembrane receptor of the mast cell growth factor that contributes to mast cells activation [Oral]	MD aPET II (Completed)

A) BACE1 inhibitors

Category	Name (Synonyms)	Company	Target type (Molecule type)	MoA – Biochemical features [Administration route]	Stage ICB Phase
	ABT-957	AbbVie	Calpain pathways (SmallM)	Inhibitor of calpain1, a cytosolic calcium-dependent cysteine protease (overactive in AD neurons) that activates pro-apoptotic pathways mediated by caspases. It also mediates Aβ toxicity by stimulating NMDR signaling cascades and has been both directly and indirectly (via GSK and cdk5) linked to hyperphosphorylation of tau [297,298] [Oral]	MD – II (Terminated)
	CT1812	Cognition Therapeutics Inc.	S2R/PGRMC1 Pathways (SmallM)	Ligand for the S2R/PGRMC1 receptor that counteracts potential perturbed calcium homeostasis causing mitochondrial dysfunction and enhanced apoptotic pathways It blocks the binding of Aβ oligomers to Sig2R/PGRMC1, thus interfering with Aβ calcium-mediated toxicity [299,300] [Oral]	MD – I (Recruiting)
	Vorinostat (suberoylanilide hydroxamic acid, Zolinza)	German Center for Neurodegenerative Diseases	Apoptotic pathways (SmallM)	Epigenetic therapy through Class I HDACs inhibitor, that normalizes, in neurons and microglia, epigenetic and transcriptional activity of HD counteracting apoptotic pathways, mitotic failure and autophagic cell death The overactivation of HDACs is also associated to microglia overactivation, insulin resistance, and sustained epigenetic post-translational modifications of Aβ [301] [Oral]	MD – I (Recruiting)
	S-Equol (Aus-131)	Ausio Pharmaceuticals, LLC	ER- β pathway (SmallM)	S-enantiomeric metabolite of daidzein, a selective agonist of the ER-β (nuclear receptor and member of ligand-regulated transcription factor family expressed on mitochondria). Downregulation of ER-β is associated to mitochondrial dysfunction, insulin resistance, and neuroinflammation (via astrocytes overactivation) [302,303] [Oral]	MD – I (Recruiting)
	LM11A-31-BHS	PharmatrophiX Inc.	p75 neurotrophin pathway (SmallM)	p-75 neurotrophin receptor ligand promotes cell survival signal mimicking the NGF in basal forebrain cholinergic neurons. It prevents Aβ1–40 to bind the receptor exerting neurotoxicity [304] [Oral]	MD CSF I-II (Recruiting)
	Allopregnanolone (3α,5α-tetrahydroprogesterone)	University of Southern California	GABA-A mediated cell signaling (SmallM)	A neurosteroid acting as allosteric positive selective modulator of ionotropic GABA-A receptors potentiating the chloride efflux resulting in depolarization of the plasma membrane followed by a rise in intracellular calcium. This, in turn, leads to the restoring of calcium homeostasis and preserved mitochondrial function with reduction of oxidative stress [305] [IV]	MCI/MD – I (Recruiting)
	BI 409306 (SUB 166499)	Boehringer Ingelheim	cGMP/NO signaling (SmallM)	Phosphodisterase9A inhibitor increasing brain levels of cGMP and NO. NO is a key molecule in anti-apoptotic/pro-survival signaling. Reduced brain cGMP levels, both basal and NMDA-coupled, are associated to AD [306] [Oral]	MD – II (Completed)
Neurotransmission modulators	Ladostigil Hemitartrate (TV-3326)	Avraham Pharmaceuticals Ltd	Acetylcholine/ IMAO A/B (SmallM)	Multi target neuroprotective with both acetylcholinesterase and brain MAO A/B inhibitor activity. The latter prevents neuronal loss by preventing mitochondria-related oxidative stress and potentiating anti-apoptotic factors like BCl2 Potential effect on the gene expression of antioxidant scavengers [307] [Oral]	MCI/MD – II (Completed)
	ORM-12741	Orion Pharma	Noradrenaline (SmallM)	Highly selective α2C-AR antagonist, a member of the GPCRs superfamily. It counteracts the presynaptic effects of the activated receptor: inhibiting the synthesis and release of NA and other amine). It counteracts the physiological event of inhibition of cAMP-	MD – II (Completed)

A) BACE1 inhibitors					
Category	**Name (Synonyms)**	**Company**	**Target type (Molecule type)**	**MoA – Biochemical features [Administration route]**	**Stage ICB Phase**
				dependent closing of the voltage-gated calcium channels, and activation of MAPK signaling cascades (which leads to tau hyperphosphorylation and amyloidogenic cellular pathways) [308] [Oral]	
	RVT-101 (SB-742457, interpiridine)	Axovant Sciences Ltd.	Serotonin (SmallM)	Selective 5-HT6 receptor antagonist. Blockade of these receptors leads to increased cholinergic firing. In addition, 5-HT6 receptors are GPCRs that positively stimulate adenylate cyclase activity, finally activating the ERK1/2 via a Fyn-dependent pathway. This, in turn, could reduce hyperphophorilated levels of tau [309–311] [Oral]	MD – III (Completed)
	SUVN-502	Suven Life Sciences Ltd	Serotonin (SmallM)	Selective 5-HT6 receptor antagonist. Blockade of these receptors leads to increased cholinergic firing. In addition, 5-HT6 receptors are GPCRs that positively stimulate adenylate cyclase activity, finally activating the ERK1/2 via a Fyn-dependent pathway. This, in turn, could reduce hyperphophorilated levels of tau [309,312] [Oral]	MD – II (Recruiting)
	SAR110894D	Sanofi	Histamine (SmallM)	Selective H3R antagonist increasing the presynaptic release of both histamine and other neurotransmitters, including acetylcholine [Oral]	MD – III (Completed)
	NorAD	Imperial College London	Noradrenaline (SmallM)	Extended-Release Guanfacine acting as postsynaptic ARs agonist modulating neuronal excitability via regulation of ion channels, including the direct modulation of Inwardly Rectifying Potassium Channels and the indirect modulation of Hyperpolarization-Activated Channels [313] [Oral]	MD – III (Not yet recruiting)

*At least heterozygosity

Abbreviations;: MoA, mechanism of action; SmallM: small molecule; ICB: inclusion criteria biomarkers; BACE1: β-site amyloid precursor protein cleaving enzyme; APP: amyloid precursor protein; BBB: blood brain barrier; AD: Alzheimer's disease; MCI: mild cognitive impairment; MD: mild AD dementia at least; AaR: asymptomatic at risk; *ε4*: apolipoprotein E *ε4* carrier; RAR/RXR: retinoid acid receptor/retinoid X receptor; ADAM-10: A disintegrin and metalloproteinase domain containing protein 10;APPs-α: soluble alfa fragment of amyloid precursor protein; PDE4: phosphodiesterase 4; cGMP: cyclic guanosine monophosphate; pCREB: phosphorylated cAMP response-element binding protein; BDNF: brain-derived neurotrophic factor; Zn: zinc; Cu: copper; Fe: ferrum; g3p: two domain fragment of the phage capsid protein; GAIM: general amyloid interaction motif; IgG: immunoglobulin G; AChEi: inhibitor of acetylcholinesterase; PPARγ: peroxisome proliferator-activated receptor γ; LXRs: liver X receptors; IV: intravenous; SC: subcutaneous; IM: intramuscular; IC: intrathecally; α-sin: α-sinuclein; IRP1: iron regulatory protein 1; ASOs: anti-sense oligonucleotides; KLH: keyhole limpet hemocyanin; P3K: phosphatidylinositol 3-kinase; GSK3β: glycogen synthase kinase-3 β; Ab: antibodies; mAb: monoclonal antibodies; 4-R tau: four microtubule binding repeats tau protein; iNOS: inducible nitric oxide synthase; COX2: cyclooxygenase-2; p38 MAPKα: the alpha isoform mitogen-activated serine/threonine protein kinase p38; Lp-PLA2: lipoprotein-associated phospholipase A2; P2Y6A: nucleotide purine metabotropic receptor; AGEs: advanced glycation end-products; RAGE: cell-surface receptor of the immunoglobulin superfamily; GLP1: glucagon-like peptide-1; mTOT: mitochondrial target of insulin sensitizers; AKT: proteine kinase B; GSK-3β: glycogen synthase kinase-3β; σ_1/Musc: sigma1/muscarinic receptors; STAT: Signal Transducer and Activator of Transcription; cdk5: cyclin-dipendent kinase5; S2R/PGRMC1: ligand for the sigma2 receptor, also known as the progesterone receptor membrane component 1; HDACs: histone deacetylases; ER-β: estrogen receptor-β; TNF-α: cytokine tumor necrosis factor alpha; GDNF: glial cell line-derived neurotrophic factor; NGF: nerve growth factor; GABA-A: inhibitory γ-aminobutyric acid; 5-HT: serotonin; GPCRs: protein-coupled receptors; pERK1/2: phosphor-extracellular signal-regulated kinase1/2; cAMP: adenylyl cyclase; VGCC: voltage-gated calcium channels; NMDA: N-methyl-D-aspartate; IMAO A/B: brain monoamine oxidase A/B inhibitor; ARs: α2c adrenergic receptor; NA: Noradrenaline; H3R: histamine 3 receptor; ROS reactive oxygen species; NO: nitric oxide; IRKC: inwardly rectifying potassium channels; HAC hyperpolarization-activated channels.

Table 2

Evolving lexicon and terminology of the Alzheimer Precision Medicine Initiative (APMI) paradigm.

Concept	Abbreviation	Definition
Biomarkers	BMs	A defined characteristic that is measured as an indicator of normal biological processes, pathogenic process, or response to an exposure or intervention, including therapeutic interventions. Molecular, histologic, radiographic, or physiological characteristics are types of biomarkers. A biomarker is not an assessment of how an individual feel, functions or survives. Categories of biomarkers include: susceptibility/risk biomarker, diagnostic biomarker, monitoring biomarker, prognostic biomarker, predictive biomarker, pharmacodynamics/response biomarker, and safety biomarker.
"Omics" or "Omic" disciplines		Exploratory high-throughput screening tools aimed at fully collecting, characterizing and quantifying comprehensive pools of biological molecules (DNA sequences, transcripts, miRNAs, proteins/peptides, metabolites/lipids) that relate to structure, function, metabolism and dynamics of an organism and/or whole organisms.
Stratified medicine		Medical model that uses the grouping of patients according to underlying biological mechanisms, biomarker-guidance, disease risk or likely treatment response, as established by diagnostic tests, to determine the course of care. Stratified medicine is a component of personalized medicine.
Precision Medicine	PM	Translational science paradigm related to both health and disease. PM is a biomarker-guided targeted medicine on systems-levels taking into account methodological advancements and discoveries of the comprehensive pathophysiological profiles of complex polygenic, multi-factorial neurodegenerative diseases (proteinopathies of the brain). It aims at optimizing the effectiveness of disease prevention and therapy, by considering (customized) an individual's specific "biological make-up" (e.g. genetic, biochemical, phenotypic, lifestyle, and psychosocial characteristics) for targeted interventions through P4M implementation.
Pathway-based therapy		A treatment developed following the systematic analysis of specific genes, their functions, and the interactions among them in relation to a specific complex disease. By using reliable exploratory strategies (i.e. GWAS, proteomics, and microarrays), the comprehensive understanding of the molecular mechanisms underlying complex diseases is realistic.
Systems Biology	SB	Evolving hypothesis-free, exploratory, holistic (non-reductionistic), global, integrative, and interdisciplinary paradigm using advances in multimodal high-throughput technological platforms that enable the examination of networks of biological pathways where elevated amounts of structurally and functionally different molecules are simultaneously explored over time at a system level (i.e., at the level of molecules and subcellular compartments, cells, group of cells, tissues, organs, apparatuses, or even whole organisms). According to systems biology, organisms are made of systems which are entities consisting in hierarchically self-organized levels with increasing structural complexity resulting in different emerging properties.
Systems Pharmacology	SP	Science of advancing knowledge about drug action at the molecular, cellular, tissue, organ, organism, and population levels" (available at http://www.aaps.org/Systems_Pharmacology/).
Precision Pharmacology	PP	Conceptual paradigm combining traditional data analyses as pharmacodynamic and pharmacokinetic data within the System biology approach. It encompasses the acquisition and integration of omics data operating at both experimental and computational level. Precision pharmacology aims at exploring and predicting the whole effect of a molecular mechanism of action at different systems levels.
Self-Organization	SO	Spontaneous (self-generated) interactions among components of an initially chaotic basis. Such a process may be triggered by random fluctuations that generate casual processes which are amplified by positive feedback. The result of self-organization is an entity of an increased structural-functional order that acquired the ability to survive the environment and self-repair after perturbation. Therefore, self-organization in organisms at each level is a key phenomenon for survival and evolutionary transitions. Self-organization, in biology, can be observed in protein folding, creation of lipid bilayer membranes, cell/tissue/organs genesis and development.
Network		Set of recurring motifs; each motif is a pathway; each pathway, in turn, carries out specific dynamical functions and can be modulated, i.e. up-down regulated, either upstream or downstream or both. There is a large *spectrum* of biological cross-talks between pathways and networks inside a level of a given system and among systems.
Feedback		Condition in which a component of a molecular pathway either activates or inhibits its own upstream regulators.
Cross-talk		Condition in which one or more signaling transductions of a pathway directly or indirectly affect one or more signaling transductions of other pathways. A component of one pathway can either positively and negatively modulate (cross-activation and cross-inhibition, respectively) an upstream component of another pathway, thus modulating the biological output.

Concept	Abbreviation	Definition
Signal-transduction cascades (pathway)		Circuit of interactions among molecular bioprocesses (molecular circuit) able to detect, amplify, and integrate different signals.
Emerging properties		Hierarchically self-organized levels with a certain degree of structural complexity that exhibit properties that levels with lower complexity do not show.
Homeostasis		It consists in a spontaneous tendency towards a condition of a dynamic *equilibrium* based on a continuous counterbalance between regulatory-defense mechanisms and disrupting stress-induced signals. Homeostasis is common to any biological system. Homeostatic signaling is hierarchically organized from subcellular to cellular level, across organs, and, finally, systems. Homeostasis is essential for protecting all core biosynthetic processes necessary to optimal functioning and survival.
Adaptation		Biological output arising from multi-level anti-stress response, generating advantageous morpho-functional alterations in cells and higher levels inside a system. Adaptation is essential for coping environmental stressful challenges aimed to prevent systems damage and finally promoting survival.
Compensation		Protective process in which a morpho-functional alteration is counterbalanced by another morpho-functional alteration without any change in biological output, thus preserving system homeostasis. Compensatory mechanisms are hierarchically organized through systems levels and aim at preserving the homeostasis under pathophysiological conditions. Example of compensation: myofribillar cardiac remodeling; cell surface receptor profiles in the immune system; membrane ionic channel in neurons.
Decompensation (Failure)		Breakdown or lack of reverse of one or more compensatory mechanisms finally resulting in maladaptive morpho-functional alterations. This, in turn, reflects a homeostatic imbalance at different levels of complexity in systems and organ systems.

Table 3

Summary of clinical trial construct to conduct trials leading to PM-based interventions.

PM	Corresponding Precision Clinical Trial Features
Right Drug	■ Target biologic known
	■ Druggable aspect of pathology identified
	■ Drug or drug/combination reflects single or multiple pathologies and stage of the process
	■ Biomarker guides drug choice
Right Dose	■ Maximum tolerated dose determined
	■ Dose-response curve constructed
	■ Multiple doses tested
	■ Doses related to disease severity
Right Patient	■ Phase of disorder ranging from preclinical without genetic/or amyloid risk factors, preclinical with genetic/amyloid risk factors, prodromal AD, AD dementia
	■ Pathology and drug target defined by biomarkers
	■ Pharmacogenetic profile of patient determined to anticipate rate of metabolism, drug-drug interactions, drug-disease interactions, and side effects
	■ Socio-demographic features of individual included in planning including age, sex, caregiver status, comorbid conditions, general health, concomitant medications, etc.
	■ Analysis of individual responses or response patterns of small groups of biologically well-defined individuals

Abbreviations: AD, Alzheimer's disease; PM, Precision Medicine.