Healthy elderly in clinical trials: how to define preclinical Alzheimer's Disease for clinical trial participation

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CHAPTER 1

Introduction

AGEING

Ageing is the process of becoming older and is an inevitable process that entails a wide variety of molecular and cellular damage over time.[1] The World Health Organization (WHO) expects 1 in 6 people in the world to be over the age of 60 by 2030. In industrialized countries, the elderly people represent the fastest growing group in the age pyramid but low- and middle income countries are following quickly.[2] Therefore, clinical research related to ageing in the elderly is important. Not only for elderly to age in a comfortable way, but also to reduce the burden on our healthcare systems. During ageing, many changes take place in the brain. Apart from neuronal cell death and brain atrophy, there are functional changes in the brain due to changes in neurotransmitter and hormone levels.[3] The ability of humans to acquire knowledge, understanding through, experience and senses is what makes us humans superior to animals. This ability is called cognitive functioning or in short, cognition.[4] Cognition depends on different brain areas to work together and combine external input (e.g., sounds, visual stimuli, touch) which may all be affected during normal aging. The cholinergic system, which is involved in memory function, consisting of cholinergic neurons in the nucleus basalis of Meynert, frontal cortex, anterior cingulate cortex, and posterior cingulate cortex, has been assumed to moderately degenerate during normal ageing of the brain.[5,6] However, cholinergic dysfunction has also been associated with several other neurodegenerative disease e.g., Alzheimer Disease (AD), Parkinson's Disease (PD) and Huntington's Disease (HD).[7] The process of ageing can be difficult to differentiate from a neurological condition as some of the processes of normal brain ageing can also be the preliminary stage of a neurological disease.[8] Usually, difficulties in performing normal daily life tasks are the first signs of change in functioning of the brain and a reason for someone to further examine if this decrease in cognitive performance is due to normal age-related decline in brain function or due to a neurological disorder.

NEURODEGENERATION

When a person ages, cognitive abilities decline, however, there is a difference in normal decline of functioning due to age and decline due to progressive loss of neurons in the context of a neurodegenerative disorder. Neurodegeneration is the progressive process of loss of structure or function of neurons, which may ultimately lead to cell death or apoptosis.[9] Accumulation or misfolding of proteins in the brain, gliosis, synaptic dysfunction, microglial activation, and inflammation

are common pathologies in neurodegenerative disorders leading to apoptosis and necrosis. These processes are involved in many neurodegenerative diseases such as AD, PD and HD, which all have a different typical age of disease onset and course of the disease.[9-11] In Alzheimer's disease, neurofibrillary tangles containing phosphorylated tau and plaques consisting of amyloid peptides are observed. In Parkinson's disease, the synaptic peptide α-synuclein aggregates as Lewy bodies in the dopaminergic neurons within the substantia nigra. In Huntington's disease, the polyglutamine protein huntingtin is present in intranuclear inclusions.[12] In most neurodegenerative diseases, the first symptoms typically appear after middle age (65 years old) and increase over time. This is also the case in AD and PD. Patients with HD have a younger average age of disease onset, between 35-45 years old.[13] Where AD is characterized by episodic memory loss in the early stages of the disease, PD is characterized by movement disorders e.g., slower movements, rather than cognitive problems, which develop in the majority of patients later in the disease process.[14] HD, which is always a hereditary genetic neurodegenerative disorder, presents with mood swings and depressive feelings at the early stage of the disease followed by movements disorders and cognitive complaints.[15] These are some examples of neurodegenerative diseases with a different pathophysiology and age of disease onset but all with cognitive decline in common. The profile of cognitive symptoms, however, differs between these diseases. Patients with AD are usually more impaired in memory functioning, while patients with PD experience more difficulties with initiation of cognitive processes.[16] In HD, cognitive decline can appear before any motor symptoms but can also be mild in advanced stages of the disease.[15] Measuring cognitive functions over time can help to determine if an elderly person is experiencing normal age-related cognitive decline or cognitive decline due to a neurodegenerative disease. As the cognitive symptoms in neurodegenerative diseases can vary in time of onset, affected cognitive function, and severity of the cognitive deficit, measuring cognitive function over time is important. This is especially so in the preclinical stage of disease where no formal diagnosis has yet been established

There are many ways of measuring cognitive functions. Overall, cognition can be divided in domains of cognitive functioning, which include sensation, perception, motor skills and construction, attention and concentration, memory, executive functioning, processing speed and language or verbal skills.[17] Different brain areas are involved in these different functions. The brain regions with major involvement in neurodegeneration are the (pre)frontal lobe for attention and behavior inhibition, the temporal and parietal lobe for e.g., language, speech and memory, the cerebellum for regulation of movement and

the occipital lobe for processing of visual stimuli.[4] Measuring cognitive brain functions can be done by neuropsychological assessments, which are traditionally performed as paper-and-pencil tasks but increasingly in computerized form since the digitalization of tests in the past decades.[18] Neuropsychological testing can be used to assess the presence or absence of cognitive dysfunction, help establish a diagnosis or to help clarify the cognitive effects of neurodegenerative diseases.[19] For most of the neuropsychological tests, normative data are available to make a distinction between normal cognitive performance of a subject and when cognitive functioning can be considered abnormal. Also, confounding factors such as age, sex and education level are taken into account to further differentiate if a subject has for instance normal age-related cognitive decline or abnormal cognitive functioning.[4] At the Centre for Human Drug Research (CHDR), a computerized neuropsychological and neurophysiological test battery was developed, the NeuroCart, for the purpose of systematically studying the effects of drugs on central nervous system (CNS) functioning in the context of early phase clinical drug studies.[20] This test battery makes neuropsychological and neurophysiological test performance less time-consuming, less prone to interrater variability, and most importantly sensitive to detect pharmacological effects with minimal learning effects. Also, it is electronically available for standardized test performance in subjects.[21]

ALZHEIMER'S DISEASE

The World Health Organization (WHO) estimates the worldwide prevalence of dementia to be approximately 50 million people (in 2020).[22] Dementia is a general term for the loss of memory, language, problem solving abilities, in other words decline in cognitive functioning that is severe enough to interfere with daily life. The most common form of dementia, which represents approximately 70% of the dementia cases, is Alzheimer's Disease (AD).[23] In 1906, the German psychiatrist and neuropathologist Alois Alzheimer first discovered the typical pathological brain alterations after studying the brain of a psychiatric patient postmortem. He found amyloid plaques surrounding cells in the brain and neurofibrillary tangles inside the cells.[24] Since then, much research has been performed focused on the role of amyloid in AD. The amyloid cascade hypothesis states that AD is caused by abnormal accumulation of the amyloid beta (Aβ) protein in the brain causing plaques of this protein in various areas of the brain.[25] The Aβ peptides 39-43 are formed through sequential enzymatic cleavage by β-secretase and γ-secretase of the amyloid precursor protein (APP).[26] $A\beta_{1-40}$ is the most prevalent amyloid

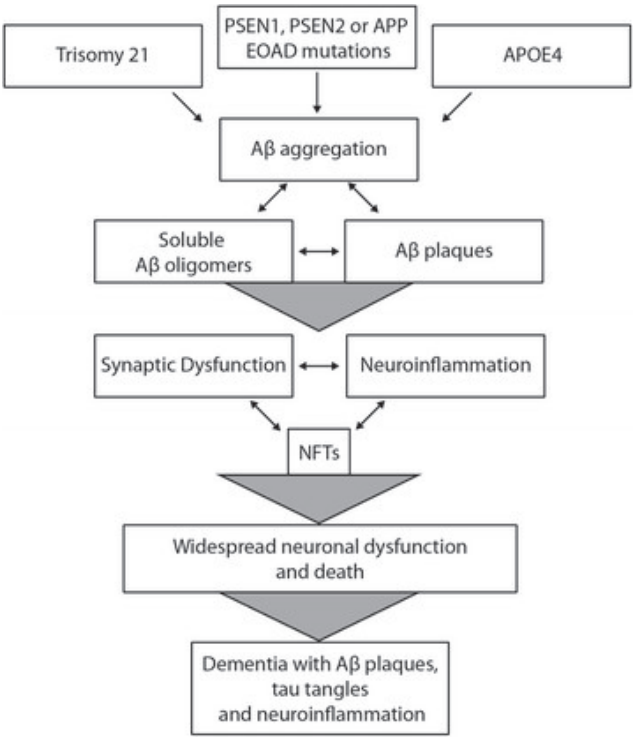
peptide in AD followed by $A\beta_{1-42}$, which aggregates faster in AD and is therefore intensively studied.[27,28] The Aβ peptides have the tendency to polymerize into toxic oligomers, which have been correlated with severity of dementia.[29] The Aβ plaque formation, in turn, leads to local microglia activation, cytokine release and astrocyte activation.[30] This toxic and inflammatory response leads to synaptic loss, neuronal loss, and gross cerebral atrophy, which in the end leads to clinical AD and to plaque and tangle pathology, as summarized in figure 1.[31]

Neurofibrillary tangles, which were similarly already described by Alois Alzheimer, also play a role in the development of AD and consist of misfolded hyperphosphorylated tau proteins. Normal, non-hyperphosphorylated tau proteins are mainly found inside neurons and play an important role in stabilization of the neuronal microtubules network.[32] Six different isoforms of tau have been identified in the adult brain. The process of hyperphosphorylation of tau causes disturbance in structural and regulatory function of the cytoskeleton, which is usually protected by the tau protein. The hyperphosphorylated tau proteins turn into neurofibrillary tangles inside the neurons and affect the normal cellular function and can lead to synaptic dysfunction and neurodegeneration.[33]

Genetics play a role in the chance of a person's development of AD and influence the progression rate. Autosomal dominant mutations of APP, presenilin1 (PSEN1) and presenilin1 (PSEN2) genes, both subunits of the γ-secretase causing an increase in $A\beta_{1-42}$ protein, cause early-onset familial AD (age <65 years old). The apolipoprotein E (ApoE) gene, specifically the ApoE ε4 allele, is a genetic risk factor for the typical late-onset (age >65 years old) variant of AD.[34,35]

AD is characterized by cognitive decline, specifically memory deficits which are not explained by age related decline. Up to 20 years before clinical onset of AD, the biological changes in Aβ plaques and tau tangles have already been observed in cerebrospinal fluid (CSF) of otherwise healthy elderly.[37] These changes may predict if a person will develop AD later in life. Especially when already experiencing subjective cognitive symptoms, 40-60% of these subjects are expected to convert to AD.[38-40] When a healthy subject with no cognitive complaints has a lowered CSF protein Aβ42 level, comparable with AD, this subject is considered to have preclinical AD according to the NIA-AA standards from 2011.[41] As having lowered CSF levels of Aβ42 does not per definition lead to developing AD, Dubois et al., (2016) recommend to use the term preclinical AD when an otherwise healthy subject has both Aβ and tau markers (CSF or PET) beyond pathological thresholds.[42] As PET and CSF are not commonly available for the qualification of a subject in the preclinical phase, the NIA-AA standards are used throughout this thesis.

Figure 1 The amyloid cascade by Morris et al., (2014).[36]



BIOMARKERS

A 'biological marker', or the portmanteau 'biomarker', is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.[43] A biomarker can be fluid (blood, CSF), imaging (magnetic resonance imaging; MRI, positron emission tomography; PET), or can be a functional measurement such as a neuropsychological test. Biomarkers can be used for various purposes. A diagnostic biomarker detects or confirms the presence of a disease but can sometimes also be used to monitor a disease. A pharmacodynamic biomarker responds to exposure to a drug. Biomarkers may also be predictive in

the sense that they may predict a subject's response to a drug. Prognostic biomarkers are used to, as the name implies, give a prognosis on the likelihood that a certain response (or disease) will occur and are useful in longitudinal clinical trials.[44] To create consensus on the use of biomarkers and the terminology, a joint task force was formed by the U.S. Food and Drug Administration (FDA) and National Institutes of Health (NIH) and created the Biomarkers EndpointS and other Tools (BEST) resource which is publicly available.[44,45]

Many different biomarkers have been associated with AD. Aβ42, the misfolded protein mainly involved in developing AD, can be measured in CSF where *lower* concentration levels are counter-intuitively associated with a higher amount of Aβ42 plaques in the brain.[46,47] The CSF Aβ42/Aβ40 ratio and CSF (p)tau/Aβ42 ratio are also considered reliable markers for either research-related diagnostics or as prognostic biomarkers.[48,49] CSF Aβ42/Aβ40 ratio has a high concordance with evidence of amyloid accumulation in the brain visualized using amyloid PET imaging.[50] In PET imaging three amyloid tracers have been approved by both the FDA and the EMA for Aβ plaque imaging; ([18F]florbetapir, [18F]flutemetamol, and [18F]florbetaben).[51] Given that CSF sampling is an invasive procedure and PET is costly and not commonly available, many have worked on finding blood-based biomarkers that can be used in the diagnosis or staging of AD. Aβ42 can be measured in blood plasma and several relatively recent studies have shown high agreement with CSF Aβ42/Aβ40 ratio and amyloid PET measurements.[52,53] Further research is needed to confirm these results and also to evaluate the course over time of Aβ42 plasma concentrations in subjects developing AD.

Neurofibrillary tangles are not specific for AD.[51] Tau protein is measurable in CSF with higher concentrations in AD patients compared to healthy controls. Higher concentrations of CSF tau have been correlated with greater cognitive impairment in preclinical and clinical AD.[54] In patients with AD, several hyperphosphorylated tau (P-TAU) isoforms can be measured in CSF, likely as a neuronal response to Aβ exposure. Therefore, an elevated CSF total tau and P-TAU concentration may be regarded as related to AD-type neurodegeneration. The only tau PET tracer approved by the FDA is 18F-Flortaucipir, which can measure the cortical tau burden in the brain.[55] Multiple other tracers are in development for measuring tau in the brain using PET. The possibility to measure P-TAU isoforms in plasma also appears to yield promising biomarkers. P-TAU181 has been reported to correlate with Aβ and tau PET and in longitudinal studies plasma P-TAU181 changed significantly before plasma Aβ, which could mean that plasma P-TAU may be used for diagnostic purposes as well as for early disease staging.[56,57]
Several biomarkers related to inflammatory and astroglial activation have been

found to be associated with AD. YKL-40 (also known as chitinase-3-like protein-1 [CHI3L1]) is a glycoprotein, which is mainly expressed in astrocytes. YKL-40 is a marker for inflammatory processes and activated astrocytes, which can be measured in CSF and in plasma and has been reported to be increased in AD compared to healthy controls.[58] Another protein that plays a role in the inflammatory response to Aβ, the soluble variant of triggering receptor expressed on myeloid cell 2 (STREM2), can be detected in CSF and can function as a biomarker for inflammation in AD. STREMS2 has been reported to increase at different stages of AD and is associated with tau pathology.[59] Glial fibrillary acidic protein (GFAP) is a marker for astrogliosis and has been reported to be increased in CSF of patients with AD and post mortem in brains of patients with AD. GFAP can also be measured in plasma and was found to be associated with brain Aβ pathology but not tau aggregation in patients with AD and in cognitively normal subjects.[60] Promising CSF biomarkers for AD related to synaptic dysfunction are neurogranin (NG), which is a post-synaptic protein and marker for synaptic loss, and neurofilament light (NFL) a marker for axonal damage.[61] None of these inflammatory or neuronal damage markers (YKL-40, STREM32, GFAP, NG, NFL) are specific for AD and elevated concentrations should be evaluated in combination with CSF and/or plasma Aβ and tau concentrations.

CSF concentrations of Aβ42, Aβ42/ Aβ40 ratio, P-TAU and total tau are considered reliable diagnostic biomarkers for AD, as are amyloid and tau PET and T1-weighted imaging MRI for overall atrophy of the brain, although all of these biomarkers are primarily used for research purposes. Other biomarkers should, at this stage, still be seen as experimental.[48] As most disease modifying therapies target the accumulation of misfolded Aβ and P-TAU proteins, or the inflammatory response to them, the classic AD biomarkers can also be regarded as potential pharmacodynamic biomarkers. CSF P-TAU may also be a prognostic biomarker as it is measurable before onset of accumulation of Aβ plaques and can predict future disease progression.[62]

Before the introduction of the above mentioned diagnostic biomarkers, a definite diagnosis of AD was only possible through autopsy of the brain to determine brain atrophy and presence of Aβ plaques and neurofibrillary tangles.[63] Fortunately, it is becoming clear that a clinical diagnosis based on neuropsychological testing combined with positive AD biomarkers corresponds highly with a diagnosis made through brain autopsy.[64] Use of CSF Aβ42 and PET Aβ as diagnostic biomarkers are now encouraged as supportive tool for the diagnosis of AD but are not yet formally approved by regulatory authorities.[62] PET and CSF sampling might not be available to all, and the NIA-Alzheimer's Association (NIA-AA) workgroups

therefore decided not to include these biomarkers as official diagnostic criteria in the last revision of the AD diagnostic criteria in 2011.[41,65] As the NIA-AA guidelines of 2011, and in particular the updated guidelines from NIA-AA in 2018, point out, biomarkers are important in biologically defining the presence of AD in humans and the use of CSF and PET biomarkers are accepted in the research framework for AD and therefore widely used in clinical trials.[66] Also, the definition of preclinical AD has been adjusted to include the use of biomarkers.[66] When a subject has no cognitive complaints but does have CSF Aβ42 levels consistent with AD, this subject is in the preclinical phase of AD, as mentioned previously.

CLINICAL TRIALS

Since the discovery of amyloid beta plaques and neurofibrillary tau tangles in the brain approximately 100 years ago, many clinical drug trials have been performed. The development of new drugs can be divided into different phases before a new drug is approved and enters the market. The first step is drug discovery, in which new technologies, new molecular compounds or existing treatments are evaluated for their potential as a medical treatment. Preclinical research follows where new compounds are being tested in vitro (in a test tube) and in vivo, in animal models to determine the pharmacological characteristics, and to determine the safety and toxicity. After extensive testing in preclinical stage, the successful new compound reaches the clinical stage of drug development, in which a compound is first administered to humans. Clinical drug research follows three phases. Phase 1 is the phase in which a compound is administered for the first time to humans and is mostly aimed at exploring the safety, tolerability, pharmacokinetics and pharmacodynamic effects of the compound. These studies are mostly performed in healthy, usually younger adult, people. Approximately 70% of all drugs move to phase 2 where the efficacy and side effects are studied in the target population. Phase 3 follows for approximately 33% of the drugs tested in phase 2. Phase 3 studies are performed in the target patient population and usually take several years, which is considerably longer than the several months needed for a typical phase 1 study. Studies in phase 2 often take several months to years to complete. Phase 3 studies are confirmatory, after phase 2 studies have already shown positive results in the target population. Phase 3 investigates the clinical efficacy of a compound in patients and monitors for adverse events. Studies in phase 3 include hundreds or thousands of patients and target to demonstrate clinical efficacy. Randomized (placebo-)controlled trials (RCT) are the preferred way to perform clinical drug studies. An RCT reduces bias and provides a rigorous tool to

examine cause-effect relationships between an intervention and outcome. This is because randomization balances participant characteristics between groups allowing attribution of any differences in outcome to the study intervention.[67] Clinical drug trials are importantly influenced by the choice of the study population as this can greatly influence the study outcome.

As the number of AD patients worldwide continues to grow, there is a huge need for disease modifying drugs. A disease modifying drug is a treatment that affects the underlying pathophysiology of the disease, in this case AD, and slows the progression of disease.[68]Despite more than a hundred phase 3 clinical trials, only one (possibly) disease modifying compound has been approved by the FDA.[69]

There may be several reasons why most clinical trials with potential disease modifying compounds in AD have so far yielded negative results. Many different pathophysiological changes play a role in the development of AD, from Aβ plaques in the brain to inflammation, glia activation and phosphorylation of tau. Targeting just one of those biomarkers may not influence the development or progression of AD sufficiently to slow disease progression. Also, not all patients with AD have the same alterations in AD related biomarkers. Better understanding of these AD related biomarkers and the complexity of the interaction between these biomarkers are needed. Combined therapy which does not focus on one single pathophysiological mechanism and therefore not one biomarker might lead to a better clinical trial outcome. As patients with AD are usually an older population with significant comorbidities, the interaction of these comorbidities with AD should be better understood. Dose selection may lead to different clinical results in patients with interacting comorbidities. The timing of the initiation of treatment in patients with AD is also of great importance. Starting treatment in AD patients with irreversible neurological damage may lead to a negative clinical trial even though the compound demonstrated positive results in an earlier phase of the disease at which less structural damage is observed.[70,71] The correct selection of trial participants is of great importance, incorporating all the above-mentioned reasons for trail failure. Pathophysiological changes such as Aβ formation and tau aggregation are already present and measurable up to 20 years before clinical disease onset and early intervention might prevent or delay a subject to become clinical.[37] Drug development in AD is shifting its attention from performing trials in patients with clinically overt AD to subjects in the preclinical phase, prior to widespread brain damage and clinical symptoms of the disease have occurred, in the hope that this will lead to positive results.[72,73]

143 agents are currently in development for AD (2022) according to the clintrials.

gov website. Mechanisms of action range from anti-inflammatory agents to the classic acetylcholinesterase inhibitors, which lead to symptomatic improvement of cognitive dysfunction, but most of them are disease modifying treatments (83.2%). The disease modifying treatments (DMT) can be divided into biologicals and small molecules. Biologicals are generally derived from living organisms and include antibodies, vaccines, antisense oligonucleotides (ASOs), and therapeutic proteins. The term small molecules refer to drugs typically taken orally that are <500 Daltons in size and can regulate a biological process.[74] In 2022, 14 trials include subjects with preclinical AD which is slightly more than in 2021 (which in turn was more than in 2020 [8 versus 4 trials]-2021). Among the phase 2 trials 7 studies recruited subjects with preclinical AD and among phase 3 trials, another 7 studies recruited subjects with preclinical AD. Table 1 gives an overview of the DMTs in development in the three clinical phases with their mechanism of action as described by Cummings et al., (2022).[74]

In June 2021 the first drug aimed at disease modification was approved for the treatment of AD. This may change the Alzheimer's Disease research field completely. Aducanumab, or Aduhelm®, promises to remove amyloid plaques from the brain that have accumulated due to AD disease process. Aducanumab is a human monoclonal antibody that selectively binds to Aβ aggregates, including soluble oligomers and insoluble fibrils and removes Aβ plaques in the brain, which has been demonstrated using florbetapir PET.[75] Two large phase 3 clinical trials, however, resulted in inconclusive results: after 18 months of treatment with aducanumab there were no reproducible clinical benefits and there was no correlation between the degree of amyloid lowering and the main clinical outcome measures.[76] The advisory board to the FDA consisting of experts in the field advised against approval of aducanumab as the studies did not prove clinical efficacy of aducanumab in the treatment of AD. Nevertheless, the FDA approved the drug in the United States of America through the 'acceleration pathway' which allows approval of drugs that do not (yet) show clinical benefit but do show effects at a biomarker level, in this case lowering of amyloid in the brain measured by PET scan. When a disease is deemed 'serious or life-threatening and a drug may provide meaningful therapeutic benefit over existing treatments by having demonstrated to influence a surrogate endpoint that is reasonably likely to predict a clinical benefit to patients the acceleration pathway can be used to approve a drug, awaiting long term clinical trial results, even when there remains some uncertainty about the drug's clinical benefit.[77] The decision of the FDA to approve the drug is considered highly debatable, as the advice of the advisory board was in this case not followed. Several members of the advisory board resigned

after this decision. The actual clinical benefit of aducanumab clearly must still be proven. If aducanumab proves not only to reduce amyloid plaques in the brain but also that long term use and removal of these plaques results in clinical benefit for AD patients, this may be considered as proof of the 'amyloid hypothesis', which states that misfolding and aggregation of beta amyloid is the primary cause of AD. Future research will then more likely target amyloid in an earlier stage to prevent the development of AD. As no drug has proven that targeting amyloid in the brain leads to clinical improvement, the amyloid hypothesis has been under discussion and it is debated if removing amyloid is the best way to move forward in AD research, causing disunity in the AD research field.

Very important for further development of aducanumab but also for other new disease modifying compounds for the treatment of AD, is correct study subject selection. For aducanumab, the discussion related to which study subjects best to enroll in further clinical trials is already ongoing. Initially the FDA approved aducanumab for all patients with AD, but they now adjusted the approval by restricting the label to patients with mild cognitive impairment or mild AD, in which the drug was also tested in the phase 3 clinical trials. The advice of the FDA to start treatment in the MCI phase or prodromal phase of AD in which only mild symptoms are present and with less severe disease pathology, is based on the expectation that treatment at these stages will lead to greater clinical benefit. Again, the importance of proper characterization of clinical trial subjects (and patients receiving newly approved treatments) is emphasized by the FDA.

OUTLINE OF THIS THESIS

This thesis comprises publications based on a several studies in healthy elderly subjects, subjects with preclinical AD and subjects with neurodegenerative diseases that were all aimed at gaining a better understanding of the difference between these subject groups and a better characterization of potential candidates for clinical trial participation in (preclinical) AD. In these studies, different biomarkers were investigated to gain more insight into healthy elderly, elderly with preclinical AD and patients with AD in order to better understand the course over time of AD biomarkers as the disease progresses and to better select the optimal potential clinical trial participants for new disease modifying treatments being developed for AD. **Chapter II** describes a large dataset analysis in which the NeuroCart, a computerized test battery to measure neuropsychological and neurophysiological performance, was used to assess age-related decline in test performance and whether test outcomes differentiate between healthy subjects

and patients with Alzheimer's Disease, Parkinson's Disease, Huntington's Disease and Vascular dementia.

Chapter III reviews animal models of AD and the translation to human AD based on diagnostic and prognostic biomarkers. Using animal models to understand AD may help to fill the knowledge gap of the pathophysiological systems involved in AD and to better define the preclinical stage of AD.

In **Chapter IV** and **Chapter V** of this thesis we discuss a study in the context of which we aimed to characterize subjects with preclinical AD and how these subjects can be selected for clinical trial participation using an algorithm including multiple neuropsychological tests and blood-based diagnostic biomarkers. The aim of this study was to reduce the need for invasive procedures (e.g., lumbar puncture) in otherwise healthy elderly trial subjects.

Chapter VI and **Chapter VII** discuss how new diagnostic biomarkers for AD behave in preclinical AD. Inflammatory responses that are expected to be involved in AD pathology were measured in plasma samples to investigate if these biomarkers are already different in subjects with preclinical AD compared to healthy elderly. Phosphorylated tau, which recently has shown to be a good predictive biomarker for the development of AD, was measured in CSF and plasma with the goal to replicate previous research.

Finally, **Chapter VIII** aims to integrate all previous chapters and discusses the issue of the selection of study participants for clinical trials with disease modifying therapies being developed for AD, putting it into in a broader perspective considering the ethical point of view of selecting preclinical subjects for trials.

Table 1 Alzheimer's Disease Drug development pipeline: Disease modifying treatment in development for Alzheimer's Disease in phase 1, 2 and 3.[74]

Phase	Mechanism class	Mechanism of action
Phase 1, 27 DMT / (90% of phase 1 compounds)/ 9 biologicals / 18 small molecules	inflammation	CSF-1R antagonist; attenuates microglial proliferation and neurodegeneration
		NRTI; reduce neuroinflammation
		Non-steroidal anti-inflammatory to reduce inflammation
		Regulatory T cells
		TNF inhibitor; reduce neuroinflammation
	epigenetic regulators	Extending telomeres may benefit AD; reduce Aβ-induced neurotoxicity; effects on multiple cellular pathways
		NNRTI; promote cholesterol removal; enhance amyloid reduction.
		10hApoE2, serotype rh. Ten AAV gene transfer vector expressing the cDNA coding for human ApoE ε2, directly to the CNS/CSF of ApoE ε4 homozygotes with AD
		Histone deacetylase (HDAC) inhibitor; enhanced synaptic plasticity
	amyloid	Monoclonal antibody targeting soluble Aβ
		Monoclonal antibody to reduce Aβ
		Anti-amyloid monoclonal antibody
	tau	O-GlycNAcase Inhibitor
		Monoclonal antibody to reduce tau
		Anti-tau monoclonal antibody
	proteostasis	Aβ and tau aggregation inhibitor; inhibits neuronal death
		Prevents Aβ and tau aggregation
		Aggregation inhibitor
	synaptic plasticity/ neuroprotection	mGluR5 allosteric modulator
		Lysine-gingipain inhibitor
		Regulates calcium dyshomeostasis; tau and Aβ reduction
	neurogenesis	GABA-A receptor modulator; promote neurogenesis and reduce inflammation
		Enhance neurogenesis; activates progenitor cells
	vasculature	Direct thrombin inhibitor; reduce neurovascular damage
		Angiotensin II receptor blocker
	autophagy	Induces autophagy and promotes clearance of aggregated proteins
	metabolism and bioenergetics	Caprylic triglyceride

(continuation Table 1)

Phase	Mechanism class	Mechanism of action
Phase 2, 71 DMT (86.6% of phase 2 compounds) / 26 biologicals / 45 small molecules / 7 studies in preclinical AD	inflammation	Monoclonal antibody targeting TREM2 receptors to promotemicroglial clearance of Aβ
		Janus kinase inhibitor; reduces neuroinflammation
		Immunomodulator
		Anti-IL-1β monoclonal antibody
		Herb with antioxidant and anti-inflammatory properties
		Monoclonal antibody targeting CD38; regulates microglial activity
		Tyrosine kinase inhibitor (dasatinib) and flavonoid (quercetin); senolytic therapy approach to reduce senescent cells and tau aggregation
		Regulatory T cells; reduce neuroinflammation
		Reduce inflammatory cytokines; modulate innate and adaptive immune responses
		Dietary amino acid; reduce brain inflammation and preserve nerve cells
		Cysteinyl leukotriene type 1 (cysLT-1) receptor antagonist; effects on inflammatory processes, neuronal injury, blood-brain-barrier integrity, and Aβ protein accumulation
		Monoclonal antibody directed at semaphoring 4D to reduce inflammation
		Granulocyte macrophage colony stimulating factor
		Calcium-activated potassium channel blocker
		Monoclonal antibody targeting galactin 3
		Immune reaction to diphtheria, pertussis, tetanus vaccine
		Antiviral against HSV-1 and -2 infection; to prevent Aβ aggregation and plaque deposition
	synaptic plasticity/ neuroprotection	PDE-4 inhibitor; prolongs cAMP activity and improves neuronal plasticity
		Protein Kinase C inhibitor; facilitates synaptogenesis
		Guanylate cyclase positive allosteric modulator
		Neurotrophic agent; activates sigma receptors to preserve synaptic plasticity; protect against Aβ toxicity
		Sigma-2 receptor antagonist; competes with oligomeric Aβ binding; protect against Aβ-induced synaptic toxicity
		Plasma transfusion from exercise-trained donors
		Activates signaling via the hepatocyte growth factor system to regenerate neurons and enhance synaptic plasticity

(continuation Table 1)

Phase	Mechanism class	Mechanism of action
Phase 2, 71 DMT (86.6% of phase 2 compounds) / 26 biologicals / 45 small molecules / 7 studies in preclinical AD		SV2A modulator; improve synaptic function; reduce Aβ-induced neuronal hyperactivity
		p38MAPK-α inhibitor
		p38MAPK-α inhibitor; enhance endolysosomal function to reduce synaptic dysfunction
		Filamin A protein inhibitor; stabilizes the interaction of soluble Aβ and the alpha7 nicotinic acetylcholine receptor, reducing Aβ and synaptic dysfunction
	amyloid	Glutamatemodulator; prodrug of riluzole; improve synaptic function
		Active immunotherapy to remove Aβ
		Alpha-secretase modulator to reduce Aβ production
		Monoclonal antibody targeting soluble Aβ oligomers
		Monoclonal antibody specific for pyroglutamate Aβ
		Monoclonal antibody directed at Aβ plaques and oligomers
		Anti-Aβ monoclonal antibody (gantenerumab) with enhanced blood-brain barrier penetration
		Monoclonal antibody directed at protofibrils
		Sirtuin-nicotinamide adenine dinucleotide stimulator to enhance alpha-secretase
		Activates transport protein ABCC1 to remove Aβ
		Prodrug of tramiprosate; inhibits Aβ aggregation into toxic oligomers
		Glutaminyl cyclase (QC) enzyme inhibitor to reduce pyroglutamate Aβ production
	tau	Active immunotherapy targeting tau
		Anti-tau monoclonal antibody
		Anti-tau monoclonal antibody
		Antisense oligonucleotide targeting tau expression; MAPT RNA inhibitor
		Monoclonal antibody targeting soluble tau
		O-GlycNAcase inhibitor; promote tau glycosylation, prevent tau aggregation
		HDAC inhibitor; to reduce tau-induced microtubule depolymerization and tau phosphorylation
		Heat shock protein 90 inhibitor; to prevent aggregation and hyperphosphorylation of tau
		Monoclonal antibody to remove extracellular tau

(continuation Table 1)

Phase	Mechanism class	Mechanism of action
Phase 2, 71 DMT (86.6% of phase 2 compounds) / 26 biologicals / 45 small molecules / 7 studies in preclinical AD	metabolism and bioenergetics	SGLT2 inhibitor; to improve insulin sensitivity and CNS glucose metabolism
		Decrease glucose resistance and increase insulin signaling in the brain
		SGLT2 inhibitor (empagliflozin) and insulin combination therapy; decrease glucose resistance and increase insulin signaling in the brain
		Dual agonist of PPARδ/γ; reduce glucose and lipid metabolism
	proteostasis	Polyphenolic compound; antioxidant; prevent aggregation of Aβ and tau
		Inhibitor of APP and α-synuclein
		mTOR inhibitor; ameliorate metabolic and vascular effects of aging
	vasculature	Polyunsaturated fatty acid; reduce damage to small blood vessels
		Angiotensin II receptor blocker (telmisartan); angiotensin converting enzyme inhibitor (perindopril)
		Cerebral blood flow enhancer
	neurotransmitter receptors	Dopamine agonist with anti-Aβ effects
		NMDA receptor antagonist
		Dual Orexin receptor antagonist; improved sleep with effects on CSF Aβ
	epigenetic regulators	hTERT peptide vaccine; mimics extra-telomeric functions to inhibit neurotoxicity, apoptosis, and reactive oxygen species
		Nucleoside reverse transcriptase inhibitor; reduces genetic rearrangements
	growth factors and hormones	GnRH receptor agonist; reduce effects of elevated GnRH and gonadotropins on the brain
		11-beta-hydroxysteroid dehydrogenase type 1 inhibitor
	neurogenesis	Allosteric modulator of GABA-A receptors
		Endothelin B receptor agonist; augments activity of neuronal progenitor cells
	cell death	Iron chelating agent; reduce damaging reactive oxygen species
	ApoE, lipids and lipoprotein receptors	Cholesteryl ester transfer protein (CETP) inhibitor
	oxidative stress	Omega 3 fatty acid; improve synaptic function; antioxidant

(continuation Table 1)

Phase	Mechanism class	Mechanism of action
Phase 3, 21 DMT (67.8% of phase 3 compounds)/ 5 biologicals / 16 small molecules / 7 trials in preclinical AD	amyloid	Monoclonal antibody directed at Aβ plaques and oligomers
		Monoclonal antibody specific for pyroglutamate form of Aβ
		Monoclonal antibody directed at Aβ plaques and oligomers
		Monoclonal antibody directed at Aβ protofibrils
		Monoclonal antibody directed at Aβ monomers
		Prodrug of tramiprosate; inhibits Aβ aggregation into toxic oligomers
	Combination of amyloid DMTs	Monoclonal antibody specific for pyroglutamate form of Aβ (donanemab); monoclonal antibody directed at plaques and oligomers (aducanumab); given in separate arms of the trial
	Combination of amyloid DMTs	Monoclonal antibody directed at Aβ plaques and oligomers (gantenerumab); Monoclonal antibody directed at Aβ monomers (solanezumab); given in separate arms of the trial
	synaptic 4 plasticity/ neuroprotection	SV2A modulator; to reduce Aβ-induced neuronal hyperactivity
		Bacterial protease inhibitor targeting gingipain produced by P. gingivalis to reduce neuroinflammation and hippocampal degeneration
		Sigma-1 receptor agonist, M2 autoreceptor antagonist; to ameliorate oxidative stress, protein misfolding, mitochondrial dysfunction, and inflammation
		Filamin A protein inhibitor; stabilizes amyloid-alpha-7 nicotinic receptor interaction
	oxidative stress	Free radical scavenger
		Purified form of the omega-3 fatty acid EPA; to improve synaptic function and reduce inflammation
		Antioxidant
	metabolism and bioenergetics	Insulin sensitizer to improve CNS glucose metabolism
		GLP-1 agonist; reduces neuroinflammation and improves insulin signaling in the brain
		Caprylic triglyceride; induces ketosis and improves mitochondrial and neuronal function
	tau	Tau protein aggregation inhibitor
	inflammation	MAPK-1/3 inhibitor; reduces proinflammatory NFκB activation
	proteostasis	Tyrosine kinase inhibitor; autophagy enhancer; promotes clearance of Aβ and tau
	vasculature	Angiotensin II receptor blocker (losartan), calcium channel blocker (amlodipine), cholesterol agent (atorvastatin)
	gut-brain axis	Algae-derived acidic oligosaccharides; changes microbiome to reduce peripheral and central inflammation

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