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## **Mechanistic early phase clinical pharmacology studies with disease-modifying drugs for neurodegenerative disorders**

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**CHAPTER 1**  
**INTRODUCTION**



Degenerative diseases of the nervous system, or neurodegenerative disorders, are often serious, progressive and can be fatal. Symptoms can present in the form of motor impairment (balance, movement, talking, breathing), cognitive impairment (memory, learning, concentration), psychiatric symptoms (depression, anxiety, hallucinations) and eventually also disturbances in consciousness. Global prevalence of these disorders is on the rise, and they currently have no cure.

Major neurodegenerative disorders include Alzheimer's disease with an estimated 150 million patients globally by 2050,<sup>1</sup> Parkinson's disease with an estimated 12 million patients by 2050,<sup>2</sup> and amyotrophic lateral sclerosis with an estimated 400 thousand patients by 2040.<sup>3</sup>

A thorough mechanistic understanding of these diseases is required to identify druggable targets that could help slow down disease progression (with disease-modifying treatments) and ultimately potentially even lead to the development of a cure. Furthermore, this mechanistic understanding can lead to the identification of valuable (pharmacodynamic) drug-response biomarkers that could be used in early clinical development to demonstrate proof-of-mechanism and support dose-finding for late-stage clinical development. Fortunately, this mechanistic understanding has recently grown tremendously, and is expected to continue to grow substantially, paving the way for the clinical development of novel treatments.

## ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a mostly sporadic neurodegenerative disorder, with genetic/familial forms accounting for <5% of cases. AD is characterized by cognitive impairment, that typically presents in mid- to late-life. Short-term memory difficulty is the most common symptom, but impairment in expressive speech, visuospatial processing and executive functions can also be presenting symptoms.<sup>4</sup> The earliest symptomatic stage of AD is often referred to as mild cognitive impairment (MCI). In this stage one or more cognitive domains are impaired to at least a mild extent, while functional capacities remain relatively preserved.<sup>5</sup> AD ultimately progresses to dementia, where more severe cognitive deficits – often accompanied by neuropsychiatric symptoms including depression, anxiety, and social withdrawal, and eventually delusions, hallucinations, emotional dyscontrol or physically aggressive behaviors<sup>6</sup> – affect daily life and impair independence. The rate of cognitive progression is highly variable between individuals, but an average

of 9.2 years has been reported for the transition from subjective memory complaint to MCI<sup>7</sup> and a conversion rate of 5% to 39% from MCI to dementia in the first year.<sup>8</sup>

On the biological level, AD is characterized by  $\beta$ -amyloid ( $A\beta$ )-containing extracellular plaques that are found in a widespread distribution throughout the cerebral cortex, and hyperphosphorylated TAU (p-TAU)-containing intracellular neurofibrillary tangles that occur initially in the medial temporal lobe. Pathophysiological biomarker changes can be observed in the preclinical AD stage, as early as 10-20 years before symptomatic cognitive impairment.<sup>9</sup> This has triggered discussion on the possibility to screen subjects with no cognitive complaints for preclinical AD. However, since there is insufficient data on improved patient outcomes and there are currently no cures for AD, screening is not recommended at present.<sup>10</sup>

$A\beta$  peptides are formed by the cleavage of amyloid precursor protein (APP) by  $\beta$ -secretases and  $\gamma$ -secretases and secreted into the extracellular space.  $A\beta$  peptides, particularly  $A\beta_{1-42}$  and to a lesser extent  $A\beta_{1-40}$ , have a high tendency to aggregate into extracellular plaques. APP is enriched in neuronal synapses, and  $A\beta$  production and release are regulated by synaptic activity.<sup>11</sup> Aggregated  $A\beta$  interacts with metabotropic glutamate receptor 5, NMDA receptors, potentially  $\alpha 7$  nicotinic acetylcholine receptor and insulin receptors and seems to cause pathological changes in dendritic spines and synaptic efficiency.<sup>4</sup>

TAU is a protein responsible for cellular microtubule stabilization and possibly involved in controlling axonal transport. Post-translational modifications can lead to TAU-aggregation and accumulation in cell bodies and dendrites. Especially hyperphosphorylation renders TAU prone to aggregation and impairs cell viability.<sup>12</sup> Synaptic activity releases TAU into the extracellular space, where it is taken up in postsynaptic neurons and glia (non-neuronal brain cells).<sup>13</sup> Aggregated p-TAU can appear intracellularly (neurofibrillary tangles), as fragments in the neuropil (neuropil threads) and in p-TAU-containing degenerated axons and dendrites surrounding  $A\beta$  plaques (dystrophic neurites).<sup>4</sup>

These pathological  $A\beta$ -plaques and TAU-tangles are accompanied by a loss of synaptic homeostasis, neurons and neuronal network integrity in AD.<sup>4</sup> Exactly how  $A\beta$  and p-TAU lead to synaptic and neuronal loss in AD is not fully understood and remains a topic of substantial investigation. Potential contributing mechanisms include neuronal/synaptic toxicity of the plaques and tangles, and alterations in basic electrophysiological homeostasis causing changes in neuron firing rates and patterns.<sup>4</sup>

Furthermore, pathophysiological alterations in the endosomal-lysosomal network and autophagy pathways can impact the clearance of extracellular material – including damaged/aggregated (A $\beta$  and p-TAU) proteins – and also affect synaptic plasticity and homeostasis. Autophagy of extracellular material should be induced following a cellular stress response, but the functioning of cellular lysosomes responsible for protein degradation is progressively corrupted due to AD pathophysiological mechanisms. This causes the cellular protein degradation process to stall and what results is a neuro-inflammatory response, with the recruitment of phagocytic microglia and release of inflammatory cytokines, spreading neurotoxicity to neighboring neurons.<sup>4</sup>

What exactly triggers A $\beta$  and p-TAU to increase to pathological levels is not yet well understood, nor is it clear if A $\beta$  and p-TAU increases are the actual underlying cause of AD. The amyloid hypothesis suggests that accumulation of A $\beta$  in the brain is the primary influence driving AD pathogenesis,<sup>14</sup> however, there are also those that argue that the aggregation of p-TAU is the most likely molecular trigger for neuronal dysfunction and death in AD.<sup>12,15</sup> Either way, our molecular understanding of AD is expanding and to date 75 genes have been identified that are associated with an increased risk of developing AD.<sup>16</sup> These discoveries have resulted in a whole array of potential new genetic and molecular drug targets, and the pipeline of new candidate drugs aimed at slowing down AD progression is growing. This is much needed, as the currently available AD treatments galantamine, rivastigmine, and donepezil (cholinesterase inhibitors aimed at improving cholinergic neurotransmission) and memantine (NMDA receptor antagonist aimed at improving glutamatergic neurotransmission) demonstrate only modest benefits in slowing decline in cognition, function, and behavior.<sup>17</sup>

Drug development efforts focus on nearly all pathophysiological processes involved in AD, including removal of A $\beta$  and p-TAU (anti-A $\beta$  antibodies/immunotherapy), inhibition of A $\beta$  production ( $\beta$ -secretase inhibitors), improvement of microglial function (TREM2 antibodies) or dampening of neuroinflammation (RIPK1-inhibitors). The first anti-A $\beta$  antibody (aducanumab) was registered as treatment for AD in patients with MCI or mild dementia by the FDA in 2021<sup>18</sup>, but following much controversy around the supportive scientific data, aducanumab's EMA application was recently retracted.<sup>19</sup> More recently, lecanemab, an investigational anti-A $\beta$  protofibril antibody, was reported to slow the rate of cognitive decline by 27% over 18 months in a clinical study of 1,795 participants with early AD, but the clinical relevance of these results is still being debated.<sup>20</sup>

## PARKINSON'S DISEASE

Parkinson's disease (PD) is a largely sporadic neurodegenerative disorder, with genetic/familial forms only accounting for 5-10% of cases. PD is characterized by motor impairment, that usually presents after the age of 50 with an increasing incidence in each subsequent decade.<sup>21</sup> The main symptoms of PD include unintended or uncontrollable movements, including tremor, rigidity, slowness of movement, and impaired balance and coordination. Additionally, PD comes with a multitude of non-motor symptoms such as cognitive impairment, autonomic dysfunction, disorders of sleep, and depression. Early motor symptoms of this disease are subtle, occur gradually, and often begin on one side of the body or even in one limb. As the disease progresses, it begins to affect both sides of the body and can lead to imbalance with falls. Further progression leads to severe disability, and ultimately a patient may become wheelchair bound or bedridden unless aided. Some patients may also develop Parkinson's disease dementia. The symptoms of PD and the rate of progression differ among individuals. Most patients go up 1 Hoehn & Yahr (H&Y) stage every two years (except for stage 2 which is 5 years), but about one-third of patients remain in stage 1 or 2 for up to 10 years. Eighty percent of patients who have had PD for 15 years have recurrent falls, and most patients with 18-20 years of PD are using a wheelchair.<sup>22</sup>

The main neuropathological features of PD are intracellular inclusions (Lewy Bodies) containing aggregates of alpha-synuclein ( $\alpha$ SYN) protein in neurons of the substantia nigra and cortex, and a loss of dopaminergic neurons in the brain substantia nigra causing striatal dopamine deficiency. The degeneration of these dopaminergic neurons can already be observed before the appearance of  $\alpha$ SYN aggregates and before the onset of motor symptoms.<sup>21,23</sup>

Based on our current understanding, the underlying molecular pathology of PD involves multiple pathways and mechanisms including  $\alpha$ SYN homeostasis, mitochondrial dysfunction, oxidative stress, and neuroinflammation.<sup>21</sup>

The exact function of  $\alpha$ SYN protein is not fully understood, but it likely plays a role in synaptic vesicle dynamics and potentially also in mitochondrial functioning and intracellular trafficking.<sup>24,25</sup>  $\alpha$ SYN accumulates and aggregates in the brain of PD patients, which may be triggered by (local) overproduction, misfolding, or impairments in degradation of the protein. In addition, pathological  $\alpha$ SYN forms have been discovered in the gut – potentially triggered by dysbiosis of the gut microbiota, infection, and inflammation – and it has been proposed that these  $\alpha$ SYN seeds may travel in

a cranial direction to the brain via the gut-brain axis via the vagus nerve and initiate prion-like spreading.<sup>26</sup>  $\alpha$ SYN accumulation and aggregation in the brain neurons in due course leads to a pathogenic process where soluble  $\alpha$ SYN monomers first form oligomers and eventually progressively combine into large, insoluble amyloid fibrils (making up the Lewy bodies) with neurotoxic properties.<sup>27</sup>

In degenerating neurons in PD,  $\alpha$ SYN aggregation is often observed together with mitochondrial dysfunction, and both processes may exacerbate each other.<sup>21</sup> Mitochondria are intracellular powerhouses that perform various cellular reactions, including the production of energy through the mitochondrial respiratory chain, the regulation of cell death, calcium metabolism and the production of reactive oxygen species (ROS). Impaired mitochondrial function leads to increased oxidative stress (OS), that in turn damages intracellular components (including depletion of lysosomes<sup>28</sup>) and activates signaling pathways leading to nigral dopamine cell death in PD.<sup>29</sup>

Additionally, neuroinflammation is likely an essential contributor to PD pathology, although maybe not the initial disease trigger. Neuroinflammation may result from an induction of both innate and adaptive immunity in reaction to  $\alpha$ SYN aggregation, and in turn neuroinflammation itself can promote  $\alpha$ SYN misfolding, forming a self-aggravating cycle.<sup>21,30</sup>

It is believed that the risk for developing sporadic PD results from an interplay of genetic, environmental and life-style factors. Exposure to pesticides and traumatic brain injury increase the risk for PD, whereas smoking and caffeine use seem to decrease the incidence of PD.<sup>31</sup> In addition, the list of identified genes that increase the life-time risk of developing PD continues to grow. The two most common genetic risk factors for PD, namely mutations in glucocerebrosidase (GBA) and leucine-rich repeat kinase 2 (LRRK2) genes, impair functioning of the lysosomal-autophagy system and therefore could affect intracellular  $\alpha$ SYN protein degradation.<sup>21</sup>

Multiple pharmacological treatment options are available for PD. These treatments mainly focus on increasing dopamine levels via administration of the dopamine-precursor amino acid L-DOPA or by inhibiting dopamine clearance (COMT and MAO-B inhibitors), or mimic dopamine activity (dopamine receptor agonists). In addition, some non-dopaminergic pharmacological treatments are available for some of the non-motor symptoms (e.g. NMDA-antagonists, choline esterase inhibitors) and there is the option of deep brain stimulation (DBS) to reduce motor fluctuations and dyskinesia in patients with advanced PD.<sup>32</sup> However, approved symptomatic PD treatments to date only temporarily reduce motor symptoms, and do not slow down disease progression.

Fortunately, genetic research over the past two decades has substantially expanded our understanding of the cellular pathogenesis of PD, and this knowledge is being used to develop a wide-array of (targeted) disease-modifying therapies for PD.<sup>33</sup> These experimental therapies generally try to restore striatal dopamine with growth factor-, gene- and cell-based approaches, or focus on reducing aggregation and cellular transport of  $\alpha$ SYN (e.g. via anti- $\alpha$ SYN antibodies or immunotherapy, or via targeted therapies focused on improving the lysosomal-autophagy protein degradation system [e.g. LRRK2-inhibitors and GCase enhancers]).<sup>21</sup> Clearly identified genetic and environmental PD risk factors also offer an opportunity to select populations with prodromal disease stages, which could facilitate an early start of 'disease-prevention/disease-modification' trials. Efforts to identify markers for prodromal disease stages are therefore a major research focus.<sup>21</sup>

## AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) is a heterogeneous neurodegenerative disorder that is characterized by the degeneration of both upper motor neurons (neurons from the cortex to the brain stem and the spinal cord) and lower motor neurons (neurons from the brainstem or spinal cord to the muscle).<sup>34</sup> ALS usually presents between the ages of 40 and 70, with an average age of 55 years at the time of diagnosis. In most patients the disease is sporadic, although approximately 10% of patients suffer from familial forms of the disease due to hereditary gene mutations. The initial presentation of ALS generally divides between spinal-onset (with muscle weakness of the limbs) or bulbar-onset disease (with difficulty with speech and swallowing). Early ALS disease symptoms usually include muscle weakness and wasting of muscles. Gradually all voluntary muscles become affected resulting in impaired movement, loss of speech, and eventually paralysis. Most patients die from respiratory failure, usually within 3 to 5 years from symptom onset, though ~10% of patients survive for  $\geq$ 10 years. During the course of the disease up to 50% of patients also develop cognitive impairment and 13% of patients develop concomitant frontotemporal dementia (FTD).<sup>34</sup>

The pathophysiological mechanisms underlying ALS are not well understood, but aggregation and accumulation of protein inclusions in motor neurons seems to be widely present also in this neurodegenerative disease. In about 97% of patients with ALS, TAR DNA-binding protein 43 (TDP-43) cytoplasmic aggregates are the major constituent, although other types of protein aggregates are seen in specific subtypes of ALS.<sup>34</sup> Historically the most well studied subtype of ALS is the autosomal dominant form caused by mutations

in the superoxide dismutase (SOD1)-gene, which occurs in approximately 2% of all patients and which lead to accumulation of misfolded SOD1 protein (and not TDP-43) in motor neurons. It is not clear if protein aggregates directly drive neurotoxicity in ALS, or if neurotoxicity already results from various processes preceding protein aggregation. Most likely, ALS results from many different interacting mechanisms that culminate in larger network disruption, and the relative extent by which these mechanisms are involved may differ from case-to-case contributing to the high heterogeneity of this disease. Some of these contributing mechanisms include impaired protein homeostasis, aberrant RNA metabolism, glutamate excitotoxicity, hyperexcitability, neuroinflammation, and mitochondrial dysfunction.<sup>34</sup>

Impaired protein homeostasis in ALS can involve misfolding of proteins, abnormal cellular localization of proteins, and/or impaired protein degradation, and several gene mutations that are involved with these processes have been identified to contribute to ALS.<sup>34</sup> Alterations in messenger RNA (mRNA) also seem to play a key role in ALS pathology.<sup>35</sup> The exact mechanisms by which this causes neurodegeneration remain to be elucidated, but processes involved include altered RNA metabolism, dysregulation of gene expression including transcription, alternative splicing of mRNA, axonal transport of mRNAs, RNA/protein toxic gain-of-function and/or protein loss-of-function, and mislocalization of RNA binding proteins (most importantly TDP-43 and FUS) from the nuclear to the cytoplasmic compartment and resulting in the formation of cellular stress granules.<sup>34-36</sup>

Excitotoxicity in motor neurons is assumed to be a mechanism common to all forms of ALS and results from calcium entry following excessive glutamate stimulation. Motor neurons are more sensitive to this type of toxicity than other neuronal subtypes due to a lower calcium buffering capacity, higher calcium permeability of AMPA receptors, and impairment of the main synaptic glutamate re-uptake transporter (EAAT2) in ALS.<sup>34</sup> Finally, similar to other neurodegenerative disorders, mitochondrial dysfunction can cause oxidative stress and DNA damage in ALS,<sup>37</sup> while also here neuroinflammation is considered an important factor in amplifying neuronal injury and enhancing disease progression.<sup>34</sup>

The result of these molecular pathophysiological processes in ALS is that motor neurons cannot maintain their axonal projections, leading to axonal retraction and denervation of the target cells. This in turn results in denervation of muscles for lower motor neurons, leading to muscle weakness, spasticity, and loss of upper motor neuron control of spinal cord motor neurons, leading to spasticity.<sup>34</sup>

In the past decades, at least 30 genetic mutations have been identified that confer a major risk for developing ALS. The most important of which are mutations in the C9ORF72 (implicated in RNA metabolism and autophagy), SOD1 (implicated in oxidative stress), TARDBP (also known as TDP-43) and FUS (both implicated in RNA metabolism) genes. These mutations likely interact with environmental risk factors such as exposure to heavy metals, organic chemicals, and cyanotoxins, smoking, participating in professional sports or occupations requiring repetitive/strenuous work, lower BMI, and viral infections, eventually leading to disease manifestation.<sup>34,38</sup>

Despite over 50 drugs with different working mechanisms having been investigated for ALS, only three compounds have been registered so far: riluzole, edaravone, and very recently the combination of sodium phenylbutyrate and taurursodiol.<sup>39</sup> The exact mode of action of all these three drugs is poorly understood, and they have limited effect sizes (riluzole is the gold standard and believed to extend survival by 3 months<sup>40</sup>). However, an extensive pipeline of potential new treatments for ALS is being tested, including antisense oligonucleotides against specific mutated proteins (SOD1, C9ORF72), cell and gene-based therapies, and compounds targeting neuroinflammation (e.g. RIPK1) or cell stress responses (e.g. EIF2B agonists).

## DISEASE-MODIFYING TREATMENTS

Most available pharmacological interventions for neurodegenerative disorders only help improve symptoms, increase mobility, or relieve pain, but do not (significantly) slow down overall disease progression. Therefore, neurodegenerative disorders currently represent one of the areas of the highest unmet medical need and there is an urgent need for novel treatments aimed at modifying disease progression. A paradigm shift from symptomatic treatment to disease-modifying treatment is rapidly taking shape, as neurodegenerative disorders are being unraveled and an array of new drug targets are being identified. This paradigm shift also requires innovative clinical drug development strategies to overcome some of the fundamental challenges of developing disease-modifying treatments for neurodegenerative disorders.

## EARLY-STAGE MECHANISTIC PROOF-OF-CONCEPT STUDIES

In *Chapter 2* several general challenges in developing drugs for neurodegenerative disorders are introduced, including poor translatability from preclinical models to human disease, disease onset well before first appear-



ance of clinical symptoms, challenges in objectifying/quantifying disease progression, and localization of the disease to a body compartment that is not easily accessible for obtaining (tissue) samples in clinical studies. Subsequently it is explained how these challenges can be (partly) overcome by using pharmacodynamic biomarkers in early mechanistic proof-of-concept studies. The goal of such studies would be to demonstrate that a novel drug reaches its intended site of action, occupies and activates or inhibits its target, and that this leads to quantifiable downstream (patho)physiological responses, often by using (purpose-developed) biomarkers. While such data-intensive early phase programs can be more costly and logistically more challenging to execute than traditional phase 1 studies that only focus on pharmacokinetics and safety, they do bring numerous advantages that justify this extra investment. Most notably:

- Proof-of-mechanism studies can help support early go/no go drug development decisions, thereby preventing heavy investments in later stage trials for drugs that are doomed to fail due to a lack of target engagement and/or target activation or inhibition in humans.<sup>41</sup>
- They can help differentiate between a negative clinical trial due to a lack of clinical effect from the targeted molecular mechanism, versus a lack of clinical effect due to insufficient drug exposure/target engagement. The former suggests diverting resources towards other molecular targets, whereas the latter could suggest still focusing on the same molecular target but with other compounds that have more favorable pharmacokinetic/pharmacodynamic properties.
- They could offer proof of pathophysiological biomarker response in a shorter timeframe than pivotal clinical trials that may take years and large numbers to demonstrate a significant clinical effect on slowing down disease progression. In fact, some pathophysiological response biomarkers could even be used as a surrogate endpoint in late-stage development to demonstrate potential disease modification by a new drug, as was recently done for aducanumab.<sup>42</sup> But this should only be done if that specific biomarker has a validated causal relation with actual disease progression.<sup>43</sup>

*Chapter 2* continues with an overview and categorization of biomarkers that were reported in early phase clinical pharmacology studies identified from a literature review of the past decade and presents considerations for biomarker selection for early clinical development. This chapter ends with a proposed roadmap for designing mechanistic, data-rich, early phase clinical pharmacology studies for disease-modifying therapies in neurodegenerative disorders.

Next, this methodology of mechanistic early phase clinical pharmacology studies is applied to the development of two novel compounds aimed at neurodegenerative disease modification: a RIPK1-inhibitor and a LRRK2-inhibitor.

## RIPK1-INHIBITOR FOR AD AND ALS

Receptor-interacting serine/threonine protein kinase 1 (RIPK1) is a master regulator of inflammatory signaling and cell death and increased RIPK1 activity is observed in several neurodegenerative disorders. RIPK1 inhibition has been shown to protect against cell death in a range of preclinical cellular and animal models of diseases.

*Chapter 3* describes the early-stage development of SAR443060 (formerly DNL747), a selective, orally bioavailable, central nervous system (CNS)-penetrant, small-molecule, reversible inhibitor of RIPK1, developed to slow disease progression in AD and ALS. This chapter includes an overview of preclinical compound safety and target engagement data, followed by three early-stage clinical trials:

- A first-in-human (FIH), randomized, placebo-controlled, double-blind, single- and multiple ascending dose study in healthy subjects to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of SAR443060 (dose-finding);
- A first-in-patient, randomized, double-blind, placebo-controlled, cross-over study in patients with AD to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of SAR443060 in patients with AD (proof-of-mechanism in target population);
- A first-in-patient, randomized, double-blind, placebo-controlled, cross-over study in patients with ALS followed by an open label extension (OLE) to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of SAR443060 patients with ALS (proof-of-mechanism in target population).

In all three studies, peripheral target engagement of SAR443060 was measured via a reduction in phosphorylation of RIPK1 at serine 166 (pRIPK1) in human peripheral blood mononuclear cells (PBMCs) compared to baseline. Additionally, SAR443060 distribution into the cerebrospinal fluid (CSF) was quantified as a surrogate for CNS drug-exposure. This data combined suggests that therapeutic modulation of RIPK1 in the CNS is possible, offering potential therapeutic promise for AD and ALS. Despite these promising initial results, SAR443060 development was discontinued due to long-term nonclinical toxicology findings. However, SAR443820, a back-up compound for SAR443060

with the same mode of action (MOA), has now successfully completed FIH studies and a phase 2 study in ALS patients has started dosing in 2022.<sup>44</sup>

## LRRK2-INHIBITOR FOR PD

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene can be a risk factor for developing PD. LRRK2 mutations associated with increased kinase activity result in lysosomal dysfunction, which could lead to impaired clearance and aggregation of toxic proteins (e.g.  $\alpha$ SYN, p-TAU). LRRK2 inhibition corrects lysosomal dysfunction and downstream neurodegeneration in preclinical models of PD.

*Chapter 4* describes investigation of candidate human safety, target engagement, pharmacodynamic and potential patient stratification biomarkers for LRRK2 pathway inhibition. To this purpose blood, PBMCs, neutrophils, and CSF were collected from PD patients with and without a LRRK2 mutation and healthy control subjects. Target engagement (total LRRK2 protein and phosphorylation of LRRK2 protein at the serine 935 residue) and downstream pathway engagement (phosphorylation of LRRK2's RAB10-substrate and  $\alpha$ SYN) biomarkers were evaluated for within- and between-subject variability and overall group level differences. The outcomes of this clinical biomarker characterization study were used to develop a robust biomarker strategy for two subsequent early-stage pharmacology studies with a novel LRRK2 inhibitor.

These follow-up studies with the CNS-penetrant LRRK2 inhibitor BIIB122 (formerly DNL151) are described in *Chapter 5*, and include:

- A FIH, randomized, placebo-controlled, double-blind, single- and multiple ascending dose study in healthy subjects to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of BIIB122 (dose-finding);
- A first-in-patient, randomized, double-blind, placebo-controlled study in patients with PD to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of BIIB122 in patients with PD (proof-of-mechanism in target population).

In both trials, dose-dependent effects on target engagement (phosphorylation of LRRK2 protein at the serine 935 residue) and pathway engagement (phosphorylation of LRRK2's RAB10-substrate) were observed, and BIIB122 concentrations in CSF reflected the unbound drug concentrations in plasma. These studies support continued investigation of LRRK2 inhibition with BIIB122, and follow-up phase 2 and 3 trials have been initiated in 2022 in PD patients with and without LRRK2 mutations.<sup>45,46</sup>

## FUTURE OUTLOOK

We are at the forefront of a paradigm shift in the treatment of neurodegenerative disorders, and many potential new disease-modifying treatments are entering the early stages of clinical development. *Chapter 6* summarizes and discusses the overarching findings of this thesis, how these learnings can be implemented in the early-stage clinical evaluation of new disease-modifying treatments, and presents considerations for ensuring optimal allocation of time and resources to address the growing burden of neurodegenerative disorders.



REFERENCES

- 1 Nichols E, Steinmetz JD, Vollset SE, et al. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Heal.* 2022;7(2):e105-e125. doi:10.1016/S2468-2667(21)00249-8
- 2 Rocca WA. The burden of Parkinson's disease: a worldwide perspective. *Lancet Neurol.* 2018;17(11):928-929. doi:10.1016/S1474-4422(18)30355-7
- 3 Arthur KC, Calvo A, Price TR, Geiger JT, Chiò A, Traynor BJ. Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. *Nat Commun* 2016 7.1. 2016;7(1):1-6. doi:10.1038/ncomms12408
- 4 Knopman DS, Amieva H, Petersen RC, et al. Alzheimer disease. *Nat Rev Dis Prim* 2021 7.1. 2021;7(1):1-21. doi:10.1038/s41572-021-00269-y
- 5 Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med.* 2004;256(3):183-194. doi:10.1111/J.1365-2796.2004.01388.X
- 6 D'Onofrio G, Sancarlo D, Panza F, et al. Neuropsychiatric Symptoms and Functional Status in Alzheimer's Disease and Vascular Dementia Patients. *Curr Alzheimer Res.* 2013;9(6):759-771. doi:10.2174/156720512801322582
- 7 Kryscio RJ, Abner EL, Cooper GE, et al. Self-reported memory complaints: Implications from a longitudinal cohort with autopsies. *Neurology.* 2014;83(15):1359. doi:10.1212/WNL.0000000000000856
- 8 Thaipisuttikul P, Jaikla K, Satthong S, Wisajun P. Rate of conversion from mild cognitive impairment to dementia in a Thai hospital-based population: A retrospective cohort. *Alzheimer's Dement Transl Res Clin Interv.* 2022;8(1):e12272. doi:10.1002/TRC2.12272
- 9 Jagust WJ, Landau SM, Initiative for the ADN. Temporal Dynamics of  $\beta$ -Amyloid Accumulation in Aging and Alzheimer Disease. *Neurology.* 2021;96(9):e1347. doi:10.1212/WNL.0000000000011524
- 10 Patnode CD, Perdue LA, Rossom RC, et al. Screening for Cognitive Impairment in Older Adults: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA.* 2020;323(8):764-785. doi:10.1001/JAMA.2019.22258
- 11 Cirrito JR, Yamada KA, Finn MB, et al. Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron.* 2005;48(6):913-922. doi:10.1016/J.Neuron.2005.10.028
- 12 Liu M, Dexheimer T, Sui D, et al. Hyperphosphorylated tau aggregation and cytotoxicity modulators screen identified prescription drugs linked to Alzheimer's disease and cognitive functions. *Sci Reports* 2020 10.1. 2020;10(1):1-14. doi:10.1038/s41598-020-73680-2
- 13 De Calignon A, Polydoro M, Suárez-Calvet M, et al. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron.* 2012;73(4):685. doi:10.1016/J.Neuron.2011.11.033
- 14 Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science (80-).* 2002; 297(5580):353-356. doi:10.1126/Science.1072994/asset/c4ed1918-4f2b-4649-ba69-684d0145f32b/assets/graphic/se2820694002.jpeg
- 15 Kametani F, Hasegawa M. Reconsideration of amyloid hypothesis and tau hypothesis in Alzheimer's disease. *Front Neurosci.* 2018;12(jan):25. doi:10.3389/fnins.2018.00025/xml/nlm
- 16 Bellenguez C, Küçükali F, Jansen IE, et al. New insights into the genetic etiology of Alzheimer's disease and related dementias. *Nat Genet* 2022 54.4. 2022;54(4):412-436. doi:10.1038/s41588-022-01024-z
- 17 Hansen RA, Gartlehner G, Webb AP, Morgan LC, Moore CG, Jonas DE. Efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer's disease: A systematic review and meta-analysis. *Clin Interv Aging.* 2008;3(2):211. Accessed October 17, 2022. /pmc/articles/PMC2546466/
- 18 US Food and Drug Administration. Aduhelm (aducanumab-avwa) injection BLA accelerated approval. BLA 761178. Published 2021. Accessed September 2, 2022. [https://www.accessdata.fda.gov/drugsatfda\\_docs/applletter/2021/761178Orig1s00ltr.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/applletter/2021/761178Orig1s00ltr.pdf)
- 19 EMA. Zemdiri: Withdrawn application. European Medicines Agency. Published 2022. Accessed September 2, 2022. <https://www.ema.europa.eu/en/medicines/human/withdrawn-applications/aduhelm>
- 20 van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in Early Alzheimer's Disease. *N Engl J Med.* Published online 2022:1-13. doi:10.1056/NEJMoa2212948
- 21 Poewe W, Seppi K, Tanner CM, et al. Parkinson disease. *Nat Rev Dis Prim.* 2017;3(1):1-21. doi:10.1038/nrdp.2017.13
- 22 'The Parkinson's Journey: Understanding Progression' - Webinar Notes Stanford PD Community Blog. Accessed October 3, 2022. <https://parkinsons-blog.stanford.edu/2021/09/the-parkinsons-journey-understanding-progression-webinar-notes/>
- 23 Dijkstra AA, Voorn P, Berendse HW, Groenewegen HJ, Rozemuller AJM, van de Berg WDJ. Stage-dependent nigral neuronal loss in incidental Lewy body and Parkinson's disease. *Mov Disord.* 2014;29(10):1244-1251. doi:10.1002/MDS.25952
- 24 Wales P, Lázaro DF, Pinho R, Outeiro TF. Limelight on alpha-synuclein: pathological and mechanistic implications in neurodegeneration. *J Parkinsons Dis.* 2013;3(4):415-459. doi:10.3233/JPD-130216
- 25 Burré J. The Synaptic Function of  $\alpha$ -Synuclein. *J Parkinsons Dis.* 2015;5(4):699. doi:10.3233/JPD-150642
- 26 Braak H, De Vos RAI, Böhl J, Del Tredici K. Gastric alpha-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neurosci Lett.* 2006;396(1):67-72. doi:10.1016/J.neulet.2005.11.012
- 27 Melki R. Role of Different Alpha-Synuclein Strains in Synucleinopathies, Similarities with other Neurodegenerative Diseases. *J Parkinsons Dis.* 2015;5(2):217. doi:10.3233/JPD-150543
- 28 Dehay B, Bové J, Rodríguez-Muela N, et al. Pathogenic lysosomal depletion in Parkinson's disease. *J Neurosci.* 2010;30(37):12535-12544. doi:10.1523/Jneurosci.1920-10.2010
- 29 Moon HE, Paek SH. Mitochondrial Dysfunction in Parkinson's Disease. *Exp Neurol.* 2015;24(2):103-116. doi:10.5607/EN.2015.24.2.103
- 30 Ransohoff RM. How neuroinflammation contributes to neurodegeneration. *Science.* 2016;353(6301):777-783. doi:10.1126/Science.AAG2590
- 31 Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. *Lancet Neurol.* 2016;15(12):1257-1272. doi:10.1016/S1474-4422(16)30230-7
- 32 Fox SH, Katzschlager R, Lim SY, et al. The Movement Disorder Society Evidence-Based Medicine Review Update: Treatments for the motor symptoms of Parkinson's disease. *Mov Disord.* 2011;26(S3):S2-S41. doi:10.1002/MDS.23829
- 33 Vazquez-Velez GE, Zoghbi HY. Parkinson's Disease Genetics and Pathophysiology. *Annu Rev Neurosci.* 2021;44:87-108. doi:10.1146/annurev-neuro-100720-034518
- 34 Hardiman O, Al-Chalabi A, Chio A, et al. Amyotrophic lateral sclerosis. *Nat Rev Dis Prim.* 2017;3(1):1-19. doi:10.1038/nrdp.2017.71
- 35 Walsh MJ, Cooper-Knock J, Dodd JE, et al. Invited review: decoding the pathophysiological mechanisms that underlie RNA dysregulation in neurodegenerative disorders: a review of the current state of the art. *Neuropathol Appl Neurobiol.* 2015;41(2):109-134. doi:10.1111/NAN.12187
- 36 Kim H, Raphael AR, Ladlow ES, et al. TDP-43 toxicity in amyotrophic lateral sclerosis disease models. *Nat Genet.* 2014;46(2):152-160. doi:10.1038/ng.2853. Therapeutic
- 37 Lopez-Gonzalez R, Lu Y, Gendron TF, et al. Poly(GR) in C9orf72-Related ALS/FTD Compromises Mitochondrial Function and Increases Oxidative Stress and DNA Damage in iPSC-Derived Motor Neurons. *Neuron.* 2016;92(2):383-391. doi:10.1016/J.Neuron.2016.09.015
- 38 Wang MD, Little J, Gomes J, Cashman NR, Krewski D. Identification of risk factors associated with onset and progression of amyotrophic lateral sclerosis using systematic review and meta-analysis. *Neurotoxicology.* 2017;61:101-130. doi:10.1016/J.Neuro.2016.06.015
- 39 FDA Approves New Treatment Option for Patients with ALS | FDA. Accessed October 9, 2022. <https://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-option-patients-als>
- 40 Lacomblez L, Bensimon G, Leigh PN, Guillet P, Meininger V. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet (London, England).* 1996;347(9013):1425-1431. doi:10.1016/S0140-6736(96)91680-3
- 41 de Visser SJ, Cohen AF, Kenter MJH. Integrating scientific considerations into R&D project valuation. *Nat Biotechnol.* 2020;38(1):14-18. doi:10.1038/s41587-019-0358-x
- 42 Budd Haerberlein S, Aisen PS, Barkhof F, et al. Two Randomized Phase 3 Studies of Aducanumab in Early Alzheimer's Disease. *J Prev Alzheimer's Dis.* 2022;9(2):197-210. doi:10.14283/jpad.2022.30/Tables/3
- 43 McGhee DJM, Ritchie CW, Zajick JP, Counsell CE. A review of clinical trial designs used to detect a disease-modifying effect of drug therapy in Alzheimer's disease and Parkinson's disease. *BMC Neurol.* 2016;16(1):92. doi:10.1186/s12883-016-0606-3

- 44 Phase 2 Study for Sar443820 in Participants With Amyotrophic Lateral Sclerosis (ALS) - Full Text View - ClinicalTrials.gov. Accessed October 12, 2022. <https://clinicaltrials.gov/ct2/show/NCT05237284>
- 45 A Study to Assess if BIIB122 Tablets Are Safe and Can Slow Worsening of Early-Stage Parkinson's Disease in Participants With Specific LRRK2 Genetic Variants Between the Ages of 30 and 80 Using the Movement Disorder Society-Unified Parkinson's Disease Rating Scale-Full Text View-ClinicalTrials.gov. Accessed October 12, 2022. <https://clinicaltrials.gov/ct2/show/NCT05418673?term=BIIB122&recrs=ab&draw=2&rank=2>
- 46 A Study to Assess the Safety of BIIB122 Tablets and if it Can Slow the Worsening of Early-Stage Parkinson's Disease in Participants Between the Ages of 30 and 80 - Full Text View - ClinicalTrials.gov. Accessed October 12, 2022. <https://clinicaltrials.gov/ct2/show/NCT05348785?term=BIIB122&recrs=ab&draw=2&rank=1>