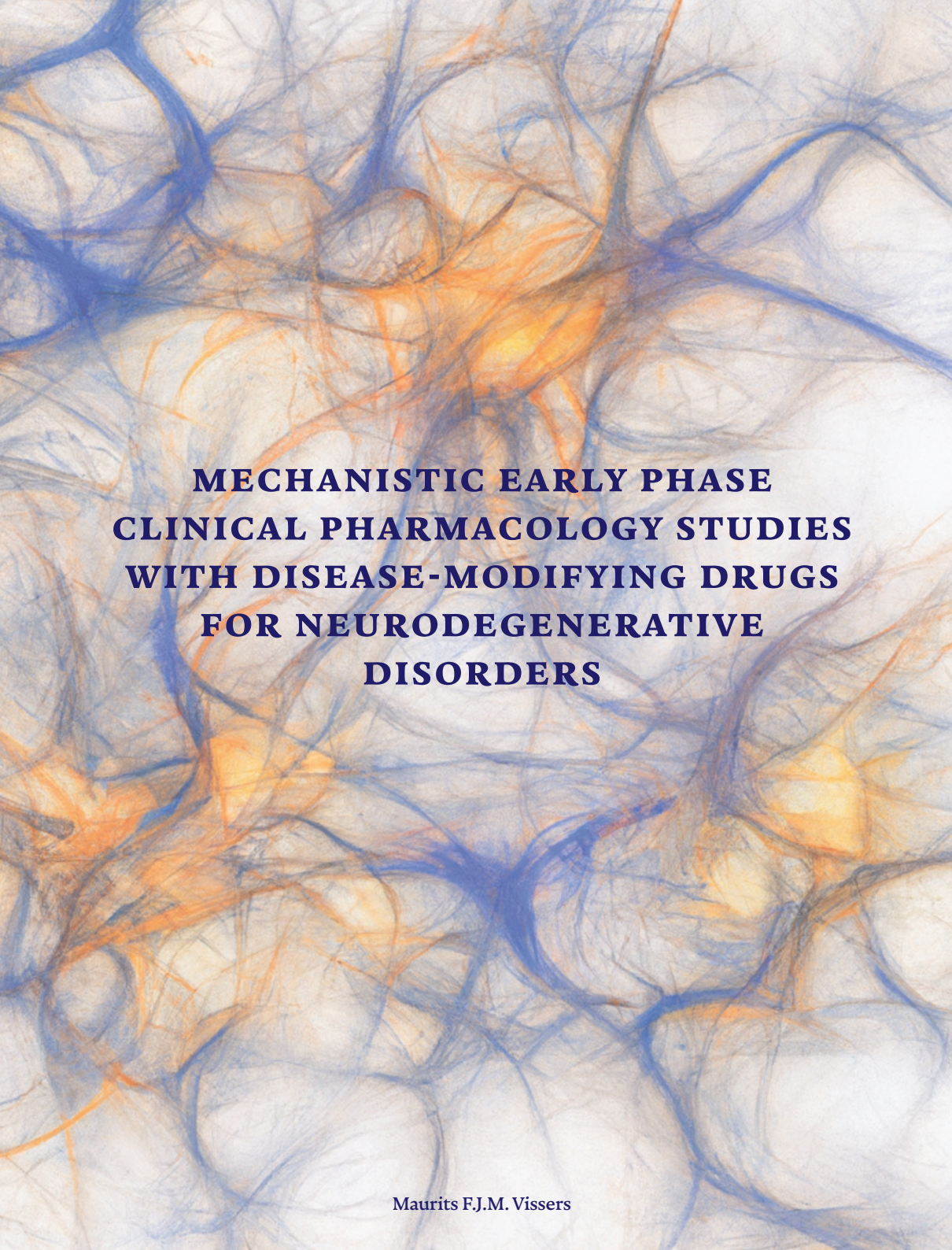

MECHANISTIC EARLY PHASE CLINICAL PHARMACOLOGY STUDIES WITH DISEASE-MODIFYING DRUGS FOR NEURODEGENERATIVE DISORDERS

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For Mia and Lotta
never stop exploring

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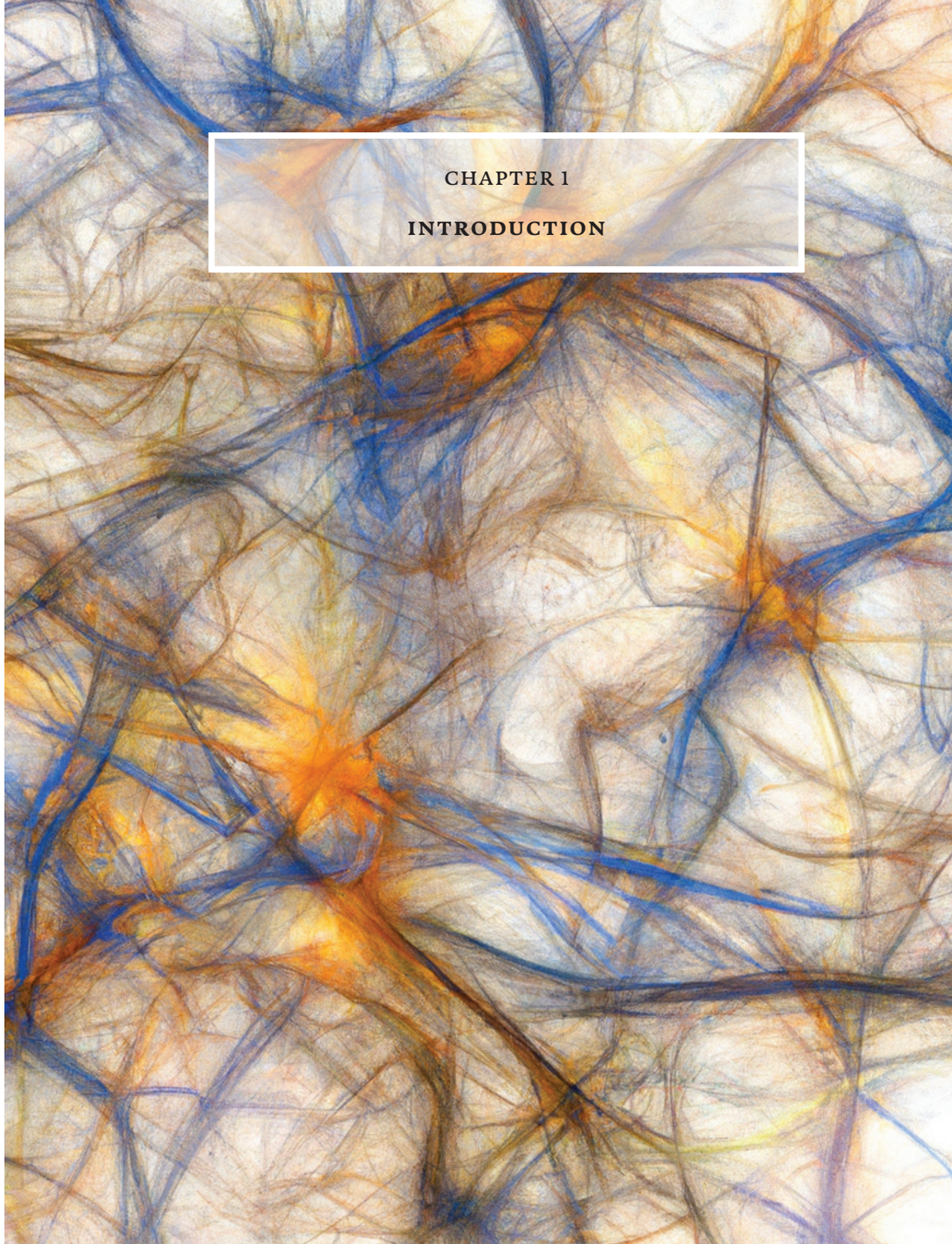

CHAPTER 1

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,[1] Parkinson's disease with an estimated 12 million patients by 2050,[2] and amyotrophic lateral sclerosis with an estimated 400 thousand patients by 2040.[3]

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.[4] 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.[5] 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[6] – 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[7] and a conversion rate of 5% to 39% from MCI to dementia in the first year.[8]

On the biological level, AD is characterized by β-amyloid (Aβ)-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.[9] 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.[10]

Aβ peptides are formed by the cleavage of amyloid precursor protein (APP) by β-secretases and γ-secretases and secreted into the extracellular space. Aβ peptides, particularly Aβ1-42 and to a lesser extent Aβ1-40, have a high tendency to aggregate into extracellular plaques. APP is enriched in neuronal synapses, and Aβ production and release are regulated by synaptic activity.[11] Aggregated Aβ interacts with metabotropic glutamate receptor 5, NMDA receptors, potentially α7 nicotinic acetylcholine receptor and insulin receptors and seems to cause pathological changes in dendritic spines and synaptic efficiency.[4]

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.[12] Synaptic activity releases TAU into the extracellular space, where it is taken up in postsynaptic neurons and glia (non-neuronal brain cells).[13] 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 Aβ plaques (dystrophic neurites).[4]

These pathological Aβ-plaques and TAU-tangles are accompanied by a loss of synaptic homeostasis, neurons and neuronal network integrity in AD.[4] Exactly how Aβ 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.[4]

Furthermore, pathophysiological alterations in the endosomal–lysosomal network and autophagy pathways can impact the clearance of extracellular material – including damaged/aggregated (Aβ 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.[4]

What exactly triggers Aβ and p-TAU to increase to pathological levels is not yet well understood, nor is it clear if Aβ and p-TAU increases are the actual underlying cause of AD. The amyloid hypothesis suggests that accumulation of Aβ in the brain is the primary influence driving AD pathogenesis,[14] 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.[12,15] 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.[16] 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.[17]

Drug development efforts focus on nearly all pathophysiological processes involved in AD, including removal of Aβ and p-TAU (anti-Aβ antibodies/immunotherapy), inhibition of Aβ production (β-secretase inhibitors), improvement of microglial function (TREM2 antibodies) or dampening of neuroinflammation (RIPK1-inhibitors). The first anti-Aβ antibody (aducanumab) was registered as treatment for AD in patients with MCI or mild dementia by the FDA in 2021[18], but following much controversy around the supportive scientific data, aducanumab's EMA application was recently retracted.[19] More recently, lecanemab, an investigational anti-Aβ 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.[20]

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.[21] 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.[22]

The main neuropathological features of PD are intracellular inclusions (Lewy Bodies) containing aggregates of alpha-synuclein (αSYN) 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 αSYN aggregates and before the onset of motor symptoms.[21,23]

Based on our current understanding, the underlying molecular pathology of PD involves multiple pathways and mechanisms including αSYN homeostasis, mitochondrial dysfunction, oxidative stress, and neuroinflammation.[21]

The exact function of αSYN protein is not fully understood, but it likely plays a role in synaptic vesicle dynamics and potentially also in mitochondrial functioning and intracellular trafficking.[24,25] αSYN 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 αSYN 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 αSYN seeds may travel in

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

In degenerating neurons in PD, αSYN aggregation is often observed together with mitochondrial dysfunction, and both processes may exacerbate each other.[21] 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[28]) and activates signaling pathways leading to nigral dopamine cell death in PD.[29]

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 αSYN aggregation, and in turn neuroinflammation itself can promote αSYN misfolding, forming a self-aggravating cycle.[21,30]

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.[31] 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 αSYN protein degradation.[21]

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.[32] 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.[33] 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 αSYN (e.g. via anti-αSYN antibodies or immunotherapy, or via targeted therapies focused on improving the lysosomal-autophagy protein degradation system [e.g. LRRK2-inhibitors and GCase enhancers]).[21] 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.[21]

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).[34] 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 ≥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).[34]

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.[34] 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.[34]

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.[34] Alterations in messenger RNA (mRNA) also seem to play a key role in ALS pathology.[35] 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.[34–36]

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.[34] Finally, similar to other neurodegenerative disorders, mitochondrial dysfunction can cause oxidative stress and DNA damage in ALS,[37] while also here neuroinflammation is considered an important factor in amplifying neuronal injury and enhancing disease progression.[34]

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.[34]

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.[34,38]

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.[39] 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[40]). 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-intense 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.[41]
- 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.[42] But this should only be done if that specific biomarker has a validated causal relation with actual disease progression.[43]

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.[44]

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. αSYN, 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 αSYN) 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.[45,46]

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.

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CHAPTER 2

TARGETING FOR SUCCESS: DEMONSTRATING PROOF-OF-CONCEPT WITH MECHANISTIC EARLY PHASE CLINICAL PHARMACOLOGY STUDIES FOR DISEASE-MODIFICATION IN NEURODEGENERATIVE DISORDERS

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ABSTRACT

The clinical failure rate for disease-modifying treatments (DMTs) that slow or stop disease progression has been nearly 100% for the major neurodegenerative disorders (NDDs), with many compounds failing in expensive and time-consuming phase 2 and 3 trials for lack of efficacy. Here, we critically review the use of pharmacological and mechanistic biomarkers in early phase clinical trials of DMTs in NDDs, and propose a roadmap for providing early proof-of-concept to increase R&D productivity in this field of high unmet medical need. A literature search was performed on published early phase clinical trials aimed at the evaluation of NDD DMT compounds using MESH terms in PUBMED. Publications were selected that reported an early phase clinical trial with NDD DMT compounds between 2010 and November 2020. Attention was given to the reported use of pharmacodynamic (mechanistic and physiological response) biomarkers. A total of 121 early phase clinical trials were identified, of which 89 trials (74%) incorporated one or multiple pharmacodynamic biomarkers. However, only 65 trials (54%) used mechanistic (target occupancy or activation) biomarkers to demonstrate target engagement in humans. The most important categories of early phase mechanistic and response biomarkers are discussed and a roadmap for incorporation of a robust biomarker strategy for early phase NDD DMT clinical trials is proposed. As our understanding of NDDs is improving, there is a rise in potentially disease-modifying treatments being brought to the clinic. Further increasing the rational use of mechanistic biomarkers in early phase trials for these (targeted) therapies can increase R&D productivity with a quick win/fast fail approach in an area that has seen a nearly 100% failure rate to date.

INTRODUCTION

While there have been successes in neuropharmacology, most central nervous system (CNS) pharmaceutical approaches treat symptoms rather than disease cause. Such symptomatic treatments can be very successful at suppressing disease symptoms at first, however, the effects eventually diminish over time and do not stop disease progression. Therefore, there is an urgent need for better treatments that can slow or stop disease progression of neurodegenerative disorders (NDDs), especially since the burden of these debilitating diseases on patients and society is on the rise as populations age.[1] Alarmingly, the clinical failure rate for such disease-modifying treatments (DMTs) for NDDs has been nearly 100% to date.[2–5] Exceptions include the approval of riluzole and edaravone as treatments for amyotrophic lateral sclerosis (ALS), however both arguably show only marginal effects.[6,7] With the recent approval of nusinersen for the treatment of spinal muscular atrophy (SMA)[8] new hope may be on the horizon.

In fact, our understanding of underlying NDD pathophysiological mechanisms is rapidly expanding,[9–13] and this has sparked a new interest in the development of (targeted) disease-modifying treatments. This is reflected for example, by the >100 compounds currently in clinical development for Alzheimer's disease[4] and close to 150 compounds in clinical development for Parkinson's disease,[14] many of which can be categorized as DMTs.

Compared to most other fields, the clinical development path of NDD DMTs faces some important additional challenges that contribute to the high failure rate experienced to date. First, preclinical and animal models have historically shown poor translatability to predict drug efficacy in human NDDs because of the complexity of the pathophysiology of neurodegenerative disorders and our incomplete understanding of these processes.[2,15,16] Secondly, in NDDs it may take a long time from disease onset to the manifestation of clinical symptoms to objectifiable disease progression and clinical trials have struggled to separate out symptomatic effects from disease-modifying effects.[2,16,17] Moreover, by the time of diagnosis significant (irreversible) damage to the CNS has often already occurred, and it has been challenging to identify robust diagnostic biomarkers to initiate treatment in earlier disease stages.[18] Thirdly, unlike diseases of most other organ systems, CNS disorders are localized to a body compartment that is not easily accessible for obtaining tissue samples in clinical studies to verify molecular pathophysiologic mechanisms and drug effects. And finally, there has been a lack of validated biomarkers as outcome measures for disease progression in disease-modification trials.[16]

However, considerable progress is being made in the development of biomarkers for NDDs,[19,20] that cannot only help diagnose or track progression of NDDs, but can also be used as tools during clinical development to demonstrate central exposure, (peripheral) target engagement and functional responses to guide dosing-decisions or facilitate patient enrichment in later stage clinical trials.[21] In particular, peripheral biomarkers for their relatively easy clinical accessibility hold a promise to help overcome some of the fundamental challenges in CNS drug development and allow for more efficient screening of drug candidates in early-phase clinical trials.[22] In a field where nearly 100% of investigational drugs fail to make it to market, the use of such biomarkers can offer an indirect yet relatively quick strategy

to confirm (peripheral) target and pathway-engagement and provide early proof-of-concept in short-duration mechanistic early-phase trials in both healthy volunteers and patients.[23,24] This quick win / fast fail approach can increase research and development (R&D) productivity and help guide dosing-decisions for maximizing success rates in later stage trials.[25]

Here we present a review and a roadmap for the use of pharmacodynamic biomarkers in early phase clinical trials of DMTs in NDDs. First, we present an introduction on NDD mechanisms, considerations for drug development of innovative disease-modifying compounds and the role of biomarkers in clinical drug development for context. Then we categorize the pharmacodynamic biomarkers that were reported in early phase clinical pharmacology studies identified from a literature review of the past decade, including an overview of bodily sources that can be used for biomarker analysis, and present considerations for biomarker selection in early clinical development. Finally, we summarize and conclude this overview with a proposal for a roadmap for designing mechanistic, data-rich early phase clinical pharmacology studies for disease-modifying therapies in neurodegenerative disorders.

NEURODEGENERATIVE DISEASE MECHANISMS

Neurodegenerative disorders, including Alzheimer's disease (AD), frontotemporal- (FTD) and Lewy body dementia (LBD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD), and spinocerebellar ataxias (SCAs), are characterized by a progressive degeneration of neurons in various regions of the brain and result in losses in cognitive and/or motor function.[26,27] As it appears, these NDDs share multiple overlapping pathological mechanisms including misfolding, aggregation, and accumulation of proteins, dysfunctional mitochondrial homeostasis, formation of stress granules, and maladaptive innate immune responses eventually leading to cellular dysfunction, loss of synaptic connections, and brain damage.[28,29] In AD amyloid-β protein fragments that cluster together and form amyloid plaques, as well as TAU proteins forming neurofibrillary tangles, disrupt neurological functioning and contribute to neurotoxicity leading to inflammation and neuronal cell death. In PD clumping of α-synuclein into so-called Lewy bodies in dopaminergic neurons is believed to play an important role in neuroinflammation and eventually neurodegeneration, while in ALS the aggregation of TAR DNA-binding protein 43 (TDP-43) in cell stress granules may contribute to disease pathology, neuroinflammation and motor neuron death. Because of an overlap in the underlying pathological mechanisms, as well as involvement of the same cell types, it is not surprising that many DMT mechanisms under development often target multiple NDDs. For example, inhibition of receptor-interacting serine/threonine-protein kinase 1 (RIPK1), a regulator of inflammation, cytokine release, and necroptotic cell death, is being investigated as treatment for AD, ALS and multiple sclerosis (MS),[30] while TAU protein is being targeted with antibodies for both progressive supranuclear palsy (PSP) and AD.[31] In addition to the more general mechanisms of neurodegeneration, genetic studies have begun identifying risk-associated alleles and disease-causing rare mutations in NDDs.[13,32] These genetic studies may pave the way for targeted therapies in selected subpopulations, such as an antisense oligonucleotide targeting the mutated superoxide dismutase (SOD1) enzyme in ALS,[33] or glucocerebrosidase (GBA)-activators or leucine-rich repeat kinase 2 (LRRK2)-inhibitors targeting disease-causing mutations in GBA or LRRK2 respectively in Parkinson's disease.[34]

INNOVATIVE DRUG DEVELOPMENT OF DISEASE-MODIFYING TREATMENTS

The development of innovative disease-modifying treatments for these NDDs with novel mechanisms of action is radically different from the development of a generic version of an existing effective drug from a well-established class.[25] For innovative compounds, the uncertainty about the different aspects of the drug is far greater, which is also reflected in the high clinical failure rate in the field of DMTs for NDDs. This uncertainty requires a high level of flexibility in the drug development program, the use of innovative methods and a high level of integration of information rather than the purely operational requirements of a generic development program.[25] Innovative drug development in essence starts with the preclinical development of assays to identify and validate a novel pharmacological target and subsequently demonstrating safety and efficacy in a (relatively standardized) battery of laboratory and animal studies. Hereafter the clinical development trajectory starts in humans and revolves around answering a set of 6 basic scientific questions in a series of what are traditionally called phase 1-3 clinical trials:

1 What is the safety and pharmacokinetic behavior of the drug?
2 Does the drug occupy the intended pharmacological target?
3 Is the drug capable of activating the target?
4 Does this target activation lead to the intended physiological response?
5 And subsequently to the intended pathophysiological response?
6 And does the drug result in a sufficient clinical response?[25]

Traditionally these questions are addressed in a chronological order, starting with small-scale phase 1 clinical studies focusing on safety and pharmacokinetics in healthy volunteers or patients and ending with large-scale, often global and multi-center, phase 3 studies to demonstrate safety and efficacy versus placebo or an active comparator in the intended drug label target population. However, as stated above, drug development does not need to take this linear approach. Especially if one considers that development becomes more and more expensive the further a compound progresses into later stage trails. In fact, for truly innovative compounds such as the development of DMTs in NDDs, there is a strong scientific and financial argument to be made to demonstrate proof-of-concept for a new compound in humans as early as possible.[35] From a scientific perspective, an early demonstration of proof-of-concept helps focus future efforts to the most promising leads. From a financial perspective early proof-of-concept contributes to a quick win / fast fail development approach thereby increasing R&D productivity and preventing investments in compounds only to fail in the most expensive later stages of drug development.

Demonstrating proof-of-concept of DMTs in early-stage trials is challenging, however. Considering the definition of a neurodegenerative DMT: 'an intervention that produces an enduring change in the clinical progression of the NDD by interfering in the underlying pathophysiological mechanisms of the disease process leading to cell death',[36] proof-of-concept for the first part of this definition is difficult to demonstrate because of the short-duration of early phase clinical trials. Moreover, traditional clinical outcomes – such as disease progression scales or patient-reported outcomes (PROs) – are not suitable for demonstrating effects of DMTs in NDDs in healthy subjects for a lack of disease, nor in patients because of the general short duration and small group sizes in phase 1 trials and large placebo-effects in PROs often seen in these patient populations. The ability of an investigational compound to 'interfere in the underlying pathophysiological mechanisms leading to cell death' on the other hand, is something that could be demonstrated with the use of pharmacodynamic biomarkers in short-duration early phase trials, even in healthy subjects.

BIOMARKERS

A biomarker (biological marker) is defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention'.[37] When the level of a biomarker changes in response to exposure to a medical product, it can be called a response or pharmacodynamic biomarker.[38] Other types of biomarkers can include diagnostic biomarkers (detecting or confirming the presence of a disease), predictive biomarkers (presence or change in the biomarker predicts an individual or group to experience a favorable or unfavorable effect from the exposure to a medical product), prognostic biomarkers (identify the likelihood of a clinical event, disease recurrence, or disease progression in untreated patients) and safety biomarkers (indicates the likelihood, presence, or extent of a toxicity as an adverse event)[38,39] – *Table 1*. In some cases a biomarker can be used as surrogate to substitute for a clinical endpoint, but to qualify as a surrogate, a biomarker must correlate with the clinical outcome and the change in the biomarker must also explain the change in the clinical outcome;[38] evidence that is currently lacking for the majority of biomarkers.

Recent reviews have described the current status of biomarkers in ALS,[40] Alzheimer's disease,[41] Parkinson's disease,[42] Huntington's disease,[43] and spinocerebellar ataxias,[44] although for most of these indications reliable indicators of disease severity, progression, and phenotype are still lacking.

EARLY PHASE PROOF-OF-CONCEPT WITH MECHANISTIC BIOMARKERS

Even without a proven correlation with clinical outcome, biomarkers are useful in early phase trials of DMTs for NDDs. At this stage of development, it is more important and feasible to demonstrate that the investigational drug engages its molecular pathway in humans as envisioned (mechanistic proof-of-concept). This can be accomplished with mechanistic biomarkers, by demonstrating pharmacologic activity of the compound both in healthy subjects as well as patients, allow for the application of mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) modelling,[46] and help define the optimal dose for phase 2/3 efficacy trials. This maximizes the eventual chance of clinical development success, or can save valuable resources by supporting an early 'no-go' decision in case the compound fails to reach or appropriately modulate its target.[21,47] In fact, disease specific regulatory guidance for drug development in NDDs also recommends the use of biomarkers in the early phases of the clinical development to:

1 Establish the pharmacological mechanism(s) on which the drug may be thought to have therapeutic activity.
2 Demonstrate target engagement and proof-of-concept.
3 Determine the PK/PD relationship and the dose-response curve.[48–50]

Additionally, by including a pharmacological effect or target engagement biomarker in a first-in-human (FIH) study, the dose-response curve in humans can be linked to the non-clinical experience, thereby supporting more informed dose escalation decisions. This is especially true for innovative drugs with a novel mode of action, where the relationship between the minimally pharmacologically active dose and a safe therapeutic dose in humans is not yet known.[51] Inclusion of a pharmacodynamic measure in FIH trials is now also recommended by the regulatory bodies for safety reasons.[52]

REPORTED USE AND CLASSIFICATION OF EARLY CLINICAL PHASE BIOMARKERS

As indicated above, biomarkers can play an important role in early phase drug development. To investigate the current use of pharmacodynamic response biomarkers for the development of DMTs for NDDs, a literature search was performed for published early phase clinical trials using medical subject headings (MESH) terms in PUBMED (*Supplement 1, available online via chapter reference*). Publications between 2010 and November 2020 were selected that reported an early phase clinical trial with NDD DMT compounds. Publications of early phase trials identified from references in the reviewed literature that were not identified by the MESH search strategy were also included. Only the first and original reports of early phase clinical trials were selected to avoid duplication (*Supplemental Figure S2*). An overview of all included trials and the reported peripheral and central pharmacodynamic biomarkers is presented in *Table 2*.

The early clinical phase pharmacodynamic response biomarkers retrieved from this search can be subdivided into proximal mechanistic biomarkers that are primarily used to demonstrate target occupancy and target activation (target engagement), and physiological and pathophysiological response (distal) biomarkers (*Table 1*).[25,46]

Overall, 89 out of 121 (74%) NDD DMT early phase trials that were published over the past decade reported the use of one or more pharmacodynamic response biomarkers (*Figure 1*). Given the significant added value of using pharmacodynamic response biomarkers in early phase trials this might not be surprising. Less than half of all trials (46%) reported the use of central pharmacodynamic biomarkers. The use of peripheral pharmacodynamic biomarkers was slightly higher at 50%. Only 65 trials (54%) reported the use of proximal mechanistic biomarkers (*Figure 1*) and there are clear differences in the use of biomarkers between different disorders and different types of drugs (*Table 2*).

Clinical outcome data was collected even more frequently in early clinical phase NDD trials (74% of all trials involving patients, or 60% of all trials) than mechanistic biomarker read-outs (54% of all trials) (*Figure 1*). This despite the fact that early phase trials are often of too short a duration and have a too limited sample size to expect a significant effect on any clinical or surrogate response biomarkers.

In the next sections we will break down the different types of identified biomarkers. For each stage of drug development, these different types of biomarkers can help answer different relevant clinical development questions, see also *Figure 2*.

Target occupancy

Only 26% of early clinical phase NDD DMT trials reported target occupancy biomarkers (*Figure 1* and *Table 2*). Target occupancy in first-in-human studies is used to demonstrate that the same target binding observed in the preclinical animal models holds true in humans.[171] The importance of this from a safety perspective is exemplified by the clinical study with the CD28 targeting immunomodulating agent, TGN1412. Because of differences in TGN1412 pharmacology between nonhuman primates and humans, the starting dose of the FIH trial directly resulted in 90% receptor occupancy, leading to life-threatening cytokine release syndrome in healthy volunteers.[172,173]

Demonstrating target engagement is also critical from the drug-development perspective. When a novel compound fails to demonstrate disease-modifying properties and no target engagement data is available, it will be difficult if not impossible to conclude whether the mechanism of action does not produce NDD disease-modification per se, or if this specific compound was just not successful in sufficiently engaging the intended target in humans.[174,175]

Ideally target occupancy is demonstrated by biomarker evidence of:

1 The compound reaching its site of action;
2 The compound binding to the intended molecular target;
3 Occupancy of the target increases with increasing dose.

Demonstrating that a compound reaches its site of action is one of the major challenges in CNS drug development, and in fact often not even possible to demonstrate directly (except post-mortem). As an alternative, often the presence of the compound at pharmacologically active concentrations in the cerebrospinal fluid (CSF) is used as a surrogate for CNS exposure.[2,23,30,54] While this is not an absolute guarantee that the compound reaches its site of action in the brain, it does provide a relatively uncomplicated method (it

can even safely be used in pediatrics)[176] to demonstrate that the compound does cross the blood-brain barrier in sufficient concentrations to expect an effect based on preclinical cellular dose-response assays. In addition, further translational approaches can be used to predict human brain distribution and target site kinetics.[177]

Besides measuring compound concentration in CSF, positron emission tomography (PET) can be used to demonstrate compound distribution into specific brain compartments and can in some cases also be used as a direct occupancy assay for receptor, transporter or enzyme targets.[178,179] However, PET imaging cannot always be applied for the lack of an appropriate radioligand or unfavorable radioligand characteristics, e.g. high non-specific binding.[159] Actual binding of the compound to the molecular target could in some cases be demonstrated in the CSF, for example for monoclonal antibodies binding to a circulating extracellular target protein such as amyloid β[54–56,60] or α-synuclein[146] (*Table 2*). However, this may not always be possible because assays are either not sensitive enough to detect the low abundance pathological target (e.g. aggregated αSYN concentrations in CSF) or drug concentrations in the CSF are not sufficient to demonstrate an effect on a more abundant surrogate biomarker (e.g. total αSYN in CSF).[145]

For (intra)cellular targets in CNS tissue it may be even more difficult to demonstrate that the compound binds the intended molecular target, mainly because of the fact that these cellular molecules are likely not present in biofluids in detectable amounts and the target neuronal cells cannot be sampled from living human beings for cell lysis and subsequent target engagement assays. In these cases, an alternative indirect strategy could be to demonstrate target engagement in peripheral cells, on the condition that the molecular target is expressed in these cells. For example, peripheral receptor occupancy on cell surfaces can be measured with the use of flow cytometry on fresh blood.[180] In a similar fashion, intracellular target occupancy can be demonstrated peripherally in blood cells such as done for LRRK2-inhibitor binding measured via the dephosphorylation of SER935 on the LRRK2 protein in lymphoblastoid cells,[181] or the reduction of phosphorylated S166 RIPK1 in peripheral blood mononuclear cells (PBMCs) after dosing of an RIPK1-inhibitor.[30] When combined with the plasma-to-CSF drug concentration ratio, such peripheral target occupancy can give an indirect indication of expected target occupancy in the CNS.

Target activation

After confirming that a novel compound occupies its molecular target, the next step in innovative clinical development is to demonstrate that upon target occupation the investigational compound activates the intended molecular pathway to a sufficient extent for possible disease modification (*Figure 2*). Such mechanistic proof-of-concept can often be demonstrated by evaluating a substrate biomarker that is downstream in the pathway of the compound's direct molecular target. When quantitatively measured, changes in such a so-called 'pathway activation biomarker' at different dose-levels can help generate a dose-response curve of the investigational compound's agonistic (stimulatory or inhibitory) molecular effects. This dose-response curve can be linked to the preclinical *in vitro* and animal model studies to determine a human dose level at which maximum disease modification can be expected in patients. Target activation biomarkers have been used more frequently than target occupation biomarkers, but still only 40% of early clinical phase NDD DMT trials reports the use of target activation biomarkers (*Figure 1*).

An example of a molecular pathway activation biomarker is the quantification of amyloid β1-42 (Aβ) concentrations in the CSF in response to BACE1-inhibitors (*Table 2*).[84–90] BACE1 (β secretase) is a protease that cleaves the amyloid precursor protein at the β-site, which eventually leads to the production and release of Aβ peptide in the brain. A decrease in Aβ brain concentrations may help prevent the progression of Alzheimer's disease.[182] However, as indicated before, such an apparently obvious relationship between the molecular pathway activation biomarker to the neurodegenerative disease that the compound is being develop is not a necessity. It is more important that the biomarker has a direct relationship to the true molecular target that the investigational compound activates or inhibits, and that the biomarker can reliably be measured with a robust and validated assay. An example is the quantification of phosphorylation of RAB10 (pRAB10), a bonafide substrate of LRRK2 kinase activity, in response to the administration of LRRK2-inhibitors under development for Parkinson's disease.[183] The fact that at the time of discovery it was not entirely clear how the activity of RAB GTPases contributes to degeneration of the nervous system[184] does not impact the usability of pRAB10 as target activation biomarker to quantify the inhibitory effects of LRRK2-inhibitors.

Similar to target occupancy, it may not always be possible to demonstrate target activation in the CNS, especially for intracellular molecular pathways, in which case an alternative strategy can also be to demonstrate target activation peripherally in blood or tissues expressing the same molecular target (*Figure 2*).[120,126,127,130]

Demonstrating target activation can be complicated by the fact that the targeted molecular pathway activation status may only be present in diseased tissue. For example, RIPK1 regulates inflammation, cytokine release, and necroptotic cell death and inhibition of RIPK1 activity protects against inflammation and cell death in multiple animal models. RIPK1 is also expressed in circulating PBMCs offering a peripheral opportunity to demonstrated target activation of RIPK1-inhibitors. However, in these non-diseased PBMCs RIPK1 activity levels will not be similar to that in the CNS of ALS and AD patients. To overcome this problem and quantify the effects of different dose levels of a RIPK1-inhibitor peripherally, PBMCs can be collected from study subjects after dosing and then be stimulated *in vitro* with e.g. the pan-caspase inhibitor ZVAD-FMK (TSZ) to stimulate these cells to increase phosphorylated RIPK1.[30] In a similar fashion, lipopolysaccharide (LPS) has been used in an early phase study in MS patients to stimulate 6-SULPHO LACNAC+dendritic cells *in vitro*, to demonstrate that laquinimod therapy is capable of reducing CD83 expression and TNF-α production.[138] The possibility to demonstrate target activation *in vitro* in human cells is supported by regulatory guidance,[50] and could be used to demonstrate target activation in first-in-human studies with healthy volunteers.[30] Some molecular targets are really only present in patients with the target disease, such as mutated huntingtin protein in patients with Huntington's disease. In such a case the best strategy may therefore be to directly include patients in the earliest clinical trials, to be able to demonstrate target activation as early as possible in the clinical development trajectory.[131]

Other types of target activation biomarkers may be used for different classes of investigational drugs (*Table 2*). For example, in the case of immunotherapy target activation could be demonstrated by the formation of antibody titers in plasma,[156] and in the case of an antisense oligonucleotide target activation may be demonstrated by a reduction in target protein levels.[33,167] For other types of drugs such as monoclonal antibodies against amyloid β[53,54,63,55–62] it may not be possible to demonstrate target activation, as the goal of these treatments is to clear the molecular target either by macrophage phagocytosis and complement activation or by altering the equilibrium of amyloid across the blood–brain barrier in favor of efflux from the brain to the blood.[185]

Physiological response

Physiological response biomarkers are reported in 23% of early phase NDD DMT clinical trials (*Figure 1*). These provide insight into more general or systemic (distal) responses to the investigational compound that are expected to contribute to, or be indicative of, possible disease modification. Examples of physiological response markers that have been used in early phase NDD DMT clinical trials include the evaluation of brain glucose metabolism after administration of nerve growth factor gene therapy[68] or deep brain stimulation[76,78] for Alzheimer's disease, and CSF cytokine production after transfusion of stem cells[101] or administration of granulocyte colony-stimulating factor (G-CSF)[115] in ALS patients (*Table 2*). However, it is important to realize that while such biomarkers can indicate that a compound exerts a physiological response, they often do not provide direct information about the actual clinical effects of the compound,[25] nor that the intervention can produce an enduring change in the clinical progression of the NDD. Nevertheless, when combined with target occupancy and activation biomarkers, physiological response biomarkers can contribute to the total amount of evidence for proof-of-concept (*Figure 2*). Additionally, physiological response markers can offer an opportunity to get a better understanding of an intervention's potential effects when no direct molecular target is involved or when the exact mechanism of action is not yet fully understood, e.g. in the case of stem cell trials in ALS patients (*Table 2*).[101,104]

Pathophysiological response

Pathophysiological response biomarkers are also distal biomarkers, and contrary to the physiological response biomarkers, should have a clear and direct link to the disease pathophysiological mechanisms. For early phase trials these biomarkers do not necessarily need to be validated surrogate substitutes for clinical endpoints, however, when available, a validated surrogate would of course provide stronger evidence for possible disease modification. It should be considered though that most early phase trials are only of a short duration and for most NDDs the disease progresses too slow to measure a significant change over a short period of time. Moreover, early phase trials usually only recruit small sample sizes and there can be significant interindividual variation in disease phenotype and progression. Therefore, chances are that it may not be possible to demonstrate a significant effect of the investigational compound on pathophysiological response biomarkers in early phase trials, which would not necessarily equal a lack of effect of the investigational compound. It is therefore not surprising that pathophysiological response biomarkers are only reported in 33% of early phase clinical trials involving patients (*Figure 1*). In healthy volunteer studies pathophysiological response biomarkers obviously cannot be included for a lack of disease presence.

Examples of pathophysiological response wet biomarkers that have been used in early phase NDD DMT trials include quantification of CSF TAU phosphorylated at threonine 181 (p-TAU181)[54,60] and evaluation of amyloid β by PET[75] for Alzheimer's disease pathology, phosphorylated neurofilament heavy chains (and post-hoc neurofilament light chain) concentrations as general axonal damage biomarker in ALS,[33] FTD,[129] and Huntington's disease,[131] and CSF mitochondrial dysfunction markers (GDF15, lactate) in MS (*Table 2*).[139] Other types of more physical pathophysiological response biomarkers include the evaluation of retinal nerve fiber layer thinning in MS[139] and electromyogram (EMG) study of the tibialis anterior muscles in ALS patients receiving stem cell treatment.[109] Also neuroimaging techniques can be used as pathophysiological response biomarkers, such as the evaluation of disease progression via dopaminergic function with the use of 18F-DOPA PET,[153] or reduction of whole brain or hippocampal atrophy (MRI) or reduction of cerebral metabolism on fluordeoxyglucose (FDG) PET,[36] although it is unlikely that an effect on these markers can be observed in short-duration trials.

Clinical response

It appears that clinical outcomes are most frequently included (74%) as exploratory endpoints in early phase trials with NDD patients (*Figure 1*). These clinical outcome measures included disease rating scales [e.g. Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-COG),[53,70,73,78] Mini-Mental State Examination (MMSE),[58,61] Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R),[33,106,119] Neuronal Ceroid Lipofuscinosis Type 2 Clinical Rating Scale (CLN2 score),[143] Unified Huntington's Disease Rating Scale (UHDRS),[132] Hammersmith Functional Motor Scale Expanded (HFMSE),[167] and Movement Disorders Society Unified Parkinson Disease Rating Scale (MDS-UPDRS)[153,154,161]], pulmonary functioning evaluation,[100,128] muscle power assessments,[99,103,113] and quality of life questionnaires.[68,120,152] We would argue, however, that due to small samples sizes in early phase trials, potentially significant placebo effects or sometimes lack of a placebo control, and the relatively low sensitivity of these disease rating scales such instruments may at best be useful as safety biomarkers but not as outcome markers at this stage of clinical development. Even in longer-duration open label extensions of early phase trials clinical outcomes are not expected to yield reliable results because of the small sample sizes and lack of a placebo control.[186] However, the high percentage of early phase trials reporting clinical outcomes may result from regulatory guidance that recommends to explore clinical outcomes in early phase trials to investigate how these can be further used in subsequent pivotal trials.[49] A more sensitive future tool for assessing exploratory clinical outcomes on disease progression could be the use of continuous digital biomarkers, such as smartphone-based assessments.[187]

BIOMARKER SOURCES

Cerebrospinal fluid (31% of trials) and blood (45% of trials) are the most frequently used biofluids for biomarker analysis in NDD research. These biofluids are relatively easily accessible in the clinical setting and well-established bioanalytical methods for these matrices are available. CSF could arguably be the most proximal source for physiological and pathological response biomarkers related to the intended CNS target. Moreover, concentrations of CNS biomarkers outside of CSF are often extremely low making them difficult to detect using standard assays, and in blood endogenous antibodies and proteases may be present that interfere with assays or shorten the lifespan of peripheral protein biomarkers.[18] However as discussed previously, mechanistic proof-of-concept of target engagement by DMT compounds can often be demonstrated very well peripherally without being hampered much by such challenges. Moreover, NDDs are found to also be influencing some peripheral tissues outside the CNS.[188] Therefore, in early stage drug development pharmacodynamic biomarkers can be used from a large variety of bodily sources (*Table 2*). Besides whole blood, plasma or serum, leukocytes and in particular the subset of PBMCs can be an easily accessible source for evaluating intracellular pathways *ex vivo*, which also offers the possibility to simulate disease states (also in heathy volunteer studies). When working with PBMCs though, it is important to realize that these cells represent a heterogeneous group that includes lymphocytes, monocytes and macrophages and the molecular target of interest may not be expressed to similar levels in all of these cells. For example, LRRK2 kinase and its direct substrate RAB10 are only abundantly expressed in monocytes and are virtually undetectable in B and T lymphocytes as well as natural killer and dendritic cells that constitute most of the PBMCs.[189] Moreover, both these proteins are expressed to an even higher degree in neutrophils, making neutrophils potentially the best source for demonstrating mechanistic proof-of-concept of LRRK2-inhibitors.[189] Another easily accessible biofluid that can be a source for biomarker analysis is urine,[190] but also more challenging matrices, such as stool samples, ocular fluids, and mucosal secretions can be considered for biomarker analyses.[191] The challenge of accurate analysis, however, is much higher in such matrices

and therefore feasibility of sampling as well as analyte extraction should be considered and demonstrated prior to implementation in clinical trials.[191] Also tissue biopsies, such as from muscle[99] or nasal olfactory neural tissue,[192] and surgical byproducts[191] can be considered as sources for biomarker analysis. And even the body surface has proven to be an easily accessible source for biomarker analysis in NDD drug development via the use of skin fibroblasts[193] and hair follicle RNA.[194]

As there may be relatively large intra- and interindividual variability in some of the biomarkers in these matrices, it could be necessary to normalize the biomarker readouts to a quantifiable reference value to draw more robust conclusions between different sampling times and individuals. This is especially important given the small numbers of subjects usually included in early phase trials. Examples of normalization factors used in biomarker analysis include normalization to total protein or creatinine to correct for the number or concentration of cells in a specific sample or matrix for gene expression analysis,[191] relating analysis of SOD1 activity in erythrocytes to the content of hemoglobin in erythrocyte lysates,[120] relating phosphorylated glycogen synthase (GS) to the total levels of GS,[129] and using the survival of motor neuron 2 full length (SMN2FL)/SMN2Δ7 mRNA ratio to reduce the confounding effects of SMN2FL and SMN2Δ7 mRNA level fluctuations for monitoring the inclusion of SMN2 exon 7 and the effect of risdiplam.[169] Also using patients as their own controls with crossover designs in early phase clinical trials helps limit the potential effects of often large intersubject variability in studies with small numbers of subjects.[81] Finally it can be worth considering using patient enrichment strategies for early phase trials,[195] to optimize the chance of success in demonstrating proof-of-concept by including the most suitable patient population (e.g. with a specific genetic mutations, disease onset state, or a slow or fast disease progression prognosis). The scientific benefit of targeting a specific subpopulation, however, should be balanced to the recruitability of the trial and potentially the targeted mode of action.

BIOMARKER SELECTION, DEVELOPMENT, AND VALIDATION

The decision to evaluate biomarkers in early phase clinical trials should be taken well in advance in order to select appropriate biomarkers to address the key scientific early phase clinical development questions and develop robust bioanalytical methods.[25,191] In fact, the biomarker strategy planning for first-in-human studies should ideally start during the preclinical development phase (*Figure 2*). Steps to consider when selecting biomarkers for use in early phase clinical trials include defining the scientific questions that the biomarker should help answer, performing a thorough literature review to select fit-for-purpose biomarker, bioanalytical method development or assay and laboratory selection, analytical model validation testing, and defining the clinical sampling, data reduction and analysis strategy.[191,196] Preferably the selected biomarkers are validated in the preclinical models used during drug development as well as in patients or patient biofluid repositories.[197] Characteristics to select a useful biomarker include that the biomarker should give a consistent response across studies and drugs with the same mode of action, must respond clearly to therapeutic doses, must have a clear dose-response relationship and ideally there should be a plausible relationship between the biomarker, pharmacology of the drug class, and disease pathophysiology (although for mechanistic biomarkers this not an absolute necessity as discussed previously).[25]

Biomarkers used in early phase clinical development do not fall under standardized regulatory requirements and therefore the clinical development team has to decide on the level of method characterization and documentation that is needed by weighing how the biomarker may provide the most value to the clinical development program goals.[191] For an early go/no-go decision a qualified assay may fit the purpose, whereas for proof-of-concept of clinical responses a fully validated method may be required.[191] Some biomarkers used in early phase trials may evolve over time to become diagnostics or surrogate endpoints, but this requires the biomarkers to become accepted for use through submission of biomarker data during the drug approval process or via the biomarker qualification program developed by the Center for Drug Evaluation and Research.[39]

LIMITATIONS

It is clear that the use of pharmacodynamic biomarkers in early phase clinical trials can help optimize clinical development in an area that has seen a near 100% failure rate to date, and that the frequency of rational use of these pharmacodynamic biomarkers should be improved (*Figure 1*). However, the use of pharmacodynamic biomarkers in itself is obviously not a guarantee for clinical development success. There are still some major challenges that the development of DMTs for NDDs faces that the use of biomarkers will not be able to solve.

DMT development has been struggling with a poor translatability of preclinical and animal models to human disease,[15] though in the past decade great advances have been with neurons derived from induced pluripotent stem cells (IPSCs) and 3D cell cultures technologies as preclinical models for neurodegenerative diseases.[198] While the use of biomarkers will not directly impact the quality of the animal models, biomarkers may help identify subsets of patients or early versus late stage disease states to better align the preclinical work with the target population for human proof-of-concept studies. Moreover, when preclinical and early stage clinical biomarker programs are well aligned, they can help demonstrate early proof-of-concept and translatability of target engagement in humans. Especially when combined with upcoming preclinical or translational PK/PD modeling and simulation (M&S) techniques,[199] mechanistic biomarkers can in this way contribute to early 'go/no-go' development decisions and thereby help improve R&D productivity in the development of NDD DMTs.

Another challenge for the development of DMTs for NDDs is that our current disease understanding or hypotheses may be wrong, and that even when biomarkers demonstrate target engagement in humans there may be no clinical disease-modifying effects of the compound.[2] However, in this case it is essential that target engagement was demonstrated in the early phase trials, as this would point towards limited clinical relevance of the targeted pathway as a whole, rather than possibly just a lack of effect of the specific compound itself.

The usefulness of biomarkers must also not be overestimated. Early phase clinical trials may be of too short a duration to demonstrate an effect on disease progression biomarkers and therefore a lack of effect on a pathophysiological response marker in early phase trials does not necessarily mean that there can be no long-term clinical effect. Another caveat to be aware of is that treating a biomarker may not treat the disease, as has become clear in the development of anti-amyloid therapies. While anti-amyloid antibodies, BACE inhibitors, and γ-secretase inhibitors all demonstrated target engagement in early phase trials, they all subsequently failed to demonstrate clinical effect in later stage trials.[200] This could potentially indicate that targeting amyloid β may after all not contribute to disease modification in Alzheimer's disease, or that amyloid β-targeting therapies need to be administered in a much earlier disease state for which we currently still lack robust diagnostic biomarkers.

Moreover, as no single one biomarker to date has been demonstrated to be indicative of NDD disease progression, it is recommended to use multiple response biomarkers when available to establish a pattern or fingerprint of treatment effects,[201,202] contributing to the overall persuasiveness of proof-of-concept for a disease-modifying effect.

Finally, it should be kept in mind that developing a robust biomarker strategy can be a very lengthy and time-consuming process, and this process should therefore already be initiated well in advance of the first-in-human studies. This requires a strong collaborative effort between the preclinical scientists and the clinical development team to ensure a seamless integration of the preclinical and early-stage clinical biomarker strategies,[25] which in the end might prove to be the most critical parameter for success in early stage NDD DMT development.

ROADMAP FOR MECHANISTIC, DATA-RICH EARLY PHASE CLINICAL PHARMACOLOGY STUDIES

Over the past decade the toolbox for early phase clinical development for NDDs has expanded significantly, which will hopefully help bring the first DMTs to patients in the decade to come. In AD (79%) and PD (71%) pharmacodynamic biomarkers by now have a well-established role in early clinical development, but in for example ALS (52%) and PSP (25%) there is still room for significant improvement (*Table 2*). In *Figure 2* we therefore propose a best-practice roadmap for mechanistic, data-rich early phase clinical pharmacology studies for disease-modifying therapies in neurodegenerative disorders. Even if modifying the course of NDDs could ultimately prove to require a multi-drug approach, it will remain essential to clearly demonstrate pathway engagement of each individual drug component to get to rational multi-drug treatment regimens.

CONCLUSION

As our understanding of NDDs is improving, there is a rise in potentially disease-modifying treatments being brought to the clinic. Further increasing the rational use of mechanistic biomarkers in early phase trials for these (targeted) therapies can increase R&D productivity with a quick win / fast fail approach in an area that has seen a nearly 100% failure rate to date.

Table 1 Biomarker categories and examples of use in NND DMT drug development (adapted from Cummings and Amur et al).[39,45]

Biomarker category	Use in drug development	Examples from NND DMT drug development
Response	Pharmacodynamic biomarker as indicator of intended drug activity • *Proximal* (molecular target occupancy and activation) • *Distal* ([patho]physiological response) Efficacy response marker as a surrogate for a clinical endpoint	CSF total amyloid-β and fragments in response to amyloid-β antibody treatments Braak staging with TAU PET as a surrogate biomarker for clinical AD (though no validated surrogate biomarkers are available yet for NDDs)
Diagnostic	Patient selection	GBA1 gene mutation in PD patients SOD1 gene mutation in ALS patients
Predictive	Patient stratification Trial enrichment via inclusion criteria	TAU PET to identify AD patients more likely to respond to anti-TAU therapies
Prognostic	Patient stratification Trial enrichment with patients likely to have disease	Percentage of weight loss at baseline for life expectancy and disease progression in ALS patients
Safety	Detect AEs and off-target drug responses	MRI for structural changes (including tumor or syrinx formation) within the brain after stem cell transplantation for ALS

Figure 1 Percentage of early clinical phase reporting the use of different categories of pharmacodynamic biomarkers and clinical outcomes. Thirty-one trials (26%) reported the use of target occupancy biomarkers and forty-eight trials (40%) reported the use of a target activation biomarkers. Sixty-five trials included at least 1 proximal (mechanistic) biomarker (target occupancy and/or activation). Twenty-eight trials (23%) reported the use of physiological response biomarkers. Thirty-two trials used patho-physiological response biomarkers, which comes down to 33% of all early phase NDD DMT trials (98) that were performed in patients. Forty-seven trials (39%) reported the use of at least 1 distal biomarker. In total 89 of 121 trials reported at least one pharmaco-dynamic biomarker and seventy-three trials reported clinical outcomes, which comes down to 74% of all early phase NDD DMT trials (98) that were performed in patients.

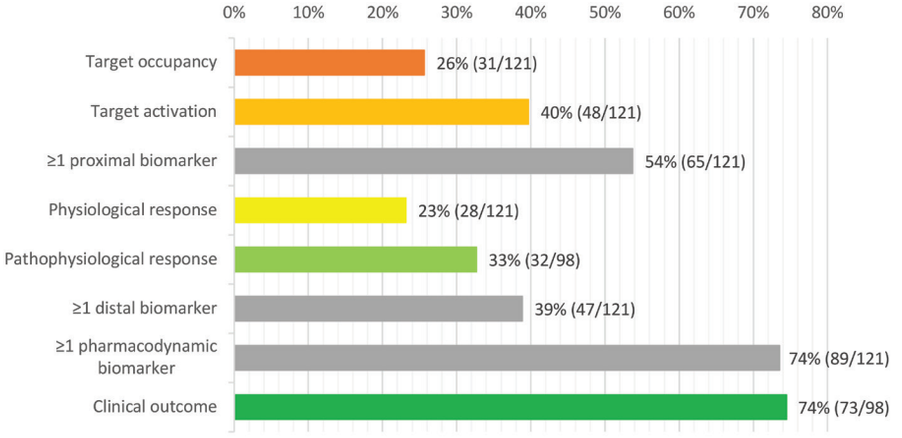


Table 2 Overview of published early phase clinical trials for disease-modifying compounds in neurodegenerative disorders between 2010 and November 2020 and reported peripheral and central pharmacodynamic biomarker outcomes.

Indication	Drug category	Drug target	Trials reporting mechanistic biomarker	Peripheral biomarkers	Central biomarkers	Types of biomarkers	Study population	References
AD	Antibody	Amyloid β	10/11 (91%)	Plasma total Aβ and Aβ fragments (Aβ1-x, Aβ1-40, Aβ1-42, Aβ3-42, Aβ1-38, Aβ18-35)	CSF Aβ species (Aβ1-x, Aβ1-40, Aβ1-42), t-TAU, and p-TAU181	Target occupancy and pathophysiological response	HVs and patients	53-63
		TAU protein	1/1 (100%)	-	CSF N-terminal TAU, mid-domain TAU, Aβ40, and Aβ42	Target occupancy and physiological response	HVs	64
	Cell therapy	Cytotropic factors, anti-inflammatory, neurogenesis	1/1 (100%)	-	CSF Aβ, t-TAU and p-TAU; PIB-PET changes in parenchymal amyloid deposition; FDG-PET metabolic changes	(patho)physiological response	Patients	65
		Nerve growth factor	0/1 (0%)	-	-	-	Patients	66
	Dietary	Xanthophyll Carotenoids, Omega-3 Fatty Acids	0/1 (0%)	-	-	-	Patients	67
	Gene therapy	Nerve growth factor	1/1 (100%)	-	PET brain glucose metabolism (post-mortem brain autopsy gene-mediated NGF expression and bioactivity)	Physiological response (and post-mortem target occupancy and activation)	Patients	68
	Growth factor	Nerve growth factor	1/1 (100%)	-	MRI for implant position; CSF Aβ1-42, t-TAU, p-TAU181, NFL, glial fibrillary acidic protein (GFAP), AChE and choline acetyltransferase (ChAT) activity and protein levels	Target occupancy, activation and (patho) physiological response	Patients	69
	Immunotherapy	Amyloid β	3/3 (100%)	Plasma anti-Aβ40 antibodies, Aβ peptides (Aβ40, Aβ42) and cytokines (IL-6, TNF-α, IL-1β, MCP-1, IL-2, sIL-2R); Serum antibody titres (Aβ IGM, Aβ IGG), Aβ1-40, Aβx-40, Aβ1-42; *In vitro* lymphocyte proliferation and cytokine production; PBMC β-specific and Qβ-specific responses of T-cells	CSF antibody titres, Aβx-40, Aβx-42, Aβ1-42, Aβn-42, t-TAU, p-TAU181; MRI brain volumetric assessment	Target activation and (patho)physiological response	Patients	70-72
		TAU protein	1/1 (100%)	IGG and IGM titre anti-vaccin peptide, anti-KLH antibody titre, anti-pathological-TAU antibody titre; Lymphocyte immunoprofiling	-	Target activation and physiological response	Patients	73,92
	Peptide	Amyloid β	0/1 (0%)	-	-	-	HVs	74
	Focused ultrasound with injected micro-bubbles	BBB-opening to amyloid β and TAU	1/1 (100%)	-	PET BBB opening and amyloid β deposition	Target occupancy and pathophysiological response	Patients	75
	DBS	Cerebral glucose metabolism	3/4 (75%)	-	PET cerebral glucose metabolism	Physiological response	Patients	76-79
	Small molecule	5-HT2A receptor	0/1 (0%)	-	-	-	HVs	80
		Amyloid precursor protein (APP) synthesis	1/1 (100%)	-	CSF sAPPα, sAPPβ, t-TAU, p-TAU, Aβ42 and inflammatory markers (complement 3, factor H, MCP-1, YKL-40, sCD14)	Target activation and (patho)physiological response	Patients	81
		Amyloid production and associated inflammatory response	0/1 (0%)	-	-	-	HVs	82
		BACE1	7/8 (89%)	Plasma total Aβ and Aβ fragments (Aβ1-37, Aβ1-38, Aβ1-40, Aβ1-42, Aβx-40), total sAPP and fragments (sAPPα, sAPPβ)	CSF total Aβ and fragments (Aβx-38, Aβx-40, Aβx-42, Aβ1-37, Aβ1-38, Aβ1-40, Aβ1-42), total sAPP and fragments (sAPPα, sAPPβ), BACE1, t-TAU, p-TAU181	Target occupancy, activation and pathophysiological response	HVs and patients	83-90
		ET(B) receptor	0/1 (0%)	-	-	-	HVs	91
		Glutaminyl cyclase (QC)	1/1 (100%)	Serum QC activity	CSF QC activity	Target occupancy and activation	HVs	92
		Glycogen synthase kinase-3β (GSK3β)	1/1 (100%)	Lymphocyte GS phosphorylation	-	Target occupancy	HVs	93
		Sigma-2 receptor complex	0/1 (0%)	-	-	-	HVs	94
		γ-secretase	2/2 (100%)	Plasma Aβx-42	CSF total Aβ and Aβ fragments (Aβ42, Aβ40, Aβ37, Aβ38)	Target activation	HVs	95, 96
		RIPK1 inhibitor*	1/1 (100%)	PBMCs reduction of ps166 RIPK1	-	Target occupancy and activation	HVs	30
		Microtubule stabilization	1/1 (100%)	-	CSF NFL, t-TAU, p-TAU, Aβ42, YKL-40	Pathophysiological response	Patients	97
	Cell therapy	Neuroprotective effects	1/1 (100%)	-	CSF t-TAU, p-TAU, Aβ42	Pathophysiological response	Patients	98
Overall use of mechanistic biomarkers in early phase AD trials			37/47 (79%)					
ALS	Antibody	Neurite outgrowth inhibitor NOGO-A	1/1 (100%)	Muscle biopsy NOGO-A RNA and protein expression; Plasma NOGO-A protein gamma sarcoglycan; EMG (MUNE)	-	Target occupancy and activation	Patients	99
	Antisense Oligonu-cleotide	SOD1	2/2 (100%)	Plasma p-NFH, NFL	CSF SOD1, p-NFH, NFL	Target activation and pathophysiological response	Patients	33, 40, 60, 100
	Cell therapy	Neurotrophic growth factors and cytokines secretion, immunomodulation and cell proliferation or replacement	5/13 (38%)	MRI muscle volume CD4+CD25+FOXP3+TREGS, proliferation of autologous responder T lymphocytes; EMG of TA muscles (CMAP, FD, SMUP, MUNE, MUNIX, MUSIX); EIM	CSF cytokines (TGF-B1, TGF-B2, TGF-B3, IL-6, IL-10, MCP-1)	(patho)physiological response	Patients	101, 102, 111-113, 103-110
	Gene therapy	Hepatocyte growth factor	1/1 (100%)	Serum HGF; Muscle circumference	-	Target activation and pathophysiological response	Patients	114
	Growth factor	Granulocyte colony-stimulating factor	1/1 (100%)	Blood cell counts, CD34+cells serum cytokines/chemokines (IL-1B, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, EOTAXIN, bFGF, FGF-2, TGF-A, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1A, MIP-1B, PDGF-BB, RANTES, TNF-α, VEGF)	CSF BMC presence, cytokines/chemokines (IL-1B, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, EOTAXIN, bFGF, FGF-2, TGF-A, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1A, MIP-1B, PDGF-BB, RANTES, TNF-α, VEGF)	Target activation and (patho)physiological response	Patients	115
		Hepatocyte growth factor	0/1 (0%)	-	-	-	Patients	116
	Small molecule	EAAT2	0/1 (0%)	-	-	-	Patients	117
		Putative mitochondrial modulation	0/1 (0%)	-	-	-	HVs	118
		Inflammatory macrophages and monocytes regulation	1/1 (100%)	Blood monocyte immune activation markers CD16, HLA-DR	-	Target activation	Patients	119
		SOD1	2/2 (100%)	Erythrocyte SOD1 enzymatic activity; Leukocyte actin-normalized SOD1	CSF SOD1 protein and enzymic activity	Target activation	Patients	120, 121
	Supplement	Lysosomal Cathepsins B and L	0/1 (0%)	-	-	-	Patients	122
		Stabilize the mitochondrial transition pore, buffer intracellular energy stores, stimulate synaptic glutamate uptake, and scavenge reactive oxygen species	1/1 (100%)	-	MRS brain glutamate and glutamine (GLX)	Physiological response	Patients	123
Overall use of mechanistic biomarkers in early phase ALS trials			14/27 (52%)*					

[*Continuation Table 2*]

Indication	Drug category	Drug target	Trials reporting mechanistic biomarker	Peripheral biomarkers	Central biomarkers	Types of biomarkers	Study population	References
ATTR amyloidosis	Antisense oligonucleotide	Transthyretin (TTR)	1/1 (100%)	Plasma TTR	-	Target activation	HVs	124
	RNA interference	Transthyretin amyloid	1/1 (100%)	Serum transthyretin, retinol-binding protein and vitamin A	-	Target occupancy and activation	HVs and patients	125
Overall use of mechanistic biomarkers in early phase ATTR trials			2/2 (100%)					
FRDA	Small molecule	FXN gene expression	1/1 (100%)	Whole blood FXN mRNA, frataxin protein; PBMC chromatin modification via H3 lysine 9 acetylation	-	Target occupancy and activation	Patients	126
	Supplement	FXN gene expression	1/1 (100%)	PBMC FXN mRNA and frataxin protein); Blood heterochromatin modifications at the FXN locus	-	Target occupancy and activation	Patients	127
	Polyunsaturated fatty acid	Lipid peroxidation	1/1 (100%)	RBC compartment D2-LA	-	Target occupancy	Patients	128
Overall use of mechanistic biomarkers in early phase FRDA trials			3/3 (100%)					
FTD	Small molecule	Progranulin protein (PGRN)	1/1 (100%)	Plasma PGRN, PGRN-related inflammatory markers (CRP, ESR), blood cytokines (IL-10, IL-2, IL-6, IL-8, TNF-α)	CSF PGRN, NFL, Aβ42, TAU, cytokines (IL-10, IL-2, IL-6, IL-8, TNF-α); MRI volumetric assessment	Target activation and (patho)physiological response	Patients	129
Overall use of mechanistic biomarkers in early phase FTD trials			1/1 (100%)					
GM2 gangliosidosis	Small molecule	β-hexosaminidase (HEX)	1/1 (100%)	Leucocyte and plasma HEX A, β-galactosidase and glucocerebrosidase activity, β-glucuronidase and acid phosphatase	-	Target activation	Patients	130
Overall use of mechanistic biomarkers in early phase GM2 gangliosidosis trials			1/1 (100%)					
HD	Antisense oligonucleotide	HTT mRNA	1/1 (100%)	-	CSF mutant HTT, NFL; MRI ventricular volume	Target activation and pathophysiological response	Patients	131
	Peptide	Cardiolipin	1/1 (100%)	MRI skeletal muscle dynamic 31P-MRS; PBMC mitochondrial membrane potential ($\Delta\Psi m$)	MRI brain 31P-MRS; CNS functional domain test battery (NeuroCart®)	Target activation and (patho)physiological response	Patients	132
Overall use of mechanistic biomarkers in early phase HD trials			2/2 (100%)					
Leber Hereditary Optic Neuropathy	Gene therapy	Mitochondrial gene encoding NADH: ubiquinone oxidoreductase subunit 4 (ND4)	1/2 (50%)	-	OCT average retinal nerve fiber layer (RNFL) thickness; Pattern electroretinogram amplitudes	Physiological response	Patients	133, 134
Overall use of mechanistic biomarkers in early phase Leber Hereditary Optic Neuropathy trials			1/2 (50%)					
MS	Antibody	Semaphorin 4D	1/1 (100%)	T-CELL CSEMA4D expression and saturation; Serum SSEMA4D	-	Target occupancy and activation	Patients	135
	Cell therapy	Neurotrophic and immunomodulatory effects, neurogenesis	2/2 (100%)	Lymphocyte subsets (CD4+, CD25+ and CD40+lymphocytes and CD83+, CD86+, and HLA-DR+myeloid dendritic cells); PBMC cytokine production	MRI labeled cell localization and volumetric assessment; OCT average retinal nerve fiber layer (RNFL); Vision (HCVA, LCLA)	Target occupancy and (patho)physiological response	Patients	136, 137
	Small molecule	Anti-inflammatory	1/1 (100%)	PBMC monocyte and 6-sulpho LACNAC+dendritic cell (slanDC) frequency, properties, and activation status	-	Target activation and physiological response	Patients	138
		Mitochondrial ATP production (coenzyme Q10)	1/1 (100%)	-	CSF mitochondrial dysfunction markers (GDF15, lactate), NFL, sCD14; BBB leakage (albumin quotient); OCT retinal nerve fiber layer thinning; MRI brain ventricular volume	(patho)physiological response	Patients	139
Overall use of mechanistic biomarkers in early phase MS trials			5/5 (100%)					

Indication	Drug category	Drug target	Trials reporting mechanistic biomarker	Peripheral biomarkers	Central biomarkers	Types of biomarkers	Study population	References
MSA	Cell therapy	Neurotrophic factors secretion	1/1 (100%)	-	CSF neurotrophic factors (NGF, GDNF, BDNF)	Physiological response	Patients	140
	Immunotherapy	α-synuclein	1/1 (100%)	Serum immunopeptide titers, α-synuclein native epitope titers	-	Target activation	Patients	141
Overall use of mechanistic biomarkers in early phase MSA trials			2/2 (100%)					
NCLS	Cell therapy	Palmitoyl-protein thioesterase 1 (PPT-1) and tripeptidyl peptidase 1 (TPP1) enzymes production	0/1 (0%)	-	-	-	Patients	142
CLN2 disease	Enzyme replacement	Lysosomal enzyme TPP1	0/1 (0%)	-	-	-	Patients	143
Overall use of mechanistic biomarkers in early phase NCLS trials			0/2 (0%)					
NPC1	Cyclodextrin	Neuronal cholesterol homoeostasis	1/1 (100%)	Serum 24(S)-hydroxycholesterol (24(S)-HC)	CSF 24(S)-hydroxycholesterol (24(S)-HC), fatty acid binding protein 3 (FABP3) and calbindin D19	Target activation and (patho)physiological response	Patients	144
Overall use of mechanistic biomarkers in early phase NPC1 trials			1/1 (100%)					
PD	Antibody	α-synuclein	3/3 (100%)	Plasma antibody/α-synuclein complexes; Serum total and free α-synuclein	CSF total and free α-synuclein, total Aβ, Aβ42, DJ-1, DAT scan	Target occupancy, activation, and pathophysiological response	HVs and patients	145-147
	Cell therapy	Neurotrophic factors to restore dopaminergic cell function	0/1 (0%)	-	-	-	Patients	148
	Gene therapy	Aromatic L-amino acid decarboxylase (AADC)	3/3 (100%)	-	PET FMT brain AADC expression and activity	Target occupancy and activation	Patients	149-151
		Tyrosine hydroxylase, AADC, cyclohydrolase 1	1/1 (100%)	-	PET cortical excitability and reflex recordings	Physiological response	Patients	152
	Growth factor	Granulocyte colony-stimulating factor (G-CSF)	0/1 (0%)	-	PET 18 F-DOPA for disease progression	Pathophysiological response	Patients	153
		Granulocyte-macrophage colony-stimulating factor (GM-CSF)	1/1 (100%)	Expression of Treg phenotype and function (CD4+Teffs (CD4+CD127hiCD25hi), CD4+Tregs (CD4+CD127loCD25hi), FOXP3+CD4+ Tregs, ICTLA4+CD4+Tregs, CD39+CD4+ Tregs, and fFAS+CD4+Tregs), T cell proliferation mRNA (GATA4, IL2, HOXA10, and KIF2C), anti-inflammatory gene expression (PPARG, LRRC32, FOSL1, IL1R2, IL13RA1, NR4A3, GFI1), tryptophan pathway targeted metabolomics	-	Target activation and physiological response	Patients	154
		rhPDGF-BB (proliferation of SOX-2/OLIG-1– POSITIVE periventricular progenitor cells)	1/1 (100%)	-	[11C]PE2I DAT binding	Pathophysiological response	Patients	155
	Immunotherapy	α-synuclein	1/1 (100%)	Serum antibody titres	CSF antibody titres, total α-synuclein, Aβ1–42, p-TAU	Target activation and pathophysiological response	Patients	156
	Deep brain stimulation (DBS)	Unknown	0/1 (0%)	-	-	-	Patients	157
	Small molecule	Glucosylceramide synthase (GCS)	1/1 (100%)	Plasma glucosylceramide (GL-1), globostriaosylceramide (GL-3), and GM3 ganglioside (GM3)	-	Target activation	HVs	158
		Myeloperoxidase	1/1 (100%)	-	PET distribution volume of 11C-PBR28 binding to microglia marker TSPO	Target occupancy	Patients	159
		Flavonoid (regulating dopaminergic system function, anti-oxidative damage and anti-inflammatory effects)	0/1 (0%)	-	-	-	HVs	160
	Supplement	Antioxidant	0/1 (0%)	-	-	-	Patients	161
Overall use of mechanistic biomarkers in early phase PD trials			12/17 (71%)					

[*Continuation Table 2*]

Indication	Drug category	Drug target	Trials reporting mechanistic biomarker	Peripheral biomarkers	Central biomarkers	Types of biomarkers	Study population	References
PSP	Antibody	TAU protein	0/2 (0%)	-	-	-	Patients	31, 162
	Cell therapy	Trophic, anti-apoptotic and regenerative effects	0/1 (0%)	-	MRI, SPECT and PET with tropanic tracers (FP-CIT and BETA-CIT) longitudinal neuroimaging	Pathophysiological response	Patients	163
	Small molecule / Blood product	Acetylation of TAU /unknown	1/1 (100%)	Plasma NFL concentrations	CSF amyloid beta Aβ, t-TAU, p-TAU181; MRI brain volumetric assessment	(patho)physiological response	Patients	164
Overall use of mechanistic biomarkers in early phase PSP trials			1/4 (25%)					
SCA	Cell therapy	Trophic factor secretion, immunomodulation	1/1 (100%)	-	PET brain glucose metabolism	Physiological response	Patients	165
	Growth factor	Antiapoptotic, antioxidative, anti-inflammatory, neurotrophic and angio- genic properties	0/1 (0%)	-	-	-	HVS	166
Overall use of mechanistic biomarkers in early phase SCA trials			1/2 (50%)					
SMA	Antisense oligonucleotide	SMN2 mRNA splicing	1/1 (100%)	-	CSF SMN protein	Target activation	Patients	167
	Small molecule	SMN2 splicing	2/2 (100%)	Blood mRNA (full-length SMN2, SMN1, SMNΔ7), SMN protein	-	Target activation	HVS and patients	168,169
	Gene therapy	SMN	0/1 (0%)	-	-	-	Patients	170
Overall use of mechanistic biomarkers in early phase SMA trials			3/4 (25%)					

*AADC = aromatic L-amino acid decarboxylase / Aβ = amyloid β / AChE = Acetylcholinesterase / AD = Alzheimer's disease / ALS = amyotrophic lateral sclerosis / AAP = amyloid precursor protein / ATP = adenosine triphosphate / ATTR = amyloid transthyretin / BACE1 = beta-secretase 1 / BBB = blood-brain barrier / BDNF = brain-derived neurotrophic factor / BETA-CIT = 18F-Fluoro-2-deoxyglucose labeled tropanic SPECT tracer / BMC = bone marrow concentrated cells / CD# = cluster of differentiation # / ChAT = choline acetyltransferase / CLN2 = classic late infantile neuronal ceroid lipofuscinosis / CMAP = compound muscle action potential / CRP = C-reactive protein / cSEMA4D = T-cell semaphorin 4D / CSF = cerebrospinal fluid / CTLA4 = cytotoxic T-lymphocyte-associated protein 4 / DAT = dopamine active transporter / DBS = deep brain stimulation / DJ-1 = protein deglycase DJ-1 (PARK7) / D2-LA = di-deutero isotopologue of linoleic acid ethyl ester / EAAT2 = excitatory amino acid transporter 2 / EIM = electrical impedance myography / EMG = electromyogram / ESR = erythrocyte sedimentation rate / ET(B). = endothelin receptor type B / FABP3 = fatty acid binding protein 3 / FD = fiber density / FDG = fluorine-18-deoxyglucose / FGF-# = fibroblast growth factor # / FMT = [18F]fluorometatyrosine / FOSL1 = FOS like 1, AP-1 transcription factor subunit / FOXP3 = forkhead box P3 / FRDA = Friedreich ataxia / FTD = frontotemporal dementia / FP-CIT = [123I] labeled tropanic SPECT tracer / FXN = frataxin / GATA4 = transcription factor GATA-4 / GCS = glucosylceramide synthase / GDF15 = growth/differentiation factor 15 / GDNF = glial cell-derived neurotrophic factor / GFAP = glial fibrillary acidic protein / GFI1 = growth factor independent 1 transcriptional repressor / GLCR = β-glucuronidase / GL-1 = glucosylceramide / GL-3 = globotriasylceramide / GLX = glutamate and glutamine / GM3 = mono-sialodihexosylganglioside / GM-CSF = granulocyte-macrophage colony-stimulating factor / GS = glycogen synthase / GSK3β = glycogen synthase kinase-3β / G-CSF = granulocyte colony-stimulating factor / HCVA = high-contrast visual acuity / HD = Huntington's disease / HEX = β-hexosaminidase / HGF = Hepatocyte growth factor / HLA-DR = human leukocyte antigen DR / HOXA10 = homeobox A10 / HTT = huntingtin / HVS = healthy volunteers / IFN-γ = interferon gamma / IGG = immunoglobulin G / IGM = immunoglobulin M / IL-# = Interleukin # / IP-10 = interferon gamma-induced protein 10 / KIF2C = kinesin Family Member 2C / KLH = keyhole limpet hemocyanin / LACNAC = N-acetyllactosamine / LCLA = low-contrast letter acuity / LRRC32 = leucine rich repeat containing 32 / MCP-1 = monocyte chemoattractant protein 1 / MIP-# = macrophage inflammatory protein # / MRI = magnetic resonance imaging / mRNA = messenger RNA / MRS = magnetic resonance spectroscopy / MS = multiple sclerosis / MSA = multiple system atrophy / MUNE = motor unit number estimation / MUNIX = motor unit number / MUSIX = motor unit size / NADH = nicotinamide adenine dinucleotide / NCLS = neuronal ceroid lipofuscinoses / ND4 = NADH-ubiquinone oxidoreductase chain 4 / NGF = nerve growth factor / NFL = neurofilament light chain / NPC1 = Niemann-Pick disease type C1 / NR4A3 = nuclear receptor subfamily 4 group A member 3 / OCT = optical coherence tomography / PBMCs = peripheral blood mononuclear cells / PD = Parkinson's disease / PDGF-BB = platelet-derived growth factor BB / PET = positron emission tomography / PGRN = progranulin protein / PIB = Pittsburgh compound B / PPARG = peroxisome proliferator-activated receptor gamma / PPT-1 = palmitoyl-protein thioesterase 1 / PSP = progressive supranuclear palsy / pS166 = phosphorylation of serine 166 / P-NFH = phosphorylated neurofilament heavy chain / p-TAU181 = TAU phosphorylated at threonine 181 / QC = glutaminyl cyclase / RANTES = regulated on activation, normal T cell expressed and secreted / RBC = red blood cells / rhPDGF-BB = recombinant human platelet-derived growth factor-BB / RIPK1 = receptor-interacting serine/threonine-protein kinase 1 / RNA = ribonucleic acid / RNFL = retinal nerve fiber layer / SAPP = soluble amyloid precursor protein / SCA = spinocerebellar ataxia / SCD14 = soluble CD14 / SIL-2R = soluble IL-2 receptor / slanDCS = 6-sulfo / LACNAC dendritic cells / SMA = spinal muscular atrophy / SMN# = survival of motor neuron # / SMNΔ7 = exon 7-deleted SMN protein / SMUP = single motor unit potential / SOD1 = superoxide dismutase 1 / SOX-2 / OLIG-1 = SRY-box transcription factor 2 / oligodendrocyte transcription factor 1 / SPECT = single photon emission computed tomography / SSEMA4D = soluble semaphorin 4D / TA = tibialis anterior / Teffs = effector T cells / TGF-# = transforming growth factor # / TNF-α = tumor necrosis factor / TPP1 = tripeptidyl peptidase 1 / Tregs = regulatory T cells / TSPO = translocator protein / TTR = transthyretin / t-TAU = total TAU / VEGF = vascular endothelial growth factor / YKL-40 = chitinase-3-like-1 protein / 24[S]-HC = α24(S)-hydroxycholesterol / 31P-MRS = 31P-magnetic resonance spectroscopy / 5-HT2A = 5-hydroxy-tryptamine 2A / ΔΨM = mitochondrial membrane potential / [11C]PE21 = selective dopamine active transporter (DAT) radiotracer / [11C]-PBR28 = 18pkD translocator protein (TSPO) radiotracer. *RIPK1 was under development for multiple indications (AD and ALS) in healthy subjects and has been added to the totals for both indications. It is only listed once AD in the Table to avoid duplication.*

Figure 2 Roadmap for early phase clinical development of disease-modification therapies in neurodegenerative disorders, focusing on demonstrating proof-of-concept with mechanistic early phase clinical pharmacology studies. Innovative clinical drug development revolves around confirming the pharmacokinetic behavior of the drug, occupation and activation of the intended pharmacological target in humans, quantifying the subsequent physiological and pathophysiological response before moving into large late-stage trials to demonstrate a clinical response (long-term disease modification). Safety evaluation is not specifically mentioned but is obviously an essential component at each stage of clinical drug development. For each stage of drug development different biomarker techniques can be used to come to an early mechanistic proof-of-concept, define the optimum dose, and facilitate a validated 'go/no-go' decision before moving into expensive late stage trials.

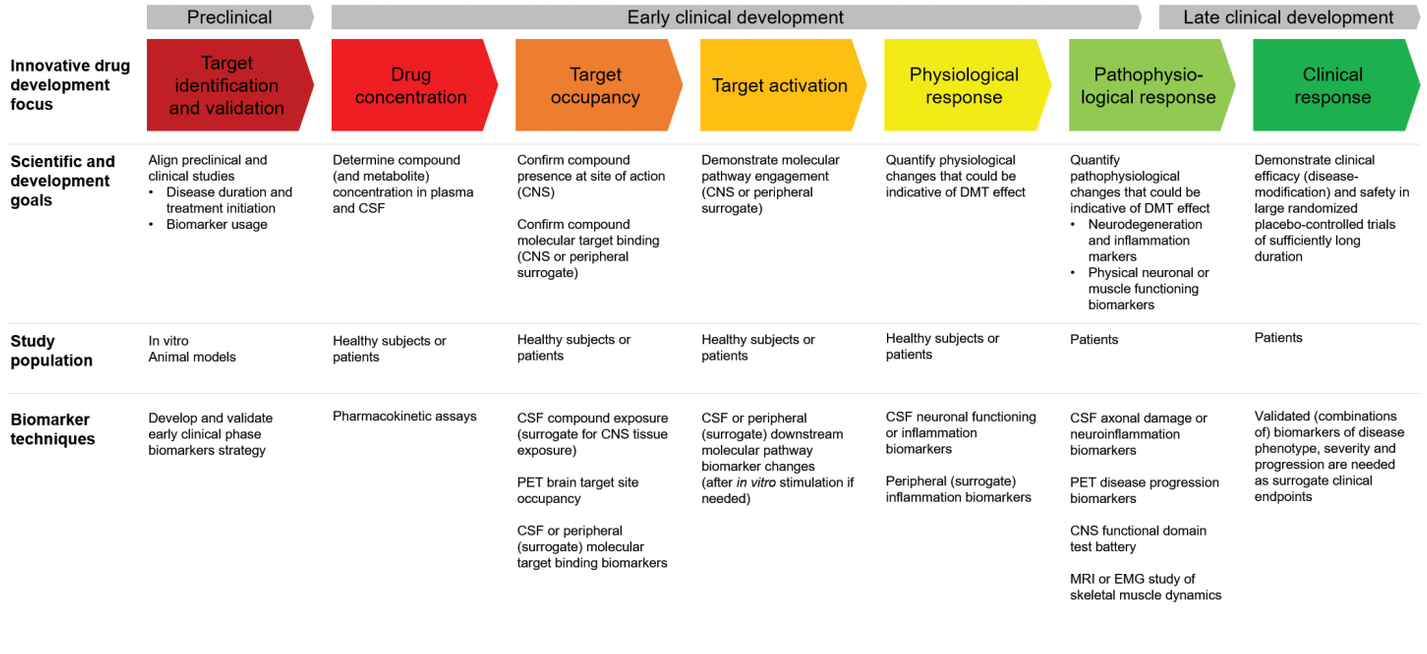
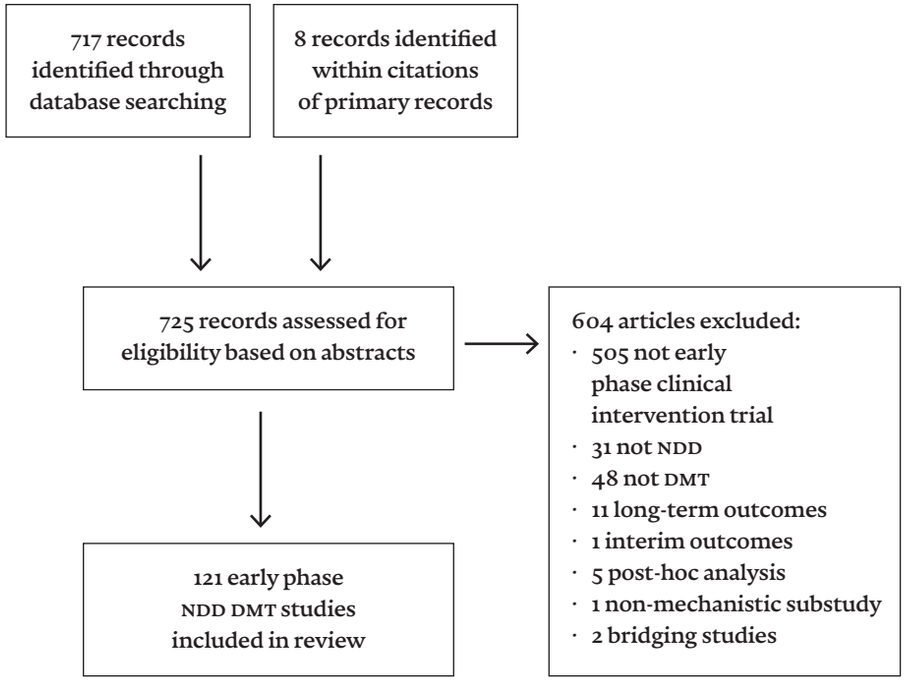


Figure S2 Study selection overview. Flow diagram of studies' screening and selection for this review.



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CHAPTER 3

SAFETY, PHARMACOKINETICS AND TARGET ENGAGEMENT OF NOVEL RIPK1 INHIBITOR SAR443060 (DNL747) FOR NEURODEGENERATIVE DISORDERS: RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND PHASE I/IB STUDIES IN HEALTHY SUBJECTS AND PATIENTS

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ABSTRACT

RIPK1 is a master regulator of inflammatory signaling and cell death and increased RIPK1 activity is observed in human diseases, including Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS). RIPK1 inhibition has been shown to protect against cell death in a range of preclinical cellular and animal models of diseases. SAR443060 (previously DNL747) is a selective, orally bioavailable, CNS–penetrant, small-molecule, reversible inhibitor of RIPK1. In three early-stage clinical trials in healthy subjects and patients with AD or ALS (NCT03757325 and NCT03757351), SAR443060 distributed into the cerebrospinal fluid (CSF) after oral administration and demonstrated robust peripheral target engagement as measured by a reduction in phosphorylation of RIPK1 at serine 166 (pRIPK1) in human peripheral blood mononuclear cells (PBMCs) compared to baseline. RIPK1 inhibition was generally safe and well tolerated in healthy volunteers and patients with AD or ALS. Taken together, the distribution into the CSF after oral administration, the peripheral proof-of-mechanism, and the safety profile of RIPK1 inhibition to date, suggest that therapeutic modulation of RIPK1 in the CNS is possible, conferring potential therapeutic promise for AD and ALS, as well as other neurodegenerative conditions. However, SAR443060 development was discontinued due to long-term nonclinical toxicology findings, although these nonclinical toxicology signals were not observed in the short duration dosing in any of the three early stage clinical trials. The dose-limiting toxicities observed for SAR443060 preclinically have not been reported for other RIPK1-inhibitors, suggesting that these toxicities are compound-specific (related to SAR443060) rather than RIPK1 pathway-specific.

INTRODUCTION

Receptor-interacting serine/threonine protein kinase 1 (RIPK1) is an intracellular protein that regulates between pro-survival NF-κB signaling and cell-death in response to inflammatory and pro-death stimuli,[1] and RIPK1 activation has been implicated in autoimmune, inflammatory and neurodegenerative diseases. RIPK1 activation most notably occurs via tumor necrosis factor alpha (TNF-α) signaling through TNF receptor 1 (TNFR1).[2] Upon activation, RIPK1 initiates a complex signaling cascade that triggers cytokine release, microglial activation, and RIPK1-dependant apoptosis or under apoptosis-deficient conditions a regulated form of necrotic cell death known as 'necroptosis'.[2–4] RIPK1 activation and necroptosis have been demonstrated in post-mortem tissue samples from patients with neurodegenerative conditions,[5–7] and RIPK1 inhibition has been shown to protect against necroptotic cell death *in vitro* across a range of cell death models.[8–13] In animal models of diseases ranging from ulcerative colitis to multiple sclerosis (MS), RIPK1 pathway inhibition protects against necroptotic cell death and also prevents the occurrence of pathologic findings.[6,12–21] These preclinical findings suggest that inhibition of RIPK1 could be beneficial in many different chronic diseases.[5–7,12,14,22,23]

RIPK1 inhibitors that do not penetrate the central nervous system (CNS), GSK2982772 and SAR443122 (DNL758), are currently in early stage clinical development for inflammatory diseases.[24–26] A CNS-penetrant inhibitor of RIPK1 may have the potential to modify the course of neurodegenerative diseases like MS, Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS).[23,27]

The results of 3 early-phase, placebo-controlled, clinical studies with SAR443060 (previously DNL747) are presented here. SAR443060 is a selective, orally bioavailable, CNS–penetrant, small-molecule reversible inhibitor of RIPK1. Presented results include assessment of safety, pharmacokinetics, and target engagement of SAR443060 following;

1 A first-in-human (FIH) single and multiple ascending dose study in healthy subjects;
2 A cross-over study in patients with AD;
3 A cross-over study in patients with ALS which was followed by an open-label long-term extension (OLE).

PRECLINICAL PHARMACOLOGY AND TOXICOLOGY

In vitro target engagement and dose-response

Reduction of phosphorylation of RIPK1 at serine 166 (pRIPK1) in human peripheral blood mononuclear cells (PBMCs) is considered a reliable biomarker for target engagement assessment and for the translation of human dose projection.[6,8,28] *In vitro*, SAR443060 blocks TNF-α–induced phosphorylation of RIPK1 in PBMCs from healthy human donors (N=4) with a geometric mean 50% maximum inhibitory concentration (IC_{50}) of 3.9 nM (*Figure 1*). When corrected for human plasma protein binding (87%), this IC_{50} corresponds to a total SAR443060 plasma concentration of approximately 0.03 μM. Based on preclinical models demonstrating that RIPK1 inhibition in astrocytes and microglia can attenuate neurodegeneration and disease progression, it was hypothesized that maintaining high levels of RIPK1 inhibition for the duration of each dosing interval could translate to slowing the clinical progression of AD and ALS.[14]

Preclinical safety

Based on 28-day Good Laboratory Practice (GLP) toxicity studies in the most sensitive species with pharmacological relevance (cynomolgus monkey), the original no observed adverse effect level (NOAEL) for SAR443060 was established at 200 mg/kg/d (mean AUC_{0-24h} of 254 μM · h and mean C_{max} of 29.2 μM). Protein binding of SAR443060 is similar across species (data on file). The key toxicity findings in the 28-day monkey study at 1000 mg/kg/d (AUC_{0-24h} of 548 μM · h) included adverse effects to the immune system (lymph nodes, bone marrow, and spleen) and skin. A subsequent 3-month toxicity study in monkeys, running in parallel to the first-in-human study (*Section Methods*), identified additional serious toxicities that were considered to be immune-mediated, including thrombocytopenia, anemia, and bleeding at doses of ≥40 mg/kg/day (20 mg/kg BID). As a result, the NOAEL was reduced from 200 mg/kg/day to 20 mg/kg/day (mean AUC_{0-24h} of 24.8 μM · h and mean C_{max} of 2.57 μM) which led to a dose reduction in the subsequent phase 1B patient studies to keep a 3-4X margin to the new NOAEL (*Section Treatments Administered*). During an extended 9-month GLP toxicity study in monkeys, anemia was also observed at 20 mg/kg/day (10 mg/kg BID) and the NOAEL was further adjusted down ~3-fold to 6 mg/kg/day. Three-month GLP toxicity studies in Sprague-Dawley rats showed good tolerability of SAR443060 to the highest doses tested at 1000 mg/kg/day (mean AUC_{0-24h} of 251 mM · h and 1040 mM · h in males and females, respectively).

METHODS

All clinical studies were conducted in accordance with the International Conference for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use, Good Clinical Practice (GCP), and the principles of the Declaration of Helsinki. The protocols and all study materials were approved by independent ethics committees (ECs)/institutional review boards (IRBs), and all subjects provided their written informed consent before participation.

Study designs and randomization

FIRST-IN-HUMAN STUDY The randomized, double-blind, placebo-controlled FIH study was conducted at a single site in the Netherlands (PRA Health Sciences, Groningen) in healthy male and female subjects, aged 18-55 years, between March and October 2018. Women had to be of nonchildbearing potential (sterilized or postmenopausal). The study consisted of two parts: a single ascending dose (SAD) PART A and a multiple ascending dose (MAD) PART B (*Figure 2A*). PART A used a sequential ascending dose cohorts design with 4 cohorts of 8 subjects randomized 3:1 SAR443060 to placebo, to evaluate the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of single ascending doses of SAR443060. The effect of food on the PK of SAR443060 was evaluated with a fixed sequence crossover food assessment in COHORTS A2 and A4. In PERIOD 1 the investigational medicinal product (IMP) was administered under fasted conditions and in PERIOD 2 after a high-fat, high-caloric breakfast in the same 8 subjects, with at least one-week wash-out between the 2 periods (*Figure 2A*).

PART B evaluated the safety, tolerability, PK, and PD of multiple ascending doses of SAR443060 administered twice daily (BID) for 14 days in 3 cohorts (*Figure 2A*) of 10 subjects randomized 4:1 SAR443060 to placebo. Cerebrospinal fluid (CSF) was sampled via lumbar punctures (LP) predose and after 12 days of dosing.

STUDIES IN PATIENTS WITH AD OR ALS Two multicenter, randomized, double-blind, placebo-controlled phase 1B studies were conducted between December 2018 and June 2020 in the Netherlands and the United States: one in patients with AD and one in patients with ALS (ClinicalTrials.gov identifiers: NCT03757325 and NCT03757351). Both studies used a similar cross-over design consisting of two 28-day treatment periods, separated by 14 days of wash-out, to evaluate the safety, tolerability, PK and PD of SAR443060 in at least 16 and up to 26 patients in each study (*Figure 2B*). Subjects were randomized 1:1 to receive either active or placebo treatment in the first treatment period and then switched to the opposite treatment assignment in the second treatment period. For the AD study, inclusion criteria included AD diagnosis per NIA-AA guidelines,[29] a Mini-Mental State Examination (MMSE) score of 16-26 points inclusive, an age of 55 to 85 years with a body mass index (BMI) between 18 to 35 kg/m^2, a Clinical Dementia Rating (CDR) score of 0.5-1.0, and a historical confirmatory amyloid positron emission tomography (PET) scan or positive CSF Amyloid β (Aβ)42 test (documented history or CSF sampling at screening).

Participants in the ALS study were male or female patients of non-childbearing potential aged between 21 and 80 years, with laboratory-supported probable, probable, or definite ALS according to the revised El Escorial criteria,[30] less than 3 years since symptom onset, a BMI between 18 and 35 kg/m^2, and a forced vital capacity (FVC) >50% of predicted.

For both patient studies use of prescription medications had to be stable for ≥1 month prior to screening and throughout the study. Moderate to strong cytochrome P450 3A (CYP3A) inducers or inhibitors, as well as antiplatelet and anticoagulation medications, apart from daily aspirin <100 mg, were not allowed. Patients were confined to the clinical research unit for 3 days at the start and end of each treatment period and returned to the clinical unit for weekly outpatient safety visits and to obtain study medication that was administered at home. CSF was sampled pre-dose in treatment PERIOD 1 and at the end of each treatment period. Safety follow-ups were conducted 1 and 2 weeks after completion of the second treatment period.

The cross-over design was selected to facilitate within-subject analysis of SAR443060-dependent biomarker changes. The sample size in both studies was based on sample size calculations for selected exploratory biomarker endpoints.

Treatments administered

FIRST-IN-HUMAN STUDY Ascending doses of 100-400 mg of SAR443060 in capsules as a spray-dried nanosuspension (SDN) formulation or a micronized drug substance (MDS) formulation or placebo were administered as single dose in PART A and BID for 14 days in PART B (*Figure 2A*). Based on the predicted human exposure, a starting dose of 100 mg was selected for the FIH study (expected to result in >90% peak RIPK1 inhibition) to enable characterization of the PD response over a dose range that encompassed the anticipated therapeutic range. This starting dose was 45-fold below the original 200 mg/kg/d NOAEL (*Section Preclinical pharmacology and toxicology*) when scaled to a human equivalent dose based on body surface area, and >90% RIPK1 inhibition was previously found to be well tolerated with another CNS-penetrant RIPK1 inhibitor.[28] Emerging safety data and available PK data from preceding cohorts were reviewed prior to dose escalation, and all cohorts used a sentinel design.

STUDIES IN PATIENTS WITH AD OR ALS A dose of 200 mg BID was initially selected for both patient studies based on adequate safety, tolerability, PK, and target engagement of SAR443060 in the previous FIH study. However, as the nonclinical NOAEL was significantly reduced following the 3-month toxicity study in monkeys (*Section Preclinical pharmacology and toxicology*), lower clinical doses were implemented via protocol amendments to maintain plasma concentrations 3-4X below the new NOAEL of 20 mg/kg/day (mean C_{max} of 2.57 µM). Patients in both studies were hence administered SAR443060 SDN 50 mg or matching placebo approximately every 12 hours (BID) in each treatment period for 28 days, followed by a final morning dose on the 29th day.

Preliminary PK/PD modeling based on the FIH study data suggested that doses at or above 50 mg BID could provide >80% inhibition of pRIPK1 throughout a BID dosing interval. This dose was expected to allow exploration of potential biomarker effects in patients with neurodegenerative diseases and provided adequate safety margins from toxicities seen in the 3-month monkey study.

Safety, pharmacokinetic and pharmacodynamic assessments

Safety and tolerability outcome measures for all 3 studies consisted of incidence and severity of adverse events (AEs), incidence of clinical laboratory abnormalities (hematology, chemistry, coagulation, and urinalysis), vital signs, electrocardiograms (ECGs), physical examinations (including lymph nodes, skin, and mucosa), and suicidal risk monitoring via the Columbia-Suicide Severity Rating Scale (C-SSRS, except for FIH PART A). Based on the preclinical toxicology studies (*Section Preclinical safety*), cutaneous or mucosal changes, lymphadenopathy, anemia, thrombocytopenia, and bleeding, petechiae, purpura, or ecchymoses were defined as AEs of special interest (AESI). PK outcomes comprised the measurement of the concentration of SAR443060 in plasma, urine (FIH PART A only) and CSF (except for FIH PART A) using a validated liquid chromatography-tandem mass spectrometry method (PRA Bioanalytical Laboratory, the Netherlands) with a lower limit of quantification (LLOQ) of 0.00247 µM. A standard set of plasma PK parameters were estimated using noncompartmental analysis, including maximum plasma concentration observed (C_{max}), time to reach C_{max} (T_{max}), half-life ($T_{½}$), area under the concentration time curve (AUC), and accumulation ratios (R_{ac}), as well as the CSF-to-unbound plasma ratio as an indication for CNS-penetration. Reduction of pRIPK1 levels in PBMCs was used to measure inhibition of RIPK1 kinase as a pharmacodynamic marker of peripheral drug target engagement, similar as described previously but without *ex vivo* stimulation.[28]

A digital clock-drawing test (DCTCLOCK™) was included in the AD study as an exploratory endpoint to gain experience with this measure in the AD population. The Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRS-R) was used an exploratory clinical endpoint in the ALS study. Both studies were not adequately powered for these exploratory endpoints.

Statistical analyses

No formal hypothesis testing was performed for these exploratory studies. All PK, PD, and safety data were listed, all data were summarized in tabular and/or graphical form, and descriptive statistics were given, as appropriate, using Statistical Analysis Software (SAS®) version 9.4 or higher. AEs were coded using the Medical Dictionary for Regulatory Activities (MEDDRA).

Noncompartmental PK analysis was performed on individual plasma and urine concentration data using PHOENIX® WINNONLIN® (Version 8.1). The PK parameters were analyzed for dose proportionality using a power model approach or analysis of variance (ANOVA) model as appropriate.

ALS open-label extension

An open label extension with SAR443060 50 mg BID up to 12 months was available for patients completing the ALS study in the Netherlands (*Figure 2B*). Routine safety assessments continued on a biweekly (up to month 6) and monthly (up to month 9) basis with a final planned visit after 12 months. The ALS OLE study part was prematurely terminated in June 2020 (9 months after first subject dosed), due to the sponsor's decision to stop the development of SAR443060.

RESULTS

Demographics and baseline characteristics

FIRST-IN-HUMAN STUDY Fifty-six healthy male and female subjects, between 18 and 55 years of age and with a BMI between 19.6 and 31.8 kg/m^2, were included in the study. In PART A, due to recruitment challenges, only 7 subjects of the planned 8 subjects were included in COHORTS A2, A3 and A4, leading to a total of 21 subjects that received a single dose of 100-400 mg SAR443060 and 8 subjects that received placebo. In PART B, only 7 subjects were included in COHORT B3 as the goals of the study had been reached. This resulted in 21 subjects receiving 100-400 mg SAR443060 BID for 14 days and 6 subjects receiving placebo in PART B (*Figure 2A*). One subject was withdrawn during the study (COHORT A2 after completion of PERIOD 1), due to a medical history of eczema which made the subject ineligible for the study and 55 (98.2%) subjects completed the study as per protocol (*Figure 2A*). Baseline characteristics (*Supplemental Table S1*) were similar across cohorts and treatments.

STUDIES IN PATIENTS WITH AD OR ALS In the AD study a total of 16 (100.0%) patients completed treatment PERIOD 1 and a total of 15 (93.8%) patients completed treatment PERIOD 2 (*Figure 2B*). One patient discontinued from the study due to AD progression during administration of placebo in PERIOD 2. Patient demographic and baseline characteristics (*Supplemental Table S1*), were comparable between both treatment sequences, except that patients randomized to the placebo/SAR443060 treatment sequence were on average older (73.4 vs 68.4 years), had a higher proportion of male patients (75% vs 50%), and a higher BMI (29.4 vs 24.1 kg/m^2) compared to subjects in the SAR443060/placebo treatment sequence, although none of these comparisons were evaluated statistically.

Treatment compliance was high (≥ 90%) in all but 1 (6.3%) patient, who received <80% of the placebo doses and discontinued the study due to the reason explained above. All 16 patients are included in the safety and PK analysis and all 15 subjects that completed both treatment periods are included in the PD analysis (*Figure 2B*). In the ALS study, only 15 of the planned 16 patients were included due to recruitment challenges. Eight patients were allocated to the treatment sequence placebo/SAR443060 and 7 patients were allocated to the treatment sequence SAR443060/placebo. The demographic and baseline characteristics (*Supplemental Table S1*) were balanced between the 2 cross-over sequences. All 15 randomized patients had high treatment compliance (≥90%). The first patient in the ALS study (sequence SAR443060/placebo) was enrolled at the original planned dose of 200 mg BID and completed 21 days in the first treatment period. This patient decided to forgo the rest of treatment PERIOD 1 without withdrawing from the study, and subsequently completed treatment PERIOD 2 (50 mg BID). No other patients were given the dose of 200 mg BID in this study. One patient in sequence SAR443060/placebo discontinued the study at the end of treatment PERIOD 2 due to ALS disease progression. All 15 patients were included in the safety analysis. One patient was excluded from the PK and PD analysis due to a protocol violation: this patient had stopped taking edaravone during the study (*Figure 2B*).

ALS OPEN-LABEL EXTENSION After completion of the double-blind ALS study, 8 patients enrolled in the ALS OLE study (*Figure 2B*, *Supplemental Table S1*). Two patients withdrew from the study prematurely due to disease progression and the other 6 patients were treated until the OLE study was terminated by the sponsor. One subject had an IMP interruption during the OLE study due to unrelated ECG abnormalities diagnosed as coronary heart disease by cardiologist consult. Duration of exposure to SAR443060 in the OLE ranged from 66 to 268 days (mean 165.5 ± 82.3 days). All 8 patients were included in the OLE safety analysis (*Figure 2B*).

Safety and tolerability

In all three clinical studies there were no SAR443060-related deaths reported or serious AEs (SAEs) and no subject withdrawals due to AEs (*Supplemental Table S2*). The safety profile and the nature of the adverse events were comparable between the SAR443060 and placebo.

FIRST-IN-HUMAN STUDY All treatment emergent AEs (TEAEs) in the FIH study were rated as mild or moderate in severity (*Supplemental Table S2*), and there were no AESIs. The most frequently reported TEAEs in PART A were medical device (ECG-electrodes) site irritation (12.1%), catheter site related reaction (6.1%), headache (6.1%), diarrhea (6.1%), and nasopharyngitis (6.1%). In PART B, the most frequently reported TEAEs were procedural pain (LP procedures) (25.0%), post-LP syndrome (15.6%), skin irritation (7.8%), catheter site related reaction (6.3%), and nausea (4.7%). No clinically significant changes from baseline or trends were observed in the clinical laboratory results, vital signs, 12-lead ECGs, physical examinations, or the C-SSRS.

STUDIES IN PATIENTS WITH AD OR ALS In the AD study there was 1 SAE of vomiting following placebo administration in the second treatment period, which required hospitalization, and was considered not related to the IMP by the investigator. There were no SAEs in the double-blind part of the ALS study (*Supplemental Table S2*). In the AD study a total of 7 AESIs were reported in 5 subjects: 2 AESIs during SAR443060 administration (mild, asymptomatic, and self-limiting anemia in 2 subjects) and 5 in subjects on placebo (mild anemia (2 subjects), thrombocytopenia (1 subject), epistaxis (1 subject), and moderate urticaria (1 subject)). All AESIs in the placebo period occurred prior to SAR443060 exposure. Two patients in the ALS study reported AESIs: seborrheic dermatitis (placebo treatment) and erythema (SAR443060 treatment). Both AESIs were of mild intensity, and only the event of erythema was considered related to the IMP by the investigator.

In the AD trial, the most common TEAEs observed in subjects during treatment with SAR443060 and in greater frequency than with placebo were confusion, headache, and procedural pain, each reported in 2 (12.5%) subjects. For the ALS trial these were headache and rhinitis, each reported in 3 (20.0%) patients. Both TEAEs of confusion following SAR443060 administration in the AD study occurred during inpatient stays and were assessed by the investigator as not related to the study drug, but likely a result from chronic AD.

In both the AD and ALS studies, other than the above reported anemias and low platelet count, there were no other clinically significant laboratory abnormalities, changes in vital signs, 12-lead ECGs, physical and neurological examinations, or changes in the C-SSRS.

ALS OPEN-LABEL EXTENSION During the ALS OLE part, one patient (12.5%) reported a treatment emergent SAE (hospitalization due to pneumonia aspiration) and one patient reported a severe TEAE (worsening of ALS), both not considered related to SAR443060 by the investigator (*Supplemental Table S2*). Four patients (50.0%) reported 5 AESIs related to skin and subcutaneous tissue disorders (seborrheic dermatitis (2x), atopic dermatitis, contact dermatitis and post inflammatory pigmentation change) and 1 patient (12.5%) experienced 2 AESIs: increased alanine and aspartate aminotransferase (ALT and AST). The increases in ALT and AST were assessed to be related to riluzole based on a de- and rechallenge with riluzole, and the skin and subcutaneous tissue findings were not considered related to SAR443060 by the investigator after consultation with a dermatologist.

Pharmacokinetics

FIRST-IN-HUMAN STUDY Following administration of single doses of 100-400 mg SAR443060 SDN in the fasted state, SAR443060 concentrations had a median T_{max} after 2 to 4 hours. Total exposure increased dose-proportionally, while the increase of C_{max} was found to be less than dose proportional (*Figure 3A*, *Table 1*). There was a 7% decrease in geometric mean C_{max} and an increase of 10% in geometric mean AUC_{0-inf} after administration of 100 mg SAR443060 SDN in fed versus fasted conditions.

A 200 mg single dose of SAR443060 MDS had a median T_{max} after 4 hours, and there was a 125% increase in geometric mean C_{max} and an increase in geometric mean AUC_{0-inf} of 13% in fed versus fasted conditions (*Figure 3A*). Geometric mean values for $T_{1/2}$ ranged from 9.7 to 11.4 hours with no clear dependence on dose, formulation or fed state (*Table 1*).

A comparison of the oral exposure of 200 mg SAR443060 MDS and SDN formulations indicated that the C_{max} of SAR443060 MDS was 61% (CI; 47-78%) of the C_{max} for SDN. The geometric mean AUC_{0-inf} after 200 mg SAR443060 MDS was 27% higher than after 200 mg SDN with a range CIs outside the 80-125% bioequivalence interval (*Figure 3A*).

During the multiple-dose period, there was an accumulation in AUC_{0-tau} of SAR443060 SDN, with an accumulation ratio (R_{ac}) ranging from 2.19 after 100 mg to 1.45 after 400 mg SAR443060, with steady state being reached at or before DAY 4 (*Figure 3C*, *Supplemental Figure S1C*). On DAY 14 of the MAD dosing period no evidence of deviation from dose proportionality was found for both C_{max} and AUC_{0-tau}. Geometric mean CSF concentrations of SAR443060 increased dose proportional (*Table 1*).

Excretion of SAR443060 in urine was very limited and not dependent on dose, with mean FE_{urine} <0.1% for all doses.

STUDIES IN PATIENTS WITH AD OR ALS After multiple dose administrations of 50 mg BID (end of treatment day 29 of PERIOD 1 or 2), the mean SAR443060 plasma concentrations increased over time and peaked at 1-1.5 hours (T_{max}) and declined gradually over 24 hours post-dose in a biphasic manner. For the total PK Population, the mean C_{max} and AUC_{0-12} were 0.670 µM and 3.62 µM · h, respectively, in the AD study and 0.638 µM and 3.12 µM · h in the ALS study (*Table 2*). Mean $T_{1/2}$ was 19.0 hours in the AD study and 14.2 hours in the ALS study. The mean R_{ac} at steady state for C_{max} was 1.77 in the AD study and 1.11 in the ALS study, while the mean R_{ac} for AUC was 2.25 and 1.48, respectively (*Table 2*). SAR443060 displayed a mean CSF-to-unbound plasma concentration ratio of 1.35 in the AD study and 1.00 in the ALS study.

PHARMACODYNAMICS – TARGET ENGAGEMENT

FIRST-IN-HUMAN STUDY SAR443060 demonstrated >90% median peripheral pRIPK1 inhibition in PBMCs at all dose levels tested. The duration that inhibition remained >90% increased with increasing doses: ≥4 hours following 100 mg, ≥8 hours following 200 mg, and for approximately 24 hours following a 400 mg single fasted dose of SAR443060 SDN (*Figure 4A*). In the multiple dose cohorts, median pRIPK1 inhibition was 79.9%, 93.7%, and 95.4% at 12 hours after the first dose on DAY 1 (trough concentration) for doses of 100 mg, 200 mg, and 400 mg respectively. Twelve hours after the last dose of SAR443060 on DAY 14, median pRIPK1 inhibition was 90.4%, 96.3%, and 96.1% following BID doses of 100 mg, 200 mg, and 400 mg respectively (*Figure 4B*).

STUDIES IN PATIENTS WITH AD OR ALS In the AD study, median (CI) percentage inhibition of pRIPK1 in PBMCs compared to baseline at steady state dosing (DAY 29) was 93.98% (CI: 95.4, 92.55, N=15) at 2 hours post dose (around T_{max}) and diminished over time to 81.83% (CI: 85.62, 78.03, N=15) at 12 hours post-dose (trough) (*Figure 5A*). In the ALS study, this was 92.34% (CI: 95.75, 68.11, N=14) at 2 hours post dose and diminished overtime to 65.92% (CI: 79.3, 45.39, N=14) at 12 hours post-dose (*Figure 5B*).

No statistically significant differences were seen with the DCTCLOCK™ for the AD study, or the ALSFRS-R clinical rating scale for the ALS study.

DISCUSSION

The results from these randomized, placebo-controlled, phase 1 and 1B clinical studies indicate that treatment with the RIPK1-inhibitor SAR443060 for up to 28 days is well tolerated with similar PK and target engagement response across healthy subjects and patients with AD or ALS. Although AESIs of dermatological findings, mild anemia and thrombocytopenia were observed during the study, none were severe or considered related to the IMP. All the hematological findings resolved spontaneously without intervention. While the OLE study in ALS patients did not identify any clear relevant safety concerns, no robust conclusion regarding the long-term safety of SAR443060 administration can be derived due to the limited sample size and early termination of the OLE trial.

SAR443060 administration demonstrated a dose-dependent effect on peripheral pRIPK1 inhibition in PBMCs from healthy subjects and patients with AD and patients with ALS. Administration of doses of 100-400 mg BID in healthy subjects led to robust target engagement ≥90% at steady state trough for all dose levels tested. However, due to dose-limiting toxicity findings in a parallel GLP 3-month preclinical study, the dose level for the AD and ALS patient studies was adjusted from 200 mg to 50 mg BID. This 50 mg BID dose level resulted in peripheral inhibition of pRIPK1 in PBMCs compared to baseline of 81.83% in the AD study and 65.92% in the ALS study, at steady state trough. We could not find any significant changes in DCTCLOCK™ for the AD study, or the ALSFRS-R clinical rating scale for the ALS study, and the study was not powered for the number and duration to allow detection of clinical change.

While it is not possible to measure pRIPK1-inhibition directly at the target site (astrocytes and microglia) in human subjects with neurodegenerative conditions, the combination of CSF-distribution and robust peripheral target engagement demonstrated in these studies with SAR443060 offers an encouraging proof-of-mechanism that therapeutic modulation of RIPK1 in the CNS may be possible.[31]

Although the long-term preclinical toxicities were not observed in the short-duration SAR443060 clinical studies, SAR443060 development was discontinued due to the potential risk of these findings from the non-clinical studies. The dose-limiting toxicities observed preclinically for SAR443060 have not been reported for other RIPK1-inhibitors with dosing periods of up to 84 days,[24,25] suggesting that these are compound specific and not common to RIPK1-pathway inhibition. Recently, it was announced that SAR443820 (DNL788), a CNS-penetrant back-up compound for SAR443060, successfully completed first in human studies and a phase 2 study (HIMALAYA) in ALS patients is expected to commence in the first quarter of 2022.[32]

Table 1 Summary statistics of pharmacokinetic parameters for SAR443060 in the FIH study (DNLI-D-0001).

Treatment	C_{max} (µM)	T_{max} (h)	AUC_{0-12} (µM · h)	AUC_{0-inf} (µM · h)	AUC_{0-tau} (µM · h)	AUC R_{ac}	$T_{1/2}$ (h)[1]	CSF (µM)[2]
SINGLE DOSE								
100 mg SDN (N=5)	0.663 (0.315-1.82)	4.03 (0.53-4.05)	2.94 (1.62-7.42)	4.75 (2.25-11.6)	-	-	10.0 (8.81-12.2)	-
200 mg SDN fasted (N=5)	1.02 (0.950-1.12)	2.12 (1.03-4.07)	5.25 (4.23-6.76)	9.26 (6.56-15.5)	-	-	11.4 (8.11-15.6)	-
100 mg SDN fed (N=4)	0.615 (0.399-0.838)	4.00 (4.00-4.00)	3.38 (2.40-4.95)	5.21 (3.50-8.28)	-	-	9.79 (8.85-10.3)	-
400 mg SDN (N=5)	1.53 (1.11-1.91)	4.03 (2.07-4.03)	9.84 (9.03-11.5)	17.8 (14.1-24.5)	-	-	9.71 (7.08-12.6)	-
200 mg MDS fasted (N=5)	0.624 (0.460-0.895)	4.05 (1.03-4.05)	4.61 (3.41-6.56)	11.8 (7.68-14.3)	-	-	8.99 (7.71-12.4)	-
200 mg MDS fed (N=5)	1.40 (0.564-2.93)	4.00 (2.02-12.03)	7.77 (4.16-14.1)	13.3 (9.09-19.1)	-	-	9.96 (7.73-15.0)	-
MULTIPLE DOSE								
100 mg SDN BID day 1 (N=8)	0.580 (0.346-1.04)	1.28 (0.50-4.05)	2.85 (1.96-3.98)	-	-	-	-	-
100 mg SDN BID day 14 (N=8)	0.966 (0.685-1.42)	1.52 (1.05-4.07)	-	-	6.24 (4.55-8.62)	2.19 (1.74-3.07)	14.2 (10.3-20.3)	0.103 (0.0711-0.157)
200 mg SDN BID day 1 (N=8)	1.07 (0.617-2.18)	2.09 (1.03-4.07)	5.34 (3.41-7.41)	-	-	-	-	-
200 mg SDN BID day 14 (N=8)	1.59 (1.06-2.47)	2.03 (1.03-4.03)	-	-	9.50 (5.50-12.8)	1.78 (1.03-2.84)	13.5 (8.38-20.5)	0.159 (0.0793-0.267)
400 mg SDN BID day 1 (N=5)	2.22 (1.44-2.90)	2.25 (1.50-4.08)	13.2 (8.94-17.2)	-	-	-	-	-
400 mg SDN BID day 14 (N=5)	3.10 (2.09-4.53)	2.05 (1.50-4.05)	-	-	19.1 (10.1-33.9)	1.45 (1.12-2.43)	12.1 (9.51-18.9)	0.234 (0.141-0.285)

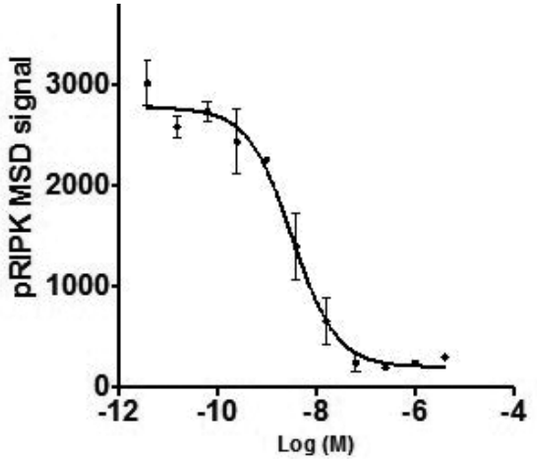
Values are presented as geometric mean (minimum-maximum), except for T_{max} where the median (range) is presented. BID = twice daily / CSF = cerebrospinal fluid / MDS = micronized drug substance / N = number of subjects receiving study medication / SDN = spray-dried nanosuspension. /1) There is uncertainty in the calculation of the geometric mean half-life for all dose levels as the sampling period was less than 5 times the half-life which is too short for a reliable half-life calculation. /2) CSF samples were collected 4 hours postdose on DAY 12.

Table 2 Summary statistics of plasma pharmacokinetic parameters for SAR443060 in the AD and ALS patient studies.

Treatment	C_{max} (µM)	C_{max} R_{ac}	T_{max} (h)	AUC_{0-12} (µM · h)	AUC R_{ac}	$T_{1/2}$ (h)	CSF-to-unbound plasma ratio
DNLI-D-0002: PHASE 1B STUDY IN AD PATIENTS							
50 mg BID single dose (SOT)	0.427 (0.222) N=16	-	1.72 (0.5; 8.0) N=16	1.64 (0.626) N=16	-	-	-
50 mg BID multiple dose (EOT)	0.670 (0.281) N=16	1.77 (0.737) N=16	1.50 (1.0; 4.2) N=16	3.62 (1.48) N=16	2.25 (0.598) N=16	19.0 (16.25) N=11	1.35 (0.538) N=15
DNLI-D-0003: PHASE 1B STUDY IN ALS PATIENTS							
50 mg BID single dose (SOT)[1]	0.581 (0.405) N=15	-	1.05 (0.47; 4.00) N=15	2.11 (1.89) N=14	-	-	-
50 mg BID multiple dose (EOT)	0.638 (0.267) N=14	1.31 (0.540) N=13	1.25 (0.50; 4.52) N=14	3.12 (1.20) N=14	1.85 (0.325) N=12	14.2 (5.18) N=14	1.00 (0.256) N=13

Values are presented as mean (standard deviation), except for T_{max} where the median (range) is presented. BID = twice daily / CSF = cerebrospinal fluid / EOT = end of treatment (DAY 28) / N = number of subjects receiving study medication / SOT = start of treatment (DAY 1). As there were only 2 patients on G-TUBE administration in the ALS OLE study, PK parameters were not analyzed for G-TUBE administration. /1) The first patient who received 200 mg twice daily for 21 days is included in the descriptive statistics of the PK parameters after the 1st administration. As a result, SOT C_{max} and AUC_{0-12} for the ALS study are likely an overestimated and AUC R_{ac} underestimated.

Figure 1 SAR443060 inhibition of RIPK1 serine 166 autophosphorylation in human PBMCs. Human peripheral blood mononuclear cell (PBMCs) from 4 healthy donors were thawed and resuspended in Roswell Park Memorial Institute (RPMI) complete medium. Cells were incubated with a range of concentrations of SAR443060 and then stimulated with a combination of TNF-α, SM-164, and zVAD-FMK (TSZ). Two hours later, cells were lysed, and phosphorylated receptor-interacting serine/threonine-protein kinase (pRIPK1) was detected by a plate-based immunoassay on the Meso Scale Discovery (MSD) platform. Increasing concentrations of SAR443060 (3.8 pM–4 µM) reduced the phosphorylation of RIPK1 at SER166 in stimulated human PBMCs in a concentration-dependent manner with a geometric mean IC_{50} value of 3.9 nM. Sample dose-response curve is from 1 donor. At each concentration tested, the mean and SD of the pS166 RIPK1 signal were calculated from the technical duplicate. Error bars are SDs.



CAPTION FIGURE 2 (NEXT PAGE) >

a. 1) One subject was withdrawn during the study (COHORT A2 after completion of PERIOD 1), due to a medical history of eczema which made the subject ineligible for the study. b. 1) One patient discontinued during administration of placebo in PERIOD 2 of the AD study due to disease progression and was not included in the PD analysis. 2) The first patient in the ALS study was enrolled at the original dose of 200 mg BID and completed 21 days in the first treatment period. This patient decided to forgo the rest of treatment PERIOD 1 without withdrawing from the study, and subsequently completed the treatment PERIOD 2 and the FFU visits. 3) One patient in the ALS study discontinued during administration of placebo in PERIOD 2 due to disease progression. 4) One patient in the ALS study was excluded from the pharmacodynamic and pharmacokinetic analysis as this patient had stopped taking edaravone 9 days prior to administration of SAR443060 in PERIOD 1 (protocol deviation). 5) Two subjects withdrew from the ALS OLE study prematurely due to disease progression. 6) For the ALS OLE study, the COVID-19 pandemic prevented the pre-planned collection of several CSF and blood samples from patients. Furthermore, the OLE study was terminated early by Sponsor decision. As a result, there was not enough PK and PD data from the OLE available for analysis. AD = Alzheimer's disease/ ALS = amyotrophic lateral sclerosis/BID = twice daily/ FIH = first-in-human/MAD = multiple ascending dose/ MDS = micronized drug substance/OLE = open-label extension/PD = pharmacodynamics/PK = pharmacokinetics/SAD = single ascending dose;SDN = spray-dried nanosuspension.

Figure 2 Study designs, randomization and analysis populations for the completed SAR443060 phase 1 and phase 1B clinical program. A. Phase 1 first-in-human study in healthy volunteers. B. Phase 1B studies in patients with AD or ALS.

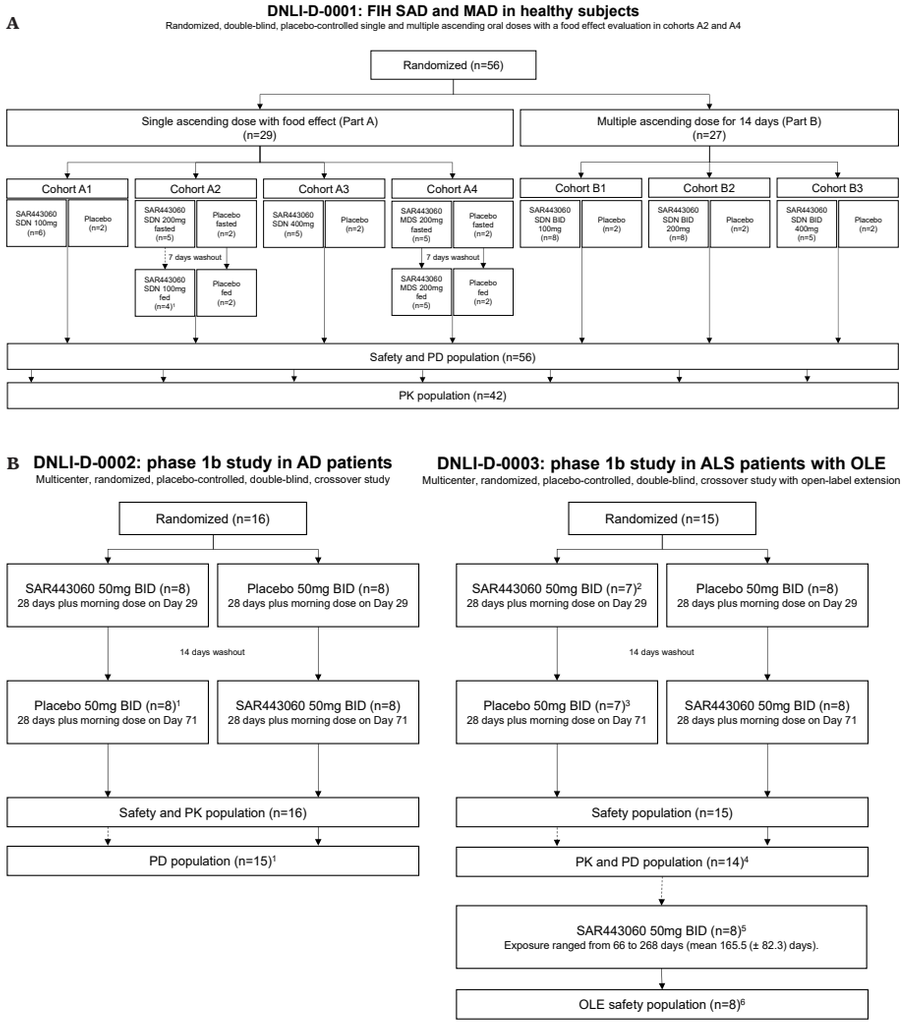


Figure 3 SAR443060 geometric mean plasma concentration-time profiles in healthy subjects. A. After administration of a single dose of the SDN and MDS formulations in fasted conditions and after a high-fat breakfast on a semi-logarithmic scale. B. DAY 1 and DAY 14 overlay for administration of twice-daily dosing (BID) on a semi-logarithmic scale. C. DAY 1 and DAY 14 full PK plasma concentration-time profiles and DAY 4, 7 and 11 predose (trough) concentrations during administration of twice-daily dosing (BID) on a semi-logarithmic scale. Mean (±SD) PK plasma concentration-time profiles on a linear scale per cohort are available in *Supplemental Figure S1.*

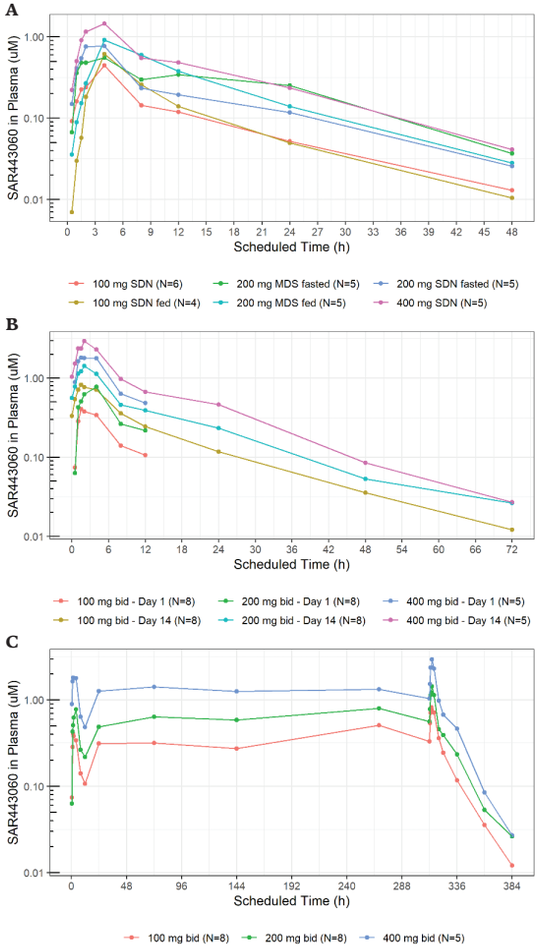


Figure 4 Median percentage of pRIPK1 inhibition compared to baseline after SAR443060 and placebo administration in healthy subjects. A. After single ascending doses and placebo up to 48 hours post dose. B. After ascending BID doses and placebo up to 48 hours post the last dose on DAY 14. Error bars represent inter quartile range (IQR). X-axis states the study days (D) and hours (HR) for each sampling timepoint. D1.0HR represents predose (baseline) measurement and D1.2HR the first measurement 2 hours post dose on DAY 1. Timepoints on the X-axis are not equally spaced in time.

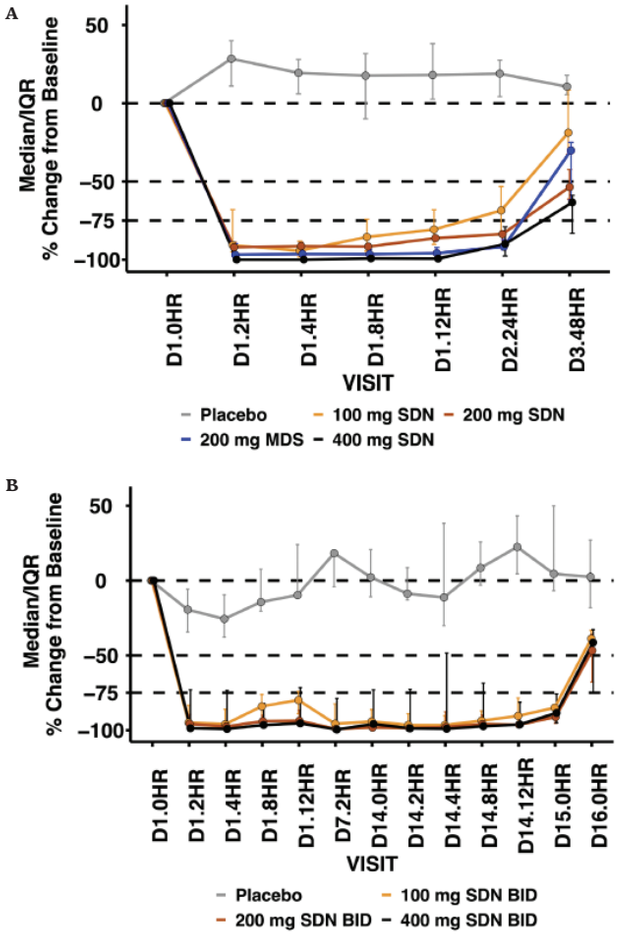


Figure 5 Median percentage of RIPK1 inhibition compared to baseline after SAR443060 and placebo administration in patients with AD (A) or ALS (B). SAR443060 50 mg or matching placebo was administered approximately every 12 hours (BID) in each treatment period for 28 days, followed by a final morning dose on the 29th day. Error bars represent inter quartile range (IQR). D1.0HR represents predose (baseline) measurement and D1.2HR the first measurement 2 hours post dose on DAY 1. Timepoints on the X-axis are not equally spaced in time.

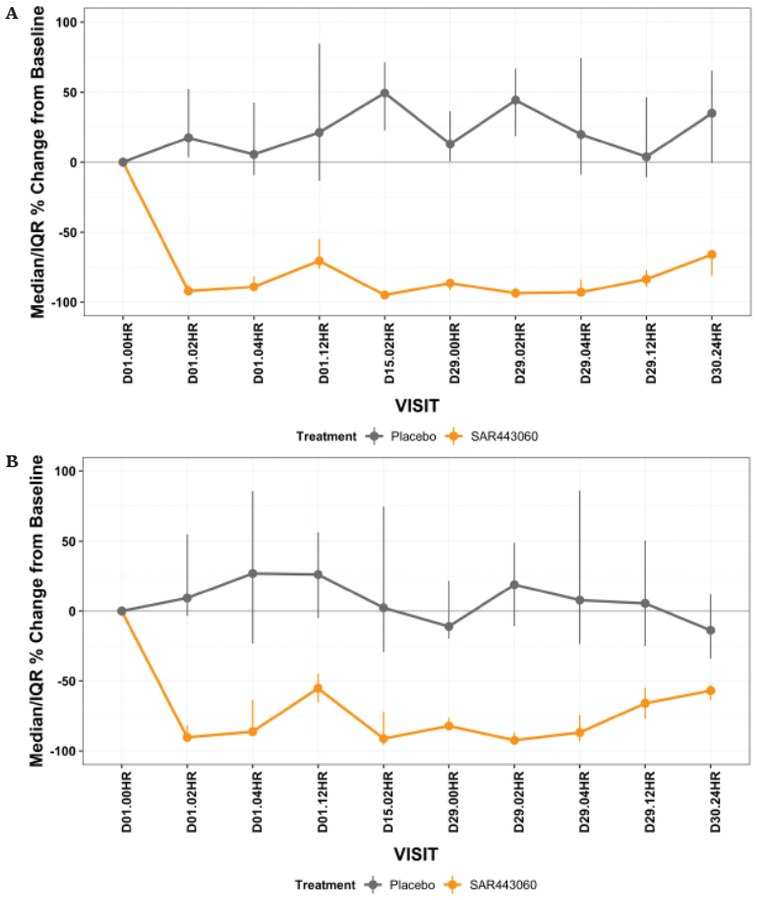


Table S1 Subject baseline characteristics at screening for the SAR443060 phase 1 and phase 1B clinical studies.

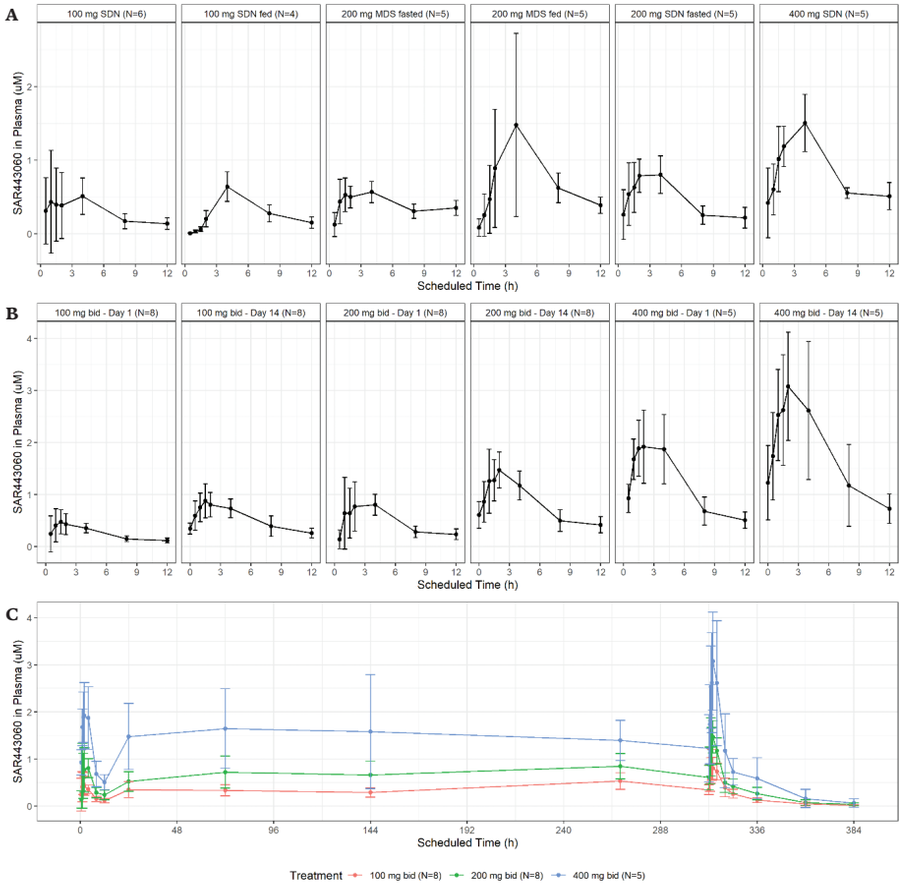
Characteristic	DNLI-D-0001: FIH SAD in healthy subjects	DNLI-D-0001: FIH MAD in healthy subjects	DNLI-D-0002: phase 1B study in AD patients	DNLI-D-0003: phase 1B study in ALS patients	DNLI-D-0003: phase 1B OLE in ALS patients
Total subjects (N)	29	27	16	15	8
Age, years, mean (SD)	31 (11)	35 (11)	70.9 (7.7)	57.5 (6.8)	59.9 (7.0)
Gender, male, N (%)	25 (86%)	25 (93%)	10 (63%)	12 (80%)	7 (75%)
BMI, kg/m^2, mean (SD)	24.6 (3.0)	25.4 (2.9)	26.8 (3.8)	26.7 (2.4)	26.6 (2.2)
Race, N (%)					
White	21 (72%)	21 (78%)	15 (94%)	15 (100)	8 (100%)
Asian	1 (3%)	1 (4%)	-	-	-
Black or African American	2 (7%)	1 (4%)	-	-	-
Other	1 (3%)	1 (4%)	1 (6%)	-	-
Mixed	4 (14%)	3 (11%)	-	-	-
Baseline MMSE, mean (SD)	N/A	N/A	20.6 (2.8)	N/A	N/A
Baseline CDR, N (%)					
0.5	N/A	N/A	7 (43.8)	N/A	N/A
1.0			9 (56.3)		
ALS disease duration at screening, months, mean (SD)	N/A	N/A	N/A	9.05 (4.58)	-
Disease medication use at baseline	N/A	N/A	8 (50%)[1]	15 (100%)[2]	8 (100%)

1) Including donepezil, galantamine, rivastigmine, and memantine /2) Most frequently reported medications by therapeutic class (>30%) in both treatment groups were other nervous system drugs, including riluzole or edaravone (100%), vitamins (53.3%) and analgesics (33.3%).

Table S2 Summary of treatment-emergent AEs for the SAR443060 phase 1 and phase 1B clinical studies.

	DNLI-D-0001: FIH SAD in healthy subjects		DNLI-D-0001: FIH MAD in healthy subjects		DNLI-D-0002: phase 1B study in AD patients		DNLI-D-0003: phase 1B study in ALS patients		DNLI-D-0003: phase 1B OLE in ALS patients
	Placebo (N=8)	*SAR443060 (N=21)*	*Placebo (N=6)*	*SAR443060 (N=21)*	*Placebo (N=16)*	*SAR443060 (N=16)*	*Placebo (N=15)*	*SAR443060 (N=15)*	*SAR443060 (N=8)*
Subject reporting ≥ 1 AE, N (%)	3 (37.5%)	13 (61.9%)	6 (100.0%)	19 (90.4%)	11 (68.8%)	12 (75.0%)	11 (73.3%)	12 (80.0%)	8 (100.0%)
Number of TEAEs, N	13	25	11	53	27	36	27	21	43
Number of mild TEAEs, N (%)	13 (100.0%)	25 (100.0%)	9 (81.8%)	48 (90.6%)	21 (81.5%)	27 (75.0%)	23 (85.2%)	18 (85.7%)	33 (76.7%)
Number of moderate TEAEs, N (%)	0	0	2 (18.2%)	5 (9.4%)	5 (18.5%)	9 (25.0%)	4 (14.8%)	3 (14.3%)	9 (20.9%)
Subjects with severe TEAE, N (%)	0	0	0	0	0	0	0	0	1 (12.5%)
Subjects with any treatment emergent SAE, N (%)	0	0	0	0	1 (6.3%)	0	0	0	1 (12.5%)
Subjects with any TEAE leading to death, N (%)	0	0	0	0	0	0	0	0	0
Subjects with any TEAE leading to study discontinuation N (%)	0	0	0	0	0	0	0	0	0
Subjects with any TEAE of special interest (AESI), N (%)	0	0	0	0	2 (12.5%)	3 (18.8%)	1 (6.7%)	1 (6.7%)	6 (62.5%)

Figure S1 SAR443060 mean (±SD) plasma concentration-time profiles in healthy subjects. A. After administration of a single dose of the SDN and MDS formulations in fasted conditions and after a high-fat breakfast. B. After administration of a multiple doses (BID) of the SDN formulations in fasted conditions on DAY 1 and DAY 14. C. DAY 1 and DAY 14 full PK plasma concentration-time profiles and DAY 4, 7 and 11 predose (trough) concentrations during administration of twice-daily dosing (BID) on a linear scale.



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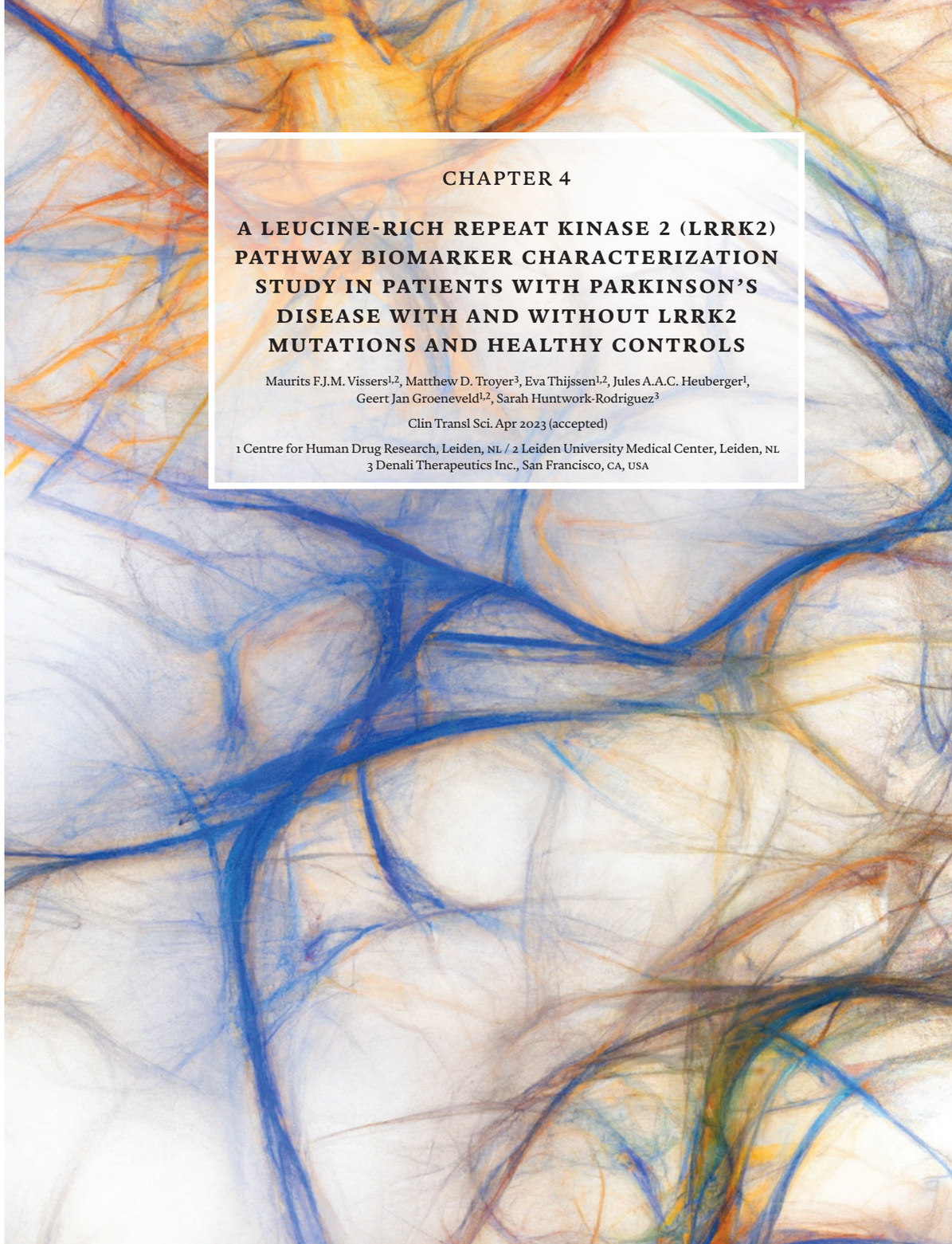

CHAPTER 4

A LEUCINE-RICH REPEAT KINASE 2 (LRRK2) PATHWAY BIOMARKER CHARACTERIZATION STUDY IN PATIENTS WITH PARKINSON'S DISEASE WITH AND WITHOUT LRRK2 MUTATIONS AND HEALTHY CONTROLS

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ABSTRACT

Increased leucine-rich repeat kinase 2 (LRRK2) kinase activity is an established risk factor for Parkinson's disease (PD), and several LRRK2-kinase inhibitors are in clinical development as potential novel disease-modifying therapeutics. This biomarker characterization study explored within- and between-subject variability of multiple LRRK2 pathway biomarkers (total LRRK2 [tLRRK2], phosphorylation of SER935 on LRRK2 [pS935], phosphorylation of RAB10 [pRAB10], and total RAB10 [tRAB10]) in different biological sources (whole blood, PBMCs, neutrophils) as candidate human target engagement and pharmacodynamic biomarkers for implementation in phase 1/2 pharmacological studies of LRRK2 inhibitors. PD patients with a LRRK2 mutation (N=6), idiopathic PD patients (N=6) and healthy matched control subjects (N=10) were recruited for repeated blood and cerebrospinal fluid (CSF) sampling split over two days. Within-subject variability (geometric CV, %) of these biomarkers was lowest in whole blood and neutrophils (range: 12.64 to 51.32%) and considerably higher in PBMCs (range: 34.81 to 273.88%). Between-subject variability displayed a similar pattern with relatively lower variability in neutrophils (range: 61.30 to 66.26%) and whole blood (range: 44.94 to 123.11%), and considerably higher variability in PBMCs (range: 189.60 to 415.19%). Group level differences were observed with elevated mean pRAB10 levels in neutrophils and a reduced mean pS935/tLRRK2 ratio in PBMCs in PD LRRK2-mutation carriers compared to healthy controls. These findings suggest that the evaluated biomarkers and assays could be used to verify pharmacological mechanisms of action and help explore the dose-response of LRRK2-inhibitors in early phase clinical studies. In addition, comparable α-synuclein aggregation in CSF was observed in LRRK2-mutation carriers compared to idiopathic PD patients.

INTRODUCTION

Gain-of-kinase-function mutations in the gene encoding the leucine-rich repeat kinase 2 (LRRK2) protein confer the highest population-attributable risk to Parkinson's disease (PD), accounting for approximately 4-5% of familial PD and 1-2% of sporadic PD.[1–3] Moreover, emerging evidence suggests that LRRK2 activity is also increased in a proportion of idiopathic PD patients,[4,5] which has sparked an interest in the development of LRRK2-kinase inhibitors as potential disease-modifying therapeutics.[6–10]

Pathogenic LRRK2 mutations associated with PD reside in the guanosine triphosphatase (GTPase) and kinase domains of the protein (*Figure 1*). The most common LRRK2 mutation, G2019S, is located in the kinase domain and increases LRRK2 kinase function approximately 2-3 fold.[6,7,11–14] Surprisingly, mutations in the neighboring GTPase domain, including the R1441C/G/H and Y1699C mutations, seem to have substantially larger (indirect) effects on LRRK2 kinase activation of up to 2-15-fold in model systems.[6,7] This effect appears to be mediated through intramolecular regulation of the kinase activity by the GTPase.[7,12,15,16]

Increased LRRK2 kinase activity results in excessive phosphorylation of substrates, including a subset of RAB GTPases. These RAB GTPases are a family of key players in intracellular trafficking events and lysosomal homeostasis.[6] Together, the LRRK2-RAB GTPase pathway is believed to play a role in regulating endo-lysosomal biology via multiple mechanisms, including endocytosis, autophagy and lysosomal functioning.[15,17–20] In PD, lysosomal damage and dysfunctioning (resulting from increased LRRK2 kinase activity) may be a central mechanism impairing degradation of proteins, ultimately resulting in the accumulation of α-synuclein (αSYN), a cardinal pathological feature of PD.[8,21–23]

Several LRRK2 pathway and inhibition biomarkers have been described in recent years including total LRRK2 (tLRRK2) protein for safety evaluation, potential pathway engagement, and normalization purposes,[7,8,10,24–29] phosphorylation of the serine 935 (pS935) residue on LRRK2 as an indirect LRRK2-inhibitor target engagement marker,[7,8,13,30,31] phosphorylation of the THR73 residue on the direct LRRK2 substrate RAB10 (pRAB10) as a direct pharmacodynamic marker, and total RAB10 (tRAB10) for pRAB10 normalization purposes (*Figure 1*).[6,8,9,13,31–33] In addition, LRRK2 autophosphorylation of serine 1292 and phosphorylation of several other RAB family substrates (e.g. RAB1B, RAB7A, RAB8A and RAB12) have been investigated as potential LRRK2 pathway biomarkers, but these carry less ideal properties for use in a clinical setting.[8,31–34] More downstream pathophysiological biomarkers that could be of interest in clinical studies with LRRK2-inhibitors include bis(mono-acylglycerol)phosphate (BMP) isoforms as a readout of lysosomal functioning,[9,35] and/or potentially direct measurement of pathologic αSYN aggregation potency.[36,37]

To establish a potential treatment effect in PD, LRRK2 inhibition would need to reach the central nervous system (CNS). CNS tissue, however, cannot be used to assess biomarker levels of target and pathway engagement in a clinical setting. Fortunately, LRRK2 is present throughout the brain and body – with the highest expression in circulating immune cells, the lungs and the kidney – , which offers an opportunity to investigate blood-based biomarkers as surrogates for CNS LRRK2 activity.[7,38]

In addition, it has recently been demonstrated that total LRRK2 can be evaluated in cerebrospinal fluid (CSF).[29] *In vitro* and *in vivo* experiments have demonstrated that LRRK2 inhibition shows a similar dose-response pattern in peripheral tissues and cells compared to the CNS.[9,13] This provides an opportunity for clinical studies with LRRK2-inhibitors to use blood (and CSF) based pathway biomarkers, combined with drug concentration measurements in blood and CSF, to predict CNS pharmacodynamic effects.[7,9,13,38] Moreover, recently new assay techniques have become available that make it possible to detect small quantities of αSYN aggregates circulating in CSF.[36] αSYN aggregation potency might provide an interesting pathophysiological response biomarker to LRRK2-inhibtion, and more relevant than total αSYN levels that can display little difference between PD patients and healthy controls.[37]

Before any anticipated pharmacological effect biomarker can be implemented in a clinical study, it is essential to understand the within- and between-subject variability as this influences the minimal detectable effect-sizes as well as the overall biomarker sampling and analysis strategy. Therefore, the purpose of this biomarker characterization study was to explore within- and between-subject variability in tLRRK2, pS935, pRAB10, and tRAB10 in different biological sources (whole blood, peripheral blood mononuclear cells [PBMCs], neutrophils) as candidate human target engagement, pharmacodynamic, and potential patient stratification biomarkers for further formal validation studies.[13] Additionally, this study aimed to explore group level differences in LRRK2 pathway activity and αSYN aggregation in CSF between PD patients with and without a LRRK2 mutation and healthy control subjects, all with the aim to develop a robust biomarker strategy for implementation in phase 1/2 pharmacological studies of novel LRRK2 inhibitors.[9,10]

METHODS

Study design and population

This single-center, non-interventional study used a design with repeated blood sampling split over two days to investigate both day-to-day within-subject (intraindividual) and between subject (interindividual) variability in LRRK2 pathway biomarkers. In addition, group level differences in biomarker levels between PD patients with a LRRK2 mutation (LRRK2+PD), idiopathic PD patients (iPD) and healthy matched control subjects (HC) without Parkinson's disease were assessed. In each group six subjects were planned to complete two visits to the clinic at least 10 days and up to 4 weeks apart, for blood sample and CSF collection after a low-fat breakfast followed by 4 hours of fasting. Four additional healthy control subjects were planned to complete only 1 clinic visit for blood sample and CSF collection in a fasted state. All biomarker samples were collected during the same part of the day (morning), at approximately the same time. Subjects had a safety follow-up visit or telephone call approximately one week after completing the last clinic visit.

The LRRK2+PD and iPD patients were recruited from a database of 3402 genotyped PD patients (CHDR, Leiden, the Netherlands) in 2018.[39] LRRK2+PD patients had to have completed genetic screening showing one of the following LRRK2 mutations: G2019S, I2020T, R1441G, R1441C, R1441H, N1437H, or Y1699C, absence of PD associated glucocerebrosidase gene (GBA) mutations, and a clinical diagnosis of PD (Hoehn & Yahr stage 1-4). The iPD patients had to have completed genetic screening showing absence of PD associated LRRK2 and GBA mutations. Healthy control subjects were matched to a LRRK2+PD or iPD patient for gender, age (+/- 5 years) and BMI (+/- 3.5 kg/m^2), had to have no clinical history or signs/symptoms of PD and no first order relatives diagnosed with PD. Subjects were allowed to maintain stable doses and regimens for concomitant medication, herbal treatments, medical marijuana, and dietary supplements during the study. Only non-smokers were included.

The sample size was based on practical considerations based on the estimated prevalence of LRRK2+PD in the Netherlands and is considered sufficient to provide descriptive information on LRRK2 pathway biomarkers.

This study was conducted in accordance with the International Conference for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use, Good Clinical Practice (GCP), and the principles of the Declaration of Helsinki. The study was registered in the Netherlands Trial Register (NTR7647), approved by an independent ethics committee (Stichting Beoordering Ethiek Biomedisch Onderzoek, Assen, the Netherlands), and all subjects provided their written informed consent before participation.

Biomarker assessments

Neutrophil and PBMC lysates were analyzed for pS935, tLRRK2, pRAB10, and tRAB10. Whole blood was analyzed for pS935 and tLRRK2 only, because RAB10 was not detectable in whole blood with available assays.

For neutrophil isolation, whole blood was collected in a K2EDTA tube and neutrophil isolation was performed within 1 hour using a Direct Human Neutrophil Isolation Kit and RoboSep device (StemCell, Germany) following the manufacturer's protocol. Frequency of neutrophils in whole blood, yield and purity (CD16+, CD66B+, CD45+) of the negative fraction containing the neutrophils were assessed by flow cytometry using a MACSQuant 10 analyser

(Miltenyi Biotec, Germany). Neutrophils were pelleted by centrifugation and then resuspended in lysis buffer. The lysates were incubated on ice for 20 min, followed by centrifugation. Supernatants were aliquoted and stored at – 80 °C for later immunoassay analysis.

For PBMC analysis, blood was collected into CPT-sodium heparin tubes and PBMCs were isolated following the manufacturer's protocol. Cells were counted on a MACSQuant 10 analyzer (Miltenyi Biotec, Germany). PBMCs were pelleted by centrifugation and resuspended in PBMC lysis buffer.[13] The lysates were incubated on ice for 20 min, followed by centrifugation. Supernatants were aliquoted and stored at – 80 °C for later immunoassay analysis.

pS935, tLRRK2, pRAB10, and tRAB10 in all samples were quantified using Meso Scale Discovery (MSD)-based assays according to methods described elsewhere,[13] with the exception that at the time of this study these assays were still in a developmental stage and not yet validated. Results were plotted with MSD arbitrary units (A.U.).

CSF was collected in polypropylene tubes following lumbar puncture with an atraumatic 22G needle, centrifuged and the supernatant was aliquoted and stored at –80°C. Samples were analyzed using an αSYN Seed Amplification Assay (αS-SAA) that uses amplification cycles and an excess of recombinant αSYN to elongate and detect misfolded αSYN aggregates (αSYN seeds) in CSF. The assay was performed by Amprion using previously published methods.[40–42] Briefly, CSF samples were blindly analyzed in triplicate (40 µL each) in a reaction mixture comprising 0.3 mg/mL recombinant αSYN (Amprion, cat# S2020), 100 mM PIPES pH 6.50, 500 mM NaCl, 10 µM Thioflavin T (THT), and one 3/32' Si3N4 bead blocked with 1% BSA. Reaction mixtures were analyzed in 96-well plates. Plates were orbitally shaken at 800 RPM for 1 minute every 29 minutes at 37 °C for 150 hours. Fluorescence readings (RFU, 440-10/490-10) were collected every 30 minutes. The results from each triplicate were combined to determine the samples result (positive/negative for αSYN seeds) using a probabilistic algorithm already described.[40]

Statistical analysis

All statistical analysis was performed using SAS statistical software (version 9.4). Within-subject variability between two visits and between-subject variability are expressed as geometric coefficient of variation (CV, %). Geometric CV was estimated within a repeated measures mixed effects model with group, visit, and group by visit as fixed factors, and visit as repeated factor within subject, and a compound symmetry variance/covariance structure within group, if possible. Where possible, pS935 was normalized to tLRRK2 (pS935/tLRRK2) and pRAB10 to tRAB10 (pRAB10/tRAB10) and to tLRRK2 (pRAB10/tLRRK2) to explore if this would reduce variability. Neutrophil and PBMC biomarker values were also normalized to cell number and glyceraldehyde 3-phosphate dehydrogenase (GADPH; PBMCs only) as an exploratory analysis to determine whether this would reduce within- and between-subject variability. Within the model, mean (95% confidence intervals [CI]) group level differences in pS935, tLRRK2, pS935/tLRRK2, pRAB10, tRAB10, pRAB10/tRAB10, and pRAB10/tLRRK2 levels were explored on log-transformed data. Inconclusive test results in the αS-SAA were treated as false negatives for calculation of the assay sensitivity and specificity, with their 95% CI (Wilson Score Intervals). The αS-SAA THT fluorescence signal between the LRRK2+PD, iPD and HC groups was analyzed with a repeated measures mixed effects model, with time as repeated factor within subject by visit, and group, visit, time, and group by time as fixed factor. The contrasts between the 3 groups were estimated within the model, with their 95% CI. Estimated means per group and timepoint were generated and graphically presented. The level of significance was set at $P < .05$.

RESULTS

Demographics and baseline characteristics

Six LRRK2+PD, six iPD and ten matched HC subjects were enrolled into the study, with an age of between 47 and 81 years and a BMI between 21.0 and 32.9 kg/m^2. Most subjects were male (86%). Baseline characteristics (except for the PD diagnosis) were generally comparable between the three groups (*Table 1*). The six LRRK2+PD patients had a shorter mean time since diagnosis (6.7±4.0 vs 9.8±3.8 years), a slightly lower mean Hoehn & Yahr score (1.8±1.2 vs 2.0±0.8), and included more subjects from North African descent (33% vs 0%) compared to the six iPD patients. Five (83%) LRRK2+PD patients carried a G2019S LRRK2 mutation and one (17%) patient carried a R1441C mutation. None of the iPD subjects had mutations in the LRRK2 and GBA genes. All patients with PD and 6 healthy control subjects completed two visits to the clinic, and four healthy control subjects only completed 1 clinic visit (*Table 1*). Two LRRK2+PD patients only completed 1 out of the 2 planned lumbar punctures.

Within-subject variability per biomarker and biological source

Within-subject variability (geometric CV, %) between VISIT 1 and 2 for each biomarker (pS935, tLRRK2, pS935/tLRRK2, pRAB10, tRAB10, pRAB10/tRAB10, and pRAB10/tLRRK2) in each biological source (whole blood, PBMCs, and neutro-

phils) is depicted in *Figure 2A*. Within-subject variability was lowest in whole blood (ranging from 12.64 to 30.37% [pS935/tLRRK2 and pS935, respectively]) and neutrophils (ranging from 43.55 to 51.32% [tRAB10 and pRAB10, respectively]). Both pS935 and tLRRK2 were below quantification limits in all neutrophil lysate samples. In PBMCs within-subject variability was considerably higher for all biomarkers (ranging from 121.49 to 273.88% [pS935/tLRRK2 and tLRRK2, respectively]), except for tRAB10 (34.81%). When normalizing pS935 to tLRRK2 (pS935/tLRRK2) and pRAB10 to tRAB10 (pRAB10/tRAB10) within-subject variability decreased in each biological source, though only marginally (*Figure 2A*). Normalizing pRAB10 to tLRRK2 (pRAB10/tLRRK2) in PBMCs resulted in the highest within-subject variability with a geometric CV of 789.46%. Normalizing to total cell number or GADPH (PBMCs only) did not decrease variability observed in PBMCs (*Supplemental Figure S1A*) or neutrophils (data not shown).

Between-subject variability within each subject group

Between-subject variability (geometric CV, %) for each biomarker in each biological source is depicted in *Figure 2B*. Between-subject variability was lowest in neutrophils (ranging from 61.30 to 66.26% [pRAB10/tRAB10 and pRAB10, respectively]), followed by whole blood (ranging from 44.94 to 123.11% [pS935 and tLRRK2, respectively]). In PBMCs between-subject variability was again considerably higher (ranging from 189.60 to 415.19% [pS935 and pRAB10/tRAB10, respectively]), except for tRAB10 (74.32%) and pS935/tLRRK2 (96.27%). Normalizing pS935 to tLRRK2 (pS935/tLRRK2) approximately halved between-subject variability of pS935 in PBMCs, but more than doubled it in whole blood. Normalizing pRAB10 to tRAB10 (pRAB10/tRAB10) did not significantly decrease between-subject variability in neutrophils and increased variability in PBMCs (*Figure 2B*). Controlling pRAB10 for tLRRK2 (pRAB10/tLRRK2) in PBMCs resulted in the highest between-subject variability with a geometric CV of 798.71%. Normalizing to total cell number or GADPH (PBMCs only) did not decrease variability observed in PBMCs (*Supplemental Figure S1B*) or neutrophils (data not shown). There were no apparent differences in between-subject variability between the three populations, except for substantially higher between-subject variability in tLRRK2, pRAB10, and pRAB10/tRAB10 in the HC group, and for pRAB10/tLRRK2 in the HC and LRRK2+PD groups (*Supplemental Figure S2E*).

Group level differences for each biomarker

No group level differences were observed for pS935, tLRRK2, and tRAB10 between the LRRK2+PD, iPD and HC groups in either whole blood, PBMCs, or neutrophils. Furthermore, no group level differences were observed for pRAB10 in PBMCs (*Supplemental Table S1*). However, the mean phosphorylation of RAB10 (pRAB10) was significantly elevated in the LRRK2+PD (4795 A.U.) compared to the HC (2595 A.U.) group in neutrophils (P=0.0404). Though numerically higher, the mean pRAB10 level in neutrophils in the iPD group (3309 A.U.) did not significantly differ from the HC group (*Figure 3A*). After correcting for tRAB10 in neutrophils, the mean pRAB10/tRAB10 ratio was approximately two-fold higher in the LRRK2+PD (1.40) compared to the HC (0.67) group (P=0.0062). The mean pRAB10/tRAB10 ratio in the iPD group (1.26) was also elevated compared to the HC group, but this difference was not significant (P=0.0698), nor was there a significant difference between the LRRK2+PD and iPD groups. The single subject with a $LRRK2^{R1441C}$ mutation had the highest pRAB10 level in neutrophils of all participants (pink dot *Figure 3A*). No group level differences were observed for pRAB10/tRAB10 or pRAB10/tLRRK2 in PBMCs.

When pS935 was corrected for tLRRK2, the mean pS935/tLRRK2 ratio was significantly lower in LRRK2+PD vs HCs (1.53 vs 3.12, P=0.0327) and in LRRK2+PD vs iPD (1.53 vs 4.46, P=0.0006) in PBMCs (*Figure 3B*), but not in whole blood.

Presence of pathogenic αSYN in CSF

Five out of six (83%) LRRK2+PD patients tested positive for αSYN seeds in all three replicates of all CSF samples, and 1 (17%, G2019S mutation) subject only tested positive in two of the three replicates in both CSF samples (result considered inconclusive). For the six iPD patients, four (67%) tested positive in all three replicates of both visits' samples, one patient (17%; 70 years; H&Y stage 2) only tested positive in one of the three replicates for both visits (result considered negative) and one patient (17%; 47 years; H&Y stage 3, DAT SPECT confirmed) did not test positive in any of the three replicates for both visits. These two αSYN seeds-negative iPD patients had the lowest mean pRAB10 and highest pS935/tLRRK2 levels within their group (blue dots *Figure 3*). Eight out of the ten (80%) HC subjects tested negative in all replicates. One HC subject (10%) tested negative in all replicates during his first visit, but positive in all three replicates is second visit, and one HC subject (10%) tested positive in all three replicates during his first and only study visit. Assuming diagnosis of the participants is correct, the calculated overall sensitivity (%, 95% CI) of the αS-SAA was 80.0% (49.0-94.3%) for the LRRK2+PD and 58.3% (32.0-80.7%) for the iPD population. Specificity was 87.5% (64.0-96.5%) in the healthy controls. The kinetics of αS-SAA aggregation (mean ± 95% CI) for all three groups are shown in *Figure 4*. The mean αS-SAA signal (RFU) was highest in the LRRK2+PD group and lowest in the HC group. Though the mean time to reach 50% aggregation

(T_{50}) was not significantly different between the LRRK2+PD and iPD groups, the total mean αS-SAA signal at the last recorded timepoint was approximately 1.3-fold higher in the LRRK2+PD group.

DISCUSSION

Variability

Reproducible, blood-based, biomarker assays are essential to allow for simple and reliable evaluation of LRRK2 pathway and inhibition levels in clinical studies with LRRK2-inhibitors. Such biomarker assays are especially helpful in early phase studies to explore the safety profile of novel compounds at different levels of LRRK2-inhibition and to help select dose levels within the anticipated therapeutic window for follow-on larger late-stage patient trials.[38]

The within-subject variability observed between two visits for pS935 and tLRRK2 (CV = 30.37 and 27.41%) in whole blood suggests that the investigated assays would be fit-for-purpose to evaluate tLRRK2 levels and LRRK2 inhibition via pS935 in whole blood in a clinical study setting. During further validation of the assays described in this manuscript, even lower within-subject variability was observed over the course of 24 hours with a CV of 16% and 7% for pS935 and tLRRK2 respectively,[13] and these assay have now successfully been used during the early stage clinical evaluation of two novel LRRK2-inhibitors.[9,10] Much higher within-subject variability with CVs >100% was observed for the investigated LRRK2 and downstream RAB10 biomarkers in PBMCs, which could make it difficult to quantitatively differentiate the lower end of the LRRK2-inhibition dose-response curve in PBMCs in a clinical setting. However, it was anticipated that in a clinical study a level of LRRK2 inhibition would be achieved that would enable characterization of the pRAB10 pharmacodynamic response to LRRK2 inhibition in PBMCs. Measurement of pRAB10 reduction in PBMCs was subsequently implemented in phase 1 and phase 1B studies of two LRRK2 inhibitors which showed a clear pharmacodynamic response alongside pS935 reduction, demonstrating that pathways downstream of LRRK2 were inhibited in those studies.[9,10] Total LRRK2 in human PBMCs can vary widely among individuals,[43] however, correcting for tLRRK2 – or tRAB10 for that matter – did not significantly reduce the pS935 or pRAB10 variability observed in PBMCs in this small-size exploratory study. Correcting for cell number or GAPDH also did not meaningfully reduce variability in PBMCs. An observed higher biomarker variability in PBMCs can be explained by the fact that PBMCs consist of a heterogeneous cell population and only a minority of these cells (monocytes, which make up 5-20% of PBMCs) express LRRK2 and RAB10.[31] LRRK2 and RAB10 are expressed roughly 2-fold higher in neutrophils compared to monocytes, which likely translates to the considerably lower within-subject variability observed for pRAB10 and tRAB10 in the neutrophils (CV = 51.32 and 43.55%). The downside of working with neutrophils in a clinical setting, however, is a more complicated isolation procedure and the fact that LRRK2 in neutrophil extracts may undergo considerable proteolytic degradation, which could explain why pS935 and tLRRK2 in this study's neutrophil assays were below the limit of detection.[31] The source of variability of pS935, tLRRK2, pRAB10, and tRAB10 in this study is likely to be due mainly to biological variability, as a subsequent characterization of the assay technical variabilities in whole blood and PBMCs showed CVs < 20% between technical replicates.[13] In addition, only two observations were used to calculate the within-subject variability which introduces a risk for overestimating the variability.

Between-subject variability was higher than within-subject variability for all investigated biomarkers in all biological sources, which is consistent with observations by others.[13,31] There are a few (counter-intuitive) exceptions to this observation when the within- and between-subject variability is broken down per subgroup (*Supplemental Figure S2B, C, E* and *F*), which likely results from the small sample size per subgroup and the fact that only two observations were used to calculate the within-subject variability. The considerable between-subject variability indicates that when assessing LRRK2 levels and inhibition in clinical studies, values should be analyzed relative to an individual's baseline value, rather than looking at absolute group level differences between active treatment and placebo, as has been done in published clinical studies with LRRK2 inhibitors.[9,10]

One disadvantage of monitoring LRRK2 biomarkers in the peripheral circulation only, is that it will always leave some uncertainty about how well this correlates to biomarker levels in the brain and peripheral organs including the lungs and kidney.[27] This caveat has been partially addressed in a recent report showing similar LRRK2 inhibition in the periphery (PBMC) and brain in cynomolgus macaques treated with DNL201, as assessed by pS935.[9] Currently, there are no clinically translatable methods to monitor LRRK2 inhibition using CSF-based methods or via imaging directly in the brain.

In addition, one caveat in using pRAB10 as a biomarker for LRRK2 kinase inhibition is that although in peripheral tissues and cells such as PBMCs, kidney, and lung LRRK2 knockout or kinase inhibition as measured by pS935 is accompanied by a reduction in pRAB10 similar in magnitude to pS935, in

brain, phosphorylation of RAB10 is only partially reduced with LRRK2 genetic ablation or kinase inhibition.[13,44] This may indicate that other kinases besides LRRK2 are also able to phosphorylate RAB10, especially in the brain where LRRK2 expression is restricted to specific cell types including microglia and oligodendrocyte precursor cells.[45] This seems to be confirmed by the recent discovery that PPM1H can act as a modulator of LRRK2 signaling via controlling dephosphorylation of RAB proteins.[46] In the absence of a clinically translatable biomarker of LRRK2 activity in CSF, peripheral pRAB10 may serve as a useful surrogate likely to be indicative of inhibition of LRRK2 in the subset of brain cells that do express LRRK2, on the condition that the investigational compound is also demonstrated to be highly CNS-penetrant with comparable peripheral and central (unbound) drug exposure levels.

Group level differences

LRRK2 kinase function has been reported to be elevated in the range of 2 to 15-fold in LRRK2 mutation carriers, while total LRRK2 levels were comparable between wildtype and LRRK2^{G2019S} mutation carriers.[6,7,11–13] These previous observations are replicated in this study, despite its small sample size, with no observed group level difference in total LRRK2 and an approximately 2-fold elevation in LRRK2's phosphorylation of RAB10 in neutrophils in the LRRK2+PD group, suggesting ≥50% LRRK2-inhibition as target for therapeutic efficacy in clinical studies.[9] Although not significant, potentially due to the small sample size, pRAB10 levels were also numerically higher the iPD group compared to HCs, which is in line with some previous reports[4,47] but has not been universally reported in studies of PBMC LRRK2 biochemical or downstream pathway activity in iPD patients.[9,13,48–51] The elevation in pRAB10 in iPD patients could potentially be explained by an increase in LRRK2 activity in response to lysosomal stress and inflammatory stimuli in idiopathic Parkinson's disease.[13] Interestingly, the single LRRK2^{R1441C} mutation carrier had the highest observed pRAB10 levels in neutrophils, which matches previous findings where an R1441C mutation displayed an approximate a 4-fold and a G2019S mutation an approximate 2-fold increase in RAB10 phosphorylation compared to LRRK2 wildtype.[6,8,33] As LRRK2 kinase is only known to affect the phosphorylation and not production of RAB10,[6] no group level differences in tRAB10 were expected or observed.

When corrected for tLRRK2, phosphorylation of LRRK2-SER935 (pS935/tLRRK2) was found to be reduced in the LRRK2 mutation carriers compared to the iPD and HC groups in PBMCs, but not in whole blood, similar to previous findings in PBMCs.[13,43] In PD, pS935 plays a role in LRRK2 binding to the 14-3-3 protein family that can regulate LRRK2 kinase activity, drive translocation of LRRK2 into exosomes followed by secretion into the urine, and protect LRRK2 from proteasomal degradation by inhibiting ubiquitylation.[52] Observations of reduced SER935 phosphorylation have also been reported in G2019S knock-in mouse astrocytes, in R1441C, R1441G, R1441H, Y1699C, and I2020T knock-in HEK-293 cells, and in the substantia nigra of iPD patients.[13,16,53]

Presence of pathogenic αSYN in CSF

The percentage of αSYN seeds positive subjects in the LRRK2+PD group (83%) was higher compared to previous reports using similar assays (40%; N=15, and 78%; N=9), which could result from assay differences and/or the low sample size in these studies, but does seem to contradict speculated αSYN structural, self-aggregation potency, and/or αSYN burden diversity between LRRK2+PD and iPD patients.[37,54]

In the αS-SAA both T_{50} and the top fluorescence value appear to be related to the concentration of αSYN seeds in the original CSF sample.[36] The T_{50} and the top fluorescence value were comparable between the LRRK2+PD and iPD groups, despite on average a shorter time since PD diagnosis in the LRRK2+PD group. This could suggest that αSYN aggregation is present at least to similar extend in LRRK2-mutation carriers compared to iPD patients, which is further supported by previous reports of elevated αSYN levels in CSF for LRRK2-mutation carriers.[37,55,56] However, at this time the αS-SAA is not validated to detect and/or quantify potential αSYN aggregation level differences and therefore these observations should be interpreted with care. But this does open up the possibility to investigate αS-SAA as a potential pharmacodynamic biomarker in the future.

Two iPD patients tested negative for αSYN with the αS-SAA, resulting in a low assay sensitivity for this population (58.3%), which is surprising considering the αS-SAA's previously reported high sensitivity of 88.5%,[36] and these patients' confirmed PD diagnosis. Interestingly, these two patients also had the lowest pRAB10 levels within the iPD group. This could support a correlation between LRRK2-activity and αSYN aggregation, which could make αS-SAA an interesting pathophysiological biomarker in future LRRK2-inhibitor studies, although this would first require confirmation in a larger population. The two HC subjects that tested positive via the αS-SAA do not have a clinical PD diagnosis to date. The high sensitivity and specificity of the αS-SAA in larger cohorts supports its continued use and investigation as a diagnostic assay in PD.[41]

To conclude, LRRK2-inhibition offers a promising therapeutic strategy for the treatment of PD patients with LRRK2-mutations and potentially also for idiopathic Parkinson's disease. While it has proved challenging to robustly demonstrate target engagement and pharmacodynamic effects of LRRK2-inhibition in the central compartment, several reliable peripheral biomarkers and assays have been identified over the past few years. Together with CSF concentrations of an investigational compound, measuring tLRRK2, pS935, tRAB10, and pRAB10 peripherally could verify pharmacological mechanisms of action and help explore the dose-response of novel LRRK2-inhibitors in early phase clinical studies. The large variability observed for pRAB10 in PBMCs indicates that this true LRRK2 pharmacodynamic marker would be useful only in situations where there is a very large effect size (e.g. as pRAB10 approaches depletion due to LRRK2 inhibition[9,10]) and is unlikely in this assay to be very useful as a method to distinguish very small changes in pRAB10 (e.g. as a patient stratification marker). pS935 and tLRRK2 in whole blood, on the other hand, seem particularly suitable to explore the full dose response curve in early phase LRRK2-inhibitor trials.

Table 1 Subject baseline characteristics at screening.

Characteristic	PD patients with LRRK2 mutation (LRRK2+PD)	Idiopathic PD patients (iPD)	Healthy controls (HC)	
	Subjects with 2 visits	*Subjects with 2 visits*	*Subjects with 2 visits*	*Subjects with 1 visit[1]*
Total subjects (N)	6	6	6	4
Age, years, mean (SD)	62.3 (11.8)	62.7 (10.7)	64.0 (10.2)	66.0 (3.1)
Gender, male, N (%)	5 (83%)	5 (83%)	5 (83%)	4 (100%)
BMI, kg/m^2, mean (SD)	27.8 (3.7)	28.5 (2.8)	27.0 (3.3)	26.8 (2.6)
Race, N (%)				
White	4 (67%)	6 (100%)	6 (100%)	3 (75%)
North African	2 (33%)	-	-	-
Mixed (White/Asian)	-	-	-	1 (25%)
Years since PD diagnosis at screening, mean (SD)	6.7 (4.0)	9.8 (3.8)	N/A	N/A
Baseline Hoehn & Yahr stage, N (%)				
Stage 1	4 (66%)	2 (33%)	N/A	N/A
Stage 2	-	2 (33%)		
Stage 3	1 (17%)	2 (33%)		
Stage 4	1 (17%)	-		
LRRK2 mutation status, N (%)				
G2019S	5 (83%)	N/A	N/A	N/A
R1441C	1 (17%)			

1) The 4 subjects that had only 1 clinic visit are not included in the analyses of within-subject variability.

Figure 1 Leucine-rich repeat kinase 2 protein schematic, downstream substrate, phosphorylation sites and common mutations. LRRK2 is a 2527 amino-acid, multidomain, protein consisting of a guanosine triphosphatase (GTPase) domain – comprised of the Ras of complex protein (ROC) terminating with a spacer domain called the C-terminal of the ROC-domain (COR) – immediately followed by a serine/threonine kinase domain and surrounded by several protein-protein interaction domains including the leucine-rich repeat (LRR) domains towards the N-terminus and WD40 domain at the C-terminus.[12] LRRK2 has a phosphorylation site at serine 935 (involved with inactive LRRK2 binding to the 14-3-3 family of proteins), that has been demonstrated to dephosphorylate upon LRRK2-inhibitor binding. The RAB10 GTPase is a direct substrate for the LRRK2 kinase domain with phosphorylation at the threonine 73 residue. Phosphorylated RAB10 is recruited onto stressed lysosomes in PD and may impact lysosomal vesicle formation/budding and exocytosis, which in turn may impair protein degradation (red cross in figure) and aggregation of misfolded proteins including pathogenic αSYN. Common pathogenic mutations associated with PD include R1441C/G/H in the GTPase domain and G2019S in the kinase domain, increasing LRRK2 kinase activity 2-3 and 2-15-fold, respectively. *Image created with BioRender.com.*

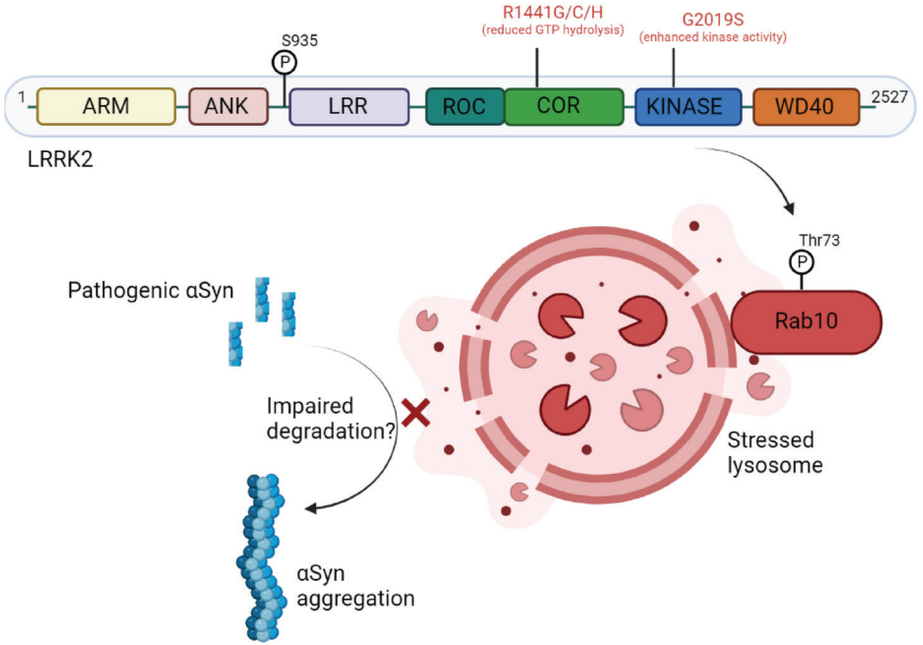

Figure 2 Variability of each biomarker in each biological source. A. Within-subject (intrasubject) variability between VISITS 1 and 2. B. Between-subject (intersubject) variability of pS935, tLRRK2, pS935/tLRRK2, pRAB10, tRAB10, pRAB10/tRAB10, and pRAB10/tLRRK2 in whole blood, PBMCs and neutrophils expressed as geometric coefficient of variation (%). Data from all 3 subpopulations (LRRK2+PD, iPD, and HC) was pooled for this analysis. Within-subject and between-subject variability were low in whole blood and neutrophils, and substantially higher in PBMCs. *Supplemental Figure S2A-F* for a breakdown per subpopulation.

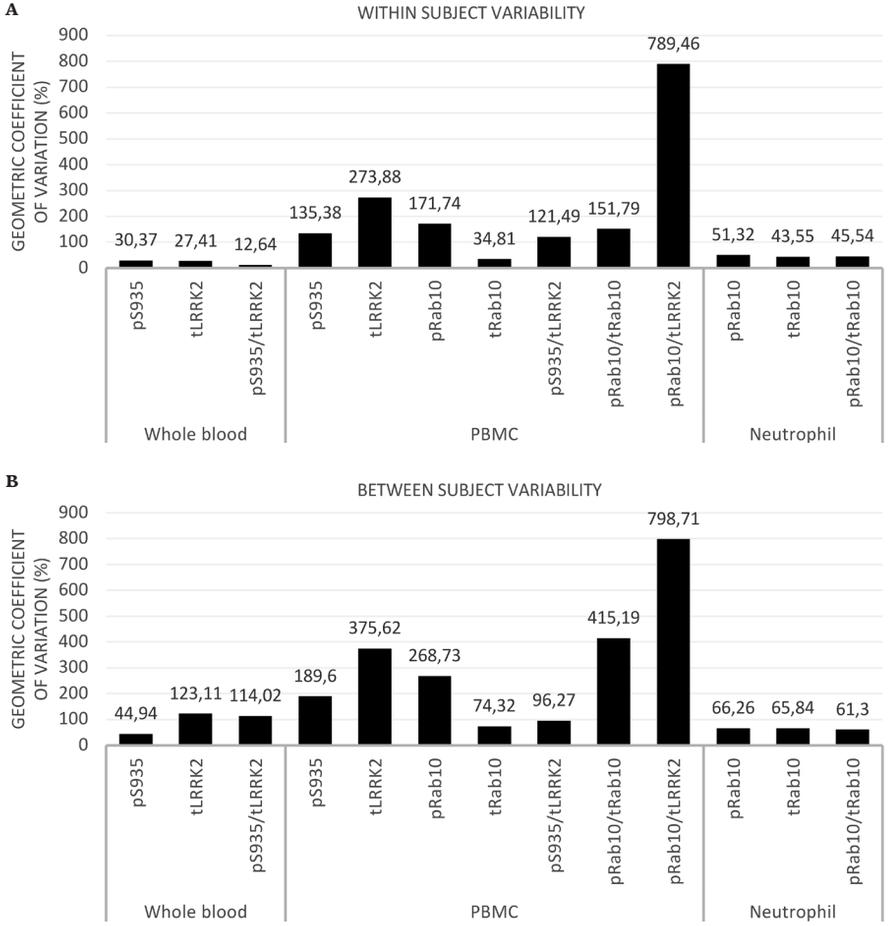

Figure 3 Group level differences in biomarker expression levels in neutrophils and PBMCs. A. Differences in phosphorylation of RAB10 in neutrophils between LRRK2+PD, iPD and HC subjects. The mean pRAB10 level was significantly higher in neutrophils in the LRRK2+PD group compared to the HC group (P = 0.0404). The mean pRAB10 levels did not significantly differ between the LRRK2+PD and iPD, and the iPD and HC groups. For subjects with two visits the values are averaged for each timepoint. B. Differences in phosphorylation of SER935, corrected for total LRRK2 (pS935/tLRRK2) in PBMCs between LRRK2+PD, iPD and HC groups. The mean pS935/tLRRK2 level was significantly lower in PBMCs in the LRRK2+PD group compared to both the iPD (P = 0.0006) and the HC group (P = 0.0327). There was no difference between the iPD and HC groups. Values expressed with interquartile range and group level mean (black square). The pink dot marks the subject with a LRRK2^{R1441C} mutation. The blue dots mark two iPD subjects that tested negative for αSYN seeds in CSF with the αS-SAA at both visits. The strength of the pRAB10 signal in the plate-based immunoassays is expressed in arbitrary units (A.U.). One timepoint for one subject in the iPD group was confirmed to be an outlier and not included in the final analysis.

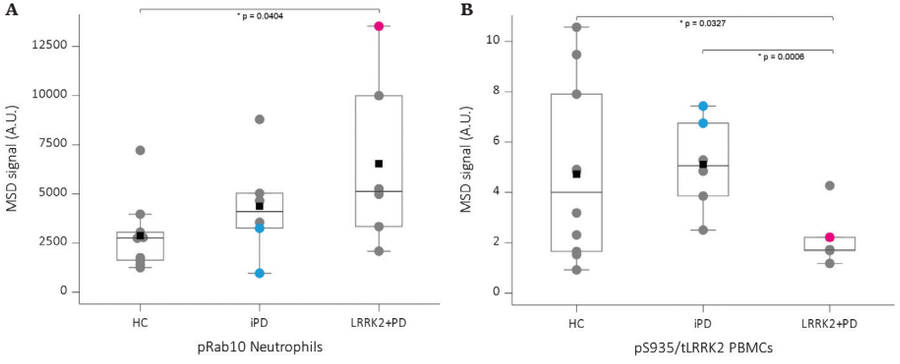

Figure 4 αSYN Seed Amplification Assay (αS-SAA) in CSF samples from LRRK2+PD, iPD and control subjects. Values correspond to the mean (±95% CI) for each subjects group, with each individual sample analyzed in triplicate. All but 2 LRRK2+PD and 4 HC subjects had CSF collected during 2 visits. Curves of LRRK2+PD and iPD groups were not significantly different as evaluated by a mixed effects model (P = 0.2882), but both PD groups did differ from the control subjects (P < .0001). Two iPD patients that tested negative for αSYN with the αS-SAA are excluded from this analysis.

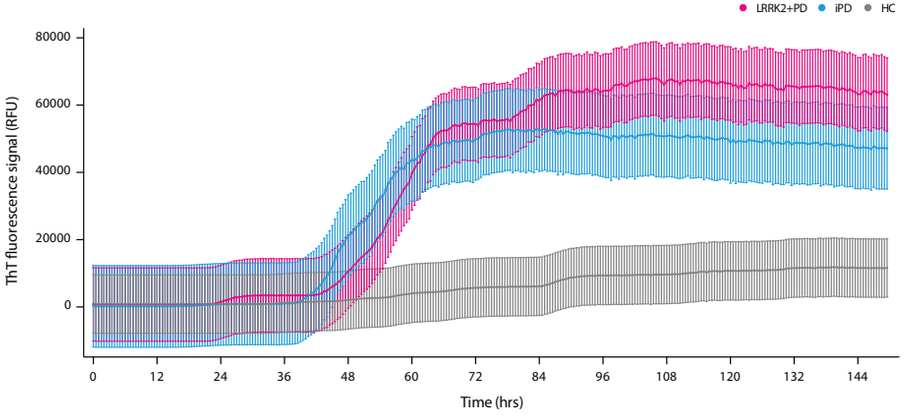

Figure S1 Variability of pS935, tLRRK2, pRAB10, and tRAB10 in PBMCs when corrected for cell number or GAPDH. A. Within-subject variability between VISITS 1 and 2. B. Between-subject variability, both expressed as geometric CV (%). Data from all 3 subpopulations (LRRK2+PD, iPD, and HC) was pooled for this analysis. Correcting for total cell number or GAPDH did not significantly reduce variability for any of the investigated biomarkers in PBMCs.

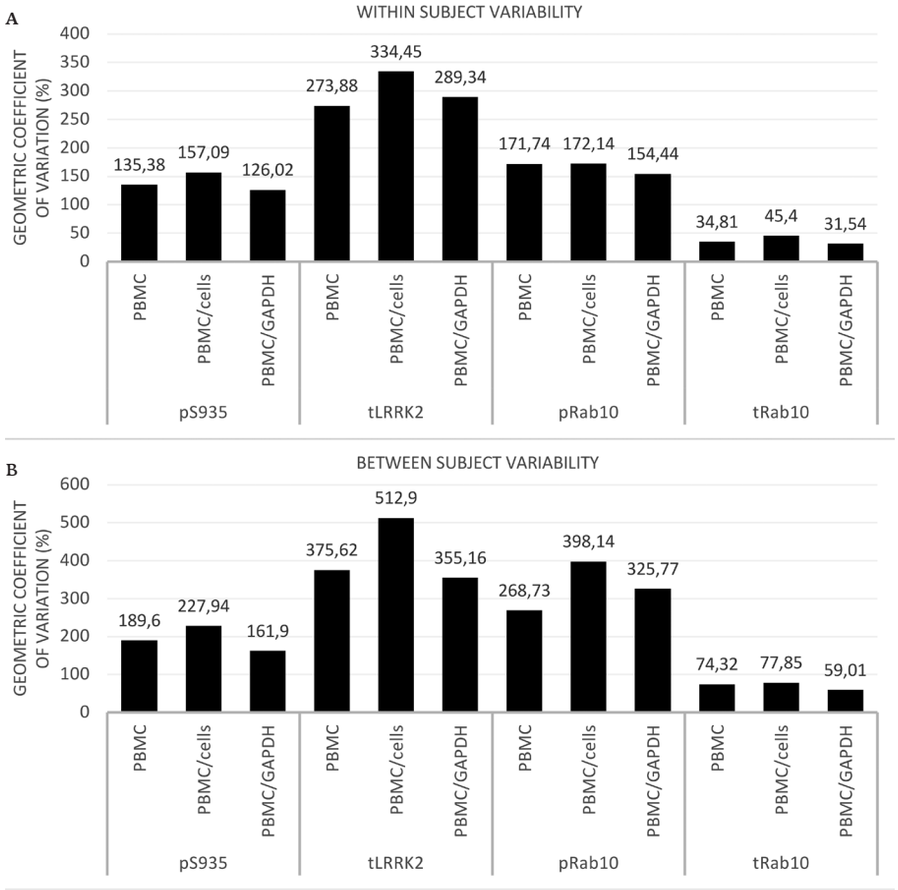

Figure S2 Variability of each biomarker in each biological source per population subgroup (LRRK2+PD, iPD and HC). Within-subject (intrasubject) variability expressed as geometric CV (%) between VISITS 1 and 2 of pS935, tLRRK2, and pS935/tLRRK2 in whole blood (A), pS935, tLRRK2, pS935/tLRRK2, pRAB10, tRAB10, pRAB10/tRAB10, and pRAB10/tLRRK2 in PBMCs (B), and pRAB10, tRAB10, and pRAB10/tRAB10 in neutrophils (C). Between-subject (intersubject) variability of pS935, tLRRK2, and pS935/tLRRK2 in whole blood (D), pS935, tLRRK2, pS935/tLRRK2, pRAB10, tRAB10, pRAB10/tRAB10, and pRAB10/tLRRK2 in PBMCs (E), and pRAB10, tRAB10, and pRAB10/tRAB10 in neutrophils (F). Within-subject variability of tLRRK2 and pS935/tLRRK2 in whole blood is pooled for all 3 subgroups because estimates per subgroup were not possible in the statistical model.

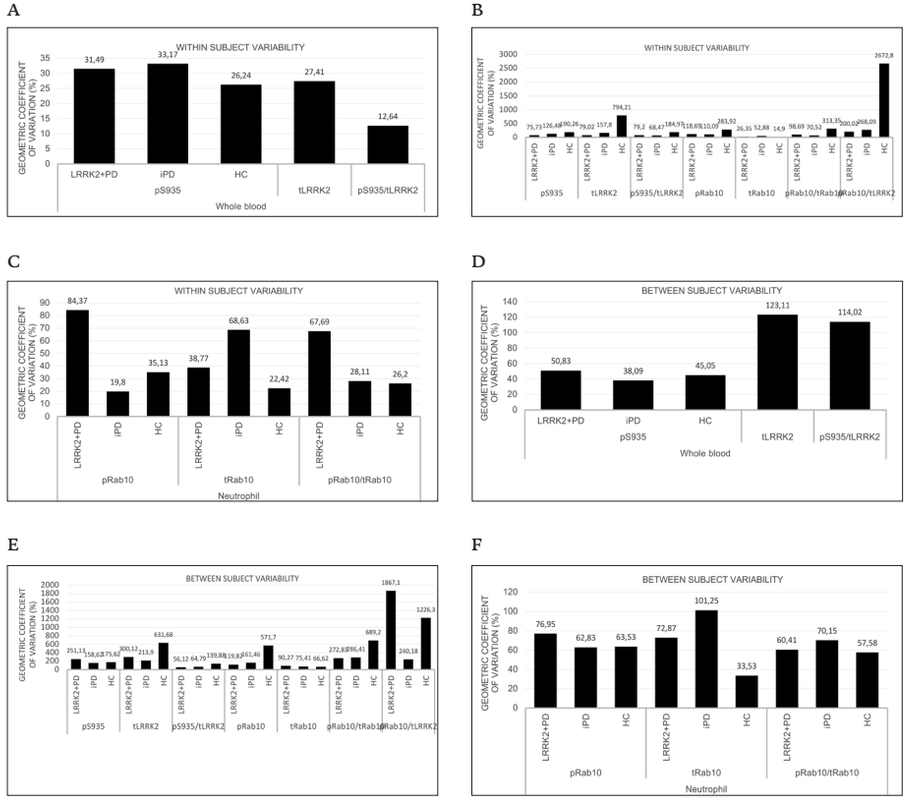

Table S1 Group level differences in biomarker expression levels. No group level differences were observed for pS935, tLRRK2, and tRAB10 between the LRRK2+PD, iPD and HC groups in either whole blood, PBMCs, or neutrophils. Furthermore, no group level differences were observed for pRAB10 in PBMCs. Group level differences were observed for pRAB10 in neutrophils (*Figure 3A*).

Biomarker	Matrix	Group	LSMEAN	95% CI lower	95% CI upper
pS935	PBMC	LRRK2+PD	2260.9	561.39	9105
pS935	PBMC	iPD	6687.7	2647.5	16893
pS935	PBMC	HC	7628.6	3798.7	15320
pS935	Whole blood	LRRK2+PD	2047.2	1307.5	3205.4
pS935	Whole blood	iPD	3124.1	2307.8	4229
pS935	Whole blood	HC	2806.2	2087.4	3772.5
tLRRK2	PBMC	LRRK2+PD	1796.5	290.04	11127
tLRRK2	PBMC	iPD	1500	500.62	4494.2
tLRRK2	PBMC	HC	2927.2	792.82	10808
tLRRK2	Whole blood	LRRK2+PD	4166.3	1863.1	9317
tLRRK2	Whole blood	iPD	5494.5	2457	12287
tLRRK2	Whole blood	HC	3144.4	1675.3	5901.6
pS935/tLRRK2	PBMC	LRRK2+PD	1.5252	1.1505	2.0219
pS935/tLRRK2	PBMC	iPD	4.4631	2.9399	6.7753
pS935/tLRRK2	PBMC	HC	3.1198	1.6582	5.8697
pS935/tLRRK2	Whole blood	LRRK2+PD	0.4908	0.2259	1.0666
pS935/tLRRK2	Whole blood	iPD	0.5688	0.2618	1.2362
pS935/tLRRK2	Whole blood	HC	0.8838	0.4838	1.6147
pRAB10	PBMC	LRRK2+PD	43997	21746	89015
pRAB10	PBMC	iPD	22291	8301.2	59858
pRAB10	PBMC	HC	17279	4867.8	61331
tRAB10	Neutrophil	LRRK2+PD	2893.7	1547.3	5411.9
tRAB10	Neutrophil	iPD	2181.3	1011.2	4705.3
tRAB10	Neutrophil	HC	3900.4	3116.2	4881.8
tRAB10	PBMC	LRRK2+PD	6896.9	3139.4	15152
tRAB10	PBMC	iPD	9971.5	5472.4	18170
tRAB10	PBMC	HC	9872.1	6406.1	15213
pRAB10/tRAB10	Neutrophil	LRRK2+PD	1.4022	0.9423	2.0864
pRAB10/tRAB10	Neutrophil	iPD	1.255	0.6515	2.4174
pRAB10/tRAB10	Neutrophil	HC	0.6743	0.4645	0.9788
pRAB10/tRAB10	PBMC	LRRK2+PD	6.3811	1.5651	26.016
pRAB10/tRAB10	PBMC	iPD	2.2367	0.5037	9.9311
pRAB10/tRAB10	PBMC	HC	1.59	0.4269	5.9212
pRAB10/tLRRK2	PBMC	LRRK2+PD	26.152	1.4453	473.19
pRAB10/tLRRK2	PBMC	iPD	14.867	5.6148	39.367
pRAB10/tLRRK2	PBMC	HC	6.5449	1.7434	24.57

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CHAPTER 5

LRRK2 INHIBITION BY BIIB122 IN HEALTHY PARTICIPANTS AND PATIENTS WITH PARKINSON'S DISEASE

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ABSTRACT

BACKGROUND Leucine-rich repeat kinase 2 (LRRK2) inhibition is a promising therapeutic approach for the treatment of Parkinson's disease (PD).

OBJECTIVE To evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of the potent, selective, CNS-penetrant LRRK2 inhibitor BIIB122 (DNL151) in healthy participants and patients with PD.

METHODS Two randomized, double-blind, placebo-controlled studies were completed. The phase 1 study (DNLI-C-0001) evaluated single and multiple doses of BIIB122 for up to 28 days in healthy participants. The phase 1B study (DNLI-C-0003) evaluated BIIB122 for 28 days in patients with mild to moderate PD. The primary objectives were to investigate the safety, tolerability, and plasma pharmacokinetics of BIIB122. Pharmacodynamic outcomes included peripheral and central target inhibition and lysosomal pathway engagement biomarkers.

RESULTS A total of 186/184 healthy participants (146/145 BIIB122, 40/39 placebo) and 36/36 patients (26/26 BIIB122, 10/10 placebo) were randomized/treated in the phase 1 and phase 1b studies, respectively. In both studies, BIIB122 was generally well tolerated; no serious adverse events were reported and the majority of TEAEs were mild. BIIB122 CSF/unbound plasma concentration ratio was ~1 (range, 0.7-1.8). Dose-dependent median reductions from baseline were observed in whole-blood pS935 LRRK2 (≤98%), PBMC pT73 pRAB10 (≤93%), CSF tLRRK2 (≤50%), and urine BMP (≤74%).

CONCLUSIONS At generally safe and well-tolerated doses, BIIB122 achieved substantial peripheral LRRK2 kinase inhibition and modulation of lysosomal pathways downstream of LRRK2, with evidence of CNS distribution and target inhibition. These studies support continued investigation of LRRK2 inhibition with BIIB122 for the treatment of PD.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease,[1,2] the prevalence of which is expected to increase as the population ages.[3] Approved symptomatic therapies temporarily reduce motor symptoms; however, the development of medications that slow disease progression remains a major unmet need for patients living with PD.[4,5]

Genetic research has expanded our understanding of the cellular pathogenesis of PD, uncovering novel therapeutic targets.[6] Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are a common cause of autosomal dominant PD, accounting for ~4% of familial and 1-2% of sporadic PD cases.[7–9] Genetic evidence also suggests that the N551K R1398H LRRK2 haplotype is associated with reduced risk of developing PD,[10,11] and may be associated with reduced LRRK2 kinase activity.[12,13] The majority of identified pathogenic variants in LRRK2 are located within its catalytic domains, including the most common pathogenic variant, G2019S.[14–17] These variants increase LRRK2 kinase activity, either through direct mechanisms within the kinase domain, or through indirect mechanisms.[18–20] LRRK2 mutations associated with increased kinase activity result in lysosomal dysfunction,[21–23] which can lead to impaired clearance and aggregation of toxic proteins, contributing to the pathology of PD.[24–26]

LRRK2 inhibitors correct lysosomal dysfunction and downstream neurodegeneration in *in vitro* and *in vivo* models of PD.[27–31] Lysosomal dysfunction is recognized as a central mechanism of PD pathogenesis; several mutations in genes, other than LRRK2, encoding lysosomal proteins and enzymes have been firmly linked to the risk of developing PD.[32–34] Increased LRRK2 kinase activity has also been observed in patients with other genetic forms of PD (e.g., VPS35 D620N-linked PD) and nonhereditary idiopathic PD.[35,36] Common noncoding variants in LRRK2 are associated with increased risk of developing PD.[37] Thus, biochemical and genetic evidence support LRRK2 kinase inhibition as a promising therapeutic approach to achieve disease modification in a broad population of patients with PD beyond those carrying an LRRK2 mutation.

In initial clinical studies with a small-molecule LRRK2 inhibitor, DNL201, dose-dependent inhibition of LRRK2 kinase activity was observed in both healthy participants and patients with PD, measured by reduction in phosphorylated serine 935 (pS935) LRRK2 in whole-blood and phosphorylated threonine 73 (pT73) RAB10,[29] a direct substrate of LRRK2, in peripheral blood mononuclear cells (PBMCs).[20] A reduction in urine di-22:6-bis (monoacylglycerol)phosphate (BMP[22:6/22:6]) was also observed, providing evidence for modulation of lysosomal pathways downstream of LRRK2.[29,38–40] At doses that demonstrated robust LRRK2 kinase inhibition and lysosomal pathway engagement, DNL201 was generally safe and well tolerated when administered for ≤28 days. The pharmacokinetic profile for the oral formulation of DNL201 requires multiple daily doses. Here, we report safety, tolerability, pharmacodynamic, and pharmacokinetic (PK) results from phase 1 healthy participant and phase 1b patient studies conducted with a second LRRK2 inhibitor, BIIB122 (also known as DNL151).

METHODS

Study design

PHASE 1 STUDY DNLI-C-0001 (Clinicaltrials.gov NCT04557800, EUDRACT 2017-003730-82) was a randomized, double-blind, placebo-controlled single ascending dose (SAD) and multiple ascending dose (MAD) study in healthy participants (*Supplemental Figure S1A*). Primary objectives were to investigate the safety, tolerability, and plasma PK of single and multiple oral doses of BIIB122. Other objectives included characterization of CSF BIIB122 concentrations, whole-blood pS935 LRRK2 levels, PBMC pT73 RAB10 levels, urine BMP(22:6/22:6), and CSF total LRRK2 (tLRRK2).

The study was conducted at two clinical research units (CRUs) in the Netherlands between 29 November 2017 and 21 February 2021, and included PART A SAD (BIIB122 10-300 mg); PART B MAD (15-300 mg once daily [QD] for 10 days); PART C single-dose elderly (40 mg); PART D multiple-dose (225 mg QD for 28 days); and PART E MAD (150-400 mg twice daily [BID] for 14 days) cohorts (*Supplemental Figure S1A*). Eligible participants were randomized to BIIB122 or placebo (3:1) in PARTS A and C (N=8/cohort planned) and PART D (N=16 planned), and PARTS B and E (N=10/cohort planned) (4:1). Study design details are provided in the *Supplemental Material*.

PHASE 1B STUDY DNLI-C-0003 (Clinicaltrials.gov NCT04056689, EUDRACT 2019-001297-28 was a randomized, placebo-controlled, double-blind, parallel-arm study in patients with PD (*Supplemental Figure S1B*). The primary objective was to evaluate the safety and tolerability of BIIB122 administered QD for 28 days. Other objectives were to characterize plasma BIIB122 PK and CSF concentrations, whole blood pS935 LRRK2 levels, PBMC pT73 RAB10 levels, urine BMP(22:6/22:6), and CSF tLRRK2.

The study was conducted at seven CRUs in the Netherlands, UK, Belgium, and US from 03 July 03 2019 to 02 December 02 2020 (*Supplemental Figure S1B*). Patients were randomized to receive placebo or BIIB122 80 mg QD in PART 1 (N=8 planned; 1:1); placebo or BIIB122 80 or 130 mg QD in PART 2 (N=16 planned; 1:1:2), and placebo or BIIB122 300 mg QD in PART 3 (N=10 planned; 1:4) for 28 days. Study design details are provided in the *Supplemental Material*.

Inclusion/exclusion criteria

PHASE 1 STUDY Eligible participants were aged 18-50 years, inclusive, for PARTS A, B, D, and E and aged 60-75 years, inclusive, for PART C. Women of childbearing potential were excluded.

PHASE 1B STUDY Eligible participants were aged 30-75 years, inclusive, with mild to moderate PD with or without PD risk genes, and modified Hoehn & Yahr Stages 1-3. Women of childbearing potential were excluded. Patients with a Montreal Cognitive Assessment (MOCA) score <24 were excluded.

Study outcomes

In both studies, safety and tolerability were assessed by adverse event (AE) monitoring, clinical laboratory tests, vital signs, ECGs, physical and neurological examinations, and neurological assessments. For the multiple-dose cohorts only, the Columbia-Suicide Severity Rating Scale (C-SSRS) and pulmonary function tests (PFTs) were performed. PK parameters, estimated from BIIB122 plasma and CSF concentrations, included area under the plasma concentration-time curve (AUC), maximum concentration (C_{max}), time to C_{max} (T_{max}), elimination half-life ($T_{1/2}$), trough plasma concentration (C_{trough}; multiple-dose cohorts only), and CSF/unbound plasma concentration ratio. Pharmacodynamic assessments included percent change from baseline in whole-blood pS935 LRRK2, PBMC pT73 RAB10, urine BMP(22:6/22:6) as a ratio to urine creatinine (ng BMP/mg creatinine), and CSF tLRRK2.

Statistical analysis

Sample sizes were not based on power calculations but were considered sufficient to achieve the study objectives. In the phase 1 and phase 1B studies, data were summarized by treatment group (placebo group and each BIIB122 dose group, pooled as appropriate).

Data were summarized using descriptive statistics. Genotyping at baseline identified 1 patient with an LRRK2 mutation (R1441C) and 3 patients with β-glucocerebrosidase (GBA) variants; no genotype-specific analyses were conducted given the small number of carriers. The incidence of treatment-emergent AEs (TEAEs) (defined as AEs that occurred or worsened after initiation of study drug) was summarized.

Bioanalytical methods for quantification of BIIB122 and pharmacodynamic measures are provided in the *Supplemental Material*.

Standard protocol approvals and participant consents

Study protocols, amendments, and informed consent forms were reviewed and approved by local institutional review boards/independent ethics committees. Written informed consent was obtained from each participant.

RESULTS

Study population

PHASE 1 STUDY Healthy participants (N=186) were randomized to BIIB122 or placebo and 96% (177/184) of treated participants completed study drug treatment (*Figure 1A*).

Overall, mean age (range) of the healthy participants in PARTS A, B, D and E was 28.7 (18-50) years and most were male (175 [99%]) (*Supplemental Table S1*). In PART C (elderly cohort), mean age (range) was 69.5 (67-74) years and 4 participants (50%) were male (*Supplemental Table S1*).

PHASE 1B STUDY Patients with mild to moderate PD (N=36) were randomized to BIIB122 or placebo and 94% (34/36) of treated patients completed study drug treatment (*Figure 1B*).

Overall, mean age (range) of patients was 61.9 (41-74) years, and 27 patients (75.0%) were male. In the BIIB122 300-mg QD group, the mean disease duration was longer and mean baseline Movement Disorder Society Parkinson's Disease Rating Scale (MDS-UPDRS) Part III score was higher (*Table 1*).

Safety

PHASE 1 STUDY BIIB122 was generally well tolerated at single doses of ≤300 mg and multiple doses of ≤400 mg BID in healthy participants. No serious AEs (SAEs) were reported, and the majority of TEAEs were mild in severity (*Supplemental Table S2* and *S3*). TEAEs leading to study drug discontinuation for BIIB122-treated participants included: 3 reported as related to study drug (moderate increased transaminases [N=1, PART D, placebo]; moderate diarrhea, nausea, headache, and disturbance in attention [N=1, 250 mg BID]; severe headache and malaise and mild myalgia [N=1, 400 mg BID]) and 3 reported as unrelated to study drug (moderate influenza-like illness [N=1, 10 mg, single dose]; mild asymptomatic COVID-19 based on COVID-19 test [N=2, 400 mg BID]).

In the single-dose cohorts, TEAEs were reported for 30 BIIB122-treated (71%) and 9 placebo-treated (64%) participants (*Supplemental Table S2*). The most common TEAE in BIIB122-treated participants was headache (12 [29%] vs 2 [14%] for placebo). In the multiple-dose cohorts, TEAEs were reported for 91 BIIB122-treated (88%) and 21 placebo-treated (84%) participants (*Supplemental Table S3*). The most common TEAE in BIIB122-treated participants was headache (53 [51%] vs 9 [36%] for placebo), the incidence and severity of which was dose dependent. TEAEs of myalgia with no associated increase in creatine phosphokinase were reported at the highest BIIB122 doses (*Supplemental Table S3*). No clinically meaningful or dose-related changes were observed in vital signs, clinical laboratory values (including renal function parameters; *Supplemental Figure S2*), physical or neurological examinations, Columbia Suicide Rating Scale (C-SSRS), or PFTs (*Supplemental Figure S3*).

PHASE 1B STUDY BIIB122 was generally well tolerated at doses of 80, 130, or 300 mg QD for ≤28 days in patients with PD. No SAEs were reported, and the majority of TEAEs were mild or moderate in severity (*Supplemental Table S4*). TEAEs leading to study drug discontinuation were reported for 2 BIIB122-treated patients: severe hypotension (asymptomatic), reported as not related to study drug (N=1, 130 mg QD), and mild hypotension (asymptomatic) reported as related to study drug (N=1, 300 mg QD); both had preexisting hypotension or orthostatic hypotension. These events resolved without intervention after study drug discontinuation. Two additional patients (1 each for 80 and 300 mg QD) had TEAEs of hypotension or orthostatic hypotension (both asymptomatic) that resolved while continuing study drug.

Overall, TEAEs were reported for 23 BIIB122-treated (89%) and 5 placebo-treated (50%) patients (*Supplemental Table S4*). The most common TEAE in BIIB122-treated patients was headache (11 [42%] vs 2 [20%] for placebo). No clinically meaningful or dose-related changes were observed in clinical laboratory values (including renal function parameters; *Supplemental Figure S2*); physical or neurological examinations; C-SSRS; PFTs (*Supplemental Figure S3*); or MDS-UPDRS PART III, Non-Motor Symptoms Scale, or MOCA scores (*Supplemental Table S5*).

Pharmacokinetics

PHASE 1 STUDY In healthy participants, BIIB122 oral absorption was rapid after single and multiple doses, with median T_{max} ranging from 1.0 to 1.5 hours (*Supplemental Figure S4*, *Supplemental Table S6*). Following multiple-dose administration of BIIB122 15-300 mg QD for 10 or 28 days or BIIB122 150-400 mg BID for 14 days in healthy participants, mean C_{max} at steady state ($C_{max(ss)}$) and AUC from time 0 through tau (AUC_{0-tau}) increased less than dose proportionally. At steady state, mean accumulation ratio (AR) based on C_{max} or AUC_{0-tau} decreased with increasing dose. After the last dose of BIIB122, mean $T_{1/2}$ ranged from 47 to 93 hours across the 15-300 mg QD and 150-400 mg BID dose ranges (*Supplemental Table S6*).

PK variability was low to moderate; percent coefficient of variation (CV) ranged from 8.9% to 31% for $C_{max(ss)}$ and from 6.7% to 40% for AUC_{0-24} (*Supplemental Table S6*). Steady state appeared to be reached after 6 days of dosing, based on C_{trough} over time.

The mean BIIB122 CSF/unbound plasma concentration ratio (calculated using a fixed unbound fraction in plasma from *ex vivo* measurements) ranged from 0.7 to 1.8 across the 30 to 300 mg QD and 150 to 400 mg BID dose ranges (*Figure 2A*). At higher doses, this ratio may be overestimated due to modest increases in BIIB122 unbound fraction at higher total plasma concentrations. Nonetheless, mean ratios were at or above unity (1.0) for most doses, indicating extensive BIIB122 CNS distribution in healthy participants.

PHASE 1B STUDY After single and multiple doses of BIIB122 80, 130, and 300 mg QD for 28 days, BIIB122 oral absorption was rapid, with median T_{max} ranging from 1.1 to 1.6 hours (*Supplemental Figure S5*, *Supplemental Table S7*). Following multiple doses, mean $C_{max(ss)}$ and AUC_{0-tau} increased less than dose proportionally, and AR based on C_{max} or AUC_{0-tau} decreased as BIIB122 dose increased from 80 to 300 mg QD. After the last dose (DAY 28), mean $T_{1/2}$ ranged from 70 to 122 hours across the 80 to 300 mg dose range (*Supplemental Table S7*). PK variability after QD dosing was low to moderate; CV ranged from 11% to 35% for $C_{max(ss)}$ and from 25% to 33% for AUC_{0-tau} (*Supplemental Table S7*). Steady-state plasma concentrations appeared to be reached after 7 days, based on C_{trough} over time.

The mean BIIB122 CSF/unbound plasma concentration ratio ranged from 0.95 to 1.2 across the 80 to 300 mg QD dose range (*Figure 2B*), indicating extensive CNS distribution of BIIB122 in patients with PD.

Pharmacodynamics

PHASE 1 STUDY After multiple-dose administration of BIIB122 in healthy participants, median whole-blood pS935 LRRK2 was reduced from baseline in a dose-dependent manner (*Figure 3A*, *Supplemental Figure S6A*, *Supplemental Figure 7A*). Likewise, median PBMC pT73 RAB10, a direct substrate of LRRK2, was reduced from baseline at all BIIB122 doses (*Figure 3B*, *Supplemental Figure S6B*, *Supplemental Figure S7B*), indicating inhibition of the biochemical pathways downstream of LRRK2. Average reduction in whole-blood pS935 LRRK2 at steady state (median) ranged from 15% to 87% for 15-300 mg QD, and from 91% to 98% for 150-400 mg BID. Average reduction in PBMC pT73 RAB10 at steady state (median) ranged from 49% to 80% for 15-300 mg QD and from 79% to 93% for 150-400 mg BID.

Total LRRK2 was recently shown to be quantifiable in CSF.[42] We hypothesized that LRRK2 inhibition in the CNS would reduce total LRRK2 in CSF, either by reducing LRRK2 levels in brain or by reducing LRRK2 secretion into CSF via exosomes.[38,39,43–45] BIIB122 dose-dependently reduced median CSF tLRRK2 levels from baseline at doses ≥150 mg QD and ≥150 mg BID by ~20% to 50% (*Figure 3C*, *Supplemental Figure S7C*), demonstrating sustained CNS kinase inhibition at these doses.

Urine BMP(22:6/22:6), a lysosomal lipid that is a mechanistic marker of modulation of the pathways downstream of LRRK2,[29,39–41] was reduced from baseline at BIIB122 doses ≥225 mg QD (median change, −45% to −52% for BIIB122 vs −3% to +9% for placebo) and ≥150 mg BID (median change, −19 to −74% for BIIB122 vs +31% for placebo) at the maximal reduction time point of 8-12 hours postdose, on DAY 10 or 28 (for QD regimen) or DAY 14 (for BID regimen), providing peripheral evidence of an effect on LRRK2-dependent lysosome function at these doses (*Figure 3D*, *Supplemental Figure S6C*, *Supplemental Figure S7D*).

PHASE 1B STUDY In patients with PD, average whole-blood pS935 LRRK2 reduction at steady state (median) was 49%, 70%, and 90% in the BIIB122 80, 130, and 300 mg QD groups, respectively (*Figure 4A*, *Supplemental Figure S8A*, *Supplemental Figure S9A*). pS935 LRRK2 levels returned to approximately baseline values on DAY 42 (*Supplemental Figure S7A*). Reduction of pT73 RAB10 was demonstrated in all dose groups, with average PBMC pT73 RAB10 reduction at steady state (median) of 70%, 72%, and 83% in the 80, 130, and 300 mg QD groups, respectively (*Figure 4B*, *Supplemental Figure S8B*, *Supplemental Figure S9B*). While the 80 and 130 mg QD dose groups did not show a reduction from baseline of tLRRK2 that was greater than the placebo group, a median reduction of 34% from baseline in CSF tLRRK2 on DAY 28 was observed in the 300 mg QD group, confirming sustained CNS kinase inhibition at that dose (*Figure 4C*, *Supplemental Figure S9C*).

Median decreases from baseline to DAY 28 in urine BMP(22:6/22:6) levels were 32%, 56%, and 63% in the 80, 130, and 300 mg QD dose groups, respectively, compared with 35% in the placebo group (*Figure 4D*, *Supplemental Figure S9D*). A larger BMP reduction with less variability was observed in the 300 mg QD group than in the lower BIIB122 doses and placebo.

DISCUSSION

In the clinical studies reported herein, the small-molecule LRRK2 inhibitor BIIB122 was generally safe and well tolerated across a broad dose range in both healthy participants and patients with PD. Biomarker results demonstrated dose-dependent peripheral LRRK2 kinase inhibition based on reduction in whole-blood pS935 LRRK2 and PBMC pT73 RAB10, modulation of the lysosomal pathway downstream of LRRK2 based on reduction in urine BMP, and central

LRRK2 kinase inhibition based on reduction in CSF tLRRK2. Thus, in these early-phase studies, LRRK2 kinase inhibition levels sufficient to modulate lysosomal pathways downstream of LRRK2 were safely achieved with daily oral dosing of BIIB122.

In healthy participants and patients with PD, BIIB122 was rapidly absorbed, with a $T_{1/2}$ that supports QD dosing. BIIB122 distributed equally to CSF and plasma, with a CSF/unbound plasma concentration ratio of ~1, reflecting extensive CNS distribution of BIIB122. Importantly, no meaningful difference in BIIB122 PK was observed between patients with PD and healthy participants (*Supplemental Figure S10*), supporting the relevance of safety and pharmacodynamic data from healthy participants to patients with PD.

In both studies, substantial, dose-dependent, peripheral LRRK2 kinase inhibition was observed, as measured by whole-blood pS935 LRRK2 and PBMC pT73 RAB10, with ≤98% reduction in pS935 LRRK2 observed on the last day of dosing. Because protein phosphorylation is frequently measured relative to the corresponding total protein amount as a normalization factor, in a subset of cohorts we measured total RAB10 in PBMC lysates as a potential normalization factor for pRAB10 reduction. There was no meaningful difference in the normalized (pT73 RAB10 as a ratio to total RAB10) vs unnormalized (pT73 RAB10) pharmacodynamic response variability, direction, or magnitude of effect (data not shown). Given that we did not collect total RAB10 data for every cohort in our studies we therefore proceeded with pharmacodynamic quantitation in PBMCs using pT73 RAB10 reduction. Similar levels of LRRK2 kinase inhibition were observed in both study populations at corresponding dose levels, indicating that pharmacodynamic data from these early-phase studies can be used to predict dose-response relationships in future patient studies. We previously demonstrated that peripheral inhibition of LRRK2 kinase activity, as measured by pS935 LRRK2, corresponds closely with that in the brain in animals treated with DNL201, another brain-penetrant LRRK2 inhibitor.[29] Together with the DNL201 data, the high CSF penetrance of BIIB122 (*Figure 4*) supports projections of strong LRRK2 kinase inhibition in the CNS, of similar magnitude to that in the periphery.

A quantitative method to measure CSF tLRRK2 levels has been recently published.[42] We hypothesized that LRRK2 kinase inhibition in the brain would reduce tLRRK2 levels in the CNS via two possible mechanisms:

1 LRRK2 kinase inhibition has been reported to reduce LRRK2 protein levels in some cellular models and in brain and other tissues in animals studies, although this effect is not universally observed across all species and tissues studied;[27,38,39,43,46]
2 LRRK2 inhibition has also been reported to reduce LRRK2 secretion into biofluids via exosomes.[44,45]

We hypothesized that LRRK2 inhibition in the CNS would be reflected by a reduction in CSF tLRRK2 either due to reduced total LRRK2 levels in the brain or reduced LRRK2 secretion into CSF in exosomes, enabling confirmation of a CNS pharmacodynamic response in humans. At steady state, BIIB122 reduced CSF tLRRK2 levels from baseline at doses ≥150 mg QD by ~20-50%, demonstrating sustained CNS LRRK2 kinase inhibition at these doses. Lower dose groups did not show a median reduction in tLRRK2, likely reflecting a need for sustained high levels of inhibition in the brain to achieve an observable reduction in CSF. This observation highlights the need for further study of the relationship between LRRK2 inhibition in the brain and CSF tLRRK2 reduction. Likewise, additional biomarker assays of lysosomal modulation in CSF are needed to provide evidence of modulation of PD pathological processes.

BMP is a phospholipid found exclusively on the intraluminal vesicles of late endosomes and lysosomes.[40] Individuals with lysosomal dysfunction, including those with the G2019S LRRK2 mutation, have increased levels of urine BMP.[41,47] LRRK2 kinase inhibition has been shown to reduce and therefore correct urine BMP in both animal models and patients with elevated BMP levels.[29,38,39] In healthy participants and patients with PD, urine BMP(22:6/22:6) was reduced at doses ≥225 mg QD. Consistent with our previous findings with DNL201,[29] reductions in urine BMP were achieved at pS935 LRRK2 inhibition levels of ≥ ~80%. BIIB122 treatment achieved peripheral LRRK2 kinase inhibition levels sufficient to modulate peripheral BMP, an effect that is likely translated to the CNS, given the high brain penetrance of the drug.[29] BIIB122 exposures demonstrating modulation of the lysosomal pathway downstream of LRRK2 are anticipated to have the highest potential for demonstrating clinical efficacy.

BIIB122 was generally well tolerated in both healthy participants and patients with PD. TEAEs of hypotension and/or orthostatic hypotension were reported for ~15% BIIB122-treated patients, whereas no such events were reported in BIIB122-treated healthy participants, despite higher BIIB122 doses administered. Hypotension and orthostatic hypotension have been reported in ~40-60% of patients with PD, and the incidence increases with longer PD duration, disease severity, and levodopa usage.[48,49] The etiology of the hypotension and orthostatic hypotension reported in our patient study remains unclear. The lack of associated symptoms (e.g., lightheadedness) suggests accommodation to hemodynamic fluctuations related to long-standing autonomic dysregulation in these patients.

Previously reported nonclinical toxicology studies evaluating multiple LRRK2 inhibitors demonstrated nonadverse, treatment-associated microscopic changes in lung (vacuolated type II pneumocytes) and kidney (pigmentation in renal tubular epithelial cells) that reversed following discontinuation of LRRK2 inhibition.[29,38,39] These nonadverse pulmonary and renal effects were attributed to on-target LRRK2 inhibition but not associated with cellular injury or inflammation and did not result in pulmonary or renal functional changes in chronic toxicology studies (exposures ≤39 weeks).[29,38,39,50,51] Preclinical toxicology studies of 6 and 9 months treatment duration with BIIB1222 in rats and monkeys, respectively, were also completed to support chronic dosing in humans. In early-phase clinical studies conducted with two LRRK2 inhibitors, DNL201[29] and BIIB122, no pulmonary or renal functional changes were observed for ≤28 days across all doses studied, providing reassurance that BIIB122 can be safely administered at doses with substantial LRRK2 kinase inhibition.

The main limitations of the early-phase studies include small sample sizes, a short duration of dosing, and a gender imbalance, with a majority of participants being male. Long-term safety of LRRK2 inhibition in patients with PD remains to be evaluated. Support for safety of chronic LRRK2 inhibition may be derived from studies of LRRK2 loss-of-function genetic variant carriers, which demonstrate no effect on life expectancy, increase in renal or pulmonary disease.[52,53] Future clinical studies with larger patient populations studied over months to years will inform long-term safety and efficacy of LRRK2 inhibition in patients with PD.

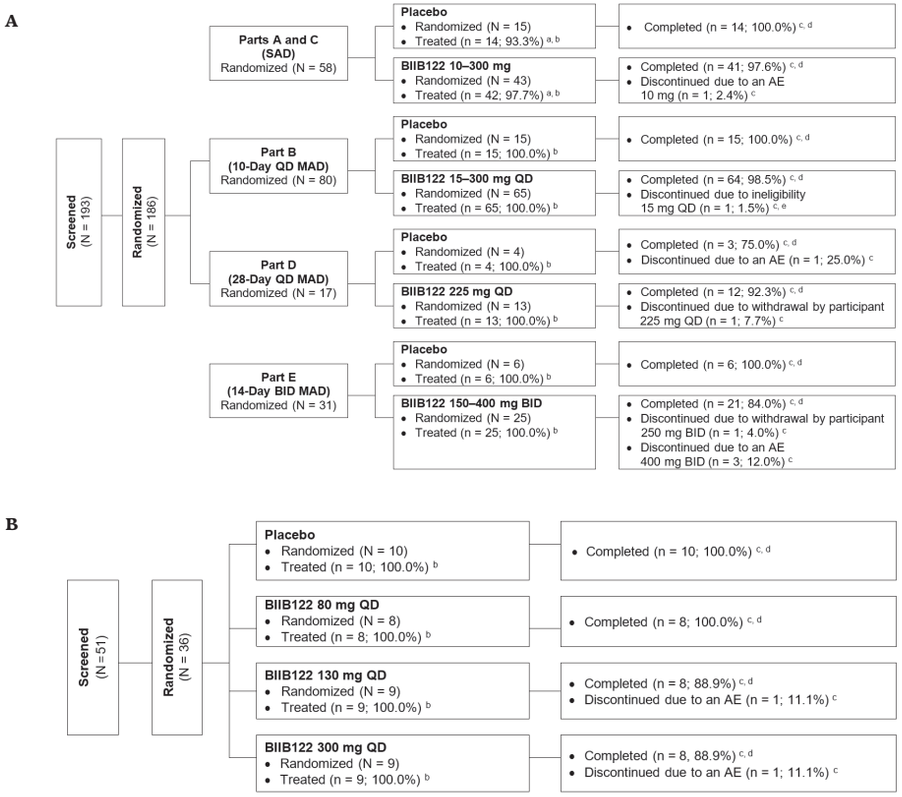
Our results support the selection of BIIB122 to advance to late-stage clinical studies in patients with PD given its favorable pharmacokinetic profile (compared to DNL201) supporting once daily dosing. Studies with two LRRK2 inhibitors (DNL201 and BIIB122) have confirmed substantial LRRK2 kinase inhibition and lysosomal pathway modulation at exposures with acceptable safety and tolerability, providing support for LRRK2 inhibition as a potential therapeutic approach to modify PD progression.

Table 1 Phase 1B study: demographics and other baseline characteristics.

Characteristic	Placebo (N = 10)	BIIB122			Total (N = 36)
		80 mg QD (N = 8)	130 mg QD (N = 9)	300 mg QD (N = 9)	
Age, Y					
Mean (SD)	61.9 (7.6)	66.9 (3.3)	60.4 (6.1)	59.1 (10.9)	61.9 (7.8)
Median (MIN, MAX)	63.0 (48, 72)	67.5 (62, 70)	59.0 (51, 69)	65.0 (41, 74)	65.0 (41, 74)
Sex, N (%)					
Male	8 (80.0)	7 (87.5)	5 (55.6)	7 (77.8)	27 (75.0)
Female	2 (20.0)	1 (12.5)	4 (44.4)	2 (22.2)	9 (25.0)
Race, N (%)					
White	10 (100.0)	8 (100.0)	9 (100.0)	9 (100.0)	36 (100.0)
Ethnicity, N (%)					
Not Hispanic or Latino	10 (100.0)	8 (100.0)	9 (100.0)	9 (100.0)	36 (100.0)
BMI, kg/m^2					
Mean (SD)	25.40 (3.17)	28.50 (4.16)	26.36 (4.04)	25.81 (4.45)	26.43 (3.96)
Baseline Parkinson's disease medication concomitant use, N (%)	10 (100.0)	8 (100.0)	7 (77.8)	9 (100.0)	34 (94.4)
Dopamine replacement agents	9 (90.0)	7 (87.5)	6 (66.7)	9 (100.0)	31 (86.1)
Dopamine agonists agents	4 (40.0)	4 (50.0)	4 (44.4)	4 (44.4)	16 (44.4)
MAOB inhibitor agents	1 (10.0)	0	2 (22.2)	0	3 (8.3)
Other Parkinson's disease medications	2 (20.0)	1 (12.5)	0	2 (22.2)	5 (13.9)
Age at Parkinson's disease diagnosis, Y					
Mean (SD)	57.4 (10.0)	62.8 (2.7)	57.1 (5.6)	53.4 (10.3)	57.5 (8.3)
Time since Parkinson's disease diagnosis, Y					
Mean (SD)	4.50 (3.03)	4.13 (2.95)	3.33 (3.16)	5.67 (4.39)	4.42 (3.39)
Modified Hoehn & Yahr assessment, N (%)					
Stage 1	4 (40.0)	3 (37.5)	4 (44.4)	3 (33.3)	14 (38.9)
Stage 1.5	2 (20.0)	0	0	1 (11.1)	3 (8.3)
Stage 2	4 (40.0)	3 (37.5)	4 (44.4)	3 (33.3)	14 (38.9)
Stage 2.5	0	2 (25.0)	1 (11.1)	2 (22.2)	5 (13.9)
Baseline MDS-UPDRS PART III score (off-state)					
Mean (SD)	29.8 (14.9)	29.9 (12.9)	26.1 (11.6)	35.8 (14.4)	30.4 (13.5)
Baseline NMSS total score					
Mean (SD)	18.0 (8.5)	36.5 (19.8)	24.3 (17.6)	38.4 (24.9)	28.8 (19.6)
Baseline MOCA total score					
Mean (SD)	27.6 (1.6)	27.5 (1.5)	27.1 (1.5)	27.1 (1.8)	27.3 (1.6)

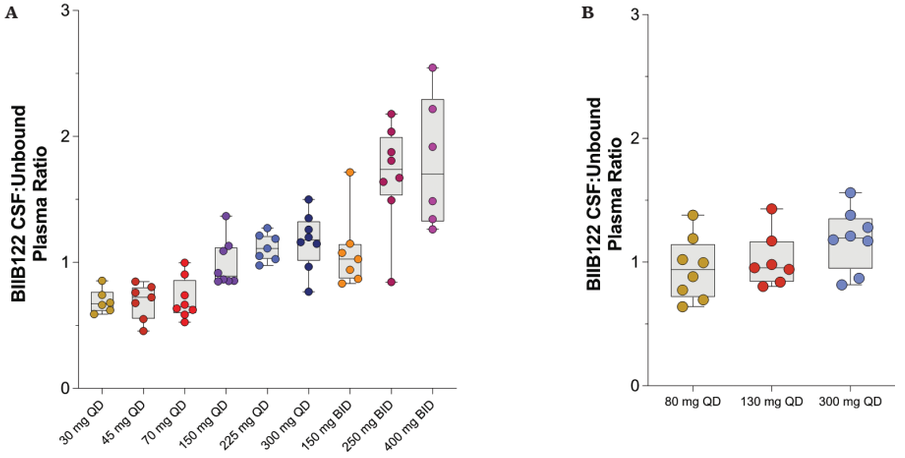
BMI = body mass index / MAOB = monoamine oxidase B / MAX = maximum / MDS-UPDRS PART III = Movement Disorders Society Unified Parkinson's Disease Rating Scale Part III / MIN = minimum / MOCA = Montreal Cognitive Assessment / NMSS = Non-Motor Symptoms Scale / PIC = powder-in-capsule / QD = once daily. / BIIB122 was administered as a PIC formulation at the 80 and 130 mg doses and as a tablet formulation at the 300 mg dose. The pooled placebo group includes patients who received placebo in either PIC or tablet form.

Figure 1 CONSORT (consolidated standards of reporting trials) diagram. A. Phase 1 study. B. Phase 1B study.



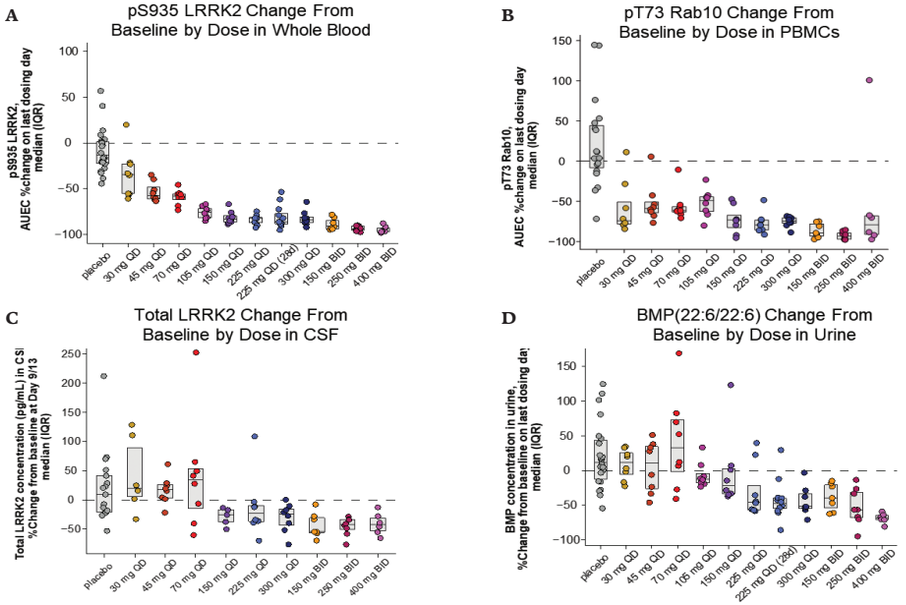
a) Two participants in PART A were discontinued post-randomization but before study drug administration: 1 participant in the placebo group due to failure to obtain the baseline CSF sample, and 1 participant in the BIIB122 10 mg group due to a vasovagal reaction following predose orthostatic testing. / b) The number of participants randomized was used as the denominator for calculation of percentages. / c) The number of participants who received each treatment was used as the denominator for calculation of percentages. / d) Completed treatment with study drug. / e) Participant was determined ineligible for the study (participant did not meet the inclusion criteria for pulmonary function test results [based on DLCO]). / AE = adverse event / BID = twice daily / MAD = multiple ascending dose / QD = once daily / SAD = single ascending dose.

Figure 2 BIIB122 CSF-to-unbound plasma concentration ratios after multiple doses in healthy participants in the phase 1 study and patients with Parkinson's disease in the phase 1B study. BIIB122 CSF-to-unbound plasma concentration ratios. A. PART B (15–300 mg QD for 10 days; N = 44) and PART E (150–400 mg BID for 14 days; N = 21) in the phase 1 study in healthy participants. B. PARTS 1 through 3 (80–300 mg QD for 28 days; N = 23) in the phase 1B study in patients with PD. CSF samples were not collected in healthy participants in PART D. Unbound plasma concentrations were calculated from total plasma concentrations by applying an unbound fraction of 0.024, which was determined from *ex vivo* measurements (using ultracentrifugation) of clinical samples from healthy participants who received BIIB122 30 mg QD (COHORT B2) and 225 mg QD (COHORT B7) in the phase 1 study (data on file). Data are described using boxplots, with the error bars representing the minimum to maximum data points.



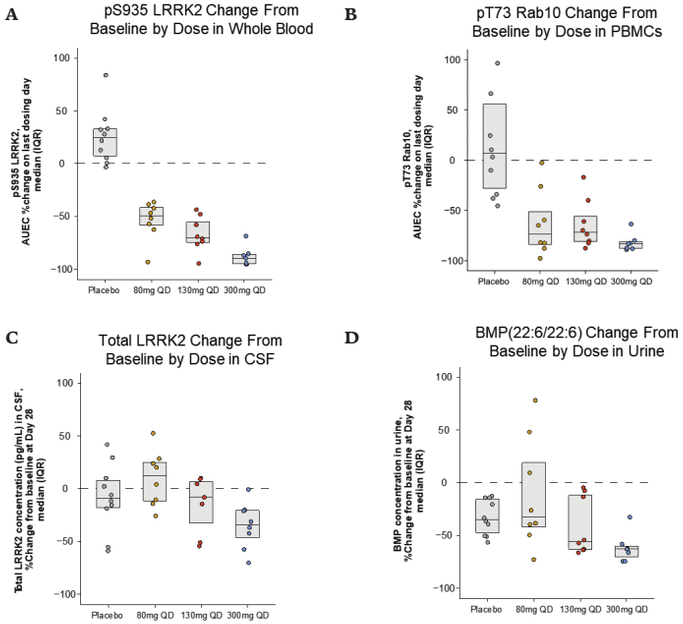
BID = twice daily / PD = Parkinson's disease / QD = once daily.

Figure 3 Phase 1 study: dose-dependent target and pathway engagement in healthy participants in multiple-dose cohorts. Pharmacodynamics of LRRK2 inhibition in healthy participants from the phase 1 multiple-dose cohorts (PARTS B, D, and E). A. pS935 LRRK2 reduction from baseline in whole blood. B. pT73 RAB10 reduction from baseline in PBMCs. Inhibition of LRRK2 over the dosing interval at steady state, as measured by average reduction in whole-blood pS935 LRRK2 and PBMC pT73 RAB10, was calculated as the median percent change from baseline time-adjusted AUEC on the last dosing day. Whole-blood and PBMC samples were collected at the following time points: DAY −1; DAY 1 predose; and on the last dosing day at predose and 1, 3, 8, and 12 hours (for BID only) or 24 hours (for QD only) postdose. Baseline was calculated as the average of DAY −1 and DAY 1 predose values. C. Total LRRK2 reduction from baseline in CSF. CSF samples were collected at the following time points: DAY -1 and DAY 9, 4 hours postdose for PART B and DAY -1 and DAY 13, 4 hours postdose for PART E. D. Urine BMP reduction from baseline in response to LRRK2 inhibition. Urine samples were collected on DAY -1, DAY 1 predose (PART E only), and 8-12 hours postdose on the last day of dosing. Urine BMP concentrations were reported as a ratio to urine creatinine concentrations (ng BMP/mg creatinine). For PART E, baseline was calculated as the average of DAY -1 and DAY 1 predose values. AUEC = area under the effect curve from time zero to 24 hours (or 12 hours for PART E).



BID = twice daily / BMP = bis(monoacylglycerol)phosphate / BMP(22:6/22:6) = di-docosahexaenoyl bis(monoacylglycerol) phosphate / IQR = interquartile range / LRRK2 = leucine-rich repeat kinase 2 / PBMC = peripheral blood mononuclear cell / pS935 = phosphorylated serine 935 / pT73 = phosphorylated threonine 73 / QD = once daily.

Figure 4 Phase 1B study: dose-dependent target and pathway engagement in patients with Parkinson's disease in multiple-dose cohorts. Pharmacodynamics of LRRK2 inhibition in patients with PD in the phase 1B study. A. pS935 LRRK2 reduction from baseline in whole blood. B. pT73 RAB10 reduction from baseline in PBMCs. One placebo outlier for pT73 RAB10 with >100% increase is not shown. Inhibition of LRRK2 over the dosing interval at steady state, as measured by average reduction in whole-blood pS935 LRRK2 and PBMC pT73 RAB10, was calculated as the median percent change from baseline time-adjusted AUEC on the last dosing day. Whole-blood and PBMC samples were collected at the following time points: DAY −1, DAY 1 predose, and on the last dosing day at predose and 1, 3, 8, and 24 hours postdose. Baseline was calculated as the average of DAY −1 and DAY 1 predose values. C. Total LRRK2 reduction from baseline in CSF in response to LRRK2 inhibition. CSF was collected at DAY −1 and DAY 28 3 hours postdose. D. Urine BMP reduction from baseline in response to LRRK2 inhibition. Urine samples were collected on DAY −1 and 1-6 hours postdose on the last day of dosing. Urine BMP concentrations were reported as a ratio to urine creatinine concentrations (ng BMP/mg creatinine). AUEC = area under the effect curve from time zero to 24 hours (or 12 hours for PART E).



BMP = bis(monoacylglycerol)phosphate / BMP(22:6/22:6) = di-docosahexaenoyl bis(monoacylglycerol)phosphate IQR = interquartile range / LRRK2 = leucine-rich repeat kinase 2 / PBMC = peripheral blood mononuclear cell / PD = Parkinson's disease / pS935 = phosphorylated serine 935 / pT73 = phosphorylated threonine 73 / QD = once daily.

Supplemental methods

STUDY CONDUCT In each part of the phase 1 study, participants were admitted to the CRU on DAY −2 and remained confined for the duration of dosing. Study drug (powder-in-capsule [PIC]) was administered in the morning in PARTS A, B, C, and D, or in the morning and evening (i.e., every 12 hours) in PART E. Participants were discharged 1 day after the last dose and returned for an outpatient follow-up visit ~1 (all parts) and 2 (PART D only) weeks after the last dose.

In each part, of the phase 1B study eligible patients were admitted to the CRU on DAY −2 for ≤5 days. Weekly outpatient visits were conducted for 3 weeks and patients were readmitted to the CRU on DAY 27 for ≤4 days for the final dose on DAY 28 and safety, PK, and pharmacodynamic assessments. Two additional outpatient safety follow-up visits were completed ~1 and ~2 weeks after the last dose.

RANDOMIZATION AND BLINDING In both clinical studies, participants were randomly assigned to treatment based on randomization lists generated using a permuted blocks randomization scheme. For the phase 1 study, for each study part and individual cohort, allocation to treatment was according to a predetermined random order. The randomization list was generated by unblinded contract research organization statisticians using a computer program. For the phase 1B study, randomization was performed using an interactive response technology (IRT) system, and the randomization list included study part–specific block sizes.

Both clinical studies were performed in a double-blind fashion, with the following controls used to maintain the double-blind status:

- For both the phase 1 and phase 1B studies, placebo capsules were identical in appearance, quantity, and packaging to the BIIB122 capsules. For the phase 1B study, placebo tablets were identical in appearance, quantity, and packaging to the BIIB122 tablets.
- The study participants, investigator's study site staff (except for the site pharmacist), medical monitors, and all other individuals involved with the study conduct remained blinded to treatment assignments until study closure.
- An unblinded pharmacist was assigned at the site and prepared the study drug for dispensing at the site. The unblinded pharmacist was also responsible for counting the returned capsules/tablets for drug accountability.

PHARMACOKINETIC SAMPLE ANALYSIS Plasma and CSF concentrations of BIIB122 were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS). Briefly, aliquots of 10 µL of calibration standards (STDS), quality-control (QC) samples, blank matrix, and study samples were transferred to a clean 96-well plate. A total of 125 µL of water:ammonium hydroxide (100:1) was added to each well. A total of 25 µL of acetonitrile:water (40:60) was added to wells containing blanks, and 25 µL of internal standard solution [750 nM of BIIB122-D6 in acetonitrile:water (40:60)] was added to all other wells. After 800 µL of methyl tert-butyl ether (MTBE) was added to each well, the samples were mixed using a Hamilton Microlab STAR automation system. After centrifugation, 100 µL of the upper organic layer was transferred to a clean 96-well plate, evaporated to dryness under purified nitrogen gas flow and reconstituted in 250 µL of acetonitrile:water:formic acid (40:60:0.2) before the samples were analyzed using LC-MS/MS. The LC-MS/MS analyses were performed on a Waters Acquity UPLC® system (Waters CO., Milford, MA) coupled with a Sciex API 4000™ mass spectrometer (AB SCIEX, Redwood City, CA). High-performance liquid chromatography (HPLC) was established on a Kinetex® XB-C18 column (2.6 mm, 50 × 2.1 mm) (Phenomenex, Torrance, CA) and the column was kept at 40°C during the run. The two mobile phases used were water:formic acid (100:0.2) and acetonitrile:formic acid (100:0.2). The multiple-reaction monitoring transition monitored for BIIB122 and BIIB122-D6 were 422.2 to 353.3 and 428.2 to 359.3, respectively. The declustering potential (DP) was at 50 V and collision energy (CE) was at 22 V. The bioanalysis method was fully validated and met the acceptance criteria for intra- and inter-run precision and accuracy defined in the 2018 Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance, with a lower limit of quantification (LLOQ) of 0.015 mM.

PHARMACODYNAMIC BIOFLUID SAMPLE COLLECTION Whole blood was collected in a tripotassium ethylenediaminetetraacetic acid (K3EDTA) blood collection tube, and the tube was inverted gently to mix the anticoagulant well with the blood. Whole-blood samples were aliquoted within 60 minutes of collection and stored at −70°C until shipment.

Human peripheral blood mononuclear cells (PBMCs) were isolated from whole blood collected in Vacutainer CPT™ sodium heparin tubes (BD 362780) following the manufacturer's protocol. PBMCs were resuspended in lysis buffer (1× cell lysis buffer with PHOSSTOP™ phosphatase inhibitor, complete

protease inhibitor, and Benzonase®) and incubated for 20 minutes. PBMC lysate was then centrifuged at 12000 × g for 20 minutes at 4°C. Supernatant was aliquoted and stored at −70°C until shipment.
CSF was collected by lumbar puncture. The first 0.5 mL of CSF was discarded, then samples were collected for pharmacokinetic and pharmacodynamic analyses. CSF samples were aliquoted and stored at −70°C until shipment.

Urine was collected either as a pooled sample or a spot sample in which participants were asked to collect a sterile, midstream urine specimen. Pooled samples were stored at 4°C until the end of the collection period. Urine samples were then centrifuged at 2500 × g at 4°C, aliquoted, and stored at −70°C until shipment.

PHARMACODYNAMIC SAMPLE ANALYSIS Whole blood was lysed by adding equal parts of lysis buffer and incubated on ice for 20 minutes. Samples were then centrifuged at 2600 × g for 20 minutes at 4°C. Phosphorylated serine 935 (pS935) leucine-rich repeat kinase 2 (LRRK2) analysis in the phase 1 study was performed as previously described.[s1,s2] pS935 LRRK2 analysis in the phase 1B study was performed at a bioanalytical laboratory using a fit-for-purpose assay. Briefly, a 96-well Meso Scale Discovery® (MSD®) Gold small-spot streptavidin plate (MSD L45SA; Rockville, MD) was washed once with ~300 mL of wash buffer per well and 25 mL of capture antibody (1 mg/mL of biotinylated pS935 Abcam AB172382) was added to each well. The plate was sealed and incubated at room temperature for 1 hour ± 6 minutes on a Heidolph plate shaker set at ~1000 RPM. Following incubation, the plate was washed 3 times with ~300 mL of wash buffer per well. Then, 25 mL of standards, blank, QC samples, and study samples were added to wells according to the plate layout. Samples collected from patients with Parkinson's disease treated with BIIB122 were analyzed alongside a recombinant full-length wild-type LRRK2 (Thermo Fisher 2082796) standard curve, and concentrations of pS935 LRRK2 in each sample were calculated. Otherwise, pS935 LRRK2 was quantified in raw luminescence units.

The plate was sealed and incubated overnight at 2°C to 8°C on a Heidolph plate shaker set at ~1000 RPM. On DAY 2, the plate was washed 3 times and 25 mL of detection antibody (0.25 mg/mL ruthenylated ANTI-LRRK2 BioLegend MC.028 in detection antibody dilution buffer) was added to each well. The plate was sealed and incubated at room temperature for 1 hour ± 6 minutes on a Heidolph plate shaker set at ~1000 RPM. Following incubation, the plate was washed 3 times. Residual wash buffer was removed as described above, and 150 mL of 2× read buffer T was added to each well. The plate was read immediately on an MSD Sector Imager 600 plate reader.

Phosphorylated threonine 73 (pT73) RAB10 in peripheral blood mononuclear cells was measured as previously described.[s1,s2] Briefly, Streptavidin-coated plates (MSD) were coated with biotinylated pT73 RAB10 antibody (Denali Therapeutics; South San Francisco, CA) for 1 hour at room temperature. Lysate was then pipetted onto these plates and incubated overnight at 4°C. Plates were washed and then ANTI-RAB10 antibody (Abcam; AB181367 with SULFO-TAG) diluted in 25% blocker A (MSD) 75% 1X TBST, D-R block (MSD), and D-M BLOCK (MSD) was added to each well. Following 1-hour room temperature incubation with shaking, 2X read buffer (diluted from 4X with water) was added to each well, and the plate was read on an MSD imager.

Total LRRK2 in CSF was detected using a slightly modified version of a previously reported anti-peptide immunoprecipitation–LC-MS assay measured as previously described.[s3] A total of 500 mL of human CSF was digested using 10 mg trypsin at 40°C for 1.5 hours in the presence of 100 fg of isotopically labeled heavy peptide (*KAEEGDLLVNPDQPR). Antipeptide immunoprecipitation was performed using biotinylated LRRK2 monoclonal antibody (MAB) N241A/34 (Antibodies Inc.). A WPS-3000 rapid-separation liquid chromatography (RSLC) system coupled to a Q EXACTIVE™ HF-X mass spectrometer (Thermo Fisher) operating in parallel-reaction monitoring mode was used to detect the ratio between light and heavy KAEEGDLLVNPDQPR peptide for absolute quantitation.

The quantitation of di-22:6-bis(monoacylglycerol)phosphate [BMP(22:6/22:6)] in human urine was conducted by Nextcea, Inc. (Woburn, MA) using a validated LC-MS/MS method, as previously described.[s2] The calibration standards and quality control samples were prepared using an authentic DI-22:6-BMP reference standard provided by Nextcea, INC. (Woburn, MA). A stable isotope labelled DI-22:6-BMP was employed as an internal standard and added during extraction. Di-22:6-BMP was extracted from urine by liquid-liquid extraction and the phospholipid layer was dried down and reconstituted for LC-MS/MS analysis. The chromatographic separation of the analyte from matrix components was achieved on a Nexera XR Ultra High Performance Liquid Chromatograph system (Shimadzu Scientific Instruments, Japan). The analyte was detected with SCIEX QTOF X500 and TripleQuad 7500 LC-MS/MS systems (SCIEX, Framingham, MA).

The intensities of DI-22:6-BMP and the internal standard were determined by integration of extracted ion peak areas using SCIEX OS software. Calibration curves were prepared by plotting the peak area ratios for each analyte to internal standard versus concentration. The model for the calibration curves was linear with (1/×2) weighting. Measured concentrations of DI-22:6-BMP in urine were divided by urine creatinine and reported in ng/mg creatinine.

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S2 Jennings D, Huntwork-Rodriguez S, Henry AG, et al. Safety, tolerability, and pharmacodynamics of LRRK2 inhibitor DNL201: from preclinical studies to Parkinson's clinical trials. *Sci Transl Med.* In press.

S3 Mabrouk OS, Chen S, Edwards AL, Yang M, Hirst WD, Graham DL. Quantitative measurements of LRRK2 in human cerebrospinal fluid demonstrates increased levels in G2019S patients. *Front Neurosci*. 2020;14:526. doi: 10.3389/fnins.2020.00526.

Table S1 Phase 1 study: demographic and other baseline characteristics.

Characteristic	SAD: PART A (N = 48)	Single-Dose Elderly: PART C (N = 8)	MAD 10 Days: PART B (N = 80)	Multiple-Dose 28 Days: PART D (N = 17)	MAD 14 Days: PART E (N = 31)	Total (PARTS A–E) (N = 184)
Age, Y						
Mean (SD)	26.8 (8.1)	69.5 (2.1)	28.9 (8.6)	29.0 (7.5)	31.0 (8.8)	30.5 (11.7)
Median (MIN, MAX)	25.0 (18, 50)	69.0 (67, 74)	26.5 (18, 50)	29.0 (18, 39)	30.0 (18, 50)	27.0 (18, 74)
Sex, N (%)						
Male	48 (100.0)	4 (50.0)	79 (98.8)	17 (100.0)	31 (100.0)	179 (97.3)
Female	0	4 (50.0)	1 (1.3)	0	0	5 (2.7)
Race, N (%)						
American Indian or Alaska Native	1 (2.1)	0	4 (5.0)	0	1 (3.2)	6 (3.3)
Asian	0	0	6 (7.5)	1 (5.9)	3 (9.7)	10 (5.4)
Black or African American	1 (2.1)	0	6 (7.5)	1 (5.9)	3 (9.7)	11 (6.0)
Native Hawaiian or other Pacific Islander	0	0	1 (1.3)	0	0	1 (0.5)
White	43 (89.6)	8 (100.0)	53 (66.3)	13 (76.5)	21 (67.7)	138 (75.0)
Multiple races reported	1 (2.1)	0	9 (11.3)	1 (5.9)	2 (6.5)	13 (7.1)
Other	2 (4.2)	0	1 (1.3)	1 (5.9)	1 (3.2)	5 (2.7)
Ethnicity, N (%)						
Hispanic or Latino	0	0	5 (6.3)	2 (11.8)	1 (3.2)	8 (4.3)
Not Hispanic or Latino	48 (100.0)	8 (100.0)	75 (93.8)	15 (88.2)	30 (96.8)	176 (95.7)
BMI, kg/m^2						
Mean (SD)	23.73 (2.66)	24.69 (3.72)	24.01 (2.62)	25.47 (3.42)	24.87 (2.72)	24.24 (2.80)

MAD = multiple-ascending dose/MAX = maximum/MIN = minimum/SAD = single-ascending dose.

Table S2 Phase 1 study: summary of treatment-emergent adverse events in healthy participants – single-dose cohorts.

	Placebo	BIIB122							BIIB122
	(N = 14)	10 mg (N = 6)	20 mg (N = 6)	40 mg (N = 6)	40 mg Elderly (N = 6)	60 mg (N = 6)	225 mg (N = 6)	300 mg (N = 6)	Total (N = 42)
Any TEAE[1]	9 (64.3)	4 (66.7)	6 (100.0)	2 (33.3)	2 (33.3)	5 (83.3)	5 (83.3)	6 (100.0)	30 (71.4)
Severe	0	0	1 (16.7)[2]	0	0	0	0	0	1 (2.4)
Moderate	4 (28.6)	1 (16.7)	5 (83.3)	0	0	1 (16.7)	0	0	7 (16.7)
Mild	5 (35.7)	3 (50.0)	0	2 (33.3)	2 (33.3)	4 (66.7)	5 (83.3)	6 (100.0)	22 (52.4)
Study drug–related TEAE	2 (14.3)	0	0	0	0	0	4 (66.7)	5 (83.3)	9 (21.4)
TEAE leading to study drug discontinuation	0	1 (16.7)	0	0	0	0	0	0	1 (2.4)
Most common TEAEs (reported for ≥5% healthy participants overall) by preferred term									
Headache	2 (14.3)	2 (33.3)	0	1 (16.7)	1 (16.7)	1 (16.7)	2 (33.3)	5 (83.3)	12 (28.6)
Procedural headache	5 (35.7)	0	6 (100.0)	0	0	1 (16.7)	0	3 (50.0)	10 (23.8)
Post procedural complication	0	2 (33.3)	3 (50.0)	0	0	1 (16.7)	3 (50.0)	1 (16.7)	10 (23.8)
Procedural pain	2 (14.3)	1 (16.7)	3 (50.0)	0	0	2 (33.3)	2 (33.3)	1 (16.7)	9 (21.4)
Fatigue	1 (7.1)	0	0	0	0	0	4 (66.7)	1 (16.7)	5 (11.9)
Dizziness	1 (7.1)	0	1 (16.7)	1 (16.7)	0	0	0	1 (16.7)	3 (7.1)
Nausea	3 (21.4)	0	2 (33.3)	0	0	0	0	1 (16.7)	3 (7.1)
Vomiting	1 (7.1)	0	3 (50.0)	0	0	0	0	0	3 (7.1)
Abdominal pain	2 (14.3)	1 (16.7)	0	0	0	0	0	0	1 (2.4)
Diarrhoea	2 (14.3)	1 (16.7)	0	0	0	0	0	0	1 (2.4)

TEAE = treatment-emergent adverse event. / Data are N (%) of participants. / TEAE Preferred Terms are presented in order of decreasing frequency in the Total BIIB122 group. / No deaths or serious adverse events were reported in the single-dose cohorts (PARTS A and C). / 1) Each participant is counted only once, in the highest severity category. / 2) Severe TEAE procedural headache, reported as not related to study drug.

Table S3 Phase 1 study: summary of treatment-emergent adverse events in healthy participants—multiple-dose cohorts.

	PART B (QD)								PART D (QD)			PART E (BID)			
	PBO	BIIB122							PBO	225 mg	BIIB122				
	(N = 15)	15 mg (N = 9)	30 mg (N = 8)	45 mg (N = 8)	70 mg (N = 8)	105 mg (N = 8)	150 mg (N = 8)	225 mg (N = 8)	300 mg (N = 8)	Total (N = 65)	(N = 4)	(N = 13)	PBO (N = 6)	150 mg (N = 7)	250 mg (N = 9)	400 mg (N = 9)	Total (N = 25)
Any TEAE (n [%])[1]	13 (86.7)	7 (77.8)	7 (87.5)	8 (100.0)	6 (75.0)	7 (87.5)	5 (62.5)	8 (100.0)	8 (100.0)	56 (86.2)	2 (50.0)	10 (76.9)	6 (100.0)	7 (100.0)	9 (100.0)	9 (100.0)	25 (100.0)
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1 (11.1)[2]	1 (11.1)[3]	2 (8.0)
Moderate	3 (20.0)	0	1 (12.5)	2 (25.0)	1 (12.5)	0	1 (12.5)	3 (37.5)	1 (12.5)	9 (13.8)	1 (25.0)	0	0	0	2 (22.2)	6 (66.7)	8 (32.0)
Mild	10 (66.7)	7 (77.8)	6 (75.0)	6 (75.0)	5 (62.5)	7 (87.5)	4 (50.0)	5 (62.5)	7 (87.5)	47 (72.3)	1 (25.0)	10 (76.9)	6 (100.0)	7 (100.0)	6 (66.7)	2 (22.2)	15 (60.0)
Study drug–related TEAE (N [%])	3 (20.0)	1 (11.1)	0	0	0	0	0	6 (75.0)	7 (87.5)	14 (21.5)	2 (50.0)	2 (15.4)	4 (66.7)	6 (85.7)	8 (88.9)	8 (88.9)	22 (88.0)
TEAE leading to study drug discontinuation (N [%])	0	0	0	0	0	0	0	0	0	0	1 (25.0)	0	0	0	1 (11.1)	3 (33.3)	4 (16.0)
Most common TEAEs (reported for ≥5% healthy participants overall) by preferred term (N [%])																	
Headache	5 (33.3)	3 (33.3)	2 (25.0)	2 (25.0)	3 (37.5)	3 (37.5)	3 (37.5)	6 (75.0)	7 (87.5)	29 (44.6)	0	3 (23.1)	4 (66.7)	6 (85.7)	7 (77.8)	8 (88.9)	21 (84.0)
Procedural pain	2 (13.3)	0	7 (87.5)	3 (37.5)	3 (37.5)	0	1 (12.5)	4 (50.0)	2 (25.0)	20 (30.8)	1 (25.0)	1 (7.7)	1 (16.7)	2 (28.6)	3 (33.3)	3 (33.3)	8 (32.0)
Procedural headache	4 (26.7)	0	4 (50.0)	4 (50.0)	5 (62.5)	0	0	1 (12.5)	0	14 (21.5)	0	0	1 (16.7)	0	1 (11.1)	3 (33.3)	4 (16.0)
Post procedural complication	4 (26.7)	1 (11.1)	1 (12.5)	1 (12.5)	2 (25.0)	3 (37.5)	1 (12.5)	1 (12.5)	0	10 (15.4)	0	2 (15.4)	1 (16.7)	2 (28.6)	1 (11.1)	0	3 (12.0)
Fatigue	2 (13.3)	1 (11.1)	0	0	0	0	1 (12.5)	4 (50.0)	6 (9.2)	1 (25.0)	1 (7.7)	2 (33.3)	3 (42.9)	0	0	3 (12.0)	
Nausea	4 (26.7)	1 (11.1)	0	1 (12.5)	2 (25.0)	0	0	1 (12.5)	1 (12.5)	6 (9.2)	0	0	0	0	1 (11.1)	3 (33.3)	4 (16.0)
Myalgia	0	1 (11.1)	1 (12.5)	1 (12.5)	0	0	0	0	0	3 (4.6)	0	0	1 (16.7)	0	4 (44.4)	5 (55.6)	9 (36.0)
Dizziness	0	1 (11.1)	0	1 (12.5)	0	0	0	0	2 (25.0)	4 (6.2)	0	0	0	1 (14.3)	2 (22.2)	1 (11.1)	4 (16.0)
Insomnia	2 (13.3)	1 (11.1)	0	1 (12.5)	0	1 (12.5)	0	0	0	3 (4.6)	0	0	0	0	2 (22.2)	0	2 (8.0)
Back pain	0	1 (11.1)	0	0	0	0	1 (12.5)	0	0	2 (3.1)	0	1 (7.7)	0	2 (28.6)	2 (22.2)	0	4 (16.0)

BID = twice daily / PBO = placebo / QD = once daily / TEAE = treatment-emergent adverse event. / Data are N (%) of participants. / TEAE Preferred Terms are presented in order of decreasing frequency overall in the multiple-dose cohorts (PARTS B, D, and E). / No deaths or serious adverse events were reported in the multiple-dose cohorts (PARTS B, D, and E). / 1) Each participant is counted only once, in the highest severity category. / 2) Severe TEAE (procedural headache) in one participant randomized to BIIB122 250 mg BID, reported as not related to study drug. / 3) Severe TEAEs (headache and malaise) in one participant randomized to BIIB122 400 mg BID, reported as related to study drug.

Table S4 Phase 1B study: summary of treatment-emergent adverse events in patients with Parkinson's disease.

	Placebo QD (N = 10)	BIIB122 QD 80 mg (N = 8)	BIIB122 QD 130 mg (N = 9)	BIIB122 QD 300 mg (N = 9)	BIIB122 Total (N = 26)
Any TEAE (N [%])[1]	5 (50.0)	8 (100.0)	8 (88.9)	7 (77.8)	23 (88.5)
Severe	0	0	1 (11.1)[2]	1 (11.1)[3]	2 (7.7)
Moderate	1 (10.0)	1 (12.5)	2 (22.2)	2 (22.2)	5 (19.2)
Mild	4 (40.0)	7 (87.5)	5 (55.6)	4 (44.4)	16 (61.5)
Study drug–related TEAE (N [%])	3 (30.0)	4 (50.0)	3 (33.3)	7 (77.8)	14 (53.8)
TEAE leading to study drug discontinuation (N [%])	0	0	1 (11.1)	1 (11.1)	2 (7.7)
Most common TEAEs (reported for ≥5% patients overall) by preferred term (N [%])					
Headache	2 (20.0)	4 (50.0)	2 (22.2)	5 (55.6)	11 (42.3)
Back pain	0	1 (12.5)	3 (33.3)	2 (22.2)	6 (23.1)
Tremor	2 (20.0)	1 (12.5)	2 (22.2)	1 (11.1)	4 (15.4)
Nasopharyngitis	0	2 (25.0)	3 (33.3)	0	5 (19.2)
Procedural pain	1 (10.0)	2 (25.0)	1 (11.1)	1 (11.1)	4 (15.4)
Nausea	0	1 (12.5)	1 (11.1)	2 (22.2)	4 (15.4)
Myalgia	1 (10.0)	1 (12.5)	1 (11.1)	1 (11.1)	3 (11.5)
Dizziness	0	0	1 (11.1)	2 (22.2)	3 (11.5)
Hypotension	0	0	1 (11.1)	2 (22.2)	3 (11.5)
Orthostatic hypotension	0	1 (12.5)	0	2 (22.2)	3 (11.5)
Hyperhidrosis	1 (10.0)	0	0	2 (22.2)	2 (7.7)
Cough	2 (20.0)	0	1 (11.1)	0	1 (3.8)
Fatigue	0	2 (25.0)	0	0	2 (7.7)
Gastrooesophageal reflux disease	0	0	1 (11.1)	1 (11.1)	2 (7.7)
Insomnia	0	1 (12.5)	0	1 (11.1)	2 (7.7)
Vomiting	0	1 (12.5)	0	1 (11.1)	2 (7.7)
Dizziness postural	1 (10.0)	1 (12.5)	0	0	1 (3.8)
Hypoacusis	1 (10.0)	0	0	1 (11.1)	1 (3.8)
Tinnitus	1 (10.0)	0	0	1 (11.1)	1 (3.8)

QD = once daily / TEAE = treatment-emergent adverse event. / Data are N (%) of patients. / TEAE Preferred Terms are shown in order of decreasing frequency overall. / No deaths or serious adverse events were reported in the study. / 1) Each patient is counted only once, in the highest severity category. / 2) Severe TEAE (asymptomatic hypotension) in one patient randomized to BIIB122 130 mg QD, reported as not related to study drug, led to early discontinuation of study drug. / 3) Severe TEAE (headache) in one patient randomized to BIIB122 300 mg QD, onset after last dose study drug, reported as not related to study drug.

Table S5 Phase 1B study: change from baseline in neurological assessments in patients with Parkinson's disease.

Neurological Assessment Time Point	Placebo (N = 10)	BIIB122 80 mg QD (N = 8)	BIIB122 130 mg QD (N = 9)	BIIB122 300 mg QD (N = 9)
MDS-UPDRS PART III off-state score				
Baseline	29.8 (14.9)	29.9 (12.9)	26.1 (11.6)	35.8 (14.4)
DAY 28	26.5 (14.4)	32.1 (12.4)	22.3 (11.7)	35.4 (15.0)[1]
Change from baseline at DAY 28	-3.3 (7.5)	2.3 (6.2)	0.0 (6.0)	-1.4 (6.0)[1]
NMSS				
Baseline	18.0 (8.5)	36.5 (19.8)	24.3 (17.6)	38.4 (24.9)
DAY 27	23.9 (15.8)	34.3 (27.7)	27.1 (23.0)[1]	35.8 (19.4)[1]
Change from baseline at DAY 27	5.9 (10.1)	-2.3 (23.0)	2.8 (12.3)[1]	2.8 (9.2)[1]
MOCA				
Baseline	27.6 (1.6)	27.5 (1.5)	27.1 (1.5)	27.1 (1.8)
DAY 27	28.1 (2.1)	28.8 (1.3)	27.6 (1.6)[1]	27.8 (1.5)[1]
Change from baseline at DAY 27	0.5 (2.2)	1.3 (1.9)	0.5 (1.2)[1]	0.8 (1.3)[1]

MDS-UPDRS PART III = Movement Disorders Society Unified Parkinson's Disease Rating Scale Part III / MOCA = Montreal Cognitive Assessment / NMSS = Non-Motor Symptoms Scale / QD = once daily. Data are mean (SD) / For each data point, N = 10 for placebo, N = 8 for the BIIB122 80 mg QD group, and N = 9 for the BIIB122 130 and 300 mg QD groups, unless otherwise indicated. / 1) N = 8.

Table S6 Phase 1 study: BIIB122 steady-state plasma pharmacokinetic parameters after once- or twice-daily administration in healthy nonelderly participants.

Parameter	PART B BIIB122 QD								PART D BIIB122 QD	PART E BIIB122 BID		
	15 mg (N = 9)	30 mg (N = 8)	45 mg (N = 8)	70 mg (N = 8)	105 mg (N = 8)	150 mg (N = 8)	225 mg (N = 8)	300 mg (N = 8)	225 mg (N = 13)	150 mg (N = 7)	250 mg (N = 9)	400 mg (N = 9)
	DAY 10 (Last Dose)								DAY 28 (Last Dose)	DAY 14 (Last Dose)		
$C_{max,\ ss}$ (mM)												
N	8	8	8	8	8	8	8	8	12	7	8	6
Mean (SD)	1.22 (0.270)	2.88 (0.713)	3.89 (0.894)	4.48 (1.17)	7.32 (1.61)	7.56 (1.93)	9.04 (2.80)	10.3 (0.918)	10.5 (2.53)	8.91 (2.29)	12.3 (2.96)	13.0 (1.35)
CV%	22.2	24.7	23.0	26.1	21.9	25.6	31.0	8.9	24.2	25.7	24.0	10.4
$T_{max,\ ss}$ (h)												
N	8	8	8	8	8	8	8	8	12	7	8	6
Median (MIN, MAX)	1.0 (0.50, 4.1)	1.5 (0.50, 2.0)	1.1 (1.0, 2.0)	1.5 (1.5, 4.0)	1.0 (0.50, 2.0)	1.5 (1.0, 3.0)	1.0 (1.0, 3.0)	1.5 (1.0, 3.0)	1.5 (0.52, 2.0)	1.1 (0.52, 2.0)	1.5 (1.0, 2.0)	1.5 (1.0, 2.0)
AUC_{0-24} (mM · h)[1]												
N	8	8	8	8	8	8	8	8	12	7	8	6
Mean (SD)	16.6 (6.61)	35.9 (13.5)	44.6 (16.0)	58.2 (11.9)	92.1 (25.1)	91.1 (22.3)	119 (34.3)	127 (16.4)	123 (39.8)	149 (44.4)	205 (71.6)	211 (14.1)
CV%	39.9	37.6	35.9	20.4	27.3	24.5	28.9	13.0	32.4	29.9	35.0	6.7
$T_{1/2}$ (h)												
N	7	7	6	8	8	7	8	8	12	7	8	6
Mean (SD)	77.0 (47.9)	53.4 (18.5)	92.6 (102)	82.9 (43.6)	86.9 (62.0)	89.0 (48.8)	66.6 (34.1)	47.1 (11.2)	71.7 (34.0)	62.0 (27.9)	73.8 (39.8)	60.7 (23.1)
C_{max} AR[2]												
N	8	8	8	8	8	8	8	8	12	7	8	6
Mean (SD)	1.60 (0.566)	1.66 (0.415)	1.79 (0.941)	1.50 (0.275)	1.36 (0.287)	1.60 (0.335)	1.10 (0.149)	1.32 (0.434)	1.26 (0.431)	1.97 (1.03)	1.51 (0.574)	1.26 (0.183)
AUC AR[2]												
N	8	8	8	8	8	8	8	8	12	7	8	6
Mean (SD)	3.16 (1.07)	2.79 (0.936)	2.84 (1.35)	2.84 (0.682)	2.85 (0.829)	2.48 (0.519)	1.89 (0.257)	1.77 (0.404)	2.02 (0.654)	3.22 (1.37)	2.42 (1.17)	1.74 (0.112)

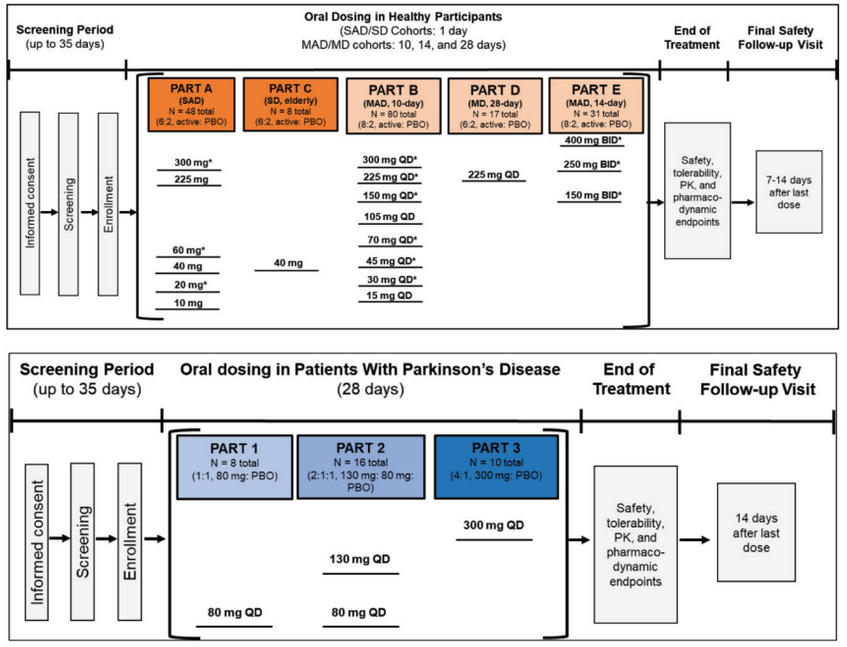
AR = accumulation ratio / AUC = area under the plasma concentration-time curve / AUC_{0-24} = area under the plasma concentration-time curve from the time of dosing to 24 hours the end of the dosing interval / AUC_{0-tau} = area under the plasma concentration-time curve from the time of dosing to the end of the dosing interval / BID = twice daily / C_{max} = maximum concentration / $C_{max,ss}$ = maximum concentration at steady state / CV = coefficient of variation / MAX = maximum / MIN = minimum / PIC = powder-in-capsule / QD = once daily / $T_{1/2}$ = elimination half-life / $T_{max,ss}$ = time of maximum concentration at steady state. / In PARTS B, D, and E, BIIB122 was administered as a PIC formulation to nonelderly participants in the fasted state. / 1) In PARTS B and D, AUC_{0-24} = AUC_{0-tau}. In PART E, AUC_{0-24} was calculated by multiplying the AUC_{0-12} (AUC_{0-tau}) value by 2. / 2) C_{max} AR = $C_{max,ss}$ [DAY 10, 14, or 28] / C_{max} [DAY 1] / AUC AR = AUC_{0-tau} [DAY 10, 14, or 28] / AUC_{0-tau} [DAY 1].

Table S7 Phase 1B study: BIIB122 steady-state plasma pharmacokinetic parameters after once-daily administration in patients with Parkinson's disease.

PK parameter	BIIB122 80 mg QD (N = 8)	BIIB122 130 mg QD (N = 8)	BIIB122 300 mg QD (N = 8)
	DAY 28 (Last Dose)		
$C_{max,ss}$ (μM)			
N	8	8	8
Mean (SD)	6.23 (2.20)	8.10 (1.67)	9.95 (1.09)
CV%	35.3	20.6	10.9
$T_{max,ss}$ (h)			
N	8	8	8
Median (MIN, MAX)	1.31 (0.5, 3.1)	1.50 (0.5, 2.0)	1.55 (1.0, 4.0)
AUC_{0-tau} (μM · h)			
N	8	8	8
Mean (SD)	76.3 (25.5)	104 (34.3)	127 (32.2)
CV%	33.4	32.9	25.4
$T_{1/2}$ (h)			
N	8	8	8
Mean (SD)	87.8 (44.0)	122 (102)	69.7 (44.6)
C_{max} AR[1]			
N	8	8	8
Mean (SD)	2.25 (1.17)	1.63 (0.320)	1.03 (0.180)
AUC AR[1]			
N	8	8	8
Mean (SD)	3.85 (1.62)	2.85 (0.961)	1.63 (0.452)

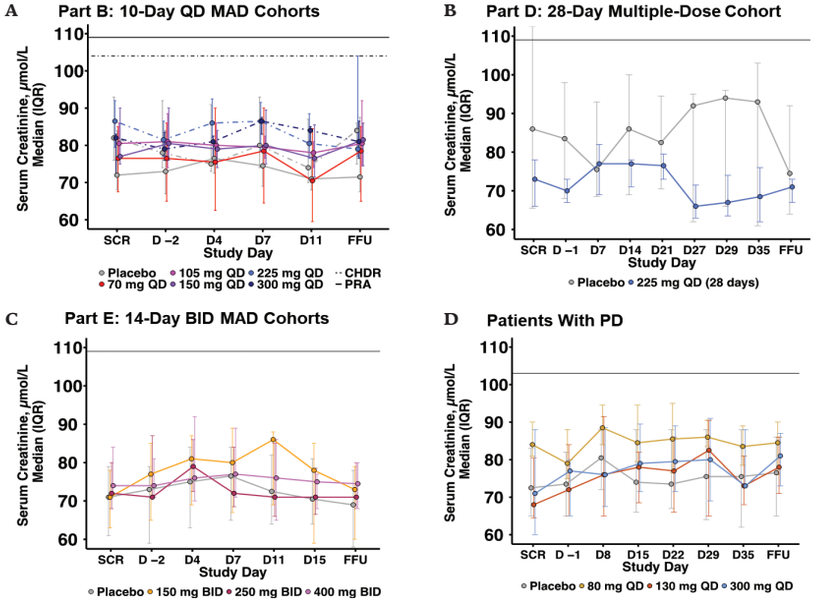
AR = accumulation ratio / AUC = area under the plasma concentration-time curve / AUC_{0-tau} = area under the plasma concentration-time curve from the time of dosing to the end of the dosing interval / C_{max} = maximum concentration / $C_{max,ss}$ = maximum concentration at steady state / CV = coefficient of variation / MAX = maximum / MIN = minimum / PIC = powder-in-capsule / PK = pharmacokinetic(s) / QD = once daily / $T_{1/2}$ = elimination half-life / $T_{max,ss}$ = time of maximum concentration at steady state. / BIIB122 was administered as a PIC formulation at the 80 and 130 mg doses and as a tablet formulation at the 300 mg dose in the fasted state. / 1) C_{max} AR = $C_{max,ss}$ [DAY 28] / C_{max} [DAY 1] / AUC AR = AUC_{0-tau} [DAY 28] / AUC_{0-tau} [DAY 1].

Figure S1 Study designs for phase 1 study and phase 1B study. A. For the phase 1 study, only cohorts designated by an asterisk (*) completed lumbar punctures for CSF collection. Sentinel dosing was used for the following cohorts: PART A 40, 60, 225, and 300 mg single-dose cohorts; PART B 30, 45 70-, 105, 150, 225, and 300 mg QD cohorts; and PART E 150, 250, and 400 mg BID cohorts. Two sentinel participants in each cohort received study drug (1 placebo and 1 BIIB122) and ≥24 hours of safety data from these participants were reviewed before the remainder of the participants in the cohort were dosed. For all cohorts, a PIC formulation was administered. B. For PART 1 of the phase 1B study, patients with PD were randomly assigned in a 1:1 ratio to receive placebo or BIIB122 80 mg QD for 28 days (PIC). In PART 2, patients were randomly assigned in a 1:1:2 ratio to receive placebo, BIIB122 80 mg, or BIIB122 130 mg, respectively, QD for 28 days (PIC). PART 2 was initiated after 8 patients were enrolled in PART 1 and after review of blinded safety data from ≥6 patients who completed ≥8 days of dosing in PART 1. In PART 3, patients were randomly assigned in a 1:4 ratio to receive placebo or BIIB122 300 mg QD for 28 days (tablet formulation). PART 3 was initiated after review of blinded safety data from PARTS 1 and 2.



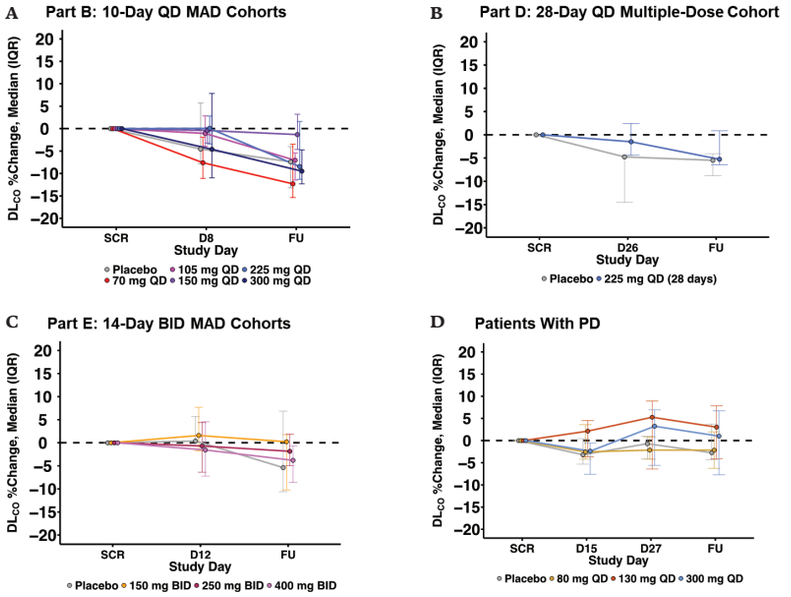
BID = twice daily / MAD = multiple ascending dose / MD = multiple dose / PBO = placebo / PD = Parkinson's disease / PIC = powder-in-capsule / PK = pharmacokinetic(s) / QD = once daily / SAD = single ascending dose / SD = single dose.

Figure S2 Phase 1 and phase 1B studies: summary of renal safety findings (serum creatinine) after multiple doses in healthy participants and patients with Parkinson's disease. Median percent change from baseline in serum creatinine in the A. PART B 10-day QD, B. PART D 28-day QD, and C. PART E 14-day BID cohorts from the phase 1 study in healthy participants and the (D) 28-day QD cohorts from the phase 1B study in patients with PD. The horizontal lines indicate the upper local laboratory limits of normal values for serum creatinine in males.



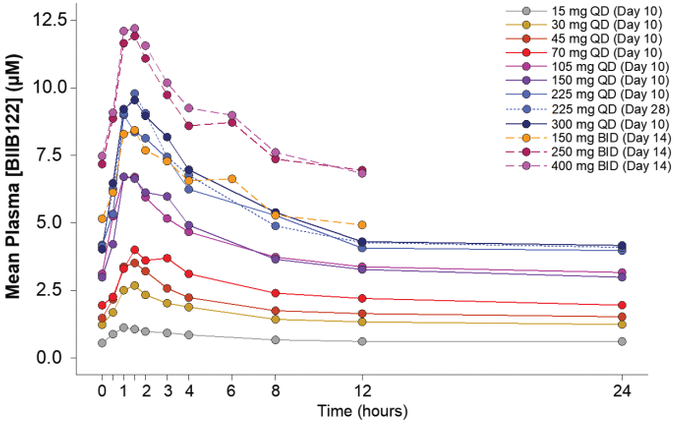
BID = twice daily / BL = baseline / CHDR = Centre for Human Drug Research / D = DAY / FFU = final follow-up / IQR = interquartile range / MAD = multiple ascending dose / PD = Parkinson's disease / PRA = PRA Health Sciences / QD = once daily / SCR = screening.

Figure S3 Phase 1 and phase 1B studies: summary of pulmonary safety findings (DLCO) after multiple doses in healthy participants and patients with Parkinson's disease. Median percent change from baseline in DLCO in the A. PART B 10-day QD, B. PART D 28 day QD, and C. PART E 14-day BID cohorts from the phase 1 study in healthy participants and the (D) 28-day QD cohorts from the phase 1B study in patients with PD. All DLCO values were adjusted for measured blood hemoglobin (closest central laboratory hemoglobin measurement to the time of DLCO measurement).



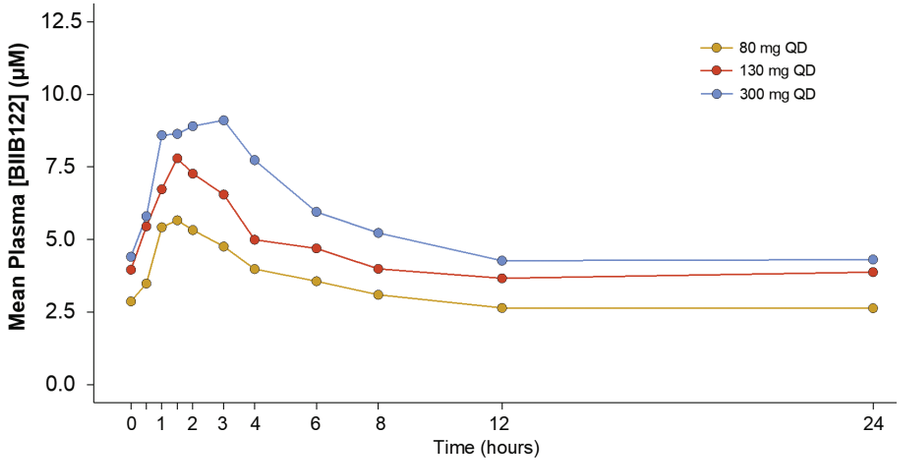
BID = twice daily / BL = baseline / D = DAY / DLCO = diffusing capacity of lungs for carbon monoxide / FU = follow-up / IQR = interquartile range / MAD = multiple ascending dose / PD = Parkinson's disease / QD = once daily / SCR = screening.

Figure S4 Phase 1 study: mean plasma concentration-time profiles of BIIB122 after multiple doses in healthy participants. Plasma pharmacokinetics of BIIB122 at steady state in healthy participants from PART B DAY 10 (15–300 mg QD), PART D DAY 28 (225 mg QD), and PART E DAY 14 (150–400 mg BID).



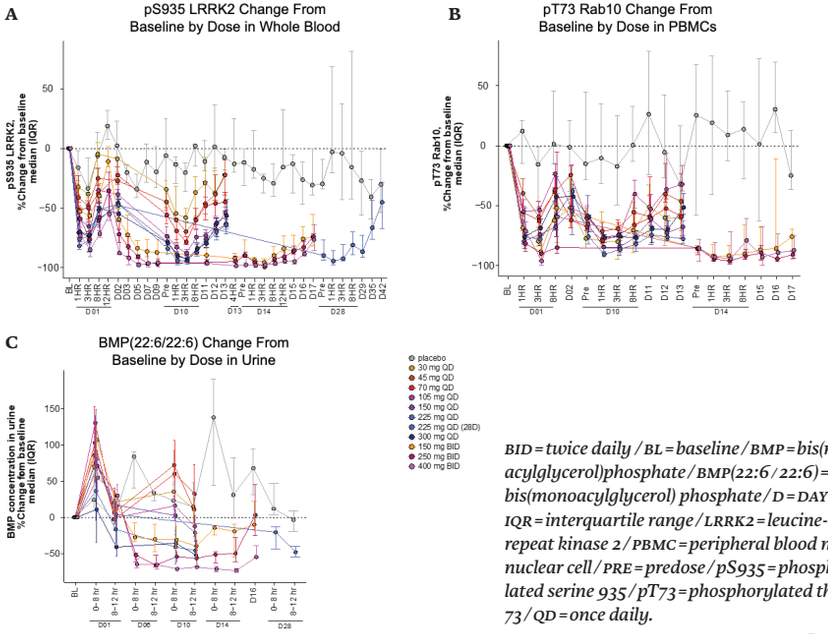
BID = twice daily / QD = once daily.

Figure S5 Phase 1B study: mean plasma concentration-time profiles of BIIB122 after multiple-doses in patients with Parkinson's disease. Plasma pharmacokinetics of BIIB122 at steady state in patients with PD from PARTS 1 through 3 (80–300 mg QD).



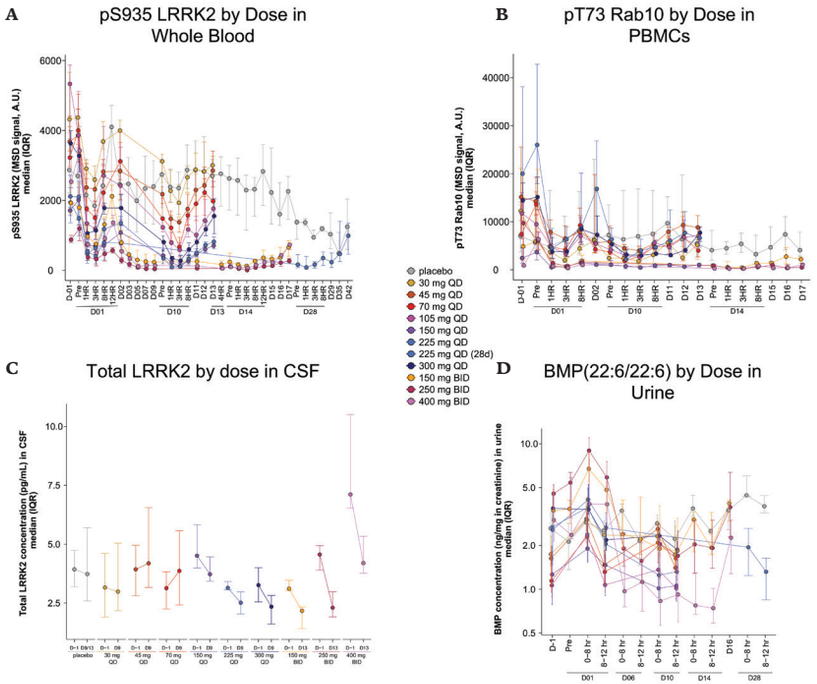
PD = Parkinson's disease / QD = once daily.

Figure S6 Phase 1 study: dose-dependent target and pathway engagement in healthy participants in multiple-dose cohorts. Pharmacodynamics of LRRK2 kinase inhibition in healthy participants from the phase 1 study (multiple-dose cohorts from PARTS B, D, and E). A. pS935 LRRK2 reduction from baseline in whole blood. B. pT73 RAB10 reduction from baseline in PBMCs. Time points of collection are denoted on the x-axis (D = day after first dose, where first dose is on D01). If no hour is specified (e.g., D02, D03), sample was collected predose or, where applicable, after the last dosing time point (e.g., D11, D12). On days on which multiple postdose time points were collected, the day of collection is indicated below the hourly time points on that day. For pS935 LRRK2 in whole blood (A), the D13 time point refers to the collection in the QD cohorts 3 days after the last dosing day, and the D13 4-hour time point refers to the collection in the BID cohorts 4 hours after the dose on D13. C. Urine BMP reduction from baseline. Time points of collection are denoted on the x-axis. On days on which multiple postdose time points were collected, the day of collection is indicated below the time periods of collection on that day (pooled sample collection over the time interval). Baseline was defined as the average of DAY −1 and DAY 1 predose measurements. A DAY 1 predose urine sample was not collected for the QD cohorts, so baseline BMP for these cohorts is defined as the DAY −1 measurement. The placebo group consists of all participants randomized to placebo across the multiple-dose cohorts in PARTS B, D, and E (variable N across time points, in particular for time points specific to PARTS D and E).



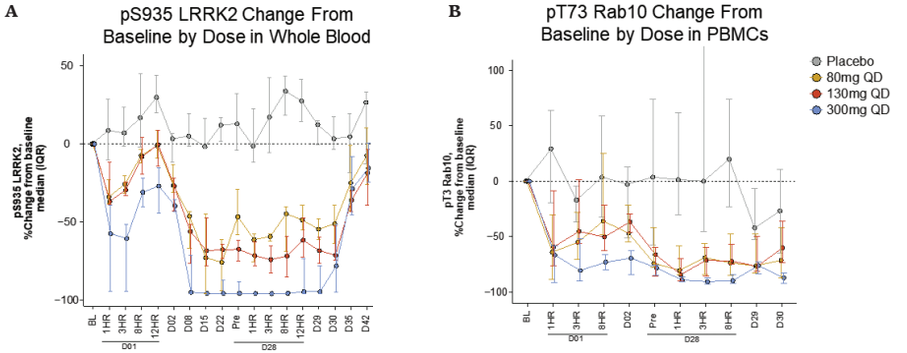
BID = twice daily / BL = baseline / BMP = bis(monoacylglycerol)phosphate / BMP(22:6/22:6) = di 22:6 bis(monoacylglycerol) phosphate / D = DAY / IQR = interquartile range / LRRK2 = leucine-rich repeat kinase 2 / PBMC = peripheral blood mononuclear cell / PRE = predose / pS935 = phosphorylated serine 935 / pT73 = phosphorylated threonine 73 / QD = once daily.

Figure S7 Phase 1 study: pharmacodynamics of LRRK2 inhibition in healthy participants in multi-dose cohorts plotted as median (IQR) value over time. A. pS935 LRRK2 in whole blood by dose group (MSD signal, A.U.) B. pT73 RAB10 (MSD signal, A.U.) in PBMCs by dose group. C. Urine BMP (ng/mg creatinine) by dose group. D. Total LRRK2 (pg/mL) in CSF by dose group. (A-D) Timepoints of collection are denoted on the x-axis (D=DAY before or after first dose where first dose is on D01 and D-01 is the day prior to the first dose). If no hour is specified (e.g. D02, D03), sample was collected predose, or where applicable, after the last dosing timepoint (e.g. D11, D12). On days where multiple postdose timepoints were collected, the day of collection is indicated below the hourly timepoints on that day. For pS935 LRRK2 in whole blood (A), the D13 timepoint refers to the collection in the QD groups three days after the last dosing day, and the D13 4 hour timepoint refers to the collection in the BID groups 4 hours after the dose on D13. For urine BMP (D), the time periods on the x-axis denote pooled sampling timepoints on the noted day of collection. The placebo group consists of all subjects randomized to placebo across PART B, D and E (variable N across timepoints, in particular for timepoints specific to PARTS D & E in the healthy volunteer study).



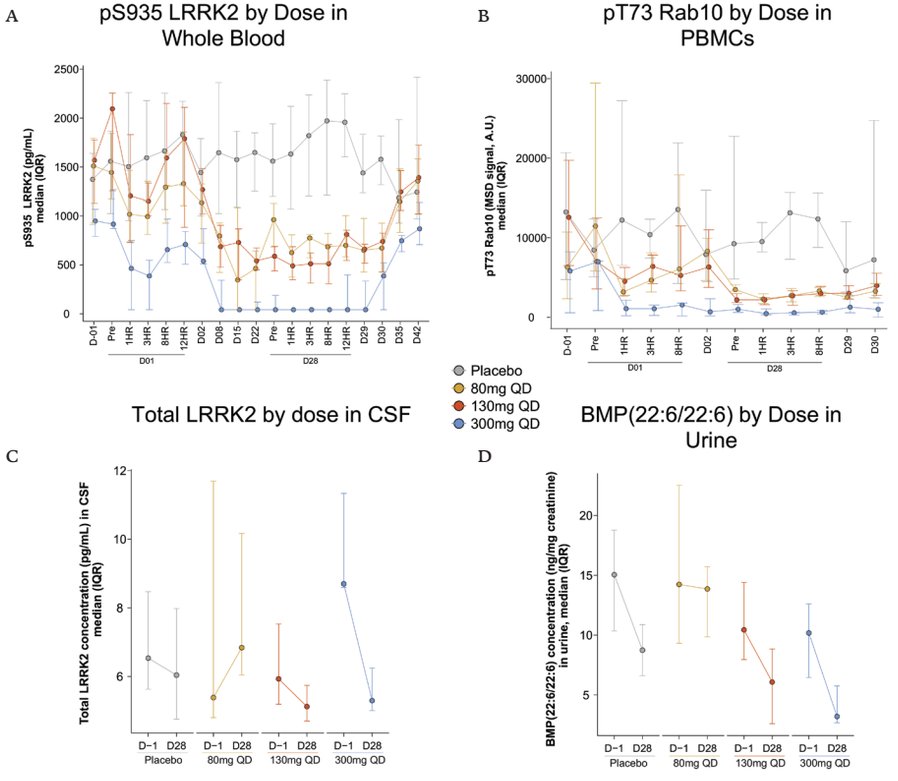
BMP=di-22:6 bis[monoacylglycerol]phosphate / IQR=interquartile range / PD=Parkinson's disease / QD=once daily / BID=twice daily.

Figure S8 Phase 1B study: dose-dependent target and pathway engagement in patients with Parkinson's disease. Pharmacodynamics of LRRK2 kinase inhibition in patients with PD in the phase 1B study. A. pS935 LRRK2 reduction from baseline in whole blood. B. pT73 RAB10 reduction from baseline in PBMCs. Collection time points are indicated on the x-axis (D=day after first dose, where first dose is on D01). If no hour is specified (e.g., D02, D08), sample was collected predose or, where applicable, after the last dosing time point (e.g., D30, D35). On days with multiple postdose time points, the day of collection is indicated below the hourly time points on that day. Baseline was defined as the average of DAY −1 and DAY 1 predose measurements.



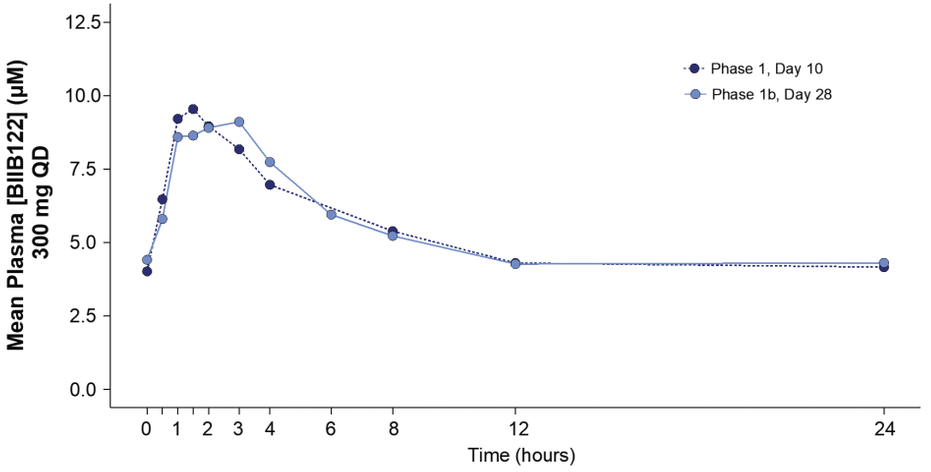
BL=baseline / D=DAY / IQR=interquartile range / LRRK2=leucine-rich repeat kinase 2 / PBMC=peripheral blood mononuclear cell / PD=Parkinson's disease / pS935=phosphorylated serine 935 / pT73=phosphorylated threonine 73 / QD=once daily.

Figure S9 Phase 1B study: pharmacodynamics of LRRK2 inhibition in PD patients, plotted as median (IQR) value over time. A. pS935 LRRK2 in whole blood by dose group. B. pT73 RAB10 (MSD signal, A.U.) in PBMCs by dose group. C. Urine BMP (ng/mg creatinine) by dose level. D. Total LRRK2 (pg/mL) in CSF by dose level. (A-D) Timepoints of collection are denoted on the x-axis (D=DAY before or after first dose where first dose is on D01 and D-01 is the day prior to the first dose). If no hour is specified (e.g. D03 and D08 for phase 1B study), sample was collected predose, or where applicable, after the last dosing timepoint (e.g. D30, D35 for phase 1B study). On days where multiple postdose timepoints were collected, the day of collection is indicated below the hourly timepoints on that day. For urine BMP in PD patients (C), the time periods on the x-axis denote pooled sampling timepoints on the noted day of collection.



BMP = di-22:6 bis[monoacylglycerol]phosphate / IQR = interquartile range / PD = Parkinson's disease / QD = once daily / BID = twice daily.

Figure S10 Phase 1 and 1B studies: mean plasma concentration-time profiles of BIIB122 300 mg once daily after multiple-doses in healthy participants and patients with Parkinson's disease. Plasma pharmacokinetics of BIIB122 300 mg QD at steady state in healthy participants from the phase 1 study and patients with PD from the phase 1B study.



PD = Parkinson's disease / QD = once daily.

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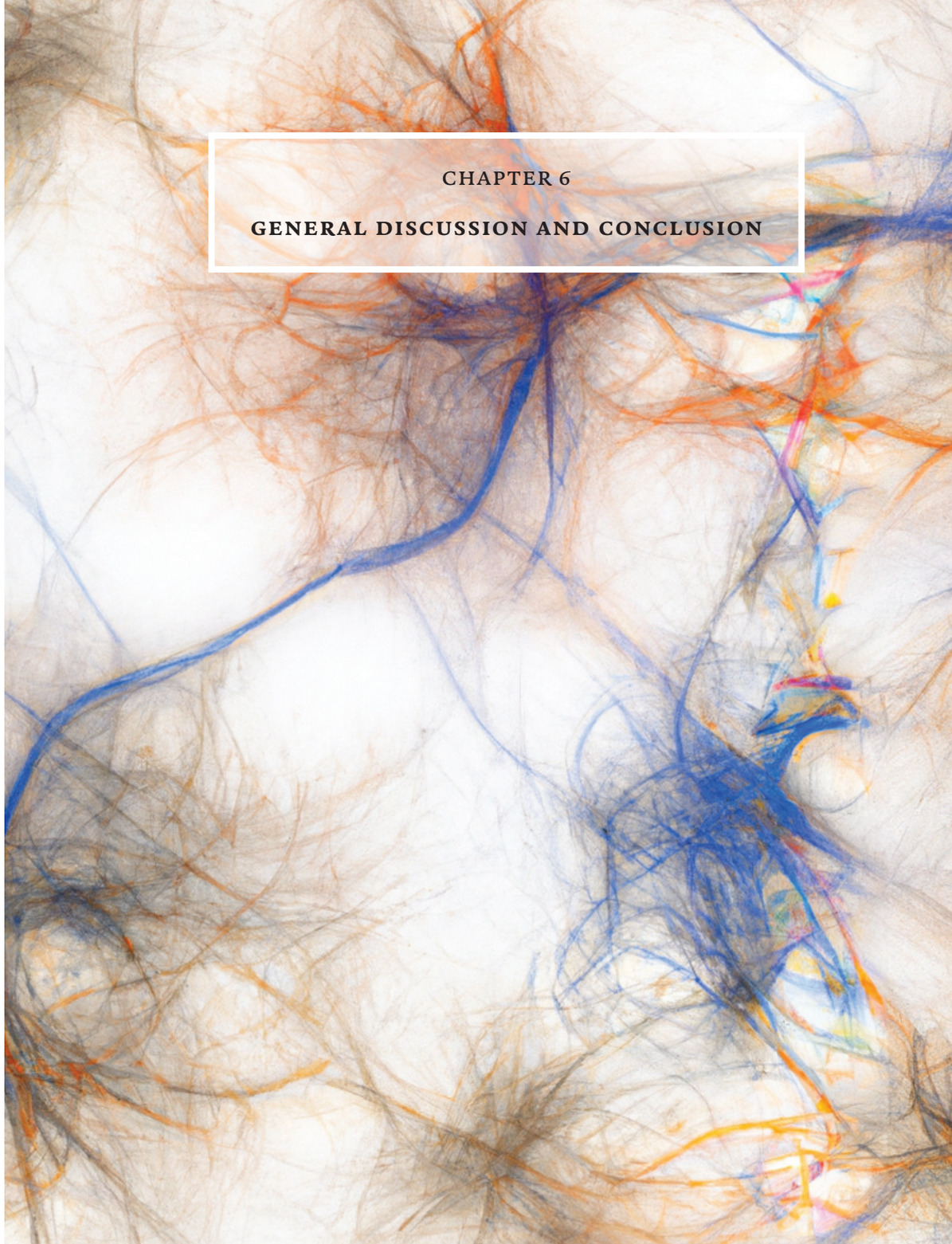

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

We are now at the forefront of a paradigm shift in the treatment of neurodegenerative disorders, driven by advances in our understanding of neurodegenerative disease mechanisms, identification of specific mutations and novel drug targets, and advances in drug development techniques over the past decades. In some ways, the advances we may expect to see in the field of neurodegeneration over the next decades could very well mimic the revolution in understanding and treatment of cancer that we have witnessed over the last four decades. The oncology revolution began with the discovery of tumor specific oncogenes and blood serum biomarkers that could be used as surrogate endpoints in clinical trials in the 1980's,[1–3] and rapidly triggered an exponential increase in identification of oncogenes that in turn led to the development of an expanding arsenal of increasingly specific targeted therapies with monoclonal antibodies, check-point inhibitors, and recently patient-personalized chimeric antigen receptor (CAR) T-CELL therapies, greatly enhancing oncology patient's chances of survival.

Since 2010, the number of identified associated genetic mutations linked to Alzheimer's disease, Parkinson's disease, and ALS has expanded from less than 10 for each indication to over 75 for AD, over 200 for PD, and over 30 for ALS today.[4–6] This has undoubtedly contributed to a rapid expanse of the pipeline of potential disease-modifying treatments for these indications, which currently holds 119 compounds for AD, 52 compounds for PD, and over 100 compounds for ALS.[7–9] The extent of this pipeline is hopeful to patients and those that carry genetic risk-factors for developing these disorders. But at the same time, this broad pipeline also offers a challenge for drug-developers and clinical trial investigators. For example, in 2022 there were over 7,900 participant slots to be filled in active phase 2 and phase 3 ALS trials alone,[9] which is higher than the total number of people being diagnosed with ALS in the United States each year (~6,000). This highlights that careful consideration is needed for how to strategically use limited resources – including funds, clinical research capacity, and participants – to focus late-stage clinical investigation towards those compounds that present the highest chance of maximum clinical benefit and overall drug-development success.

This thesis discusses one way to support such strategic drug development decisions in the field of neurodegenerative diseases, by using (pharmacodynamic) biomarkers to demonstrate proof-of-mechanism in early phase clinical pharmacology studies (*Chapter 2*). When utilized in early clinical development, these biomarkers can help select the best drug candidates, their anticipated effective dose levels, optimize trial designs, guide decisions to move forward into late-stage development, and/or terminate unsuccessful compounds early to facilitate optimal use of scarce resources. Moreover, there is a strong ethical argument to be made; to only initiate trials with compounds that have a demonstrated reasonable chance of efficacy in patients suffering from these debilitating and progressive diseases.

In addition, many of the potential disease-modifying treatments in development for neurodegenerative disorders target completely new pharmacologic targets (first-in-class). This makes these compounds and their clinical development different, with larger uncertainty (as reflected in a high development-failure rate), compared to non-first-in-class compounds for relatively well-understood therapeutic areas. The use of pharmacological biomarkers in early-stage clinical development therefore also helps link the dose-response curve in humans to the pre-clinical data, which is essential to uncover the relationship between the minimally pharmacologically active dose and a safe therapeutic dose in humans.

The importance of uncovering this relation between the pharmacologically active dose and a safe therapeutic dose is highlighted in *Chapter 3*, that describes the early clinical development trajectory of the RIPK1 inhibitor SAR443060 (DNL747). Although the exact level of RIPK1-inhibition that would be required for potential clinical efficacy in human AD and ALS is still under investigation, recent reports suggest that inhibition levels of >95% may be required.[10] That level of inhibition is significantly higher than the median 66% to 82% of RIPK1-inhibition that was achieved with 50 mg BID SAR443060 at trough concentrations in PBMCs of ALS and AD patients, respectively. Higher dose levels of SAR443060 (up to 400 mg BID) did lead to median RIPK1-inhibition of >95% in PBMCs in healthy subjects, but these higher dose levels were not deemed safe for chronic dosing in patients due to serious thrombocytopenia and anemia findings in long-term toxicity studies in monkeys at these higher dose levels. Consequently, SAR443060 development was discontinued. However, as other (non-CNS-penetrant) RIPK1-inhibitors have achieved higher levels of RIPK1-inhibition with dosing periods of up to 84 days,[11] the dose limiting toxicities observed are most likely compound specific and not common to RIPK1-pathway inhibition. This led to the decision to further pursue RIPK1-inhibition with SAR443820 (DNL788), a CNS-penetrant back-up compound for SAR443060, as a potential disease-modifying treatment strategy for ALS in the HIMALAYA study that is currently enrolling.[12] These insights and the subsequent strategic drug-development decisions would not have been possible without the use of phosphorylation of RIPK1 in PBMCs as a target engagement biomarker in SAR443060's early clinical development program. Or worse, without these target engagement insights, late-stage RIPK1-inhibi-

tion trials could have been initiated with inadequate SAR443060 dose-levels, potentially eventually leading to a discontinuation of the pursuit of RIPK1-inhibition as a potential treatment strategy for AD and ALS for a lack of clinically efficacy of potentially inadequate dose levels.

One important challenge, however, that remains for the further development of CNS-penetrant RIPK1-inhibitors for neurodegenerative diseases is that direct measurement of RIPK1-inhibition levels in CNS-tissue (the actual target site) is not possible as of today. While preclinical data suggests that peripheral RIPK1-inhibiton demonstrates similarities with brain RIPK1-inhibition,[13] and SAR443060 unbound-plasma and CSF drug concentrations were similar, these are still only surrogate markers for the pharmacologic situation in the target astrocytes and microglia in the CNS. Moreover, it has been demonstrated that lumbar CSF drug concentration may not always be an accurate surrogate of brain extracellular fluid drug concentrations, particularly in CNS diseases, and that systems approaches accounting for multiple levels of CNS complexity may be needed to better predict brain pharmacokinetics.[14]

Chapters 4 and *5* demonstrate the benefits of expanding an early-phase biomarker strategy beyond target-engagement biomarkers alone. For the development of LRRK2-inhibitor BIIB122 (DNL151) as potential targeted disease-modifying treatment for Parkinson's disease patients with a LRRK2 mutation, besides peripheral and central target engagement biomarkers (whole blood pS935 and CSF tLRRK2), also down-stream kinase substrate (PBMC pRAB10) and lysosomal functioning (urine BMP) were used to explore the compound dose-response curve. This combination of biomarkers offers an even stronger pharmacologic proof-of-mechanism, as it not only demonstrates that the compound affects its direct target, but it also helps explore the dose response curve of downstream pathway effects that do not necessarily correlate linearly with the level of target engagement (as demonstrated by the differences in dose-response curves for the biomarkers in *Figure 3* and *4* in *Chapter 5*). These additional biomarker insights helped to define the anticipated optimal therapeutic dose level of BIIB122 (225 mg oral tablets QD) for further clinical evaluation in the recently initiated phase 3 LIGHTHOUSE study in PD patients carrying a LRRK2 mutation.[15]

This study will need to tell us if LRRK2-inhibition ultimately provides clinical benefit in the form of slowing Parkinson's disease progression. Because, despite the promising LRRK2 pathway biomarker readouts for BIIB122, that piece of the puzzle still remains to be confirmed in humans. However, if LRRK2-inhition can provide therapeutic benefit, then based on the data-rich early-stage clinical development program, BIIB122 is optimally positioned to be successful.

Another benefit of the biomarker-intense development program for BIIB122 is that it provided confirmation that LRRK2 kinase activity also appears to be elevated in PD patients without a LRRK2 mutation, tough to a lesser extent than in those carrying a LRRK2-mutation (*Chapter 4*). This provides a strong rationale for the recent initiation of the phase 2B LUMA study investigating the clinical effects of LRRK2 inhibitor BIIB122 in PD patients *without* a LRRK2 mutation.

What these pharmacological biomarkers can't tell us, however, is the optimal timing for initiating disease-modifying treatment. The LUMA study will be enrolling early-stage (H&Y stages 1 and 2) PD patients, but it could still turn out that we may need to treat even earlier (e.g. already prior to symptom onset) to achieve meaningful long-term disease-modification. This ultimately would require identification and validation of prognostic biomarkers and screening programs for those at risk (which, from an ethical perspective, should only be initiated if an effective treatment is available).

The biomarker strategy for the early-stage development of LRRK2 inhibitor BIIB122 proved to be very valuable in the clinical development of this compound. However, it may not always be possible to use such extensive biomarker characterization for every novel compound, simply because of technical (assay) limitations, incomplete understanding of newly unraveled disease pathways, high within-subject variability in candidate biomarkers hindering reliable interpretation of results, and/or time and money constraints. Nonetheless, the RIPK1 and LRRK2 examples provided in this thesis do suggest that we should always strive to include a pharmacodynamic biomarker in early-stage development of potentially disease-modifying compounds for neurodegenerative disorders.

What challenges still lie ahead

Although there has been great progress in linking subpopulations with neurodegenerative disorders to specific genetic mutations, in many other cases molecular defects underlying the disease have not yet been identified. Without a better understanding of these disease processes and the underlying key molecular defects, it remains difficult to develop effective targeted therapies aimed at disease-modification. And based on the high clinical development failure rate we have seen to date for compounds targeting general pathological processes, such as amyloid-β in AD or α-synuclein in PD, targeted therapies may eventually be our best shot at significantly slowing down disease-progression. Which brings us back to the comparison to the field of oncology, where the discovery of very specific molecular defects in different types of cancer has led to highly effective drugs specifically targeting these

defects. And a similar trend is now visible in the neurodegenerative space, with distinct targeted treatments being developed for e.g. PD patients with a LRRK2 versus a GBA mutation, or ALS patients with a SOD1 versus C9ORF72 mutation.

Another remaining challenge in drug development for neurodegenerative disorders lies in the translational animal models, that are not as advanced, or predictive of human clinical efficacy, as in other therapeutic areas. This is not surprising, since these models are human-engineered to reproduce the initial proteinopathy and/or make use of specific genetic mutations, and therefore may not be able to fully mimic entire sequence of pathophysiologic events that occur in human disease as long as our molecular understanding of these diseases remains incomplete.[16] Some limitations of animal models may not be easily overcome, such as the short life-span of rodents that may lead to incomplete development of pathological hallmarks and/or neurodegeneration. On the other hand, advances in genome editing and our expanding understanding of neurodegenerative disease mechanisms will undoubtedly help improve and validate new preclinical models. This increased disease-understanding will also help better understand the utility and limitations of various animal models, so that the best-fitting and most-predictive models (and treatment-timing) can be selected for the preclinical development of each specific compound for each specific disease subtype.[16]

Finally, the uncertainty around the timing of the molecular onset of the disease and best time for intervention, the large heterogeneity in disease-progression between patients, and the lack of validated biomarkers for the rate of disease-progression all make it difficult to precisely evaluate clinically relevant responses to novel compounds without the use of very large and lengthy trials. To overcome this challenge the neurodegenerative disorders research community is exploring innovative clinical trial design approaches, including platform and adaptive designs to maximize the statistical power of trials and minimize the duration and overall number of patients required for these trials.[17,18]

Additionally, efforts are being undertaken in developing risk-based inclusion criteria for trials to reduce participant-exclusion rates and improve generalizability of trial results.[19]

Future outlook

With an increasing understanding of disease mechanisms and a drug development pipeline fuller than ever, it is an exciting time for the neurodegenerative field. This is perhaps best illustrated by the recent readouts of the phase 3 lecanemab (a soluble amyloid-β protofibrils antibody) study in early AD, that demonstrated a reduction of markers of amyloid in early Alzheimer's disease and resulted in moderately slower decline on measures of cognition and function than placebo at 18 months.[20]

The consistency of all endpoints in this trial being in the same direction suggests that the amyloid hypothesis may hold true after all, and that anti-amyloid-β therapies could slow down progression of AD.

On the other hand, lecanemab was only able to slow the rate of cognitive decline by 27% at 18 months. This could suggest that the administered lecanemab dose may have been too low (only 0.1-0.3% of the administered IV dose of lecanemab is recovered in CSF[21]), or that intervening at the stage of early AD is already too late. However, it could also indicate that targeting amyloid-β alone may not be enough to achieve meaningful disease-modification. In fact, given that there are more people at risk of developing neurodegenerative disorders, e.g. due to the presence of disease-related genetic mutations, than there are people that actually develop disease symptoms, it is not unlikely that development of these conditions may require simultaneous activation of more than one pathogenic pathway, and that certain cellular defense mechanisms fail concomitantly.[22]

This could imply that to achieve clinically meaningful disease-modification it may eventually require a combination of drugs targeting multiple affected disease pathways in parallel. And, similar to the field of oncology, we may eventually need a combination of genetic screening and prognostic biomarkers to be able to define the optimal combination of disease-modifying drugs for each individual patient.

In the end disease-modifying treatments are only expected to be able to slow down disease progression and not to lead to reversal of disease. In this aspect the neurodegenerative field is very different from oncology. Where in oncology the goal is to eliminate tumor cells, in neurodegeneration the goal is to protect from neuronal cell death. This fight has proven to be even more challenging so far, especially given the fact that neurons have very limited capacity to regenerate and disease symptoms only present when a majority of neurons has already been lost. At this moment it is too early to tell if we will ever be able to cure neurodegenerative disorders. However, based on neuron's limited capacity to regenerate, a cure may eventually only be possible via prophylactic gene therapy for people at risk and/or via neuro-regenerative cell therapies.

While such a potential cure may sound like a faraway future, the preparations for its development are actually already happening today with new disease mechanisms being unraveled, new genetic mutations being identified and dozens of potential disease-modifying therapies entering early clinical development. Each of these discoveries will expand our understanding and bring us one step closer to a cure for these debilitating diseases. In fact, the pace at which these developments are evolving is an indication that we are heading into a phase of exponential growth. Disease-modifying treatments sounded like a faraway future not too long ago, but today we are testing them in the clinic. In data-rich mechanistic early-phase studies these disease-modifying treatments help us further understand and validate disease mechanism and potential treatment options. And as we have seen in other areas of research, when knowledge starts to expand exponentially, this will attract more resources and innovation starts taking place at an unprecedented speed. And soon a paradigm can shift.

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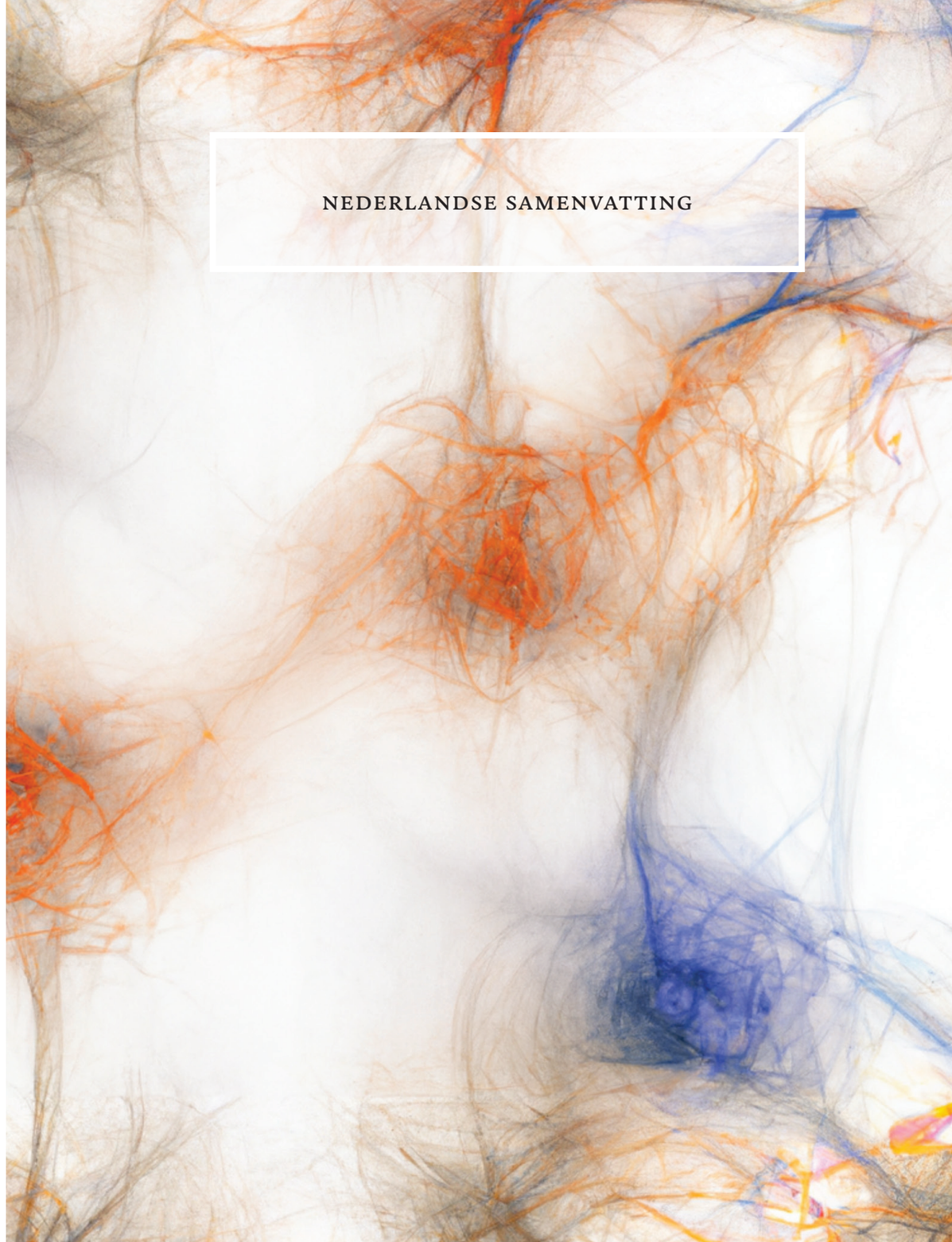

NEDERLANDSE SAMENVATTING

Degeneratieve ziekten van het zenuwstelsel, ofwel neurodegeneratieve aandoeningen, zijn vaak ernstig, progressief en kunnen fataal zijn. Symptomen uiten zich meestal in de vorm van motorische stoornissen (zoals evenwichts-, bewegings-, spraak- en ademhalingsproblemen), cognitieve stoornissen (zoals geheugenverlies, verminderd leervermogen en concentratieproblemen), psychiatrische symptomen (zoals depressie, angst en hallucinaties) en uiteindelijk ook verstoringen in het bewustzijn. Enkele van de bekendste neurodegeneratieve aandoeningen zijn de ziekte van Alzheimer (AD), de ziekte van Parkinson (PD) en amyotrofische laterale sclerose (ALS). Wereldwijd neemt de prevalentie van deze aandoeningen toe en op dit moment zijn ze niet te genezen.

De meeste farmacologische interventies (geneesmiddelen) voor neurodegeneratieve aandoeningen verlichten alleen tijdelijk de symptomen, vergroten de mobiliteit of verzachten de pijn, maar vertragen de ziekteprogressie niet. Neurodegeneratieve aandoeningen zijn daarom momenteel een van de indicatiegebieden met de grootste onvervulde medische behoefte en er wordt dringend gezocht naar behandelingen die de ziekteprogressie kunnen vertragen.

Het is alarmerend dat tot op heden bijna 100% van de onderzoeksmiddelen gericht op het vertragen van ziekteprogressie bij neurodegeneratieve aandoeningen in het klinisch onderzoek is mislukt. Dit komt voornamelijk door een aantal fundamentele uitdagingen in de ontwikkeling van geneesmiddelen voor neurodegeneratieve aandoeningen, zoals een slechte vertaalbaarheid van preklinische modellen naar het ziekteproces bij mensen, het feit dat het ziekteproces al begint lang voordat de eerste klinische symptomen waarneembaar zijn, het beperkt kunnen objectiveren en kwantificeren van de ziekteprogressie en de lokalisatie van het ziekteproces in een lichaamscompartiment (het brein/zenuwstelsel) dat niet gemakkelijk toegankelijk is voor het verkrijgen van (weefsel)monsters tijdens klinisch onderzoek.

Niettemin lijkt er toch een paradigmaverschuiving te gaan plaatsvinden in de behandeling van neurodegeneratieve aandoeningen. Deze verschuiving wordt gedreven door vooruitgang in ons begrip van de ziektemechanismen die ten grondslag liggen aan neurodegeneratieve aandoeningen over de afgelopen decennia, de identificatie van genetische mutaties en nieuwe aangrijpingspunten voor geneesmiddelen, en de algemene technische vooruitgang in geneesmiddelonderzoek.

Sinds 2010 is het aantal geïdentificeerde genetische mutaties die geassocieerd zijn met de ziekte van Alzheimer, de ziekte van Parkinson en ALS gestegen van minder dan 10 mutaties voor elke indicatie tot respectievelijk meer dan 75, meer dan 200 en meer dan 30 mutaties. Dit heeft geleid tot een snelle groei van de ontwikkelingspijplijn van potentiële ziekte-modificerende behandelingen voor deze aandoeningen. Begin 2023 omvatte deze pijplijn 119 onderzoeksmiddelen voor Alzheimer, 52 voor Parkinson en meer dan 100 voor ALS. Dit biedt hoop voor patiënten en dragers van genetische risicofactoren voor deze ziekten. Echter, deze volle pijplijn vormt ook een uitdaging voor geneesmiddelenontwikkelaars en klinische onderzoekers. In 2022 werden er bijvoorbeeld al meer dan 7.900 deelnemers gezocht voor alle lopende fase 2 en fase 3-onderzoeken naar nieuwe middelen voor ALS. Dit aantal is hoger dan het totale aantal mensen dat jaarlijks de diagnose ALS krijgt in de Verenigde Staten (~6.000), waar de meeste klinische onderzoeken plaatsvinden. Dit laat zien dat strategische keuzes nodig zijn om onderzoeksmiddelen die de grootste kans bieden op maximaal klinisch effect en ontwikkelingssucces voldoende toegang of zelfs voorrang te geven tot de beperkt beschikbare middelen, inclusief financiering, klinische onderzoekscapaciteit en patiënten voor fase 2 en 3 studies.

Dit proefschrift beschrijft een belangrijke methode om dergelijke strategische beslissingen over de ontwikkeling van geneesmiddelen op het gebied van neurodegeneratieve ziekten te faciliteren, namelijk door het gebruik van farmacodynamische biomarkers om mechanistische effecten aan te tonen in vroege klinisch-farmacologische studies (*hoofdstuk 2*). Wanneer deze biomarkers in de vroege klinische ontwikkeling worden gebruikt, kunnen ze helpen bij het selecteren van de beste kandidaat-geneesmiddelen en hun verwachte therapeutische dosering, het optimaliseren van de opzet van (vervolg)onderzoeken en het maken van gefundeerde keuzes om een geneesmiddel door te ontwikkelen in grote fase 2 en 3 (registratie) studies. Anderzijds zouden zij aanleiding kunnen geven tot de beslissing om de ontwikkeling van een niet-kansrijk middel zo vroeg mogelijk te staken. Naast de keuze met betrekking tot de optimale inzet van beperkte middelen is er ook een sterk ethisch argument te maken om alleen studies in patiënten toe te laten met onderzoeksmiddelen waarvan is aangetoond dat ze een redelijke kans hebben op werkzaamheid tegen deze slopende en progressieve ziekten.

Veel van de potentiële ziekte-modificerende behandelingen die momenteel in ontwikkeling zijn voor neurodegeneratieve aandoeningen, zijn gericht op volledig nieuwe farmacologische aangrijpingspunten (*first-in-class*). Dit maakt de klinische ontwikkeling van deze middelen wezenlijk anders dan die van geneesmiddelen voor relatief goed begrepen therapeutische gebieden en aangrijpingspunten, wat leidt tot grotere onzekerheid gedurende het gehele ontwikkeltraject. Dit wordt gereflecteerd door het hoge percentage

middelen dat faalt tijdens klinisch onderzoek. Het gebruik van farmacodynamische biomarkers in een vroeg stadium van de klinische ontwikkeling helpt om de dosis-responscurve bij de mens te koppelen aan preklinische gegevens. Deze kennis is essentieel om de relatie tussen de minimaal farmacologisch actieve dosis en een verwachte veilige therapeutische dosis bij de mens in kaart te brengen.

Het gebruik van farmacodynamische biomarkers kan ook helpen bij het onderscheid maken tussen een negatieve klinische studie-uitkomst als gevolg van een gebrek aan klinisch effect van het beoogde moleculaire mechanisme versus een mogelijk gebrek aan klinisch effect als gevolg van onvoldoende blootstelling aan het geneesmiddel of onvoldoende modulatie van het beoogde aangrijpingspunt. Het eerste zou suggereren dat de focus verlegd moet worden naar andere moleculaire aangrijpingspunten, terwijl het tweede kan suggereren dat men zich zou kunnen blijven focussen op hetzelfde moleculaire aangrijpingspunt, maar met andere of betere middelen die gunstiger farmacokinetische of farmacodynamische eigenschappen hebben.

Hoofdstuk 2 geeft een overzicht en categorisering van biomarkers die in de literatuur zijn gerapporteerd in vroege fase klinisch-farmacologische studies in de afgelopen tien jaar. Daarnaast geeft dit hoofdstuk overwegingen voor de selectie van biomarkers voor vroege fase klinische geneesmiddelstudies. *Hoofdstuk 2* eindigt met een voorgestelde routekaart voor het ontwerpen van vroege fase, mechanistische, klinisch-farmacologische studies voor ziekte-modificerende therapieën voor neurodegeneratieve aandoeningen.

Deze methodologie van vroege fase, mechanistische, klinisch-farmacologische studies is vervolgens toegepast bij de ontwikkeling van twee potentiële nieuwe middelen gericht op ziekte-modificatie bij neurodegeneratieve aandoeningen: een RIPK1-remmer en een LRRK2-remmer.

RIPK1-remmer voor Alzheimer en ALS

Receptor-interacting serine/threonine protein kinase 1 (RIPK1) is een belangrijke regulator van ontstekingssignalering en celdood. Verhoogde RIPK1-activiteit wordt waargenomen bij verschillende neurodegeneratieve aandoeningen. RIPK1-remming blijkt te beschermen tegen celdood in preklinische cellulaire en dierlijke ziektemodellen.

Hoofdstuk 3 beschrijft de vroege klinische fase van de ontwikkeling van SAR443060 (voorheen DNL747), een selectieve, oraal bio-beschikbare, centraal zenuwstelsel(CZS)-penetrerende, reversibele remmer van RIPK1. SAR443060 is ontwikkeld om de ziekteprogressie bij Alzheimer en ALS te vertragen.

Hoofdstuk 3 begint met een overzicht van de preklinische gegevens over de veiligheid van SAR443060 en de *ex vivo* dosis-respons curve. Dit wordt gevolgd door een eerste-in-de-mens, gerandomiseerd, placebogecontroleerd, dubbelblind (RCT), enkel- en meervoudige oplopende dosisstudie bij gezonde proefpersonen en twee eerste-in-de-patiënt, RCT, cross-over studies bij patienten met Alzheimer en ALS. Het doel van deze studies was om de veiligheid, verdraagbaarheid, farmacokinetiek en farmacodynamiek van SAR443060 te evalueren. De farmacologische activiteit van SAR443060 werd bepaald door de autofosforylering van RIPK1 op serine 166 (pRIPK1) in perifeer bloed mononucleaire cellen (PBMCs) te meten en te vergelijken met de gemeten uitgangswaarde voorafgaand aan dosering. De distributie van SAR443060 in liquor (CSF) werd ook gekwantificeerd als surrogaat voor de verwachtte concentraties van het onderzoeksmiddel in het CZS. De gecombineerde resultaten van deze studies suggereren dat therapeutische modulatie van RIPK1 in het CZS mogelijk is, wat mogelijk een therapeutisch effect zou kunnen hebben bij Alzheimer en ALS.

Desondanks werd in deze studies benadrukt hoe belangrijk het is om in een vroeg stadium van de ontwikkeling de relatie tussen de farmacologisch actieve dosis en een veilige therapeutische dosis te onderzoeken. Hoewel het exacte niveau van RIPK1-inhibitie dat nodig is voor potentiële klinische werkzaamheid bij Alzheimer en ALS bij de mens nog onvoldoende duidelijk is, suggereert de meest recente literatuur dat inhibitieniveaus van >95% wellicht nodig zijn. Dit niveau van benodigde inhibitie is aanzienlijk hoger dan de RIPK1-inhibitie in PBMCs (mediaan 66% tot 82%) die werd bereikt met de dalconcentraties na tweemaal daags 50 mg SAR443060 bij respectievelijk ALS- en Alzheimerpatiënten. Hogere doseringen van SAR443060 (tot 400 mg tweemaal daags) leidden wel tot mediane RIPK1-remming van >95% in PBMCs bij gezonde proefpersonen, maar deze hogere doseringen worden niet veilig beschouwd voor chronische dosering bij patiënten na bevindingen van ernstige trombocytopenie en anemie in lange-termijn toxiciteitsstudies bij apen. Daarom is de ontwikkeling van SAR443060 gestopt. Aangezien andere (niet-CZS-penetrerende) RIPK1-remmers hogere niveaus van RIPK1-remming hebben bereikt met doseringsperioden tot 84 dagen, zijn de waargenomen dosis beperkende bijwerkingen hoogstwaarschijnlijk specifiek voor het middel SAR443060 en niet voor RIPK1-inhibitie in het algemeen. Dit heeft bijgedragen aan de keuze om RIPK1-inhibitie als een potentiële ziekte-modificerende therapie voor ALS verder te onderzoeken met SAR443820 (DNL788), een alternatief middel dat lijkt op SAR443060, in de HIMALAYA-studie.

Deze inzichten en de daaropvolgende strategische beslissing om de ontwikkeling van SAR443060 te staken zouden niet mogelijk zijn geweest zonder het gebruik van pRIPK1 in PBMCs als biomarker voor de farmacologische activiteit van SAR443060 zo vroeg in het klinische ontwikkelingsprogramma. Als deze inzichten over de ontoereikende binding aan het moleculaire aangrijpingspunt niet aan het licht waren gekomen, zouden late-fase RIPK1-studies kunnen zijn gestart met inadequate doseringen van SAR443060, wat uiteindelijk in het ergste geval had kunnen leiden tot het staken van de verdere ontwikkeling van RIPK1-inhibitie als een potentiële behandelingsstrategie voor Alzheimer en ALS vanwege een gebrek aan klinische werkzaamheid van potentieel inadequate doseringen.

LRRK2-remmer voor de ziekte van Parkinson

Mutaties in het leucine-rich repeat kinase 2 (LRRK2) gen kunnen een risicofactor zijn voor het ontwikkelen van de ziekte van Parkinson. LRRK2-mutaties gaan vaak gepaard met een verhoging van de LRRK2-kinase-activiteit en verstoren verderop in de moleculaire cascade het functioneren van lysosomen. Deze lysosomale disfunctie kan de klaring van eiwitten verstoren en uiteindelijk leiden tot aggregatie van toxische eiwitten zoals αSYN en p-TAU, beide karakteristiek voor de ziekte van Parkinson. In preklinische modellen voor Parkinson corrigeert remming van LRRK2 lysosomale disfunctie en de daaruit voortvloeiende neurodegeneratie.

Hoofdstuk 4 beschrijft een onderzoek naar kandidaat-biomarkers voor potentiële patiëntstratificatie en het karakteriseren van de veiligheid, interactie met het moleculair aangrijpingspunt en farmacodynamiek van LRRK2-remmers. Hiervoor zijn bloed, PBMCs, neutrofielen en liquor verzameld van Parkinsonpatiënten met en zonder LRRK2-mutatie en gezonde controlepersonen. Daarbij is specifiek gekeken naar de variabiliteit in biomarker niveaus binnen één-en-dezelfde proefpersoon over de tijd, tussen verschillende proefpersonen en op groepsniveau, voor biomarkers gericht op de interactie met het moleculair aangrijpingspunt (totaal LRRK2-eiwit [tLRRK2] en fosforylering van LRRK2-eiwit op het serine 935 residu [pS935]) en biomarkers verderop in de moleculaire cascade (fosforylering van LRRK2's RAB10-substraat [pRAB10] en αSYN). De resultaten van deze biomarkerstudie zijn vervolgens gebruikt om een robuuste biomarkerstrategie te ontwerpen voor twee vroege-fase klinisch-farmacologische studies met een nieuwe LRRK2-remmer.

Deze vervolgstudies met de CZS-penetrerende LRRK2-remmer BIIB122 (voorheen DNL151) worden beschreven in *hoofdstuk 5* en bestaan uit een eerste-in-de-mens RCT-studie met enkel- en meervoudige oplopende doseringen bij gezonde proefpersonen en een eerste-in-de-patiënt RCT bij Parkinsonpatiënten om de veiligheid, verdraagbaarheid, farmacokinetiek en farmacodynamiek van BIIB122 te evalueren. In beide onderzoeken werden dosisafhankelijke effecten gezien voor BIIB122's interactie met het moleculair aangrijpingspunt (pS935) en verderop in de moleculaire cascade (pRAB10). Bovendien bleek dat BIIB122-concentraties in het liquor vergelijkbaar waren met de ongebonden geneesmiddelconcentraties in het plasma, wat duidt op goede blootstelling in het CZS.

Deze LRRK2-studies tonen de voordelen aan van een biomarkerstrategie die nog verder gaat dan alleen het meten van de interactie met het moleculair aangrijpingspunt in vroege-fase geneesmiddelonderzoeken. Naast perifere en centrale interactie met het moleculair aangrijpingspunt (gemeten via pS935 in volbloed en tLRRK2 in CSF), werden ook het LRRK2-kinase substraat (pRAB10 in PBMCs) en het algemeen functioneren van lysosomen (via BMP in urine) gebruikt om de dosis-responscurve van BIIB122 uitvoerig in kaart te brengen. Deze combinatie van biomarkers biedt sterk farmacologisch-mechanistisch bewijs, omdat het niet alleen aantoont dat BIIB122 zijn directe doelwit (LRRK2) bindt, maar ook een dosis-effect relatie verderop in de moleculaire cascade in beeld brengt. Dit is belangrijk omdat effecten verderop in de moleculaire cascade niet noodzakelijkerwijs lineair correleren met de mate van directe binding van BIIB122 aan LRRK2, zoals blijkt uit de verschillen in dosis-responscurves voor de biomarkers in *figuur 3* en *4* van *hoofdstuk 5*.

Deze aanvullende biomarker-gedreven inzichten hebben geholpen bij het bepalen van de verwachte optimale therapeutische dosering van BIIB122 (225 mg orale tabletten eenmaal daags) voor verdere klinische evaluatie in de onlangs gestarte fase 3 studie LIGHTHOUSE bij Parkinsonpatiënten met een LRRK2-mutatie. Deze studie zal ons moeten vertellen of LRRK2-remming naast moleculair-mechanistische effecten uiteindelijk ook klinisch voordeel oplevert in de vorm van vertraging van de progressie van de ziekte van Parkinson. Want ondanks de veelbelovende LRRK2-biomarkerresultaten met BIIB122, moet het klinische effect van LRRK2-inhibitie bij mensen nog worden aangetoond. Als LRRK2-remming inderdaad therapeutisch voordeel kan opleveren, dan is BIIB122 op basis van het biomarker-intensieve vroege klinische ontwikkelingsprogramma wel optimaal gepositioneerd voor het behalen van succes.

Een ander voordeel van het biomarker-intensieve ontwikkelingsprogramma voor BIIB122 is dat het verder bewijs heeft geleverd voor het feit dat de LRRK2-kinaseactiviteit ook verhoogd lijkt te zijn bij Parkinsonpatiënten zonder LRRK2-mutatie, zij het in mindere mate dan bij patiënten met een LRRK2-mutatie (*hoofdstuk 4*). Dit was een belangrijke reden voor de recente start

van de fase 2b LUMA-studie waarin de klinische effecten van LRRK2-remmer BIIB122 worden onderzocht bij Parkinsonpatiënten zonder LRRK2-mutatie.

Wat deze farmacologische biomarkers ons echter niet hebben kunnen vertellen, is wat de optimale timing is voor het starten van deze potentieel ziekte-modificerende behandeling. De LUMA-studie zal Parkinsonpatiënten in een vroeg stadium (H&Y-stadia 1 en 2) includeren, maar het zou nog steeds kunnen blijken dat we eigenlijk nog vroeger moeten behandelen, bijvoorbeeld vóór het begin van de eerste ziektesymptomen, om op lange termijn klinisch-relevante ziekte-modificatie te kunnen bewerkstellingen. Dit vereist mogelijk uiteindelijk identificatie en validatie van prognostische biomarkers voor de ziekte van Parkinson en screeningprogramma's voor risicopatiënten. Zulke screeningsprogramma's moeten vanuit ethisch oogpunt echter pas gestart worden als er een effectieve behandeling beschikbaar is.

De uitgebreide biomarker strategie in de vroege fase van de klinische ontwikkeling van de LRRK2-remmer BIIB122 is zeer waardevol gebleken. Het is echter niet altijd mogelijk om voor elk nieuw onderzoeksmiddel een dergelijke uitgebreide biomarker karakterisering toe te passen, simpelweg vanwege technische (assay)beperkingen, een nog onvolledig begrip van nieuw ontrafelde ziektemechanismen, grote natuurlijke variabiliteit in kandidaat-biomarker niveaus die een betrouwbare interpretatie van de resultaten belemmeren en/of beperkingen in tijd en geld voor het ontwikkelprogramma. Niettemin suggereren de voorbeelden van RIPK1 en LRRK2 in dit proefschrift dat men er altijd naar zou moeten streven om tenminste één farmacodynamische biomarker op te nemen in het vroege stadium van de klinische ontwikkeling van potentieel ziekte-modificerende middelen voor neurodegeneratieve aandoeningen.

Welke uitdagingen liggen er nog voor ons?

Hoewel er grote vooruitgang is geboekt in het identificeren van specifieke genetische mutaties die bijdragen aan het ontstaan van neurodegeneratieve aandoeningen in specifieke (sub)populaties, zijn in veel gevallen de moleculaire defecten die aan de ziektes ten grondslag liggen nog niet geïdentificeerd. Zonder een beter begrip van deze ziekteprocessen en de specifieke onderliggende moleculaire pathologie blijft het moeilijk om doeltreffende ziekte-modificerende therapieën te ontwikkelen. Op basis van het hoge percentage mislukte klinische ontwikkelingen dat we tot nu toe hebben gezien voor middelen die gericht zijn op relatief algemene pathologische processen, zoals amyloïde-β bij Alzheimer of α-synucleïne bij Parkinson, zijn doel-specifieke therapieën, zoals het remmen van LRRK2, uiteindelijk misschien wel onze beste kans om ziekteprogressie aanzienlijk te kunnen vertragen. Een andere belangrijke uitdaging bij de ontwikkeling van geneesmiddelen voor neurodegeneratieve aandoeningen ligt in de translationele diermodellen, die niet zo geavanceerd of voorspellend blijken te zijn voor klinische werkzaamheid bij de mens als in andere therapeutische gebieden. Dit is niet verrassend omdat deze diermodellen vaak zijn ontworpen om de oorspronkelijke proteïnopathie (abnormaal-eiwit pathologie) te reproduceren en/of gebruik maken van specifieke genetische mutaties. Ze zijn daarom waarschijnlijk niet in staat om de volledige opeenvolging van pathofysiologische gebeurtenissen die bij de mens optreden volledig na te bootsen, zolang ons begrip van het fundamentele moleculaire proces bij deze ziekten onvolledig blijft. Een deel van de beperkingen van diermodellen zijn wellicht niet gemakkelijk te overwinnen, zoals de korte levensduur van knaagdieren die kan leiden tot een onvolledige ontwikkeling van pathologische kenmerken en/of neurodegeneratie. Anderzijds zal de vooruitgang op het gebied van genetische modificatie en ons groeiende inzicht in de mechanismen van neurodegeneratieve ziekten op termijn ongetwijfeld bijdragen aan verbeterde nieuwe preklinische modellen. Onze toegenomen kennis over deze ziekten zal ook bijdragen aan een beter begrip van het nut en de beperkingen van verschillende diermodellen, zodat de best passende en meest voorspellende modellen (en het tijdstip van het starten van de behandeling) kunnen worden geselecteerd voor de preklinische ontwikkeling van elk specifiek nieuw middel voor elk specifiek (genetisch) subtype van deze aandoeningen.

Ten slotte maken verschillende factoren het moeilijk om klinisch relevante effecten van nieuwe behandelingen voor neurodegeneratieve aandoeningen nauwkeurig te evalueren anders dan via zeer grote en langlopende studies. Hierin spelen de onzekerheid over de moleculaire oorsprong van de ziekte, het ideale tijdstip voor interventie, de grote heterogeniteit in ziekteprogressie tussen patiënten en het ontbreken van gevalideerde biomarkers om de snelheid van ziekteprogressie nauwkeurig te meten, een belangrijke rol. Om deze uitdagingen te adresseren, verkent de onderzoeksgemeenschap onder andere innovatieve benaderingen voor de opzet van klinische studies, waaronder platform- en adaptieve studieontwerpen. Op deze manier kan de statistische kracht van de studies worden gemaximaliseerd en de duur en het aantal patiënten dat nodig is voor studies worden geminimaliseerd. Daarnaast wordt ook gewerkt aan de ontwikkeling van op risico gebaseerde inclusiecriteria voor studies, om het aantal deelnemers dat momenteel uitgesloten wordt te verminderen en de generaliseerbaarheid van de onderzoeksresultaten te verbeteren.

Toekomstperspectief

Met een groeiend begrip van de ziektemechanismen en een geneesmiddelenpijplijn die voller is dan ooit tevoren, beleven we momenteel een boeiende tijd in het neurodegeneratieve veld. Dit wordt het best geïllustreerd door recente resultaten van een fase 3-studie met lecanemab, een antilichaam tegen oplosbare amyloïde-β-protofibrillen, bij de vroege ziekte van Alzheimer. Deze studie liet een vermindering van amyloïde biomarkers zien en resulteerde na 18 maanden in een matig tragere achteruitgang op cognitieve en functiemaatstaven dan placebo. De consistentie van alle eindpunten in deze studie suggereert dat de *amyloïdhypothese* bij Alzheimer mogelijk standhoudt en dat anti-amyloïd-β therapieën de progressie van de ziekte kunnen vertragen.

Aan de andere kant was lecanemab slechts in staat de cognitieve achteruitgang na 18 maanden met 27% te vertragen. Dit zou kunnen wijzen op een te lage dosis lecanemab (slechts 0,1-0,3% van de toegediende IV-dosis lecanemab wordt teruggevonden in liquor), of dat ingrijpen in het stadium van vroege Alzheimer mogelijk al te laat is. Het zou er echter ook op kunnen wijzen dat het verminderen van amyloïd-β alleen wellicht niet voldoende is om klinisch-relevante ziekte-modificatie te bereiken. Omdat meer mensen het risico lopen op neurodegeneratieve aandoeningen, bijvoorbeeld door de aanwezigheid van ziekte-gerelateerde genetische mutaties, dan dat er mensen daadwerkelijk ziektesymptomen ontwikkelen, is het waarschijnlijk dat de ontwikkeling van deze aandoeningen gelijktijdige activering van meer dan één pathogene cascade vereist en dat meerdere cellulaire afweermechanismen tegelijkertijd falen. Dit zou kunnen betekenen dat voor een klinisch-relevante ziekte-modificatie uiteindelijk een combinatie van geneesmiddelen nodig zal zijn die gelijktijdig op meerdere moleculaire cascades is gericht. En dat we misschien een combinatie van genetische screening en prognostische biomarkers nodig hebben om elke individuele patiënt met een optimale combinatie van ziekte-modificerende geneesmiddelen te kunnen behandelen.

Uiteindelijk is het de verwachting dat ziekte-modificerende behandelingen alleen de ziekteprogressie kunnen vertragen en niet kunnen leiden tot herstel bij deze aandoeningen. Dit komt doordat zenuwen een beperkt vermogen hebben om te regenereren en ziekteverschijnselen meestal optreden wanneer het merendeel van de zenuwen al verloren is gegaan. Op dit moment is het nog te vroeg om te zeggen of neurodegeneratieve aandoeningen ooit genezen kunnen worden. Mogelijk kan genezing alleen bereikt worden door profylactische gentherapie voor mensen die risico lopen en/of via neuro-regeneratieve celtherapieën.

Hoewel dit soort therapieën momenteel nog als verre toekomstmuziek klinken, zijn er al voorbereidingen aan de gang voor de ontwikkeling ervan. Er worden nieuwe ziektemechanismen ontrafeld, nieuwe genetische mutaties geïdentificeerd en tientallen potentiële ziekte-modificerende therapieën beginnen aan de klinische ontwikkeling. Elk van deze ontdekkingen zal ons begrip vergroten en ons dichter bij een remedie voor deze slopende ziekten brengen. Het tempo waarin deze ontwikkelingen zich voltrekken geeft aan dat we mogelijk een fase van exponentiële groei tegemoet gaan. Hoewel ziekte-modificerende behandelingen nog niet zo lang geleden een verre toekomst leken, worden ze nu al getest in de kliniek. Farmacologisch-mechanistische studies in een vroeg ontwikkelstadium met deze behandelingen helpen ons om ziektemechanismen en potentiële behandelingsopties beter te begrijpen en te valideren. Zoals we in andere onderzoeksgebieden hebben gezien, zal kennisuitbreiding meer middelen aantrekken en kan hierdoor innovatie met ongekende snelheid gaan plaatsvinden. Dit kan hopelijk snel leiden tot een paradigmaverschuiving in de behandeling van neurodegeneratieve aandoeningen.

Curriculum Vitae

Maurits Frederick Johan Maria Vissers (Amsterdam, 1987) graduated from secondary school (Gymnasium, OSG Sevenwolden, Heerenveen), in 2005, and thereafter spent one year at Elizabethtown College, PA, USA as a non-degree international student. In 2006 he started studying Pharmacy at the University of Groningen and graduated *cum laude* for his master's degree in 2013. As part of his master's, he spent six months as a visiting researcher at the Massachusetts Institute of Technology, MA, USA to complete a thesis on microfluidics and organ-on-a-chip technology. At the end of his master's, he completed an internship in Pharmaceutical Manufacturing at Astellas BV, Meppel, and was asked to extend this internship as a temporary contractor Pharmaceutical Technology for three months to help resolve an antibiotic manufacturing issue. Maurits started his professional career in 2013 as a Global Project Manager Compounding and Global Management Trainee at Fagron BV, Rotterdam. In 2016 he joined the Medical Affairs department of AbbVie BV, Hoofddorp, where he worked in the field of biotherapeutics and later hepatitis C. In 2018 he was recruited for AbbVie's High Performing Leadership Program and that same year he completed a post-graduate program on Clinical Development of the Paul Janssen Future Lab, Leiden. This program sparked an enthusiasm for Clinical Development and in 2018 Maurits decided to join the Centre for Human Drug Research (CHDR, Leiden) as a Clinical Scientist and pursue a PHD in early phase clinical pharmacology studies with disease-modifying drugs for neurodegenerative disorders at Leiden University under the supervision of prof. dr. G.J. Groeneveld. During the COVID pandemic in 2020 and 2021, Maurits co-authored an ethical framework to evaluate and support the restart of clinical research at the time of the pandemic, completed research on the impact of COVID on immunosuppressant therapy pharmacokinetics in kidney transplant recipients, and led a potential COVID treatment trial in patients alongside his PHD-related trials. In 2021, Maurits transitioned to the position of Experienced Clinical Scientist and in 2022 he completed training and registered as a Clinical Pharmacologist. Since 1 January 2023, Maurits holds the position of Clinical Operations Director and has joined CHDR's Management Team. He currently lives in Amsterdam with his fiancé Mei-An Middelkoop and their two daughters Mia (2021) and Lotta (2022).

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