EARLY PHASE CLINICAL STUDIES WITH DISEASE-MODIFYING THERAPIES IN PARKINSON'S DISEASE

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CHAPTER 1

INTRODUCTION

CHALLENGES OF CLINICAL TRIALS FOR NEURODEGENERATIVE DISORDERS

Neurodegenerative disorders are marked by a deliberate loss of neurons and synaptic connections, usually occurring later in life. These disorders include, among others, Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, and Huntington's disease.[1] Currently, there are therapies approved to treat the symptoms of several of these disorders. To date, only a few therapies have been approved that are considered to target components of the underlying etiological processes. These include lecanemab in Alzheimer's disease, which targets amyloid-β pathology, and riluzole and edaravone in amyotrophic lateral sclerosis, which are thought to modulate glutamatergic excitotoxicity and oxidative stress, respectively. These treatments may represent an initial step towards disease-modifying therapy to limit disease progression; however, they are not expected to reverse neuronal loss. Despite these advances, there are still major challenges in developing disease-modifying therapies, particularly due to the complexity of neurodegeneration and the difficulty of intervening at the right time. Consequently, there are currently no therapies approved to stop or even slow disease progression in Parkinsonian disorders, which is further described in **chapter 2**. Here, several obstacles encountered in clinical trials for neurodegenerative diseases will be discussed, with a focus on Parkinson's disease, and explore potential strategies to overcome them.

Ever since scientific research started to be focused on disease-modifying therapies for neurodegenerative disorders, the interval between actual disease onset defined by initiation of neurodegeneration and start of clinical symptoms was acknowledged to form a major challenge. For Parkinson's disease, by the time the disease manifests clinically, an estimated 35% to 45% of dopamine-producing neurons in the substantia nigra have already degenerated.[2] A major treatment goal for disease-modifying therapeutics would be to halt further neuronal degeneration, with restoration of degenerated neurons, which is generally regarded to be impossible. Because of this, it is thought that it is crucial to intervene early, potentially even at a presymptomatic stage, as this would allow investigational compounds to target as many neuronal cells as possible to preserve. However, for sporadic forms of Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis, there are currently no validated tests for early or presymptomatic diagnosis, making such early intervention inherently challenging. This contrasts with Huntington's disease, for which genetic testing allows for identification of individuals at risk before symptom onset.[3]

The first studies in humans for a novel investigational molecule are designed to answer questions such as; "does the molecule arrive at the target site?", "does the molecule bind its intended receptor?" and "does receptor binding result in the appropriate downstream molecular response?".[4] Phase 1(b) are designed to assess the safety and tolerability of a novel investigational molecule, and therefore these clinical trials are not designed to effectively measure disease progression and treatment efficacy (e.g. "does the therapy actually help the patient?"). The sample size of these trials in general and the trials in this thesis specifically range between 20 and 50 participants, and the trial duration is considered short. The primary objective of phase 1 clinical trials is to assess safety and tolerability in the target population, which justifies this small sample size. If designed well, these trials can also provide insights into proof-of-mechanism as well as proof-of-concept.[5] Given the sample size and length, they are not powered to measure an effect on disease progression or clinical efficacy. The low participant numbers result in higher variability in clinical efficacy measures as well as a higher risk for type I and type II errors when performing pharmacodynamic assessments. Consequently, these limitations lead to either overestimation or underestimation of the clinical efficacy of the drug and therefore should be interpreted with caution during the developmental program. Strategies to partially overcome these difficulties are evaluating interim results during the trial, and looking for dose dependent changes, irrespective of statistical significance.[6] Insights from proof-of-mechanism or proof-of-concept studies can help reduce uncertainty by optimizing dose-ranging in later-phase trials. In the following, we will focus specifically on Parkinson's disease to illustrate the challenges and opportunities in later-phase clinical trials.

CHALLENGES AND OPPORTUNITIES IN LATER-PHASE CLINICAL TRIALS IN PARKINSON'S DISEASE

Moving forward from early phase clinical trials, phase 2 and 3 trials are often powered to demonstrate statistically significant effects on either the total (Movement Disorder Society (MDS)) Unified Parkinson's Disease Rating Scale (UPDRS) score, or the total motor score of part 3. A 2024 systematic review reports that a change of only between 1/40 and 1/20 points on the score is needed to detect a clinically meaningful change.[7] However, multiple factors can influence the measurement of the MDS-UPDRS, and this variability in MDS-UPDRS scores is challenging to reduce due to daily symptom fluctuations, multiple subscales assessing different disease aspects, and minimal clinically important values that vary with disease severity, resulting in substantial error variance.[7,8] Therefore, it is challenging to extract a clinically meaningful signal due to the noise generated by this variability.

Selecting the appropriate trial population is crucial for the success of clinical trials, especially in diseases with diverse etiological mechanisms like Parkinson's disease. Increasing evidence emerges that different underlying etiological mechanisms can lead to the same clinical phenotype of a hypokinetic rigid syndrome.[9] Parkinson's disease pathogenesis involves multiple interconnected mechanisms, with alpha-synuclein aggregation thought to be central to the disease. Misfolded alpha-synuclein forms toxic oligomers that propagate pathology and impair mitochondrial function, contributing to oxidative stress and neuronal damage.[10,11] Mitochondrial dysfunction is implicated in both idiopathic and familial cases, with genetic mutations affecting mitophagy-related proteins such as parkin.[12] Impaired protein clearance via the ubiquitin-proteasome system and autophagy-lysosome pathway leads to alpha-synuclein accumulation, further exacerbating neurodegeneration.[13] Additionally, neuroinflammation, driven by microglial activation and immune system dysregulation, plays a role in disease progression.[14] A striking example of the disease's heterogeneity is that the classical view of alpha-synuclein as central to Parkinson's disease pathology does not hold true for a minority of patients – specifically those with certain genetic mutations (e.g., LRRK2 or PRKN).[15] Although these individuals present with the clinical phenotype of Parkinson's disease and may exhibit nigrostriatal degeneration, they notably lack alpha-synuclein inclusions.[15] Genetic factors are known to play a primary, causal role in approximately 5% to 10% of Parkinson's disease cases, particularly involving mutations in GBA1, SNCA, LRRK2, and PARK2.[16,17] However, it is likely that genetic contributions extend beyond these monogenic forms, with modifying variants and yet-undiscovered loci influencing disease risk and phenotype in a larger subset of patients. As seen in for example amyotrophic lateral sclerosis, the genetic landscape is likely more complex than currently understood, with both known and yet-undiscovered genetic modifiers playing roles of varying magnitude in many patients.[18]

In addition to genetic factors, environmental differences contribute to the heterogeneity. While less well understood, environmental factors also influence disease development and progression. Epidemiological studies have linked rural living, farming, and pesticide exposure to an increased risk of Parkinson's disease.[19,20] Emerging evidence suggests that pesticide exposure may lead to changes in epigenetic markers, particularly in DNA methylation patterns.[21] Specific pesticides, such as paraquat and rotenone, are chemically similar to 1-methyl-4-fenyl-1,2,3,6-tetrahydropyridine, also known as MPTP, a toxic compound for mitochondria. These pesticides have been associated with Parkinson's disease, and can also be used to replicate its pathology in animal models.[22,23] Other environmental factors, including well water consumption, exposure to industrial chemicals, and heavy metals, have also been implicated.[24,25] Based on an individual's genetic profile (which may include not only causal mutations but also multiple variants that confer smaller or larger degrees of risk) as well as environmental exposures, different molecular mechanisms are thought to underlie disease development in different patient subgroups.[21] Therefore, the selection of a particular subgroup of patients with disease biology in line with the mechanism of action of an investigational compound becomes increasingly important in the design of a clinical trial.

BIOMARKERS RELATED TO DISEASE PROGRESSION

This complexity in underlying disease mechanisms due to genetic and environmental heterogeneity is also shown in the clinical presentation of patients. The variability in clinical disease presentation, such as tremor dominant versus bradykinesia dominant, complicates the selection of appropriate biomarkers to measure disease progression.[26] This heterogeneity causes difficulties in detecting the efficacy of a treatment, which is predominantly a primary objective in phase 2 and 3 trials. Trials have included validated clinical, radiological and wet biomarker endpoints and account for genetic status and patient related factors in the analysis.[27,28] For example, the phase 3 trial of lecanemab, an approved disease-modifying therapy for Alzheimer's disease, included both a clinical and radiological endpoint to assess disease modification.[29] A promising strategy to improve the sensitivity of detecting treatment effects is the collection of longitudinal, continuous, remote biomarker data, allowing for a more individualized tracking of disease progression over time, and the use of machine learning to analyze data from several sources.[30] This approach reduces the impact of short-term variability, considerably increases the resolution to detect treatment effects compared to clinical rating scales and provides a more accurate representation of disease progression.[30] Potential biomarkers are wearable sensors for Parkinson's disease, such as smartwatch to measure movement and sleep behavior. [31–33] Once the disease has manifested, the rate of progression is also variable between subjects.[34] More rapid progression in Parkinson's disease is usually predicted by older age at onset, more rapidly declining cognitive and motor function, and the presence of rapid eye movement sleep behavior disorder.[35] Genetic and environmental factors contribute to this heterogeneity as well, influencing disease onset and rate of progression.[9,36,37] In **chapter 3,** we describe a phase 0 trial in which we investigate fatty acids as a biomarker for stearoyl-CoA-desaturase inhibitors. A clinical trial investigating a stearoyl-CoA-desaturase inhibitor is subsequently evaluated in **chapter 4.**

Alpha-synuclein seed amplification assay

An interesting biomarker is the seed amplification assay, which is used in the clinical study that will be described in **chapter 5**. It was developed as a diagnostic tool used to detect misfolded proteins through their prion properties, particularly in neurodegenerative disorders.[38] The assay works by adding the misfolded proteins, also called seeds, from body fluid from patients with a specific disorder to a solution containing normal monomeric proteins. Under assay conditions, the seeds cause the normal proteins to misfold and aggregate, amplifying a measurable signal. Therefore, this amplification allows for the detection of very low levels of pathologically misfolded proteins.[39] Specifically for Parkinson's disease, the alpha-synuclein seed amplification assay has been validated as a diagnostic tool with a sensitivity and specificity up to 95% and 98% respectively, and can distinguish between healthy persons, participants with Parkinson's disease and with multiple system atrophy.[40,41] Also, the assay provides information about the molecular heterogeneity and detects prodromal individuals before diagnosis, although long term data is still needed to validate this assay for a quantitative purposes.[42] In **chapter 5**, we describe a phase 1b trial in which this assay has been used to measure the target engagement of an investigational compound quantitively. Interestingly, the Food and Drug Authority has issued a 'Letter of Support' to encourage the use of the assay in drug development and is expected to be used more frequently in the future.

Accessing the central nervous system

The central nervous system compartment is not easily accessible for sampling fluids or tissue for pharmacokinetic or pharmacodynamic assessments. This raises the challenge of measuring disease progression based on biomarkers and understanding how these biomarkers translate between the peripheral compartment, primarily blood, and the central nervous system. The blood-brain barrier prevents the free passage of most molecules between the central nervous system and blood compartments.[43] This is one of the several factors that explain why brain derived biomarkers are usually detected at considerably lower levels in blood, compared to the cerebrospinal fluid.[44] For example, the concentration of neurofilament light chain, a general marker of neurodegeneration, is approximately 10 to 15-times higher in cerebrospinal fluid than in plasma.[45,46] Also, patients with neurodegenerative disorders have generally higher levels of neurofilament light chain in cerebrospinal fluid, compared to healthy controls.[47] For Alzheimer's disease, recent advances in biomarkers have allowed blood based differentiation between healthy and diseased states and have enabled the detection of blood-based biomarkers that correlate with disease progression.[46,48,49] For example, pTau217 detects Alzheimer's Disease (based on amyloid-beta positivity on PET-scans) with a 95% sensitivity and specificity and detects longitudinal changes in these patients.[49] Several plasma biomarkers have been compared to radiologic markers, such as amyloid plaques in Alzheimer's disease. Specifically, the plasma Aβ42/Aβ40 ratio can accurately detect amyloid plaques by positron emission tomography.[48] However, the actual relationship with brain concentrations is only measurable post-mortem.[48] For Parkinson's disease, blood-based biomarkers are currently not yet available to aid in diagnosis or to predict disease course.

Translating animal models to human data

Another challenge arises from the translation of animal models to human data. Although animal models have provided insights into the underlying mechanisms and have been important in preclinical drug development, there are inherent differences between these models and the human disease condition.[50,51] Neurodegenerative disorders are the result of complex biological processes, and replicating the full spectrum of the symptoms and disease progression in animal models is not often possible.[50,51] Moreover, the response to potential therapies can differ significantly between species, and the factors driving disease progression in humans may not be reflected accurately in animal models.[50,51] Also, most animal studies are exploratory in nature and therefore have very low statistical power due to the small sample size. A few solutions have been proposed recently to improve animal research, and to add information on the relative levels of evidence for the hypothesis of the trial.[52–55] In the context of this thesis, all investigational medicinal products administered in the studies described, were previously tested in a translational animal models, the results of which were described in the investigators brochure that supported the clinical development.

In conclusion, neurodegenerative disorders, including Alzheimer's and Parkinson's disease, are characterized by progressive neuronal loss. Early intervention is likely to be crucial but is complicated by the uncertainty of actual pathophysiological disease onset and by the variability in disease progression, making it difficult to design clinical trials that accurately measure treatment efficacy. Small sample sizes in early-phase trials lead to statistical challenges, while heterogeneity in disease presentation complicates the selection of appropriate biomarkers. Well-designed early phase clinical studies incorporating target engagement and pathway engagement biomarkers can partly overcome these challenges. Further advances in biomarker research, such seed amplification assays and wearable sensors, offer

additional improvements in demonstrating target engagement or tracking disease progression respectively. However, challenges remain in translating findings from animal models to humans, necessitating better experimental designs and translational approaches.

Treating non-motor complaints in Parkinson's disease

For Parkinson's disease, current symptomatic therapies primarily reduce the motor symptoms. Non-motor complaints, however, make up a significant part of the disability and reduction in quality of life due to Parkinson's disease. In general, there are treatment options for various non-motor symptoms originating from the central nervous system or peripherally.[56] Non-motor complaints include cognitive dysfunction, anxiety, gastro-intestinal symptoms, depression and sleep disorders.[57–59] The most prevalent complaint is depression, which occurs in 50% of the patients at some point during the disease, and is usually treated with standard antidepressants.[56,60] Cognitive disfunction is also frequently observed, with 40 percent of patients experiencing mild cognitive impairments.[61] There are currently no treatment options specifically designed to reduce cognitive dysfunction.

Participants with Parkinson's disease have an increased risk of cognitive dysfunction and dementia, influenced by age, motor symptoms, genetics, and predictive biomarkers like basal ganglia atrophy and elevated alpha-synuclein levels.[62–66] Beta-agonistic acting drugs have been identified as potential therapies to improve cognitive dysfunction. These drugs could enhance activity in the locus coeruleus, the center of adrenergic function in the central nervous system, by activating beta adrenergic receptors. This activation increases neuronal excitability and modulates the release of noradrenaline to other brain regions, influencing arousal, attention, and stress responses. [67] In **chapter 6**, we describe a phase 1 trial that evaluates the effects of three beta-agonistic acting drugs on the cognition of participants with Parkinson's disease. Likewise, antidiabetic medication such as pioglitazone has been identified as a potential drug to modify cognitive dysfunction.[66]

THIS THESIS

Neurodegenerative disorders such as Parkinson's disease and related synucleinopathies pose a growing challenge, with limited effective treatments that can modify the underlying disease process. Although lecanemab has been approved by the Food and Drug Administration as disease modifying treatment for Alzheimer's disease, the clinical benefits are marginal for individual patients. This thesis aims to explore novel therapeutic approaches for disease modification in Parkinson's disease, ranging from the modulation of metabolic pathways to innovative immunotherapies. By examining both the current overview of therapies as well as presenting new clinical findings, this work contributes to the ongoing search for therapies that slow or halt neurodegenerative disease progression.

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CHAPTER 2

A SYSTEMATIC REVIEW ON DISEASE-MODIFYING THERAPIES IN PARKINSONIAN DISORDERS

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INTRODUCTION

Parkinsonian disorders, including Parkinson's disease and Lewy Body Dementia, are progressive neurodegenerative conditions characterized by the gradual loss of neurons. In Parkinson's disease, the primary loss occurs in dopaminergic neurons, leading to both motor and non-motor symptoms. In Lewy Body Dementia, the affected neurons include both dopaminergic and cholinergic types, resulting in a broader range of symptoms such as cognitive decline, visual hallucinations, and fluctuations in alertness and attention. While symptomatic treatments can provide relief, there is a growing emphasis on developing disease-modifying therapies aimed at slowing or halting the underlying neurodegenerative processes. A disease-modifying therapy is defined as one that treats the pathogenic mechanisms of disease to prevent disease progression, or a sustained reduction in symptoms and disease activity beyond the temporary effects of other interventions.[1]

The pathophysiology of Parkinsonian disorders revolves around the aggregation of alpha-synuclein protein, a hallmark feature of the disease.[2,3] Alpha-synuclein can undergo conformational changes leading to the formation of oligomers, which gradually progress into insoluble fibrils. These fibrils exhibit prion-like properties, meaning they can induce misfolding in normal proteins and propagate their abnormal structure. This expansion of aggregates facilitates cell-to-cell spread within the central nervous system. The consequences of alpha-synuclein aggregation extend beyond mere protein misfolding, impacting crucial cellular processes. Dysfunctional lysosomal and age-associated glymphatic clearance mechanisms fail to degrade or clear misfolded alpha-synuclein efficiently leading to accumulation within neurons. Mitochondrial dysfunction follows, compromising cellular energy metabolism and exacerbating oxidative stress. Neuroinflammation is triggered as a response to aggregated alpha-synuclein, further exacerbating neuronal damage. Consequently, these pathological processes contribute to neuronal degeneration and eventual loss of function. Notably, similar alpha-synuclein aggregation pathology is implicated in Lewy body dementia, albeit with differences in disease presentation and progression. Additionally, other neurodegenerative diseases such as multiple system atrophy also involve alpha-synuclein aggregation, albeit with distinct pathophysiological and clinical manifestations. Understanding the shared and unique mechanisms underlying alpha-synuclein aggregation in Parkinsonian's disorders is crucial for the development of effective disease-modifying therapies.

In recent years, an increasing number of studies have investigated various therapeutic interventions with the potential to modify the course of the Parkinsonian disorders, especially Parkinson's disease.[4,5] However, no disease-modifying therapy for any Parkinsonian disorder has been approved up to this date. This review aims to provide a comprehensive synthesis of the existing literature on disease-modifying therapy for Parkinsonian disorders, focusing on interventions that have shown promise in clinical settings.

METHODS

Prior to initiating data collection, we registered our review protocol on International Prospective Register of Systematic Reviews (PROSPERO) (ID: CRD42023382886). Reporting follows the 2020 Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.

Search strategy and selection criteria

For this systematic review without meta-analysis, studies were eligible if they included study results from clinical trials with a drug that was considered disease modifying, with healthy volunteers and/or patients with Parkinson's disease, Lewy body dementia, multiple system atrophy, progressive supranuclear palsy, or corticobasal syndrome up to May 1st, 2025. A disease-modifying therapy was considered a drug that would slow the natural progression of the disorder. We included both clinical trials as well as population studies. We excluded preclinical studies, (systematic) reviews, meta-analysis, case reports, non-English articles and studies without a disease-modifying therapy. Duplicates and trial registers were also removed. Data from unpublished studies were identified and included, if available.

Four electronic databases were searched, including PubMed, Embase, Web of Science, and the Cochrane Library. Reference lists in review articles identified during this search, and the final included articles were checked to identify additional potentially eligible studies. The search strategy contained terms related to the intervention and the population (see Supplement 1 for the full search strategy).

Data analysis

Two reviewers (PE and LS) assessed articles independently by title and abstract, and if needed also the article, by using the online Rayyan.io tool. Thereafter, in case of conflicts, a third reviewer (PK) independently determined the final decision to include or exclude. No formal heterogeneity test was performed, but the included studies were assessed for risk of bias on individual study level during the interpretation of the results. Articles were grouped based on their therapeutic effect.

Due to journal guidelines limiting the number of references cited in the main text, we selected 150 references to include based on their direct relevance to the key findings and discussion points of this review. The remaining 65 references, which were deemed supportive but not essential for in-text citation, have been provided as

Supplemental Material to ensure transparency and allow interested readers full access to the evidence base underpinning our analysis.

RESULTS

Monoamine oxidase-B inhibitors levodopa and dopamine agonists

Monoamine oxidase-B inhibitors levodopa and dopamine agonists have been extensively studied as both symptomatic and disease-modifying therapies as our search identified 62 trials. Historically there were concerns that levodopa accelerated disease progression prompting trials of dopamine agonists as levodopa-sparing therapies and studying their suggested neuroprotective properties. More recently in 2004 the Unified Parkinson's Disease Rating Scale (UPDRS) results of the ELLDOPA study suggested a potential disease-modifying effect of Levodopa yet neuroimaging showed a potential neurotoxic effect. This led to the LEAP study of 2019 that showed that treatment with levodopa in combination with carbidopa had no disease modifying effect nor was disease progression exacerbated. Moreover to date all phase 3 clinical trials with MAO-B inhibitors or dopamine agonists have failed to demonstrate disease modifying effects in Parkinsonian disorders such as the LEAP study of 2019. Due to the amount of trials (n = 62) as well as the lack of effects details of the trials were not included in Table 1.

Alpha-synuclein therapies

The soluble, disordered, monomeric protein alpha-synuclein is abundant in presynaptic neurons and is implicated in several important aspects of neuronal functioning. (Figure 2) The primary structure of alpha-synuclein includes three regions: the basic N-terminal domain (residues 1–60), which forms an alpha helix when bound to negatively charged membranes; the hydrophobic non-amyloid-β-component domain (residues 61-95), which is prone to aggregation and fibril formation; and the flexible C-terminal domain (residues 96–140), which is rich in negatively charged residues and interacts with cations and polyamines.[7–9] The functional description of alpha-synuclein epitopes highlights the contrasting roles of the N- and C-termini. The N-terminus promotes aggregation and interactions with membranes and chaperones, while the C-terminus acts as an auto-chaperone inhibiting aggregation.[10,11] While the N-terminus has therapeutic potential, the C-terminus engages in transient long-range interactions with the N-terminal region, maintaining alpha-synuclein in a compact, soluble conformation. The C-terminus disrupts these interactions, leading to a more extended, aggregation-prone structure.[12] There is no consensus on which terminus has the most potential as a target. Moreover a total of 14 distinct truncation variants of alpha-synuclein have been identified including N-terminal truncations (5–140, 39–140, 65–140, 66–140, 68–140, 71–140) and C-terminal truncations (1–139, 1–135, 1–133, 1–122, 1–119, 1–115, 1–110, and 1–103). However it seems that specifically the truncation of the C-terminal leads to the formation of aggregation-prone species.

Several therapies targeting alpha-synuclein directly have been evaluated or are currently being evaluated in clinical trials. These include small molecules that inhibit alpha-synuclein misfolding, aggregation or translation, as well as active and passive immunotherapies aimed at enhancing the clearance of alpha-synuclein.

The most advanced small molecule is buntanetap, which suppresses the translation of messengerRNAs involved in the production of neurotoxic, aggregation-prone proteins. After demonstrating safety (the molecule carried the name posiphen back then), buntanetap was investigated in a 25-day study in 54 patients with early Parkinson's disease and 14 with Alzheimer's disease.[13,14] The study showed a statistically significant improvement compared to baseline of the Movement Disorder Society (MDS) - Unified Parkinson's Disease Rating Scale (UPDRS) total and part III scores for two dose levels. However, these improvements were not dose-dependent, and no significant differences were found between the highest dose levels and placebo. In addition, the study found non-significant reductions in several biomarkers, including total alpha-synuclein in cerebrospinal fluid (CSF). Based on these results a phase 3 study in early Parkinson's disease has since been initiated and completed. The results are not yet published, but in a press release it was announced that the highest dose level significantly improved the MDS-UPDRS Part II, III and total scores, with especially pronounced effects in patients with postural instability and gait difficulties. Furthermore, buntanetap was able to stop cognitive decline in all patients and improved cognition in patients with a MMSE score between 20 and 26 with normal ranges of 24 to 30. It was hypothesized that the improvements over a short duration of treatment were due to buntanetap's ability to rescue and restore neuronal function. Whether these results will reshape our current understanding of the potential disease modification in Parkinson's disease will depend on a more rigorous evaluation of these findings once the peer-reviewed publication is available.

Two other small molecules, UCB0599 and anle138b, were evaluated in placebo-controlled phase 1 trials for their ability to inhibit pathological alpha-synuclein aggregation.[15,16] UCB0599 prevents the initial misfolding and aggregation of alpha-synuclein on lipid membranes whereas Anle138b destabilizes toxic oligomers prevents the formation of oligomer pores in membranes and blocks the prion-like propagation of alpha- synuclein aggregation. Both agents were well tolerated in up to 68 healthy volunteers, and UCB-0599 also showed a favorable safety profile in 31 patients with Parkinson's disease. UCB-0599, rebranded as minzasolmin, was later tested in a proof-of-concept study of 18 months in 450 patients with early Parkinson's disease.

According to a late-2024 press release, the trial did not meet its endpoints, including the primary outcome of disease progression based on the combined MDS-UPDRS parts I to III score. Anle138b has been evaluated in a phase 1b study in Parkinson's disease patients, but no results are reported yet (NCT04685265). The ongoing TOPAS-MSA study assesses the safety and efficacy of anle138b (TEV-56286) in 200 patients with multiple system atrophy over 27 months.

In addition to small molecules, which aim to interfere with alpha-synuclein synthesis or aggregation, another major therapeutic strategy involves leveraging the immune system to target alpha-synuclein more directly. A passive immunotherapy that is still being assessed in two open-label extension studies is prasinezumab. This is a humanized monoclonal antibody with a high affinity for C-terminus region of both aggregated alpha-synuclein and monomers. It was first evaluated in the PASADENA study and its primary endpoint (change from baseline in total scores on the MDS-UPDRS) was not met.[17] Similarly, no significant differences were observed in imaging of nigrostriatal terminal degeneration using dopamine transporter scans. However, a post-hoc analysis in patients with faster disease progression suggested less worsening in the MDS-UPDRS part III in the prasinezumab group compared to placebo.[18] A long-term follow up was conducted as an open-label extension of the PASADENA study.[19] Both the early-start and delayed-start groups showed reduced clinical progression in the MDS-UPDRS part II scores and in part III scores in both the OFF and ON-state compared to patients enrolled in the Parkinson's Progression Markes Initiative (PPMI) observational study. Therefore, prasinezumab may be effective specifically in Parkinson's disease patients with faster disease progression. However, interpretation is limited by methodological constraints, including the use of 80% confidence intervals and the absence of a concurrent placebo group. This study remains ongoing. In parallel, the PADOVA trial was launched to evaluate the investigational therapy in early-stage Parkinson's disease patients. Topline results presented during MDS 2025 showed that although the PADOVA study did not meet its primary endpoint prasinezumab demonstrated signals of slowing disease progression in early-stage Parkinson's disease when added to effective symptomatic therapy supporting continued investigation in future trials.

Another passive immunotherapy is cinpanemab, a human-derived monoclonal antibody with a high affinity for the N-terminus of aggregated alpha-synuclein. A phase 1 study showed increases in the total plasma alpha-synuclein levels in a dose-dependent manner in 18 Parkinson's disease patients, which suggested proof of biological activity as binding to alpha-synuclein prolongs its half-life by limiting clearance.[20–22] In contrast, CSF levels remained unchanged, which aligns with the predominance of monomeric over aggregated alpha-synuclein in CSF. A phase 2 trial (SPARK) evaluating cinpanemab in 398 patients with Parkinson's disease over a 112-week period did not meet its primary objective, a change in clinical scores on the MDS-UPDRS.[20] Similarly, imaging of the dopamine transporter via single-photon emission computed tomography yielded no treatment-related differences. Due to the lack of efficacy, the trial was terminated early. Post-hoc analyses raised concerns about the suitability of imaging endpoints such as dopamine transporter scans and structural magnetic resonance imaging (MRI) for assessing disease progression beyond 96 weeks.[23] These modalities were limited by nonlinear symptom progression, increased dropout rates, and a higher likelihood of patients initiating symptomatic therapy. In addition, the authors emphasized that changes in total alpha-synuclein levels in early Parkinson's disease primarily reflect proximal pharmacodynamic effects and may not correlate with long-term disease modification, particularly in the absence of dose-consistent changes in CSF. No further clinical development of the investigational therapy is ongoing.

When comparing investigational therapies, prasinezumab and cinpanemab aim to inhibit alpha-synuclein aggregation. While cinpanemab primarily binds aggregated forms via the N-terminus, prasinezumab can target monomers, oligomers and aggregated forms via the C-terminus. This could explain the difference in potential therapeutic effect as prasinezumab might act earlier in the aggregation process compared to cinpanemab. In December 2024, a press release stated that prasinezumab had not met its primary endpoint in the PADOVA study but showed favorable trends across multiple secondary and exploratory outcomes. Both the PADOVA and PASADENA studies are continuing to assess the long-term potential therapeutic effect of prasinezumab.

Other passive immunization therapies include MEDI1341, Lu AF82422 and exidavnemab.[24–26] All are human monoclonal antibodies with preferential binding to a broad spectrum of aggregated alpha-synuclein. For MEDI1341 there is no peer-reviewed article available, however a 2023 conference abstract showed that there was a dose-dependent suppression of free alpha-synuclein in the CSF of 25 Parkinson's disease patients after 3 monthly infusions. Both Lu AF82422 and exidavnemab reduced free-to-total alpha-synuclein ratios in a dose-dependent manner in Parkinson's disease patients in phase 1 studies, and Lu AF82422 also reduced CSF free-to-total alpha-synuclein ratios at the highest dose level. Currently, Lu AF82422 is being investigated as amlenetug in multiple system atrophy patients, although top line findings stated that amlenetug failed to show a statistical effect compared to placebo. For exidavnemab, a phase 2 trial in Parkinson's disease patients is ongoing. Notably, exidavnemab has received orphan drug designation from the US FDA for the treatment of multiple system atrophy. MEDI1341 is currently investigated as TAK-341 in a phase

2 trial including only multiple system atrophy patients, assessing effects on disease severity measured by the unified multiple system atrophy rating scale over 52 weeks.

While passive immunization depends on recurrent administration of monoclonal antibodies, active immunization leverages the immune system to generate a long-lasting immune response consisting of a variety of antibodies targeting a predefined epitope. Three investigational therapies are in development that elicit an antibody response against various epitopes in the C-terminal: PD01A, PD03A and UB-312.27–32 Specifically, PD01A targets the 118-126 region, PD03A targets the 110-130 region, and UB-312 targets the 97-135 region.

PD01A and PD03A use different short peptides as antigenic components. In a 36-week placebo-controlled study involving 30 individuals with multiple system atrophy, neither therapy showed a significant improvement in clinical scores. In Parkinson's disease, PD01A was studied in a 128-week, single-blinded, non-placebo-controlled trial in 24 patients, while PD03A was tested in a 36-week, placebo-controlled study in 36 patients. PD01A led to a 89% serum seroconversion rate and PD03A to a 58% seroconversion rate. Furthermore, there was a 0.3% CSF to serum ratio for PD01A compared to a 0.1% ratio for PD03A. Therefore, it seems that PD01A generated a higher antibody response. In both trials, there was no effect on part III of the MDS-UPDRS, and no effect on imaging for PD03A, and no effect on several CSF biomarkers for PD01A, including total alpha-synuclein. However, a post-hoc analysis revealed a 51 percent reduction in oligomeric alpha-synuclein at the highest tested dose of PD01A, an indication of central target engagement. Currently, a phase 2 trial with ACI-7104.056, an optimized formulation of PD01A, is ongoing. A press-release in November 2024 announced that it was safe and well tolerated and induced anti-alpha-synuclein antibodies, though no pharmacodynamic results were shared.

The UB-312 early phase trial included seed amplification assay to detect misfolded, self-propagating alpha-synuclein aggregates in CSF. This assay, performed directly on CSF, involves adding recombinant alpha-synuclein to the fluid in a reaction mixture and subjecting it to cycles of shaking and resting at body temperatures. The formation of aggregates is then tracked by monitoring thioflavin fluorescence, which increases as misfolded protein seeds trigger further aggregation. In Parkinson's disease patients, UB-312 delayed the seeding kinetics of the alpha-synuclein seed amplification assay compared to placebo, which could be interpreted as evidence for target engagement. No differences were observed on the MDS-UPDRS part III. A post-hoc analysis suggested that patients with detectable alpha-synuclein antibodies in CSF showed greater improvements in activities of daily living (MDS-UPDRS part II), compared to those without antibody detection. Given the small sample size and short trial duration, these findings are considered exploratory. Currently, there are no trials ongoing.

In multiple system atrophy patients, rifampicin and epigallocatechin gallate did not modify disease progression measured as by the Unified Multiple System Atrophy Rating Scale.[33,34] While rifampicin, an alpha-synuclein fibril inhibitor, was considered safe, epigallocatechin gallate, an alpha-synuclein oligomer modulator (400 mg, three times daily) led to liver toxicity in 20 patients, and there were 4 deaths, while in the placebo group there were 5 instances of liver toxicity and there were 2 deaths. An explanation for the high mortality in both groups has not been provided. No trials are currently ongoing.

Despite several efforts, alpha-synuclein remains a challenging therapeutic target, with most investigational therapies showing limited or no clinical efficacy in Parkinson's disease or multiple system atrophy. Buntanetap showed preliminary improvements in motor and cognitive symptoms in early Parkinson's disease patients, especially those with gait issues. Other agents demonstrated peripheral or central pharmacodynamic activity, but these effects did not consistently translate into meaningful clinical outcomes. Notably, cinpanemab and prasinezumab both showed target engagement, yet only prasinezumab yielded exploratory evidence of benefit in patients with faster progressing disease. In multiple system atrophy, none of the therapies evaluated to date, including rifampicin, epigallocatechin gallate, and Lu AF82422 (amlenetug), have demonstrated disease modifying effects. Together, these findings underscore the need for more selective patient stratification and robust biomarker strategies to guide the future development of alpha-synuclein–targeted therapies.

Glucocerebrosidase therapies

The glucocerebrosidase 1 gene (GBA1) encodes lysosomal glucocerebrosidase (GCASE), a pivotal enzyme in the degradation of glucosylceramide (GLUCER) into glucose and ceramide. GBA1 mutations influence GCASE activity and have been strongly associated with the risk of developing Parkinson's disease in genome-wide association studies.[35] Decreased GCASE activity is thought to impair lysosomal function and has been linked to heightened alpha-synuclein aggregation.[36] This aggregation, coupled with impaired lysosomal function, may contribute to neuronal damage and apoptosis, particularly in dopaminergic neurons of the substantia nigra.[36] Consequently, augmenting GCASE levels or replacing dysfunctional GCASE emerges as a potential strategy to modify the progression in Parkinsonian disorders. Several trials are currently ongoing.

The most advanced molecule is ambroxol, an inhibitory chaperone that promotes the trafficking of mutant GCASE from the endoplasmic reticulum to the lysosome by binding to the enzyme's active site, inducing a conformational change that facilitates its transport.[37,38] After demonstrating safety in patients with Gaucher's disease and Parkinson's disease with GBA1 mutations over 19 months, ambroxol was

investigated in a 19-month open-label phase 2 trial involving 24 patients with moderate Parkinson's disease (Hoehn and Yahr stage less than 3).[39,40] Ambroxol led to an increase in CSF GCASE and alpha-synuclein levels compared to baseline, while CSF tau and GLUCER levels remained unchanged. The increased CSF GCASE levels were suggestive of target engagement, and the authors hypothesized that the increased CSF alpha-synuclein may reflect enhanced extracellular export from the brain parenchyma. Clinically, a reduction in total MDS-UPDRS scores was observed, primarily driven by improvements in part III. However, interpretation is limited by the open-label design and the absence of a placebo group. Currently, several trials are ongoing in Parkinson's disease: the GREAT phase 2/3 trial in patients with a GBA1 mutation assessing effects on the MDS-UPDRS part III score over 60 weeks, the ASPro-PD phase 3 trial assessing the change in MDS-UPDRS part I to III over 104 weeks, a phase 1/2 trial assessing the safety and tolerability of high-dose (up to 1.2 gram per day) ambroxol over 52 weeks, the phase 2 AiM-PD trial assessing central nervous system (CNS) and CSF penetration, and two phase 2 trials including the AMBITIOUS trial assessing effects on cognition and dementia over 52 weeks. Also, currently ongoing is the ANeED phase 2 study in patients with prodromal or early dementia with Lewy bodies.

BIA 28-6156, previously known as LTI-291, is an allosteric modulator of GCASE designed to enhance the activity of both wild-type and mutant enzyme variants. Following demonstration of central nervous system penetration and a favorable safety profile in healthy individuals, BIA 28-6156 was evaluated in a 28-day study involving 40 Parkinson's disease patients with GBA1 mutations.[41,42] Treatment resulted in a significant but transient increase in four of five intracellular glucosylceramide isomers within peripheral blood mononuclear cells. No corresponding changes were detected in glucosylceramide levels in plasma or CSF, and there was no observed correlation between intracellular and plasma levels. These findings were unanticipated, as activation of glucocerebrosidase was expected to reduce glucosylceramide levels. The authors proposed that the increase in systemic ceramide and glucosylceramide concentrations may lead to feedback inhibition of de novo synthesis, a process known to be sensitive to ceramide levels.[43–46] Also, the CSF to plasma ratios was below 0.02, which could also explain the lack of effect in CSF. Currently, the phase 2 ACTIVATE trial is ongoing in patients with a GBA1 mutation, assessing efficacy in delaying clinical meaningful motor progression over 78 weeks.

Recombinant GCASE is also being explored as a form of supplementation. Magnetic resonance–guided focused ultrasound (MRGFUS) is an emerging technique that enables transient, localized permeabilization of the blood-brain barrier, allowing for anatomically targeted delivery of therapeutics. In a proof-of-concept, open-label phase 1 study, recombinant GCASE was administered to four Parkinson's disease patients using MRGFUS in combination with microbubbles.[47] The procedure was considered safe and well tolerated; however, two of the four patients experienced transient worsening or emergence of dyskinesia. No group-level efficacy analysis was performed. A phase 1/2 study is currently ongoing in Parkinson's disease, assessing safety as well as the feasibility of the procedure over 12 months.

Therapies targeting GCASE offer a promising disease modifying approach for Parkinson's disease, particularly in patients carrying GBA1 mutations. These strategies aim to restore lysosomal function and reduce alpha-synuclein accumulation, a key pathological hallmark of the disease. Ambroxol, the most advanced candidate, has shown target engagement and clinical improvement in early-phase trials, although placebo-controlled data are pending. BIA 28-6156 revealed unexpected metabolic effects together with questionable CNS penetration, highlighting the complexity of modulating GCASE activity in the brain. The use of MRGFUS to deliver recombinant GCASE represents an innovative and feasible approach, though safety and efficacy remain under investigation. While all therapies have demonstrated initial safety and tolerability, robust efficacy data from ongoing randomized, controlled trials will be critical to determine their potential to alter disease progression.

Glucagon-Like Peptide-1 receptor therapies

Glucagon-like peptide 1 receptor (GLP-1R) agonists have recently emerged as a potential disease-modifying therapy for Parkinsonian disorders. These therapies, originally developed for diabetes management, have exhibited neuroprotective properties in preclinical studies.[48,49] The potential neuroprotective effect of GLP-1R activation is attributed to its ability to protect against cytokine-mediated apoptosis and to stimulate neurogenesis, providing a promising avenue for targeted intervention in the underlying neurodegenerative processes.[48,49] Currently, exenatide and similar molecules are under investigation.

Exenatide is a synthetic analogue of exendin-4, a naturally occurring analogue of human GLP-1. After demonstrating safety in two phase 1 trials and showing a statistically significant effect on the MDS-UPDRS part III off-score at 60 weeks as well as enhancing pathways related to brain insulin in a phase 2 study, exenatide was further evaluated in a 96-week phase 3 trial in 194 Parkinson's disease patients.[50–54] In contrast to the earlier findings, the phase 3 trial did not demonstrate any benefit of exenatide over placebo on the MDS-UPDRS part III off-score or on any secondary outcome measures, including MDS-UPDRS subset scores and striatal binding ratios. No differential effects were observed in subgroup analyses based on age or BMI. The phase 2 and 3 study populations were demographically comparable, and plasma and CNS exenatide concentrations were consistent across studies (11.3 pg/mL and 11.7 pg/mL, respectively).

Although the phase 3 findings did not support a disease modifying effect of exenatide, the authors plan post-hoc analyses to explore potential explanations. These analyses will also investigate whether subgroups defined by biochemical profiles, such as mildly elevated glycated haemoglobin A1c levels, show distinct clinical, target engagement, or biochemical responses to exenatide. There are currently no trials ongoing.

Lixisenatide, a modified version of exenatide with higher affinity for GLP-1R, showed a statistically significant improvement in MDS-UPDRS part III scores in the ON-state in a 12-month phase 2 trial involving 126 Parkinson's disease patients.[55] In contrast, NLY01, a brain-penetrant pegylated analogue, did not demonstrate improvement in MDS-UPDRS part III scores in the OFF-state in a 36-week phase 2 trial including 255 Parkinson's disease patients.[56] Currently there are no trials ongoing.

Kinase inhibitor therapies

Kinase inhibitors show promise as disease-modifying therapy by targeting dysregulated signaling pathways linked to neurodegeneration. By selectively inhibiting specific kinases associated with oxidative stress, neuroinflammation, and mitochondrial dysfunction, these inhibitors may attenuate molecular signaling pathways leading to neuronal degeneration.[57–59] Additionally, their ability to modulate cellular processes, including autophagy and protein aggregation, enhances their potential as disease-modifying therapies.[57–59] Through this targeted approach, kinase inhibitors offer a novel avenue for developing therapeutic strategies to interrupt the neurodegenerative trajectory of Parkinsonian disorders.

Nilotinib is a multikinase inhibitor that selectively targets Abelson and discoidin domain receptors. After demonstrating safety in an open-label phase 1 study involving Parkinson's disease and Lewy body dementia patients, nilotinib was further investigated in two randomized, placebo-controlled phase 2 trials, each with approximately 75 patients and a duration of 6 to 12 months.[60–64] Both trials reported acceptable safety profiles, although the study by Pagan et al. showed a significant increase in severe adverse events, including falls, hip fractures, urinary tract infections, and orthostatic hypotension, which were attributed to Parkinson's disease-related factors, while the study by Simuni et al. did not observe this. Exploratory biomarkers in the study by Pagan et al. suggested treatment-related changes in CSF levels of Triggering Receptor Expressed on Myeloid Cells 2 (TREM2), a receptor involved in microglial activation, as well as homovanillic acid and 3,4-dihydroxyphenylacetic acid. However, these effects were observed using 90 percent confidence intervals and did not exhibit a dose-dependent pattern. Simuni et al.'s study did not replicate these findings, and neither study found any effect on (MDS-)UPDRS scores. A 2022 meta-analysis of both trials reported favorable safety and tolerability across various doses of nilotinib, with a slight improvement in some CSF biomarker levels at 300 mg but concluded that nilotinib showed no advantage in improving motor outcomes.[65] Currently, there are no trials ongoing.

CEP-1347, an inhibitor of mixed lineage kinases, was evaluated in a 24-month phase 2 trial including 806 Parkinson's disease patients not yet requiring dopaminergic therapy, following demonstration of safety in a phase 1 study.[66,67] A preplanned interim analysis at 21 months showed futility, with 57% of patients in the placebo group and 59% to 65% of patients receiving 10 to 50 mg CEP-1347 reaching the primary endpoint of disability requiring dopaminergic therapy, and no effect observed on UPDRS scores or striatal dopamine transporter uptake. Despite earlier positive findings in in vitro and in vivo MPTP-induced models in rodents and nonhuman primates, the authors attribute the lack of efficacy to limited relevance of mixed lineage kinase pathways in human Parkinson's disease or compensatory activation of alternative cell-death mechanisms. There are currently no trials ongoing.

Tideglusib, a glycogen synthase kinase-3 inhibitor targeting tau pathology in progressive supranuclear palsy, was safe in phase 1 but did not improve disease severity or other clinical outcomes in a 52-week phase 2 trial involving 146 patients.[68–70] Notably, an MRI substudy revealed significantly slower progression of brain atrophy in the treatment group, despite the absence of clinical benefit. According to the authors this effect is unlikely to be a false-positive due to selection bias, as MRI findings were consistent across brain regions and baseline characteristics were balanced. The authors suggest that MRI may have been more sensitive than clinical scales in detecting treatment effects. Currently, there are no trials ongoing.

Four investigational therapies have been evaluated in phase 1 trials. DNL201 (also known as BIIB122) a small-molecule LRRK2 inhibitor, and terazosin, a phosphoglycerate kinase 1 stimulator, were both safe and well tolerated, and both demonstrated proof of target engagement.[71,72] However, both studies were limited by short durations (28 days for DNL201 and 12 weeks for terazosin) and small sample sizes (28 and 13 patients, respectively). The LRRK2-PD phase 2 trial is ongoing in patients with a LRRK2 mutation to assess the safety of BIIB122 over 12 weeks. For terazosin, a phase 2 trial is ongoing assessing the gait of Parkinson's disease patients, as well as the TZ-DLB phase 2 trial assessing safety and brain adenosine triphosphate levels in Lewy body dementia patients. Interestingly, a phase 3 trial with BIIB122 was discontinued due to the trial's long timeline and complexity, according to a press release. Risvodetinib and vodobatinib are selective inhibitors of activated non-receptor Abelson tyrosine kinases. While phase 1 trials indicated a favorable safety profile for both investigational therapies, press releases reported that neither demonstrated clinical efficacy, and there are currently no ongoing trials.[73,74]

Kinase inhibitors have shown potential as disease-modifying therapies for Parkinson's disease by targeting pathways linked to neurodegeneration, such as oxidative stress, neuroinflammation, and mitochondrial dysfunction. To date, no kinase inhibitors have managed to meet the primary endpoints in phase 2 or phase 3 trial.

Neurotrophic factor related therapies

Neurotrophic factor therapy holds promise as a disease-modifying therapy for Parkinsonian disorders by promoting the survival and function of dopaminergic neurons in the brain. By delivering neurotrophic factors directly to affected brain regions, these therapies aim to counteract the progressive degeneration of dopaminergic neurons. Additionally, neurotrophic factor therapy may stimulate neuronal growth and repair, offering potential long-term benefits in slowing or halting the neurodegenerative process.

Isradipine, a dihydropyridine calcium-channel blocker approved for hypertension, has been investigated for Parkinson's disease by inhibiting plasma membrane CAV-1 L-type calcium channels. After demonstrating safety in a phase 1 trial and potential disease modifying effects in a phase 2 trial, isradipine did not affect Parkinson's disease progression over a 36-month period in a phase 3 trial with 336 patients, nor did it impact any secondary outcomes.[75–79] The authors suggest that the selected dose of 5 mg twice daily might have been too low, as it was based on tolerability data of the phase 2 study and not on pharmacodynamic target or pathway engagement data. Additionally, the absence of a method to directly measure calcium-channel engagement in humans raises the possibility of lower brain bioavailability than observed in preclinical models. No ongoing trials are currently ongoing.

Glial-cell-derived neurotrophic factor (GDNF) and its structural and functional analogue, neurturin, have been investigated as investigational therapies for Parkinson's disease. Both GDNF and neurturin are delivered using an adeno-associated virus type-2 (AAV2) vector, which has been genetically engineered to express and secrete the human gene for these neurotrophic factors. In all trials, AAV2-GDNF or neurturin (also known as CERE-120) were delivered either by surgery, MRI-guided infusion or direct injection.[80–87] In the phase 1 trials, both therapies were considered safe and well tolerated, although their effects on disease progression were inconsistent. In subsequent phase 2 trials lasting between 6 and 24 months with approximately 50 patients per trial, neither GDNF nor neurturin showed any impact on UPDRS part III scores. Currently, the REGENERATE-PD phase 2 trial in moderate stage Parkinson's disease is ongoing, assessing the effect of AAV2-GDNF on normalized on and off time over 18 months.

Melatonin has been proposed as a potential disease-modifying therapy in Parkinson's disease due to its antioxidant properties and its ability to counteract alpha-synuclein-induced cytotoxicity.[88–90] In a 12-week placebo-controlled phase 2 trial including 51 patients, melatonin significantly reduced high-sensitivity C-reactive protein levels by 0.94 mg/L compared to placebo, although the absolute difference at 12 weeks (4.3 mg/L for placebo vs. 3.5 mg/L for melatonin) suggests limited clinical relevance.[91] Among secondary outcomes, only the UPDRS part I score, covering nonmotor symptoms such as sleep disturbances, depression, and anxiety, improved significantly, as did sleep quality and mood-related scores; however, no effects were observed on UPDRS total or part III scores. Melatonin also led to statistically significant reductions in biomarkers associated with its antioxidant activity, including total antioxidant capacity and growth-stimulating hormone. A 2023 meta-analysis pooled data from five clinical trials investigating melatonin in Parkinson's disease, including the previously mentioned study and four trials focused on sleep disturbances.[92] The analysis found that melatonin significantly slowed Parkinson's disease progression when administered at doses of 10 mg/day or more for at least 12 weeks and reported beneficial effects on both motor symptoms and sleep. However, as sleep quality itself can influence motor function, these effects may be interrelated. The so-called "sleep benefit" (an improvement in motor function upon waking) occurs in over 40% of Parkinson's disease patients and is thought to result from enhanced dopaminergic function due to increased dopamine storage during sleep.[93] Currently, there are no trials ongoing.

CNM-Au8 is a catalytic nanotherapeutic that promotes the oxidation of nicotinamide adenine. thereby increasing intracellular levels of oxidized nicotinamide adenine and adenosine triphosphate. In the 12-week non-placebo-controlled phase 2 REPAIR-PD and REPAIR-MS trials including 13 Parkinson's disease and 11 multiple sclerosis patients, CNM-Au8 increased the brain nicotinamide adenine oxidation ratio by 10.4% from baseline as measured by 7 Tesla 31 phosphorous magnetic resonance spectroscopy in a pooled analysis, although this effect was not statistically significant at the group level.[94] Notably, the nicotinamide adenine oxidation ratio returned to pre-treatment levels after cessation, supporting a treatment-related effect. Despite limitations including small sample size, short duration, and absence of a placebo group, the results were deemed positive for further development in the phase 2 VISIONARY-MS trial in 150 multiple sclerosis patients. A 2024 press release stated that CNM-Au8 improved both visual acuity and performance on the symbol digit modality test, a marker of cognition, over 144 weeks compared to placebo. Currently, no trials are ongoing.

Preclinical studies have demonstrated neuroprotective effects of nicotinamide riboside in Parkinson's disease.[95] In a 30-day phase 1 trial including 30 patients, oral administration of nicotinamide riboside was safe and well tolerated, increased cerebral nicotinamide adenine levels, and upregulated transcriptional pathways related to mitochondrial, lysosomal, and proteasomal function in blood and skeletal muscle.[96] Additionally, nicotinamide riboside reduced inflammatory cytokine levels in both serum and CSF. Despite these promising findings, the study was limited by its short duration and small sample size, and no trials are currently ongoing.

Recombinant human erythropoietin, commonly used to treat anemia in various conditions, has shown potential neuroprotective effects in Parkinson's disease.[97] Two small, five-week, open-label proof-of-concept studies demonstrated that subcutaneous and intravenous administration was safe and well tolerated, with mixed effects on motor and non-motor symptoms. The smaller study showed statistically significant improvements in total UPDRS and motor scores (off-state) in 10 patients, while the larger study only reported improvements in non-motor scores in 26 patients.[98,99] A subsequent randomized, placebo-controlled phase 1 trial confirmed safety and tolerability but did not assess disease modification.[100] Given the limitations in sample size, duration, and lack of placebo in earlier studies, further trials are needed to confirm potential neuroprotective effects. Currently, no trials are ongoing.
Fas associated factor 1 (FAF1), a protein linked to Fas-mediated apoptosis, is upregulated in Parkinson's disease brains, and reducing its expression in animal models inhibits cell death, making it a promising therapeutic target.[101–103] KM-819, an oral inhibitor of FAF1, was safe and tolerable as single dose in healthy participants in a phase 1 clinical trial.[104] Currently a phase 2 trial assessing the safety, tolerability and effect on activities of daily living of multiple doses in healthy elderly and Parkinson's disease patients is ongoing. A 2023 press release stated that multiple doses were safe and tolerated in healthy elderly.

For progressive supranuclear palsy, davunetide was investigated for its ability to ameliorate deposition of hyperphosphorylated, insoluble forms of tau. After demonstrating safety in a 12-week phase 2 trial in 144 mild cognitive impairment patients, davunetide was investigated in a 52-week phase 3 trial with 213 patients with progressive supranuclear palsy.[105,106] Davunetide did not have an effect on the primary objective, namely disability severity measured by two rating scales, or any other secondary endpoint. The authors were unsure if sufficient concentrations entered the CNS to exert an effect due to the lack of pharmacokinetic data in this trial and the lack of CNS biomarkers for davunetide. There are currently no trials ongoing.

Neurotrophic factor therapy represents a promising avenue for disease modification in Parkinsonian disorders by supporting dopaminergic neuron survival and function. Melatonin showed modest effects on non-motor symptoms and inflammatory markers, with meta-analyses suggesting benefits at higher doses, with results possibly mediated by sleep-related improvements. Nicotinamide riboside and erythropoietin showed neuroprotective signals in early trials, but larger studies are needed. KM-819, a FAF1 inhibitor, is currently under investigation in a phase 2 trial. Davunetide failed to show benefit in progressive supranuclear palsy, possibly due to poor CNS penetration. While several therapies have demonstrated safety and biological activity, robust evidence of disease modification remains lacking, underscoring the need for further controlled trials.

Glutathione therapies

Oxidative stress is implicated in Parkinson's disease, contributing to dopaminergic neuron degeneration. Insufficient production of the antioxidant glutathione (GSH) is an early event in Parkinson's disease progression. Postmortem analysis of substantia nigra tissue from Parkinson's disease patients revealed that GSH is depleted early.[107] There is also a correlation between whole blood glutathione concentrations and clinical severity of Parkinson's disease.[108] Therefore, GSH supplementation could be a potential treatment strategy in Parkinsonian disorders.
GSH was found to be safe and well tolerated across four early-phase studies using intranasal, intravenous, or oral supplementation in Parkinson's disease patients (approximately 10 to 30 patients, with a duration of 4 weeks to 3 months).[109–112] Intranasal GSH was further evaluated in a 3-month phase 2 trial with 45 patients, showing no significant effect on UPDRS total or motor subscores compared to placebo.[113] However, the highest dose (600 mg/day) showed improvement from baseline, possibly due to increased CNS GSH concentrations observed only at this dose. The authors suggest this could reflect that intranasal administration only increases CNS GSH levels by a limited margin. However, magnetic resonance spectroscopy data showed evidence that intranasal administration of glutathione resulted in a rapid and significant increase in brain GSH levels without a corresponding rise in blood levels, suggesting direct uptake through nose-to-brain pathways.[114] These findings support the intranasal route as a noninvasive method for targeted delivery to the central nervous system. Interestingly, whole blood GSH concentrations declined across all groups, which the authors attributed to general study participation. No ongoing trials are currently ongoing.

GM1 ganglioside therapies

GM1-ganglioside (GM1), an endogenous neural membrane molecule prevalent in the brain and nervous system, is being explored as a potential disease-modifying therapy

for Parkinson's disease. GM1 prevents dopaminergic cell damage in Parkinson's disease by stabilizing neurotrophic factor receptors, maintaining alpha-synuclein in a non-aggregating form, and promoting autophagy.[115] Additionally, GM1 is implicated in maintaining mitochondrial health, supporting cellular energy production, and reducing oxidative stress associated with Parkinson's disease pathology.[115] Therefore, GM1 could be a promising therapeutic agent for Parkinson's disease.

GM1 was investigated in multiple trials led by Jay S. Schneider between 1995 and 2015. After demonstrating safety and mixed efficacy results from phase 1 trials and a 5-year open-label extension, GM1 was assessed in a 120-week phase 2 trial using an early versus delayed start design in 77 Parkinson's disease patients.[116–120] During the first 24 weeks, GM1 significantly improved UPDRS motor scores in both on and off states compared to placebo, possibly due to functional enhancement of residual dopaminergic neurons. After the delayed start group started with GM1 at week 24, the early start group continued to show sustained benefit, with motor scores remaining below baseline by treatment end. In contrast, the delayed group experienced only symptomatic improvements, though both groups differed significantly from placebo. During the washout period, symptoms worsened in all participants. An additional analysis indicated a slower decline in striatal dopamine transporter binding in GM1-treated patients. These findings suggest that long-term GM1 treatment may slow disease progression, although the study's small sample size is a limitation, and symptoms worsened in all participants during washout, which could suggest a more symptomatic effect. Currently, a phase 1 trial is ongoing in which the safety is investigated of talineuren, a combination of GM1 and a proprietary lipid formulation assembled as liposomes.

Stem cell therapies

Stem cell therapy could slow Parkinsonian disorder progression by replacing damaged neurons, particularly in the substantia nigra where dopamine-producing cells are lost. It is thought that these stem cells differentiate into mature neurons, replenishing depleted dopamine levels crucial for motor function, or may offer neuroprotection by modulating inflammation and releasing trophic factors supporting existing neurons. This multifaceted approach could foster regeneration and provide a supportive environment, offering a potential avenue for disease modification and improved patient outcomes in Parkinsonian disorders.

Stem cell therapy has been explored in three small-scale (all less than 20 patients), open-label phase 1 trials in Parkinson's disease, involving transplants of peripheral nerve grafts, human embryonic stem cells, and induced pluripotent stem cell-derived dopaminergic progenitors.[121–123] All studies demonstrated safety and tolerability, though results on motor and non-motor symptoms were mixed. Due to limitations in sample size, study duration, and absence of placebo controls, further research is needed to confirm potential neuroprotective effects. Currently, no trials are ongoing.

Allogeneic and mesenchymal stem cells have also been studied following peripheral administration in two small-scale (approximately 20 patients), open-label trials.[124,125] Both approaches were safe and well tolerated and appeared to improve motor and non-motor symptoms in Parkinson's disease. As with previous studies, these trials were limited by small sample sizes, short duration, and lack of placebo control, underscoring the need for further research to confirm potential neuroprotective effects. Currently for Parkinson's disease only, two phase 2 trials are ongoing assessing the effect of mesenchymal stem cells on MDS-UPDRS scores over 52 to 78 weeks, and three phase 1 trials assessing the safety and efficacy of nasally administered mesenchymal stem cells over 1 to 4 years.

Anti-inflammatory therapies

Anti-inflammatory therapy may function as a disease-modifying therapy for Parkinsonian disorders by targeting neuroinflammation, a key component of its pathogenesis. By suppressing inflammatory responses in the brain, anti-inflammatory agents could potentially halt or slow down the progression of dopaminergic neuron degeneration.[126,127] Additionally, reducing neuroinflammation may mitigate oxidative stress and protein aggregation, further attenuating neurodegeneration.[126,127]

Sargramostim enhances regulatory T-cell function to reduce neuroinflammation and protect dopaminergic neurons.[128,129] In an open-label 12 month trial with a 30 month extension in five Parkinson's disease patients, sargramostim was safe and well tolerated, increased regulatory T cell counts, and had no effect on UPDRS part II and part III scores.[130] A placebo controlled 56 day phase 1 trial confirmed these findings in 17 patients.[131] No biomarkers representing central nervous system effects of the therapy were measured. Given the small sample size and short duration, the absence of UPDRS changes was anticipated; larger and longer studies are needed to assess effects on disease progression. Two larger phase 1 trials in Parkinson's disease on safety and tolerability are currently completed, without public results available.

Intravenous immunoglobulin was investigated for multiple system atrophy in a 6-month open-label phase 1 trial involving 9 patients.[132] Treatment was safe and well tolerated and was associated with reduced disease severity on the unified multiple system atrophy rating scale, compared to an external control group. C-reactive protein levels, considered a marker of systemic inflammation in this trial, did not change significantly, likely due to two participants experiencing viral infections at the final visit. Excluding these cases, average C-reactive protein levels decreased compared to baseline. Although limited by sample size, duration, and the lack of placebo,

intravenous immunoglobulin decreased systemic inflammation and reduced disease severity, and therefore the results may warrant further investigation. No trials are currently ongoing.

Vinpocetine, a semisynthetic derivative of vincamine, has demonstrated anti-inflammatory and neuroprotective effects in vitro by inhibiting NF-kappaB activation and cytokine production.[133] In a 14-day placebo-controlled phase 1 trial with 89 Parkinson's disease patients, vinpocetine reduced inflammatory markers including IL-8 and IL-10 and improved cognitive function on the mini mental state examination.[134] Although the improved cognitive function is likely more a symptomatic effect due to the short study duration, this could be a signal of target engagement. No trials are currently ongoing.

Repurposed drugs

Several drugs have been investigated as disease-modifying therapy for Parkinsonian disorders. The majority of the drugs have failed to demonstrate either an effect on disease progression in phase 2 or were only investigated in phase 1 trials.[135–145] Below, we have highlighted a few interesting trials.

There is mixed evidence that rheumatoid arthritis has been associated with a higher risk of Parkinson's disease, and the use of a disease-modifying therapies for rheumatoid arthritis may explain this association.[146–149] Minocycline acts as an anti-inflammatory independent of its antimicrobial activity, while creatine supports mitochondrial function by stabilizing cellular energy through phosphocreatine-mediated regeneration of adenosine triphosphate.[150,151] After demonstrating non-futility in a 12-month futility phase 2 trial with 207 Parkinson's disease patients in a combined trial, minocycline and creatine were subsequently evaluated for disease modifying effects in separate phase 3 trials.[152–154] The 5-year NET-PD phase 3 trial of creatine in 955 Parkinson's disease patients was terminated early for futility based on results of a planned interim analysis of measures of function, activities of daily living, ambulation, cognition, and quality of life. The 1-year MEMSA phase 3 trial of minocycline in 63 multiple system atrophy did not show an effect on motor function or progression rate. No phase 3 trial of minocycline was conducted in Parkinson's disease, and no trials are currently ongoing.

Several population studies have investigated the effect of non-steroidal anti-inflammatory drugs (NSAID), with mixed results reported. Some showed reduced Parkinson's disease incidence with non-aspirin NSAIDs, while others found no effect or even an increased dementia risk. Cognition was not excessively impaired in patients with Lewy body dementia.[155–158] Preclinical data showed that celecoxib, a selective cyclooxygenase-2 inhibitor, could attenuate Parkinson's disease symptoms and inflammation.[159,160] In a 6-month phase 2 trial with 60 Parkinson's disease patients, the UPDRS total score was statistically significantly lower in celecoxib group compared to placebo, as well as serum biomarkers related to the oxidative stress and neuroinflammation.[161] More specifically, the authors argue that celecoxib may offer neuroprotection in Parkinson's disease by inhibiting cyclooxygenase-2 inhibitor mediated inflammation, reducing toxic alpha-synuclein aggregation, increasing brain-derived neurotrophic factor expression to support neuronal survival, and enhancing antioxidant responses through nuclear factor erythroid 2–related factor 2 activation. No further trials are currently ongoing.

Thiazolidinedione has been approved for type II diabetes, but could potentially act as disease-modifying therapy for Parkinson's disease. By activating the peroxisome proliferator-activated receptor ɣ, pioglitazone might provide neuroprotection by inhibiting microglia and astrocyte activation and inhibiting the production of pro-inflammatory cytokines and nitric oxide.[162] Two population studies in diabetic cohorts found mixed evidence linking glitazone use to reduced Parkinson's disease incidence.[163,164] Pioglitazone was evaluated in a 44-week phase 2 trial involving 210 Parkinson's disease patients but showed no effect on total UPDRS scores or peripheral biomarkers including proliferator–activated receptor ɣ coactivator-1α, a downstream target of engagement.[165,166] The authors attribute the lack of efficacy due to the relatively short study duration or the possibility that peripheral biomarkers do not accurately reflect CNS drug levels. There are currently no trials ongoing.

Depression affects approximately 40 to 50 percent of Parkinson's disease patients and worsens disability and quality of life. Some antidepressants may have disease modifying potential, with preclinical studies showed that paroxetine and fluoxetine protected against dopaminergic neuron loss, and a population study linked tricyclic antidepressants, especially amitriptyline, to delayed dopaminergic treatment, without changes in UPDRS scores.[167,168] Lithium was evaluated in a 24-week open-label phase 1 trial in 19 patients.[169] It was safe and well tolerated, with the medium dose 45 milligram showing the strongest effect on blood and MRI markers of disease progression. Non-dose-dependent findings were likely due to formulation and individual variability. No trials are currently ongoing

Memantine, a N-methyl D-aspartate receptor antagonist approved for Alzheimer's disease, modulates glutamatergic signaling and protects against glutamate-induced toxicity.[170] It was studied in a 24-week phase 2 trial with open-label extension and a 36-month phase 2 trial, each involving approximately 72 patients with Parkinson's disease dementia or Lewy body dementia. In the shorter trial, 27 percent of memantine-treated patients improved on the clinical global impression scale versus none on placebo, though 57 percent experienced symptom recurrence after washout compared

to 25 percent on placebo. According to the authors of the trial, this deterioration upon drug withdrawal was a drug effect that is not yet evident. In the longer trial, memantine significantly improved survival, particularly in patients who initially responded to treatment, while UPDRS scores remained unchanged. The authors hypothesize that symptomatic improvement may have led to better physical health and lower mortality, such as fewer infections, thromboses, or falls, or that responder status reflects a generally better prognosis. However, the absence of similar effects in the placebo group argues against these interpretations. A phase 3 trial in 50 Parkinson's disease patients has been completed, focusing on cognitive outcomes and alpha-synuclein transmission measured by MRI, but results are not yet available.

Riluzole, an N-methyl D-aspartate receptor antagonist approved for amyotrophic lateral sclerosis, was evaluated in phase 1 trials involving 20 Parkinson's disease patients over 6 months and 10 multiple system atrophy patients over 4 weeks.[171,172] Both studies demonstrated safety and tolerability, but no differences in UPDRS scores were observed compared to placebo. Given the small sample sizes, short durations, and exploratory design, larger trials are needed to determine any potential neuroprotective effects. No trials are currently ongoing.

In conclusion, while population studies suggest that NSAIDs, rheumatoid arthritis treatments, and antidiabetic drugs may lower the risk of developing Parkinson's disease, clinical trials have not confirmed these effects. Notably, memantine and riluzole, both approved for other neurodegenerative conditions, have shown more encouraging findings, though definitive evidence from clinical trials is still awaited. This may reflect a closer alignment of the underlying disease mechanisms in Parkinsonian disorders with those of Alzheimer's disease and amyotrophic lateral sclerosis, rather than with pain, rheumatoid arthritis, or diabetes.

Conclusion

In recent years, there has been a surge in the development of disease-modifying therapies for Parkinson's disease, with between 55 and 60 new candidates yearly in the years 2020 to 2024.[4,5,173] However, a similar surge is not seen for the other Parkinsonian disorders. Despite this influx of new investigational treatments for Parkinson's disease, success in clinical trials has been elusive and no disease-modifying therapy has been registered up to this date. Several therapies have reached phase 3 clinical development for Parkinson's disease, including buntanetap (an anti-alpha-synuclein therapy), ambroxol (a GCASE enhancer), exenatide (a GLP1 receptor agonist), prasinezumab , and cinpanemab (both anti-alpha-synuclein monoclonal antibodies). Of these, buntanetap showed improvements in motor function during phase 2 studies. However, prasinezumab and cinpanemab did not meet their primary endpoints in phase 2 and are no longer in active development. No candidates have been progressed for the other disorders. The diversity in the mode of action of these disease-modifying therapies offers promising avenues, often substantiated by target engagement evidenced through biomarkers. However, translating these promising preliminary results into tangible improvements in motor function remains a challenge for most new disease-modifying therapies.

Most aforementioned clinical trials incorporate the MDS-UPDRS as a clinical marker for disease modification in Parkinson's disease, encompassing both biomarker and treatment effect aspects. However, the MDS-UPDRS predominantly reflects dopaminergic system influence, with motor symptoms (parts II and III) and motor complications (part IV) receiving greater emphasis than non-motor symptoms (part I).[174] The annual change in UPDRS ranges between 6 to 12 points initially, with a general estimate of 4.7 points per year.[175,176] A mean change of +2 points in the MDS-UPDRS part III score after one year may signify clinically significant disease progression slowing, based on a sample size of 142 with 90% power.[176] Notably, many described disease-modifying therapies do not directly target the dopaminergic system or MDS-UPDRS score. Nevertheless, all disease-modifying therapies share the objective of slowing Parkinson's disease progression, where the MDS-UPDRS serves as a pertinent clinical biomarker, providing comprehensive clinical status information.

Therefore, the manuscript authors would like to stress that blood or CSF-based biomarker-driven research is pivotal in advancing drug development by enabling precise diagnostics, prognostics, and therapeutic interventions.[177] Blood or CSF biomarkers facilitate the identification of disease subtypes, allowing for tailored treatment plans that enhance efficacy and minimize adverse effects. This approach improves patient outcomes and optimizes resources by avoiding one-size-fits-all treatments. Moreover, biomarkers can accelerate drug development by serving as surrogate endpoints in clinical trials, thus reducing time and costs. Their role in early disease detection and monitoring response to treatment underscores their value in clinical practice.

Looking ahead, the landscape of disease-modifying therapies for Parkinson's disease appears promising. We anticipate that more therapeutic candidates will progress to phase 3 clinical trials, driven by advances in biomarker research and a deeper understanding of the disease's pathophysiology. This progression reflects a growing optimism within the scientific community that the first disease modifying treatments will soon be registered. Such breakthroughs would mark a significant milestone in Parkinson's care, offering hope for improved patient outcomes and potentially altering the disease's natural course.

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CHAPTER 3

FATTY ACIDS AS POTENTIAL BIOMARKERS OF STEAROYL-COA DESATURASE INHIBITION: VARIATION IN HEALTHY PARTICIPANTS AND PARKINSON'S DISEASE PATIENTS

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ABSTRACT

This study aimed to assess the naturally occurring variation in plasma fatty acids in healthy participants and participants with Parkinson's disease.

Alpha-synuclein plays a major role in Parkinson's disease. Inhibition of stearoyl-CoA desaturase (SCD) reduces levels of mono-unsaturated C16 and C18 fatty acids, which are involved in alpha-synuclein toxicity in vitro and in vivo. The ratio of mono-unsaturated to saturated fatty acids (fatty acid desaturase index (FA-DI)) in plasma after SCD-inhibition correlates with effects on brain FA-DI. However, the FA-DI normal values and the inter- and intra-day variation in participants with Parkinson's disease and healthy participants is unknown.

Ten participants with Parkinson's disease (54 –73 years) and ten age-matched healthy participants were included. On three consecutive days, fatty acids fractions and concentrations were measured throughout the day. Outcomes are expressed as estimated mean, and the coefficient of variation (CV%) in percentage.

For C16 FA-DI, the inter-subject CV% was 20.7% in healthy participants, and 37.7% in participants with Parkinson's disease. The intra-subject CV% over days was 14.0% in healthy participants, and 14.2% in participants with Parkinson's disease, and within days 5.1% in healthy participants, and 5.9% in participants with Parkinson's disease. For C18 FA-DI, the inter-subject CV% was 14.8% in healthy participants, and 16.0% in participants with Parkinson's disease. The intra-subject CV% over days was 11.0% in healthy participants, and 8.6% in participants with Parkinson's disease, and within days 8.4% in healthy participants, and 6.7% in participants with Parkinson's disease.

The observed extent of variability in healthy participants and participants with Parkinson's disease support C16 and C18 FA-DI as suitable biomarkers to demonstrate target engagement in plasma, of for example SCD-inhibitors, in both healthy participants and participants with Parkinson's disease.

INTRODUCTION

Abnormally aggregated alpha-synuclein is a principal component of Lewy bodies, a pathological hallmark of Parkinson's disease. Stearoyl-CoA desaturase (SCD) is an endoplasmic reticulum enzyme that catalyses the biosynthesis of monounsaturated fatty acids from saturated fatty acids that are either synthesized de novo or derived from the diet.[1] Humans express two δ-9 desaturase genes designated SCD-1 and SCD-5, with the former exhibiting nearly ubiquitous expression and the latter being restricted primarily to the brain and pancreas.[1]

It has been demonstrated in multiple in vitro[2,3] and in vivo[4,5] studies that inhibitors of the enzyme SCD decreases cellular levels of monounsaturated fatty acids, which decreases toxicity associated with accumulation of alpha-synuclein, and improves cell survival. The precise mechanism of action has not yet been entirely defined.[6] Possibly, a toxic increase in fatty acid desaturation could be directly reversed by SCD inhibition. Alternatively, reduced fatty acid desaturation could directly antagonize toxic effects of alpha-synuclein on membrane properties or trafficking. Lastly, reduced fatty acid desaturation could ameliorate a direct toxic interaction of alpha-synuclein with membranes.[6]

The extent of SCD inhibition in plasma can be quantified using the fatty acid desaturase index (FA-DI), which is the ratio of unsaturated to saturated fatty acids,[7] and has been demonstrated for SCD1 in the liver.[8] For C16 FA-DI, the ratio consists of fatty acids C16:0 (saturated) and C16:1n7 (unsaturated), and for C18 FA-DI, the ratio consists of C18:0 (saturated) and C18:1n9 (unsaturated). Preclinically, changes in plasma C16 and C18 FA-DI after SCD inhibition correlate strongly with changes in brain FA-DI, such that it can be hypothesized that the plasma FA-DI is a surrogate biomarker for drug-induced changes in brain FA-DI.[5] It should be noted that could only apply to the specific dosing route used; alternative routes may result in less plasma–brain concordance. Strong SCD inhibition may also affect levels of fatty acid profiles in the tissues where SCD is expressed, as well as in plasma. It is hypothesized that changes in the levels of these secondary fatty acids may play a role in the adverse effects associated with constitutive SCD inhibition, notably dryness in the skin and eyes.[7] However, for FA-DI, the inter- and intra-subject variability in both healthy participants and participants with Parkinson's disease are currently unknown. To evaluate whether FA-DI could indeed be relevant markers of target engagement, a first step is to investigate the intra-subject variability in FA-DI levels in plasma over the course of a day and between days within each subject among both populations.

It has been demonstrated that food increases the variability of C18 FA-DI in healthy participants compared to fasted state.[9] In Parkinson's disease, it was shown

that patients with a heterozygous glucocerebrosidase mutation had lower levels of specific fatty acids, such as C16:0, and lower levels of total fatty acids, compared to both idiopathic participants with Parkinson's disease and control subjects.[10] Determining the variability of these fatty acids is essential to evaluate the target engagement of drugs aimed at influencing FA-DI and SCD, and thereby potentially attenuating alpha-synuclein toxicity and altering disease progression rate of Parkinson's disease.

The objective of this study was to evaluate the variability in plasma fatty acid concentrations, focusing on C16 FA-DI and C18 FA-DI and their associated fatty acids in healthy participants and participants with Parkinson's disease, in order to assess whether these may serve as markers of target engagement of the effects of drugs targeting these processes in healthy participants and participants with Parkinson's disease.

MATERIAL AND METHODS

Study Population

The study was performed in two gender and aged matched groups. The first group consisted of healthy participants aged between 35 and 80 years. The second group consisted of participants with Parkinson's disease aged 40 to 75 years and with a Hoehn and Yahr grade of 1-4. Each of the groups consisted of 10 participants and the participants from both groups were age-matched with a range of ± five years. As this is an exploratory study, no sample size calculation was performed. For a phase 0 biomarker study, 10 participants per group is commonly accepted.

The main exclusion criteria were clinically significant laboratory or physical examination abnormalities during screening, any clinically significant medical condition, except the presence of Parkinson's disease-related abnormalities for the patients or being on a diet composed of relevantly altered amounts of fat, protein, or carbohydrates, that may affect triglycerides and fatty acids levels. Participants were recruited from a patient database as well as via advertisements between 18-Nov-2019 and 27-Jan-2020. The study was conducted between 28-Nov-2019 and 05-feb-2020.

Healthy participants received a standardized meal approximately 1.5 to 2 hours prior to the 10.00 and 14.00 measurements and a snack approximately 1.5 to 2 hours prior to the 19.00 measurement. participants with Parkinson's disease had a non-standardized meal at home at least 1 hour prior to the 10.00 measurement and received a standardized meal approximately 1.5 to 2 hours prior to the 14.00 measurement.

SAMPLE COLLECTION AND BIOMARKER ASSAY

For fatty acid measurements, 2 mL of blood was collected in a K2EDTA tube via an intravenous catheter placed in an antecubital vein in the arm and placed on ice until processing. The sample was centrifuged within 30 minutes of collection for 10 minutes at 2000 G at 4 °C and plasma was stored at -80 °C. The samples were drawn on days 1 through 3. For healthy participants, the samples were drawn at 08.00, 10.00, 12.00, 14.00, 17.00 and 19.00, with a 0.5-hour window. For participants with Parkinson's disease, the samples were drawn at 10.00, 12.00, 14.00 and 17.00 with a 0.5-hour window.

The following profile was assessed for the fatty acid measurements, however only the FA-DI and associated fatty acids will be described in the results. For the individual fatty acids, results were provided as concentration (ng/mL) and as fraction of the total amount of fatty acids.

- C16 FA-DI (the ratio between C16:1n7 and C16:0)
- C18 FA-DI (the ratio between C18:1n9 and C18:0)
- Saturated fatty acids: C14:0, C16:0, C18:0, C20:0, C22:0, C24:0
- Unsaturated fatty acids: C16:1n7t, C16:1n9, C16:1n7, C18:1t, C18:1n7, C18:1n9, C18:2n6t, C18:2n6, C18:3n6, C20:1n9, C18:3n3, C20:2n6, C20:3n6, C20:4n6, C20:5n3, C24:1n9, C22:4n6, C22:5n6, C22:5n3, C22:6n3

Samples were analyzed by OmegaQuant (Sioux Falls, South Dakota, United States (USA)) as previously described.[11]

Data Collection

Demographic and clinical data, including age, height, weight, gender, ethnicity and Hoehn and Yahr rating (participants with Parkinson's disease only) were collected for each participant at one site in the Netherlands (Centre for Human Drug Research, Leiden, the Netherlands). There were no important changes to the methods after trial commencement.

Statistical Analysis

Baseline characteristics, including continuous variables like age, height, weight, gender, ethnicity, and body mass index, were summarized using descriptive statistics for each study group.

Descriptive statistics were calculated for baseline triglyceride levels, stratified by health status (healthy participants versus participants with Parkinson's disease). Means and ranges for gender, age and triglyceride levels were compared across groups to assess baseline similarity. To evaluate potential group differences

in triglyceride levels by age and gender, a Kruskal-Wallis test was performed across four groups (healthy participants-male, healthy participants-female, Parkinson's disease patient-male, Parkinson's disease patient-female), as well as a linear regression analysis with baseline triglyceride level as the dependent variable and age and gender as independent variables.

For all individual mono-unsaturated fatty acid concentrations related to C16 and C18 (C16:1n7t, C16:1n9, C16:1n7, C18:1t, C18:1n7, C18:1n9), the ratio compared to the unsaturated fatty acid (C16:0 or C18:0) was calculated as well.

Repetitively measured endpoints related to fatty-acid profiles were summarized, and if needed, log-transformed for statistical analysis. Inter- and intra-subject variability was derived from mixed effects models. To assess the variability, the overall estimated mean and 95% confidence interval (95%CI) was used to determine the coefficient of variability (CV%).

None of the statistical tests were corrected for inflated alpha due to multiple testing since the study had an exploratory nature. All statistical analyses were conducted with SAS 9.4 for Windows or newer (SAS Institute Inc., Cary, NC, USA).

Ethical Considerations

The study was conducted under ethical principles that have ethical origins in the Declaration of Helsinki according to the Dutch Medical Research in Human Subjects Act. The study was performed in compliance with Good Clinical Practice. The protocol of this study was submitted to and reviewed by the independent ethics committee (Stichting Beoordeling Ethiek Biomedisch Onderzoek [PO Box 1004, 9400 BA Assen, The Netherlands]). The committee approved the protocol on 04 November 2019. The study did not commence before formal approval was granted. The trial was registered under NL71599.056.19 at the Dutch Health Care Authority.

RESULTS

Baseline characteristics

Baseline demographic characteristics of the healthy participants and participants with Parkinson's disease were comparable (Table 1). The mean (standard deviation) age was 62 years (7.7) in the healthy participants and 62 years (7.0) in participants with Parkinson's disease. In both groups, 10% of the participants were considered of Asian ethnicity, and 90% were considered of white ethnicity. The Hoehn and Yahr staging was 2 (unilateral involvement only) for 5 participants with Parkinson's disease (50.0%), 3 (bilateral involvement without balance impairment) for 3 participants with Parkinson's disease (30.0%), and 4 (mild to moderate involvement) for 2 participants with Parkinson's disease (20.0%). All 10 participants in both cohorts were included in the analysis. All participants with Parkinson's disease used dopamine agonists. None of the participants used concomitant medication that could impact lipid balance.

Influence of age and gender on triglyceride levels

Age and baseline triglyceride levels were comparable across all four groups (healthy participants-male, healthy participants-female, Parkinson's disease patient-male, Parkinson's disease patient-female) with similar means and ranges observed (Table 1). To confirm that there were no statistically significant differences in baseline triglyceride levels between groups, a Kruskal-Wallis test was performed, which revealed no statistically significant group differences ($H = 0.63$, $p = 0.89$). Additionally, a linear regression analysis showed that neither age ($p = 0.31$) nor gender ($p = 0.93$) statistically significantly influenced triglyceride levels. These findings support the decision not to stratify further by age or gender in subsequent analyses, given the small sample size and comparable baseline characteristics.

FA-DI

We observed notable variations in the coefficient of CV% across different subject groups and fatty acid derivatives. Specifically, for C16 FA-DI, the inter-subject CV% was found to be 20.7% in healthy participants and 37.7% in participants with Parkinson's disease, highlighting distinct metabolic profiles between the two cohorts (Figure 1 and Table 2). Moreover, the intra-subject CV% exhibited variations over days, with values of 14.0% and 14.2% in healthy participants and participants with Parkinson's disease, respectively, indicating differential metabolic stability within each group (Table 2). Notably, the within-day CV% for C16 FA-DI was lower in healthy participants (5.1%) compared to participants with Parkinson's disease (5.9%), underscoring potential physiological differences in fatty acid metabolism (Table 2).

Similarly, for C18 FA-DI, we observed differing inter-subject CV% values of 14.8% in healthy participants and 16.0% in participants with Parkinson's disease, suggesting distinct metabolic responses to this fatty acid derivative among the two subject groups (Figure 2 and Table 2). Intra-subject CV% values over days also demonstrated variability, with 11.0% and 8.6% in healthy participants and participants with Parkinson's disease, respectively, and within-day CV% of 8.4% in healthy participants and 6.7% in participants with Parkinson's disease (Table 2). These findings emphasize the intricate interplay between fatty acid metabolism and disease state.

Pooling data from healthy participants and participants with Parkinson's disease revealed no statistically significant difference in the fed versus fasted state for

both C16 FA-DI (p = 0.749) and C18 FA-DI (p = 0.758), indicating consistent metabolic responses across nutritional conditions (Figure 1, Figure 2 and Table 2).

Further analysis revealed distinct estimated means for C16 FA-DI and C18 FA-DI in healthy participants and participants with Parkinson's disease. Specifically, the estimated mean (95% CI) for C16 FA-DI was 0.071 (0.057 – 0.085) in healthy participants and 0.081 (0.057 – 0.106) in participants with Parkinson's disease (Table 2). For C18 FA-DI, the estimated mean (95% CI) was 3.495 (3.025 – 3.966) in healthy participants and 3.683 (3.157 – 4.208) in participants with Parkinson's disease (Table 2). For C16 FA-DI, there was no statistically significant difference between healthy participants and patients with Parkinson's disease overall (p = 0.426) or specifically on Day 1 (p = 0.403), Day 2 (p = 0.407) or Day 3 (p = 0.524). Similarly for C18 FA-DI, there was no statistically significant difference between healthy participants and patients with Parkinson's disease overall (p = 0.570) or specifically on Day 1 (p = 0.423), Day 2 (p = 0.473) or Day 3 (p = 0.908). These results underscore the importance of considering both subject group and specific fatty acid derivatives when investigating metabolic dynamics, providing valuable insights into the underlying physiological mechanisms.

Concentrations and fractions

In Table 2 we present the variability of individual fatty acid concentrations and fractions involved in the FA-DI. Inter-subject coefficient of variation (CV%) for concentrations ranged between 13.8% and 23.7% in healthy participants and between 17.7% and 52.9% in Parkinson's disease patients. Intra-subject CV% over days ranged between 10.3% and 20.1% in healthy participants and between 10.0% and 20.8% in participants with Parkinson's disease. Within days, the intra-subject CV% ranged between 10.0% and 16.8% in healthy participants and between 10.6% and 14.9% in participants with Parkinson's disease. Summary graphs and tables and variability data for all individual fatty acid concentrations are available in supplemental material.

Similarly, for individual fatty acid fractions, inter-subject and intra-subject variability over days and within days are provided in Table 2. Inter-subject CV% ranged between 5.4% and 23.5% in healthy participants and between 6.5% and 40.8% in participants with Parkinson's disease. Intra-subject CV% over days ranged between 4.5% and 14.4% in healthy participants and between 3.4% and 14.8% in participants with Parkinson's disease. Within days, the intra-subject CV% ranged between 2.8% and 5.4% in healthy participants and between 2.6% and 5.4% in participants with Parkinson's disease. Summary graphs and tables and variability data for all individual fatty acid fractions are provided as supplemental material.

Calculated ratios

In Table 2 we present the calculated ratio of each mono-unsaturated fatty acid to the corresponding saturated fatty acid.

C16:1n7 was the most abundant, with a ratio of 0.070 in healthy participants and 0.085 in participants with Parkinson's disease (Table 2). For C16:1n7t, the ratios were 0.007 and 0.010, and for C16:1n9, the ratio were 0.019 and 0.020, respectively (Table 2). The distinction between C16:1n7 and C16:1n7t lies in the stereochemistry of the double bond. C16:1n7 contains a cis double bond, which induces a bend or "kink" in the hydrocarbon chain due to the spatial orientation of the hydrogen atoms on the same side of the double bond. In contrast, C16:1n7t possesses a trans double bond, where the hydrogen atoms are on opposite sides, resulting in a more linear and extended molecular conformation.

For C18, C18:1n9 was the most abundant, with a ratio of 3.521 in healthy participants and 3.710 in participants with Parkinson's disease (Table 2). For C18:1n7, the ratios were 0.216 and 0.208, and for C18:1t, the ratios were 0.062 and 0.052, respectively (Table 2).

DISCUSSION AND CONCLUSION

The objective of this study was to evaluate naturally occurring levels and variation in plasma fatty acids, focusing on C16 FA-DI and C18 FA-DI in healthy participants and participants with Parkinson's disease, in order to assess whether these may have potential as biomarkers for future clinical trials involving SCD-inhibitors in both populations. In this study we observed that for C16 and C18 FA-DI, the inter- and intra-subject coefficients of variation were low. Noteworthy, the inter-subject CV% of the C16:1n7 concentration and fraction in participants with Parkinson's disease was high compared to the other fatty acids. In early-phase clinical trials, understanding target engagement is important, particularly in a first in human study.[12,13] These effects help explain how a drug interacts with the body and its target molecules, providing valuable insights into dosing, efficacy, and safety, but also assessing the physiological or biochemical responses induced by a drug.[12,13] In this context, biomarkers for target engagement are distinctive, as they are specific to these responses and serve as indicators of drug activity or proof of mechanism.[12–18] Unlike conventional biomarkers, which can encompass a broad range of measurable biological entities, biomarkers for target engagement are needed to reflect the drug's specific mechanism of action, aiding in early assessment of its intended effects in patients.[12–18] First in patient studies further emphasize the importance of proving the mechanism of action, enabling researchers to validate these effects and pave the way for subsequent phases of clinical development.[12,13]

In the context of biomarkers, a low CV% is generally considered to be below 10-20%. This suggests that the biomarker measurements are relatively stable and consistent, which is important in assessing the reliability and precision of the biomarker in a particular study or context. E.g. for the neuroendocrine tumour mRNA genomic biomarker (NETEST) in blood, the CV% is less than 5%, and therefore an excellent test to diagnose neuroendocrine tumors.[19] In contrast, for endothelial function within 48 hours after cardiac surgery, a CV% between 24% and 48% was not correlated with the endothelial function, as measured by serum endocan levels.[20] Therefore, maintaining a CV% below 20% may allow to observe discernible effects, while a higher CV% could decrease the usefulness of the biomarker.

Using the variability data of the C16 FA-DI, we can calculate the minimal effect size for changes in FA-DI that can robustly be found in study involving an SCD inhibitor, given different sample sizes. Based on the square root of the intra- and inter-subject CV% of C16 FA-DI (the standard deviation of the response variable), there is an 80% probability for a one-sided 0.05 significance level if the observable difference in C16 FA-DI is 0.017 units for a study with 20 healthy participants. The observable difference ranges from 0.026 units for 10 participants with Parkinson's disease, to 0.017 units for 100 participants with Parkinson's disease and to 0.007 units for 500 participants with Parkinson's disease. The estimated mean (95%CI) in our study was 0.081 (0.057 – 0.106) for participants with Parkinson's disease. Based on preclinical trials, it is hypothesized that a 20 to 50% decrease in brain C16 FA-DI may be efficacious in reducing alpha-synuclein aggregation.[6,11,21] Therefore, the estimated difference that can be observed seems to be in line with the estimated decrease that may be required based on preclinical data. The observed extent of variability of fatty acids in healthy participants and participants with Parkinson's disease support C16 FA-DI as suitable biomarkers to demonstrate target engagement, of SCD inhibitors in clinical trials, in both healthy participants and participants with Parkinson's disease. For C18 FA-DI, the CV% is similar to C16 FA-DI, and therefore similar conclusions can be drawn.

There are several possible limitations to this study. Firstly, fatty acids can be measured in various media, including erythrocytes, plasma and adipose tissues. Fatty acids levels in adipose tissue may reflect dietary intake over the years[22] and levels in erythrocyte in months,[23] whereas plasma fatty acids levels are known to be a marker of a shorter time span, approximately over the course of two weeks.[7,18,24] Therefore, the observed circadian variability in these fatty acids may be reflective of the dietary habits of the subjects. More specifically, the breakfast was not completely standardized, which may have led to additional variability in plasma fatty acid levels as well. However, as the population that participates in an early phase clinical trial, whether healthy participants or participants with Parkinson's disease, will not have had a standardized diet for months or years, the cause of the variability is irrelevant. The magnitude of the variability determines the usefulness of the biomarker. Also, fatty acids were measured in plasma only, and may not reflect levels in the brain accurately. Lastly, the number of participants in the trial was only 10 per group, therefore more data should confirm these results.

In conclusion, the observed concentrations and extent of variability of fatty acids in healthy participants and participants with Parkinson's disease support C16 and C18 FA-DI as suitable biomarkers to demonstrate target engagement of SCD inhibitors in both healthy participants and participants with Parkinson's disease.

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FIGURE 1 MEAN (STANDARD DEVIATION) OF PLASMA C18 FA-DI (THE RATIO BETWEEN C18:1N9 AND C18:0) DURING EACH OF THE THREE DAYS IN HEALTHY PARTICIPANTS AND PARKINSON'S DISEASE PATIENTS. Healthy participants received a meal approximately 1.5 to 2 hours prior the 10.00 and 14.00 measurements and a snack approximately 1.5-2 hours prior to the 19.00 measurement. Parkinson's disease patients had a meal at home at least 1 hour prior to the 10.00 measurement and received a meal approximately 1.5 to 2 hours prior to the 14.00 measurement. There was no statistically significant difference between healthy participants and patients with Parkinson's disease overall (p = 0.570) or specifically on Day 1 (p = 0.423), Day 2 (p = 0.473) or Day 3 (p = 0.908).

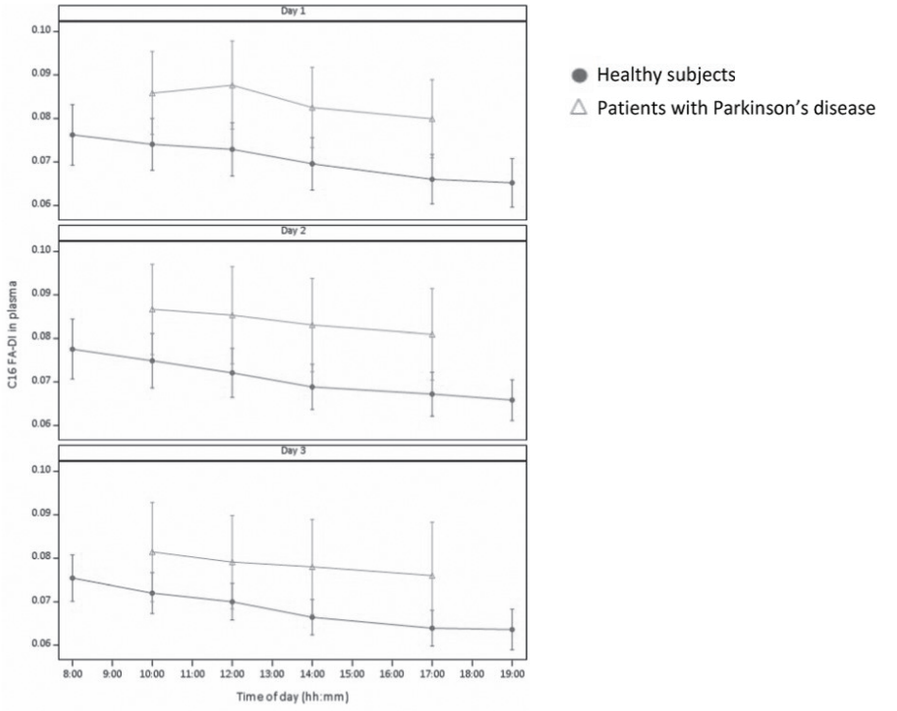

FIGURE 2 MEAN (STANDARD DEVIATION) OF PLASMA C18 FA-DI DURING EACH OF THE THREE DAYS IN HEALTHY PARTICIPANTS AND PARTICIPANTS WITH PARKINSON'S DISEASE. Healthy participants received a meal approximately 1.5 to 2 hours prior the 10.00 and 14.00 measurements and a snack approximately 1.5-2 hours prior to the 19.00 measurement. participants with Parkinson's disease had a meal at home at least 1 hour prior to the 10.00 measurement and received a meal approximately 1.5 to 2 hours prior to the 14.00 measurement.

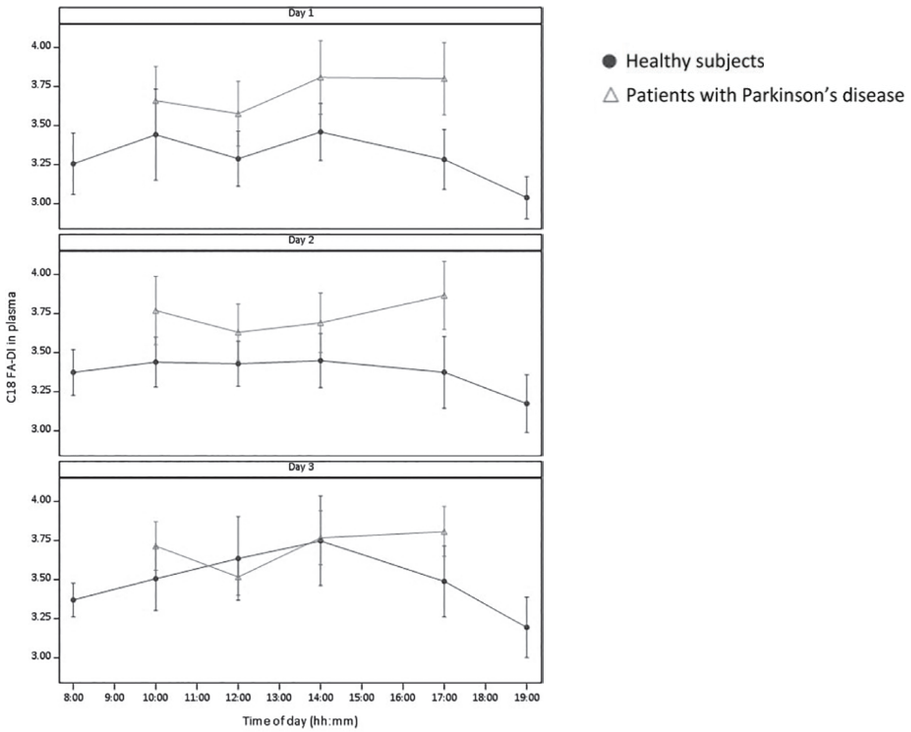

TABLE 1 DEMOGRAPHIC CHARACTERISTICS FOR HEALTHY PARTICIPANTS AND PARTICIPANTS WITH PARKINSON'S DISEASE.

	Group		
	Healthy participants	Participants with Parkinson's disease	All Subjects
Number per group	10	10	20
Age (years)	62 (7.7)	62 (7.0)	62 (7.2)
Height (cm)	177.2 (11.1)	174.13 (12.1)	175.6 (11.4)
Weight (kg)	72.33 (9.25)	79.58 (9.74)	75.95 (9.96)
BMI (kg/m2)	23.1 (2.3)	26.3 (3.3)	24.7 (3.2)
Gender (female)	4 (40.0%)	4 (40.0%)	8 (40.0%)
Female (%)	4 (40.0%)	4 (40.0%)	8 (40.0%)
RACE			
Asian	1 (10.0%)	1 (10.0%)	2 (10.0%)
White	9 (90.0%)	9 (90.0%)	18 (90.0%)
Triglyceride level (mmol/L)	1.16 (0.28)	1.17 (0.56)	1.17 (0.43)
Male	1.20 (0.29)	1.20 (0.70)	1.12 (0.30)
Female	1.10 (0.30)	1.14 (0.35)	1.20 (0.51)
HOEHN & YAHR			
1. Asymptomatic / healthy subject	10 (100%)	0	10 (50.0%)
2. Unilateral involvement only	0	5 (50.0%)	5 (25.0%)
3. Bilateral involvement without balance impairment	0	3 (30.0%)	3 (15.0%)
4. Mild to moderate involvement	0	2 (20.0%)	2 (10.0%)
CONCOMITANT MEDICATION USE			
Statins	0	0	0
Dopamine agonist	0	10 (100%)	10 (50%)
Beta-blockers	0	0	0
Thiazide diuretics	0	0	0
Hormone-replacement therapy	0	0	0

Values are mean (standard deviation) or n (%). There were no meaningful differences between treatment groups.
Abbreviations: BMI = Body Mass Index.

TABLE 2 INTER- AND INTRA-SUBJECT VARIABILITY AND COEFFICIENT OF VARIATION FOR FATTY ACID DESATURASE INDICES, FATTY ACIDS CONCENTRATIONS AND PROPORTIONS, FOR BOTH HEALTHY PARTICIPANTS AND PARTICIPANTS WITH PARKINSON'S DISEASE. Estimated means are provided to put the variability in perspective. Only fatty acids that are part of a fatty acid desaturase index are reported. All individual fatty acids are provided as supplementary data.

Parameter	Group	Estimated Mean	Calculated ratio compared to saturated concentration (either C16:0 or C18:0)	95% Confidence Interval		Inter-subject variability and CV%		Intra-subject variability CV% over days		Intra-subject variability CV% within day	
				Lower limit	Upper limit	Variability	CV% (%)	Variability	CV% (%)	Variability	CV% (%)
FATTY ACIDS CONCENTRATION (NG/ML) AND FA-DI IN PLASMA											
C16 FADI in plasma	HS	0.071	N.A.	0.057	0.085	2.15E-04	20.7%	9.90E-05	14.0%	1.30E-05	5.1%
	PD	0.081	N.A.	0.057	0.106	9.39E-04	37.7%	1.34E-04	14.2%	2.30E-05	5.9%
C16:0 concentration in plasma (ng/ml)	HS	766	N.A.	672	860	11169	13.8%	9762	12.9%	7087	11.0%
	PD	630	N.A.	501	758	27272	26.2%	5782	12.1%	6618	12.9%
C16:1n7 concentration in plasma (ng/ml)	HS	54	0.070	41	66	162	23.7%	117	20.1%	32.380	10.6%
	PD	53	0.085	31	75	794	52.9%	123	20.8%	32.068	10.6%
C16:1n7t concentration in plasma (ng/ml)	HS	5.694	0.007	4.601	6.786	1.359	20.5%	1.289	19.9%	0.874	16.4%
	PD	5.991	0.010	3.871	8.112	7.845	46.7%	1.182	18.1%	1.243	18.6%
C16:1n9 concentration in plasma (ng/ml)	HS	14	0.019	12	17	11	23.1%	3.906	13.8%	2.934	12.0%
	PD	13	0.020	10	16	15	30.4%	2.749	13.1%	2.362	12.2%
C18 FADI in plasma	HS	3.495	N.A.	3.025	3.966	0.266	14.8%	0.147	11.0%	0.086	8.4%
	PD	3.683	N.A.	3.157	4.208	0.347	16.0%	0.101	8.6%	0.061	6.7%
C18:0 concentration in plasma (ng/ml)	HS	237	N.A.	208	266	1291	15.2%	600	10.3%	562	10.0%
	PD	191	N.A.	163	218	1141	17.7%	360	10.0%	408	10.6%
C18:1n7 concentration in plasma (ng/ml)	HS	51	0.216	45	58	55	14.5%	33	11.3%	41	12.5%
	PD	40	0.208	32	48	104	25.6%	25	12.7%	22	11.8%
C18:1n9 concentration in plasma (ng/ml)	HS	833	3.521	675	992	35945	22.7%	20143	17.0%	19637	16.8%
	PD	707	3.710	552	862	36624	27.1%	11807	15.4%	11177	14.9%
C18:1t concentration in plasma (ng/ml)	HS	15	0.062	11	18	11	23.2%	16	27.3%	9	20.7%
	PD	10	0.052	6	14	25	50.3%	7	26.9%	8	27.9%

[CONTINUATION TABLE 2]

Parameter	Group	Estimated Mean	Calculated ratio compared to saturated concentration (either C16:0 or C18:0)	95% Confidence Interval		Inter-subject variability and CV%		Intra-subject variability CV% over days		Intra-subject variability CV% within day	
				Lower limit	Upper limit	Variability	CV% (%)	Variability	CV% (%)	Variability	CV% (%)
FATTY ACIDS FRACTION IN PLASMA											
C16:0 fraction in plasma	HS	0.221	N.A.	0.210	0.232	1.41E-04	5.4%	1.01E-04	4.5%	4.80E-05	3.1%
	PD	0.209	N.A.	0.192	0.227	4.79E-04	10.4%	6.00E-05	3.7%	4.80E-05	3.3%
C16:1n7 fraction in plasma	HS	0.016	N.A.	0.012	0.019	1.40E-05	23.5%	5.00E-06	14.4%	1.00E-06	4.8%
	PD	0.017	N.A.	0.011	0.023	4.80E-05	40.8%	6.00E-06	14.8%	1.00E-06	5.4%
C16:1n7t composition in plasma (%)	HS	0.002	N.A.	0.001	0.002	0.00E+00	18.2%	0.00E+00	16.2%	0.00E+00	12.8%
	PD	0.002	N.A.	0.001	0.002	1.00E-06	37.2%	0.00E+00	13.1%	0.00E+00	11.6%
C16:1n9 composition in plasma (%)	HS	0.004	N.A.	0.004	0.004	0.00E+00	10.8%	0.00E+00	6.0%	0.00E+00	4.1%
	PD	0.004	N.A.	0.004	0.005	0.00E+00	13.4%	0.00E+00	6.3%	0.00E+00	4.3%
C18:0 fraction in plasma	HS	0.068	N.A.	0.065	0.072	1.50E-05	5.7%	1.10E-05	4.9%	4.00E-06	2.8%
	PD	0.064	N.A.	0.061	0.068	1.70E-05	6.5%	5.00E-06	3.4%	3.00E-06	2.6%
C18:1n7 composition in plasma (%)	HS	0.015	N.A.	0.013	0.016	4.00E-06	12.7%	1.00E-06	5.4%	0.00E+00	3.9%
	PD	0.013	N.A.	0.012	0.015	4.00E-06	14.1%	1.00E-06	6.8%	0.00E+00	4.3%
C18:1n9 fraction in plasma	HS	0.238	N.A.	0.216	0.259	5.83E-04	10.2%	2.71E-04	6.9%	2.13E-04	6.1%
	PD	0.233	N.A.	0.207	0.260	1.01E-03	13.6%	2.64E-04	7.0%	1.31E-04	4.9%
C18:1t composition in plasma (%)	HS	0.004	N.A.	0.003	0.005	0.00E+00	16.7%	1.00E-06	20.3%	0	14.4%
	PD	0.003	N.A.	0.002	0.004	2.00E-06	38.8%	0.00E+00	17.9%	0.00E+00	16.0%

Abbreviations: CI = Confidence interval; CV% = coefficient of variability; HS = Healthy participants group; N.A. = Not Applicable fwor ratio calculation; PD = Parkinson's disease group.

SUPPLEMENTAL MATERIALS

Analysis table providing inter- and intra-subject variability for all individual fatty acid concentrations and compositions, and FA-DI. Summary graphs and tables for all individual fatty acid concentrations and compositions, and FA-DI.

CHAPTER 4

SAFETY, PHARMACOKINETICS AND MARKERS OF TARGET ENGAGEMENT OF A NOVEL SCD INHIBITOR (YTX-7739): RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND PHASE 1/1B STUDIES IN HEALTHY PARTICIPANTS

submitted

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ABSTRACT

Parkinson's disease is a progressive neurodegenerative disorder for which no therapies currently exist that can modify the course of the disease. YTX-7739 was a novel, orally administered compound that inhibits the enzyme stearoyl-CoA desaturase, which plays a role in the formation of fatty acids associated with the toxicity of the protein alpha-synuclein (a hallmark of Parkinson's pathology).

This paper presents results from two clinical studies in healthy volunteers: one with single increasing doses and one with multiple increasing doses. YTX-7739 was administered in doses ranging from 5 to 400 milligrams (single dose) and from 15 to 25 milligrams per day for up to 28 days (multiple dose). The compound was well tolerated, and no serious adverse events were reported.

Pharmacokinetic analysis showed a highly variable absorption, a clear food effect and cerebrospinal fluid penetration. Target engagement was demonstrated by a significant reduction in the ratio of unsaturated to saturated plasma fatty acids. No significant changes were observed in cerebrospinal fluid, likely due to low baseline levels and the absence of cells in this fluid.

INTRODUCTION

Parkinson's disease is a progressive neurodegenerative disorder that affects both motor function and cognition. The core pathology of Parkinson's disease is degeneration of the dopaminergic neurons in the midbrain (substantia nigra, pars compacta). Available pharmacological therapies are symptomatic and aimed at either temporarily replenishing dopamine or mimicking the action of dopamine such as with the dopamine precursor levodopa. Currently, there are no disease-modifying therapies for Parkinson's Disease. Parkinson's disease is distinguished by abnormally aggregated oligomeric alpha-synuclein, a principal component of Lewy bodies, a pathological hallmark of the disease.[1,2] The neuronal cell loss occurs in association with the formation of intraneuronal Lewy inclusion bodies consisting of alpha-synuclein protein aggregates. Non-pathogenic alpha-synuclein is concentrated in nerve terminals and plays a role in neurotransmitter release and synaptic plasticity.

It has been demonstrated in vivo that unsaturated fatty acids promote oligomerization of alpha-synuclein,[3–6] which is a key feature of Parkinson's disease. Furthermore, fatty acid-binding proteins, which are members of a superfamily and are essential in fatty acid trafficking, were reported to trigger alpha-synuclein oligomerization in neurons and glial cells and to target the mitochondrial outer membrane, thereby causing mitochondrial loss.[7] The brain contains six saturated fatty acids: myristic acid, palmitic acid (C16:0), stearic acid (C18:0), arachidic acid, behenic acid, and lignoceric acid.[8] Elevated levels of C16:0 were found in the frontal cortex of participants with Parkinson's disease and Lewy Body Dementia,[9,10] with equal levels in Alzheimer's Disease compared to healthy controls.[11] The ratio of unsaturated to saturated 16- and 18-carbon fatty acids are the fatty acid desaturation indices (FA-DI); C16 FA-DI and C18 FA-DI. Previous research found differences in naturally occurring levels in plasma fatty acids and ceramide, as well as variation in their metabolism, in healthy participants and participants with Parkinson's disease.[12–16] The observed concentrations and extent of variability of fatty acids in healthy participants and participants with Parkinson's disease supported C16 and C18 FA-DI as suitable biomarkers to demonstrate target engagement of stearoyl-CoA desaturase (SCD) inhibitors in both healthy participants and participants with Parkinson's disease (*not yet published data)*.

It has been demonstrated in multiple in vitro studies that inhibitors of the enzyme SCD decreased cellular levels of monounsaturated fatty acids, reduced toxicity associated with accumulation of alpha-synuclein, and increased cell survival.[17] Profiling data demonstrates that SCD1 has widespread expression throughout the body with highest levels in the liver and adipose tissues.[18] SCD5 expression is more restricted and, with highest levels in the brain and pancreas.[19] In vitro and in vivo

inhibition of SCD5 and SCD1 reduces production of monounsaturated fatty acids (palmitoleic and oleic acids, respectively).[20] YTX-7739 is a novel, orally active inhibitor of SCD enzymatic activity. A yeast indirect growth assay showed preferential inhibition of the SCD5 isoenzyme compared to the SCD1 isozyme (*unpublished data)*.

In this paper, we summarize the results from both the single ascending dose (SAD) study in healthy participants, as well as the multiple ascending dose (MAD) study in healthy participants. We aim to explore the safety, tolerability, and pharmacokinetic properties of YTX-7739, as well as target engagement on C16 and C18 FA-DI after administration of YTX-7739.

METHODS

Both clinical studies were conducted at a single site in the Netherlands (Centre for Human Drug Research (CHDR), Leiden) in accordance with the International Conference for Harmonization of Technical Requirements for Pharmaceuticals for Human Use, Good Clinical Practice, and the principles of the Declaration of Helsinki. The protocols and study materials were approved by independent ethics committee (Stichting Beoordeling Ethiek Biomedisch Onderzoek, Assen, NL, and all subjects provided their written informed consent before participation.

When applicable, subjects were required to eat a high-fat breakfast or low caloric breakfast 30 minutes prior to study drug administration. The high-fat breakfast included approx. 1,000 kilocalories, of which approx. 56% was fat in line with FDA guidelines. The low caloric breakfast included between 280 and 441 kcals, of which approx. 9% to 37% was fat. Differences in caloric properties of the breakfast were caused due to providing different breakfast options for the subjects.

STUDY DESIGN

SAD in healthy participants

The first in human study (NL8258) was conducted between 13-Sep-2019 and 06-Jan-2021 in healthy male and female subjects (aged 18-45 years). Female subjects of childbearing potential were eligible if they agreed to use a highly effective method of barrier contraception for the duration of the study and for at least 90 days after their last dose of study treatment. Per protocol, the study consisted of three parts (Figure 1). Part A was a randomized, double-blind, placebo-controlled single ascending dose study in fasted condition with five cohorts of eight subjects. Subjects in cohorts 4 and 5 additionally participated in Part B, in which the food effect was investigated). Part C was an open label study with two cohorts of eight subjects. Cerebrospinal fluid (CSF) was sampled only in the second cohort of Part C via lumbar puncture predose and 6-hours postdose.

MAD in healthy participants

Following the first in human study, a randomized, double-blind, placebo-controlled, multiple dose study (NL9172) was conducted in healthy participants between 04-Sep-2020 and 27-Feb-2022 (Figure 2). Per protocol, the study consisted of up to four cohorts of eight healthy male and female subjects (aged 18-55 years). CSF was sampled via lumbar puncture predose and on the day after the final dose.

RANDOMIZATION AND TREATMENTS ADMINISTERED

Participants were administered three strengths of active, 5 mg, 25 mg and 100 mg YTX-7739 or matching placebo capsules. All doses were produced by Aptuit (Verona, Italy) Srl, (Evotec company), and were dispensed by the Hospital Pharmacy of Leiden University Medical Centre, The Netherlands according to Good Manufacturing Practice. Randomization was generated by an independent statistician using SAS 9.4. CHDR study physicians enrolled participants and assigned them with a participant number, which corresponded to a blinded treatment allocation.

PRECLINICAL DATA AND DOSE SELECTION

The conducted toxicology studies indicated that the main organs for toxicity were eyes, fur and skin, which is consistent with the known pharmacology of YTX-7739. Results from toxicology studies have been published previously.[21]

Doses for the SAD study were derived by a combination of allometric and pharmacokinetic scaling using data obtained from preclinical animal studies as well as the GLP toxicology studies for YTX-7739. The starting dose was set using a 10x safety margin versus the no-adverse events level derived from the most sensitive toxicology species.

Before advancing to the next cohort, the study team discussed the interim safety, PK and if available, target engagement data, selected a dose for the next cohort in both the SAD and MAD.

SAD in healthy participants

In Part A and B, six subjects were randomized to receive YTX-7739 and two to placebo. In Part C, all eight subjects received YTX-7739 in an open label fashion.

In Part A, YTX-7739 or placebo was administered following a 10-hour period of fasting with 240 mL of still water. For the fed administration in part B and C, subjects received high-calorie, high-fat meals 30 minutes prior to YTX-7739 administration according to the Food and Drug Agency (FDA) guidelines.[22]

MAD IN HEALTHY PARTICIPANTS

Based on pre-clinical research, we selected a starting daily dose of 25 mg for the MAD, based on pre-clinical and clinical data of the SAD. The calculated starting dose of 25 mg would result in an expected 50% target reduction of brain C16 FA-DI. Based on emerging safety, pharmacokinetic (PK) target engagement data, the additional daily dose levels of 15 mg was explored (Figure 2). In both cohorts subjects received high-calorie, high-fat meals 30 minutes prior to YTX-7739 administration according to the FDA guidelines.[22] Six subjects were randomized to receive YTX-7739 and two to placebo.

SAFETY, PK, TARGET ENGAGEMENT ASSESSMENT AND BIOMARKERS

Safety and tolerability outcome measures for both studies consisted of incidence and severity of adverse events (AES), incidence of clinical laboratory abnormalities (hematology, chemistry, and urinalysis), vital signs, electrocardiograms.

PK outcomes comprised the measurements of the concentration of YTX-7739 in plasma, urine (SAD part A only) and CSF (SAD only cohort 7 and MAD both cohorts) using a validated LC-MS/MS method (Ardena/ABL, Assen, The Netherlands). A standard set of plasma PK parameters were estimated using noncompartmental analysis, including observed maximum concentration (C_{max}), time to reach C_{max} (T_{max}), terminal half-life ($T_{1/2}$), Area Under the Curve (AUC), and accumulation ratios (RAC), as well as the CSF-to-unbound plasma ratio as an indication for central nervous system-penetration.

SCD inhibition by YTX-7739 was quantified by analyzing the reduction in FA-DI and individual fatty acid levels in plasma and CSF.

Statistical analysis

The studies were not tested by formal hypothesis due to the exploratory nature. All PK, target engagement, and safety data were listed, all data were summarized in tabular and/or graphical form, and descriptive statistics were provided, as appropriate, using Statistical Analysis Software (SAS) version 9.4 or higher. AES were coded using the Medical Dictionary for Regulatory Activities. Individual plasma, CSF and urine concentration data were analyzed by noncompartimental PK analysis using R 3.5.3 or newer. The PK parameters were analyzed for dose proportionality using a power model approach or an analysis of variance model as appropriate. Log transformation was utilized to stabilize variance, normalize skewed data, and linearize relationships when dealing with variables that exhibited multiplicative effects or heavily right-skewed distributions. For the target engagement data and exploratory biomarkers, we also reported estimated difference (ED), 95% confidence interval (95%CI) and p-value.

RESULTS

Demographic and baseline characteristics

SAD IN HEALTHY PARTICIPANTS

Healthy participants in the first in human trial were predominantly white. On average, 44% [range 12.5% to 66.7%] was male, and the mean age was 24.2 years [range 19 to 39]. Demographics and other baseline characteristics were generally similar among treatment arms (Supplementary Table 1).

MAD IN HEALTHY PARTICIPANTS

Healthy participants were predominantly male and white. Demographics and other baseline characteristics were generally similar among treatment arms, except for age. The mean age of healthy participants was 30.6 years [range 18 to 53]). Age did not significantly differ between active and placebo treatment arms (Supplementary Table 1).

Safety and tolerability

SAD IN HEALTHY PARTICIPANTS

Single oral doses between 5 mg and 400 mg YTX-7739 were safe and well tolerated in Healthy participants. There were no severe adverse events or deaths.

The most frequently reported AES were headache, fatigue, gastro-intestinal complaints and musculoskeletal symptoms among the subject who received YTX-7739 at single dose levels up to 25 mg in the double-blinded part of the study. Frequencies of these adverse events were comparable in the placebo group, except for gastro-intestinal complaints, which occurred less frequently in the placebo group (Supplementary Table 2). We identified no clinically relevant changes in blood chemistry, hematology, urinalysis, vital signs or ECG tests after single doses of YTX-7739.

MAD IN HEALTHY PARTICIPANTS

Administration of YTX-7739 at multiple ascending oral daily doses up to 25 mg for up to 28 days was generally safe and well tolerated in healthy participants.
There were no deaths or severe adverse events in the dose level cohorts up to 25 mg in healthy participants.

Early in the study in cohort A1, treatment was discontinued due to hepatic laboratory findings in 3 healthy participants, of which 2 were administered YTX-7739 25 mg, and 1 was administered placebo. Treatment in 2 of these 3 healthy participants was discontinued due to hyperbilirubinemia and in one (1) healthy subject due to concomitant hyperbilirubinemia and increased alanine aminotransferase (ALAT),

which was considered unlikely related to YTX-7739 administration after reviewing the unblinded results. One of these subjects already had elevated bilirubin levels prior to dosing.

The most frequently reported AEs were headache, fatigue, puncture site pain (all after lumbar puncture) and post lumbar puncture syndrome among the subjects who received YTX-7739 at any multiple dose level. Frequencies of these adverse events were comparable in the placebo group, except for dry eyes, puncture site pain and myalgia which occurred less frequently in the placebo group (Supplementary Table 3).

We identified no clinically relevant changes in blood chemistry, hematology, urinalysis, vital signs or ECG tests after multiple doses of YTX-7739.

PHARMACOKINETICS

SAD in healthy participants

Absorption was highly variable between individuals. T_{max} ranged from 4 to 96 hours in fasted subjects. Plasma concentrations increased in a biphasic manner at all dose levels in fasted subjects (Table 3 and Figure 3), although this was especially pronounced at the higher dose levels (100 – 400 mg). There was an initial concentration peak around 4-6 hours, followed by a decline in concentration until 8-12 hours and a subsequent increase in concentration to a second, typically higher, peak concentration at 24-36 hours in most subjects. The mean half-life following fasted dosing varied between 47.3 and 78 hours. Plasma exposure of YTX-7739 following fasted administration increased approximately dose proportionally in the 10 mg – 100 mg dose range and less than dose proportionally in the 100 – 400 mg dose range (Table 3).

Following fasted dosing, the inter-individual variability (%CV) of the C_{max} was higher in the 10-100 mg dose range compared to the 250 – 400 mg dose range; the %CV for the $AUC_{0\text{-}last}$ was comparable across all dose levels (Table 3). Administration of single doses of YTX-7739 following a high-fat meal in the 5-250 mg dose range resulted in monophasic absorption with a median T_{max} of 8h, increased C_{max} and AUC_{last}, and reduced variability, when compared to fasted administration (Table 3). C_{max} and AUC_{24H} increased dose-proportionally in the 10 – 250 mg dose range, when administered following a high-fat meal (Table 3). Administration of 400 mg of YTX-7739 following a high-fat meal resulted in increased C_{max} and AUC_{24H} compared to fasted administration. However, the C_{max} and AUC_{24H} of 400 mg YTX-7739 administered in a fed state were less than dose-proportional and more variable, when compared to fed administration in the 5 – 250 mg dose range (Table 3). The mean CSF-to-unbound-plasma ratio was 0.849 (0.166) in the 30 mg fed cohort (Table 3).

MAD IN HEALTHY PARTICIPANTS

Following multiple oral doses of YTX-7739, the median T_{max} was 4.01 to 10.06 hours. After daily administration of 25 mg, the mean (standard deviation) AUC_{24H} was 26,144 (8,390) ng*h/mL and C_{max} was 1,408 (477) ng/mL at steady state. The variability of AUC_{24H} was 32.09% at steady state after daily administration of 25 mg. Steady state was reached after Day 15 of daily dosing. (Table 3, Figure 5 and Supplementary Figure 1). $T_{1/2}$ ranged from 60 to 121 hours after multiple doses of YTX-7739. The mean CSF-to-unbound-plasma ratio ranged between 0.74 to 0.85.

Markers of Target engagement and Exploratory Biomarkers

In the SAD study, we observed a significant overall reduction for C16 FA-DI in plasma after administration in fed state of 250 mg ($p < 0.001$) and 400 mg fed ($p = 0.002$), compared to placebo. Also, we observed a significant overall reduction for C18 FA-DI in the 250 mg fed treatment group only ($p = 0.011$).

Following multiple oral doses of YTX-7739, there was a statistically significant reduction after daily administration of 25 mg in C16 FA-DI ($p < 0.001$) and C18 FA-DI ($p = 0.018$), as well as after 15 mg in C16 FA-DI ($p = 0.001$) and C18 FA-DI ($p = 0.001$) compared to placebo. There were no significant changes in the fatty acids included in the C16 FA-DI (C16:0 and C16:1n7) and C18 FA-DI (C18:0 and C18:1n9) after administration of multiple oral doses of YTX-7739 (Table 4) . Across both treatment levels, C16:0 and C18:0 levels did not change statistically significantly ($p < 0.05$), nor was there any trend ($0.05 < p < 0.10$). There was a statistically significant reduction after administration of both dose levels in C16:1n7. However, there was only a statistically significant reduction after daily administration of 15 mg in C18:1n9 ($p = 0.009$), compared to placebo.

In CSF, the same desaturation indices or its associated fatty acids did not change significantly following multiple oral doses of YTX-7739 during a treatment period of up to 28 days. In the associated fatty acids, there was only a trend towards a significant reduction of C18:0 in CSF after administration of YTX-7739 15 mg in healthy participants ($p = 0.070$) (Table 4). Also, we compared the median values C16:0, C18:0, C16:1n7 and C18:1n9 levels. All median individual fatty acid levels were much lower in CSF compared to plasma. This difference ranged approximately between 109 and 325-fold (Table 5). The overall treatment effects for the C16 and C18 FA-DI and the included fatty acids are noted in Table 4. Following multiple oral doses of YTX-7739, there was no statistically significant reduction any of the exploratory biomarkers.

DISCUSSION

YTX-7739 was in development as an oral SCD-inhibitor with potential disease-modifying effects for the treatment of Parkinson's disease. At both dose levels of the MAD study, YTX-7739 has demonstrated target engagement by decreasing C16 and C18 FA-DI in plasma compared to placebo in healthy participants. In this study, administration of YTX-7739 at multiple ascending daily oral doses up to 25 mg for up to 28 days was generally safe and well tolerated in healthy participants.

In this study, YTX-7739 has demonstrated reductions in FA-DI in plasma in healthy participants, which is a measure for target engagement also for participants with Parkinson's disease. There are differences in naturally occurring levels of plasma fatty acids and ceramide in healthy participants and participants with Parkinson's disease.[12–16] Moreover, levels of C16 and C18 FA-DI are similar between healthy participants and participants with Parkinson's disease.[12] In a single individual, C16 and C18 FA-DI are stable both within and between days.[12]

The mean YTX-7739 CSF-to-unbound-plasma ratio ranged between 0.74 and 0.85, which indicates robust brain penetration. In contrast to the difference observed in plasma, there was no change in C16 and C18 FA-DI between treatment and placebo groups in CSF. This finding is similar to preclinical observations for C16 FA-DI in CSF of monkeys (C18 not investigated). Presumably, CSF FA-DI correlates less well to cellular changes following SCD inhibition, as CSF is a cell-free matrix.[21] YTX-7739 reduced C16 FA-DI in monkey brain tissue after administration of YTX-7739, further supporting this explanation.[21] C16:0, C18:0, C16:1n7 and C18:1n9 levels were much lower in CSF compared to plasma, which is consistent with previous data.[27] These lower baseline levels reduce the sensitivity for the assay to detect a change in treatment versus placebo groups. Furthermore, it has been demonstrated that the levels of C16:0 and C18:0 fatty acids in CSF in idiopathic PD (iPD), glucocerebrosidase (GBA) PD, and healthy controls do not differ significantly, however C16:1n7 and C18:1n9, the products of SCD, are significantly lower in GBA-PD compared to iPD and healthy controls.[28]

SCD inhibition prevented alpha-synuclein aggregation in vitro,[17,29–31] and development of Parkinson's disease phenotypes in alpha-synuclein mutant and alpha-synuclein overexpressing mouse models.[29] YTX-7739 did not reduce the PD related biomarkers in healthy participants in this study. It is plausible that these matrices do not reflect the intraneuronal environment in the 14 to 28-day time course of this study.[32,33]

In both the SAD and MAD study, YTX-7739 has demonstrated a significant food effect. The absorption in blood of YTX-7739 seems to be limited by both the total amount of YTX-7739 in the gut, as well as by the availability of an adequate amount of orally ingested fat.

TABLE 3 PHARMACOKINETIC PARAMETERS OF YTX-7739 IN PLASMA AND CSF FOLLOWING ADMINISTRATION OF YTX-7739 UP TO 400 MG IN HEALTHY PARTICIPANTS

	Mean (SD) or median (range)												
	SAD							MAD					
	Healthy participants							Healthy participants					
Cohort												A2	A1
Dose level	5 mg	10 mg	10 mg	30 mg	30 mg	100 mg	250 mg	250 mg	400 mg	400 mg	400 mg	15 mg	25 mg
Study day	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 1	Day 28	Day 14
Number of subjects in group	N = 4	N = 6	N = 4	N = 6	N = 8	N = 6	N = 6	N = 6	N = 6	N = 5	(N = 6)	(N = 6)	(N = 6)
Breakfast	High-fat	None	High-fat	None	High-fat	None	None	High-fat	None	High-fat	High-fat	High-fat	High-fat
C_{max} (ng/mL)	45.38 (9.732)	64.35 (36.696)	82.35 (8.123)	179.17 (60.141)	265.38 (49.399)	418.17 (86.08)	626 (117)	2208 (234)	843 (166)	2744 (716)	757 (403)	1,408 (477)	
%CV of C_{max} (%)	21.45	57.03	9.86	33.57	18.61	20.59	18.72	10.61	19.7	26.08	53.22	33.84	
T_{max}(h)[1]	8 (5-10)	7 (4-36)	8 (4-10)	30 (6-36)	8 (5-36)	30 (8-36)	36 (24-96)	8 (5-10)	30 (4-36)	7 (5-24)	10.06 (6.00-23.42)	6.00 (4.00-8.00)	
AUC 24H (ng*h/mL)	739.0 (160.58)	1247.0 (684.17)	1538.4 (206.96)	3317.1 (1128.41)	4436.5 (917.12)	8041.7 (2120.36)	10767 (2223)	39353 (5673)	15600 (1626)	50526 (14148)	16,775 (8,818)	26,144 (8,390)	
%CV of AUC_{24H} (%)	21.73	54.86	13.45	34.02	20.67	26.37	20.65	14.42	10.42	28	52.57	32.09	
AUC_{last} (ng*h/mL)	2946.7 (1434.95)	1785.4 (1033.05)	8190.7 (1199.3)	17816.8 (7323.52)	17051.9 (6836.65)	48757.4 (10516.5)	66441 (24806)	169994 (49049)	125424 (55855)	232153 (91210)	130,840 (117,339)	163,645 (110,914)	
T½ (h)	55.030 (30.309)	N.Q.[3]	N.Q.[3]	50.185 (218.774)[3]	48.818 (28.592)[3]	72.137 (59.76)[3]	51 (121)	53 (70)	78 (345)	59 (298)	121 (48)	74 (34)	
Rac	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	7.86 (5.06)	5.96 (2.01)
dnC_{max} (ng/ml/mg)	9.075 (1.946)	6.435 (3.670)	8.235 (0.812)	5.972 (2.005)	8.846 (1.647)	4.182 (0.861)	2.5 (0.469)	8.83 (0.938)	2.11 (0.664)	6.86 (2.862)	50 (27)	56 (19)	
dnAUC 24H (ng*h/mL/mg)	147.80 (32.116)	124.70 (68.417)	153.84 (20.696)	110.57 (37.614)	147.88 (30.571)	80.42 (21.204)	43.07 (8.893)	157.41 (22.694)	39 (6.504)	126.31 (56.592)	1,118 (588)	1,046 (336)	
CCSF[2] (ng/mL)	N.A.[5]	N.A.[5]	N.A.[5]	N.A.[5]	9.995 (3.373)	N.A.[5]	N.A.[5]	N.A.[5]	N.A.[5]	N.A.[5]	35 (23)	45 (26)	
Unbound CSF/ plasma ratio[4]	N.A.[5]	N.A.[5]	N.A.[5]	N.A.[5]	0.80 (0.15)	N.A.[5]	N.A.[5]	N.A.[5]	N.A.[5]	N.A.[5]	0.76 (0.12)	0.83 (0.13)	

Abbreviations: AUC_{24H} = area under the concentration-time curve from time 0 to 24 hours; AUC_{last}= area under the concentration-time curve from time 0 to the last measurement; Ccsf = YTX-7739 concentration in CSF; CSF = Cerebrospinal Fluid; C_{max} = maximum concentration; dn = dose normalized; N.A. = Not Applicable; N.Q. = Not Quantifiable; T_{max} = time to maximum concentration; T½ = half-life. / 1. T_{max} data is provided as median (range). / 2. CSF was obtained at day – 1 in the SAD and MAD. The postdose sample was obtained in the SAD 6 hours post dose, and in the MAD on the day after the final dose (day 15 or 29). / 3. In the 10 mg cohorts, there was insufficient data to quantify T½. In the 30 mg fasted cohort, the PK data of 4 subjects was used to calculate T½, in the 30 mg fasted cohort, 7 subjects, and in 100 mg fasted, 3 subjects. / 4. Unbound CSF/plasma ratio was only assessed in the MAD in 4 of the 6 healthy participants in cohort A1 (25 mg) due to treatment discontinuation. / 5. CSF was not collected in these cohorts.

TABLE 4 TREATMENT EFFECT OF MULTIPLE DOSES OF YTX-7739 ON C16 AND C18 FA-DI AND ITS ASSOCIATED FATTY ACIDS IN PLASMA AND CSF IN HEALTHY PARTICIPANTS. For comparison, the estimated mean of the placebo groups was added to put the estimated difference in perspective. In plasma, all estimated differences of the individual fatty acids were log transformed and therefore presented as percentage. In CSF, the estimated differences of the FA-DI were log transformed and therefore presented as percentage. All bold values are statistically significant ($p < 0.05$). All italic values are trends towards a statistically significant difference ($0.05 < p < 0.10$).

	healthy participants placebo	15 mg vs placebo (Cohort A2, healthy participants, 28 days)			25 mg vs placebo (Cohort A1, healthy participants 14 days)		
Parameter (index)	Estimated mean	ED	95% CI	p-value	ED	95% CI	p-value
C16 FA-DI in plasma	0.078	-0.017	-0.025; -0.008	0.001	-0.03	-0.039; -0.022	<0.001
C16:0 in plasma (ng/mL)*	659.860	-13.0%	-28.9%; 6.6%	0.168	-13.9%	-29.8%; 5.5%	0.140
C16:1n-7 in plasma (ng/mL)*	49.475	-31.5%	-49.8%; -6.3%	0.020	-47.6%	-61.0%; -29.6%	<0.001
C18 FA-DI in plasma	3.561	-0.593	-0.93; -0.261	0.001	-0.422	-0.764; -0.080	0.018
C18:0 in plasma (ng/mL)*	195.934	-7.2%	-20.6%; 8.6%	0.335	-3.2%	17.5%; 13.4%	0.670
C18:1n-9 in plasma (ng/mL)*	689.217	-23.5%	-36.8%; -7.2%	0.009	-15.7%	-30.8%; 2.7%	0.086
C16 FA-DI in CSF*	0.015	61.2%	-39.6%; 330.1%	0.288	-32.4%	-79.6%; 124.2%	0.466
C16:0 in CSF (ng/mL)	5.830	-1.750	-4.153; 0.654	0.132	-0.658	1.961; 0.654	0.578
C16:1n-7 in CSF (ng/mL)	0.113	-0.002	-0.114; 0.109	0.966	-0.061	0.054; 0.109	0.256
C18 FA-DI in CSF*	1.583	27.8%	-36%; 155.2%	0.437	9.9%	-41.4%; 106.1%	0.739
C18:0 in CSF (ng/mL)	3.247	-1.482	-3.114; 0.151	0.070	-0.106	1.632; 0.151	0.892
C18:1n-9 in CSF (ng/mL)	5.007	-0.852	-2.678; 0.975	0.314	-0.204	1.837; 0.975	0.824

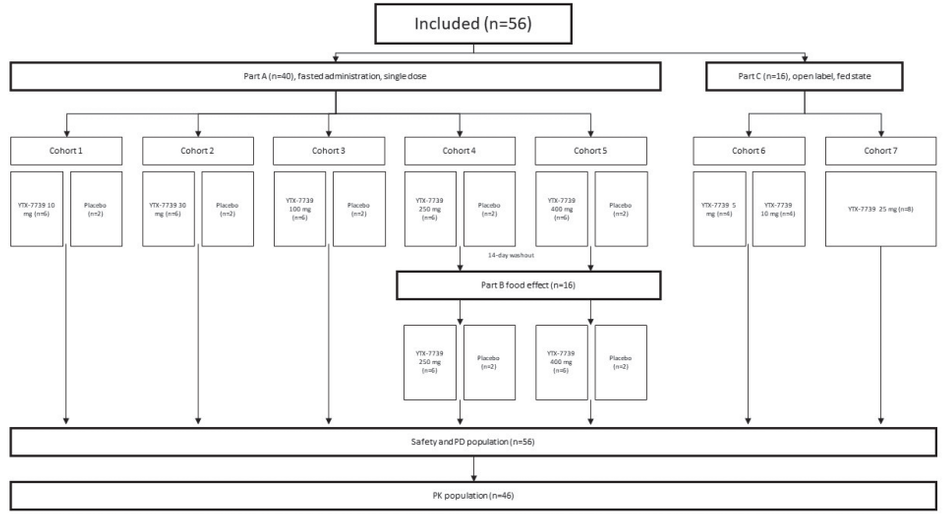
*Note: Values with * were log transformed and therefore provided as percentage (%). Abbreviations: CI = Confidence Interval; CSF = Cerebrospinal fluid; ED = Estimated Difference; FA-DI = Fatty Acid Desaturase Index.*

TABLE 5 MEDIAN (RANGE) OF CSF AND PLASMA FADI ASSOCIATED FATTY ACIDS AND PLASMA TO CSF RATIO'S IN HEALTHY PARTICIPANTS.

	Median (range) or plasma to CSF ratio healthy participants (baseline) n = 6				
Fatty acid	CSF (ng/uL)		Plasma (ng/uL)		ratio
C16:0	5.319	3.555; 10.715	580.454	527.263; 1113.226	109
C16:1n7	0.160	0.027; 0.054	51.955	35.931; 95.008	325
C18:0	1.436	0.759; 2.981	189.901	150.202; 273.449	132
C18:1n9	5.588	4.042; 13.522	641.061	621.273; 1616.782	115

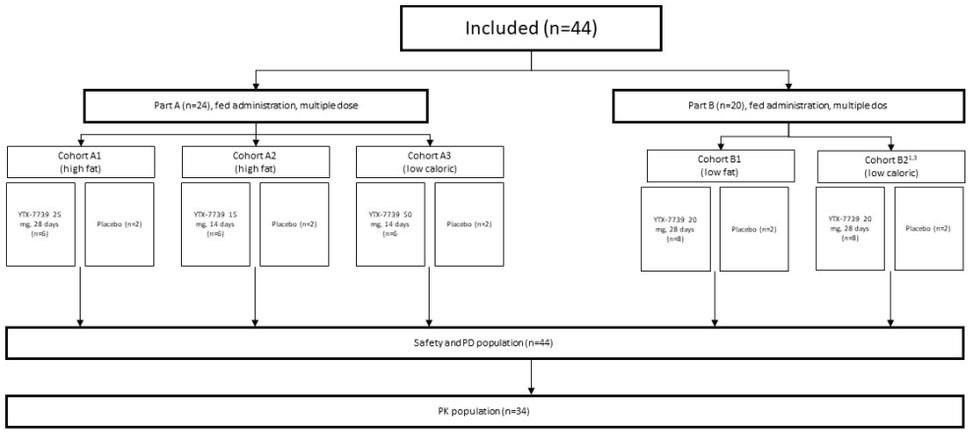
Abbreviations: CI = Confidence Interval; CSF = Cerebrospinal Fluid; ED = Estimated Difference; FA-DI = Fatty Acid Desaturase Index.

FIGURE 1 OVERVIEW OF SINGLE DOSE STUDY (CHDR1911; 7739-01-001). Part A was the double-blind, placebo-controlled, randomized study; YTX-7739 was administered in fasted state. Part B consisted of 2 cohorts of 16 healthy participants, in which the highest dose levels of 250 mg and 400 mg was compared in fasted and fed state. Part C was the open label part, and YTX-7739 was administered in fed state.



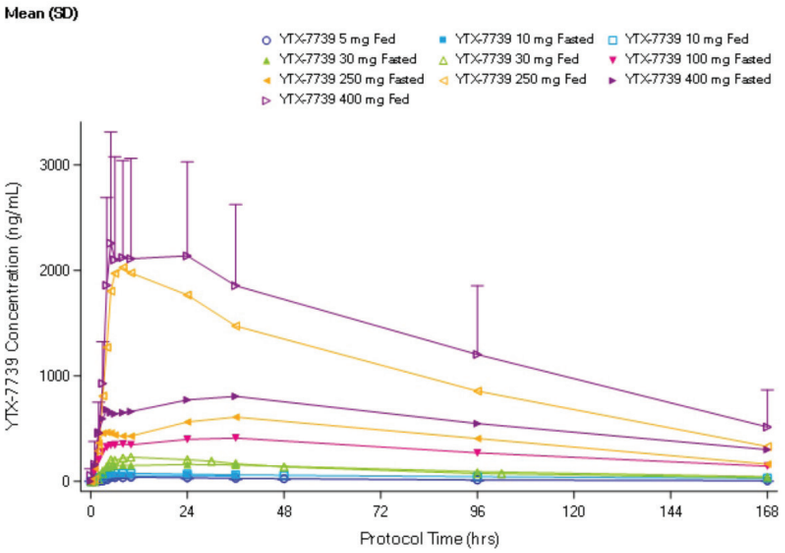
Abbreviations: n = number; PK = pharmacokinetic.

FIGURE 2 OVERVIEW OF MULTIPLE DOSE STUDY (CHDR1916; 7739-01-002). All participants were administered YTX-7739 in fed state. Both cohorts A1 and A2 received a high-fat breakfast.



Abbreviations: n = number; PK = pharmacokinetic.

FIGURE 3 MEAN (SD) PLASMA YTX-7739 CONCENTRATION AGAINST TIME FOLLOWING A SINGLE ORAL DOSE OF 10, 30, 100, 250 AND 400 MG YTX-7739 IN A FASTED STATE AND 5, 10, 30, 250 AND 400 MG YTX-7739 IN A FED STATE. After administration of a single dose of YTX-7739 in fed and fasted condition on a linear scale per cohort.



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Supplementary Tables

SUPPLEMENTARY TABLE 1 DEMOGRAPHICS OF STUDY PARTICIPANTS IN THE SINGLE ASCENDING DOSE AND MULTIPLE ASCENDING DOSE STUDY OF YTX-7739 IN HEALTHY PARTICIPANTS AND PARTICIPANTS WITH PARKINSON'S DISEASE.

	SAD									MAD		
	Part A			Part B food effect			Part C			Part A		
Dose	10 mg	30 mg	100 mg	250 mg	400 mg	5 mg fed	10 mg fed	25 mg fed	Placebo	15 mg fed	25 mg fed	Placebo
Number of subjects	(N=6)	(N=6)	(N=6)	(N=6)	(N=6)	(N=4)	(N=4)	(N=8)	(N=10)	(N=6)	(N=6)	(N=4)
M/F	2/4	2/4	3/3	2/4	4/2	1/3	2/2	4/4	2/8	2/4	3/3	02-Feb
AGE, YEARS												
Mean	24.7 (4.4)	24.8 (2.4)	28 (6.5)	25.3 (2.6)	22.5 (4.4)	19.8 (1)	25.8 (6.4)	23.9 (2.2)	22.8 (3.2)	29.5 (10.1)	26.7 (5.8)	29.5 (5.4)
Median	26	26	26	25	21	20	24	24	23	25	26	31
Min, max	19, 30	21, 27	21, 39	23, 29	19, 31	19, 21	21, 35	20, 27	19, 30	21, 45	19, 34	21, 35
WEIGHT (KG)												
Mean	71.1 (14.4)	62.3 (11.1)	75.1 (8.5)	60.1 (7.7)	76 (11.2)	64.6 (8.6)	78.8 (8.6)	72.2 (10.5)	69.8 (13.4)	66.13 (9.06)	74.81 (11.95)	75.84 (7.0)
Median	72.93	59.98	76.28	57.9	72.82	67.25	78.4	72.65	64.48	68.4	78.93	75.65
Min, max	54.10,,89.05	50.30,,80.85	65.15,,86	51.30, ,74.25	63.10, ,93.2	52.40, 71.50	68.70, 89.50	56.10, 85.90	54.90,,99.4	54.75, 78.40	53.05, 84.40	67.65, 84.40
HEIGHT (CM)												
Mean	173.2 (9.4)	174.1 (11.6)	175.8 (5.1)	167.5 (5.3)	178.5 (3.6)	170.5 (7.6)	176.6 (10.5)	176.5 (8.6)	175.3 (9.8)	167.4 (5.6)	177.7 (9.9)	170.6 (8.5)
Median	174.7	170.2	175.1	168.9	178	168.9	174.9	175.4	171	166.6	179.5	169.8
Min, max	161.6, 184.8	162.4, 189.9	169.4, 183.3	157.1, 172.2	174.7, 183.9	163.2, 181.1	165.8, 190.6	165.6, 187.9	160.6, 192.5	161.7, 175.8	164.1, 190.7	160.8, 182.0
BMI (KG/M2)												
Mean	23.5 (2.9)	20.5 (2.7)	24.5 (3.7)	21.4 (2.3)	23.8 (2.7)	22.2 (2.2)	25.2 (1.1)	23.1 (1.8)	22.5 (2.1)	23.60 (2.83)	23.63 (3.06)	26.03 (0.30)
Median	23.4	19.9	24.7	20.7	23.5	22.1	24.8	23.4	22.5	24.1	24.1	26.2
Min, max	20.1, 27.6	18.0, 24.8	19.4, 30.0	20.2, 26.0	20.6, 27.6	19.7, 24.8	24.3, 26.8	19.6, 25.1	18.5, 26.8	19.6, 26.6	18.5, 26.6	25.5, 26.20

Abbreviations: BMI = Body Mass Index; MAD = Multiple Ascending Dose; SAD = Single Ascending Dose.

SUPPLEMENTARY TABLE 2 ADVERSE EVENTS PER DOSE LEVEL IN THE SINGLE DOSE STUDY IN HEALTHY VOLUNTEERS. Headache, fatigue, gastro-intestinal complaints and musculoskeletal symptoms were the most frequently reported adverse events among the subject who received YTX-7739 at any single dose in the double-blinded part of the study. Frequencies of these adverse events were comparable in the placebo group, except for gastro-intestinal complaints, which occurred less frequently in the placebo group.

Dose level	5 mg		10 mg		10 mg		30 mg		30 mg		100 mg		250 mg		250 mg		400 mg		400 mg		Placebo	
Fed or fasted	Fed		Fasted		Fed		Fasted		Fed		Fasted		Fasted		Fed		Fasted		Fed		Both	
Number of s. in group	N = 4		N = 6		N = 4		N = 6		N = 8		N = 6		N = 6		N = 8		N = 6		N = 6		N = 10	
System Organ Class	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects
Preferred Term	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)
Total	15	4(100.0)	5	5(83.3)	6	4(100.0)	11	6(100.0)	9	5(62.5)	12	4(66.7)	12	6(100.0)	7	4(66.7)	14	5(83.3)	6	4(66.7)	23	9(90.0)
CARDIAC DISORDERS																						
	1	1(25.0)	1	1(16.7)													1	1(16.7)			2	2(20.0)
Palpitations	1	1(25.0)																			1	1(10.0)
Supraventricular tachycardia			1	1(16.7)																		
Ventricular tachycardia																	1	1(16.7)			1	1(10.0)
EYE DISORDERS																						
													1	1(16.7)			2	2(33.3)			1	1(10.0)
Dry eye																	1	1(16.7)			1	1(10.0)
Ocular hyperaemia													1	1(16.7)								
Photopsia																	1	1(16.7)				
GASTROINTESTINAL DISORDERS																						
					1	1(25.0)	1	1(16.7)			2	2(33.3)	2	2(33.3)			2	2(33.3)	2	2(33.3)	2	1(10.0)
Abdominal discomfort							1	1(16.7)														
Abdominal pain											1	1(16.7)	1	1(16.7)								
Burn oesophageal													1	1(16.7)								
Gastroenteritis																			1	1(16.7)		
Nausea																	2	2(33.3)				
Oropharyngeal pain																					2	1(10.0)
Periodontitis					1	1(25.0)																
Vomiting											1	1(16.7)							1	1(16.7)		
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS																						
	2	2(50.0)	1	1(16.7)	1	1(25.0)	3	3(50.0)	1	1(12.5)			2	2(33.3)	2	1(16.7)	2	2(33.3)			4	3(30.0)
Catheter site inflammation									1	1(12.5)												
Catheter site pain	1	1(25.0)																				
Fatigue	1	1(25.0)	1	1(16.7)	1	1(25.0)	3	3(50.0)					2	2(33.3)	1	1(16.7)	2	2(33.3)			3	3(30.0)
Feeling cold																					1	1(10.0)
Swelling															1	1(16.7)						
IMMUNE SYSTEM DISORDERS																						
			1	1(16.7)			1	1(16.7)			2	2(33.3)	1	1(16.7)							3	2(20.0)
Dermatitis contact			1	1(16.7)			1	1(16.7)			2	2(33.3)	1	1(16.7)							3	2(20.0)
INFECTIONS AND INFESTATIONS																						
							1	1(16.7)									1	1(16.7)				
Nasopharyngitis							1	1(16.7)									1	1(16.7)				
INJURY, POISONING AND PROCEDURAL COMPLICATIONS																						
			1	1(16.7)																	1	1(10.0)
Ligament sprain			1	1(16.7)																		
Procedural dizziness																					1	1(10.0)
INVESTIGATIONS																						
							1	1(16.7)							1	1(16.7)	1	1(16.7)				
Menstruation normal							1	1(16.7)									1	1(16.7)				
Nitrite urine															1	1(16.7)						
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS																						
	1	1(25.0)									1	1(16.7)					2	2(33.3)	2	2(33.3)	2	2(20.0)
Muscle spasms	1	1(25.0)																				
Muscle twitching																					1	1(10.0)
Musculoskeletal stiffness																	1	1(16.7)				
Myalgia																	1	1(16.7)	2	2(33.3)	1	1(10.0)
Restless legs syndrome											1	1(16.7)										

[CONTINUATION SUPPLEMENTARY TABLE 2]

Dose level	5 mg		10 mg		10 mg		30 mg		30 mg		100 mg		250 mg		250 mg		400 mg		400 mg		Placebo	
Fed or fasted	Fed		Fasted		Fed		Fasted		Fed		Fasted		Fasted		Fed		Fasted		Fed		Both	
Number of s. in group	N = 4		N = 6		N = 4		N = 6		N = 8		N = 6		N = 6		N = 8		N = 6		N = 6		N = 10	
System Organ Class	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects	Events	Subjects
Preferred Term	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)	N	N(%)
NERVOUS SYSTEM DISORDERS																						
	5	2(50.0)			3	2(50.0)	3	3(50.0)	5	3(37.5)	5	3(50.0)	4	2(33.3)	1	1(16.7)	1	1(16.7)	2	2(33.3)	5	5(50.0)
Dizziness											1	1(16.7)	1	1(16.7)					1	1(16.7)	1	1(10.0)
Headache	4	2(50.0)			3	2(50.0)	3	3(50.0)	5	3(37.5)	4	3(50.0)	3	2(33.3)	1	1(16.7)	1	1(16.7)	1	1(16.7)	4	4(40.0)
Migraine	1	1(25.0)																				
REPRODUCTIVE SYSTEM AND BREAST DISORDERS																						
	2	2(50.0)	1	1(16.7)			1	1(16.7)									1	1(16.7)				
Menstrual discomfort	2	2(50.0)	1	1(16.7)			1	1(16.7)									1	1(16.7)				
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS																						
									2	2(25.0)												
Cough									1	1(12.5)												
Nasopharyngitis									1	1(12.5)												
SKIN AND SUBCUTANEOUS TISSUE DISORDERS																						
	4	3(75.0)							1	1(12.5)	1	1(16.7)	2	2(33.3)	3	3(50.0)					3	3(30.0)
Alopecia													1	1(16.7)							1	1(10.0)
Dermatitis	1	1(25.0)													2	2(33.3)						
Dermatitis contact	1	1(25.0)																				
Dry skin													1	1(16.7)							2	2(20.0)
Erythema	2	2(50.0)							1	1(12.5)												
Pruritus											1	1(16.7)			1	1(16.7)						
VASCULAR DISORDERS																						
					1	1(25.0)					1	1(16.7)					1	1(16.7)				
Dizziness postural											1	1(16.7)										
Increased tendency to bruise																	1	1(16.7)				
Presyncope					1	1(25.0)																

SUPPLEMENTARY TABLE 3 ADVERSE EVENTS PER DOSE LEVEL IN THE MULTIPLE DOSE STUDY IN HEALTHY VOLUNTEERS AND PATIENTS WITH PARKINSON'S DISEASE. Headache, fatigue, puncture site pain (all after lumbar puncture) and post lumbar puncture syndrome were the most frequently reported AEs among the subjects who received YTX-7739 at any multiple dose level. Frequencies of these adverse events were comparable in the placebo group, except for dry eyes, puncture site pain and myalgia which occurred less frequently in the placebo group.

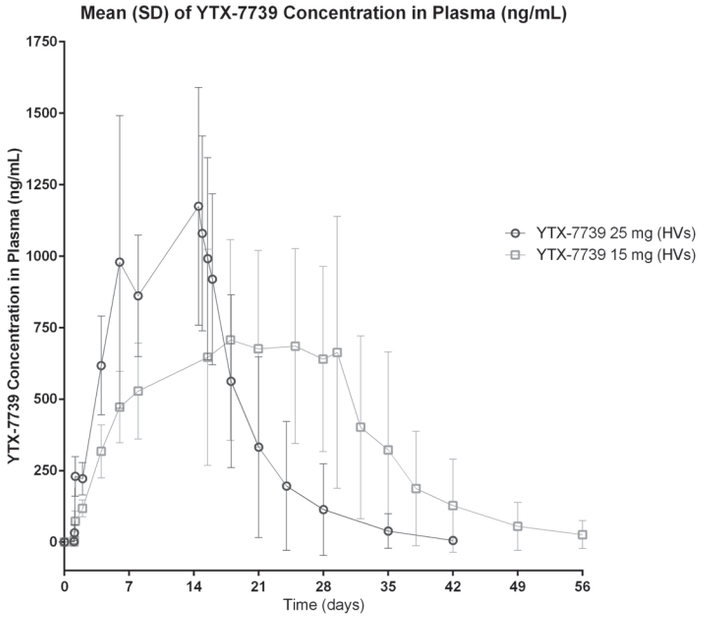
Dose level	YTX-7739 15 mg (healthy participants)		YTX-7739 25 mg (healthy participants)		Placebo (healthy participants)	
Breakfast type	High fat		High fat		Both	
Number of subjects in group	N = 6		N = 6		N = 4	
System Organ Class/	Events	Subjects	Events	Subjects	Events	Subjects
Preferred Term	N	N (%)	N	N (%)	N	N (%)
Total	44	5 (83.3)	62	6 (100.0)	15	4 (100.0)
BLOOD AND LYMPHATIC SYSTEM DISORDERS						
Anaemia			2	2 (33.3)		
EYE DISORDERS						
Asthenopia	1	1 (16.7)	5	3 (50.0)	4	3 (50.0)
Dry eye	1	1 (16.7)	3	2 (33.3)	3	1 (16.7)
Eye irritation			1	1 (16.7)		
Ocular hyperaemia					1	1 (16.7)
GASTROINTESTINAL DISORDERS						
Abdominal discomfort	3	2 (33.3)	3	2 (33.3)		
Constipation			1	1 (16.7)		
Diarrhoea	1	1 (16.7)	1	1 (16.7)		
Dry mouth	1	1 (16.7)				
Lip dry	1	1 (16.7)				
Mouth ulceration			1	1 (16.7)		
Nausea			5	2 (33.3)		
Toothache						
Vomiting			1	1 (16.7)		
GENERAL DISORDERS & ADMINISTRATION SITE CONDITIONS	6	4 (66.7)	17	5 (83.3)	1	1 (16.7)
Chest discomfort			1	1 (16.7)		
Fatigue	1	1 (16.7)	10	5 (83.3)		
Feeling cold	1	1 (16.7)	1	1 (16.7)		
Feeling hot			3	1 (16.7)		
Feeling of relaxation					1	1 (16.7)
Puncture site pain	4	4 (66.7)	2	2 (33.3)		
Vaccination site pain						
INFECTIONS AND INFESTATIONS					1	1 (16.7)
Corona virus infection					1	1 (16.7)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	1	1 (16.7)	6	3 (50.0)		

[CONTINUATION SUPPLEMENTARY TABLE 2]

Dose level	YTX-7739 15 mg (healthy participants)		YTX-7739 25 mg (healthy participants)		Placebo (healthy participants)	
Post lumbar puncture syndrome	1	1 (16.7)	6	3 (50.0)		
INVESTIGATIONS			2	2 (33.3)	2	2 (33.3)
Alanine aminotransferase increased					1	1 (16.7)
Blood bilirubin increased			2	2 (33.3)	1	1 (16.7)
METABOLISM AND NUTRITION DISORDERS	1	1 (16.7)	1	1 (16.7)		
Decreased appetite	1	1 (16.7)	1	1 (16.7)		
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	2	2 (33.3)				
Back pain	1	1 (16.7)				
Musculoskeletal pain	1	1 (16.7)				
NERVOUS SYSTEM DISORDERS	13	4 (66.7)	14	5 (83.3)	2	1 (16.7)
Dizziness					2	1 (16.7)
Dreamy state	1	1 (16.7)	2	1 (16.7)		
Dysgeusia	2	1 (16.7)				
Headache	7	3 (50.0)	10	5 (83.3)		
Paraesthesia	3	2 (33.3)				
Somnolence			1	1 (16.7)		
Syncope			1	1 (16.7)		
PSYCHIATRIC DISORDERS	1	1 (16.7)			2	2 (33.3)
Abnormal dreams	1	1 (16.7)				
Insomnia					1	1 (16.7)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	4	3 (50.0)	1	1 (16.7)	2	1 (16.7)
Dry throat	2	2 (33.3)				
Rhinorrhoea	2	2 (33.3)	1	1 (16.7)	2	1 (16.7)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	9	3 (50.0)	1	1 (16.7)		
Alopecia	1	1 (16.7)				
Dry skin	6	2 (33.3)				
Eczema			1	1 (16.7)		
Erythema	1	1 (16.7)				
Pruritus	1	1 (16.7)				
VASCULAR DISORDERS			1	1 (16.7)	1	1 (16.7)
Orthostatic hypotension			1	1 (16.7)	1	1 (16.7)

Supplementary Figures

SUPPLEMENTARY FIGURE 1 MEAN (SD) PLASMA YTX-7739 CONCENTRATION AGAINST TIME FOLLOWING MULTIPLE ORAL DOSES OF 15 AND 25 YTX-7739 IN FED STATE IN HEALTHY PARTICIPANTS. After administration of multiple doses of YTX-7739 in fed condition on a linear scale per cohort. Breakfast types were high-fat for both cohorts.



Abbreviations: SD = Standard Deviation; HVs = Healthy Volunteers

CHAPTER 5

TARGET ENGAGEMENT AND IMMUNOGENICITY OF UB-312 IN PATIENTS WITH PARKINSON'S DISEASE: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE 1 TRIAL WITH BIOMARKER ANALYSES

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ABSTRACT

Investigational therapeutics that target toxic species of alpha-synuclein aim to slow down or halt disease progression in patients with Parkinson's disease. Here this 44-week, randomized, placebo-controlled, double-blind, single-center phase 1 study investigated safety, tolerability and immunogenicity of UB-312, an active immunotherapeutic targeting pathological alpha-synuclein, in patients with Parkinson's disease. The primary outcome measures were adverse event frequency and change in anti-alpha-synuclein antibody titers in blood and cerebrospinal fluid (CSF). Exploratory outcomes were changes in clinical scales and biomarker-based target engagement as measured by seed amplification assays. Twenty patients were randomized 7:3 (UB-312:placebo) into 300/100/100 µg or 300/300/300 µg (weeks 1, 5 and 13) intramuscular prime-boost dose groups. Safety was similar across groups; adverse events were mostly mild and transient. Two patients experienced three serious adverse events in total, one possibly treatment related; all resolved without sequalae. Anti-alpha-synuclein antibodies in serum from 12/13 and CSF from 5/13 patients who received three UB-312 doses confirmed immunogenicity. Mean serum titers (in log-dilution factor) increased from baseline by 1.398 and 1.354, and peaked at week 29 at 2.520 and 2.133, for 300/100/100 µg and 300/300/300 µg, respectively. CSF titers were 0 at baseline and were 0.182 and 0.032 at week 21, respectively. Exploratory analyses showed no statistical differences in clinical scales but a significant reduction of alpha-synuclein seeds in CSF of a subset of UB-312-treated patients. These data support further UB-312 development. ClinicalTrials.gov:NCT04075318.

INTRODUCTION

Parkinson's disease (PD) is characterized by progressive deterioration of motor, cognitive, behavioral, and autonomic function.[1] Mechanisms of dopaminergic cell loss in PD are not fully understood; however, alpha-synuclein has a central role in neurodegeneration. Expressed primarily in presynaptic terminals, alpha-synuclein is involved in synaptic vesicle trafficking and modulation, and neurotransmitter regulation.[2,3] Duplications, point mutations or single nucleotide polymorphisms in SNCA, encoding alpha-synuclein, contribute to PD susceptibility; the mutated forms of the protein have altered structural configurations that promote pathological aggregation.[4,5] While such mutations are rare, alpha-synuclein aggregates in the form of Lewy bodies (LB) are common neuropathological hallmarks of PD. Moreover, preformed fibrils of alpha-synuclein can induce formation of LB-like inclusions and cellular dysfunction in cell-based assays and preclinical animal models.[3,6] Together, these data strongly suggest that targeting pathological, aggregation-prone forms of alpha-synuclein has therapeutic potential.

There are no approved disease-modifying therapies for PD. Passive and active immunotherapies targeting alpha-synuclein (that is, delivery of monoclonal antibodies or vaccination to raise an endogenous immune response, respectively) can ameliorate alpha-synuclein pathology and functional deficits in mouse models of PD, and both approaches are now in clinical development.[7–9] These approaches have exhibited promising results in phase 1 clinical trials.[7,8,10,11] Two phase 2 clinical trials recently failed to demonstrate efficacy of monoclonal antibodies against alpha-synuclein,[12,13] but as was experienced in Alzheimer's disease , early failure to demonstrate clinical efficacy does not necessarily invalidate the therapeutic target or investigational drug.[14] Trial design is crucial and elements such as appropriate patient selection, choice of clinical scales, and inclusion of relevant biomarkers require careful refinement. The importance of biomarkers to test target engagement in Alzheimer's disease drug development was illustrated with validation of amyloid positron emission tomography imaging to monitor effects on brain pathology,[15] which accelerated decision making in many anti-amyloid drug development programs. However, convincing biomarkers of target engagement to support clinical trials in patients with PD have so far been lacking.

Successful vaccination against endogenous targets requires overcoming immune tolerance to generate a humoral antibody response, while avoiding T-cell-mediated cytotoxicity; both are dependent on how the relevant epitope is presented to the immune system. A novel vaccine carrier platform utilizing proprietary synthetic T-helper peptides linked to target epitopes demonstrated potential to

achieve these aims, inducing a targeted B-cell humoral response without T-cell-mediated toxicity.[16] UB-312 was selected from over 60 synthetic peptide immunogens, and has demonstrated high immunogenicity in preclinical studies across species (unpublished data). Notably, antibodies induced by UB-312 selectively targeted pathological oligomeric and fibrillar alpha-synuclein forms, binding specifically to alpha-synuclein inclusions in postmortem brain sections from patients with PD, dementia with LBS, and multiple system atrophy (MSA).[17] Furthermore, UB-312-derived antibodies exhibited neuroprotective effects in vitro, reduced alpha-synuclein in the brain and the gut, and prevented motor function deficits in a transgenic synucleinopathy mouse model.[18]

In Part A of this phase 1 study, escalating doses of UB-312 were tested versus placebo in healthy volunteers aged 40 to 85 years, as previously reported.[19] UB-312 was considered safe and well tolerated up to 300/300/300 mg three-dose 'prime–boost' regimen, with most adverse events being mild and transient. UB-312 triggered dose- and time-dependent antibody production, with anti-alpha-synuclein antibodies detectable in both serum and cerebrospinal fluid (CSF) for all participants receiving the 300/300/300 mg prime–boost UB-312 regimen, and an average CSF/serum ratio of 0.2%. Serum and CSF alpha-synuclein concentrations were not altered by treatment, consistent with preferential binding of UB-312-derived antibodies to pathological forms of alpha-synuclein and poor binding to monomeric alpha-synuclein.[17] The 300/100/100 mg and 300/300/300 mg prime–boost dose regimens were selected based on safety and immunogenicity profile for further evaluation in patients with PD.

This phase 1 study Part B was designed to assess the safety, tolerability and immunogenicity of the two chosen UB-312 regimens in patients with PD. To address the unmet need of a biomarker for target engagement by immunotherapies in clinical trials in patients with PD, we also investigated use of the alpha-synuclein seed amplification assay (ASYN-SAA), which is able to detect small amounts of pathological alpha-synuclein and is increasingly used to support diagnosis of PD.[20] Application of alpha-synuclein-SAA to the assessment of target engagement by UB-312-induced antibodies was an exploratory biomarker endpoint. Other exploratory outcome measurements included cognitive and PD-related clinical efficacy assessment.

RESULTS

Patient disposition

Recruitment occurred from 27 October 2021 to 06 April 2022. Among 41 participants screened, 15 were ineligible based on inclusion/exclusion criteria, and four withdrew after screening (Fig. 1). Between 11 January 2022 and 27 April 2022, 21 participants were randomized to either UB-312 or placebo, and one was planned as reserve participant. One participant was excluded before the first vaccination due to an elevated C-reactive protein level. Twenty participants received the first and second injection, and 19 received the third; one participant in the 300/100/100 mg cohort did not, due to a serious adverse event (SAE). All participants completed the follow up visit. The modified intention-to-treat (MITT) population consisted of all 20 participants. The per-protocol (PP) population comprised 20 participants up to week 13; after one participant did not receive the third vaccination the PP population was 19 of 20 participants. Baseline characteristics were comparable between groups, including Hoehn and Yahr (H&Y) stage and PD duration in years (Table 1).

Primary outcomes: safety, tolerability and immunogenicity

Headache, local pain after lumbar puncture, and fatigue were the most frequently reported treatment-emergent adverse events (TEAEs) (Table 2). TEAEs appeared to occur equally after administration of UB-312 (14 of 14 participants) and placebo (5 of 6 participants) (Table 2). Most TEAEs were considered either mild or moderate after administration of UB-312, comparable to placebo. Three SAEs were reported, of which one—a deep venous thrombosis of the left leg 50 days after the second administration of UB-312 300/100/100 mg—was considered possibly related, due to the timing of onset. There were no safety signals in assessments including electrocardiogram (ECG), vital signs, and blood and urine assessments. There was no difference in either physician or participant reported tolerability within 7 days after each administration of UB-312 compared with placebo. No postvaccination brain magnetic resonance imagings (MRI) were performed.

UB-312 vaccination generated robust and time-dependent serum antibodies against the C-terminal epitope ASYN97-135, with titers peaking at week 29 and remaining greater than baseline values at week 45 (Fig. 2A).

The definition of seroconversion was met for 5 of 6 participants after administration of 300/100/100 mg, and all 7 participants after administration of 300/300/300 mg who received all three doses of UB-312, for an overall seroconversion rate of 12/13.

Epitope-specific anti-alpha-synuclein antibodies were also detectable in CSF, in a total of 5/13 participants who received all three doses; 4 of 6 in the 300/100/100 µg group and 1 of 7 in the 300/300/300 mg group. After peaking at week 21 (Fig. 2B), CSF titers at week 45 were measurable in only two participants. UB-312 vaccination did not lead to the generation of antibodies against full length alpha-synuclein compared with placebo.

Exploratory outcomes

Total scores of the Montreal Cognitive Assessment (MOCA) and the Movement Disorder Society–Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part II (MDS-UPDRS-II) and part III (MDS-UPDRS-III) were generally stable during the study, with no statistical differences between groups (Supplementary Figs. 1 and 2).

Postimmunization IgG fractions and affinity purified antibodies isolated from sera of healthy volunteers collected in Part A of this study demonstrated strong binding to aggregated alpha-synuclein (MSA-derived, PD-derived and recombinant-derived aggregates) and low binding to recombinant monomeric alpha-synuclein (Extended Data Fig. 1a). Furthermore, when spiked into a saline solution containing alpha-synuclein oligomers or into a CSF sample from a patient with PD, postimmunization IgG fractions readily altered the kinetics of alpha-synuclein aggregation (Extended Data Fig. 1b,c).

In Part B, CSF samples were collected at weeks 1 (baseline), 21 and 45, except one patient in the 300/100/100 µg group who provided CSF only at baseline, and another only at baseline and week 21. Nineteen out of 20 baseline CSF samples tested positive in the Amprion alpha-synuclein-SAA. Excluding the negative sample, the median dilution factor was 32.40 (range 197.1) (Fig. 3a), indicating for subsequent analyses an optimal dilution of 1:5 to retain positivity status and prevent natural inhibitors such as lipoproteins from interfering with the alpha-synuclein-SAA seeding kinetics21. Individual seed amplification curves obtained at baseline and end of study (EOS) are illustrated in Supplementary Figs. 3–5. The patient who provided CSF only at baseline and the patient who was negative for alpha-synuclein-SAA were not included in the final analysis.

As illustrated in Fig. 3b, the maximum fluorescence (F_{max}) assessed longitudinally indicated a significant change from baseline (CFB) ($F=6.622$ (1.541–22.35), $P=0.009$), with placebo showing a nonsignificant 2.8% increase, UB-312 (300/100/100 µg) showing a significant 19.8% decrease ($P<0.05$) and UB-312 (300/300/300 µg) showing a nonsignificant 15.2% decrease at week 45. At week 45, F_{max} was significantly lower in patients treated with UB-312 300/100/100 µg versus placebo ($P<0.05$). Interestingly, from a qualitative standpoint, one patient in the 300/300/300 µg group was alpha-synuclein-SAA positive at baseline and end of treatment, but alpha-synuclein-SAA negative at the end of study.

Post hoc analyses

An unplanned post hoc analysis to evaluate whether the reduction of F_{max} was related to CSF antibody titers (Fig. 3c,d) showed it was indeed more pronounced and statistically significant in individuals with detectable CSF antibody titers (as measured at week 21, n=5) than those without (n=13) at week 21 (time effect: $F=12.77$ (1.73–26.82), $P=0.0002$; treatment×time effect: $F=6.755$ (2–31), $P=0.0037$). Interestingly, a significant difference in CSF levels of pS129-alpha-synuclein was also observed between patients with or without detectable CSF antibodies (Fig. 4), further supporting an effect of UB-312 on pathological alpha-synuclein. Similarly, while there was no significant difference between treatment groups on the MDS-UPDRS-II and MDS-UPDRS-III (Supplementary Fig. 2), CFB on the MDS-UPDRS-II scale indicated a statistically significant improvement in individuals with detectable CSF antibody titers compared with other patients ($F=12.94$ (1.569–24.32), $P=0.0004$; treatment×time effect: $F=4.739$ (2–31), $P=0.016$; Fig. 3e,f).

DISCUSSION

This phase 1 Part B trial in patients with PD met its primary prespecified outcomes and showed that UB-312 was generally safe and well tolerated, and generated a time-dependent increase in anti-alpha-synuclein antibodies in both serum and CSF in patients with PD. No substantial differences in TEAEs were observed between the UB-312 groups and the placebo group, with most TEAEs being transient and self-resolving.

In healthy volunteers, UB-312 was generally safe and well tolerated up to a dose regimen of 300/300/300 mg without SAEs.[19] In the higher dose regimens in healthy volunteers, influenza-like symptoms were observed in two participants.[19] These events were not observed in patients with PD. The frequency and intensity of TEAEs were comparable between healthy volunteers and patients with PD, and similar TEAEs seemed to occur in both populations (headache, lumbar puncture site pain and fatigue). Local injection-site reactions did not increase after subsequent administrations, and no severe local reactions were observed (as seen previously with other active immunotherapies targeting alpha-synuclein.[7,8,10,22] One participant reported a deep venous thrombosis 50 days after administration of a second dose of UB-312 for which no etiological cause could be found, and which was therefore considered possibly related to study drug. Nevertheless, this venous thrombo-embolism does not follow the venous thrombo-embolism events observed after other vaccinations, and therefore may not be related.[23–25] Further research should investigate the potential relationship.

In both healthy volunteers and patients with PD, UB-312 vaccination generated similar time-dependent serum antibodies against the C-terminal epitope. In healthy volunteers, antibody levels were similar to those achieved in other active and passive immunization studies (i.e., typically requiring serum antibody concentrations

of ~10–20 mg/mL for target engagement).[7,19] The levels were lower in patients with PD. Potentially, this difference could be attributed to comorbidities,[26,27] but a more likely cause may be that redistribution of antibodies to pathological tissues and target-mediated clearance differs in patients with PD, compared with healthy volunteers, which has been demonstrated in preclinical studies.[18,28] That is, UB-312 does not induce antibodies against normal alpha-synuclein. Higher levels of UB-312-induced antibodies in healthy volunteers could therefore result from lack of target-mediated clearance in this population, in comparison with patients with PD in whom aggregated alpha-synuclein is present in both peripheral and central nervous system tissues. The elicited antibodies could conceivably be sequestered in pathological peripheral tissues after leaving the blood. Another possibility is that patients with PD may have a compromised immune system less able to respond to immunization,[29] which is supported by a recent report suggesting that B-cell numbers in peripheral blood are reduced in patients with PD.[30] This potential alteration in immune response will be investigated in future clinical development studies in patients with PD.

Dosing regimens are less straightforward for active compared with passive immunotherapies. Rather than escalating based solely on total amount of drug product administered over time, factors including time between administrations and the relationship between prime–boost doses can also be varied. The dose-response observed in Part A suggested that a low prime dose followed by high boost doses may be less immunogenic.[19] In Part B, the two selected regimens had the same high prime dose, followed by two different boost-dose regimens. Interestingly, the high prime dose followed by lower boost doses seemed to be more immunogenic, albeit not statistically significant. Clearly, further investigations of different doses and additional boosts will be required to better understand the pharmacological response to an active immunization against endogenous proteins.

We also investigated the use of ASYN-SAA. Originally developed to detect small amounts of pathological misfolded proteins in biological samples,[20] ASYN-SAA leverages the intrinsic self-replicative nature of misfolded alpha-synuclein aggregates (alpha-synuclein-seeds), which can initiate polymerization of monomeric protein. Study samples are combined with excess monomeric alpha-synuclein and subjected to cycles of fragmentation and elongation, which can amplify the biomarker to detectable levels.[31] ASYN-SAA has demonstrated excellent sensitivity and specificity for detection of pathological forms of alpha-synuclein in CSF from patients with PD,[32–34] can discriminate CSF samples from patients with PD, patients with MSA and healthy individuals,[35] and is becoming progressively accepted as a supporting tool for diagnosis of PD.

To our knowledge, this is the first report showing a positive effect of an investigational therapy on the ASYN-SAA signal. UB-312-induced antibodies, purified from immunized healthy volunteers, preferentially bound aggregated alpha-synuclein and delayed the seeding kinetics of alpha-synuclein whether in a CSF sample from a patient with PD or in a solution containing recombinant aggregates of alpha-synuclein. We found that active immunization with UB-312 was associated with reductions in the ASYN-SAA signal in CSF as measured by F_{max}, which can be interpreted as a sign of *in vivo* target engagement. The reduction indicates that the Alpha-synuclein seeds are not recruiting the whole pool of alpha-synuclein monomers that were spiked in, and could be the result of reduced number of alpha-synuclein seeds, type of seeds, and/or a reduction of a specific pool of seeds more prone to aggregate. The F_{max} readings were stable between triplicate measurements from individual patients (Supplementary Figs. S4–S6). Interestingly, one patient who had ASYN-SAA positivity at baseline no longer did at end of study, after treatment with UB-312 300/300/300 mg. Moreover, that the MDS-UPDRS-II scores also improved in patients with CSF antibody titers, in line with the change in ASYN-SAA signal, is intriguing, though anecdotal given the small cohort size. A much larger trial will be required to show clinical efficacy. It is also important to note that ASYN-SAA has not yet been validated as a quantitative measure, and the rate of conversion from a reduction in F_{max} to reduction in misfolded synuclein is unknown, as fluorescence is measured in arbitrary units. However, in the absence of a valid biomarker of target engagement, these data suggest a potential route to quantifiable assessment of an effect of treatment on pathology in future clinical trials, using an assay that is already increasingly deployed in a diagnostic setting.

There are several limitations to this study. First, the sample size was small; 13 patients with PD completed vaccination with UB-312. However, it is not an abnormally low number for a first-in-patient study.[36,37] Second, demographic characteristics were comparable between groups, and all patients were on stable medication for PD before enrolling. Nevertheless, as the study aimed to determine antibody responses to vaccination, this relatively homogeneous population was deemed suitable. Patients were not screened for ASYN-SAA positivity or genetic predispositions, biomarkers that might facilitate selection of a relevant patient population for alpha-synuclein-targeted therapies. We also did not evaluate lipoprotein levels in the CSF to confirm stability and lack of interference with alpha-synuclein aggregation;[21] however, the dilution factor of 1:5 was sufficient to avoid this effect, and it is unlikely that any CSF constituents could have influenced the signal only at the end of the study. Additionally, CSF antibodies could be detected using a titer assay;

however, CSF antibody concentrations were all below the lower limit of quantification. A difference in processing or analysis method could potentially contribute to this discrepancy between the two assays. Either way, our results suggest that antibodies in CSF must reach detectable levels to have an effect on ASYN-SAA. Future dose optimization will be needed to achieve greater CSF antibody titer exposures and to confirm the effects of UB-312 on ASYN-SAA.

UB-312 is not the only immunotherapy targeting alpha-synuclein to enter clinical development in PD; clinical data have been published for active and passive immunotherapies.[8,11,12,13] Compared with passive immunization therapy, which typically requires frequent administration at high costs, vaccination is likely to offer greater accessibility and convenience, requiring administration only every 3 – 12 months after the priming regimen to sustain an antibody response.[38] This minimized burden of administration and visits to a clinic is particularly advantageous for patients with advanced PD. Regardless of approach, this study of UB-312, which is to be corroborated in future phases of clinical development, is the first to assess an immunotherapeutic effect on pathological forms of alpha-synuclein as measured by ASYN-SAA.

In conclusion, this first-in-patient trial of an active immunotherapeutic targeting aggregated alpha-synuclein in patients with PD met its primary outcomes of safety, tolerability and immunogenicity. UB-312 was observed to safely overcome immune tolerance, inducing antibodies specific against pathological forms of alpha-synuclein and importantly able to cross the blood brain barrier. The results are consistent with conclusions from Part A of the phase 1 study, and with preclinical studies. Together, these data support continued development of UB-312 as a disease-modifying treatment for PD. Future trials should focus on optimizing dose and antibody exposure in CSF over longer treatment periods, and further assess the safety and efficacy of UB-312 in synucleinopathies.

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FIGURE 1 PATIENT DISPOSITION. Enrolled patients were randomized to placebo (n=6), UB-312 300/100/100 µg (n=7) and UB-312 300/300/300 µg (n=7) treatment groups. The PP population comprised 20 participants up to week 13 and 19 thereafter.

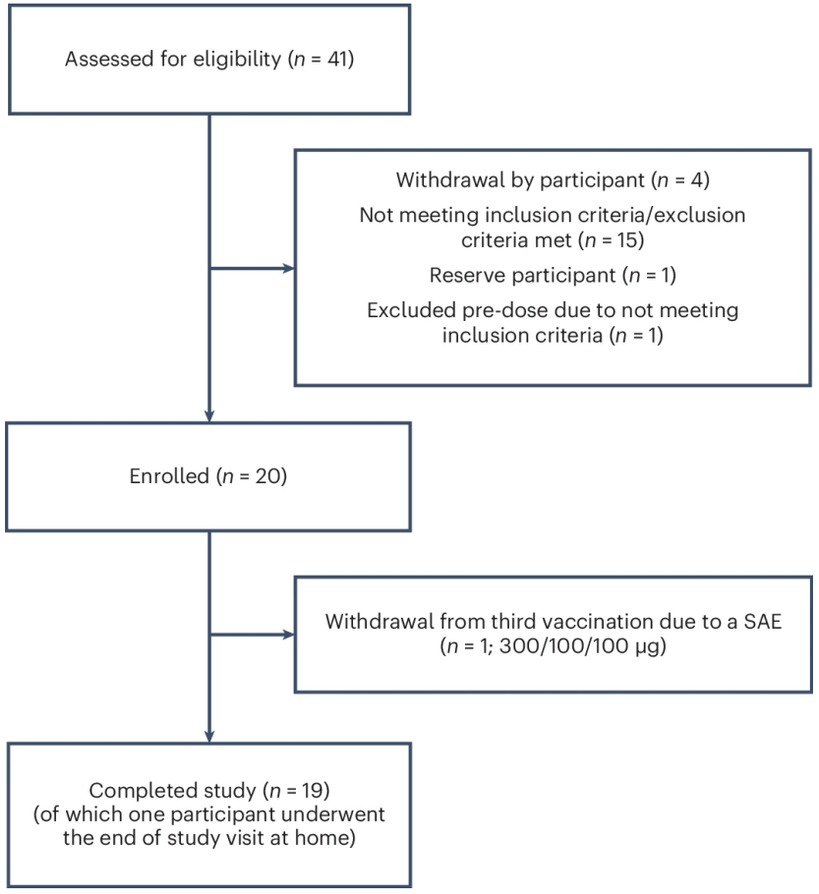
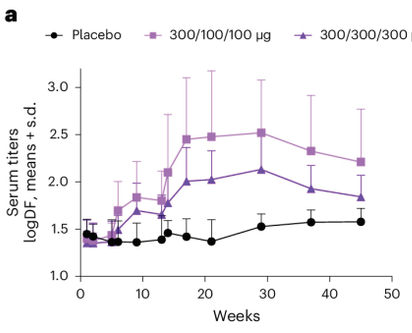
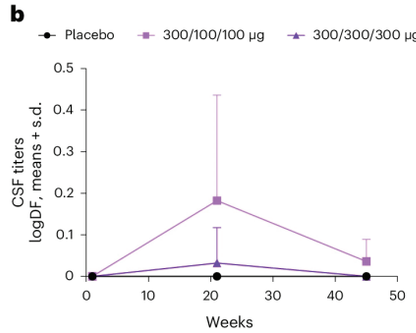


FIGURE 2 EPITOPE-SPECIFIC ANTI-ALPHA-SYNUCLEIN ANTIBODY TITERS IN BLOOD AND CSF. UB-312 or placebo were administered at weeks 1, 5 and 13. a, Serum antibody titers increased predominantly after the third vaccination. The definition of seroconversion was met in 12/13 participants receiving all three doses (5/6 in 300/100/100 mg group and 7/7 in 300/300/300 mg group). Increases were more pronounced in the 300/100/100 mg treatment cohort compared to the 300/300/300 mg treatment cohort. Samples were analyzed in duplicate and data are expressed as logDF mean+s.d. b, CSF antibody titers were more pronounced in the 300/100/100 µg treatment group compared with the 300/300/300 µg cohort; levels were detectable in 4/6 and 1/7 participants, respectively. Samples were analyzed in duplicate and data are expressed as logDF mean+s.d. Numbers per group: placebo, n=6; 300/100/100 µg, n=7 up to week 13 and thereafter n=6 until week 45; 300/300/300 µg, n=7. logDF, log-dilution factor.





	Serum titers, logDF mean (s.d.)		
Cohort	Baseline	Week 29 (peak)	Week 45
300/100/100 µg	1.398 (0.079)	2.520 (0.557)	2.212 (0.561)
300/300/300 µg	1.354 (0.243)	2.133 (0.366)	1.842 (0.228)
Placebo	1.448 (0.156)	1.528 (0.134)	1.578 (0.142)

	CSF titers, logDF mean (s.d.)		
Cohort	Baseline	Week 21	Week 45
300/100/100 µg	0 (0)	0.182 (0.253)	0.036 (0.053)
300/300/300 µg	0 (0)	0.032 (0.085)	0 (0)
Placebo	0 (0)	0 (0)	0 (0)

FIGURE 3 EVALUATION OF ALPHA-SYNUCLEIN SEEDS IN CSF SAMPLES FROM PATIENTS WITH PD. a, CSF samples collected before treatment (baseline) were serially diluted and tested in the alpha-synuclein-SAA (n=20). Data presented are maximal dilution factor achieved before losing the signal in the alpha-synuclein-SAA; the bar represents median±95% CI. b, Maximum fluorescence (%CFB, mean±s.e.m.) in CSF samples collected at baseline, week 21 and week 45 in patients treated with placebo (n=6), UB-312 300/100/100 µg (n=6, one patient provided CSF only at baseline and is excluded) or UB-312 300/300/300 µg (n=6, one individual who was alpha-synuclein-SAA negative at baseline is excluded) showed a significant difference between placebo and 300/100/100 µg (two-way ANOVA, time×treatment interaction, P=0.0343). c, A comparison of the maximum fluorescence (%CFB, mean±s.e.m.) in CSF samples collected at baseline, week 21 and week 45 showed a significant difference (two-way ANOVA, time×treatment interaction, P=0.0037) between individuals with (n=5) versus without (n=13) detectable antibody titers. d, Maximum fluorescence at EOS (%CFB means of three technical replicates per individual; bar represents median±95% CI) shows a significant between-group difference (two-tailed unpaired t-test, P=0.0094). e, A comparison in CFB (mean±s.e.m.) in MDS-UPDRS-II score showed a significant difference (unpaired t-test, time×group interaction, P=0.016) between patients with (n=5) versus without (n=13) detectable CSF antibody titers. The subcomponent that showed the greatest difference in this change was 'Getting out of bed, getting out of a car or standing up from a low chair' (Supplementary Table 1). A two-way ANOVA with a mixed-effect model was followed by within-group analysis per Benjamini, Krieger and Yekitieli. f, A comparison in the CFB (mean±s.e.m.) in MDS-UPDRS-III score between patients with (n=5) versus without (n=13) detectable CSF antibody titers. A two-way ANOVA was used as per e. In a–f, *P<0.05 and **P<0.01. CI, confidence interval.

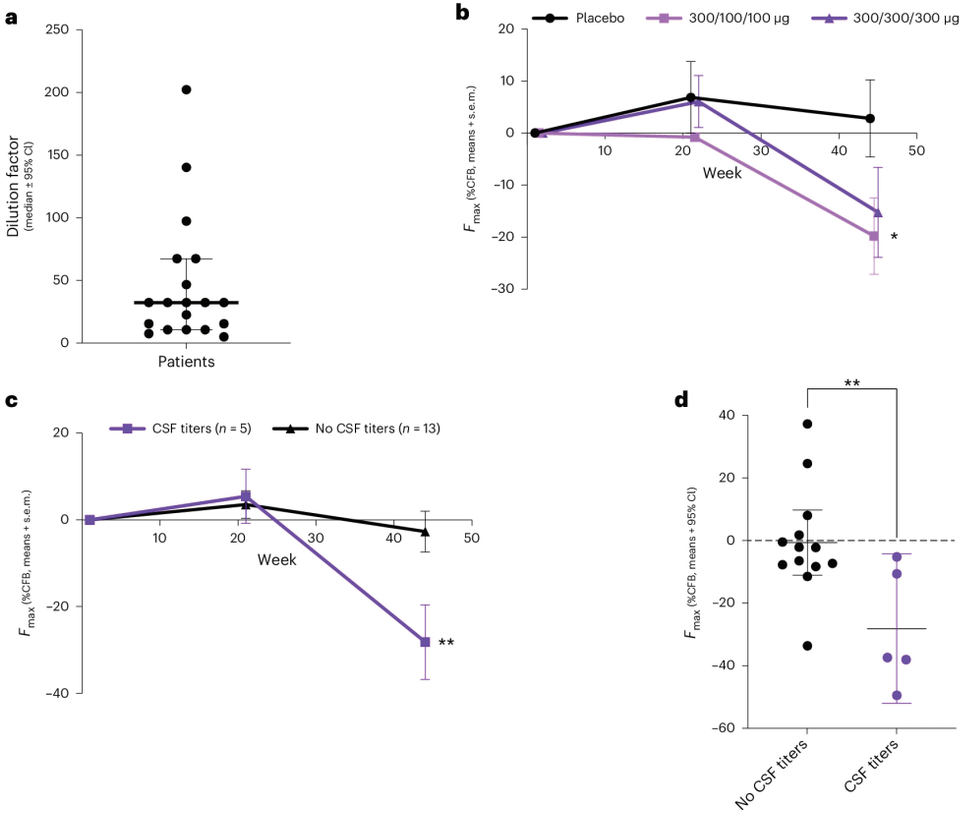


FIGURE 4 LEVELS OF PS129-ALPHA-SYNUCLEIN IN THE CSF OF PATIENTS WITH DETECTABLE CSF ANTIBODIES AFTER IMMUNIZATION WITH UB-312. CSF samples were analyzed in duplicate using immunomagnetic reduction as described in the methods, in patients with (n=5) and without (n=13) detectable CSF antibody titers. Data are expressed as percent CFB, mean±s.e.m. A Bonferroni multiple comparisons test confirmed a significant difference between groups at EOS, *P=0.0154.

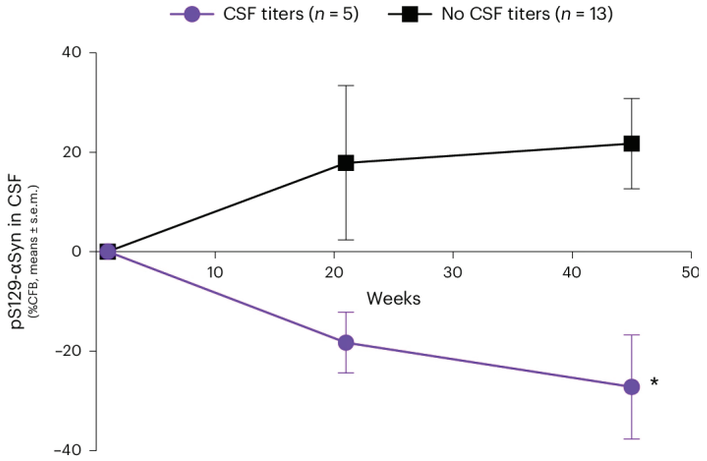


TABLE 1 DEMOGRAPHIC BASELINE DATA IN ALL PARTICIPANTS AND PER TREATMENT COHORT. Baseline characteristics were comparable between study groups, including H&Y stage and duration of PD. aMeasured prevaccination on day 1.

	All participants	UB-312 300/100/100 mg	UB-312 300/300/300 mg	Placebo
N	20	7	7	6
AGE, YEARS				
Mean (SD)	64.1 (9.9)	67.4 (12.3)	63.4 (9.0)	61.0 (8.0)
Min; Max	44; 83	44; 83	50; 75	51; 73
HEIGHT, CM				
Mean (SD)	177.8 (7.9)	177.4 (4.8)	176.1 (10.3)	180.1 (8.4)
Min; Max	159.8; 192.2	169.4; 182.0	159.8; 188.7	166.1; 192.2
WEIGHT, KG				
Mean (SD)	79.7 (10.5)	81.5 (10.3)	75.6 (11.1)	82.3 (10.3)
Min; Max	62.0; 101.6	69.3; 101.6	62.0; 96.3	66.2; 90.9
BMI, KG/M^2				
Mean (SD)	25.2 (2.9)	25.9 (2.6)	24.4 (3.1)	25.4 (3.1)
Min; Max	19.7; 30.7	22.8; 30.7	20.8; 29.4	19.7; 28.1
SEX, N (%)				
Female	4 (20.0)	1 (14.3)	2 (28.6)	1 (16.7)
Male	16 (80.0)	6 (85.7)	5 (71.4)	5 (83.3)
RACE, N (%)				
White	20 (100)	7 (100)	7 (100%)	6 (100)
HOEHN & YAHR,* N (%)				
0 (Asymptomatic)	0 (0)	0 (0)	0 (0)	0 (0)
1 (Unilateral involvement only)	2 (10.0)	0 (0)	2 (28.6)	0 (0)
2 (Bilateral involvement without balance impairment)	16 (80.0)	7 (100)	4 (57.1)	5 (83.3)
3 (Mild to moderate involvement)	2 (10.0)	0 (0)	1 (14.3)	1 (16.7)
MDS-UPDRS PART II TOTAL SCORE*				
Mean (SD)	10.5 (6.23)	14.1 (6.77)	8.4 (5.38)	8.5 (5.32)
Min; Max	1; 27	6; 27	1; 15	1; 16
MDS-UPDRS PART III TOTAL SCORE*				
Mean (SD)	33.5 (15.37)	37.1 (14.72)	37.4 (19.92)	24.7 (5.28)
Min; Max	8; 60	23; 62	8; 60	18; 31
MOCA TOTAL SCORE*				
Mean (SD)	27.1 (2.17)	26.9 (2.12)	27.0 (2.71)	27.5 (1.87)
Min; Max	21; 30	23;29	21; 29	25; 30
PARKINSON DURATION, YEARS				
Mean (SD)	6.8 (4.3)	7.4 (3.8)	7.6 (5.4)	5.0 (3.5)
Min; Max	1; 16	2;13	2; 16	1; 11
ANTI-PARKINSON DRUGS, N (%)				
Levodopa	18 (90.0)	7 (100)	5 (71.4)	6 (100)
Dopamine agonists	10 (50.0)	5 (71.4)	3 (42.9)	2 (33.3)
MAO-B inhibitors	0 (0)	0 (0)	0 (0)	0 (0)

**Measured pre-vaccination on day 1 / BMI, Body Mass Index; MAO-B, monoamine oxidase B inhibitor; MDS-UPDRS, Movement Disorder Society – Unified Parkinson's Disease Rating Scale; MoCA, Montreal Cognitive Assessment; SD, Standard Deviation.*

TABLE 2 SUMMARY OF ALL TEAES PER TREATMENT COHORT AND POOLED UB-312 COHORTS. Specific TEAEs are listed by total number of events (N), as well as number and percentage (N (%)) of participants reporting the specific adverse event. Headache, local pain after lumbar puncture, and fatigue were the most frequently reported TEAEs after vaccination with UB-312. TEAEs appeared to occur equally after administration of UB-312 (14 of 14 participants) and placebo (5 of 6 participants).

	UB-312						Placebo	
Treatment cohort	**300/100/100 mg**		**300/300/300 mg**		**Pooled**			
Number of participants	**7**		**7**		**14**		**6**	
System Organ Class/ Preferred Term	**Events**	**Partici-pants**	**Events**	**Partici-pants**	**Events**	**Partici-pants**	**Events**	**Partici-pants**
	N	N (%)	N	N (%)	N	N (%)	N	N (%)
ANY EVENTS	59	7 (100.0)	42	7 (100.0)	101	14 (100.0)	20	5 (83.3)
CARDIAC DISORDERS	1	1 (14.3)	2	1 (14.3)	3	2 (14.3)	2	1 (16.7)
Arrhythmia	..	..	..	..	..	..	1	1 (16.7)
Atrial fibrillation	..	..	..	..	..	..	1	1 (16.7)
Palpitations	1	1 (14.3)	2	1 (14.3)	3	2 (14.3)	..	..
EYE DISORDERS	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Vision blurred	1	1 (14.3)	..	..	1	1 (7.1)	..	..
GASTROINTESTINAL DISORDERS	2	2 (28.6)	2	1 (14·3)	4	3 (21·4)	1	1 (16.7)
Diarrhea	2	2 (28.6)	1	1 (14.3)	3	3 (21.4)	..	..
Nausea	..	..	1	1 (14.3)	1	1 (7.1)	1	1 (16.7)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	8	4 (57.1)	4	2 (28.6)	12	6 (42.9)	5	3 (50.0)
Chest pain	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Fatigue	4	3 (42.9)	1	1 (14.3)	5	4 (28.6)	2	1 (16.7)
Injection site pain	3	2 (28.6)	1	1 (14.3)	4	3 (21.4)	3	2 (33.3)
Malaise	..	..	2	1 (14.3)	2	1 (7.1)	..	..
INFECTIONS AND INFESTATIONS	9	4 (57.1)	10	5 (71.4)	19	9 (64.3)	1	1 (16.7)
Acute sinusitis	..	..	1	1 (14.3)	1	1 (7.1)	..	..
COVID-19	2	2 (28.6)	2	2 (28.6)	4	4 (28.6)	..	..
Gastroenteritis	..	..	1	1 (14.3)	1	1 (7.1)	..	..
Pharyngitis	..	..	1	1 (14.3)	1	1 (7.1)	..	..
Pneumonia	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Post-acute COVID-19 syndrome	1	1 (14.3)	1	1 (14.3)	2	2 (14.3)		
Respiratory tract infection	..	..	1	1 (14.3)	1	1 (7.1)	..	..
Rhinitis	..	..	1	1 (14.3)	1	1 (7.1)	..	..
Upper respiratory tract infection	2	2 (28.6)	1	1 (14.3)	3	3 (21.4)	1	1 (16.7)
Urinary tract infection	1	1 (14.3)	1	1 (14.3)	2	2 (14.3)	..	..
Urosepsis	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Viral infection	1	1 (14.3)	..	..	1	1 (7.1)	..	..
INJURY. POISONING AND PROCEDURAL COMPLICATIONS	8	6 (85.7)	1	1 (14.3)	9	7 (50)	3	2 (33.3)
Clavicle fracture	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Concussion	1	1 (14.3)	..	..	1	1 (7.1)	1	1 (16.7)

[CONTINUATION TABLE 2]

	UB-312						Placebo	
Treatment cohort	**300/100/100 mg**		**300/300/300 mg**		**Pooled**			
Number of participants	**7**		**7**		**14**		**6**	
System Organ Class/ Preferred Term	**Events**	**Partici-pants**	**Events**	**Partici-pants**	**Events**	**Partici-pants**	**Events**	**Partici-pants**
Muscle strain	..	..	..	..	..	..	1	1 (16.7)
Post lumbar puncture syndrome	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Local pain after lumbar puncture	4	4 (57.1)	1	1 (14.3)	5	5 (35·7)	1	1 (16.7)
Skin laceration	1	1 (14.3)	..	..	1	1 (7.1)	..	..
INVESTIGATIONS	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Blood pressure systolic increased	1	1 (14.3)	..	..	1	1 (7.1)	..	..
METABOLISM AND NUTRITION DISORDERS	..	..	1	1 (14.3)	1	1 (7.1)	..	..
Hypercholesterolemia	..	..	1	1 (14.3)	1	1 (7.1)	..	..
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	8	2 (28.6)	2	2 (28.6)	10	4 (28.6)	..	..
Myalgia	7	2 (28.6)	2	2 (28.6)	9	4 (28.6)	..	..
Plantar fasciitis	1	1 (14.3)	..	..	1	1 (7.1)	..	..
NERVOUS SYSTEM DISORDERS	14	6 (85.7)	13	7 (100.0)	27	13 (92.9)	4	4 (66.7)
Carpal tunnel syndrome	..	..	..	..			1	1 (16.7)
Dizziness	2	2 (28.6)	1	1 (14.3)	3	3 (21.4)	..	..
Headache	10	5 (71.4)	10	6 (85.7)	20	11 (78.6)	3	3 (50.0)
Presyncope	1	1 (14.3)	1	1 (14.3)	2	2 (14.3)	..	..
Sedation	..	..	1	1 (14.3)	1	1 (7.1)	..	..
Somnolence	1	1 (14.3)	..	..	1	1 (7.1)	..	..
PSYCHIATRIC DISORDERS	1	1 (14.3)	2	2 (28.6)	3	3 (21.4)	..	..
Insomnia	1	1 (14.3)	1	1 (14.3)	2	2 (14.3)	..	..
Stress	..	..	1	1 (14.·3)	1	1 (7.1)	..	..
RENAL AND URINARY DISORDERS	..	..	..	..	..	..	1	1 (16.7)
Renal cyst	..	..	..	..	..	..	1	1 (16.7)
RESPIRATORY THORACIC AND MEDIASTINAL DISORDERS	1	1 (14.3)	2	2 (28.6)	3	3 (21.4)	1	1 (16.7)
Cough	..	..	1	1 (14.3)	1	1 (7.1)	..	..
Dyspnea	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Epistaxis	..	..	..	..			1	1 (16.7)
Sneezing	..	..	1	1 (14.3)	1	1 (7.1)	..	..
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	2	1 (14.3)	..	..	2	1 (7.1)	1	1 (16.7)
Erythema	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Photosensitivity reaction	..	..	..	..	..	..	1	1 (16.7)
Rash	1	1 (14.3)	..	..	1	1 (7.1)	..	..
VASCULAR DISORDERS	3	3 (42.9)	3	3 (42.9)	6	6 (42.9)	1	1 (16.7)
Deep vein thrombosis	1	1 (14.3)	..	..	1	1 (7.1)	..	..
Orthostatic hypotension	..	..	3	3 (42.9)	3	3 (21.4)	1	1 (16.7)
Peripheral venous disease	2	2 (28.6)	..	..	2	2 (14.3)	..	..

METHODS

Study Design

This was a phase 1, single-center, randomized, double-blind, placebo-controlled clinical trial (Part B) conducted at the Centre for Human Drug Research (CHDR), the Netherlands, in accordance with the Declaration of Helsinki and International Council for Harmonisation Good Clinical Practice guidelines. Independent ethics approval for the protocol was granted by the Beoordeling Ethiek Biomedisch Onderzoek, Assen, the Netherlands, and all participants provided written informed consent. Participants received an allowance for participation and were reimbursed for travel expenses. There were no important protocol changes after trial commencement. Samples from the healthy volunteer cohort from Part A of the study (methods previously published[19]) were used in biomarker analyses. Study details are available at ClinicalTrials.gov, NCT04075318.

Participants

Recruitment to Part B used an existing database and advertisements. Males (n=16) and females (n=4) (sex/gender was determined based on self-report and collected on the case report form) with a diagnosis of PD (as confirmed by a treating general practitioner or neurologist, and including dopaminergic deficiency (see below)), H&Y Stage ≤III at screening, aged 40–85 years, with a body mass index (BMI) of 18–32 kg m−2, who were postmenopausal, surgically sterile or using adequate contraception, with no clinical abnormalities based on medical history, physical examination, clinical laboratory evaluations and 12-lead ECGs were eligible if they were expected to be able to undergo all study procedures. As part of the screening, a baseline MRI was performed to exclude patients with structural brain abnormalities, and a dopamine transporter (DAT) scan was performed if no historic DAT scan was available to confirm the loss of dopaminergic activity as part of the PD diagnosis. Participants were allowed to use concomitant medication for PD or other comorbidities if the regimen was stable before first injection (from 30 days before first study drug administration for permitted antiparkinsonian medications or 60 days for monoamine oxidase B inhibitor (MAO-B) inhibitors), and expected to remain stable throughout the study. An unscheduled MRI could be requested per the investigator's judgment for safety evaluation. Participants were excluded if they had clinical abnormalities or history of medical, neurological or psychiatric conditions that the investigator judged might compromise their safety or scientific value of the study; acute or chronic infection with human immunodeficiency virus, hepatitis C virus or hepatitis B virus; history or evidence of

an autoimmune disorder; history of anergy; and history of allergy or any confirmed allergic reactions. Participants were also ineligible if they had participated in any clinical trial with monoclonal antibodies or vaccines directed at alpha-synuclein, had any other known or suspected cause of Parkinsonism besides idiopathic PD; had history or evidence at screening of PD-related freezing episodes, falls or orthostatic hypotension; had a DAT scan inconsistent with dopamine transporter deficit; or had any neurological disease other than PD.

Randomization and blinding

Eligible participants were randomized by code (SAS version 9.4) to UB-312 or placebo within treatment cohort 1 (300/100/100 µg or placebo), cohort 2 (300/300/300 µg) or placebo) by an independent statistician, without restrictions or stratifications, in consecutive order and numbered according to treatment cohort. Both cohorts consisted of ten participants and were randomized 7:3 (UB-312:placebo). Individual randomization codes were placed in a single sealed envelope, labeled 'emergency decoding 'envelopes' and kept in a safe cabinet at the clinical site.

Syringes with either UB-312 or placebo were prepared by an independent, unblinded pharmacist at the Leiden University Medical Centre. Both had an identical white, opaque appearance. Participants and clinical staff at the site were blinded to the treatment during the clinical conduct of the study.

Procedures

UB-312 or placebo was administered intramuscularly in the deltoid muscle on weeks 1, 5 and 13, and return visits were planned on weeks 2, 6, 9, 14, 21 (considered end of treatment), 29, 37 and 45 (considered EOS). A review of concomitant medication, TEAEs and vital signs were done at every visit. Physical and neurological assessments were done at weeks 1, 5, 13, 21, 29, 37 and 45. A triplicate of ECG was done at weeks 1, 5 and 13 (both 45 min preadministration, as well as 6 h postadministration) and week 21. Safety blood and urine tests including full blood count, coagulation, electrolytes, liver and renal function, C-reactive protein and erythrocyte sedimentation rate were assessed at weeks 1, 5, 13, 17 and 21, and if applicable, all results were available before the administration. A pregnancy test was done at weeks 1, 5 and 13 (all preadministration). A urine drug test and alcohol breath test were done at week 1 only, preadministration. A safety telephone contact was conducted the day after each administration of UB-312 or placebo.

CSF was sampled at weeks 1 (preadministration), 21 and 45, and was used both to assess safety as well as free and total alpha-synuclein and anti-alpha-synuclein antibodies. CSF was collected using CHDR standard operating procedures, processed on ice, analyzed for white and red blood cell counts, protein, glucose, albumin and hemoglobin within 2 h of collection, and discarded if red blood cells were present. Within 60 min of collection, CSF samples were aliquoted and frozen immediately on dry ice, and then stored upright at −80 °C. Antibodies against full length alpha-synuclein and against the C-terminal epitope, alpha-synuclein antibodies against components of the vaccine (CpG1 and the T-helper peptide) and free and total alpha-synuclein in blood were sampled at every study visit. Cytokines in blood were sampled at weeks 1, 5, 13 and 21. T cell enzyme-linked immunosorbent spot in peripheral blood mononuclear cells was sampled at weeks 1 and 17. Human leukocyte antigen in blood was sampled at week 1.

The MOCA and the MDS–UPDRS parts II and III, including H&Y classification, were done at weeks 1 (preadministration), 21 and 45. An individual H&Y classification was also conducted at screening. All MDS–UPDRS assessments were performed in the ON state. Before the actual assessments, the assessor confirmed verbally with the patient if the patient was indeed in the ON state.

Participants were provided with a paper diary for self-recording of solicited local vaccination-site reactions (that is, pain, tenderness, erythema/redness and induration/swelling) and systemic reactions (that is, fever, nausea/vomiting, diarrhea, headache, fatigue, myalgia and illness) during a 7-day period after each administration.

Outcomes

The primary endpoints were to evaluate the safety and tolerability as determined by the assessment of TEAEs, safety blood and urine tests, neurological and physical examinations, ECG, and immunogenicity as determined by anti-alpha-synuclein antibodies in blood and CSF. The exploratory objectives for the study including Part A were to determine the immunogenicity of UB-312 against components of the vaccine, and differences in total alpha-synuclein and free alpha-synuclein in blood and CSF, while exploratory outcomes specific to Part B comprised effects on MDS-UPDRS and MOCA, and target engagement by ASYN-SAA. Bioanalytical and biomarker methods were previously fully described.[19]

Antibody purification

Sera collected pre- or postimmunization from the healthy volunteer cohort were pooled using 200 µl per sample. Protein A plus spin columns (Thermo Fisher, 89960) were equilibrated with protein A IgG binding buffer (Thermo Fisher, 21001) at room

temperature followed by centrifugation at 1,000g for 1 min. Sera samples were applied to the column and incubated at room temperature with end-over-end mixing for 10 min. Columns were then placed in a new 50 ml collection tube and centrifuged for 1 min at 1,000g. Columns were then washed 3× by adding 10 ml of binding buffer and centrifuged for 1 min. Next, 500 µl of neutralization buffer (Thermo Fisher, 89948) was added to three 50 ml collection tubes. Columns were then placed into one of the collection tubes and 5 ml of elution buffer (Thermo Fisher, 21004) was added to the column and centrifuged for 1 min into the first of the three collection tubes with neutralization buffer. Spin columns were transferred to another tube that contains neutralization buffer, saving the collected solution as the first elution fraction. These steps were repeated to obtain three fractions. Pooled IgG fractions were then buffer exchanged and concentrated using an Amicron Ultra-15 centrifugal filter 50 kDa molecular weight cutoff (Millipore Sigma, UFC905024) per the manufacturer's instructions. For affinity purification, epitope-specific peptide-linked columns were washed with wash buffer 3× at room temperature. Sample IgG fractions were then added and incubated at room temperature, gently mixing end-over-end. Immunodepleted samples were then collected and set aside to evaluate potential residual binding efficiency. The column was then washed 6× with 2 ml of wash buffer. Samples were eluted by centrifuging into a clean tube with neutralization solution 5× with 2 ml of elution buffer. Affinity purified antibodies were then buffer exchanged as described above for IgG fractions.

Seed amplification assay

The alpha-synuclein-SAA methodology was performed according to Shahnawaz et al.[34] Briefly, human CSF samples (40 µl) were added to the wells of an opaque 96-well plate (Costar, 3916). Thereafter, seed-free alpha-synuclein at a concentration of 1 mg ml−1 in 100 mM piperazine-N,N'-bis(2-ethanesulfonic acid (PIPES), pH 6.5 and 500 mM NaCl was added to each well in the presence of 5 µM of thioflavin T at a final volume of 200 µl. Samples were subjected to cyclic agitation (1 min at 500 r.p.m. followed by 29 min without shaking) at 37 °C on a SpectraMax Gemini EM Microplate Reader (Molecular Devices). The increase in thioflavin T fluorescence was monitored at excitation of 435 nm and emission of 485 nm periodically.

To determine the optimal dilution across samples and for the evaluation of the kinetic parameters after UB-312 immunization, SAA was performed as described in Concha-Marambio et al.31, with modifications. The assay included 40 µl of sample and 60 µl of reaction mixture for a final 100 µl reaction comprising 0.3 mg ml−1 of recombinant alpha-synuclein (Amprion, S2020), 500 mM NaCl, 100 mM PIPES pH 6.5, 0.1% sarkosyl and 2 1/8' silicone nitride beads (Tsubaki Nakashima). To assess the optimal dilution, CSF samples were threefold serially diluted in synthetic CSF (Amprion, S2022) up to 1:729 and evaluated in the assay. For the assessment of alpha-synuclein-SAA kinetics, CSF samples underwent a single fivefold dilution in synthetic CSF and were tested in triplicate.

Dot blot analyses

First, 2 µl of purified alpha-synuclein protein either from recombinant or patient-derived preparations were spotted onto nitrocellulose membranes (Amersham Biosciences) and air dried for 30 min at room temperature. Patient-derived preparations were obtained from CSF samples that were submitted to two rounds of amplification in the alpha-synuclein-SAA, as described above. Membranes were blocked with 5% w/v nonfat dry milk in Tris-buffered saline–Tween 20 (20 mM Tris, pH 7.2, 150 mM NaCl and 0.05% (v/v) Tween 20) at room temperature for 2 h. After blocking, the membranes were probed with either a pan anti-alpha-synuclein antibody (BD Bioscience, 610787), an oligomer-specific anti-alpha-synuclein antibody (clone MJFR-14-6-4-2, Abcam, ab209538), IgG fractions or affinity purified antibodies isolated from sera at week 17 postimmunization. Species-relevant horseradish peroxidase-conjugated secondary antibodies (1:5,000) were then applied and blots were visualized using the enhanced chemiluminescence plus western blotting detection kit (Amersham Biosciences).

Measurements of pS129-alpha-synuclein in CSF samples

CSF samples were collected as described above. The concentrations of CSF pS129-alpha-synuclein were measured using the Phospho-a-Synuclein S129 kit from MagQu (MagQu, MF-PS1-0060) and immunomagnetic reduction (IMR). Before measurement, CSF samples were thawed on ice and reagents were brought to room temperature. CSF was first diluted 20 times with PBS. Thereafter, 60 ml of diluted CSF sample were added to 60 ml of IMR reagent for IMR analysis. Each sample was assessed in duplicates using an IMR analyzer (XacPro-S, MagQu).

Statistical Analysis

The sample size was considered adequate to characterize the safety, tolerability and dose–response profile of UB-312's immunogenicity, based on data from Part A in healthy volunteers. The trial was not powered for statistical comparisons between regimens, and results presented for safety and immunogenicity analyses are descriptive.

No interim analysis was planned for Part B. An analysis of immunogenicity and selected safety data for Part B was performed when the last patient in Part B complete the end of treatment (week 21) visit. The study continued as planned. The study team remained blinded to the treatment of individual patients until the end of the study.

Safety and tolerability were analyzed based on the safety population, defined as all participants randomized and exposed to at least one vaccination, identical to the MITT population. Analyses of immunogenicity and pharmacodynamic endpoints were performed by treatment allocation based on the PP population (all participants who received all planned vaccinations, up to the point of a protocol violation, if applicable, fulfilled all entry criteria and had no critical or major protocol deviations). There were no critical or major protocol deviations.

Baseline data were described by summary statistics of the MITT and PP populations. Immunogenicity and pharmacodynamic endpoints included in the analysis were concentrations of anti-alpha-synuclein (full length and C-terminal epitope); anti-CpG1 and anti-T-helper peptide antibodies; inflammatory markers; T cell enzyme-linked immunosorbent spot assay results; free and total alpha-synuclein in CSF and blood; and the total scores for MOCA, MDS–UPDRS part II and part III.

For immunogenicity, the seroconversion rate was provided as the percentage of participants that had no measurable (under the lower limit of quantification) full length and C-terminal epitope-specific anti-alpha-synuclein antibody levels before the first vaccination and subsequently developed quantifiable antibodies after the first vaccination.

Full length anti-alpha-synuclein antibody concentration data were provided by one individual laboratory (QPS). Data for the epitope-specific anti-alpha-synuclein antibodies were provided by two laboratories. QPS provided antibody concentrations in ng ml–1 and Vaxxinity Laboratories provided antibody titers in log-dilution factor, which are provided in the results.

For exploratory outcomes, changes in F_{max} (relative fluorescence units), MDS–UPDRS and MOCA were analyzed. To analyze F_{max}, samples were run in triplicate and average values utilized. Percentage CFB was calculated per individual from their week 1 value. For MDS–UPDRS-II and MDS–UPDRS-III, CFB was calculated per individual as the difference in score from their week 1 value. MOCA was summarized. An unplanned post hoc analysis was performed to evaluate F_{max}, pS129-alpha-synuclein, MDS–UPDRS-II and MDS–UPDRS-III differences in individuals with and without detectable CSF antibody titers. A two-way analysis of variance (ANOVA) with a mixed-effect model, due to one missing sample at week 45, was used with time and treatment as factors. A significant time effect ($P<0.05$) was followed by a within-group analysis using the method of Benjamini, Krieger and Yekitieli. Unpaired t-tests were used to compare differences between groups at each time point. Data are presented as means±s.e.m.

Safety and statistical programming were conducted with SAS 9.4 for Windows (SAS Institute Inc.). Exploratory biomarker analyses were conducted with GraphPad Prism 10.1.1 for macOS (GraphPad Software).

There was an independent medical monitor. There was no data monitoring committee. This trial is registered with ClinicalTrials.gov: NCT04075318.

CHAPTER 6

COGNITIVE EFFECTS OF THREE β-ADRENOCEPTOR ACTING DRUGS IN HEALTHY PARTICIPANTS AND PARTICIPANTS WITH PARKINSON'S DISEASE

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ABSTRACT

Noradrenergic signaling declines in Parkinson's disease (PD) following locus coeruleus neurodegeneration. Epidemiologic studies demonstrate that β-acting drugs slow PD progression.

The primary objective was to compare the safety and effects of 3 β-adrenoceptor (β-AR) acting drugs on central nervous system (CNS) function after a single dose in healthy participants, and evaluate the effects of multiple doses of β-AR acting drugs in healthy participants and participants with Parkinson's disease.

In Part A, healthy participants received single doses of 32mg salbutamol, 160µg clenbuterol, 60mg pindolol and placebo administered in a randomized, 4-way cross-over study. In Part B (randomized cross-over) and Part C (parallel, 2:1 randomized), placebo and/or clenbuterol (20µg on Day 1, 40µg on Day 2, 80µg on Days 3-7) were administered. CNS functions were assessed using the NeuroCart test battery, including pupillometry, adaptive tracking and recall tests.

Twenty-seven healthy participants and 12 participants with Parkinson's disease completed the study. Clenbuterol improved and pindolol reduced the adaptive tracking and immediate verbal recall performance. Clenbuterol and salbutamol increased and pindolol decreased pupil-to-iris ratios. Clenbuterol was selected for Parts B and C.

In Part B, clenbuterol significantly increased performance in adaptive tracking with a tendency toward improved performance in immediate and delayed verbal recall. In Part C trends toward improved performance in immediate and delayed verbal recall were observed in participants with Parkinson's disease. Typical cardiovascular peripheral β2-AR effects were observed with clenbuterol.

This study demonstrates the pro-cognitive effects of clenbuterol in healthy participants with similar trends in participants with Parkinson's disease. The mechanism of action is likely activation of β2-ARs in the CNS.

INTRODUCTION

The adrenergic system plays a crucial role in regulating physiological processes such as heart rate, blood pressure, and metabolism via two transmitters, adrenaline and noradrenaline that also play important stimulatory roles in central nervous system (CNS) function.[1] Recent research has suggested that early dysfunction of the adrenergic system of the brain arising from the locus coeruleus may contribute to the development and progression of neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease.[2–7]

Parkinson's disease is a common, progressive, and debilitating neurodegenerative disorder classically characterized by both motor and non-motor symptoms. Motor symptoms include tremor, rigidity, bradykinesia, and postural and balance disorders. There is a growing appreciation of the burden and poor medical management of non-motor symptoms, including cognitive dysfunction, neuropsychiatric symptoms, autonomic dysfunction, sleep disorders, and sensory dysfunction.[8-19]

Neuroanatomical staging studies show that neurodegeneration in the locus coeruleus occurs very early in PD.[20,21] Consistent with this, positron emission tomography studies with ^{11}C-MeNER, a highly selective tracer for noradrenaline transporters, shows reduced binding, and by inference, reduced density of noradrenergic axon projections in the brain of participants with PD compared with age-matched healthy controls.[22] Consequently, decline of this locus coeruleus projection system may be causally associated with loss of cognition, mood, and brain health, and revitalizing the influence of adrenergic receptor stimulation represents a potentially important pharmacological approach for the treatment of PD symptoms and disease progression.

Several pharmacoepidemiologic studies suggest that chronic treatment with β-adrenoceptor (β-AR) acting drugs is associated with a significantly reduced incidence of PD.[23–28] Additional data also suggest that the effect of β-AR acting drugs may generalize to other neurodegenerative disorders such as AD.[29,30] The concept is further supported by evidence of pro-cognitive and/or disease modifying effects of β-AR acting drugs in rodent models of neurodegenerative diseases. [31–33]

This study investigated the effects of three β-AR acting drugs—salbutamol (also known as albuterol), pindolol, and clenbuterol—on CNS function in both healthy participants and participants with PD. Since it is not clear which β-AR subtype is likely to be the ideal one to target for the treatment of neurodegenerative disorders, this study investigated β-AR acting drugs that have a distinct pharmacology from one another and are able to cross the blood-brain barrier. Salbutamol is a potent β2-AR agonist with modest CNS absorption, normally used as short acting β-agonist

to treat pulmonary disorders. Pindolol acts as a β2-AR antagonist and a β1-AR partial agonist with moderate to high CNS absorption. Clenbuterol is a selective β2-AR agonist with high CNS absorption, normally used as long acting β-agonist to treat pulmonary disorders (unpublished data).[27,34–36] The study aimed to (a) characterize and compare the effects of these three agents with placebo on CNS function and safety parameters such as adverse events and effects on heart rate and blood pressure after a single oral dose; (b) assess the safety and CNS effects of multiple doses of one agonist selected from observations in the single-dose cohort in healthy participants; and (c) similarly evaluate the safety and CNS effects in a small cohort of participants with PD.

MATERIAL AND METHODS

Participants

This 3-part, randomized, placebo-controlled study was conducted at a single site in the Netherlands in accordance with the International Conference for Harmonization of Good Clinical Practice, the principles of the Declaration of Helsinki (1964) and ethical principles as referenced in EU Directive 2001/20/EC. The protocol and all study materials were approved by the Medical Research Ethics Committee of the BEBO foundation (Assen, the Netherlands) and prospectively registered in the Dutch Trial Register (number NL8002), and all volunteers provided their written informed consent before participation. The study was conducted between September 2019 and March 2020 at a single site in the Netherlands (Centre for Human Drug Research, Leiden). The study was completed when the planned enrolment had been reached and all enrolled participants completed the scheduled study assessments.

Inclusion and exclusion criteria

In Part A and Part B, male or female healthy participants 35 – 60 years of age were eligible to participate if they weighed ≥ 50 kg, with BMI 18-35 kg/m,^{2} and were free from clinically significant abnormalities based on medical history, physical examination, 12-lead electrocardiogram (ECG), and laboratory tests (serum chemistry, hematology, coagulation, urine drug screen, and urinalysis). Use of any prescription- or over-the-counter medication or herbal supplements were excluded, except paracetamol/acetaminophen up to 4g/day, oral contraceptives, and hormone replacement therapy. In Part C, male or female patients with PD 40 – 75 years of age with a confirmed diagnosis of PD (defined as bradykinesia in addition to resting tremor, rigidity, or impairment of postural reflexes with no known or suspected cause), were eligible if they were assessed to be at Hoehn & Yahr stage ≤ 3, had a mini-mental status examination score ≥26 and no clinically significant abnormalities based on medical history, physical examination, ECG, and laboratory tests as deemed by the investigator. In these participants, use of dopamine agonists or catechol-O-methyltransferase inhibitors for treatment of PD was allowed if stable for ≥60 days and stable use (≥ 3 months) of medications for treatment of e.g., hypertension, dyslipidemia blood pressure, and vitamin E (up to 400 IU daily), estrogens, aspirin (81-300 mg daily).[37-39] Use of adrenergic agents was not permitted during the study except for β-AR blockers for treatment of tremors which were discontinued at least 48 hours prior to NeuroCart testing. Pregnant or nursing women were excluded from participation in all parts of this study. Participants or their partners, unless confirmed sterile or postmenopausal, were required to use a condom or two effective birth control methods during penile-vaginal intercourse throughout the study.

Dose selection

In Part A, healthy participants were randomly assigned to receive single oral doses of salbutamol (32 mg), clenbuterol (160 µg), pindolol (20 mg in the first five healthy participants and 60 mg in the subsequent healthy participants) and placebo in 1 of 4 treatment sequences (using a William's square, 4 period crossover design) with a washout period of approximately 7 days between doses. The doses selected for the three active drugs conform with the upper ranges of daily allowed doses/exposures approved for use in humans for treatment of pulmonary or cardiovascular diseases.[40–42] The decision to increase the dose of pindolol was based on emerging observations in a separate study, in which pindolol had no effect on cerebral blood flow in any of the brain regions of interest analyzed using Arterial Spin Labeling (unpublished data). Also, in this study, pindolol was tolerated well up to 20 mg (unpublished data). Commercially available oral tablets of salbutamol (4 mg tablets, GlaxoSmithKline), clenbuterol (Spiropent tablets, 20 µg; Boehringer Ingelheim), and pindolol (10 mg tablets, Teva Canada) and placebo were administered.

After interim review of safety and CNS data from Part A, one of the three acting drugs, clenbuterol was selected to be administered in the subsequent parts of the study (Part B and Part C). Based on the long plasma half-life of clenbuterol, an oral dose of up to 80 µg clenbuterol hydrochloride daily for 7 days was selected as this was anticipated to deliver a steady-state plasma concentration similar to the exposure achieved in Part A following a single dose of 160 µg clenbuterol hydrochloride. In Part B, 16 healthy participants received 80 µg clenbuterol and placebo in a double-blind, 2-period crossover design. Healthy participants were randomized 1:1 to the treatment sequence (active-then-placebo, vs placebo-then-active).

Clenbuterol hydrochloride was up titrated over the dosing period as follows: 20 µg on Day 1, 40 µg on Day 2 and 80 µg on Day 3 to Day 7. The two treatment periods were separated by a washout period of at least 14 days. Finally, in Part C of this study, 12 participants with PD were randomized 8:4 to receive 80 µg clenbuterol or placebo, administered once daily for 7 days. Clenbuterol hydrochloride was up titrated over the dosing period as follows: 20 µg on Day 1, 40 µg on Day 2 and 80 µg on Day 3 to Day 7. The final study visit was completed 7 (±2) days after the last study drug administration.

Randomization and blinding

For all parts of this study, randomizations codes were generated using SAS version 9.1.3 (SAS Institute Inc. Cary, NC, USA; 2004) by a statistician who was otherwise not involved in this study. Parts A and B were conducted in a double-blind fashion, while Part C of the study was single-blind (investigator blinded). In Part A, an unblinded physician administered the different tablets to the healthy participants who were blindfolded during drug administration to avoid recognition. Participants, study staff and investigators who evaluated safety and treatment effects were blind to treatment assignment; randomization codes were known only to the unblinded pharmacist who dispensed study medications and an unblinded physician who administered the doses.

Outcome measurements

In all parts of the study, safety and tolerability (including adverse events (AES) vital signs, electrocardiograms, physical examinations, routine clinical chemistry, and hematology assessments), plasma drug concentrations and pharmacokinetics, and CNS effects were evaluated. CNS effects were measured using the NeuroCart test battery, a validated set of tests used to measure the CNS effects of drugs in a standardized manner.[43] The tests included in the NeuroCart test battery and their related CNS domains are described in Table 1. The NeuroCart test battery included the following tests: saccadic eye movement, smooth pursuit, adaptive tracking, body sway, pupil size, visual verbal learning test, Stroop color-word interference task, visual analogue scale (VAS) Bond and Lader, VAS Bowdle, and pharmaco-electroencephalography.

In addition in Part C, subjects were also provided with a smartwatch (Withings, Withings Steel HR France) to measure heart rate during night and day, and polysomnography (Trackit Mk3, Lifelines, USA) was used to measure the effect on sleep, including number of awakenings, R latency, sleep latency, time in bed, total sleep time, duration of N1, N2, N3 and R sleep, and sleep efficiency.

Safety was evaluated throughout the study by routine assessments of adverse events, blood levels of potassium and glucose, ECG (Marquette 2000 or 5500, GE Healthcare, USA), and vital signs including supine blood pressure and pulse rate, respiratory rate, and temperature. Plasma drug concentrations were measured using LC/MS-MS methods and drug pharmacokinetics were evaluated using standard non-compartmental analyses.

Statistical analysis

This was an exploratory study and therefore, we did not perform a formal sample size estimation. The sample size was based on practical considerations and is standard for this type of study.[43] The study collected data on pharmacodynamic parameters for each subject, visit, and time point of treatment. Mixed model analysis of covariance and analysis of variance were used to determine whether there were significant treatment effects on pharmacodynamic parameters, heart rate, and blood pressure. The models included factors such as treatment, time, and period, as well as random factors such as subject. Also, the Kenward-Roger approximation and the restricted maximum likelihood method was used to estimate model parameters and reported treatment effects with the estimated difference (ED) compared to placebo of the least squared means (LSM), 95% confidence intervals (95%CI), and p-values. P-values <0.05 were assessed as statistically significant. These ED, LSM and p-values will be reported in the results section. In Part A, specific contrasts were also calculated to compare the effects of specific treatments to a placebo. Parameters were initially analyzed without transformation, but if the data suggest otherwise, log transformation was applied. Residual Q-Q plots were produced to check the assumption of normality of the error term in the mixed effects models. This was done by visual inspection and the Shapiro-Wilk test statistic.

Pharmacodynamic, pharmacokinetic and safety analyses were performed with SAS 9.4 for Windows (SAS Institute Inc. Cary, NC, USA, 2013). All available data were included in the analyses with no imputation of missing data and no correction for multiplicity. Data from Part A was evaluated prior to the initiation of the other parts of the study to inform which β-AR drug to take forward into Part B and Part C.

RESULTS

Disposition and demographics

A total of 79 healthy participants were screened for participation in the first 2 parts of this study, of whom 35 were enrolled. A total of 35 healthy participants received at least 1 dose of the study drug, and 27 completed the scheduled dosing and assessments. In

Part C, 23 participants with PD were screened for participation, of whom 13 were enrolled. All 12 participants with PD were administered all doses during the study and completed the study. The numbers of participants who were screened, enrolled, and withdrawn/completed each of the 3 parts of the study is summarized in Figure 1. The demographic characteristics of participants enrolled in this study are summarized in Table 2. No important differences were noted between treatment groups within each part, except for a difference in age groups as expected from the enrolment criteria between healthy participants in Parts A and B, and participants with Parkinson's disease in Part C.

Pharmacodynamic effects

PART A

In Part A, administration of a single dose of clenbuterol to healthy participants significantly increased the number of correctly recalled words during the first immediate recall trial test (ED of the LSM 1.30 [95%CI 0.10; 2.60], N=15, p = 0.04, Figure 2A) increased the number of correctly recalled positive words during the delayed recall test (ED of the LSM 8.79 [95%CI 0.17; 17.41], p = 0.05, Figure 2B) increased the performance in the adaptive tracking test during the first 4 hours (ED of the LSM 2.23 [95%CI 0.51; 3.95], p = 0.01, Figure 2C), increased the pupil/iris ratio in both the left eye (ED of the LSM 0.02 [95%CI 0.01; 0.04], p = 0.01, Figure 2D) and the right eye (ED of the LSM 0.02 [95%CI 0.01; 0.04], p = 0.01), and decreased the smooth pursuit parameter of saccadic eye movements albeit non-significantly (ED of the LSM -0.97% [95%CI -3.10; 1.15]). In these participants, responses following administration of a single dose of salbutamol were qualitatively similar to clenbuterol, although a significant effect was observed only in the immediate word recall test (ED of the LSM 1.3 [95%CI 0.1; 2.6], p = 0.03, Figure 1). Conversely, among the subset of participants (N=10) who received 60 mg pindolol, there was a tendency towards worse performance in the cognitive tasks, while the pupil/iris ratio was significantly decreased in the left eye (ED of the LSM -0.03 [95%CI -0.05; -0.02], p < 0.01), and the right eye (ED of the LSM -0.03 [95%CI -0.05; -0.02], p < 0.01). Also, saccadic eye movement during smooth pursuit was significantly decreased (ED of the LSM -3.19 [95%CI -5.68; -0.71], p = 0.01). Data for the 5 participants who received 20 mg pindolol are not presented but generally consistent with the observations with 60 mg pindolol. In the presence of each of the three active drugs administered in Part A, there was a decrease in the number of correct responses in incongruent trials compared to placebo which was statistically significant with clenbuterol (ED of the LSM -0.2 [95%CI -0.40; -0.00], p = 0.04).

PART B

As with the single-dose assessments, a general, albeit non-significant, improvement in visual verbal learning tests was observed in healthy participants who received once-daily oral clenbuterol hydrochloride for 7 days in Part B (20 µg on Day 1, 40 µg on Day 2 and 80 µg on Day 3 to Day 7, Figure 3A). In addition, a significant improvement in performance was observed in the adaptive tracking test over the first 4 hours after dosing on Day 7 (ED of the LSM 1.58% [95%CI 0.22; 2.94], p = 0.03, Figure 3B), compared to placebo. Also, administration of clenbuterol significantly increased parieto-occipital delta-power with eyes open (ED of the LSM 11.10% uV^2 [95%CI 5.20%; 17.30%], p = 0.01) and closed (ED of the LSM 9.50% uV^2 [95%CI 3.20%; 16.10%], p = 0.01), compared to placebo.

PART C

Unlike in the larger, crossover cohorts in Parts A and B of this study, evaluation of the effects of treatment in participants with PD enrolled in Part C of the study was significantly confounded by the small sample size, especially in the placebo group (N=4) of this parallel-group assessment. Nonetheless, a tendency toward increased recall and recognition of words was observed (ED of the LSM for number of words correct over all test days in immediate word recall [trial 1] 1.10 [95%CI -2.80; 5.10], and delayed word recall 0.90 [95%CI -3.30; 5.10]) albeit without attaining statistical significance. Administration of clenbuterol did not have significant effects on EEG frequency bands. Polysomnography evaluations in Part C showed that administration of clenbuterol in participants with PD significantly decreased the time before waking up after sleep onset compared to placebo (ED of the LSM -68.59 min [95%CI -135.55; -1.635], p = 0.05).

Safety and Pharmacokinetics

Clenbuterol, salbutamol and pindolol have been approved for use in humans for several decades, and the safety profile observed for these drugs was similar to previously reported experience. The treatment emergent AES that were reported most frequently while receiving the β2-AR acting drugs, salbutamol or clenbuterol, were typical for the drug class: headache, tremor, palpitations, tachycardia, fatigue, nausea, and dizziness.[44] The most commonly reported adverse events with the β2-AR antagonist/β1-AR partial agonist, pindolol, were headache, dizziness and nausea.

Of the 43 healthy participants or participants with PD who were enrolled and received active drug, 5 healthy participants discontinued due to AES, most frequently dizziness, nausea, vomiting, cold sweat, headache, paraesthesia, muscular

weakness, palpitations, somnolence, tremor, and/or fatigue while on salbutamol or clenbuterol. One healthy subject withdrew from study due to adverse events of syncope, somnolence, nausea, cold sweat while receiving pindolol. All withdrawn healthy participants recovered from the adverse events within seven days without intervention. No deaths or serious AEs were observed. Paracetamol was occasionally used as concomitant medication for headache or malaise.

Also, in keeping with the β2-AR agonist mechanism of action, increases in supine heart rate were observed following repeat administration of 80 µg clenbuterol to healthy participants over multiple measurements during Days 4 through 7 compared to placebo (ED of the LSM 11.50 bpm [95%CI 8.00; 14.90], p = 0.01), with a modest increase in systolic blood pressure compared to placebo (ED of the LSM 1.80 mmHg [95%CI 0.40; 3.20], p = 0.02) and no effect on diastolic blood pressure (ED of the LSM -0.2 mmHg [95%CI -2.7; 2.2], p = 0.83). Similarly, significant increases in supine heart rate were observed with clenbuterol compared with placebo in participants with PD during waking hours (ED of the LSM 13.30 bpm [95%CI 8.20; 18.40], p = 0.01 on Day 7) as well as during sleep (ED of the LSM in the median percentile of 11.10 bpm [95%CI 7.05; 15.15], p = 0.01, across all dosing days), without significant changes in supine systolic (ED of the LSM -4.4 mmHg [95%CI -16.9; 8.1], p = 0.45) or diastolic blood pressure (ED of the LSM -3.7 mmHg [95%CI -10.7; 3.3], p = 0.27).

Plasma drug concentrations were measured by LC/MS-MS and submitted to non-compartmental analyses for determination of pharmacokinetic parameters, including the observed time to maximal plasma concentration (T_{max}) and half-file. The observations generally conformed with published values. After administration of 160 µg clenbuterol hydrochloride, the T_{max} was 3 hours and longer, and half-life longer than could be estimated based on the amount of samples in the study (prior studies report > 30 hour half-lives for clenbuterol).[45] After administration of 32 mg salbutamol, T_{max} was 6-7 hours and half-life was 1-2 hours. After administration of 60 mg pindolol, T_{max} was 1 hour and half-life was 4 hours.

DISCUSSION

Degeneration of the locus coeruleus neurons likely contributes to the loss of cognitive function in diseases such as Parkinson's disease and Alzheimer's disease.[46,47] Although a role of the locus coeruleus in attention, learning and memory has been known for over 50 years, the specific adrenergic receptor(s) that produce these effects are not well-established.[48] This study sought to evaluate the effects of two potent β2-AR acting drugs (clenbuterol and salbutamol) and a non-selective β1-AR agonist/β2-AR antagonist (pindolol) on CNS measures with the aim to select a drug for future trials investigating the potential to reduce cognitive dysfunction in PD. In this study, we observed enhancements in working memory, attention, visuomotor coordination and pupil/iris ratios among healthy participants with the CNS penetrating β2-AR acting drug, clenbuterol. Responses to salbutamol were similar to clenbuterol, but weaker in magnitude, presumably due to its lower CNS absorption, while the effects were not observed for the β1-AR agonist/β2-AR antagonist, pindolol. These findings suggest that centrally acting β2-AR agonists may improve cognitive function in individuals affected by neurodegenerative diseases like PD. In addition, we demonstrated the safety of single and repeat doses of clenbuterol in healthy participants and participants with PD.

The observations in the present study are in line with a prior study in approximately 40 healthy participants, in which salbutamol produced pro-cognitive effects on emotional memory and attention (unpublished data, NCT01957293).[23] The observations are additionally consistent with the pro-cognitive effects of direct stimulation of β-ARs using clenbuterol and indirect stimulation using the norepinephrine reuptake inhibitor, atomoxetine, in a rat model of visuospatial attention.[49] However, this is the first study in humans to compare different β-AR properties, including β1- vs β2 selectivity, and CNS absorption to enable identification of the key properties for a potential future drug for treating cognitive decline. While significant improvements in immediate word recall were observed with both clenbuterol and salbutamol, the improvements in other cognitive tasks that were observed with clenbuterol but not salbutamol suggest that penetration into the CNS may contribute to the broader range of observed effects. The contrasting tendency of pindolol to worsen performance in word recall and adaptive tracking lend further support to the hypothesized pro-cognitive role of β2-AR activation, as pindolol is an antagonist at this receptor and may be blocking endogenous stimulation.[50] However, whether the latter effect may also be the result of partial agonism of β1-AR, or some other nonselective mechanism cannot be concluded definitively from the present study.

The data from Part A suggest that CNS-penetrating β2-AR acting drugs may mediate the effects of locus coeruleus innervation on memory and alertness. This informed the decision to evaluate clenbuterol in Part B and Part C of this study. The observed effects of a single dose of clenbuterol in Part A were corroborated following once-daily administration of clenbuterol to healthy participants for 1 week in Part B. Specifically, there was a trend towards improved performance in the immediate and delayed word recall tests, and statistically significant improvement in performance in the adaptive tracking task. Similar evaluation of the effects of clenbuterol in patients with PD in Part C was confounded by sample size, especially in

the placebo comparator arm (N=4). In addition, motor deficits in the PD population may impact measurement of response, especially in tasks with greater motor involvement such as the adaptive tracking task. Since cognitive impairment varies not only with disease severity but also between different neurodegenerative diseases, presumably reflecting the different brain regions, pathways and cell types involved, we cannot expect a single drug target to rescue all cognitive domains.[51] In the case of clenbuterol, some of the improvements in performance of cognitive tasks may reflect effects on attention and alertness, while non-neuronal changes in metabolism, inflammation and perfusion mediated by β2-adrenergic receptors expressed on astrocytes and microglia may provide more sustained benefit.[52]

In Part A of this study, the two β2-AR agonists, clenbuterol and salbutamol, resulted in qualitatively similar effects on cognition in healthy participants, including significant improvements in the immediate verbal recall test, and a tendency toward improved performance in delayed verbal recall. Performance in visual verbal tests has been correlated with short-term memory and is known to be improved after administration of caffeine.[53–55] In addition, clenbuterol significantly improved performance in the adaptive tracking task. The adaptive tracking test is a marker of drug effects on coordination and vigilance.[56,57] The performance during the adaptive tracking test is worsened by sedatives such as diazepam and temazepam,[58,59] and increased by psychoactive drugs such as meta-chlorophenyl piperazine.[60] In keeping with its lower absorption into the CNS, the observed effects of salbutamol in this study were qualitatively similar but typically more modest compared with those of clenbuterol.[36] In contrast, the effects of pindolol were directionally different from clenbuterol and salbutamol, including worsening of performance in the immediate and delayed verbal recall tests as well as in adaptive tracking.

When administered in single or repeat doses, the peripheral effects observed for clenbuterol were similar to those often reported for the β2-AR agonist drug class.[61,62] The most frequently reported AEs by healthy participants were palpitations and tremor with increases in mean supine heart rate of more than 10 bpm that led to 5 withdrawals and that likely impacted the evaluations of cognition undertaken in this study. There were no withdrawals of participants with Parkinson's disease. Chronic dosing with clenbuterol, as would be anticipated if used for treatment of cognitive decline due to PD or Alzheimer's disease, will necessitate better control of these effects in the periphery, e.g., by using strategic co-administration of a selective β2-AR antagonist that achieves minimal penetration into the CNS. However, this needs to be confirmed in future trials.

This study had some limitations. Firstly, this study involved a small number of participants with Parkinson's disease, which may not fully represent the diverse patient populations that will ultimately use the drug. Consequently, the findings may not accurately predict how clenbuterol will affect patients with various medical conditions or demographics. Secondly, this study assessed the short-term effects of the treatment. Similar effects on the long term will need to be corroborated in a follow-up study. Additionally, the controlled environment of this study may not fully reflect real-world conditions, which is inherent to an early phase clinical study. Factors such as patient compliance, concomitant medications, and environmental influences can affect drug outcomes differently.

Taken together, the observations from this study identify the β2-AR as a candidate receptor that may result in pro-cognitive effects. Moreover, the superior effects of the CNS-penetrating drug, clenbuterol, compared with salbutamol reveal the potential utility of selective, CNS-penetrant β2-AR agonism, especially if the agonistic effect can be limited to the brain. These data support further investigation of the effects of clenbuterol on measures of cognition and alertness in patients with a neurodegenerative disease such as PD, especially under conditions where the peripheral effects of β2-AR agonism are controlled.

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FIGURE 1 FLOWCHART SHOWING DISPOSITION OF ALL CONSENTING PARTICIPANTS

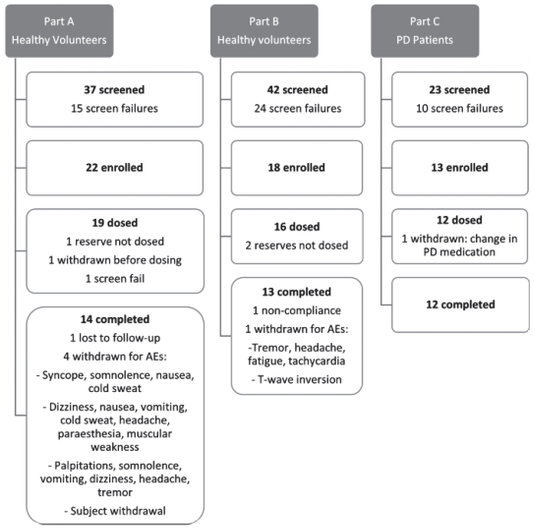
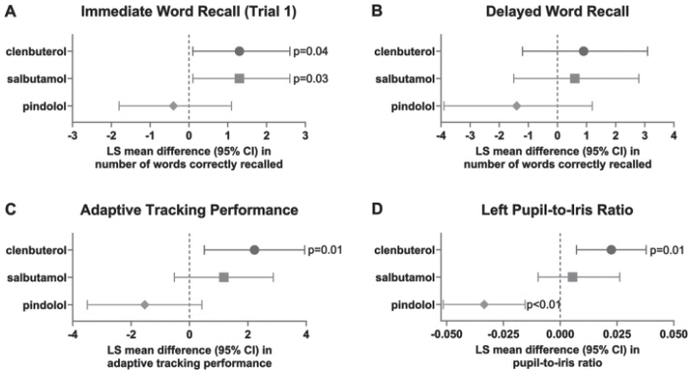
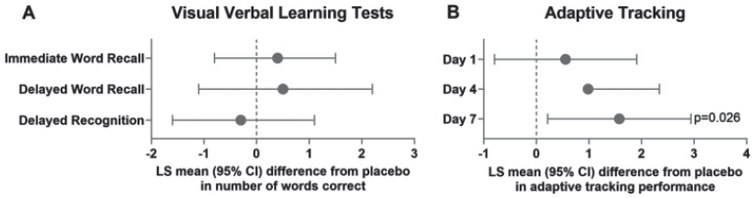


FIGURE 2 EFFECTS OF SINGLE DOSES OF CLENBUTEROL, SALBUTAMOL AND PINDOLOL ON IMMEDIATE WORD RECALL, DELAYED WORD RECALL, ADAPTIVE TRACKING AND PUPIL/IRIS RATIO IN PART A



Abbreviations: LS = Least Square; CI = Confidence Interval. Data for the cohort of healthy participants in Part A who received a single dose of placebo, clenbuterol (160 µg, N=16), salbutamol (32 mg, N=17) and pindolol (60 mg, N=10) in a randomized sequence separated by an approximately 7-day washout. LS mean (95%CI) differences between drug and placebo are plotted for repeat observations over the first 4 hours after dosing on Day 1 for immediate word recall (trial 1, A), delayed word recall (B), adaptive tracking (C) and left pupil-to-iris ratio (D).

FIGURE 3 EFFECTS OF REPEAT, ONCE-DAILY ORAL DOSES OF CLENBUTEROL (80 MG) IN HEALTHY PARTICIPANTS IN IMMEDIATE WORD RECALL (A) AND ADAPTIVE TRACKING (B) IN PART B



Abbreviations: LS = Least Square; CI = Confidence Interval. Data for the cohort of 14-15 healthy participants in Part B who received placebo and clenbuterol (up-titrated over the dosing period from 20 µg on Day 1, 40 µg on Day 2 and 80 µg on Days 3-7) in a 7-day crossover are presented. LS mean (95%CI) differences between clenbuterol and placebo are plotted for repeat observations at 1, 2, and 8 hours after dosing on Days 1, 4 and 7 for the number of correct responses for the immediate word recall (trial 1), delayed word recall and delayed word recognition (B), and repeat observations over the first 4 hours after dosing on Days 1, 4 and 7 for adaptive tracking (B).

TABLE 1 NEUROCART TEST BATTERY, EEG AND RELATED CNS DOMAINS

NeuroCart test	Targeted function and Description of the test	Related CNS areas
Saccadic eye movement	ALERTNESS, VIGILANCE 58,63 The measurement of saccadic eye movements, specifically saccadic peak velocity, is highly sensitive for assessing sedation. Saccadic eye movements are captured using a moving dot displayed on a computer screen. Parameters such as latency, saccadic peak velocity, and inaccuracy of saccades were measured and analysed.	Superior colliculus, substantia nigra, amygdala
Smooth pursuit	VISUOMOTOR COORDINATION 58,63 In the case of smooth pursuit eye movements, the target moved at frequencies ranging from 0.3 to 1.1 Hz, in increments of 0.1 Hz. The amplitude of target displacement corresponded to a rotation of 22.5 degrees for both sides of the eyeball. Four cycles were recorded for each stimulus frequency. The duration of time during which the eyes tracked the target in smooth pursuit was calculated for each frequency and expressed as a percentage of the stimulus duration. The average percentage of smooth pursuit across all stimulus frequencies was used as a parameter.	Midbrain
Adaptive tracking	ATTENTION, VISUOMOTOR COORDINATION 64 The adaptive tracking test was conducted using custom equipment and software, which was developed by Hobbs and Strutt, according to specifications of Borland and Nicholson. Analysis involved the average performance and standard deviation of scores over a 3.5-minute period, excluding the initial 0.5-minute run-in time. The task required the subject to track a moving circle on the screen by controlling a joystick to keep a dot within the target circle. Performance was deemed to be successful if the participant tracked the target at high speeds, while failure occurred when the velocity for tracking the target was low.	Neocortex, basal nuclei, brain stem, cerebellum
Body sway	MOTOR COORDINATION, POSTURAL BALANCE 65 Postural stability was assessed using a body sway meter, which measured body movements in a single plane to determine postural stability. The measurement of body sway was conducted using a pot string meter based on the Wright ataxiameter. A string was attached to the subject's waist, capturing all body movements over a specific time period and quantifying them as millimetres of sway. The total measurement duration for body sway was two minutes.	Cerebellum, brain stem
Pupil size	BRAIN STEM FUNCTION. A digital camera was used to measure the size of subject's pupils at different time points. One picture was taken from both eyes simultaneously, and the ratio between the pupil- and iris diameter was measured.	Brain stem, medulla oblongata
Visual verbal learning test (VVLT)	EPISODIC MEMORY. Subjects that perform the VVLT were presented 30 words in three consecutive word trials, i.e. word learning test. Each trial ended with a free recall of the presented words (Immediate Recall) test to determine acquisition and consolidation of information). Approximately 2.5 hours after start of the first trial, the subjects were asked to recall as many words as possible (Delayed Recall – this test measures active retrieval from long term memory). Immediately thereafter, the subjects underwent memory recognition test, which consisted of 15 presented words and 15 'distractors' (Delayed Recognition-testing memory storage). A different word list was presented to each subject during the different occasions, in this way a subject was presented with a new word list each measurement.	Hippocampus
Stroop color-word interference task	A two-trial version of the colour-word Stroop task was presented to the test subjects. In the first trial, 6 coloured items were presented at random. The subjects were asked to respond as fast and as accurately as possible by pressing the keys 1, 2 or 3 on the number pad with the index finger, middle finger and ring finger of the dominant hand, corresponding with the correct answer. In the second trial, which appeared directly after the first trial, 34 colour and word pairs were presented randomly to the subject, forming either congruent or incongruent matches. The subjects were again asked to respond as fast as possible by pressing the keys 1, 2 or 3 on the numpad, corresponding with the correct answer. Three colours were shown, which are green, red and blue. The coloured items were presented in a random order.	
Visual Analogue Scale (VAS) Bond and Lader	ALERTNESS, MOOD, CALMNESS 66 Participants were asked to indicate with a mouse click on the computer screen how they felt on sixteen visual analogue scales from which the following 3 main factors were calculated as described by: alertness (from nine scores), contentedness (often called mood; from five scores), and calmness (from two scores).	Cortex, prefrontal cortex
Visual Analogue Scale (VAS) Bowdle	FEELING HIGH, INTERNAL AND EXTERNAL PERCEPTION 67–69 Potential subjective psychotomimetic (psychedelic) effects of antiglutamatergic agents can be evaluated using specific VAS. Bowdle Psychotomimetic Effects Scores consisted of thirteen 10 cm visual analogue lines ranging from 0 ('not at all') to 100 mm ('extremely'), addressing various abnormal states of mind. The Bowdle VAS was administered electronically and took approximately 2 minutes to complete.	Cortex, prefrontal cortex, amygdala
Pharmaco-electro_ encephalo-graphy (PEEG)	CNS ACTIONS OF PHARMACOLOGICAL SUBSTANCES 70 Resting-state PEEG recordings were conducted on subjects with open and closed eyes for 5 minutes each. Subjects were instructed to avoid staring, head and eye movements, and suppress eye blinks while facing a featureless wall. PEEG was recorded using a 40-channel system, with electrode placement following the international 10-20 system. Ocular artifacts were detected using vertical and horizontal PEEG recordings. The recorded signals were processed by applying filters, calculating power spectrum density, and analysing specific electrode sites of interest.	All brain regions

CNS = Central Nervous System; pEEG = pharmaco-electroencephalography; VAS=visual analogue scale; VVLT = Visual Verbal Learning Test

TABLE 2 DEMOGRAPHIC CHARACTERISTICS OF ALL PARTICIPANTS WHO RECEIVED STUDY DRUG

	Healthy participants		PD: multiple dose		
	Part A: single dose	Part B: multiple dose	Part C – overall	Part C – clenbuterol	Part C – placebo
N	19	16	12	8	4
Age (years)	49.0 (4.7)	51.6 (6.7)	63.4 (6.6)	64.1 (6.1)	62 (8.3)
Height (cm)	178.47 (9.03)	177.74 (9.98)	177.93 (9.57)	180.55 (5.59)	172.68 (14.43)
Weight (kg)	81.52 (14.84)	84.53 (10.97)	77.68 (8.07)	78.656 (6.753)	75.73 (11.16)
BMI (kg/m2)	25.46 (3.35)	26.84 (3.55)	24.56 (1.98)	24.16 (2.34)	25.35 (0.58)
Gender, male (%)	68.4	56.3	83.3	100	50
MMSE	n.a.	n.a.	29.3 (0.8)	29.2 (0.9)	29.3 (0.77)
Hoehn and Yahr	n.a.	n.a.	1.7 (0.9)	1.8 (0.7)	1.5 (1.3)

BMI = Body Mass Index; MMSE = Mini-Mental State Examination. n.a. = not applicable; PD = Parkinson's disease / Results are either presented as mean (standard deviation), or percentages.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

SUMMARY OF THIS THESIS

This thesis describes the investigation of novel therapeutic strategies for Parkinson's disease and related disorders, with a focus on disease-modifying therapies. By systematically reviewing the literature on current disease-modifying therapies in development, describing metabolic and neurobiological targets, and evaluating novel pharmacological interventions, this thesis describes both the challenges and potential near-future breakthroughs in the field. Key findings include the validation of plasma fatty acids as biomarkers for target engagement, the modulation of alpha-synuclein pathology through immunotherapy, and the cognitive effects of beta-adrenoreceptor modulation. Together, these studies contribute to a deeper knowledge of clinical trials on disease-modifying therapies for Parkinson's disease.

In **chapter 2**, we reviewed the status of disease-modifying therapies for Parkinson's disease, Lewy body dementia, multiple system atrophy, progressive supranuclear palsy, and corticobasal syndrome. This review highlighted the vast number of molecules under investigation converse to the limited number of approved drugs. Among the candidates currently in phase 3 trials for Parkinsonian disorders, several therapies have reached phase 3 clinical development for Parkinson's disease, including buntanetap (an anti-alpha-synuclein therapy), ambroxol (a glucocerebrosidase enhancer), prasinezumab (an anti-alpha-synuclein monoclonal antibody), and cinpanemab (another anti-alpha-synuclein monoclonal antibody). Notably and interestingly, a 2024 press release states that in the phase 3 trial buntanetap improved Movement Disorder Society (MDS) Unified Parkinson's Disease Rating Scale (UPDRS) total, part 2, part 3, and the combined part 2 and 3 scores, compared to placebo and baseline.[68] A peer-reviewed publication in a scientific journal was not yet available at the time of writing.

As target engagement biomarkers are pivotal for adequately profiling the dose-response curve in clinical studies, studies validating biomarkers can be performed prior to inclusion of that biomarker in a clinical trial. In **chapter 3**, we demonstrate that plasma fatty acids have a low intra-subject variability in both healthy participants as well as participants with Parkinson's disease in a phase 0 study. It has been demonstrated in vitro and in vivo that fatty acids are involved in alpha-synuclein toxicity via stearoyl-CoA-desaturase.[69–71] Our study provides evidence supporting the use of plasma fatty acids as a biomarker to demonstrate target engagement of a stearoyl-CoA-desaturase inhibitor in early phase clinical trials.

In the study described in **chapter 4**, we investigated YTX-7739, a novel stearoyl-CoA-desaturase inhibitor, in both healthy participants as well as participants with Parkinson's disease Parkinson's disease. While this novel medicine could be considered a remarkable candidate target to slow disease progression in Parkinson's disease, the effects of fatty acids in alpha-synuclein toxicity has been well established.[69–71] Building on our findings from chapter 3, we evaluated whether targeting stearoyl-CoA-desaturase could modulate plasma fatty acid levels in vivo. We demonstrate that YTX-7739 successfully reduces plasma fatty acids and is generally well tolerated and safe in both populations.

In the study described in **chapter 5**, we investigated UB-312, an active immunotherapeutic targeting pathological alpha-synuclein in participants with Parkinson's disease.[72] An active immunotherapy has multiple advantages over passive immunotherapy such as greater accessibility and convenience, and multiple efforts are underway to develop such therapies. In healthy participants, UB-312 was considered safe and well tolerated up to a certain dose level, but dose escalation was limited due to the development of influenza-like symptoms in the higher dose ranges.[73] In participants with Parkinson's disease, UB-312 was considered generally safe and well tolerated, without the development of influenza-like symptoms. Importantly, UB-312 generated anti-alpha-synuclein antibodies in both serum and cerebrospinal fluid, and an exploratory analysis showed a significant reduction of alpha-synuclein seed amplification in cerebrospinal fluid of a subset of UB-312-treated patients, suggesting preliminary evidence of target engagement.[72] There were no statistical differences in clinical rating scales.

In the study described in **chapter 6**, we investigated the effects of beta-adrenoreceptor acting drugs on the central nervous system in both healthy participants and participants with Parkinson's disease.[74] Shifting focus in the thesis from disease-modifying strategies to a more symptomatic treatment, we explored the potential enhancement of cognition by beta-adrenoreceptor modulation. In this study, we compared beta-adrenoreceptor full- and partial agonists with different levels of central nervous system penetration. Clenbuterol was the most promising drug and was considered safe and well tolerated in all participants. Interestingly, we observed pro-cognitive effects in healthy participants, as measured by increased performance in adaptive tracking and word recall tests. Although similar trends were noted in the participants with Parkinson's disease, these did not reach statistical significance, likely due to the small sample size.

SUMMARY AND FUTURE OUTLOOK

Slowing the progression of neurodegenerative disease with pharmacological interventions has proven to be a hard nut to crack. For example, despite advances in research, no disease-modifying therapy for Parkinson's disease has yet received regulatory approval. For Alzheimer's disease, approved therapies have minor clinical relevance at moderate risks for complications. Key issues remain our incomplete understanding of molecular mechanisms of the disease and the underlying etiological factors, as well as poor translatability of animal models, making the development of effective treatments difficult.

The complexity of Parkinson's disease pathophysiology stems from the intricate interplay of genetic, molecular, and environmental factors, many of which remain poorly understood.[75,76] Therefore, a trial population included based on the clinical diagnosis of Parkinson's disease, is inherently heterogenous from the perspective of the molecular etiology. For future trials it could be considered to focus on one specific (pathophysiological) subpopulation, for example participants with a glucosylceramidase beta 1, or GBA1, mutation (5 to 15% of the Parkinson's disease population).[77] Participants with Parkinson's disease with variants in the GBA1 gene have reduced activity of the glucocerebrosidase protein and tend to be younger and have a higher risk for the development of cognitive impairment.[78] Another example could be to focus on rapid eye movement sleep behavior disorder status, a common status and disorder that occurs in 15 to 75% of the participants with Parkinson's disease.[79] These patients are commonly male and older, and have a longer disease duration, and high motor scores on the UPDRS.[79] Eventually, the trial population can be selected based on the mechanism of action of the intervention, when multi-omics approaches allow the identification of patient subgroups with alpha-synuclein, neuroinflammatory, mitochondrial or lysosomal pathology as primary driver of their disease. Focusing on one specific subpopulation could decrease the heterogeneity of clinical trials and increase the chance of demonstrating a clinically significant effect of a drug that likely differentially influences disease progression in different subpopulations depending on the expression of specific pathophysiology.

Given that Parkinson's disease arises from multiple converging molecular mechanisms, an alternative approach to drug development could involve combination treatments. This would allow the targeting of several pathophysiological aspects of Parkinson's disease simultaneously, for example by targeting the development of toxic oligomers of alpha-synuclein, improving mitochondrial and/or lysosomal function inhibiting neuro-inflammation.

However, combining multiple therapies could introduce scientific, regulatory, and operational challenges. From a scientific standpoint, drug combinations may result in pharmacokinetic or pharmacodynamic interactions that alter efficacy or safety, potentially leading to unexpected adverse effects not observed with individual therapies. Regulatory agencies such as the United States Food and Drug Administration and the European Medicines Agency require each combination to be tested independently to demonstrate its unique efficacy, identify specific risks, and confirm that all components contribute meaningfully to the clinical benefit. From a developmental perspective, each added therapy multiplies the number of potential trial scenarios, increasing the complexity of study design, lengthening timelines, raising costs, and introducing ethical considerations regarding patient exposure to untested combinations. As a result, although rational combination therapy is a compelling approach, it necessitates rigorous evaluation to ensure safety and efficacy, and to meet regulatory standards.

As described more extensively in **Chapter 2**, a range of investigational therapies are currently under development for Parkinson's disease, targeting diverse pathological mechanisms. Among the most advanced are buntanetap, ambroxol, and exenatide, all of which have progressed to phase 3 clinical trials. Buntanetap, which targets alpha-synuclein translation, showed improvements in motor and cognitive symptoms in phase 2, and a phase 3 trial has been completed, though results are pending. Ambroxol, which enhances glucocerebrosidase activity and lysosomal function, is under investigation in multiple phase 2 and 3 trials, including studies focused on participants with GBA-mutations and those with dementia with Lewy bodies. Exenatide, a glucagon-like peptide 1 receptor agonist, is also being evaluated in a 96-week phase 3 trial after earlier studies suggested motor benefit and potential engagement of brain insulin signaling. Other therapies such as prasinezumab and cinpanemab, both monoclonal antibodies targeting alpha-synuclein, reached phase 2 trials but did not meet primary endpoints and have not advanced further. In addition, several earlier-stage therapies are under active investigation in phase 1 or 2 trials, including DNL201 (also known as BIIB122, a leucine-rich repeat kinase 2 inhibitor), terazosin (a phosphoglycerate kinase 1 stimulator), KM-819 (a Fas-associated factor 1 inhibitor), and CNM-Au8 (a catalytic nanotherapeutic that promotes the oxidation of nicotinamide adenine dinucleotide and increases intracellular adenosine triphosphate levels). While these candidates have shown biological activity or target engagement, most lack definitive evidence of clinical efficacy. For other Parkinsonian disorders such as multiple system atrophy and progressive supranuclear palsy, clinical development remains limited, and no

therapies have progressed beyond early-phase trials. Overall, the therapeutic landscape is expanding, but robust, placebo-controlled data from ongoing and future trials will be essential to determine which approaches can meaningfully alter disease progression.

While challenges persist, this thesis provides insights in the development of disease-modifying therapies for neurodegenerative disorders, specifically Parkinson's disease. Continued innovation, combined with targeted and individualized approaches, holds promise for addressing both motor and non-motor symptoms of Parkinson's disease with the potential to reduce the rate of progression and thereby improve patient outcomes.

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APPENDICES

NEDERLANDSE SAMENVATTING

SAMENVATTING VAN DEZE THESIS

Deze thesis beschrijft het onderzoek naar nieuwe therapeutische strategieën voor de ziekte van Parkinson en aanverwante aandoeningen, met een focus op ziekte-modificerende therapieën. Door systematisch de literatuur te beoordelen over huidige ziekte-modificerende therapieën in ontwikkeling, metabole en neurobiologische doelwitten te beschrijven, en nieuwe farmacologische interventies te evalueren, worden zowel de uitdagingen als potentiële doorbraken op korte termijn in dit vakgebied besproken. Belangrijke bevindingen zijn onder andere de validatie van plasma-vetzuren als biomarkers voor target engagement, de modulatie van alfa-synucleïne pathologie via immunotherapie, en de cognitieve effecten van bèta-adrenoreceptor modulatie. Samen dragen deze studies bij aan een diepere kennis van klinische studies naar ziekte-modificerende therapieën voor de ziekte van Parkinson.

In **hoofdstuk 2** beoordeelden we de status van ziekte-modificerende therapieën voor de ziekte van Parkinson, Lewy body dementie, multipele systeematrofie, progressieve supranucleaire parese en corticobasale syndroom. Deze review benadrukte het grote aantal moleculen dat onderzocht wordt, tegenover het beperkte aantal goedgekeurde geneesmiddelen. Onder de kandidaten die zich momenteel in fase 3 klinische ontwikkeling bevinden voor Parkinson-achtige aandoeningen, zijn er verschillende therapieën voor de ziekte van Parkinson, waaronder buntanetap (een anti-alfa-synucleïne therapie), ambroxol (een glucocerebrosidase-versterker), exenatide (een glucagon-like peptide 1 receptor agonist), prasinezumab (een anti-alfa-synucleïne monoklonaal antilichaam), en cinpanemab (nog een anti-alfa-synucleïne monoklonaal antilichaam). Opmerkelijk is dat volgens een persbericht uit 2024 buntanetap in een fase 3-studie verbetering liet zien op de Movement Disorder Society (MDS) Unified Parkinson's Disease Rating Scale (UPDRS) totaal, deel 2, deel 3, en de gecombineerde deel 2 en 3 scores, vergeleken met placebo en baseline. Een peer-reviewed publicatie in een wetenschappelijk tijdschrift was op het moment van schrijven nog niet beschikbaar.

Aangezien biomarkers voor target engagement essentieel zijn voor het adequaat profileren van de dosis-responscurve in klinische studies, kunnen studies ter validatie van biomarkers worden uitgevoerd vóór opname in een klinische trial. In **hoofdstuk 3** tonen we aan dat plasma-vetzuren een lage intra-individuele variabiliteit hebben bij zowel gezonde deelnemers als deelnemers met de ziekte van Parkinson in een fase 0-studie. In vitro en in vivo is aangetoond dat vetzuren betrokken zijn bij alfa-synucleïne toxiciteit via stearoyl-CoA-desaturase. Onze studie levert bewijs ter ondersteuning van het gebruik van plasma-vetzuren als biomarker om target engagement van een stearoyl-CoA-desaturase remmer aan te tonen in vroege fase klinische studies.

In de studie beschreven in hoofdstuk 4 onderzochten we YTX-7739, een nieuwe stearoyl-CoA-desaturase remmer, bij zowel gezonde deelnemers als deelnemers met de ziekte van Parkinson. Hoewel dit nieuwe geneesmiddel als een veelbelovende kandidaat kan worden beschouwd om de ziekteprogressie bij Parkinson te vertragen, zijn de effecten van vetzuren op alfa-synucleïne toxiciteit goed vastgesteld. Voortbouwend op onze bevindingen uit hoofdstuk 3, evalueerden we of het richten op stearoyl-CoA-desaturase de plasma-vetzurenniveaus in vivo kon moduleren. We tonen aan dat YTX-7739 succesvol plasma-vetzuren verlaagt en over het algemeen goed verdragen en veilig is in beide populaties.

In de studie beschreven in **hoofdstuk 5** onderzochten we UB-312, een actieve immunotherapie gericht op pathologische alfa-synucleïne bij deelnemers met de ziekte van Parkinson. Een actieve immunotherapie heeft meerdere voordelen ten opzichte van passieve immunotherapie, zoals betere toegankelijkheid en gebruiksgemak, en er zijn meerdere inspanningen gaande om dergelijke therapieën te ontwikkelen. Bij gezonde deelnemers werd UB-312 als veilig en goed verdragen beschouwd tot een bepaalde dosering, maar dosisverhoging werd beperkt door het optreden van griepachtige symptomen bij hogere doseringen. Bij deelnemers met Parkinson werd UB-312 over het algemeen als veilig en goed verdragen beschouwd, zonder het optreden van griepachtige symptomen. Belangrijk is dat UB-312 anti-alfa-synucleïne antilichamen genereerde in zowel serum als hersenvocht, en een verkennende analyse toonde een significante vermindering van alfa-synucleine zaad-amplificatie in het hersenvocht van een subset van UB-312-behandelde patiënten, wat wijst op preliminair bewijs van target engagement. Er waren geen statistisch significante verschillen in klinische beoordelingsschalen.

In de studie beschreven in **hoofdstuk 6** onderzochten we de effecten van bèta-adrenoreceptor werkende geneesmiddelen op het centrale zenuwstelsel bij zowel gezonde deelnemers als deelnemers met de ziekte van Parkinson. Door de focus in de thesis te verschuiven van ziekte-modificerende strategieën naar meer symptomatische behandeling, onderzochten we de mogelijke verbetering van cognitie door bèta-adrenoreceptor modulatie. In deze studie vergeleken we bèta-adrenoreceptor volledige en gedeeltelijke agonisten met verschillende niveaus van penetratie in het centrale zenuwstelsel. Clenbuterol was het meest veelbelovende geneesmiddel en werd als veilig en goed verdragen beschouwd bij

alle deelnemers. Interessant genoeg observeerden we pro-cognitieve effecten bij gezonde deelnemers, gemeten door verbeterde prestaties in adaptieve tracking en woordherinneringstests. Hoewel vergelijkbare trends werden opgemerkt bij deelnemers met Parkinson, bereikten deze geen statistische significantie, waarschijnlijk vanwege de kleine steekproefgrootte.

SAMENVATTING EN TOEKOMSTPERSPECTIEF

Het vertragen van de progressie van neurodegeneratieve aandoeningen met farmacologische interventies blijkt een bijzonder moeilijke opgave. Ondanks vooruitgang in onderzoek is er bijvoorbeeld nog geen ziekte-modificerende therapie voor de ziekte van Parkinson goedgekeurd door regelgevende instanties. Voor de ziekte van Alzheimer zijn er wel goedgekeurde therapieën, maar deze hebben slechts beperkte klinische relevantie en brengen matige risico's op complicaties met zich mee. Belangrijke knelpunten blijven het onvolledige begrip van de moleculaire mechanismen van de ziekte en de onderliggende etiologische factoren, evenals de beperkte overdraagbaarheid van diermodellen naar de mens, wat de ontwikkeling van effectieve behandelingen bemoeilijkt.

De complexiteit van de pathofysiologie van de ziekte van Parkinson komt voort uit de ingewikkelde wisselwerking tussen genetische, moleculaire en omgevingsfactoren, waarvan vele nog onvoldoende begrepen zijn. Daarom is een trialpopulatie die wordt geselecteerd op basis van de klinische diagnose van Parkinson per definitie heterogeen vanuit moleculair etiologisch perspectief. Voor toekomstige studies zou overwogen kunnen worden om te focussen op een specifieke (pathofysiologische) subpopulatie, bijvoorbeeld deelnemers met een mutatie in het glucosylceramidase beta 1-gen (GBA1), wat voorkomt bij 5 tot 15% van de Parkinsonpopulatie.

Deelnemers met varianten in het GBA1-gen hebben een verminderde activiteit van het glucocerebrosidase-eiwit, zijn vaak jonger en hebben een verhoogd risico op cognitieve achteruitgang. Een ander voorbeeld is het focussen op de status van REM-slaapgedragsstoornis, een veelvoorkomende aandoening die voorkomt bij 15 tot 75% van de Parkinsonpatiënten. Deze patiënten zijn vaak mannelijk, ouder, hebben een langere ziekteduur en hogere motorscores op de UPDRS. Uiteindelijk kan de trialpopulatie worden geselecteerd op basis van het werkingsmechanisme van de interventie, wanneer multi-omics benaderingen het mogelijk maken om patiëntsubgroepen te identificeren waarbij alfa-synucleïne, neuro-inflammatie, mitochondriale of lysosomale pathologie de primaire drijvende kracht achter de ziekte is. Door te focussen op één specifieke subpopulatie kan de heterogeniteit van klinische studies worden verminderd en de kans worden vergroot om een klinisch significant effect aan te tonen van een geneesmiddel dat waarschijnlijk verschillend werkt afhankelijk van de uiting van specifieke pathofysiologie.
Aangezien de ziekte van Parkinson voortkomt uit meerdere convergerende moleculaire mechanismen, zou een alternatieve benadering van geneesmiddelenontwikkeling kunnen bestaan uit combinatietherapieën. Hiermee kunnen meerdere pathofysiologische aspecten van de ziekte gelijktijdig worden aangepakt, bijvoorbeeld door het tegengaan van toxische oligomeren van alfa-synucleïne, het verbeteren van mitochondriale en/of lysosomale functie, en het remmen van neuro-inflammatie.

Het combineren van meerdere therapieën brengt echter wetenschappelijke, regelgevende en operationele uitdagingen met zich mee. Wetenschappelijk gezien kunnen geneesmiddelencombinaties leiden tot farmacokinetische of farmacodynamische interacties die de werkzaamheid of veiligheid beïnvloeden, met mogelijk onverwachte bijwerkingen die niet optreden bij individuele therapieën. Regelgevende instanties zoals de Amerikaanse FDA en het Europese EMA vereisen dat elke combinatie afzonderlijk wordt getest om de unieke werkzaamheid aan te tonen, specifieke risico's te identificeren en te bevestigen dat alle componenten daadwerkelijk bijdragen aan het klinisch voordeel. Vanuit ontwikkelingsperspectief verhoogt elke extra therapie het aantal mogelijke trialscenario's, wat de complexiteit van het studiedesign vergroot, de tijdlijnen verlengt, de kosten verhoogt en ethische overwegingen oproept over blootstelling van patiënten aan niet-geteste combinaties. Hoewel rationele combinatietherapie een aantrekkelijke benadering is, vereist deze een rigoureuze evaluatie om veiligheid en werkzaamheid te waarborgen en te voldoen aan de regelgeving.

Zoals uitgebreider beschreven in **hoofdstuk 2**, zijn er momenteel diverse experimentele therapieën in ontwikkeling voor de ziekte van Parkinson, gericht op uiteenlopende pathologische mechanismen. De meest gevorderde zijn buntanetap, ambroxol en exenatide, die allen fase 3 klinische studies hebben bereikt. Buntanetap, dat zich richt op de translatie van alfa-synucleïne, liet in fase 2 verbetering zien in motorische en cognitieve symptomen, en een fase 3-studie is afgerond, al zijn de resultaten nog niet bekend. Ambroxol, dat de activiteit van glucocerebrosidase en lysosomale functie versterkt, wordt onderzocht in meerdere fase 2- en 3-studies, waaronder studies gericht op deelnemers met GBA-mutaties en patiënten met Lewy body dementie. Exenatide, een GLP-1 receptoragonist, wordt geëvalueerd in een 96-weken durende fase 3-studie na eerdere aanwijzingen voor

motorisch voordeel en mogelijke betrokkenheid van insulinesignalering in de hersenen. Andere therapieën zoals prasinezumab en cinpanemab, beide monoklonale antilichamen gericht op alfa-synucleïne, bereikten fase 2 maar voldeden niet aan de primaire eindpunten en zijn niet verder ontwikkeld. Daarnaast zijn er verschillende therapieën in een vroeg stadium van ontwikkeling (fase 1 of 2), waaronder DNL201 (ook bekend als BIIB122, een LRRK2-remmer), terazosine (een PGK1-stimulator), KM-819 (een FAS-associatiefactor 1-remmer), en CNM-Au8 (een katalytische nanotherapeutische die de oxidatie van NAD bevordert en de intracellulaire ATP-niveaus verhoogt). Hoewel deze kandidaten biologische activiteit of target engagement hebben aangetoond, ontbreekt bij de meeste nog overtuigend bewijs van klinische werkzaamheid. Voor andere Parkinson-achtige aandoeningen zoals multipele systeematrofie en progressieve supranucleaire parese blijft de klinische ontwikkeling beperkt, en zijn er nog geen therapieën verder gekomen dan vroege fase studies.

Over het geheel genomen breidt het therapeutische landschap zich uit, maar robuuste, placebogecontroleerde gegevens uit lopende en toekomstige studies zullen essentieel zijn om te bepalen welke benaderingen daadwerkelijk de ziekteprogressie kunnen beïnvloeden.

Hoewel er nog steeds uitdagingen zijn, biedt deze thesis inzichten in de ontwikkeling van ziekte-modificerende therapieën voor neurodegeneratieve aandoeningen, in het bijzonder de ziekte van Parkinson. Voortdurende innovatie, gecombineerd met gerichte en geïndividualiseerde benaderingen, biedt perspectief om zowel motorische als niet-motorische symptomen aan te pakken, met het potentieel om de progressiesnelheid te verlagen en daarmee de uitkomsten voor patiënten te verbeteren.

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Eijsvogel P, Gorbenko AA, Tardiff DF, Skupien M, Rhodes K, Scannevin RH, et al. *Fatty acids as potential biomarkers of stearoyl-CoA desaturase inhibition: Variation in healthy subjects and Parkinson's disease patients.* Biomarkers Neuropsychiatry. 2025. https://doi.org/10.1016/j.bionps.2025.100132

Eijsvogel P, Smits LMG., Kremer PHC, Groeneveld GJ. *A systematic review on disease-modifying therapies in Parkinsonian disorders*. Submitted

Eijsvogel P, Gorbenko AA, Vissers MFJM, Scannevin RH, Groeneveld GJ, Kremer PHC. *Safety, Pharmacokinetics and Markers of Target Engagement of a SCD-inhibitor (YTX-7739): Randomized, Placebo-Controlled, Double-Blind Phase 1/1b Studies in Healthy Participants*. Submitted.

CURRICULUM VITAE

Pepijn Percival Nicolaas Maria Eijsvogel was born in Haarlem on December 9th 1992 and attended grammar school at the Stedelijk Gymnasium Haarlem, graduating in 2011. He subsequently studied medicine at the University of Hasselt, Belgium, and University of Maastricht, graduating in 2018. After a period in surgery, he joined the Centre for Human Drug Research in Leiden as research physician and project leader in 2020, later progressing to the role of (Associate) Clinical Study Manager. The research presented in this thesis was conducted during this period, alongside his contributions to multiple clinical trials across other therapeutic areas. During his tenure at CHDR, he qualified as a board-certified Clinical Pharmacologist. In 2026, he started working as Clinical Lead Early Clinical & Experimental Therapeutics (ECET) at Sanofi. Pepijn currently lives in Boskoop with his wife Lucia and their sons Hugo and Boudewijn.

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