

Clinical pharmacology studies
investigating novel formulations
of dopaminergic drugs

Eva Thijssen

CLINICAL PHARMACOLOGY STUDIES INVESTIGATING NOVEL
FORMULATIONS OF DOPAMINERGIC DRUGS

CLINICAL PHARMACOLOGY
STUDIES INVESTIGATING
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OF DOPAMINERGIC DRUGS

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CHAPTER 1

Introduction

PARKINSON'S DISEASE

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects millions of people worldwide, significantly impacting their quality of life. It is the fastest growing neurological disorder.¹ Whereas in 1990, 2.5 million people were affected by PD worldwide, this number had increased to 6.1 million in 2016.¹ Projections indicate that this will further increase to 13-14 million people in 2040.² This increase can be attributed to the aging worldwide population but environmental factors linked to industrialization are expected to contribute as well.¹

Early motor symptoms of PD can be subtle and may go unnoticed. As the condition progresses, more pronounced motor signs become apparent, such as slowness of movement (bradykinesia), tremors, rigidity, and impaired balance. Before the appearance of these characteristic motor symptoms, patients may have already experienced non-motor symptoms like hyposmia, sleep disturbance (e.g., rapid eye movement (REM) sleep behavior disorder (RBD)), depression and constipation for several years.³ Similar to the motor symptoms, the non-motor symptoms will progress as the disease advances, and cognitive impairment and autonomic dysfunction will become more common in the later disease stage.³

The exact cause of PD is unknown, but it is believed to result from an interplay between genetic, environmental and lifestyle factors. The two most common genetic risk factors linked to PD are mutations in the glucocerebrosidase (*GBA1*) and the leucine-rich repeat kinase 2 (*LRRK2*) genes. Ongoing research continues to unveil a growing number of common and rare genetic variants linked to the disease.⁴ Exposure to pesticides has been linked to a higher likelihood of developing PD, while smoking, caffeine intake, and physical activity have been associated with a decreased risk.⁵

Neuropathologically, PD is characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta. At the time of diagnosis, it is estimated that approximately 30% of the dopaminergic neurons of the substantia nigra and 50-60% of their axon terminals have degenerated.⁶ This degeneration

causes striatal dopamine deficiency resulting in the cardinal PD motor symptoms. The second pathological hallmark of PD is the accumulation of alpha-synuclein aggregates in neurons, called Lewy bodies and Lewy neurites. Under physiological circumstances, alpha synuclein is thought to play a role in synaptic vesicle dynamics, mitochondrial function and intracellular trafficking. However, upon its misfolding and aggregation, it becomes neurotoxic. It has been hypothesized that the initial misfolding and aggregation of alpha-synuclein may start in the gut enteric nerves and the olfactory bulb, and from there spread in a prion-like fashion to other areas, ultimately affecting the dopaminergic neurons in the substantia nigra.⁷ Despite the importance of nigrostriatal neurodegeneration in the clinical motor presentation, degeneration is certainly not limited to dopaminergic neurons, but also affects for example GABAergic, glutamatergic, and cholinergic neurons.⁸ The pathophysiological mechanisms underlying the abovementioned PD hallmarks involve a complex interplay of alpha-synuclein aggregation and spreading, mitochondrial dysfunction and oxidative stress, lysosomal dysfunction, and neuroinflammation.⁷

To date, no disease-modifying therapies for PD are on the market yet. Available treatment options are focused on symptom control to improve the quality of life for patients. This is typically achieved by either indirectly boosting dopamine levels in the brain or mimicking dopamine's effects through medications such as levodopa, dopamine agonists, catechol-O-methyltransferase (COMT) inhibitors, and monoamine oxidase B (MAO-B) inhibitors. Moreover, some non-dopaminergic and non-pharmacological treatment options are available, such as anticholinergics, amantadine, physiotherapy, occupational therapy, and speech and language therapy.⁹

SYMPTOM FLUCTUATIONS

A large proportion of patients develop motor complications within a few years after disease onset and dopaminergic therapy initiation.^{10,11} Complications consist of motor fluctuations and abnormal involuntary movements (dyskinesias). Motor fluctuations cause the

patient to alternate between periods of favorable response to medication (*ON* phase) and periods of inadequate response (*OFF* phase). These fluctuations in therapeutic effects can be predictable (e.g., end of dose 'wearing *OFF*') or unpredictable, and do not only involve fluctuations in motor symptoms but also in non-motor symptoms like anxiety, panic attacks, mood changes, slowness of thinking, and pain.¹² Fluctuating symptoms impact activities of daily living and worsen quality of life.^{11,13}

The development of motor complications results from pre- and postsynaptic dopaminergic changes, as well as secondary changes to non-dopaminergic systems.¹⁴ As the disease progresses an increasing number of dopaminergic nigrostriatal neurons degenerate, resulting in reduced endogenous dopamine synthesis, presynaptic dopamine storage capacity and dopamine release.¹⁵ Due to the loss of the presynaptic dopaminergic terminals, there is a reduced capacity to regulate the fluctuations in plasma levodopa levels.^{15,16} Moreover, dopamine release is further dysregulated by serotonergic neurons taking over the function of dopaminergic neurons in the striatum.¹⁴ Whereas serotonergic neurons can store and release dopamine, they do so in an uncontrollable manner since they lack presynaptic autoreceptors and the ability for dopamine reuptake.¹⁴ This leads to unphysiological fluctuations in extracellular dopamine and hence in unphysiological postsynaptic dopamine receptor stimulation. Similarly, dopaminergic treatment leads to non-physiological, pulsatile stimulation of post-synaptic dopamine receptors.¹⁷⁻¹⁹ This non-physiological stimulation results in post-synaptic changes affecting receptor sensitivity and intracellular signal processing, ultimately affecting the postsynaptic response to dopamine and the striatal output activity.^{7,15} Lastly, the combined impact of dopaminergic neurodegeneration and pulsatile receptor stimulation leads to pathophysiological changes to non-dopaminergic systems, such as the glutamatergic and serotonergic system.¹⁴ Together these changes result in impaired dopaminergic control leading to *ON-OFF* fluctuations and/or dyskinesia.

When a patient is using levodopa but motor complications persist despite optimized oral levodopa therapy, other treatments can

be chosen or added. First options are adding oral or transdermal drugs, like non-ergot dopamine agonists and enzyme inhibitors that prolong the effect of levodopa, i.e., COMT and MAO-B inhibitors.²⁰ If dyskinesia is the main problem, then if possible, dopaminergic medication should be reduced and amantadine or clozapine can be added.²⁰ If the abovementioned adjustments are insufficient, advanced device-aided treatments are available, i.e., deep brain stimulation (DBS), continuous subcutaneous apomorphine infusion and levodopa-carbidopa intestinal infusion.²¹⁻²³ The infusion pump therapies provide continuous drug administration resulting in fewer fluctuations in drug plasma levels and hence more continuous dopaminergic stimulation. However, if a patient has less than five *OFF* periods per day, intermittent treatment is often preferred over a continuous therapy. For relief of these sudden and intermittent *OFF* periods, subcutaneous apomorphine injections have long been the only treatment option. Its onset of action has been reported to be between 5-15 minutes,²⁴⁻²⁶ with maximum motor improvements as assessed by part III of the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) after 20-40 minutes.^{25,27,28} Despite its efficacy and fast onset of action, the use of intermittent injections is often limited by injection site reactions, pain and difficulty self-administering the injection during an *OFF* period.²⁹ With the FDA approval in 2018 of Inbrija, a breath-actuated inhaler of levodopa powder, and in 2020 of KYNMOBI, an apomorphine sublingual film, the treatment options for on-demand therapy of *OFF* periods increased.³⁰ Inhalable levodopa and sublingual apomorphine have an initial onset of effect based on MDS-UPDRS III reduction around 10 minutes and 15 minutes respectively, and show maximum MDS-UPDRS III improvements at 30 and 60 minutes respectively.³¹⁻³³ Both are considered less invasive treatment options than subcutaneous apomorphine injections. Unfortunately, only three years after sublingual apomorphine was introduced to the market, Sunovion announced its discontinuation in the US and Canada due to "limited utilization" and "business reasons". As of September 2023, it is no longer available, and hence the treatment options for *OFF* periods remain limited.

APOMORPHINE

The historical use of apomorphine is believed to have its origins in ancient cultures like that of the Maya civilization and ancient Egyptians. Depictions in tombs and papyrus scrolls dating back to 1400 BC portray the *Nymphaea caerulea* flower. Based on the drawings, it seems these civilizations used *Nymphaea* plants in religious-magical ceremonies, likely because of their aphrodisiac and hallucinogenic properties, as well as in purifying rituals because of their emetic effects. We now know that the bulbs and roots of this water lily species contains various aporphins, including apomorphine.³⁴ The synthesis of apomorphine did not occur until 1868 when Matthiessen and Wright produced apomorphine by heating morphine with concentrated hydrochloric acid.³⁵ This advanced and intensified the research into the effects of apomorphine. In 1884, it was suggested by Weil for the first time that apomorphine might be a potential treatment for PD.³⁶ Nonetheless, it took until 1951 for apomorphine to be administered to PD patients for the first time by Schwab et al.³⁷ They demonstrated that subcutaneous injections had positive effects on PD motor symptoms, but also resulted in side effects like nausea, vomiting and hypotension. These peripheral side effects impeded its broader use. Therefore, the discovery that a peripheral dopamine receptor antagonist (domperidone) could reduce apomorphine-induced side effects like nausea, drowsiness, sedation and hypotension, was a major breakthrough in 1979.³⁸ More years of research followed, resulting in apomorphine's first European marketing authorization in the UK in 1993.³⁹ It was approved for use as subcutaneous intermittent injections (APO-go) to treat OFF episodes in PD patients. Approval in other EU countries followed in the subsequent years. In the US, intermittent subcutaneous apomorphine injections (APOKYN) earned FDA approval in 2004.⁴⁰ In the EU, apomorphine is also available as a continuous subcutaneous infusion via a percutaneous pump. Again, this formulation received its first marketing approval in the UK in 2004 and was approved in other EU countries in the following years.⁴¹ This formulation is currently not (yet) authorized by the FDA. The FDA

did authorize the use of apomorphine sublingual film (ΚΥΝΜΟΒΙ) in 2020.³⁰ Unfortunately, the drug was discontinued about three years later due to "limited utilization".

Apomorphine is a potent broad spectrum dopamine agonist, activating both D1-like (D1, D5) and D2-like (D2, D3, D4) receptor subtypes.⁴² Moreover, based on in vitro studies, it was demonstrated to have modest agonistic activity at 5-HT_{1A} receptors, and acts as an antagonist at α₂-adrenergic, 5-HT_{2A} and 5-HT_{2C} receptors.^{43,44} The potential influence of apomorphine on the adrenergic system has not yet been well explored.⁴⁵ As for the serotonergic system, apomorphine might have a lower tendency to induce visual hallucinations compared to other dopamine agonists due to its 5-HT_{2A} antagonism.⁴⁶ Apomorphine's molecular formula is C₁₇H₁₇NO₂ and it has a molecular weight of 267.32 g/mol.⁴⁵ In clinical practice, apomorphine is used as its hydrochloride salt. It is a chiral molecule, meaning it exists in two distinct mirror-image forms called R- and S-enantiomers. The R-enantiomer is the biologically active form responsible for its pharmacological effects.⁴⁵ Therefore, the R-enantiomer is utilized in clinical practice.⁴⁷ In vivo, there is no interconversion to the S-form.⁴⁸ Apomorphine's polycyclic structure makes it highly lipophilic, allowing apomorphine to easily cross the blood-brain barrier. Its ortho-catechol group has less favorable effects since it renders apomorphine sensitive to oxidation, making it unstable in aqueous solutions. Exposure to light and air triggers this spontaneous oxidation, turning apomorphine solutions green. Apomorphine oxidation results in a loss of pharmacological activity and the formation of quinones and reactive oxygen species.⁴⁵ These molecules can be cytotoxic due to the induction of oxidative stress and damage to lipids, proteins, and DNA.^{49,50} Therefore, antioxidants like sodium bisulfite and L-ascorbic acid are often part of apomorphine formulations to enhance apomorphine's stability in solution. Moreover, it is protected from light.⁴⁵

Apomorphine has a poor oral bioavailability (<4%) due to its nearly complete first-pass hepatic metabolism.⁵¹ Hence, apomorphine formulations are administered subcutaneously or sublingually (recently discontinued), thereby effectively bypassing the first-pass effect. The bioavailability of subcutaneous apomorphine is approximately

100%,^{52,53} and the relative bioavailability of sublingual to subcutaneous apomorphine is 17.2 (13.7–21.6)%.⁵⁴ For subcutaneous and sublingual apomorphine, median time to reach maximum plasma concentrations (T_{max}) varies across studies, but usually ranges between 15–23 and 38–51 minutes respectively.^{54–56} Maximum concentrations in cerebrospinal fluid lag approximately 10–20 minutes behind, and correlate with the onset of clinical effect.⁵⁷ Apomorphine is rapidly cleared from the body with a terminal elimination half-life ($T_{1/2}$) ranging between 30–60 minutes.⁵⁸ This rapid clearance results in a short duration of effect, making apomorphine a suitable rescue medication that can be used in addition to standard PD medication.

Since apomorphine's pharmacokinetics (PK) and pharmacodynamics are subject to high interindividual variability, individual dose titration under medical supervision is required.⁵⁷ A low dose is given initially (usually 1 or 2 mg for subcutaneous apomorphine,^{59,60} and 10 mg for sublingual apomorphine), and increased until the 'optimal' dose is found. The 'optimal' dose is considered the dose with the shortest latency to effect and the longest effect duration while minimizing side effects. While the optimal dose differs for each patient, most patients require 3 mg apomorphine when given as a subcutaneous administration.⁶¹ During dose titration, special focus should be on blood pressure (supine and standing) and ECGs to monitor for potential (orthostatic) hypotension and QT prolongation, respectively. Apomorphine, especially at high doses, may induce QT prolongation.⁵⁹ This risk is increased with the concomitant use of domperidone, which is often co-prescribed to prevent the peripheral side effects of apomorphine. Therefore, monitoring the QT interval prior to domperidone initiation and during apomorphine treatment initiation is recommended, and as clinically indicated thereafter.^{60,62} Especially in patients that are at risk for torsades de pointes arrhythmia. Other commonly reported side effects are nausea, vomiting, yawning, drowsiness, somnolence, dizziness, and dyskinesia.^{59,60,62} Also local side effects should be monitored, i.e., injection site reactions (subcutaneous apomorphine) or oropharyngeal side effects (sublingual apomorphine).

With the recent discontinuation of sublingual apomorphine (KYNMOBI), subcutaneous apomorphine remains the only available apomorphine administration route for the treatment of *OFF* episodes.

The need for a less invasive and therefore more user-friendly treatment option remains high. Over the years, other administration routes have been investigated, such as transdermal, intranasal, rectal and inhaled routes. Initial studies investigating transdermal delivery of apomorphine were hampered by poor/absent bioavailability.^{63,64} However, a study in 2004 showed relevant plasma concentrations could be reached when an apomorphine microemulsion was administered.⁶⁵ Single administration of this microemulsion often caused local erythema (71.4%) and its PK (T_{max} 5.1 hours; $T_{1/2}$ 10.8 hours) was unfavorable for use as a treatment of acute *OFF* episodes. It might be suitable as an add on sustained-release formulation for e.g., nocturnal fluctuations. To date, no further PD patient studies using transdermal apomorphine have advanced in clinical development. Intranasal administration, although fast and efficacious,^{66–71} resulted in local side effects like nasal irritation, nasal congestion, vestibulitis and nasal crusting.^{67,69–71} Hence, it is no longer in development. In the 1990's, three studies investigated rectal apomorphine delivery.^{72–74} While this route demonstrated clinical efficacy, its further development was halted, likely due to difficulty self-administering apomorphine rectally during an *OFF* episode, as well as its longer latency to effect compared to subcutaneous delivery. Inhalation of apomorphine dry powder (VRO40) has been investigated in three studies published in 2013. It showed favorable PK with maximum plasma concentrations between 1–7 minutes post-dose,^{75,76} and a mean latency to an *ON* state of 8 and 10 minutes reported in two studies.^{76,77} No local side effects or effects on lung function were reported. The observed side effects were limited to dopaminergic side effects, consistent with those seen with other apomorphine formulations.^{75–77} Despite these positive outcomes, no further studies on this dry powder apomorphine formulation have been reported.

Apomorphine is currently underutilized,⁷⁸ likely because the subcutaneous injection has several impracticalities like the need to inject oneself while being *OFF*, whereas also the occurrence of injection site reactions and fear for needles may play a role. The sublingual apomorphine film that was available between 2020 and 2023 in the US and Canada often caused oropharyngeal side effects and had a later T_{max} than subcutaneous apomorphine.⁵⁶ Furthermore, the administration of subcutaneous injections and

the handling of the sublingual film requires good finger dexterity and muscle coordination that is often impaired in patients with PD.⁷⁹ Therefore, there is a need for a non-invasive and easy to administer apomorphine formulation. In this thesis, we focus on two new apomorphine formulations for the management of acute *OFF* episodes. In **chapter 2 and 3**, we will evaluate AZ-009, which is a breath-actuated, oral inhalation device using the Staccato technology.^{80,81} This technology was already previously approved by the FDA and EMA for the administration of loxapine.⁸²⁻⁸⁴ A single breath through the device leads to rapid heating (<0.5 second) of a metal substrate coated with a thin film of excipient-free apomorphine. As a result pure drug vapor is formed that rapidly cools and condenses into aerosol particles appropriate for deep lung delivery and subsequent systemic exposure. Consequently, AZ-009 has the potential to induce a quick transition from *OFF* to *ON*, potentially even faster than subcutaneous apomorphine. In **chapter 4**, we describe a study that evaluated APORON, an oromucosal solution that is administered to the buccal area using a dispenser. Since apomorphine is dissolved in a solution (as opposed to the sublingual film), it is hypothesized to have a reduced risk of apomorphine particles lingering in the oropharyngeal space for a prolonged time after dosing. The dose that is not buccally absorbed is anticipated to be swallowed with the saliva and become subject to first-pass metabolism. This would minimize the chance of apomorphine degradation into reactive oxygen species in the oropharyngeal space, thereby reducing toxicity.

SHOWING ACUTE DOPAMINERGIC MEDICATION EFFECTS IN CLINICAL TRIALS

To evaluate whether a drug is suitable as a rescue medication for managing acute *OFF* episodes, it should not only be efficacious, but also have a rapid onset of action. Its onset, as reflected by T_{max} , should be faster than the standard PD medication like levodopa/carbidopa immediate release tablets, which has a reported median T_{max} in advanced PD patients of 1.25 to 1.5 hours after repeat dosing.^{85,86}

The Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) can be seen as the gold standard for evaluating various aspects of Parkinson's disease, and is often used in clinical trials to show medication effects. The scale is composed of four parts.⁸⁷ Part I assesses the non-motor impact on experiences of daily living, e.g., the effect of cognitive impairment, mood and sleep disturbances, pain, urinary and gastrointestinal problems, and orthostatic hypotension on daily living. Part II assesses the motor impact on experiences of daily living, e.g., the effect of problems with speech, swallowing/chewing, dressing, writing and other mobility problems on daily living. Part III is a motor examination performed by a trained rater assessing speech, facial expression, rigidity, finger/toe tapping, hand movements, gait, balance, posture, bradykinesia, and tremor. Part IV investigates the presence and functional impact of motor complications, i.e., dyskinesia, motor fluctuations and painful *OFF* state dystonia. Especially part III of the MDS-UPDRS is often used in clinical trials to show motor improvements after (dopaminergic) medication intake.^{31,32,88} However, part III requires a trained rater who preferably assesses a patient throughout the entire trial to avoid inter-rater variability. Additionally, the assessment takes relatively long (approximately 15 minutes, dependent on the patient's clinical state). This makes accurate time-response assessment of fast-acting agents challenging, especially when safety and pharmacokinetic measurements also need to be performed. Hence, a short, rater-independent and quantitative measurement would be ideal for use in clinical trials.

As technology continues to advance, we are increasingly capable of objectively and quantitatively measuring core PD motor symptoms like tremor, bradykinesia, gait disturbances and dyskinesia. These symptoms and their severity can be assessed using wearable sensors such as accelerometers, gyroscopes and inertial measurement units; with tests on tablets and mobile phones; and by using video-based movement analysis.⁸⁹ The recent advances in wearable sensors and smart devices make at-home monitoring of PD symptoms possible. At-home monitoring can be useful to monitor a patient's symptom severity over time to facilitate medication adjustments, or to evaluate whether a disease-modifying drug can slow down disease

progression. The focus of this thesis, however, is on demonstrating acute (dopaminergic) medication effects. For that, we evaluated three different finger tapping tasks while patients were confined to the clinical research unit. Finger tapping tasks are well suited for the evaluation of bradykinesia, which is one of the cardinal features of PD and defined as 'slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions'.⁹⁰ Bradykinesia, unlike tremor, usually responds well to dopaminergic treatment.⁹⁰ While quantification of finger tapping is not new, the methods available are improving and growing in numbers, ranging from rudimentary to more sophisticated methods. Described methods for the assessment of repetitive index finger tapping or alternate index-middle finger tapping include the use of arcade buttons,⁹¹ computer keyboards,^{92,93} keyboards with a musical instrument digital interface,⁹⁴ and touchscreen devices (smartphones, tablets).^{23,37,71,95} An advantage of touchscreen devices is that the precise location (x, y coordinates) of each tap can be registered and hence tapping accuracy can be quantified precisely. Despite the abundance of available methods, there is a lack of standardization in task configurations. Differences between tasks include the finger(s) used for tapping (e.g., index finger tapping, or alternate index-middle finger tapping), the distance between targets (if applicable), the test duration, and whether it is given with or without a (visual) cue. Thumb-index finger tapping is part of the MDS-UPDRS III (item 3.4) and includes the evaluation of tapping rhythm, slowing of movement and tapping amplitude. These various aspects result in a combined score between 0 and 4 points. Methods for the quantification of thumb-index finger tapping are also available such as video-based motion-analysis systems,^{96,97} and sensors like accelerometers,⁹⁸ gyrosopes,⁹⁹ magnetic sensors,¹⁰⁰ or combinations of these sensors known as inertial measurement units.¹⁰¹ Electronic goniometers are angular sensors and can quantify joint movement when the end blocks of the goniometer are placed on either side of the center of the joint. It has been shown useful for this purpose in measuring for example the flexion and extension of the index finger, wrist and elbow.¹⁰² To the best of our knowledge, an electrogoniometer has never been used to quantify thumb-index finger tapping in PD patients.

Literature has shown that finger tapping tasks can differentiate between healthy controls and PD patients^{92,103-110} and between medication states (*ON/OFF*).^{94,105,107,109-113} However, the set-up and devices used for these tapping tasks vary among studies, and it is unclear which is most suitable for the determination of (dopaminergic) medication effects in randomized placebo-controlled trials. Therefore, in this thesis we evaluate different tapping tasks alongside the gold standard MDS-UPDRS III. In [chapter 5](#), we describe a study that evaluated the within- and between-day repeatability of touchscreen-based tapping tasks in different configurations in healthy volunteers. Configurations included alternate index and middle finger tapping with 2.5 cm between targets and repetitive alternate index finger tapping with 20 cm between targets. Both of these tasks were tested with and without a visual cue. In [chapter 6](#), we describe a randomized, double-blind, placebo-controlled crossover study in PD patients to assess the ability of three different finger tapping tasks to detect dopaminergic medication effects. Moreover, we evaluated whether finger tapping outcomes correlate with the gold standard MDS-UPDRS III. The tapping tasks being compared include the two uncued touchscreen tasks described in chapter 5 and a thumb-index finger tapping task using an electronic goniometer. In [chapter 7](#), the data from the clinical study in chapter 6 were used to train machine learning algorithms to select the optimal combination of finger tapping task features ('composite biomarker') to predict treatment effect (i.e., did the patient receive active or placebo treatment?) and estimate the disease severity (i.e., MDS-UPDRS III score).

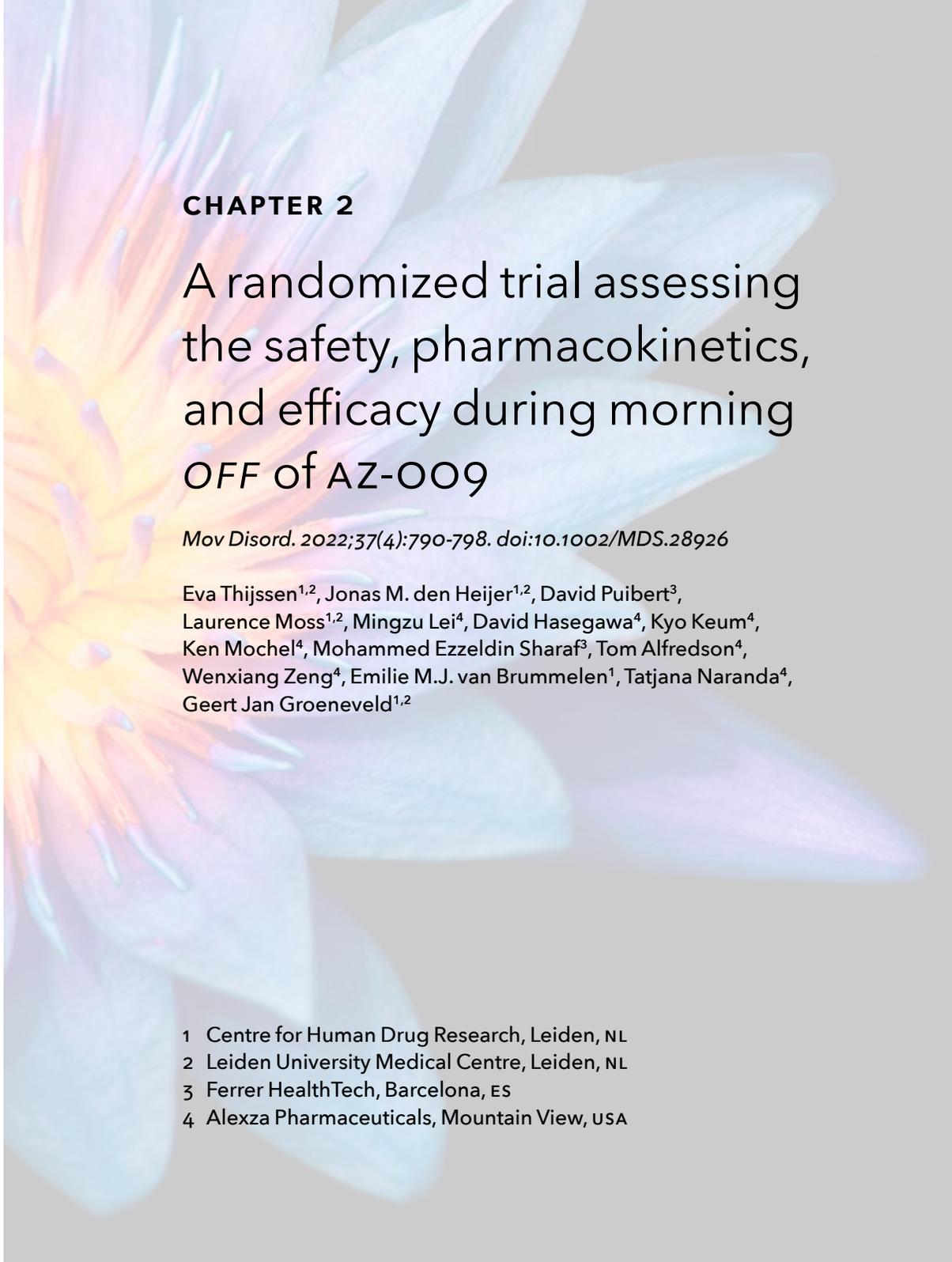
Summarizing, this thesis investigates two novel apomorphine formulations for the acute treatment of *OFF* episodes, aiming to provide a more user-friendly alternative than the currently available subcutaneous injections. Moreover, we evaluated the usefulness of different finger tapping tasks as quick and quantitative pharmacodynamic measures for assessing the efficacy of fast-acting dopaminergic compounds. With the results described in this thesis, we hope to improve the quality of early phase clinical trials with novel fast-acting apomorphine formulations, ultimately resulting in better symptomatic treatment of PD.

REFERENCES

- 1 Ray Dorsey E, Elbaz A, Nichols E, et al. Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 2018;17(11):939–953. doi:10.1016/S1474-4422(18)30295-3.
- 2 Dorsey ER, Bloem BR. The Parkinson Pandemic—A Call to Action. *JAMA Neurol.* 2018;75(1):9–10. doi:10.1001/JAMANEUROL.2017.3299.
- 3 Schapira AHV, Chaudhuri KR, Jenner P. Non-motor features of Parkinson disease. *Nat Rev Neurosci* 2017;18(7):435–450. doi:10.1038/nrn.2017.62.
- 4 Blauwendraat C, Nalls MA, Singleton AB. The genetic architecture of Parkinson's disease. *Lancet Neurol.* 2020;19(2):170–178. doi:10.1016/S1474-4422(19)30287-X.
- 5 Ascherio A, Schwarzschild MA. The epidemiology of Parkinson's disease: risk factors and prevention. *Lancet Neurol.* 2016;15(12):1257–1272. doi:10.1016/S1474-4422(16)30230-7.
- 6 Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. *Ann Neurol.* 2010;67(6):715–725. doi:10.1002/ANA.21995.
- 7 Poewe W, Seppi K, Tanner CM, et al. Parkinson disease. *Nat Rev Dis Prim.* 2017;3:1–21. doi:10.1038/NRDP.2017.13.
- 8 Miguez C, De Deurwaerdère P, Sgambato V. Editorial: Non-Dopaminergic Systems in Parkinson's Disease. *Front Pharmacol.* 2020;11:593822. doi:10.3389/fphar.2020.593822/BIBTEX.
- 9 Bloem BR, Okun MS, Klein C. Parkinson's disease. *Lancet.* 2021;397(10291):2284–2303. doi:10.1016/S0140-6736(21)00218-X/ATTACHMENT/04055C61-9DC5-416D-9929-D92B8F68DF58/MMC2.PDF.
- 10 Ahlskog JE, Muenter MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov Disord.* 2001;16(3):448–458. doi:10.1002/MDS.1090.
- 11 Stocchi F, Antonini A, Barone P, et al. Early DEtection of wEaring off in Parkinson disease: the DEEP study. *Parkinsonism Relat Disord.* 2014;20(2):204–211. doi:10.1016/J.PARKRELDIS.2013.10.027.
- 12 Martínez-Fernández R, Schmitt E, Martínez-Martin P, Krack P. The hidden sister of motor fluctuations in Parkinson's disease: A review on nonmotor fluctuations. *Mov Disord.* 2016;31(8):1080–1094. doi:10.1002/MDS.26731.
- 13 Chapuis S, Ouchchane L, Metz O, Gerbaud L, Durif F. Impact of the motor complications of Parkinson's disease on the quality of life. *Mov Disord.* 2005;20(2):224–230. doi:10.1002/MDS.20279.
- 14 Cenci MA, Skovgård K, Odin P. Non-dopaminergic approaches to the treatment of motor complications in Parkinson's disease. *Neuropharmacology.* 2022;210:109027. doi:10.1016/J.NEUROPHARM.2022.109027.
- 15 Stocchi F, Jenner P, Obeso JA. When do levodopa motor fluctuations first appear in Parkinson's disease? *Eur Neurol.* 2010;63(5):257–266. doi:10.1159/000300647.
- 16 De La Fuente-Fernández R, Sossi V, Huang Z, et al. Levodopa-induced changes in synaptic dopamine levels increase with progression of Parkinson's disease: implications for dyskinesias. *Brain.* 2004;127(Pt 12):2747–2754. doi:10.1093/BRAIN/AWH290.
- 17 Nutt JG, Obeso JA, Stocchi F. Continuous dopamine-receptor stimulation in advanced Parkinson's disease. *Trends Neurosci.* 2000;23(10 Suppl). doi:10.1016/S1471-1931(00)00029-X.
- 18 Chase TN, Baronti F, Fabbrini G, Heuser JJ, Juncos JL, Mouradian MM. Rationale for continuous dopaminomimetic therapy of Parkinson's disease. *Neurology.* 1989;39(11 Suppl 2):7–10; discussion 19. Accessed July 26, 2023. <https://europepmc.org/article/med/2685653>.
- 19 Obeso JA, Rodriguez-Oroz M, Marin C, et al. The origin of motor fluctuations in Parkinson's disease: importance of dopaminergic innervation and basal ganglia circuits. *Neurology.* 2004;62(1 Suppl 1). doi:10.1212/WNL.62.1_SUPPL_1.S17.
- 20 Armstrong MJ, Okun MS. Diagnosis and Treatment of Parkinson Disease: A Review. *JAMA.* 2020;323(6):548–560. doi:10.1001/JAMA.2019.22360.
- 21 Katzenschlager R, Poewe W, Rascol O, et al. Apomorphine subcutaneous infusion in patients with Parkinson's disease with persistent motor fluctuations (TOLEDO): a multicentre, double-blind, randomised, placebo-controlled trial. *Lancet Neurol.* 2018;17(9):749–759. doi:10.1016/S1474-4422(18)30239-4.
- 22 Pahwa R, Aldred J, Merola A, et al. Long-term results of carbidopa/levodopa enteral suspension across the day in advanced Parkinson's disease: Post-hoc analyses from a large 54-week trial. *Clin Park Relat Disord.* 2023;8:100181. doi:10.1016/J.PRDOA.2022.100181.
- 23 Hariz M, Blomstedt P. Deep brain stimulation for Parkinson's disease. *J Intern Med.* 2022;292(5):764–778. doi:10.1111/JOIM.13541.
- 24 Frankel JP, Lees AJ, Kempster PA, Stern GM. Subcutaneous apomorphine in the treatment of Parkinson's disease. *J Neurol Neurosurg Psychiatry.* 1990;53(2):96–101. doi:10.1136/JNPP.53.2.96.
- 25 Pfeiffer RF, Gutmann L, Hull KL, Bottini PB, Sherry JH. Continued efficacy and safety of subcutaneous apomorphine in patients with advanced Parkinson's disease. *Parkinsonism Relat Disord.* 2007;13(2):93–100. doi:10.1016/J.PARKRELDIS.2006.06.012.
- 26 Stibe CMH, Kempster PA, Lees AJ, Stern GM. Subcutaneous apomorphine in parkinsonian on-off oscillations. *Lancet (London, England).* 1988;1(8582):403–406. doi:10.1016/S0140-6736(88)91193-2.
- 27 Trosch RM, Silver D, Bottini PB. Intermittent subcutaneous apomorphine therapy for “off” episodes in Parkinson's disease: a 6-month open-label study. *CNS Drugs.* 2008;22(6):519–527. doi:10.2165/00023210-200822060-00005.
- 28 Pahwa R, Koller WC, Trosch RM, Sherry JH. Subcutaneous apomorphine in patients with advanced Parkinson's disease: a dose-escalation study with randomized, double-blind, placebo-controlled crossover evaluation of a single dose. *J Neurol Sci.* 2007;258(1–2):137–143. doi:10.1016/J.JNS.2007.03.013.
- 29 Carbone F, Djamshidian A, Seppi K, Poewe W. Apomorphine for Parkinson's Disease: Efficacy and Safety of Current and New Formulations. *CNS Drugs.* 2019;33(9):905–918. doi:10.1007/S40263-019-00661-Z.
- 30 Hauser RA, LeWitt PA, Comella CL. On demand therapy for Parkinson's disease patients: Opportunities and choices. *Postgrad Med.* 2021;133(7):721–727. doi:10.1080/00325481.2021.1936087.
- 31 Olanow CW, Factor SA, Espay AJ, et al. Apomorphine sublingual film for off episodes in Parkinson's disease: a randomised, double-blind, placebo-controlled phase 3 study. *Lancet Neurol.* 2020;19(2):135–144. doi:10.1016/S1474-4422(19)30396-5.
- 32 LeWitt PA, Hauser RA, Pahwa R, et al. Safety and efficacy of CVT-301 (levodopa inhalation powder) on motor function during off periods in patients with Parkinson's disease: a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet Neurol.* 2019;18(2):145–154. doi:10.1016/S1474-4422(18)30405-8.
- 33 LeWitt PA, Hauser RA, Grosset DG, et al. A randomized trial of inhaled levodopa (CVT-301) for motor fluctuations in Parkinson's disease. *Mov Disord.* 2016;31(9):1356–1365. doi:10.1002/MDS.26611.
- 34 Bertol E, Fineschi V, Karch SB, Mari F, Riezzo I. Nymphaea cults in ancient Egypt and the New World: a lesson in empirical pharmacology. *J R Soc Med.* 2004;97(2):84. doi:10.1258/JRSM.97.2.84.
- 35 Mathiessen A, Wright CRA. III. Researches into the chemical constitution of the opium bases. Part I.—On the action of hydrochloric acid on morphia. *Proc R Soc London.* 1869;17:455–460. doi:10.1098/RSP.1868.0094.
- 36 Weil E. De l'apomorphine dans certain troubles nerveux. *Lyon Méd.* 1884;(48):411–419.
- 37 Schwab R, Amador L, Lettvin J. Apomorphine in Parkinson's disease. *Trans Am Neurol Assoc.* 1951;(56):251–253. <https://pubmed.ncbi.nlm.nih.gov/14913646/>.
- 38 Corsini GU, Zompo M Del, Gessa GL, Mangoni A. Therapeutic efficacy of apomorphine combined with an extracerebral inhibitor of dopamine receptors in Parkinson's disease. *Lancet.* 1979;313(8123):954–956. doi:10.1016/S0140-6736(79)91725-2.
- 39 Medicines and Healthcare products Regulatory Agency. Public Assessment Report APO-Go PFS 5mg/ml Solution (UK/H/0342/03/MR.); 2008. Accessed August 9, 2023. <https://www.geneesmiddeleninformatiebank.nl/pars/h35295.pdf>.
- 40 Apomorphine-subcutaneous - Bertek/ Britannia. *Drugs R D.* 2004;5(4):211–212. doi:10.2165/00126839-200405040-00004/METRICS.
- 41 APO-go PFS 5mg/ml Solution for Infusion in Pre-filled Syringe - Summary of Product Characteristics (SmPC) - (emc). Published 2018. Accessed August 9, 2023. <https://www.medicines.org.uk/emc/product/3908/smpc>.
- 42 Millan MJ, Maiorini L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A. Differential Actions of

- Antiparkinson Agents at Multiple Classes of Monoaminergic Receptor. I. A Multivariate Analysis of the Binding Profiles of 14 Drugs at 21 Native and Cloned Human Receptor Subtypes. *J Pharmacol Exp Ther.* 2002;303(2):791-804. doi:10.1124/JPET.102.039867.
- 43 Newman-Tancredi A, Cussac D, Audinot V, et al. Differential Actions of Antiparkinson Agents at Multiple Classes of Monoaminergic Receptor. II. Agonist and Antagonist Properties at Subtypes of Dopamine D2-Like Receptor and $\alpha 1/\alpha 2$ -Adrenoceptor. *J Pharmacol Exp Ther.* 2002;303(2):805-814. doi:10.1124/JPET.102.039875.
- 44 Newman-Tancredi A, Cussac D, Quentric Y, et al. Differential Actions of Antiparkinson Agents at Multiple Classes of Monoaminergic Receptor. III. Agonist and Antagonist Properties at Serotonin, 5-HT1 and 5-HT2, Receptor Subtypes. *J Pharmacol Exp Ther.* 2002;303(2):815-822. doi:10.1124/JPET.102.039883.
- 45 Auffret M, Drapier S, V erin M. Pharmacological Insights into the Use of Apomorphine in Parkinson's Disease: Clinical Relevance. *Clin Drug Investig* 2018 384. 2018;38(4):287-312. doi:10.1007/S40261-018-0619-3.
- 46 Borgemeester RWK, Lees AJ, van Laar T. Parkinson's disease, visual hallucinations and apomorphine: A review of the available evidence. *Parkinsonism Relat Disord.* 2016;27:35-40. doi:10.1016/J.PARKRELDIS.2016.04.023.
- 47 Neef C, Van Laar T. Pharmacokinetic-pharmacodynamic relationships of apomorphine in patients with Parkinson's disease. *Clin Pharmacokinet.* 1999;37(3):257-271. doi:10.2165/00003088-199937030-00004/METRICS.
- 48 Van der Geest R, Van Laar T, Kruger PP, et al. Pharmacokinetics, enantiomer interconversion, and metabolism of R-apomorphine in patients with idiopathic Parkinson's disease. *Clin Neuropharmacol.* 1998;21(3):159-168. Accessed August 16, 2023. <https://europepmc.org/article/med/9617507>.
- 49 Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinones in toxicology. *Chem Res Toxicol.* 2000;13(3):135-160. doi:10.1021/TX9902082.
- 50 Dos Santos El-Bach a R, Daval JL, Kozziel V, Netter P, Minn A. Toxic effects of apomorphine on rat cultured neurons and glial C6 cells, and protection with antioxidants. *Biochem Pharmacol.* 2001;61(1):73-85. doi:10.1016/S0006-2952(00)00524-4.
- 51 Gancher ST, Nutt JG, Woodward WR. Absorption of apomorphine by various routes in parkinsonism. *Mov Disord.* 1991;6(3):212-216. doi:10.1002/MDS.870060304.
- 52 Gancher ST, Woodward WR, Boucher B, Nutt JG. Peripheral pharmacokinetics of apomorphine in humans. *Ann Neurol.* 1989;26(2):232-238. doi:10.1002/ANA.410260209.
- 53 Nicolle E, Pollak P, Serre-Debeauvais F, et al. Pharmacokinetics of apomorphine in parkinsonian patients. *Fundam Clin Pharmacol.* 1993;7(5):245-252. doi:10.1111/J.1472-8206.1993.TB00238.X.
- 54 Agbo F, Isaacson SH, Gil R, et al. Pharmacokinetics and Comparative Bioavailability of Apomorphine Sublingual Film and Subcutaneous Apomorphine Formulations in Patients with Parkinson's Disease and "OFF" Episodes: Results of a Randomized, Three-Way Crossover, Open-Label Study. *Neurol Ther.* 2021;10(2):693-709. doi:10.1007/S40120-021-00251-6/TABLES/6.
- 55 Nomoto M, Kubo SI, Nagai M, et al. A randomized controlled trial of subcutaneous apomorphine for Parkinson disease: A repeat dose and pharmacokinetic study. *Clin Neuropharmacol.* 2015;38(6):241-247. doi:10.1097/WNF.000000000000111.
- 56 Chen YL, Shi L, Agbo F, Yong SH, Tan PS, Ngounou Wetie AG. LC-MS/MS simultaneous quantification of apomorphine and its major metabolites in human plasma: Application to clinical comparative bioavailability evaluation for the apomorphine sublingual film and a subcutaneous product. *J Pharm Biomed Anal.* 2020;190:113493. doi:10.1016/J.JPBA.2020.113493.
- 57 LeWitt PA. Subcutaneously administered apomorphine. *Neurology.* 2004;62(6 suppl 4):S8-S11. doi:10.1212/WNL.62.6_SUPPL_4.S8.
- 58 Caughman CY, Factor S, Factor Jean S, Amos Parkinson P. A critical review of apomorphine hydrochloride sublingual film for the treatment of Parkinson's disease 'OFF' episodes. <https://doi.org/10.1080/1473717520201855145>. 2020;21(2):169-177. doi:10.1080/14737175.2020.1855145.
- 59 APOKYN (apomorphine hydrochloride injection) for subcutaneous use only - Summary of Product Characteristics (SmPC) - (FDA). Published 2017. Accessed August 20, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/021264s014lbl.pdf.
- 60 APO-go Pen 10mg/ml Solution for Injection - Summary of Product Characteristics (SmPC) - (emc). Published 2022. Accessed August 20, 2023. <https://www.medicines.org.uk/emc/product/2232/smpc#gref>.
- 61 Castillo-Torres SA, Lees AJ, Merello M. Intermittent Apomorphine Use for off Period Rescue in Parkinson's Disease: A Pragmatic Review of over Three Decades of Clinical Experience. *Mov Disord Clin Pract.* 2023;10(2):190-208. doi:10.1002/MDC3.13593.
- 62 Kynmobi 30 mg sublingual film - Summary of Product Characteristics (SmPC) - (emc). Published 2023. Accessed August 20, 2023. <https://www.medicines.org.uk/emc/product/14890/smpc/print>.
- 63 Van Der Geest R, Van Laar T, Gubbens-Stibbe JM, Bodd e HE, Danhof M. Iontophoretic delivery of apomorphine. II: An in vivo study in patients with Parkinson's disease. *Pharm Res.* 1997;14(12):1804-1810. doi:10.1023/A:1012152401715.
- 64 Gancher ST, Nutt JG, Woodward WR. Absorption of apomorphine by various routes in parkinsonism. *Mov Disord.* 1991;6(3):212-216. doi:10.1002/MDS.870060304.
- 65 Priano L, Albani G, Brioschi A, et al. Transdermal apomorphine permeation from microemulsions: A new treatment in Parkinson's disease. *Mov Disord.* 2004;19(8):937-942. doi:10.1002/MDS.20054.
- 66 Kapoor R, Turjanski N, Frankel J, et al. Intranasal apomorphine: a new treatment in Parkinson's disease. *J Neurol Neurosurg Psychiatry.* 1990;53(11):1015. doi:10.1136/JNPN.53.11.1015.
- 67 Van Laar T, Jansen ENH, Essink AWG, Neef C. Intranasal apomorphine in parkinsonian on-off fluctuations. *Arch Neurol.* 1992;49(5):482-484. doi:10.1001/ARCHNEUR.1992.00530290064013.
- 68 Dewey RB, Maraganore DM, Ahlskog JE, Matsumoto JY. Intranasal apomorphine rescue therapy for parkinsonian "off" periods. *Clin Neuropharmacol.* 1996;19(3):193-201. doi:10.1097/00002826-199619030-00001.
- 69 Esteban Mu oz J, Mart ı MJ, Mar ın C, Tolosa E. Long-term treatment with intermittent intranasal or subcutaneous apomorphine in patients with levodopa-related motor fluctuations. *Clin Neuropharmacol.* 1997;20(3):245-252. doi:10.1097/00002826-199706000-00009.
- 70 Dewey RB, Maraganore DM, Ahlskog JE, Matsumoto JY. A double-blind, placebo-controlled study of intranasal apomorphine spray as a rescue agent for off-states in Parkinson's disease. *Mov Disord.* 1998;13(5):782-787. doi:10.1002/MDS.870130505.
- 71 Kleedorfer B, Turjanski N, Ryan R, Lees AJ, Milroy C, Stern GM. Intranasal apomorphine in Parkinson's disease. *Neurology.* 1991;41(5):761-762. doi:10.1212/WNL.41.5.761-A.
- 72 Hughes AJ, Bishop S, Lees AJ, Stern GM, Webster R, Bovingdon M. Rectal apomorphine in Parkinson's disease. *Lancet (London, England).* 1991;337(8733):118. doi:10.1016/0140-6736(91)90780-S.
- 73 Van Laar T, Jansen ENH, Essink AWG, Rutten WJ, Neef C. Rectal apomorphine: a new treatment modality in Parkinson's disease. *J Neurol Neurosurg Psychiatry.* 1992;55(8):737-738. doi:10.1136/JNPN.55.8.737-A.
- 74 van Laar T, Jansen ENH, Neef C, Danhof M, Roos RAC. Pharmacokinetics and clinical efficacy of rectal apomorphine in patients with Parkinson's disease: A study of five different suppositories. *Mov Disord.* 1995;10(4):433-439. doi:10.1002/MDS.870100405.
- 75 Grosset KA, Malek N, Morgan F, Grosset DG. Phase IIa randomized double-blind, placebo-controlled study of inhaled apomorphine as acute challenge for rescuing "off" periods in patients with established Parkinson's disease. *Eur J Neurol.* 2013;20(11):1445-1450. doi:10.1111/ENE.12091.
- 76 Grosset KA, Malek N, Morgan F, Grosset DG. Inhaled dry powder apomorphine (vR040) for 'off' periods in Parkinson's disease: an in-clinic double-blind dose ranging study. *Acta Neurol Scand.* 2013;128(3):166-171. doi:10.1111/ANE.12107.
- 77 Grosset KA, Malek N, Morgan F, Grosset DG. Inhaled apomorphine in patients with "on-off" fluctuations: a randomized, double-blind, placebo-controlled, clinic and home based, parallel-group study. *J Parkinsons Dis.* 2013;3(1):31-37. doi:10.3233/JPD-120142.
- 78 Auffret M, Drapier S, V erin M. The Many Faces of Apomorphine: Lessons from the Past and Challenges for the Future. *Drugs R D.* 2018;18(2):91-107. doi:10.1007/S40268-018-0230-3.
- 79 Chahine LM, Edison B, Daeschler M, et al. The Most Bothersome Aspects of Off Periods Reported by

- Individuals with Parkinson's Disease. *Mov Disord Clin Pract.* 2020;7(3):284-292. doi:10.1002/MDC3.12915.
- 80 Rabinowitz JD, Wensley M, Lloyd P, et al. Fast onset medications through thermally generated aerosols. *J Pharmacol Exp Ther.* 2004;309(2):769-775. doi:10.1124/JPET.103.062893.
- 81 Rabinowitz JD, Lloyd PM, Munzar P, et al. Ultra-fast absorption of amorphous pure drug aerosols via deep lung inhalation. *J Pharm Sci.* 2006;95(11):2438-2451. doi:10.1002/JPS.20694.
- 82 Drug Approval Package: Adasuve (loxapine) NDA #022549. Accessed December 14, 2021. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/022549_adasuve_toc.cfm.
- 83 Adasuve | European Medicines Agency. Accessed December 14, 2021. <https://www.ema.europa.eu/en/medicines/human/EPAR/adasuve#authorisation-details-section>.
- 84 Allen MH, Feifel D, Lesem MD, et al. Efficacy and Safety of Loxapine for Inhalation in the Treatment of Agitation in Patients With Schizophrenia: A Randomized, Double-Blind, Placebo-Controlled Trial. *J Clin Psychiatry.* 2011;72(10):0-0. doi:10.4088/JCP.10Mo6011YEL.
- 85 Ferreira JJ, Poewe W, Rascol O, et al. Effect of Opicapone on Levodopa Pharmacokinetics in Patients with Fluctuating Parkinson's Disease. *Mov Disord.* 2022;37(11):2272-2283. doi:10.1002/MDS.29193.
- 86 Othman AA, Dutta S. Population pharmacokinetics of levodopa in subjects with advanced Parkinson's disease: Levodopa-carbidopa intestinal gel infusion vs. oral tablets. *Br J Clin Pharmacol.* 2014;78(1):94-105. doi:10.1111/BCP.12324/SUPINFO.
- 87 Goetz CG, Tilley BC, Shaftman SR, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. *Mov Disord.* 2008;23(15):2129-2170. doi:10.1002/MDS.22340.
- 88 Hattori N, Mochizuki H, Hasegawa K, et al. Ropinirole Patch Versus Placebo, Ropinirole Extended-Release Tablet in Advanced Parkinson's Disease. *Mov Disord.* 2020;35(9):1565-1573. doi:10.1002/MDS.28071.
- 89 Chandrabhatla AS, Pomeranec IJ, Ksendzovsky A. Co-evolution of machine learning and digital technologies to improve monitoring of Parkinson's disease motor symptoms. *npj Digit Med* 2022 51. 2022;5(1):1-18. doi:10.1038/s41746-022-00568-y.
- 90 Kalia L V., Lang AE. Parkinson's disease. *Lancet* (London, England). 2015;386(9996):896-912. doi:10.1016/S0140-6736(14)61393-3.
- 91 Nutt JG, Woodward WR, Hamerstad JP, Carter JH, Anderson JL. The "On-Off" Phenomenon in Parkinson's Disease. <https://doi.org/10.1016/NEJM198402233100802>. 2010;310(8):483-488. doi:10.1056/NEJM198402233100802.
- 92 Akram N, Li H, Ben-Joseph A, et al. Developing and assessing a new web-based tapping test for measuring distal movement in Parkinson's disease: a Distal Finger Tapping test. *Sci Reports* 2022 121. 2022;12(1):1-11. doi:10.1038/s41598-021-03563-7.
- 93 Nikolaus Homann C, Suppan K, Wenzel K, et al. The Bradykinesia Akinesia Incoordination Test (BRAIN TEST), an Objective and User-Friendly Means to Evaluate Patients With Parkinsonism. Published online 2000. doi:10.1002/1531-8257.
- 94 Tavares ALT, Jefferis GSXE, Koop M, et al. Quantitative measurements of alternating finger tapping in Parkinson's disease correlate with UPDRS motor disability and reveal the improvement in fine motor control from medication and deep brain stimulation. *Mov Disord.* 2005;20(10):1286-1298. doi:10.1002/MDS.20556.
- 95 Broeder S, Roussos G, De Vleeschhauwer J, D'Cruz N, de Xivry JJO, Nieuwboer A. A smartphone-based tapping task as a marker of medication response in Parkinson's disease: a proof of concept study. *J Neural Transm.* 2023;130(7):937-947. doi:10.1007/S00702-023-02659-W/FIGURES/3.
- 96 Khan T, Nyholm D, Westin J, Dougherty M. A computer vision framework for finger-tapping evaluation in Parkinson's disease. *Artif Intell Med.* 2014;60(1):27-40. doi:10.1016/J.ARTMED.2013.11.004.
- 97 Bologna M, Leodori G, Stirpe P, et al. Bradykinesia in early and advanced Parkinson's disease. *J Neurol Sci.* 2016;369:286-291. doi:10.1016/J.JNS.2016.08.028.
- 98 Stamatakis J, Ambroise J, Crémers J, et al. Finger tapping clinimetric score prediction in Parkinson's disease using low-cost accelerometers. *Comput Intell Neurosci.* 2013;2013. doi:10.1155/2013/717853.
- 99 Kim JW, Lee JH, Kwon Y, et al. Quantification of bradykinesia during clinical finger taps using a gyrosensor in patients with Parkinson's disease. *Med Biol Eng Comput.* 2011;49(3):365-371. doi:10.1007/S11517-010-0697-8/TABLES/2.
- 100 Sano Y, Kandori A, Shima K, et al. Quantifying Parkinson's disease finger-tapping severity by extracting and synthesizing finger motion properties. *Med Biol Eng Comput.* 2016;54(6):953-965. doi:10.1007/S11517-016-1467-Z/FIGURES/9.
- 101 Martinez-Manzanera O, Roosma E, Beudel M, Borgemeester RWK, Van Laar T, Maurits NM. A Method for Automatic and Objective Scoring of Bradykinesia Using Orientation Sensors and Classification Algorithms. *IEEE Trans Biomed Eng.* 2016;63(5):1016-1024. doi:10.1109/TBME.2015.2480242.
- 102 Wang PT, King CE, Do AH, Nenadic Z. A durable, low-cost electrogoniometer for dynamic measurement of joint trajectories. *Med Eng Phys.* 2011;33(5):546-552. doi:10.1016/J.MEDENGGPHY.2010.12.008.
- 103 Lalvay L, Lara M, Mora A, et al. Quantitative Measurement of Akinesia in Parkinson's Disease. *Mov Disord Clin Pract.* 2017;4(3):316. doi:10.1002/MDC3.12410.
- 104 Mitsi G, Mendoza EU, Wissel BD, et al. Biometric Digital Health Technology for Measuring Motor Function in Parkinson's Disease: Results from a Feasibility and Patient Satisfaction Study. *Front Neurol.* 2017;8(JUN). doi:10.3389/FNEUR.2017.00273.
- 105 Ling H, Massey LA, Lees AJ, Brown P, Day BL. Hypokinesia without decrement distinguishes progressive supranuclear palsy from Parkinson's disease. *Brain.* 2012;135(Pt 4):1141-1153. doi:10.1093/BRAIN/AWS038.
- 106 Yokoe M, Okuno R, Hamasaki T, Kurachi Y, Akazawa K, Sakoda S. Opening velocity, a novel parameter, for finger tapping test in patients with Parkinson's disease. *Parkinsonism Relat Disord.* 2009;15(6):440-444. doi:10.1016/J.parkreldis.2008.11.003.
- 107 Stavrakoudis A, Larkin S, López Castellanos JR, et al. Tablet-Based Application for Objective Measurement of Motor Fluctuations in Parkinson Disease. *Digit Biomarkers.* 2017;1(2):126. doi:10.1159/000485468.
- 108 Lee CY, Kang SJ, Hong SK, Ma H II, Lee U, Kim YJ. A Validation Study of a Smartphone-Based Finger Tapping Application for Quantitative Assessment of Bradykinesia in Parkinson's Disease. *PLoS One.* 2016;11(7):e0158852. doi:10.1371/JOURNAL.PONE.0158852.
- 109 Hasan H, Burrows M, Athauda DS, et al. The BRadykinesia Akinesia INcoordination (BRAIN) Tap Test: Capturing the Sequence Effect. *Mov Disord Clin Pract.* 2019;6(6):462-469. doi:10.1002/MDC3.12798.
- 110 Bologna M, Guerra A, Paparella G, et al. Neurophysiological correlates of bradykinesia in Parkinson's disease. *Brain.* 2018;141(8):2432-2444. doi:10.1093/BRAIN/AWY155.
- 111 Espay AJ, Giuffrida JP, Chen R, et al. Differential response of speed, amplitude, and rhythm to dopaminergic medications in Parkinson's disease. *Mov Disord.* 2011;26(14):2504-2508. doi:10.1002/MDS.23893.
- 112 De Vleeschhauwer J, Broeder S, Janssens L, Heremans E, Nieuwboer A, Nackaerts E. Impaired Touchscreen Skills in Parkinson's Disease and Effects of Medication. *Mov Disord Clin Pract.* 2021;8(4):546-554. doi:10.1002/MDC3.13179.
- 113 Lipp MM, Batycky R, Moore J, Leinonen M, Freed MI. Preclinical and clinical assessment of inhaled levodopa for OFF episodes in Parkinson's disease. *Sci Transl Med.* 2016;8(360). doi:10.1126/scitranslmed.AAD8858/SUPPL_FILE/8-360RA136_SM.PDF.



CHAPTER 2

A randomized trial assessing the safety, pharmacokinetics, and efficacy during morning *OFF* of AZ-009

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ABSTRACT

Background Inhalation of apomorphine could be a faster-acting and more user-friendly alternative to subcutaneous injection for treating *OFF* periods in PD.

Objectives To compare the safety and pharmacokinetics of inhaled apomorphine (AZ-009) with subcutaneous apomorphine (APO-go PEN) in healthy volunteers, and to examine the safety, pharmacokinetics, and efficacy of AZ-009 in PD patients.

Methods In part A of this study, 8 healthy volunteers received 1 mg AZ-009 and 2 mg subcutaneous apomorphine in a randomized crossover manner. In the subsequent single ascending dose parts in healthy volunteers (part B, n=16) and PD patients (part C, n=25), participants were randomized to placebo or AZ-009 up to 4 mg. In patients, after medication withdrawal, MDS-UPDRS III and *ON/OFF* states were assessed pre- and post-dose.

Results AZ-009 was rapidly absorbed with peak plasma concentrations at 2 minutes, as compared to 30 minutes for subcutaneous apomorphine. Adverse events for AZ-009 were comparable to subcutaneous apomorphine, except for mild and transient throat irritation. Adverse events limited AZ-009 dose escalation in healthy volunteers to 3 mg. Patients tolerated up to 4 mg. In PD patients, 2, 3, and 4 mg AZ-009 reduced mean MDS-UPDRS III score (standard deviation) by 10.7 (13.6), 12.8 (7.9) and 10.3 (3.7) points respectively, compared to 4.8 (4.9) after placebo at 10 minutes post-dose. The percentage of patients achieving full *ON* within 45 minutes post-dose increased dose-dependently: 0% (placebo), 17% (2 mg), 50% (3 mg), 83% (4 mg).

Conclusions AZ-009 appears to be a rapid-acting and reasonably well-tolerated formulation for treating *OFF* periods.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting movement, cognition, emotion, and autonomic activity. PD patients are usually treated with dopaminergic drugs such as levodopa and/or a direct-acting dopamine agonist. Initial therapy is selected based upon a number of criteria including patient age, comorbid conditions, disease severity and degree of functional disability.¹⁻³ However, most patients eventually require levodopa therapy and a large proportion of patients develop motor complications within a few years of starting its use.⁴⁻⁶ Complications consist of predictable end of dose *OFF* episodes ('wearing *OFF*'), prolonged latency to *ON*, inability to turn *ON*, sudden *ON/OFF* fluctuations and/or dyskinesia. These fluctuations in therapeutic effects can be predictable or unpredictable and do not only involve fluctuations in motor symptoms but also in non-motor symptoms such as anxiety/panic attacks, mood changes, slow thinking, and pain.⁷

A number of strategies have been investigated to increase *ON* time while reducing disabling *OFF* time, e.g., dosing more often with a lower levodopa dose, adding dopamine agonists, giving catechol-O-methyltransferase or monoamine oxidase B inhibitors, administering controlled- or sustained-release drug formulations, or following a protein redistribution diet.^{2,8,9} However, despite optimal oral therapy, patients often continue to experience *OFF* periods that severely compromise quality of life and daily activities.¹⁰ Subcutaneous apomorphine provides rapid and effective relief from such *OFF* periods and has been indicated for use in advanced PD for approximately two decades. Often reported side effects include injection site reactions, hallucinations, sedation, somnolence, dizziness, yawning, nausea and vomiting. In addition, there is an increased risk of orthostatic hypotension in the elderly population especially during initiation of therapy.¹¹ To diminish the risk of nausea, vomiting, and (orthostatic) hypotension, patients are usually pretreated with domperidone or another antiemetic for at least 2 days prior to initiation of apomorphine.¹¹⁻¹³ Although the subcutaneous formulation of apomorphine is efficacious, it has disadvantages such as difficulty self-administering

a subcutaneous injection while *OFF* and a high incidence of injection site reactions.¹⁴ A more user-friendly formulation would allow for a broader use of apomorphine. This unmet medical need is recognized by the medical community, and research has been focused on finding more suitable formulations.^{14,15} Recently, sublingual apomorphine has been approved by the FDA, providing a more user-friendly formulation, albeit still requiring a film strip under the tongue for up to 3 minutes.¹⁶ It is expected that apomorphine inhalation will not only be more user-friendly, but also result in an even faster action.

AZ-009, also called Staccato® apomorphine, is a single-use, disposable, breath-actuated drug-device combination product for oral inhalation. It has been developed to deliver apomorphine hydrochloride as a thermally generated, condensation aerosol to the deep lung for rapid systemic exposure. We performed a 3-part phase 1 trial to evaluate the pharmacokinetics (PK) of AZ-009 and compare it with a registered subcutaneous apomorphine injection (part A), and to study the safety and PK of single ascending doses of AZ-009 in healthy volunteers (HVs) (part B) and PD patients (part C). The last study part also evaluated AZ-009's efficacy during an induced morning *OFF* state.

METHODS

The study was conducted in accordance with European Medicines Agency guidelines for Good Clinical Practice and registered in ClinicalTrials.gov (NCT03822364). The protocol was approved by the Independent Ethics Committee of Foundation Beoordeling Ethiek Biomedisch Onderzoek. Prior to any study-related activity, all participants provided written informed consent. The study was conducted at the Centre for Human Drug Research between October 2018 and May 2019.

Study design

This study was divided into three parts: part A, B and C. Refer to Supplemental Figure 1 for a schematic overview of the study designs.

The randomization code was generated separately for each part using SAS version 9.4 by a study-independent CHDR statistician. No formal sample size calculations were performed. Part A of the study was a randomized, open-label crossover study assessing single doses of AZ-009 (1 mg) and subcutaneous apomorphine (2 mg) in 8 HVs. The washout between the two study periods was at least three days (apomorphine half-life is approximately 30-50 minutes).^{17,18} Safety data were examined during a dose level evaluation meeting before proceeding to study part B. Part B was a randomized, double-blind, placebo-controlled, single-ascending dose study of AZ-009 with planned doses of 2, 3, and 4 mg in HVs. The 4 mg cohort was cancelled due to incidence of adverse events (AEs) in the 3 mg cohort. Each cohort was composed of 8 HVs of which 6 were randomized to receive active treatment and 2 to receive placebo. Before advancing to the next cohort, safety data were evaluated. Part C had the same study design as part B, but was performed in PD patients after overnight anti-Parkinson medication withdrawal. Patients were dosed the next morning only when they were in an *OFF* state as assessed by a physician.

The study consisted of:

- a screening visit;
- at-home pretreatment with an antiemetic (domperidone) three times daily (TID);
- a single stay of 7, 3, or 2 days (part A, B and C respectively) at the clinical research unit;
- and a follow-up telephone call.

In part A, participants received 10 mg domperidone TID from 3 days prior to dosing until after last dose. In part B, domperidone dose was increased to 20 mg on the evening and morning prior to dosing. At other time points domperidone intake remained 10 mg as in part A. In part C, participants received 20 mg domperidone TID from 2 days prior to dosing until after dosing.

Participants

In study parts A and B, healthy non-smoking men and women aged 18-60 years with a body mass index of 18-32 kg/m² were eligible

to participate. In study part C, non-smoking PD patients with recognizable OFF periods aged 30-85 years with Hoehn and Yahr stage I-IV were eligible for participation. Patients were excluded if their systolic blood pressure (BP) was below 100 mmHg at screening or baseline, they had symptomatic clinically relevant and medically uncontrolled orthostatic hypotension, or a history of long QT syndrome and/or a QTcF of >470 ms (male) or >480 ms (female).

Investigational drugs

AZ-009 was available in two dose strengths (1 and 2 mg apomorphine hydrochloride). A dose of 3 mg was delivered by 3 consecutive oral inhalations of 1 mg, and a dose of 4 mg by 2 consecutive inhalations of 2 mg. Matching Staccato placebo (including number of devices inhaled) was identical to AZ-009, but without a coated apomorphine film. AZ-009 and matching placebo were manufactured by Alexza Pharmaceuticals, Inc. Participants were instructed to inhale through the mouthpiece with a steady deep breath and to hold their breath for as long as possible, up to 10 seconds.

Inhalation through the product initiates the controlled rapid heating of a thin film of excipient-free apomorphine to form a thermally generated drug vapor. The vapor condenses into aerosol particles with a particle size distribution appropriate for efficient delivery to the deep lung, i.e., with a mass median aerodynamic diameter in the range of 0.5 to 3.5 μm .

In study part A, apomorphine was also administered subcutaneously with the APO-go PEN. APO-go was provided as the commercially available product with the appropriate country-specific labeling by the Leiden University Medical Centre pharmacy. A volume of 0.2 mL (2 mg) was injected in the thigh.

Safety

For all study parts, a medical screening was performed to assess eligibility based on medical history, concomitant medications, ECG, vital signs, routine hematology, chemistry and urinalysis, and physical examination. Electrolytes and QTcF were assessed at screening (prior

to domperidone initiation) and again at baseline (after domperidone initiation and prior to apomorphine administration). During the study, safety was evaluated by monitoring of AEs (classified by Medical Dictionary for Regulatory Activities (MedDRA) version 20.1), vital signs, ECGs, physical examination, and clinical laboratory tests. Orthostatic hypotension was defined as a systolic BP drop of ≥ 20 mmHg or a diastolic BP drop ≥ 10 mmHg upon standing. Postural dizziness was defined as dizziness upon standing that was not accompanied by a drop in BP (at the scheduled measurement time) as defined for orthostatic hypotension.

Pharmacokinetics

Blood samples for PK analysis were obtained pre-dose and 1, 2, 5, 10, 20, 30, and 45 minutes, and 1, 2, 4, 8 and 24 hours post-dose in parts A/B. In part C, samples were obtained pre-dose and 2, 5, 15, 30, and 45 minutes, and 1, 1.5, 4 and 5 hours post-dose. A lower sampling frequency and shorter sampling duration were chosen in part C to allow time for efficacy measurements and to reduce patient burden. Plasma samples were analyzed for apomorphine using a validated liquid chromatography-tandem mass spectrometry method.

Plasma concentrations of apomorphine were analyzed using non-compartmental analysis in Phoenix™ WinNonlin® version 8.1. PK parameters that were calculated include maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), apparent terminal elimination half-life ($T_{1/2}$) and area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\text{inf}}$).

For part A, the comparison of the dose-normalized log-transformed PK parameters C_{max} and $AUC_{0-\text{inf}}$ for apomorphine across treatments (1 mg AZ-009 inhalation vs. 2 mg subcutaneous apomorphine) was performed using an analysis of variance (ANOVA) model and the two one-sided t-tests procedure. The ANOVA model included factors for sequence, subject within sequence, treatment, and period. Point estimates and 90% confidence intervals for the geometric mean ratios (AZ-009/subcutaneous apomorphine) were calculated for PK parameters by back transformation to the original scale.

For parts A-C combined, C_{\max} and $AUC_{0-\infty}$ for apomorphine were compared across dose levels (1-4 mg) to assess dose proportionality. Statistical analyses were conducted using a power model with mixed effects.¹⁹

Efficacy

Motor function was assessed using part III of the licensed Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS). Physicians administering the scale were trained and certified in its use. To the degree feasible, the same physician evaluated a patient at Day -1 (day before dosing) and Day 1 pre-dose and 10-, 30- and 60-minutes post-dose. Mean change from baseline (CFB) MDS-UPDRS III total score was calculated and presented graphically.

The disease state of a patient was assessed by a physician pre-dose and 2, 10, 20 and 45 minutes post-dose. Possible categories were *ON* with disabling dyskinesia, *ON* with non-disabling dyskinesia, *ON* with no dyskinesia and normal motor function, partial *ON* and *OFF*. The first 3 categories were combined, classified as full *ON*, and presented graphically as percentage of patients turning full *ON*.

RESULTS

Demographics

See Supplemental Figure 2-4 for CONSORT flow diagrams providing an overview of number of participants screened, randomized, completed, and analyzed per study part. Table 1 outlines the demographics and disposition of all participants enrolled in the study. Eight HVs completed the comparative PK study part (part A), and two cohorts of eight HVs (6 AZ-009: 2 placebo) completed the single ascending dose study part (part B). Demographics of HVs in part A and B were comparable, only the median age was higher in part A compared to part B (40 and 26 years, respectively). In part C of the study, a total of 25 PD patients were included, divided over three cohorts receiving 2, 3, or 4 mg AZ-009, or placebo in a 6:2

ratio. The 2 mg AZ-009 group contained one additional patient due to a replacement in cohort 1 (see Supplemental Figure 4). The age of PD patients was higher than that of HVs. All groups contained males and females, except for the placebo group, which was composed of males only.

Pharmacokinetics

PART A: COMPARATIVE PK IN HVs

Apomorphine was rapidly absorbed into the systemic circulation following administration of AZ-009 and subcutaneous apomorphine in HVs (Figure 1). Descriptive statistics of the PK parameters are summarized in Supplemental Table 1. AZ-009 inhalation resulted in peak plasma apomorphine concentrations (C_{\max}) 1-2 min after dosing and showed a bi-exponential elimination phase. In contrast, apomorphine concentrations after subcutaneous apomorphine injection increased over time with a median T_{\max} of 30 minutes. When normalized for dose, the C_{\max} and $AUC_{0-\infty}$ geometric mean ratios (90% confidence interval) of AZ-009/subcutaneous apomorphine were 2.9 (1.6-5.4) and 0.8 (0.5-1.2) respectively. Mean apomorphine $T_{1/2} \pm$ standard deviation (SD) of AZ-009 was shorter (39 ± 7 min) than that of subcutaneous apomorphine (55 ± 22 min). Inter-subject variability (CV%) in apomorphine C_{\max} and $AUC_{0-\infty}$ was higher for AZ-009 (53.7% and 47.2%) than for subcutaneous apomorphine (36.4% and 22.7%).

PARTS B AND C: SINGLE ASCENDING DOSES IN HVs AND PD PATIENTS

AZ-009 was rapidly systemically absorbed in HVs (Figure 2A), as well as in PD patients (Figure 2B). Median T_{\max} in HVs was similar as in part A, i.e., 1 minute. The first PK sample in PD patients was taken at 2 minutes post-dose. Median T_{\max} in PD patients was 2 or 3 minutes depending on the dose group (Supplemental Table 2). C_{\max} and $AUC_{0-\infty}$ after 2 and 3 mg AZ-009 were similar for HVs and PD patients. $T_{1/2}$ in both HVs and PD patients was similar as was reported for 1 mg AZ-009 in part A. In PD patients, $AUC_{0-\infty}$ increased from 2 to 3 mg, but not from 3 to 4 mg, i.e., mean (SD) $AUC_{0-\infty}$ was 5.1 (1.5), 12.6 (4.5) and 11.3 (5.1) h·ng/mL for 2, 3 and 4 mg AZ-009 respectively.

Dose proportionality was assessed on the combined data of part A-C. The estimated exponent (90% confidence interval) was 0.57 (0.15-1.00) for the C_{max} and 0.77 (0.41-1.13) for the AUC_{0-inf} .

Safety and tolerability

The incidence of moderate AEs was 62.5% after AZ-009 and 100% after subcutaneous apomorphine treatment (Table 2). The most frequently reported TEAEs were nausea and presyncope (despite pretreatment with 10 mg domperidone TID), and somnolence and headache. Participants who received subcutaneous apomorphine reported the first AEs around 20 minutes post-dose, whereas for AZ-009 this was after 2-3 minutes (data not shown).

In part B, the domperidone dose was increased to 20 mg on the evening and morning prior to dosing in HVs. At other time points, domperidone intake remained 10 mg. A dose of 2 mg AZ-009 combined with this higher domperidone dose was better tolerated than 1 mg AZ-009 combined with a lower dose of domperidone (Table 2). The most frequently reported TEAEs were somnolence and yawning. The number of TEAEs, and in particular the frequency of moderate TEAEs, increased from 2 to 3 mg AZ-009. Nausea, orthostatic hypotension, somnolence, and yawning were reported most often in the 3 mg group. Standing BPs as low as 70/34 mmHg were measured and 5 out of 6 participants in the 3 mg group needed to lie down until symptoms subsided. Due to the dose-dependent increase in incidence of TEAEs, it was decided not to escalate to 4 mg in HVs and to increase the domperidone dose to 20 mg TID from 2 days prior to dosing in part C of the study in PD patients^{12,13}.

AZ-009 was relatively well tolerated by PD patients at 2, 3, and 4 mg with mostly mild TEAEs (Table 2). The most frequently reported TEAEs in the AZ-009-treated groups were throat irritation, orthostatic hypotension, and yawning. Orthostatic hypotension was mostly asymptomatic and was also reported in the placebo group. Some patients reported an increase in their PD symptoms in the days after the overnight Parkinson's medication withdrawal and dosing with placebo or AZ-009. No increase in incidence and severity of TEAEs was observed with an increase in dose. Most TEAEs resolved without

treatment, except for one case of severe hypotension in the 3 mg group which was treated with ephedrine, and two cases where the number of Parkinson's medication doses was increased for several days after study participation because of increased PD symptoms.

No consistent or clinically relevant QTcF prolongation or clinical laboratory changes were reported in any of the participants.

Efficacy

PD patients in part C were dosed during an *OFF* state after overnight medication withdrawal. All three AZ-009-treated dose groups showed a reduction from baseline in mean MDS-UPDRS part III total score at the first assessment 10 minutes post-dose (Figure 3A). The mean MDS-UPDRS III change from baseline (CFB) with SD at this time point was -10.7 (13.6) for the 2 mg group, -12.8 (7.9) for the 3 mg group, -10.3 (3.7) for the 4 mg group, and -4.8 (4.9) for the placebo group. The effect observed in the AZ-009-treated groups started to decrease at 30 minutes post-dose and further decreased at 1 hour post-dose to less than half of the maximum effect observed at 10 minutes post-dose. In contrast, the placebo group no longer showed a reduction compared to baseline at 1 hour post-dose.

All patients were assessed by a physician as being in an *OFF* state prior to dosing (Figure 3B). None of the placebo-treated patients achieved a full *ON* response at any of the time points. In contrast, the first patients converted to a full *ON* as early as 2 minutes after AZ-009 dosing. The highest percentage of patients in an *ON* state occurred 10 minutes post-dose for the 3 mg AZ-009 group and 20 minutes post-dose for the 2 and 4 mg AZ-009 groups. The percentage of patients achieving a full *ON* at any time point within 45 minutes post-dose increased with dose from 17% (2 mg) to 50% (3 mg) to 83% (4 mg). No patients presented with disabling dyskinesias.

DISCUSSION

Subcutaneous apomorphine injections have long been used by PD patients for the treatment of sudden or early morning *OFF* periods.

Even though subcutaneous apomorphine is efficacious, it can be painful and/or difficult to self-administer, and often results in injection site reactions.¹¹ Moreover, maximal motor improvements have been shown to occur only after about 20 to 40 minutes following subcutaneous apomorphine.²⁰⁻²² This formulation of inhalable apomorphine, AZ-009, could provide an easier and faster-acting formulation for the treatment of *OFF* periods. This 3-part study was designed to evaluate the PK of AZ-009 and compare it with the subcutaneous injection, and to examine the safety and PK of ascending doses of AZ-009 in HVs and PD patients. The last study part also aimed to evaluate AZ-009's efficacy in PD patients during an induced morning *OFF* state.

AZ-009 led to rapid systemic exposure with a median T_{max} of 2 minutes based on the combined data of HVs and PD patients. In contrast, the subcutaneous apomorphine injection resulted in a T_{max} of 30 minutes. AZ-009's PK profile makes it especially suitable for fast onset of action which is preferential in the treatment of sudden *OFF* periods. Dosing with 1 mg AZ-009 resulted in a mean (SD) C_{max} of 14.3 (7.7) ng/mL and 2 mg subcutaneous apomorphine in 8.6 (3.1) ng/mL. A difference in total exposure (AUC_{0-inf}) between inhalable and subcutaneous apomorphine could not be confirmed due to the relatively high variability and small sample size. Similarly, no definitive conclusions could be drawn on dose proportionality. Future larger trials will need to be conducted to gain more information on this.

Despite comparable PK, AZ-009 resulted in a less favorable safety profile in HVs than in PD patients. This was not unexpected since PD patients are likely to have developed tolerance because of daily dopaminergic medication use. Also, PD patients were administered a higher domperidone dose compared to HVs. The most frequently reported AEs in PD patients were throat irritation, orthostatic hypotension, and yawning. Throat irritation occurred immediately after dosing and usually resolved within minutes. Orthostatic hypotension was mostly asymptomatic and was observed in the placebo group as well. This can likely be partly explained by autonomic dysregulation in PD.

One PD patient receiving 3 mg AZ-009 presented with severe hypotension shortly after dosing that was treated with ephedrine.

Hypotension is a known side effect of apomorphine^{12,23} and moderate hypotension was also reported by one healthy volunteer receiving 2 mg subcutaneous apomorphine in study part A. All participants that presented with reduced blood pressure spontaneously recovered after lying down or lying in Trendelenburg position. However, in the context of patient comfort, ephedrine was more readily administered during the patient part of the study. Moreover, AZ-009 gives higher peak apomorphine concentrations than subcutaneous apomorphine and this patient was immediately given 3 mg AZ-009. In clinical practice, subcutaneous apomorphine is initiated under medical supervision at 2 mg and titrated up to a dose that is both tolerable and effective. The same should be done with AZ-009 when used in clinical practice. For some patients, AZ-009 might not be tolerable at effective doses, as is now also the case for some patients receiving subcutaneous injections.

During this trial a prototype of the inhalation device was used. Of 25 PD patients, 23 (92.0%) indicated they liked how the drug was delivered. Whether they also found the device easy to use could not be adequately evaluated due to the prototype being used. Future trials should therefore focus on ease of use of the commercial device in PD patients.

Treatment with 2, 3 and 4 mg AZ-009 showed promise in controlling morning *OFF* periods in PD patients after overnight medication withdrawal. At 10 minutes post-dose, all three AZ-009 dose groups showed a clear reduction (10.3-12.8 points) from baseline in mean MDS-UPDRS III score that was greater than for placebo (4.8 points). These reductions were larger than 3.25 points, which has been described as the minimal, but clinically relevant improvement.²⁴ Moreover, the difference in MDS-UPDRS III response between placebo and apomorphine was comparable to that reported in another apomorphine inhalation study (8.4 points (95% confidence interval: 1.2-15.5)).²⁵ MDS-UPDRS III improvement did not seem to correlate with AZ-009 dose. This is likely the result of inter-patient variability in exposure and MDS-UPDRS III response. From literature, it was already known that the minimally effective apomorphine concentration differs widely between patients,²⁶ and that the degree of response is (partly) dependent on disease

severity.²⁷ The fast onset of action and relatively short duration of action would make this formulation ideal for patients suffering from sudden and unpredictable *OFF* periods or from delayed *ON*. Findings on the MDS-UPDRS III were supported by the physician's *ON/OFF* state assessment. Whereas none of the placebo patients achieved a full *ON* response, the AZ-009-treated patients dose-dependently converted from *OFF* to full *ON*. For future studies, assessing *ON/OFF* states after 45 minutes is advised to determine duration of clinical effect. Since patients were randomized to their AZ-009 dose, it is likely that they did not reach their maximum possible improvement. In clinical practice, the dose of apomorphine is titrated to reach a dose with optimal efficacy and minimal side effects. Whereas this study demonstrates a beneficial effect of AZ-009 over placebo, future studies should further investigate the efficacy of AZ-009 at the patient's optimal dose.

Taken together, AZ-009 is reasonably well tolerated by PD patients pretreated with domperidone. AZ-009 is rapidly absorbed into the systemic circulation and can provide rapid relief from early morning *OFF* periods.

TABLE 1 Demographics of participants in study parts A to C.

Demographic variables for healthy volunteers	PART A		PART B		
	All participants (N=8)	All participants (N=16)	2 mg AZ-009 (N=6)	3 mg AZ-009 (N=6)	Placebo (N=4)
Age (years)					
Median (range)	40 (19-58)	26 (19-60)	29 (21-39)	24 (21-60)	40 (19-58)
BMI (kg/m ²)					
Median (range)	25 (20-31)	24 (19-30)	24 (19-28)	24 (21-27)	26 (24-30)
Sex (n/n (%/%)					
Female/Male	5/3 (62.5/37.5)	12/4 (75.0/25.0)	5/1 (83.3/16.7)	5/1 (83.3/16.7)	2/2 (50.0/50.0)
Race (n (%))					
Asian	0 (0)	2 (12.5)	1 (16.7)	1 (16.7)	0 (0)
Mixed	2 (25.0)	2 (12.5)	2 (33.3)	0 (0)	0 (0)
White	6 (75.0)	12 (75.0)	3 (50.0)	5 (83.3)	4 (100.0)
Demographic variables for patients with PD	PART C				
	All participants (N=24) ^a (N=25) ^b	2 mg AZ-009 (N=6) ^a (N=7) ^b	3 mg AZ-009 (N=6)	4 mg AZ-009 (N=6)	Placebo (N=6)
Age (years)					
Median (range)	62 (44-83)	63 (58-75)	55 (53-67)	67 (56-71)	58 (44-83)
BMI (kg/m ²)					
Median (range)	25 (20-31) ^a 25 (20-32) ^b	27 (20-30) ^a 27 (20-32) ^b	26 (22-29)	24 (22-27)	24 (22-31)
Sex (n/n (%/%)					
Female/Male	7/17 (29.2/70.8) ^a 7/18 (28.0/72.0) ^b	3/3 (50.0/50.0) ^a 3/4 (42.9/57.1) ^b	1/5 (16.7/83.3)	3/3 (50.0/50.0)	0/6 (0/100.0)
Race (n (%))					
Other ^c	1 (4.2 ^a ; 4.0 ^b)	0 (0)	1 (16.7)	0 (0)	0 (0)
White	23 (95.8) ^a 24 (96.0) ^b	6 (100.0) ^a 7 (100.0) ^b	5 (83.3)	6 (100.0)	6 (100.0)
MMSE					
Median (range)	29 (25-30)	30 (27-30)	29 (27-30)	30 (25-30)	29 (26-30)

[continuation of Table 1]

Demographic variables for patients with PD	PART C				
	All participants (N=24) ^a (N=25) ^b	2 mg AZ-009 (N=6) ^a (N=7) ^b	3 mg AZ-009 (N=6)	4 mg AZ-009 (N=6)	Placebo (N=6)
Hoehn and Yahr stage at Day -1 (when using regular medication) (n (%))					
Stage 1	1 (4.2)	0 (0)	0 (0)	0 (0)	1 (16.7)
Stage 2	15 (62.5)	5 (83.3)	3 (50.0)	4 (66.7)	3 (50.0)
Stage 3	6 (25.0)	1 (16.7)	2 (33.3)	2 (33.3)	1 (16.7)
Stage 4	2 (8.3)	0 (0)	1 (16.7)	0 (0)	1 (16.7)
MDS-UPDRS III total score at Day -1 (when using regular medication)					
Median (range)	33 (13-76)	30 (15-38)	36 (19-73)	30 (22-50)	32 (13-76)
Concomitant PD medication (n (%))					
Levodopa-containing agents	23 (95.8)	6 (100.0)	6 (100.0)	6 (100.0)	5 (83.3)
Dopamine agonists	16 (66.7)	5 (83.3)	5 (83.3)	2 (33.3)	4 (66.7)
COMT inhibitors	7 (29.2)	3 (50.0)	2 (33.3)	2 (33.3)	0 (0)
MAO-B inhibitors	3 (12.5)	2 (33.3)	1 (16.7)	0 (0)	0 (0)
Amantadine	4 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)	0 (0)

In part C, when the pharmacodynamics population differed from the pharmacokinetics/safety population in age, BMI, sex, and/or race, information is provided for both; remaining variables are presented for the pharmacodynamics population only.

a. Information given for pharmacodynamics analysis population. / b. Information given for pharmacokinetics and safety analysis population. / c. North African.

BMI, body mass index; MMSE, Mini Mental State Examination; MDS-UPDRS, Movement Disorder Society-Unified Parkinson's Disease Rating Scale; PD, Parkinson's disease, COMT, catechol-O-methyltransferase; MAO-B, monoamine oxidase B.

TABLE 2 Summary of the number of TEAEs and the number and percentage of participants (n (%)) with any, mild, moderate and severe TEAEs and with a specific TEAE as indicated per treatment group and study part.

	PART A Crossover study in HVs				PART B SAD study in HVs				PART C SAD study in PD patients				
	2 mg scapo (N=8)	1 mg AZ-009 (N=8)	n (%)	n (%)	2 mg AZ-009 (N=6)	3 mg AZ-009 (N=6)	Placebo (N=4)	n (%)	2 mg AZ-009 (N=7)	3 mg AZ-009 (N=6)	4 mg AZ-009 (N=6)	Placebo (N=6)	n (%)
#TEAEs ^a	43	35	13	43	0	0	0	23	24	24	10	10	
Any TEAEs	8 (100.0)	7 (87.5)	6 (100.0)	6 (100.0)	-	-	-	6 (85.7)	5 (83.3)	6 (100.0)	5 (83.3)	5 (83.3)	
Mild TEAEs	7 (87.5)	7 (87.5)	5 (83.3)	6 (100.0)	-	-	-	6 (85.7)	5 (83.3)	6 (100.0)	4 (66.7)	4 (66.7)	
Moderate TEAEs	8 (100.0)	5 (62.5)	3 (50.0)	5 (83.3)	-	-	-	3 (42.9)	1 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)	
Severe TEAEs	-	-	-	-	-	-	-	-	1 (16.7)	-	-	-	
Most common TEAEs ^b													
Lacrimation increased	-	-	1 (16.7)	2 (33.3)	-	-	-	1 (14.3)	-	-	1 (16.7)	-	-
Nausea	6 (75.0)	5 (62.5)	1 (16.7)	5 (83.3)	-	-	-	1 (14.3)	1 (16.7)	2 (33.3)	1 (16.7)	-	
Vomiting	4 (50.0)	1 (12.5)	-	1 (16.7)	-	-	-	-	-	-	-	-	
Throat irritation	-	-	1 (16.7)	2 (33.3)	-	-	-	2 (28.6)	2 (33.3)	5 (83.3)	-	-	
Fatigue	2 (25.0)	1 (12.5)	1 (16.7)	1 (16.7)	-	-	-	1 (14.3)	2 (33.3)	-	-	1 (16.7)	
Feeling hot	3 (37.5)	2 (25.0)	-	1 (16.7)	-	-	-	1 (14.3)	2 (33.3)	-	-	-	
Sluggishness	1 (12.5)	1 (12.5)	-	-	-	-	-	-	-	-	-	-	
Dizziness	3 (37.5)	2 (25.0)	-	2 (33.3)	-	-	-	1 (14.3)	1 (16.7)	1 (16.7)	-	-	
Headache	3 (37.5)	4 (50.0)	-	-	-	-	-	-	-	-	-	-	
Orthostatic hypotension	-	-	-	-	-	-	-	-	-	-	-	-	
· Asymptomatic	1 (12.5)	-	-	-	-	-	-	2 (28.6)	2 (33.3)	2 (33.3)	4 (66.7)	-	
· Symptomatic	2 (25.0)	-	-	4 (66.7)	-	-	-	1 (14.3)	-	-	-	-	
Dizziness postural ^c	2 (25.0)	3 (37.5)	-	1 (16.7)	-	-	-	1 (14.3)	1 (16.7)	1 (16.7)	1 (16.7)	-	
Increased PD symptoms	-	-	-	-	-	-	-	2 (28.6)	-	-	2 (33.3)	2 (33.3)	
Presyncope	5 (62.5)	3 (37.5)	-	3 (50.0)	-	-	-	2 (28.6)	-	-	1 (16.7)	-	
Somnolence	5 (62.5)	3 (37.5)	3 (50.0)	5 (83.3)	-	-	-	2 (28.6)	1 (16.7)	1 (16.7)	1 (16.7)	-	
Syncope	1 (12.5)	1 (12.5)	-	-	-	-	-	-	1 (16.7)	-	-	-	
Time perception altered	1 (12.5)	2 (25.0)	-	2 (33.3)	-	-	-	-	1 (16.7)	-	-	-	
Yawning	-	1 (12.5)	2 (33.3)	4 (66.7)	-	-	-	2 (28.6)	2 (33.3)	1 (16.7)	-	-	

a. Not expressed as n (%). This parameter describes the total number of TEAEs reported, and hence is unitless. / b. TEAEs reported by at least two (part A/B) or three (part C) of the apomorphine-treated participants. / c. Dizziness on standing but no significant blood pressure decline measured at scheduled standing blood pressure measurement.

TEAE, treatment-emergent adverse event; sc apo, subcutaneous apomorphine; HV, healthy volunteer; SAD, single ascending dose; PD, Parkinson's disease.

FIGURE 1 Mean (standard deviation) apomorphine concentration time profiles after single-dose administrations of 1 mg AZ-009 and 2 mg subcutaneous (sc) apomorphine on semilogarithmic scale to healthy volunteers up to 8 hours (A) and 1 hour (B) postdose.

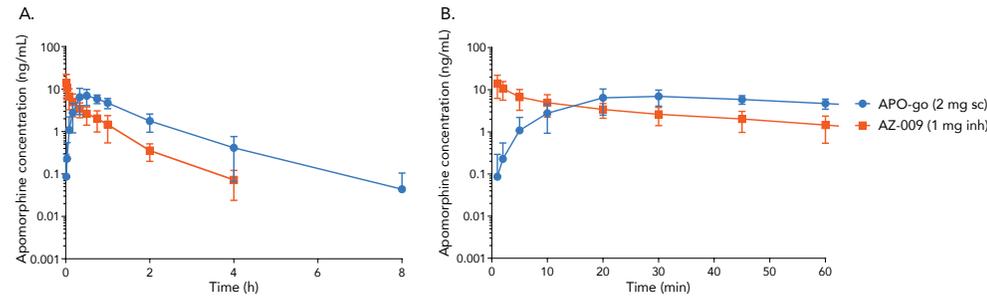


FIGURE 2 Mean (standard deviation) apomorphine concentration time profiles after single-dose administrations of 2 or 3 mg AZ-009 to healthy volunteers (part B) (A) and 2, 3, or 4 mg AZ-009 to patients with PD (part C) (B) on a semilogarithmic scale.

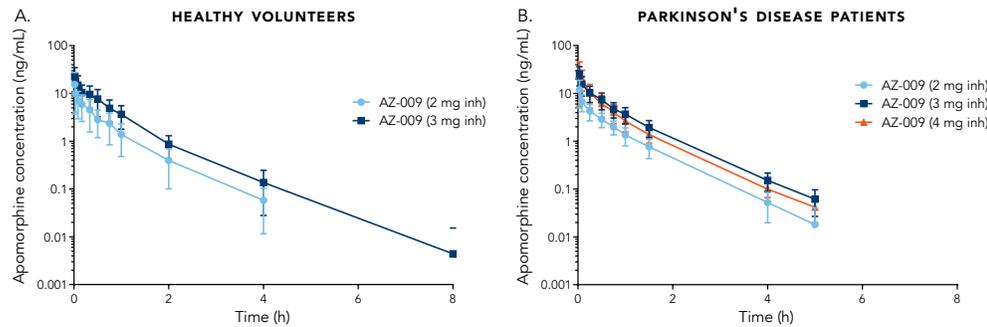
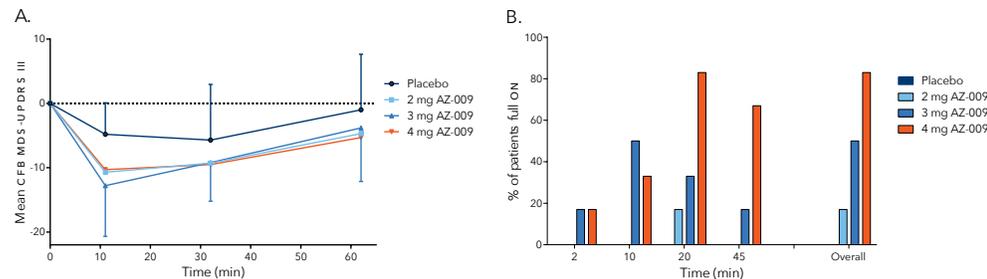


FIGURE 3 Mean change from baseline (CFB) Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III total score with standard deviation (A) and percentage (%) of patients achieving a full ON response (B) after the indicated treatment in patients with PD during an induced OFF state.



SUPPLEMENTARY MATERIAL

SUPPLEMENTAL TABLE 1 Pharmacokinetic parameters of apomorphine after single-dose administrations of 1 mg AZ-009 inhalation and 2 mg subcutaneous injection to healthy volunteers.

	2 mg sc apomorphine (N=8)	1 mg AZ-009 (N=8)
T_{max} (min)		
Median (range)	30 (20-60)	1 (1-2)
C_{max} (ng/mL)		
Mean (SD)	8.6 (3.1)	14.3 (7.7)
Median (range)	6.8 (5.2-12.9)	14.5 (3.7-23.7)
AUC_{0-inf} (h·ng/ml)		
Mean (SD)	11.4 (2.6)	4.9 (2.3)
Median (range)	11.8 (7.6-14.6)	4.9 (2.3-9.1)
$T_{1/2}$ (min)		
Mean (SD)	55 (22)	39 (7)
Median (range)	49 (33-95)	39 (25-48)

T_{max} , time to maximum plasma concentration; C_{max} , maximum plasma concentration; SD, standard deviation; AUC_{0-inf} , area under the plasma concentration-time curve from zero to infinity; $T_{1/2}$, apparent terminal elimination half-life; sc, subcutaneous.

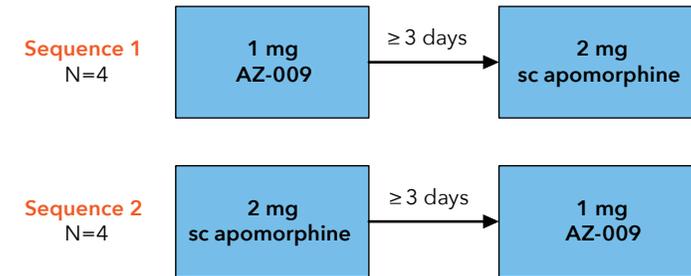
SUPPLEMENTAL TABLE 2 Pharmacokinetic parameters of apomorphine after single-dose administrations of 2 and 3 mg AZ-009 to healthy volunteers (part B) and 2, 3, and 4 mg AZ-009 to Parkinson's disease patients (part C).

	Healthy volunteers (PART B)		Parkinson's disease patients (PART C)		
	2 mg AZ-009 (N=6)	3 mg AZ-009 (N=6)	2 mg AZ-009 (N=7)	3 mg AZ-009 (N=6)	4 mg AZ-009 (N=6)
<i>T</i> _{max} (min)					
Median (range)	1 (1-10)	1 (1-5)	2 (2-6)	2 (2-2)	3 (2-5)
<i>C</i> _{max} (ng/mL)					
Mean (sd)	16.2 (11.1)	25.0 (9.5)	12.0 (6.8)	25.3 (11.0)	26.5 (16.6)
Median (range)	15.2 (1.3-29.3)	26.6 (11.0-38.2)	10.4 (4.4-22.7)	29.4 (6.0-36.7)	23.6 (10.3-54.2)
AUC _{0-inf} (h·ng/mL)					
Mean (sd)	5.3 (3.1)	11.8 (5.4)	5.1 (1.5)	12.6 (4.5)	11.3 (5.1)
Median (range)	5.3 (0.7-9.9)	12.7 (2.5-17.6)	5.0 (3.3-7.1)	14.3 (3.7-15.6)	10.3 (6.5-20.6)
<i>T</i> _{1/2} (min)					
Mean (sd)	38 (4)	40 (15)	38 (10)	42 (3)	40 (5)
Median (range)	39 (32-42)	35 (28-68)	38 (20-50)	42 (38-45)	39 (34-48)

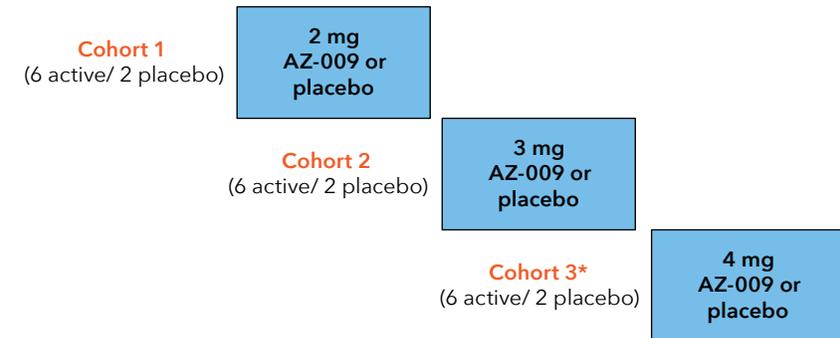
*T*_{max}, time to maximum plasma concentration; *C*_{max}, maximum plasma concentration; sd, standard deviation; AUC_{0-inf}, area under the plasma concentration-time curve from zero to infinity; *T*_{1/2}, apparent terminal elimination half-life.

SUPPLEMENTAL FIGURE 1 Overview of study designs.

STUDY PART A – CROSSOVER STUDY IN HEALTHY VOLUNTEERS

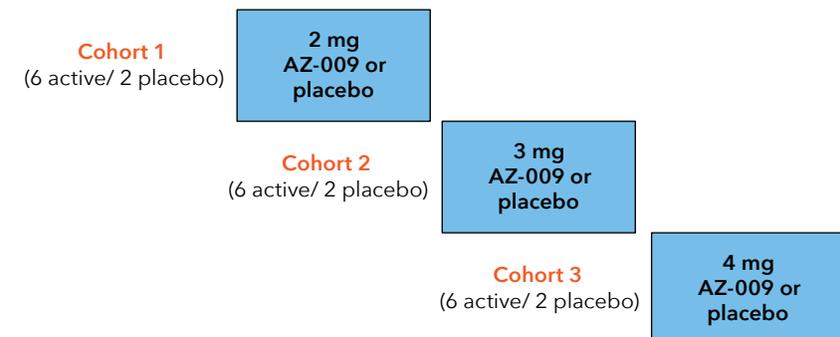


STUDY PART B – SINGLE ASCENDING DOSE STUDY IN HEALTHY VOLUNTEERS

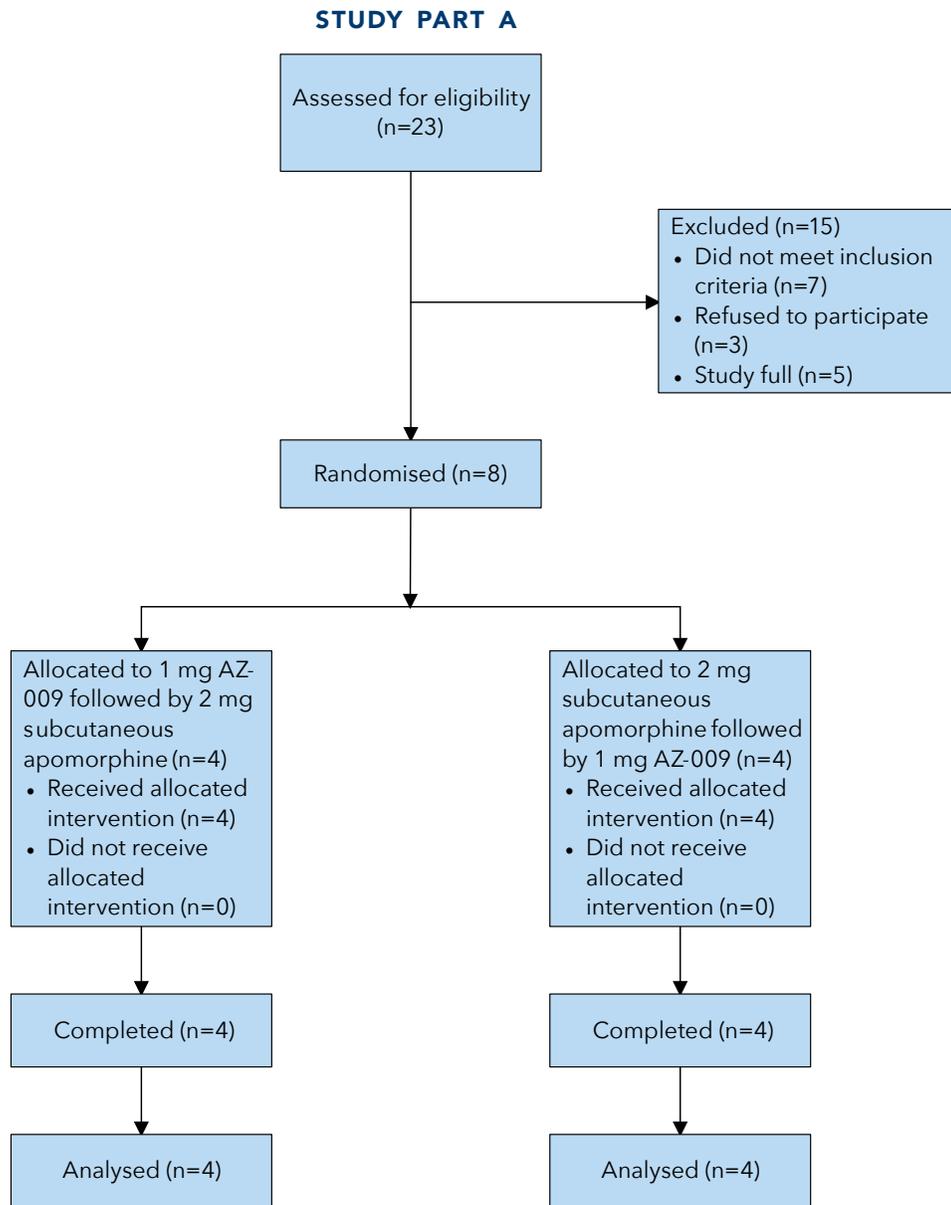


*Cohort planned but not started due to incidence/severity of AEs in the previous cohort.

STUDY PART C – SINGLE ASCENDING DOSE STUDY IN PARKINSON'S DISEASE PATIENTS

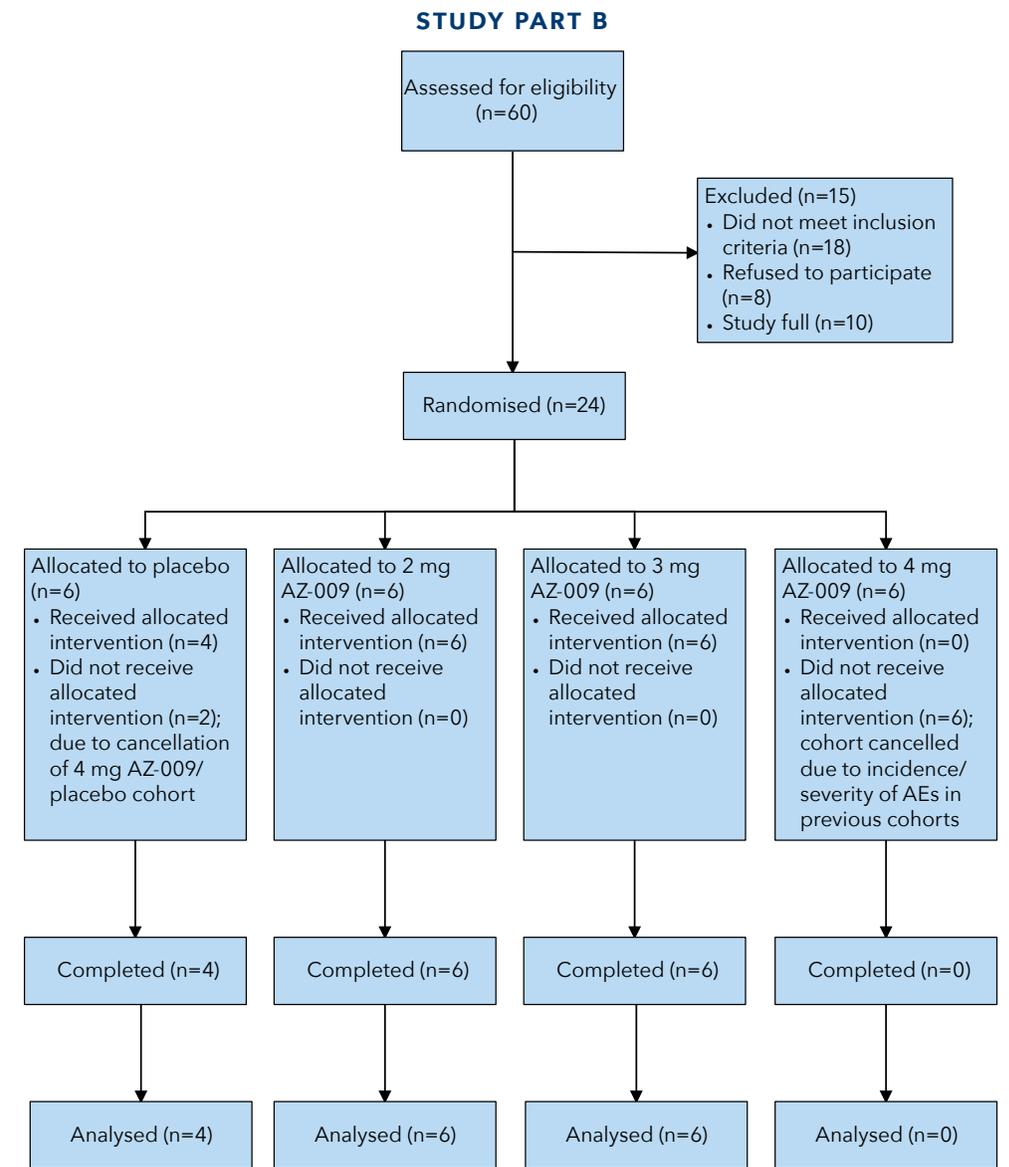


SUPPLEMENTAL FIGURE 2 CONSORT flow diagram for study part A.



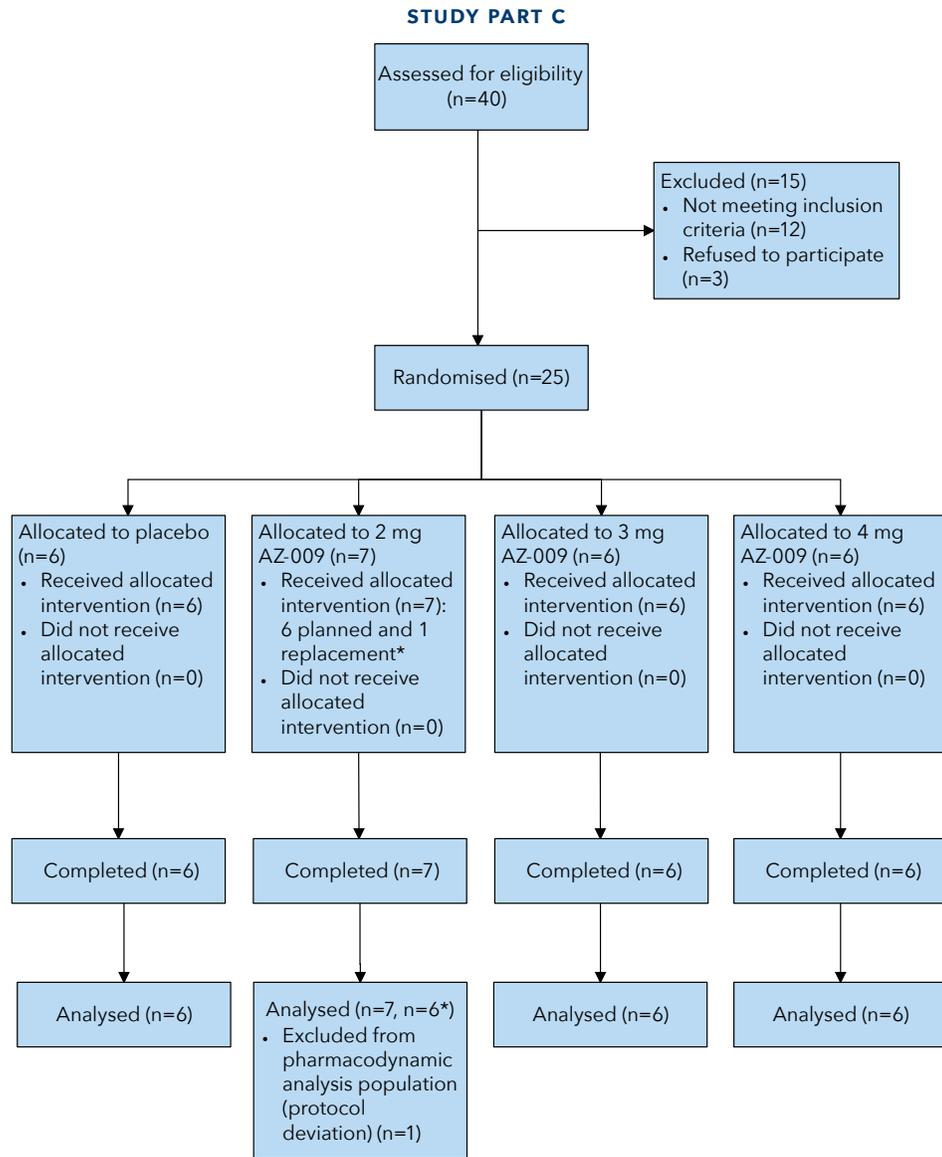
CONSORT, Consolidated Standards of Reporting Trials.

SUPPLEMENTAL FIGURE 3 CONSORT flow diagram for study part B.



CONSORT, Consolidated Standards of Reporting Trials.

SUPPLEMENTAL FIGURE 4 CONSORT flow diagram for study part C.



* A patient in the 2 mg AZ-009 group was replaced due to a protocol deviation (patient confessed post-dose that pramipexole was taken prior to dosing with the study drug). The patient was excluded from the pharmacodynamic analysis population (N=6) but not from the safety and PK analysis population (N=7).
CONSORT, Consolidated Standards of Reporting Trials.

REFERENCES

- Olanow CW, Stern MB, Sethi K. The scientific and clinical basis for the treatment of Parkinson disease (2009). *Neurology*. Published online 2009. doi:10.1212/WNL.0b013e3181a1d44c.
- Dietrichs E, Odin P. Algorithms for the treatment of motor problems in Parkinson's disease. *Acta Neurol Scand*. Published online 2017. doi:10.1111/ane.12733.
- de Bie RMA, Clarke CE, Espay AJ, Fox SH, Lang AE. Initiation of pharmacological therapy in Parkinson's disease: when, why, and how. *Lancet Neurol*. 2020;19(5):452-461. doi:10.1016/S1474-4422(20)30036-3.
- Ahlskog JE, Muentner MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov Disord*. 2001;16(3):448-458. doi:10.1002/mds.1090.
- Holloway R, Shoulson I, Kieburtz K, et al. Pramipexole vs Levodopa as initial treatment for Parkinson disease: A randomized controlled trial. *J Am Med Assoc*. 2000;284(15):1931-1938. doi:10.1001/jama.284.15.1931.
- Sweet RD, McDowell FH. Five years' treatment of Parkinson's disease with levodopa. Therapeutic results and survival of 100 patients. *Ann Intern Med*. 1975;83(4):456-463. doi:10.7326/0003-4819-83-4-456.
- Chou KL, Stacy M, Simuni T, et al. The spectrum of "off" in Parkinson's disease: What have we learned over 40 years? *Park Relat Disord*. 2018;51:9-16. doi:10.1016/j.parkrelidis.2018.02.001.
- Cereda E, Barichella M, Pedrolli C, Pezzoli G. Low-protein and protein-redistribution diets for Parkinson's disease patients with motor fluctuations: A systematic review. *Mov Disord*. 2010;25(13):2021-2034. doi:10.1002/mds.23226.
- Guebila M Ben, Thiele I. Model-based dietary optimization for late-stage, levodopa-treated, Parkinson's disease patients. *npj Syst Biol Appl*. 2016;2(1):1-8. doi:10.1038/npjbsa.2016.13.
- Antonini A. Apomorphine and Levodopa Infusion Therapies for Advanced Parkinson's Disease. *J Mov Disord*. 2009;2(1):4-9. doi:10.14802/jmd.09002.
- APO-go Pen 10mg/ml Solution for Injection - Summary of Product Characteristics (SmPC) - (emc). Accessed April 16, 2021. <https://www.medicines.org.uk/emc/product/2252/smpc#gref>.
- Bhidayasiri R, Garcia Ruiz PJ, Henriksen T. Practical management of adverse events related to apomorphine therapy. *Park Relat Disord*. 2016;33:S42-S48. doi:10.1016/j.parkrelidis.2016.11.017.
- ABN recommendations for domperidone. Accessed April 16, 2021. <https://gallery.mailchimp.com/7f92f52090d776e2c33>.
- Carbone F, Djamshidian A, Seppi K, Poewe W. Apomorphine for Parkinson's Disease: Efficacy and Safety of Current and New Formulations. *CNS Drugs*. 2019;33(9):905-918. doi:10.1007/s40263-019-00661-z.
- Titova N, Chaudhuri KR. Apomorphine therapy in Parkinson's and future directions. *Park Relat Disord*. 2016;33:S56-S60. doi:10.1016/j.parkrelidis.2016.11.013.
- Drug Approval Package: KYNMOBI. Accessed April 16, 2021. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/210875Orig1s000TOC.cfm.
- Gancher ST, Woodward WR, Boucher B, Nutt JG. Peripheral pharmacokinetics of apomorphine in humans. *Ann Neurol*. 1989;26(2):232-238. doi:10.1002/ana.410260209.
- Nomoto M, Kubo S, Nagai M, et al. A Randomized Controlled Trial of Subcutaneous Apomorphine for Parkinson Disease. *Clin Neuropharmacol*. 2015;38(6):241-247. doi:10.1097/WNF.000000000000111.
- Smith BP, Vandenhende FR, DeSante KA, et al. Confidence interval criteria for assessment of dose proportionality. *Pharm Res*. 2000;17(10):1278-1283. doi:10.1023/A:1026451721686.
- Pahwa R, Koller WC, Trosch RM, Sherry JH. Subcutaneous apomorphine in patients with advanced Parkinson's disease: A dose-escalation study with randomized, double-blind, placebo-controlled crossover evaluation of a single dose. *J Neurol Sci*. 2007;258(1-2):137-143. doi:10.1016/j.jns.2007.03.013.
- Pfeiffer RF, Gutmann L, Hull KL, Bottini PB, Sherry JH. Continued efficacy and safety of subcutaneous apomorphine in patients with advanced Parkinson's disease. *Park Relat Disord*. 2007;13(2):93-100. doi:10.1016/j.parkrelidis.2006.06.012.
- Trosch RM, Silver D, Bottini PB. Intermittent subcutaneous apomorphine therapy for "off" episodes in Parkinson's disease: A 6-month open-label study. *CNS Drugs*. 2008;22(6):519-527.

- doi:10.2165/00023210-200822060-00005.
- 23 FDA, CDER. Prescribing information Apokyn® (apomorphine hydrochloride injection). Published online March 2017. Accessed July 12, 2021. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/021264s014lbl.pdf.
- 24 Horváth K, Aschermann Z, Ács P, et al. Minimal clinically important difference on the Motor Examination part of MDS-UPDRS. *Park Relat Disord.* 2015;21(12):1421-1426. doi:10.1016/j.parkrelidis.2015.10.006.
- 25 Grosset KA, Malek N, Morgan F, Grosset DG. Inhaled Apomorphine in Patients with "on-off" Fluctuations: A Randomized, Double-blind, Placebo-controlled, Clinic and Home Based, Parallel-group Study. *J Parkinsons Dis.* 2013;3:31-37. doi:10.3233/JPD-120142.
- 26 T van L, R van der G, M D, HE B, PH G, RA R. Stepwise intravenous infusion of apomorphine to determine the therapeutic window in patients with Parkinson's disease. *Clin Neuropharmacol.* 1998;21(3):152-158. Accessed November 5, 2021. <https://europepmc.org/article/med/9617506>.
- 27 Verhagen Metman L, Locatelli ER, Bravi D, Mouradian MM, Chase TN. Apomorphine responses in Parkinson's disease and the pathogenesis of motor complications. *Neurology.* 1997;48(2):369-372. doi:10.1212/WNL.48.2.369.

CHAPTER 3

Safety and pharmacokinetics of multiple dosing with inhalable apomorphine (AZ-009), and its efficacy in a randomized crossover study in Parkinson's disease patients

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ABSTRACT

Background Apomorphine is used to treat *OFF* periods in Parkinson's disease (PD) patients. AZ-009 is a novel apomorphine formulation that delivers a thermally-generated aerosol to the deep lung via inhalation with a single breath.

Methods Part A was a randomized, placebo-controlled, double-blind study investigating the safety and pharmacokinetics of multiple ascending doses of AZ-009. PD patients (n=24) received placebo or 2, 3 or 4 mg AZ-009 once daily for 5 days, followed by three times daily for 2 days with 2 hours between doses. Part B was a double-blind crossover study in 8 PD patients who experience *OFF* periods. During an *OFF* state, patients received 4 mg AZ-009 and placebo on two consecutive days in a randomized order. MDS-UPDRS III and *ON/OFF* state were assessed pre- and post-dose.

Results Three times daily dosing with 2, 3 and 4 mg AZ-009 was relatively well tolerated with no apparent accumulation or changes in safety profile. Mild and transient throat irritation and cough were reported most often. AZ-009 was rapidly absorbed with median T_{max} between 1-2 minutes. When corrected for placebo response, the maximum effect of 4 mg AZ-009 based on MDS-UPDRS III scores was observed at 10 and 30 minutes post-dose with mean (SD) reductions of 6.8 (9.4) and 6.1 (9.1) points respectively. Whereas 0% of patients turned *ON* after placebo, 50% turned *ON* 10 minutes after 4 mg AZ-009 treatment.

Conclusion AZ-009 is rapidly systemically absorbed and safe to dose three times daily. AZ-009 could provide a faster-acting and easier to use formulation than currently available therapies.

INTRODUCTION

Parkinson's disease (PD) patients can begin to experience motor and/or non-motor fluctuations within a few years of disease onset.^{1,2} Fluctuating symptoms impact activities of daily living and worsen quality of life.³

When motor fluctuations persist despite optimized oral levodopa therapy, other treatment options can be sought or added. First-line treatments are usually oral or transdermal drugs, such as dopamine agonists and enzyme inhibitors that prolong the effect of levodopa, i.e., COMT and MAO-B inhibitors. When the above interventions are insufficiently effective, advanced treatment options are available, such as intermittent or continuous subcutaneous apomorphine administration, continuous percutaneous infusion of levodopa-carbidopa intestinal gel and deep brain stimulation (DBS) surgery.⁴ For relief of sudden and intermittent *OFF* periods, subcutaneous apomorphine injections have long been the only treatment option. Its onset of action has been reported between 5-15 minutes,⁵⁻⁷ with maximum motor improvements as assessed by part III of the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) after 20-40 minutes.^{6,8,9} Despite its efficacy, the use of intermittent injections is sometimes limited by injection site reactions, pain and difficulty self-administering the injection during an *OFF* period.¹⁰ With the FDA approval of inhalable levodopa (2018) and sublingual apomorphine (2020), the treatment options for on-demand therapy of *OFF* periods have increased.¹¹ Inhalable levodopa and sublingual apomorphine have an initial onset of effect at 10 and 15 minutes respectively, and show maximum MDS-UPDRS III improvements at 30-60 and 60 minutes respectively.¹²⁻¹⁴ Both are considered less invasive treatment options than subcutaneous apomorphine injections. Inhalation of apomorphine could be another user-friendly alternative with potentially a faster action than already available therapies.

AZ-009 is a breath-actuated, oral inhalation device using the Staccato technology.^{15,16} Inhalation leads to the thermal generation of fine apomorphine aerosol particles that are appropriate for rapid

deep lung delivery and subsequent systemic exposure. The aim of this study was to assess the safety and pharmacokinetics (PK) of multiple (daily) dosing with 2, 3 and 4 mg AZ-009 in PD patients. Patients received AZ-009 or placebo once daily for 5 days, followed by three times daily for 2 days. Moreover, efficacy of AZ-009 relative to placebo was evaluated in PD patients during an induced *OFF* state in a separate crossover design study.

METHODS

The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The study was registered in ClinicalTrials.gov (NCT04157933) and approval was obtained by the Independent Ethics Committee of Foundation Beoordeling Ethiek Biomedisch Onderzoek (BEBO) (Assen, The Netherlands). All patients provided written informed consent prior to their participation. The study was conducted at the Centre for Human Drug Research (CHDR) (Leiden, the Netherlands) between September 2019 and March 2020.

Study design

The study was composed of two parts: part A and B. Part A was a randomized, placebo-controlled, double-blind study investigating multiple ascending doses (MAD) of AZ-009 (2, 3 and 4 mg). Patients were dosed once daily for 5 days, followed by three times daily for 2 days with 2 hours between doses. Each cohort was composed of 8 PD patients (6 active: 2 placebo). This 6:2 ratio was not based on formal sample size calculations, but is common for phase 1 studies for an initial evaluation of safety and PK. Before commencing to the next cohort, safety data were evaluated. Part B was a randomized, double-blind, placebo-controlled crossover study in 8 PD patients who experience *OFF* periods. Patients received 4 mg AZ-009 and placebo on Day 1 and 2 in a randomized order during an *OFF* state. *OFF* was induced by overnight medication withdrawal. No formal sample size calculation was performed for part B due to its exploratory nature.

The study was composed of a screening visit, pretreatment with 20 mg domperidone three times daily from 2 days prior to dosing until last dose, 4 visits of 1 day each followed by 1 visit of 3 days or if preferred by the patient 1 visit of 7 days (part A) or 1 visit of 3 days (part B) at the clinical research unit, and a follow-up telephone call.

Patients

In both study parts, non-smoking PD patients between 30-85 years with a body mass index of 18-32 kg/m² were eligible for participation. Patients in part A had to be classified as Hoehn and Yahr stage I-IV in the *ON* state, and patients in part B as stage I-III in the *ON* state and experiencing motor fluctuations with recognizable *OFF* periods. Main exclusion criteria were use of 5-HT₃ antagonists, use of apomorphine (historical use was allowed), systolic blood pressure (BP) <100 mmHg at screening or baseline, symptomatic clinically relevant and medically uncontrolled orthostatic hypotension, history of long QT syndrome and/or a QTcF of >450 ms (male) or >470 ms (female), history of clinically significant pulmonary (e.g., asthma, COPD) conditions, previous significant complication from oral dopamine agonist therapy including hospitalization, hallucinations, or any other clinically relevant neuropsychiatric adverse event (AE). In addition, in part B, a MMSE score <18 rendered a patient ineligible.

Investigational product

AZ-009 / Staccato apomorphine was administered as a single nominal dose of 1 or 2 mg apomorphine hydrochloride per inhalation device. Doses of 3 mg were achieved by 3 inhalations of 1 mg, and doses of 4 mg by 2 inhalations of 2 mg. Matching placebo was identical to AZ-009 but contained no apomorphine. Devices were packaged in heat-sealed multi-laminate pouches to protect apomorphine from light and moisture. Devices were marked with patient and visit number to maintain the blind. The device manufacturer was Alexza Pharmaceuticals, Inc. (Mountain View, CA, USA). Instructions to the participant were to first exhale, then inhale through the mouthpiece with a steady deep breath, and finally to hold the breath for as long as possible for up to 10 seconds.

The device makes use of the Staccato technology,^{15,16} which is already FDA and EMA approved for the administration of loxapine.¹⁷⁻¹⁹ The device is breath-actuated, a single breath through the device leads to rapid heating (<0.5 second) of a metal substrate coated with a thin film of excipient-free apomorphine. As a result pure drug vapor is formed that rapidly cools and condenses into aerosol particles appropriate for deep lung delivery.

Assessments

SAFETY

Safety was evaluated by AE monitoring (classified by Medical Dictionary for Regulatory Activities (MedDRA) version 21.1), and assessment of ECGs, vital signs, physical examinations, and laboratory measurements. A drop in systolic BP ≥ 20 mmHg and/or a drop in diastolic BP ≥ 10 mmHg upon standing was classified as orthostatic hypotension. QTcF was reviewed after domperidone pretreatment and prior to first dose, as well as prior to each subsequent dose.

PHARMACOKINETICS

Single dose PK has been described previously²⁰ and therefore we only report PK of the MAD study here. AZ-009's PK profile was evaluated in the MAD study (part A) on Day 1, 3 and 5 (once-daily dosing), and on Day 7 (three times daily dosing; dosing at $t=0$, $t=120$ and $t=240$ minutes). Blood samples were collected pre-dose and at 1-, 2-, 5-, 10-, 20-, 30-, 45-, 60-, 120- and 240-minutes post-dose on Day 1, 3 and 5. On Day 7, samples were taken pre-dose and at 2, 5, 10, 30, 60 and 120 minutes after each dose, where the 120-minute sample was taken just prior to the next dose. Apomorphine plasma concentrations were determined by a liquid chromatography-tandem mass spectrometry method (LC-MS/MS) validated for a range of 0.0263-13.1 ng/mL. Concentration-time data were analyzed by non-compartmental methods in Phoenix™ WinNonlin® (Version 8.1, Certara, L.P.). Actual sample times were used in the analysis. Maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), apparent terminal elimination half-life ($T_{1/2}$) and area under the concentration-time curve from zero to infinity (AUC_{0-inf}) values were derived.

Inter- and intra-individual variability (CV%) of C_{max} and AUC_{0-inf} were calculated for each dose group using data of Day 1, 3 and 5. For C_{max} , also data of the first dose of Day 7 were used. The variance of the natural logarithm of C_{max} and AUC_{0-inf} was computed for each day and for each patient, these were averaged to obtain inter- and intra-variance, and subsequently used to calculate the CV% as follows: $100 * \sqrt{\exp(\text{variance}) - 1}$.

EFFICACY

In study part B, efficacy was assessed by the motor examination part of the MDS-UPDRS, i.e., part III. The same trained physician assessed a patient throughout the study. In one patient this was not possible, but all pre- and post-dose assessments of Day 1 were performed by the same physician, and all assessments of Day 2 were performed by another physician. MDS-UPDRS III was performed on Day -1, the day before first dosing and when patients were still using their own anti-Parkinson medication, and on Day 1 and Day 2 pre-dose and 10-, 30- and 60-minutes post-dose. Mean change from baseline (CFB) MDS-UPDRS III total score, with and without correction for placebo, was calculated and presented graphically.

In addition, a patient's disease state was assessed by a physician pre-dose and 10-, 20- and 45-minutes post-dose on Day 1 and 2. Percentage of patients being *ON* (with or without dyskinesia), partial *ON* and *OFF* at each time point were presented in a stacked bar graph. Partial *ON* was defined as a partial response, i.e., the patient showed some improvement after drug administration but did not reach a full *ON* state.

Statistical analysis

Only descriptive statistics were conducted.

RESULTS

Demographics

Demographic characteristics of the patients enrolled in both studies are described in Table 1. In the MAD study (part A), 26 PD patients were

enrolled of which 2 patients discontinued early. One patient withdrew consent after the second dosing day and one was discontinued early due to an AE (atrial fibrillation). In total, 24 patients completed the full study. In the crossover study (part B), 9 PD patients were enrolled of which 1 patient only completed the first (placebo) dosing day. In total, 8 patients completed the full study. Refer to Supplemental Figures 1 and 2 for CONSORT flow diagrams providing an overview of number of participants screened, randomized, completed, and analyzed per study part.

Pharmacokinetics of multiple (daily) dosing

AZ-009 was rapidly absorbed with median t_{max} between 1-2 minutes during once-daily dosing in PD patients (Figure 1). Descriptive statistics of the PK parameters are summarized in Supplemental Table 1. Mean $t_{1/2}$ ranged from 38 to 44 minutes. There was no carryover of apomorphine across study days. Mean C_{max} increased with an increase in dose on Day 1, 3 and 5. On Day 1 and 3, mean AUC_{0-inf} increased from 2 to 3 mg, but was comparable between 3 and 4 mg AZ-009. On Day 5, mean AUC_{0-inf} increased with dose from 2 to 3 to 4 mg AZ-009.

On Day 6 and 7, AZ-009 was administered three times daily with 2 hours between doses. PK sampling took place on Day 7 (Figure 1). Descriptive statistics are summarized in Supplemental Table 2. After the first administration on Day 7, mean C_{max} increased with an increase in dose. After the second and third administration, mean C_{max} increased from 2 to 3 mg, but not from 3 to 4 mg AZ-009. Mean C_{max} after each 2 mg dose, and after each 4 mg dose (at $t=0$, $t=2$ and $t=4$ hours) was comparable. In contrast, there was an increase in concentration with multiple dosing in the 3 mg cohort, where mean C_{max} after the second and third dose was higher compared to C_{max} after the first dose. Following three times daily dosing, mean AUC_{0-inf} increased with dose from 2 to 3 mg, but was comparable after 3 and 4 mg AZ-009. Mean $t_{1/2}$ ranged from 34 to 38 minutes.

Inter-individual variability (CV%) of C_{max} was 160%, 152% and 72%, and of AUC_{0-inf} 118%, 81% and 43% in the 2, 3 and 4 mg AZ-009 group respectively. Intra-individual variability (CV%) of C_{max} was 77%, 59%

and 57%, and of AUC_{0-inf} 32%, 32% and 42% in the 2, 3 and 4 mg AZ-009 group respectively.

Safety of multiple (daily) dosing

In the AZ-009-treated groups more treatment-emergent AEs (TEAEs) (68-87) were reported than in the placebo group (12) (Table 2). The most frequently reported TEAEs by patients receiving 2, 3 or 4 mg AZ-009 were cough and throat irritation (incidence between 71.4-100%) and fatigue (50.0-57.1%). Most TEAEs were mild and all were transient. The number and severity of TEAEs was not affected when dosed three times daily as opposed to once daily. One TEAE, classified as possibly related to AZ-009, led to early discontinuation of a patient. The patient developed first onset atrial fibrillation as detected on ECG approximately 15 minutes after the first 4 mg AZ-009 inhalation. Approximately 4 hours post-dose, the patient spontaneously converted back to sinus rhythm. One serious AE was reported in a patient receiving 4 mg AZ-009. The serious AE, tooth abscess, was assessed as being unrelated to the study drug. No consistent or clinically relevant QTcF prolongation was reported.

Efficacy in a crossover study with placebo

When patients received placebo, they showed a mean deterioration over time, i.e., an increase of mean MDS-UPDRS III (SD) compared to baseline of 1.6 (7.4) points, 2.8 (9.0) points and 4.1 (9.8) points at 10-, 30- and 60-minutes post-dose respectively (Figure 2A). In contrast, 4 mg AZ-009 led to a mean reduction in MDS-UPDRS III total score at 10- and 30-minutes post-dose of 4.8 (5.7) and 6.3 (6.0) points respectively (Figure 2A). At 60-minutes post-dose, the patients treated with 4 mg AZ-009 no longer showed an improvement compared to baseline (-0.7 (10.6) points) (Figure 2A). At 10 minutes post-dose, MDS-UPDRS III could only be assessed in half of the AZ-009-treated patients, since known AEs for apomorphine, i.e., presyncope and hypotension, prevented its conduct. At 30 minutes post-dose, the patients treated with AZ-009 all recovered sufficiently to perform the assessment again, except for one patient ($n=7$). When

corrected for individual placebo response, the maximum effect of 4 mg AZ-009 was observed at 10 minutes post-dose with a mean (placebo-corrected) reduction (SD) of 6.8 (9.4) points (Figure 2B). This effect was comparable to the effect observed at 30 minutes post-dose, i.e., -6.1 (9.1) points.

A physician evaluated whether a patient was *ON*, partial *ON* or remained *OFF* pre-dose and 10-, 20- and 45-minutes post-dose (Figure 2C). Prior to dosing all patients were *OFF*. None of the placebo-treated patients achieved a full *ON* response, but 22% did turn partial *ON* from 10 minutes post-dose onwards. In contrast, at 10 minutes post-dose, 25% of the patients receiving 4 mg AZ-009 transitioned to a partial *ON* state and 50% of patients to a full *ON* state. At 45 minutes post-dose, there were no AZ-009-treated patients still in an *OFF* state (12% (1 patient) was not evaluable).

DISCUSSION

Here, we report the first safety and PK data of multiple dosing with AZ-009, a new apomorphine inhalation device to treat *OFF* periods in PD patients. AZ-009 was rapidly absorbed with median T_{max} between 1-2 minutes, which is considerably faster than currently available on-demand therapies for *OFF* periods. For subcutaneous and sublingual apomorphine, median T_{max} differs between studies, but usually ranges between 15-23 and 38-51 minutes respectively.²¹⁻²³ For inhaled levodopa, a median T_{max} of 15 minutes has been reported.^{24,25} Another inhalable apomorphine formulation (VRO40) shows more comparable PK, i.e., T_{max} ranging between 1-7 minutes.^{26,27} Since group sizes in this study were relatively small and inter-individual variability relatively high, no conclusions could be drawn on dose proportionality over the dose range 2-4 mg. Future larger trials are needed for this assessment. Inter-individual variability in exposure parameters (CV%) ranged between 72-160% for C_{max} , and 43-118% for AUC_{0-inf} , with a trend towards decreased variation with an increase in AZ-009 dose. This is higher than reported for subcutaneous apomorphine injections where CV% for C_{max} has been reported between 20-71% and for AUC_{0-inf} between 20-32%.^{21,22,28-30}

For sublingual apomorphine, also relatively high inter-individual variability has been reported, i.e., 73% for C_{max} and 68% for AUC_{0-inf} in a study with larger sample size ($n=19$ and $n=16$ for C_{max} and AUC_{0-inf} respectively) than in this study.²¹ As expected, intra-individual variability was lower than inter-individual variability.

5-Day once-daily dosing, followed by 2 days three times daily dosing (every 2 hours) with AZ-009 at doses of 2, 3 and 4 mg was relatively well tolerated by PD patients. Most patients reported mild throat irritation and cough directly after inhalation which usually resolved within minutes. No apparent accumulation or changes in safety profile during three times daily dosing were observed. Mean $T_{1/2}$ of apomorphine ranged between 34-44 minutes, meaning that after 2 hours on average 3 half-lives have passed. Therefore, theoretically some accumulation will occur, but with the observed variability and the sample size used, it is not surprising that this accumulation was not objectified.

One patient developed first onset atrial fibrillation as detected on ECG approximately 15 minutes after the first 4 mg AZ-009 inhalation. The patient was asymptomatic and spontaneously converted back to sinus rhythm approximately 4 hours post-dose. The patient was a 65-year old male, diagnosed with Parkinson's disease for 14 years and treated with levodopa/benserazide 100/25 mg 7 times daily and pramipexole 1.5 mg once daily. In addition, the patient had a DBS for 7 years. The patient had no history of cardiovascular disease. In literature, a few cases of atrial fibrillation in PD patients after apomorphine administration have been described.³¹⁻³³ It has been hypothesized that atrial fibrillation is caused by an imbalance of autonomic tone with predominance of vagal activity.^{31,34,35}

Usability of this specific Staccato device has not yet been adequately investigated in the PD population. Future studies will investigate the usability of the commercial device by PD patients while *OFF*. Previous research with a dry powder inhaler has shown that most PD patients after adequate training are able to handle a dry powder inhaler, have sufficiently high inspiratory flow rates and are able to hold their breath for up to 5 seconds after inhalation.³⁶ Another study evaluated the ability of PD patients to correctly open a pouch wherein the inhaler was stored and to prepare the inhaler for

use.³⁷ The study showed that 58% of PD patients in an *OFF* state were able to open pouch 1 as intended (via the tear notch), whereas this was much higher (75%) for pouch 2, indicating that pouch 2 would be better suited for use in a PD population. This underlines that evaluation of device packaging, preparation and use in the target population is crucial. Encouraging results on inhalation device use have been reported in a phase 2b study with inhalable dry powder levodopa: patients were able to prepare and self-administer the treatment, even though some indicated concerns about inhaler system use during telephone contact (7% placebo, 14% levodopa).¹² Similarly, in a phase 2a study with inhalable apomorphine (VRO40), 23 out of 24 patients were able to load and use the device correctly.²⁶

When corrected for individual placebo response, the maximum effect of 4 mg AZ-009 was observed at 10 and 30 minutes post-dose with mean (SD) reductions of 6.8 (9.4) and 6.1 (9.1) points at 10 and 30 minutes respectively. This is in line with results from a phase 1, parallel design study, where 4 mg AZ-009 led to reduction of 10.3 (3.7) points and placebo to 4.8 (4.9) points at 10 minutes post-dose.²⁰ In contrast, subcutaneous apomorphine reaches its maximum MDS-UPDRS III response after 20-40 minutes,^{6,8,9} sublingual apomorphine after 60 minutes¹⁴, and inhalable levodopa after 30-60 minutes.^{12,13} The time to maximum effect of AZ-009 resembles that reported for another apomorphine inhaler (VRO40) under clinical investigation, i.e., maximum MDS-UPDRS III response at 20 minutes post-dose.^{27,38} Mean MDS-UPDRS III differences between apomorphine dry powder inhalation and placebo in these studies were 8.4 (95% CI 1.2-15.5) and 11.6 (95% CI 2.3-20.9). Both were ascending dose titration studies which might explain the larger effects found. The observed effect in the present study is expected to be an underestimation since the administered dose was not optimized per individual after up titration, as is done in the clinical setting for subcutaneous and sublingual apomorphine. This likely led to suboptimal dosing, where for some the dose was too high and therefore resulted in AEs (known for apomorphine) preventing the conduct of MDS-UPDRS III, and for others might have been too low to reach optimal efficacy. Therefore, in clinical practice, AZ-009 would have to be initiated at a lower dose and titrated to a dose that balances efficacy and side effects.

Future studies should address AZ-009's efficacy when administered at a patient's optimal dose. Nevertheless, this study clearly showed a conversion from *OFF* to partial or full *ON* after 4 mg AZ-009 treatment. At 10 minutes post-dose, 75% of patients turned partial or full *ON*, and at 45 minutes no patients (1 patient not evaluable) were left in an *OFF* state. In contrast, none of the placebo-treated patients achieved a full *ON* response, even though 22% did turn partial *ON* from 10 minutes post-dose onwards.

With AZ-009's median T_{max} of 1-2 minutes and expected maximum MDS-UPDRS III improvements at 10 and 30 minutes post-dose, this inhalable apomorphine formulation could provide an easy and fast-acting formulation for rescue of *OFF* periods.

TABLE 1 Demographics.

	MAD study					Crossover study
	All patients (N=26)	2 mg AZ-009 (N=6)	3 mg AZ-009 (N=7)	4 mg AZ-009 (N=7)	Placebo (N=6)	All patients (N=9)
Age (years)						
Mean (SD)	64.2 (9.2)	63.2 (11.1)	64.6 (11.2)	60.9 (8.3)	68.7 (4.6)	63.3 (6.3)
Median (range)	65 (48-83)	61 (51-81)	60 (50-83)	65 (48-68)	69 (62-75)	66 (55-70)
BMI (kg/m ²)						
Mean (SD)	25.5 (2.7)	25.9 (2.0)	25.0 (2.8)	25.6 (3.1)	25.8 (3.4)	23.2 (3.4)
Median (range)	25 (21-31)	26 (23-29)	24 (23-30)	24 (22-31)	26 (21-30)	23 (19-31)
Sex (n/n (%/%))						
Female/Male	7/19 (27/73)	2/4 (33/67)	3/4 (43/57)	1/6 (14/86)	1/5 (17/83)	5/4 (56/44)
Race (n (%))						
Asian	1 (4)	0 (0)	0 (0)	0 (0)	1 (17)	0 (0)
Black or African American	1 (4)	0 (0)	0 (0)	1 (14)	0 (0)	0 (0)
White	24 (92)	6 (100)	7 (100)	6 (86)	5 (83)	9 (100)
Hoehn and Yahr stage (n (%)) ^a						
Stage 1	5 (19)	2 (33)	1 (14)	2 (29)	0 (0)	0 (0)
Stage 2	4 (15)	2 (33)	2 (29)	0 (0)	0 (0)	8 (89)
Stage 3	9 (35)	1 (17)	0 (0)	4 (57)	4 (67)	1 (11)
Stage 4	8 (31)	1 (17)	4 (57)	1 (14)	2 (33)	0 (0)
Concomitant PD medication (n (%))						
Levodopa-containing agent	25 (96)	5 (83)	7 (100)	7 (100)	6 (100)	9 (100)
Dopamine agonist	20 (78)	5 (83)	5 (71)	6 (86)	4 (67)	4 (44)
COMT inhibitor	3 (12)	0 (0)	1 (14)	1 (14)	1 (17)	3 (33)
MAO-B inhibitor	2 (8)	0 (0)	0 (0)	1 (14)	1 (17)	0 (0)
Amantadine	5 (19)	1 (17)	3 (43)	0 (0)	1 (17)	1 (11)
Deep brain stimulator	5 (19)	2 (33)	2 (29)	1 (14)	0 (0)	1 (11)

a. Hoehn and Yahr stage defined at screening (MAD study) or at the day prior to dosing while still on regular anti-Parkinson medication (crossover study).

SD, standard deviation; BMI, body mass index; PD, Parkinson's disease; COMT, catechol-O-methyltransferase; MAO-B, monoamine oxidase B; MAD, multiple ascending dose.

TABLE 2 Summary of the number of treatment-emergent adverse events (TEAEs) and the number and percentage of participants (n (%)) with any, mild, moderate and severe TEAE and with a specific TEAE as indicated per treatment group in the multiple ascending dose study (part A).

	2 mg AZ-009 (N=6) n (%)	3 mg AZ-009 (N=7) n (%)	4 mg AZ-009 (N=7) n (%)	Placebo (N=6) n (%)
#TEAEs ^a	87	68	69	12
Any TEAEs	6 (100)	7 (100)	7 (100)	5 (83.3)
Mild TEAEs	6 (100)	7 (100)	7 (100)	5 (83.3)
Moderate TEAEs	1 (17)	0 (0)	1 (14)	0 (0)
Severe TEAEs	0 (0)	0 (0)	0 (0)	0 (0)
Most common TEAEs ^b				
Throat irritation	6 (100.0)	7 (100.0)	5 (71.4)	1 (16.7)
Cough	6 (100.0)	6 (85.7)	5 (71.4)	0 (0)
Fatigue	3 (50.0)	4 (57.1)	4 (57.1)	2 (33.3)
Headache	1 (16.7)	4 (57.1)	2 (28.6)	0 (0)
Yawning	2 (33.3)	2 (28.6)	2 (28.6)	0 (0)
Dizziness	1 (16.7)	1 (14.3)	1 (14.3)	1 (16.7)

a. Not expressed as n (%). This parameter describes the total number of TEAEs reported, and hence is unitless. / b TEAEs reported by ≥ 15% of participants

FIGURE 1 Mean (standard deviation) apomorphine concentration-time profiles of the multiple ascending dose study (part A) on a linear scale depicting Day 1, 3 and 5 after once daily dosing, and Day 7 after three times daily dosing (every 2 h) with 2, 3 or 4 mg AZ-009. For Day 7, only the mean concentration-time profile is shown for legibility.

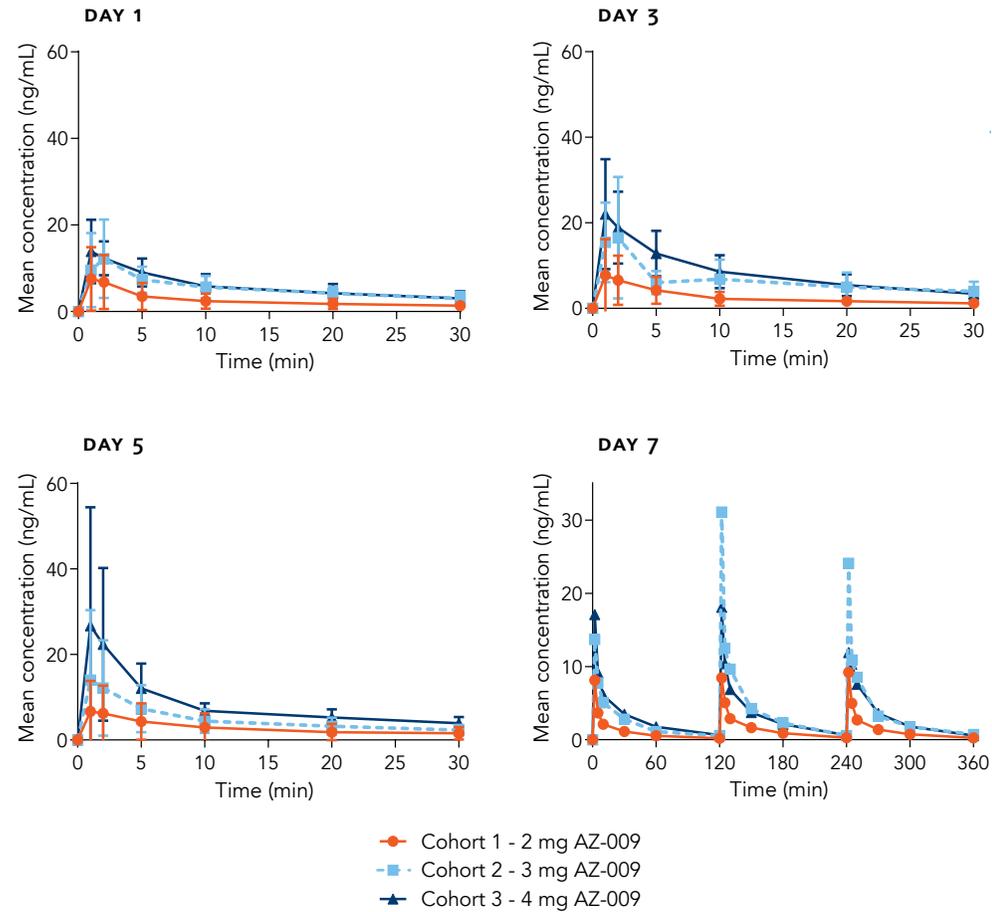
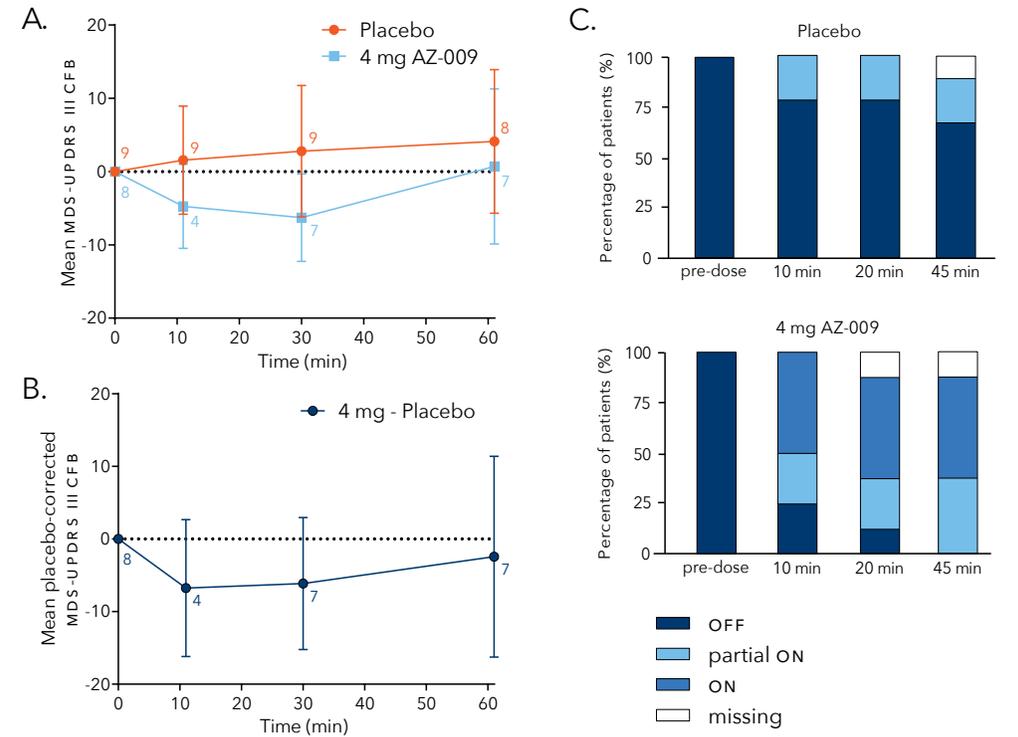


FIGURE 2 Parkinson's disease patients received placebo and 4 mg AZ-009 (crossover) during an induced OFF state. Efficacy is shown as mean change from baseline (CFB) MDS-UPDRS III total score with standard deviation without (A) and with individual correction for placebo response (B). Number of patients assessed at each time point are indicated in the graph. Figure 2C presents the percentage (%) of patients that are OFF, partial ON and full ON at the indicated time points.



MDS-UPDRS III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III.

SUPPLEMENTARY MATERIAL

SUPPLEMENTAL TABLE 1 Pharmacokinetic parameters of apomorphine after once daily dosing with 2, 3 and 4 mg AZ-009 on Day 1, 3 and 5.

	2 mg AZ-009			3 mg AZ-009			4 mg AZ-009		
	Day 1 (N=6)	Day 3 (N=6)	Day 5 (N=6)	Day 1 (N=7) ^b	Day 3 (N=5) ^c	Day 5 (N=6)	Day 1 (N=7)	Day 3 (N=6)	Day 5 (N=6)
T_{max} (min)									
Median	1.5	1.5	1.5	2	1	2	1	1	1.5
(range)	(1-10)	(1-5)	(1-2)	(2-6)	(1-2)	(1-3)	(1-5)	(1-2)	(1-5)
C_{max} (ng/mL)									
Mean	7.8	9.6	6.9	12.0	19.3	14.7	14.9	23.5	28.1
(SD)	(7.2)	(8.7)	(7.0)	(8.3)	(13.8)	(15.8)	(5.8)	(11.7)	(26.8)
Median	5.2	8.8	4.3	11.3	21.7	10.0	17.1	18.5	14.0
(range)	(1.4-18.7)	(0.7-21.3)	(1.2-20.3)	(2.9-29.1)	(6.5-39.8)	(1.5-43.9)	(7.4-21.9)	(12.7-41.9)	(6.7-67.1)
AUC_{0-inf} (h·ng/mL)									
Mean	2.5	2.3	2.6	5.6	7.1	4.5	5.7	7.3	8.0
(SD)	(1.9)	(1.4)	(2.8) ^a	(2.2)	(4.2)	(2.8)	(2.7)	(2.8)	(3.4)
Median	1.7	2.2	1.7	5.9	6.6	4.4	4.2	6.5	7.1
(range)	(0.4-5.8)	(0.6-4.6)	(0.6-7.3) ^a	(1.8-8.3)	(1.4-12.6)	(1.6-8.4)	(3.4-10.3)	(4.1-12.4)	(3.9-13.0)
T_{1/2} (min)									
Mean	41	39	39	44	38	44	41	42	39
(SD)	(4)	(9)	(7)	(9)	(7)	(12)	(5)	(7)	(4)
Median	39	40	38	40	40	41	43	41	38
(range)	(38-46)	(27-50)	(28-48)	(36-61)	(27-47)	(29-60)	(34-46)	(33-52)	(34-46)

a. N=5. For one patient the AUC_{0-inf} acceptance criteria were not met (> 20% of the AUC was extrapolated) and therefore the AUC_{0-inf} of this patient was excluded from summary statistics. / b. One patient erroneously received 2 instead of 3 mg AZ-009 on Day 1. / c. N=5. One patient was not dosed due to too low pre-dose blood pressure at Day 3. T_{max}, time to maximum plasma concentration; C_{max}, maximum plasma concentration; SD, standard deviation; AUC_{0-inf}, area under the plasma concentration-time curve from zero to infinity; T_{1/2}, apparent terminal elimination half-life.

SUPPLEMENTAL TABLE 2 Pharmacokinetic parameters of apomorphine after three times daily dosing (every 2 hours at T=0, T= 120, and T=240 minutes) with 2, 3 and 4 mg AZ-009 on Day 7.

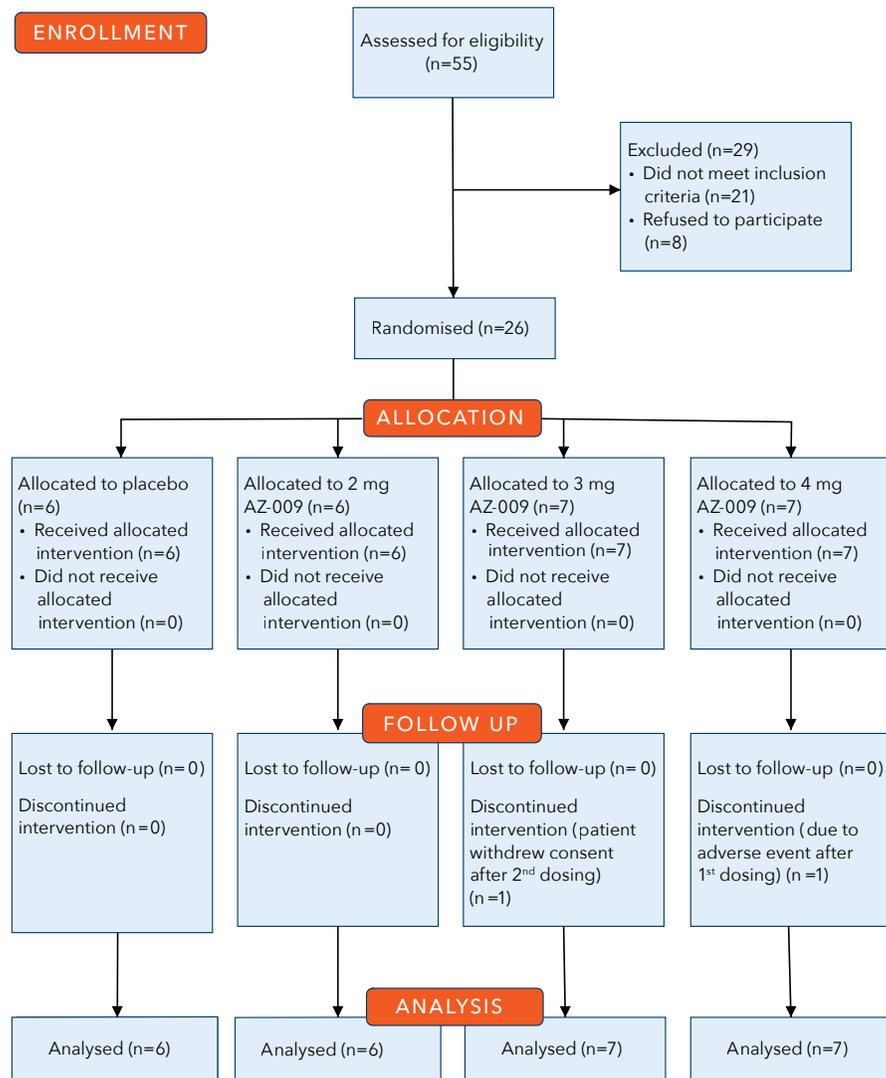
	2 mg AZ-009			3 mg AZ-009			4 mg AZ-009		
	Day 7, dose 1 (N=6)	Day 7, dose 2 (N=6)	Day 7, dose 3 (N=6)	Day 7, dose 1 (N=6)	Day 7, dose 2 (N=6)	Day 7, dose 3 (N=6)	Day 7, dose 1 (N=6)	Day 7, dose 2 (N=6)	Day 7, dose 3 (N=6)
T_{max} (min)^a									
Median (range)	2 (2-5)	123 (122-125)	242 (242-245)	2.5 (2-3)	124 (122-150)	247 (242-274)	2 (2-2)	122 (122-125)	243 (242-275)
C_{max} (ng/mL)									
Mean	8.4	8.5	9.3	13.7	22.4	24.4	17.1	18.4	12.0
(SD)	(13.3)	(11.1)	(9.1)	(15.0)	(25.3)	(23.2)	(8.7)	(12.6)	(5.8)
Median	3.2	2.9	4.8	8.8	12.6	20.0	17.3	15.2	11.6
(range)	(1.7-35.5)	(1.5-29.8)	(1.9-25.6)	(0.6-41.5)	(0.3-68.5)	(2.2-58.0)	(7.0-29.3)	(4.9-40.9)	(4.6-20.7)
AUC_{0-inf} (h·ng/mL)									
Mean (SD)	7.9 (6.9)			22.8 (13.2) ^b			18.8 (7.7)		
Median (range)	6.5 (1.7-21.1)			19.9 (5.8-40.7) ^b			17.5 (10.9-32.4)		
T_{1/2} (min)									
Mean (SD)	-	-	37 (7)	-	-	38 (2) ^b	-	-	34 (5)
Median (range)	-	-	38 (26-45)	-	-	38 (36-41) ^b	-	-	34 (28-42)

a. Calculated from the time of first dosing on Day 7. / b. N=5. For one patient the acceptance criteria for reporting the terminal elimination rate constant were not met (adjusted R² < 0.800). Therefore, AUC_{0-inf} and T_{1/2} could not be determined.

T_{max}, time to maximum plasma concentration; C_{max}, maximum plasma concentration; SD, standard deviation; AUC_{0-inf}, area under the plasma concentration-time curve from zero to infinity; T_{1/2}, apparent terminal elimination half-life.

SUPPLEMENTAL FIGURE 1 CONSORT flow diagram for the multiple ascending dose study.

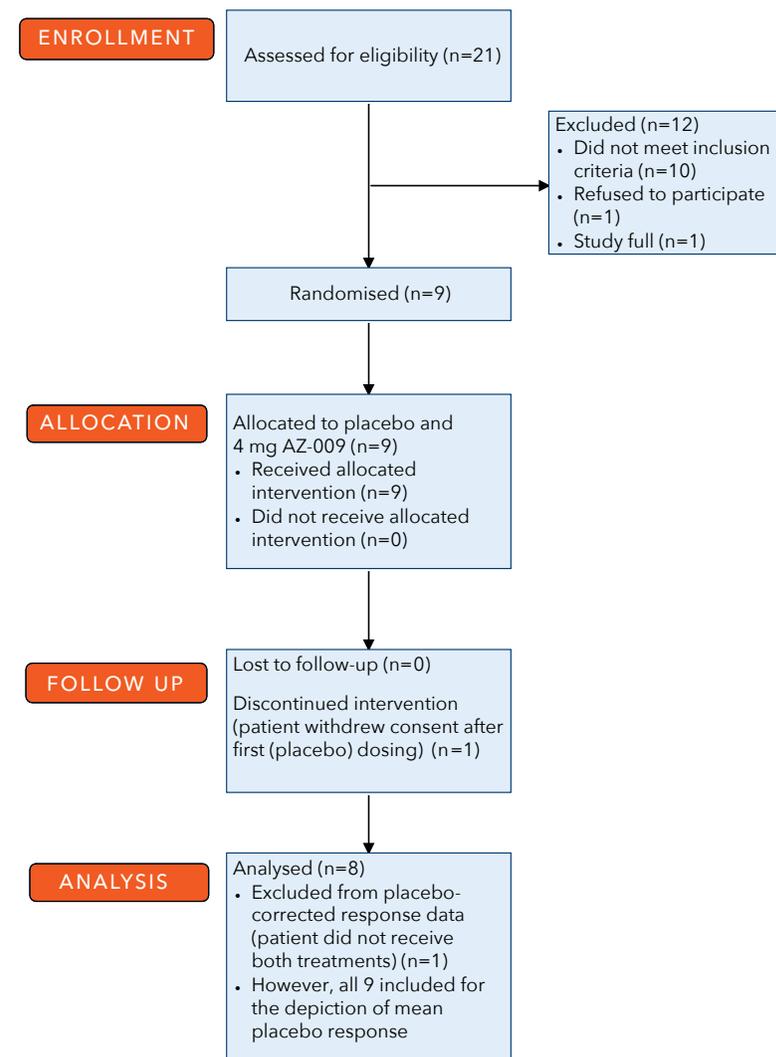
STUDY PART A - MULTIPLE ASCENDING DOSE STUDY



CONSORT, Consolidated Standards of Reporting Trials

SUPPLEMENTAL FIGURE 2 CONSORT flow diagram for the crossover study.

STUDY PART B - CROSSOVER STUDY



CONSORT, Consolidated Standards of Reporting Trials.

REFERENCES

- 1 F. Stocchi, A. Antonini, P. Barone, M. Tinazzi, M. Zappia, M. Onofri, S. Ruggieri, L. Morgante, U. Bonuccelli, L. Lopiano, P. Pramstaller, A. Albanese, M. Attar, V. Posocco, D. Colombo, G. Abbruzzese, DEEP study group, Early DEtection of wEaring off in Parkinson disease: The DEEP study, *Park. Relat. Disord.* 20 (2014) 204–211. <https://doi.org/10.1016/j.parkreldis.2013.10.027>.
- 2 J.E. Ahlskog, M.D. Muentner, Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature, *Mov. Disord.* 16 (2001) 448–458. <https://doi.org/10.1002/mds.1090>.
- 3 M. Rodríguez-Violante, N. Ospina-García, N. Merari Dávila-Avila, D. Cruz-Fino, A. De La Cruz-Landero, A. Cervantes-Arriaga, Motor and non-motor wearing-off and its impact in the quality of life of patients with Parkinson's disease, *Arq. Neuropsiquiatr.* 76 (2018) 517–521. <https://doi.org/10.1590/0004-282X20180074>.
- 4 S.H. Fox, R. Katzenschlager, S.Y. Lim, B. Barton, R.M.A. de Bie, K. Seppi, M. Coelho, C. Sampaio, International Parkinson and movement disorder society evidence-based medicine review: Update on treatments for the motor symptoms of Parkinson's disease, *Mov. Disord.* 33 (2018) 1248–1266. <https://doi.org/10.1002/mds.27372>.
- 5 J.P. Frankel, A.J. Lees, P.A. Kempster, G.M. Stern, Subcutaneous apomorphine in the treatment of Parkinson's disease, *J. Neurol. Neurosurg. Psychiatry.* 53 (1990) 96–101. <https://doi.org/10.1136/jnnp.53.2.96>.
- 6 R.F. Pfeiffer, L. Gutmann, K.L. Hull, P.B. Bottini, J.H. Sherry, Continued efficacy and safety of subcutaneous apomorphine in patients with advanced Parkinson's disease, *Park. Relat. Disord.* 13 (2007) 93–100. <https://doi.org/10.1016/j.parkreldis.2006.06.012>.
- 7 C.M.H. Stibe, P.A. Kempster, A.J. Lees, G.M. Stern, Subcutaneous apomorphine in parkinsonian on-off oscillations, *Lancet.* 331 (1988) 403–406. [https://doi.org/10.1016/S0140-6736\(88\)91193-2](https://doi.org/10.1016/S0140-6736(88)91193-2).
- 8 R.M. Trosch, D. Silver, P.B. Bottini, Intermittent subcutaneous apomorphine therapy for “off” episodes in Parkinson's disease: A 6-month open-label study, *CNS Drugs.* 22 (2008) 519–527. <https://doi.org/10.2165/00023210-200822060-00005>.
- 9 R. Pahwa, W.C. Koller, R.M. Trosch, J.H. Sherry, Subcutaneous apomorphine in patients with advanced Parkinson's disease: A dose-escalation study with randomized, double-blind, placebo-controlled crossover evaluation of a single dose, *J. Neurol. Sci.* 258 (2007) 137–143. <https://doi.org/10.1016/j.jns.2007.03.013>.
- 10 F. Carbone, A. Djamshidian, K. Seppi, W. Poewe, Apomorphine for Parkinson's Disease: Efficacy and Safety of Current and New Formulations, *CNS Drugs.* 33 (2019) 905–918. <https://doi.org/10.1007/s40263-019-00661-z>.
- 11 R.A. Hauser, P.A. LeWitt, C.L. Comella, On demand therapy for Parkinson's disease patients: Opportunities and choices, <https://doi.org/10.1080/00325481.2021.1936087>. 133 (2021) 721–727. <https://doi.org/10.1080/00325481.2021.1936087>.
- 12 P.A. LeWitt, R.A. Hauser, D.G. Grosset, F. Stocchi, M.H. Saint-Hilaire, A. Ellenbogen, M. Leinonen, N.B. Hampson, T. DeFeo-Fraulini, M.I. Freed, K.D. Kiebertz, A randomized trial of inhaled levodopa (CVT-301) for motor fluctuations in Parkinson's disease, *Mov. Disord.* 31 (2016) 1356–1365. <https://doi.org/10.1002/MDS.26611>.
- 13 P.A. LeWitt, R.A. Hauser, R. Pahwa, S.H. Isaacson, H.H. Fernandez, M. Lew, M. Saint-Hilaire, E. Pourcher, L. Lopez-Manzanares, C. Waters, M. Rudzinska, A. Sedkov, R. Batycky, C. Oh, Safety and efficacy of CVT-301 (levodopa inhalation powder) on motor function during off periods in patients with Parkinson's disease: a randomised, double-blind, placebo-controlled phase 3 trial, *Lancet Neurol.* 18 (2019) 145–154. [https://doi.org/10.1016/S1474-4422\(18\)30405-8](https://doi.org/10.1016/S1474-4422(18)30405-8).
- 14 C.W. Olanow, S.A. Factor, A.J. Espay, R.A. Hauser, H.A. Shill, S. Isaacson, R. Pahwa, M. Leinonen, P. Bhargava, K. Sciarappa, B. Navia, D. Blum, xx xx, Apomorphine sublingual film for off episodes in Parkinson's disease: a randomised, double-blind, placebo-controlled phase 3 study, *Lancet Neurol.* 19 (2020) 135–144. [https://doi.org/10.1016/S1474-4422\(19\)30396-5](https://doi.org/10.1016/S1474-4422(19)30396-5).
- 15 J.D. Rabinowitz, P.M. Lloyd, P. Munzar, D.J. Myers, S. Cross, R. Damani, R. Quintana, D.A. Spyker, P. Soni, J. V. Cassella, Ultra-Fast Absorption of Amorphous Pure Drug Aerosols via Deep Lung Inhalation, *J. Pharm. Sci.* 95 (2006) 2438–2451. <https://doi.org/10.1002/JPS.20694>.
- 16 J.D. Rabinowitz, M. Wensley, P. Lloyd, D. Myers, W. Shen, A. Lu, C. Hodges, R. Hale, D. Mufson, A. Zaffaroni, Fast Onset Medications through Thermally Generated Aerosols, *J. Pharmacol. Exp. Ther.* 309 (2004) 769–775. <https://doi.org/10.1124/jpet.103.062893>.
- 17 Drug Approval Package: Adasuve (loxapine) NDA #022549, (n.d.). https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/022549_adasuve_toc.cfm (accessed December 14, 2021).
- 18 Adasuve | European Medicines Agency, (n.d.). <https://www.ema.europa.eu/en/medicines/human/EPAR/adasuve#authorisation-details-section> (accessed December 14, 2021).
- 19 M.H. Allen, D. Feifel, M.D. Lesem, D.L. Zimbhoff, R. Ross, P. Munzar, D.A. Spyker, J. V. Cassella, Efficacy and Safety of Loxapine for Inhalation in the Treatment of Agitation in Patients With Schizophrenia: A Randomized, Double-Blind, Placebo-Controlled Trial, *J. Clin. Psychiatry.* 72 (2011) 0–0. <https://doi.org/10.4088/JCP.10Mo6011YEL>.
- 20 E. Thijssen, J. den Heijer, D. Puibert, L. Moss, M. Lei, D. Hasegawa, K. Keum, K. Mochel, M.E. Sharaf, T. Alfredson, W. Zeng, E. van Brummelen, T. Naranda, G.J. Groeneveld, A Randomized Trial Assessing the Safety, Pharmacokinetics, and Efficacy During Morning Off of AZ-009, *Mov. Disord.* (2022). <https://doi.org/10.1002/MDS.28926>.
- 21 Y.L. Chen, L. Shi, F. Agbo, S.H. Yong, P.S. Tan, A.G. Ngounou Wetie, LC-MS/MS simultaneous quantification of apomorphine and its major metabolites in human plasma: Application to clinical comparative bioavailability evaluation for the apomorphine sublingual film and a subcutaneous product, *J. Pharm. Biomed. Anal.* 190 (2020) 113493. <https://doi.org/10.1016/J.JPBA.2020.113493>.
- 22 M. Nomoto, S.I. Kubo, M. Nagai, T. Yamada, A. Tamaoka, Y. Tsuboi, N. Hattori, A randomized controlled trial of subcutaneous apomorphine for Parkinson disease: A repeat dose and pharmacokinetic study, *Clin. Neuropharmacol.* 38 (2015) 241–247. <https://doi.org/10.1097/WNF.000000000000111>.
- 23 F. Agbo, S.H. Isaacson, R. Gil, Y.Y. Chiu, S.J. Brantley, P. Bhargava, B. Navia, Pharmacokinetics and Comparative Bioavailability of Apomorphine Sublingual Film and Subcutaneous Apomorphine Formulations in Patients with Parkinson's Disease and “OFF” Episodes: Results of a Randomized, Three-Way Crossover, Open-Label Study, *Neurol. Ther.* 10 (2021) 693–709. <https://doi.org/10.1007/S40120-021-00251-6>.
- 24 M.M. Lipp, R. Batycky, J. Moore, M. Leinonen, M.I. Freed, Preclinical and clinical assessment of inhaled levodopa for OFF episodes in Parkinson's disease, *Sci. Transl. Med.* 8 (2016). https://doi.org/10.1126/SCITRANSLMED.AAD8858/SUPPL_FILE/8-360RA136_SM.PDF.
- 25 B.E. Safirstein, A. Ellenbogen, P. Zhao, H.R. Henney, D.M. Kessler-Ebo, C. Oh, Pharmacokinetics of Inhaled Levodopa Administered With Oral Carbidopa in the Fed State in Patients With Parkinson's Disease, *Clin. Ther.* 42 (2020) 1034–1046. <https://doi.org/10.1016/J.CLINTHERA.2020.04.004>.
- 26 K.A. Grosset, N. Malek, F. Morgan, D.G. Grosset, Phase IIa randomized double-blind, placebo-controlled study of inhaled apomorphine as acute challenge for rescuing ‘off’ periods in patients with established Parkinson's disease, *Eur. J. Neurol.* 20 (2013) 1445–1450. <https://doi.org/10.1111/ENE.12091>.
- 27 K.A. Grosset, N. Malek, F. Morgan, D.G. Grosset, Inhaled dry powder apomorphine (vR040) for ‘off’ periods in Parkinson's disease: An in-clinic double-blind dose ranging study, *Acta Neurol. Scand.* 128 (2013) 166–171. <https://doi.org/10.1111/ANE.12107>.
- 28 E. Nicolle, P. Pollak, F. Serre-Debeauvais, P. Richard, C. Gervason, E. Broussolle, M. Gavend, Pharmacokinetics of apomorphine in parkinsonian patients, *Fundam. Clin. Pharmacol.* 7 (1993) 245–252. <https://doi.org/10.1111/j.1472-8206.1993.tb00238.x>.
- 29 S.T. Gancher, W.R. Woodward, B. Boucher, J.G. Nutt, Peripheral pharmacokinetics of apomorphine in humans, *Ann. Neurol.* 26 (1989) 232–238. <https://doi.org/10.1002/ana.410260209>.
- 30 T. Van Laar, C. Neef, M. Danhof, K.I. Roon, R.A.C. Roos, A new sublingual formulation of apomorphine in the treatment of patients with Parkinson's disease, *Mov. Disord.* 11 (1996) 633–638. <https://doi.org/10.1002/mds.870110607>.
- 31 F. Stocchi, M.F. De Pandis, F.A. Delfino, T. Anselmo, D. Frongillo, Transient atrial fibrillation after subcutaneous apomorphine bolus, *Mov. Disord.* 11 (1996) 584–585. <https://doi.org/10.1002/MDS.870110520>.

- 32 G. Ardolino, E. D'Adda, E. Nobile-Orazio, Recurrent atrial fibrillation after subcutaneous apomorphine, *Parkinsonism Relat. Disord.* 14 (2008) 173-174. <https://doi.org/10.1016/J.PARKRELDIS.2007.05.012>.
- 33 P.A. LeWitt, W.G. Ondo, B. Van Lunen, P.B. Bottini, Open-label study assessment of safety and adverse effects of subcutaneous apomorphine injections in treating "off" episodes in advanced Parkinson disease, *Clin. Neuropharmacol.* 32 (2009) 89-93. <https://doi.org/10.1097/WNF.0B013E31816D91F9>.
- 34 D. Cannata, N.B. Narbone, Clinical Observations on the Role of the Vegetative Nervous System in the Pathogenesis of Atrial Fibrillation, *Cardiology.* 32 (1958) 329-345. <https://doi.org/10.1159/000165836>.
- 35 P. Coumel, Cardiac Arrhythmias and the Autonomic Nervous System, *J. Cardiovasc. Electrophysiol.* 4 (1993) 338-355. <https://doi.org/10.1111/J.1540-8167.1993.TB01235.X>.
- 36 M. Luinstra, A.W.F. Rutgers, H. Dijkstra, F. Grasmeyer, P. Hagedoorn, J.M.J. Vogelzang, H.W. Frijlink, A.H. De Boer, Can Patients with Parkinson's Disease Use Dry Powder Inhalers during Off Periods?, *PLoS One.* 10 (2015) e0132714. <https://doi.org/10.1371/JOURNAL.PONE.0132714>.
- 37 M. Luinstra, V. Isufi, L. de Jong, A.W.F. Rutgers, P. Hagedoorn, J. Puttenstein, T. van Laar, H.W. Frijlink, Learning from Parkinson's patients: Usability of the Cyclops dry powder inhaler, *Int. J. Pharm.* 567 (2019) 118493. <https://doi.org/10.1016/J.IJPHARM.2019.118493>.
- 38 K.A. Grosset, N. Malek, F. Morgan, D.G. Grosset, Inhaled Apomorphine in Patients with "on-off" Fluctuations: A Randomized, Double-blind, Placebo-controlled, Clinic and Home Based, Parallel-group Study, *J. Parkinsons. Dis.* 3 (2013) 31-37. <https://doi.org/10.3233/JPD-120142>.

CHAPTER 4

Clinical trial evaluating apomorphine oromucosal solution in Parkinson's disease patients

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ABSTRACT

Background Apomorphine is used to treat *OFF* episodes in patients with Parkinson's disease (PD). Unlike subcutaneous injections, administration of an oromucosal solution is a non-invasive, easy route of administration.

Objectives To assess the safety, tolerability, pharmacokinetics (PK), and dose proportionality of a novel apomorphine hydrochloride (HCl) oromucosal solution, as well as its relative bioavailability to subcutaneous apomorphine injection and apomorphine sublingual film.

Methods In part A of the study, 12 patients with PD received 2 mg oromucosal apomorphine (4% weight/volume) and 2 mg subcutaneous apomorphine in a randomized order, followed by 4 and 8 mg oromucosal apomorphine. In part B of the study, 13 patients with PD received 7 mg oromucosal apomorphine (7% weight/volume) and 30 mg sublingual apomorphine in a randomized order, followed by 14 mg oromucosal apomorphine. Washout between dose administrations in both study parts was at least 2 days. Safety, tolerability and PK were assessed pre- and post-dose.

Results Oromucosal apomorphine was generally well tolerated. Observed side effects were typical for apomorphine administration and included asymptomatic orthostatic hypotension, yawning, fatigue and somnolence. Oromucosal apomorphine exposure increased with dose, although less than dose proportional. The mean (SD) maximum exposure reached with 14 mg oromucosal apomorphine was 753.0 (298.6) ng·min/mL for AUC_{0-inf} and 8.0 (3.3) ng/mL for C_{max} . This was comparable to exposure reached after 2 mg subcutaneous apomorphine and approximately half of the exposure observed with 30 mg sublingual apomorphine.

Conclusions Apomorphine oromucosal solution was generally well tolerated and resulted in clinically relevant plasma concentrations in PD patients.

INTRODUCTION

Apomorphine is a non-ergoline dopamine agonist. Subcutaneous injections of apomorphine are indicated for the acute, intermittent treatment of "*OFF*" episodes in patients with Parkinson's disease (PD). In general, apomorphine treatment is initiated when patients suffer from *OFF* periods despite optimized oral/transdermal dopaminergic treatment. Notwithstanding its well-known safety and efficacy profile, apomorphine is currently underutilized.¹ This is likely because the available licensed products are mostly administered as subcutaneous injections (APOKYN® and APO-go®), presenting several impracticalities. These include the challenge of self-administering injections during an *OFF* state, along with the potential for needle phobia. Another delivery mode of apomorphine is the sublingual film (KYNMOBI®), which can cause oropharyngeal side effects and takes longer to reach maximum plasma concentrations than subcutaneous administration.² The administration of both formulations requires good finger dexterity and muscle coordination, which is often impaired in patients with PD.³

Over the years, other non-invasive apomorphine administration routes have been investigated, such as oral, transdermal, intranasal, and inhaled routes.⁴⁻⁸ Due to the extensive first-pass metabolism of apomorphine, oral administration results in too low bioavailability (< 4%) to allow for clinically relevant apomorphine exposures.⁹ Furthermore, due to the delayed absorption from the gastrointestinal tract, oral administration is not suited for use as rescue medication. Transdermal delivery has not been developed so far, although administration via an iontophoretic patch showed promising results and the use of lipophilic di-ester prodrugs may hold promise for the future.^{7,10} Intranasal administration, although efficacious, results in nasal irritation.⁶ Lastly, inhaled apomorphine looks promising with a rapid absorption and onset of effect.^{4,5,8} However, collection of long-term safety data of pulmonary exposure to apomorphine is essential to confirm suitability for long-term use.

To overcome the disadvantages associated with the available licensed and experimental apomorphine formulations, a highly concentrated apomorphine hydrochloride (HCl) oromucosal solution

(APORON) has been developed which is intended for self-administration by the patient, using an easy-to-operate device. This formulation is designed to enable an easy buccal delivery of efficacious doses of oromucosal apomorphine without local side effects. With the recent global discontinuation of the sublingual apomorphine film, the development of a novel non-invasive apomorphine administration has become even more relevant.

In this two-part crossover study, the safety, tolerability, pharmacokinetics (PK), and dose proportionality of the novel apomorphine HCl oromucosal solution was assessed, as well as its relative bioavailability compared to subcutaneous apomorphine injection and apomorphine sublingual film. In the first study part, three ascending doses of apomorphine oromucosal solution (4% weight/volume (w/v)) and a single dose of 2 mg subcutaneous apomorphine were evaluated. In the second study part, a higher percentage (7% w/v) apomorphine oromucosal solution was evaluated at two dose levels, and compared with apomorphine sublingual film.

METHODS

The study is registered in the Netherlands Trial Register (Trial NL9540), and was conducted at the Centre for Human Drug Research (Leiden, the Netherlands) between May and August 2021 (study part A), and between June and August 2022 (study part B). The study was conducted in accordance with Good Clinical Practice guidelines and approved by the Independent Ethics Committee of Foundation Beoordeling Ethiek Biomedisch Onderzoek. Prior to any study-related activity, all participants provided written informed consent.

Study design

This study consisted of two sub-studies, part A and part B. For a depiction of their study design, refer to Figure 1. Part A of the study was an open-label, two-way crossover study in 12 patients with Parkinson's disease to characterize and compare the PK of apomorphine after oromucosal and subcutaneous administration, and to assess the

dose proportionality of oromucosal apomorphine. Patients received 2 mg oromucosal and 2 mg subcutaneous apomorphine during two different visits in a randomized order, followed by 4 mg and lastly 8 mg oromucosal apomorphine (the highest dose (6 or 8 mg depending on emerging data) was chosen after review of PK and safety/tolerability data of at least 6 patients). The washout period between dose administrations was a minimum of 3 days and a maximum of 3 weeks. A sample size of 12 was chosen since this could confirm dose proportionality of oromucosal apomorphine with 80% power (one-sided $\alpha=0.05$), assuming an intra-individual CV of 20%.¹¹ A one-sided alpha was chosen since higher dose levels (i.e., volume) are expected to increase the chance of swallowing and thereby result in lower oromucosal absorption and exposure.

Safety, tolerability and PK data were examined during a dose level evaluation meeting before proceeding to study part B. Based on the oromucosal apomorphine exposure observed in part A, a percentage of 7% apomorphine w/v in the oromucosal solution was selected for use in part B.

Part B was an open-label comparative PK study evaluating the more concentrated oromucosal apomorphine solution and a sublingual apomorphine film in 12 patients with Parkinson's disease. Patients first received 7 mg oromucosal and 30 mg sublingual apomorphine in a randomized order, followed by 14 mg oromucosal apomorphine. There was a 2-day washout period between dose administrations. No formal sample size calculation was performed due to the exploratory nature of the study.

Participation in the trial consisted of a screening visit, pretreatment with 20 mg domperidone three times daily^{12,13} from 2 days prior to dosing until last dose (max. 9 consecutive days in part A, and max. 7 days in part B), followed by 4 visits (part A) or 3 visits (part B) of 1 day each to the clinical research unit, and a follow-up phone call.

Participants

In study parts A and B, PD patients aged 30-85 years with Hoehn and Yahr stage I-IV and with clear, self-described motor fluctuations as assessed by the 9-symptom Wearing-off Questionnaire,¹⁴ were eligi-

ble for participation. Patients were excluded if they had symptomatic clinically relevant and/or medically uncontrolled orthostatic hypotension, a history of long QT syndrome and/or a QTcF of >450 ms (male) or >470 ms (female), or aphthous ulcers or mouth sores within 6 months prior to the screening visit.

Investigational drugs

Apomorphine HCl oromucosal solutions (APORON[®], Supplemental Figure 1) containing 2.0 mg (4% weight/volume) or 3.5 mg (7% weight/volume) apomorphine HCl per 50 μ L-spray pump actuation, were administered to alternating buccal cheeks. Varying the number of spray device actuations allowed for the administration of doses ranging from 2 mg to 14 mg apomorphine HCl. APORON is a non-aqueous solution containing apomorphine HCl, one or more organic solvents of which at least 50% is propylene glycol, and one or more antioxidants (US patent 9,737,526 B2). The 4% solution was used in part A, and the 7% in part B of the study.

In part A, patients received 2, 4 and 8 mg oromucosal apomorphine HCl. Patients were instructed to swallow their saliva prior to the buccal administration of the oromucosal solution, and to not swallow or speak for 2 minutes after dosing. In part B, patients received 7 and 14 mg respectively. Patients were given the same instructions as in part A, with the exception that they should not swallow or speak for 3 minutes after dosing (increased to 3 minutes to be consistent with the instructions in the patient leaflet of apomorphine sublingual film). When 14 mg was administered, the first 7 mg was administered at $t=0$, and the second 7 mg at $t=4$ minutes.

In part A, 2 mg subcutaneous apomorphine HCl (APO-go[®] 10 mg/ml solution for injection) was injected in the abdomen as active comparator. In part B, 30 mg apomorphine HCl sublingual film (KYNMOBI[®]) was used as active comparator. When receiving sublingual film, patients were instructed to moisten their mouth just prior to administration and to not swallow or speak for 3 minutes after administration. If not fully dissolved after 3 minutes, patients were instructed to not swallow or speak for 1 additional minute.

In clinical practice, subcutaneous apomorphine is initiated at a dose of 2 mg. Hence, 2 mg was considered a safe dose for study part A.

As apomorphine oromucosal solution was expected to have lower bioavailability than subcutaneous apomorphine, 2 mg apomorphine oromucosal solution was considered a safe starting dose. Furthermore, in clinical practice, sublingual apomorphine is initiated at 10 mg and titrated to a dose that is both safe and efficacious (max. 30 mg). In study part B, a dose of 30 mg was considered to be safe, since it was expected to result in a similar or lower maximum plasma concentration (C_{max}) than 2 mg subcutaneous apomorphine in part A (which was well tolerated).^{2,15,16}

Safety

Patients were medically screened to confirm their eligibility. Screening included an assessment of the patient's medical history, concomitant medications, electrocardiogram (ECG), vital signs, routine laboratory assessments, and physical and neurological examination. QTcF was assessed at screening (prior to domperidone initiation) and again at baseline (after domperidone initiation and prior to apomorphine administration). During the study, safety was evaluated by monitoring of adverse events (AEs) (classified by Medical Dictionary for Regulatory Activities (MedDRA) version 25.0), vital signs, ECGs, physical and neurological examination, and clinical laboratory tests. The buccal mucosa was assessed by a physician pre-dose, 1 hour post-dose and prior to leaving the clinical research unit. For AE reporting, a decrease in systolic blood pressure (BP) of ≥ 20 mmHg or in diastolic BP of ≥ 10 mmHg upon standing accompanied by dizziness was documented as symptomatic orthostatic hypotension. A decrease in BP of ≥ 20 mmHg or in diastolic BP of ≥ 10 mmHg upon standing without dizziness was documented as asymptomatic orthostatic hypotension. If a patient reported dizziness upon standing without the abovementioned drop in BP, this was documented as postural dizziness.

Pharmacokinetics

In part A, whole blood was collected pre-dose and 4, 8, 12, 16, 20, 24, 28, 32, 40, 50, 60, 90, 120 and 240 min post-dose. In part B, collection took place pre-dose and 5, 10, 15, 20, 25, 35, 40, 45, 50, 55, 60, 75,

90, 120, 240 and 360 min post-dose. A longer sampling duration was used in part B to ensure that the apparent terminal half-life ($T_{1/2}$), and hence the exposure as area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\text{inf}}$), could be calculated for all patients since the $T_{1/2}$ of sublingual and oromucosal apomorphine was expected to be longer than for subcutaneous apomorphine.^{2,15} Ascorbic acid was added to the plasma samples prior to freezing to prevent apomorphine oxidation.

Apomorphine was extracted from plasma by Liquid Liquid Extraction, after which apomorphine was quantified using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Non-compartmental analysis was used to calculate apomorphine's C_{max} , time to C_{max} (T_{max}), $T_{1/2}$ and $AUC_{0-\text{inf}}$. Dose proportionality of oromucosal apomorphine over the dose range 2-8 mg in part A was tested by regression of $\text{Ln}(\text{dose})$ on $\text{Ln}(AUC_{0-\text{inf}})$ and $\text{Ln}(C_{\text{max}})$ with subject as random factor where the 90% confidence interval (CI) of the slope of the regression had to fall between $1 + (\text{Ln}(0.8)/\text{Ln}(\text{dose ratio})) < \text{slope} < 1 + (\text{Ln}(1.25)/\text{Ln}(\text{dose ratio}))$ to conclude for dose proportionality.¹⁷ In part B, dose proportionality between 7 and 14 mg oromucosal apomorphine was tested for dose-normalized $\text{Ln}(C_{\text{max}})$ and $\text{Ln}(AUC_{0-\text{inf}})$ with an ANOVA with treatment as fixed factor and subject as random factor. The 90% CI of the ratio of the geometric means between the two dose levels needed to be within 0.8 and 1.25 to conclude dose proportionality.¹⁷ Bioequivalence between sublingual and oromucosal apomorphine was tested for $\text{Ln}(C_{\text{max}})$ and $\text{Ln}(AUC_{0-\text{inf}})$ with a linear model analysis with treatment as fixed factor and subject as random factor. The 90% CI of the ratio between the two treatments needed to be within 0.8 and 1.25 to be bioequivalent. In this analysis, only patients receiving both treatments were included.

RESULTS

Demographics

Table 1 provides an overview of baseline patient characteristics, and Supplemental Figure 2 and 3 for CONSORT flow diagrams of both study parts. In part A, 12 PD patients were enrolled in the study,

of whom 10 patients completed the full study. Two patients were discontinued early, i.e., after the second and third dosing day, due to adverse events that were considered unrelated to apomorphine (refer to Supplemental Figure 2). In part B, 12 PD patients completed the full study, and 1 patient completed only the first study visit. After this visit, the patient withdrew consent due to increased PD symptoms and fatigue. In part A, the majority of participants (83%) had Hoehn and Yahr stage I, 8% had stage II and 8% had stage III. In part B, 15% had stage I, 54% stage II and 31% stage III. No other differences in demographic and baseline characteristics were noted between participants in part A and B.

Pharmacokinetics

In part A, a single dose of subcutaneous apomorphine and three ascending doses of 4% apomorphine oromucosal solution were compared (Figure 2A, Table 2). Subcutaneous apomorphine was more readily available systemically than oromucosal apomorphine; the median T_{max} (range) was 19 (8-40) and 32 (16-120) minutes respectively. The ratio of the geometric least squares mean (90% CI) of 2 mg oromucosal to 2 mg subcutaneous apomorphine was 0.28 (0.24-0.33) for $AUC_{0-\text{inf}}$ and 0.20 (0.15-0.26) for C_{max} . Over the dose range 2-8 mg, oromucosal apomorphine exposure increased less than dose-proportionally (Figure 2C-D), resulting in 8 mg oromucosal apomorphine having a lower exposure than 2 mg subcutaneous apomorphine.

In part B, a 7% apomorphine oromucosal solution was compared to sublingual apomorphine (Figure 2B, Table 2). These administrations showed a similar PK profile with a median T_{max} (range) of 45 (15-75) minutes for sublingual, and 45 (25-77) minutes for oromucosal apomorphine (7 mg). Moreover, oromucosal apomorphine had a similar dose-normalized exposure as sublingual apomorphine (Figure 2C-D). The ratio of the geometric least squares mean (90% CI) of 7 mg oromucosal apomorphine to 30 mg sublingual apomorphine was 0.30 (0.25-0.36) for $AUC_{0-\text{inf}}$ and 0.28 (0.23-0.34) for C_{max} . The ratio of the geometric least squares mean (90% CI) of 14 mg oromucosal apomorphine to 30 mg sublingual apomorphine was 0.51 (0.45-0.57) for $AUC_{0-\text{inf}}$ and 0.52 (0.45-0.61)

for C_{max} . Oromucosal apomorphine exposure increased less than dose-proportionally from 7 to 14 mg for C_{max} and AUC_{0-inf} , while C_{max} showed a trend towards dose proportionality with a 90% CI of the geometric mean ratio of both dose levels of 0.77-1.12.

Safety and local tolerability

Oromucosal apomorphine administration was generally well tolerated with mostly mild and transient AEs (Table 3). The type of AEs that were reported were comparable with subcutaneous and sublingual apomorphine, and are typically associated with apomorphine administration. Asymptomatic orthostatic hypotension, yawning and fatigue and/or somnolence were most frequently reported. There was no clear dose-dependent or exposure-dependent increase in the incidence and severity of AEs. No oropharyngeal AEs were reported and no abnormalities of the buccal and sublingual mucosa were observed after the administration of oromucosal apomorphine for up to 3 treatment days and a single dose of 30 mg sublingual apomorphine.

Seven AEs that were moderate in severity were reported. In two subjects the AEs were considered possibly or probably related to apomorphine administration. One patient reported somnolence moderate in severity approximately 30 minutes after sublingual apomorphine administration that resolved spontaneously within 1.5 hours. Another patient reported postural dizziness and vomited repeatedly, starting 1.5 hours after 14 mg oromucosal apomorphine administration. In addition, the patient's 6 hours post-dose lab showed thrombocytopenia that was moderate in severity (pre-dose 219; 6 hours post-dose $136 \cdot 10^9/L$). Upon retest approximately 1 week later, the thrombocyte count had returned to normal. The patient had tolerated both 30 mg sublingual and 7 mg oromucosal apomorphine well except for some mild asymptomatic orthostatic hypotension and somnolence.

Almost all patients reported that the oromucosal solution had a bitter taste. Two patients also reported a slightly sweet taste on one occasion, and 4 patients reported a (bitter/sour) taste. Taste did not affect the patients' ability to follow the dosing instructions.

DISCUSSION

For over two decades, subcutaneous apomorphine injections and infusions have been used as an efficacious treatment for managing *OFF* episodes.^{18,19} However, intermittent subcutaneous administration is still a suboptimal delivery route, especially since it requires a good dexterity, which is typically impaired during an *OFF* episode. Moreover, the need to self-administer injections in areas like the arms, legs and abdominal wall, all typically covered by clothing, makes the use of injections less practical in public settings. The buccal administration of the oromucosal apomorphine solution is expected to solve most of these impractical issues of apomorphine delivery.

After the buccal administration of oromucosal apomorphine, maximum plasma concentrations were observed between 32 and 53 minutes (median T_{max} over dose groups). The median T_{max} in study part A (32 minutes) was lower than in study part B (45 minutes (7 mg) and 53 minutes (14 mg)). This is likely a chance finding due to variability in PK, i.e. T_{max} in part A ranged from 16 to 120 minutes, and in part B from 25 to 82 minutes. The somewhat later T_{max} following 14 mg compared to 7 mg oromucosal apomorphine can be partly ascribed by the 14 mg dose being administered as 2x 7 mg at $t=0$ and $t=4$ minutes. T_{max} is calculated counting from the first dose administration. Sublingual apomorphine had a median T_{max} of 45 minutes, which was comparable to the T_{max} of oromucosal apomorphine. As expected, subcutaneous apomorphine was more readily absorbed with a median T_{max} of 19 minutes and had a higher bioavailability compared to oromucosal and sublingual apomorphine.^{15,16} Moreover, as has also been described for sublingual apomorphine, oromucosal apomorphine exposure increased less than dose-proportionally.¹⁶ This might be attributed to the fact that higher doses are administered as larger volumes. These volumes are closer to the volume that triggers a swallowing reflex,²⁰⁻²² and hence more of the drug might be swallowed prematurely at the higher oromucosal doses. Since apomorphine has a low oral bioavailability, swallowing may have significantly contributed to the lower bioavailability at higher doses.

Based on the observed bioavailability of oromucosal apomorphine in part A of the study, the formulation in part B was changed by increasing the apomorphine concentration from 4 to 7%. Moreover, patients in part B who received 4 spray pump actuations were given these as 2 actuations at $t=0$ and 2 actuations at $t=4$ minutes, as opposed to 4 consecutive spray pump actuations in part A. Figure 2 shows that the increased apomorphine concentration led to an increased apomorphine exposure in part B. Four spray pump actuations with the 7% oromucosal apomorphine formulation (14 mg) resulted in a comparable exposure as 2 mg subcutaneous apomorphine, and about half of the exposure to 30 mg sublingual apomorphine. The exposure to 30 mg sublingual apomorphine observed in this study was higher than reported in the literature.^{15,16} However, the sample size of our study was relatively small and apomorphine exposure is known to be highly variable between patients receiving identical doses. Furthermore, sublingual apomorphine in our study was administered to a non-titrated patient population, whereas the literature reports patients being titrated to an effective and tolerable dose.¹⁶ Therefore, titration can lead to enrichment of patients with lower PK exposure in the higher dose groups. This hypothesis is supported by a different dose- C_{max} relationship reported by Agbo et al. in titrated PD patients with *OFF* episodes versus untitrated healthy volunteers.¹⁶ The titrated patient population had a lower regression coefficient (i.e., less steep dose- C_{max} relationship), and their exposure tended to plateau at higher doses. In our study, the C_{max} values of 30 mg sublingual apomorphine administered to untitrated patients followed the dose- C_{max} relationship described for untitrated healthy volunteers receiving 10 to 25 mg sublingual apomorphine.¹⁶ This implies that PK exposure in healthy volunteers and untitrated PD patients is comparable, and differences reported between healthy volunteers and PD patients are likely the result of dose titration, but not pathophysiological differences.

The apomorphine concentration at which a patient shows clinical improvement is also subject to high inter-patient variability. However, a previous study in a small group of PD patients has reported a mean minimal effective concentration (MMEC) of 4.7 ng/mL.²³ Using this cut-off, 11 out of 12 PD patients treated with 14 mg oromucosal

apomorphine reached plasma concentrations exceeding this MMEC. Moreover, 8 out of 12 patients remained above this MMEC for ≥ 40 minutes. Therefore, this study has provided clinically relevant plasma concentrations after the administration of 14 mg oromucosal apomorphine. The time to reach the MMEC varied between patients. For subcutaneous apomorphine it ranged between 8-20 minutes and for oromucosal apomorphine between 14-59 minutes. This indicates that not all patients using subcutaneous apomorphine will show a similar onset of efficacy with oromucosal apomorphine. The PK exposure of oromucosal apomorphine is comparable to the exposure of efficacious sublingual apomorphine doses as reported in literature.²⁴ Consequently, it is expected that the onset of efficacy of oromucosal apomorphine will be comparable to that of sublingual apomorphine in most patients.

In this two-part study, PD patients received oromucosal apomorphine solution in ascending doses up to 14 mg, and its safety was compared with subcutaneous and sublingual apomorphine. Oromucosal apomorphine up to 14 mg was generally well-tolerated with AEs comparable to those observed after single doses of apomorphine sublingual film and subcutaneous injection. All AEs that were considered at least possibly related to oromucosal apomorphine were mild in severity, with the exception of the observation of thrombocytopenia, postural dizziness, and vomiting which was reported by one patient in the 14 mg group. Nausea, vomiting and (postural) dizziness are known side effects of apomorphine.¹⁸ For this reason, all patients were instructed to take an anti-emetic three-times daily from two days prior to dosing.

Apomorphine undergoes autooxidation in aqueous environments at neutral pH such as saliva.²⁵ This autooxidation process results in the formation of quinone derivatives and reactive oxygen species which have been associated with cytotoxicity.^{26,27} Therefore, apomorphine that remains in the oropharyngeal space long enough to undergo autooxidation in the saliva, has the potential to induce oropharyngeal irritation via the formed apomorphine quinones. For apomorphine sublingual film, it is known that oropharyngeal AEs occur after repeated exposure. A phase 3 study with apomorphine sublingual film reported oropharyngeal AEs as the most common

AEs with an incidence of 31% for sublingual apomorphine compared to 7% in the placebo group. These oropharyngeal AEs led to treatment discontinuation in the 12-week maintenance phase in 17% of the patients in the sublingual apomorphine group compared to 2% in the placebo group.²⁸ The side effects of sublingual apomorphine are likely related to apomorphine particles in the dual layer film that can remain in the vallecula where they degrade into reactive oxygen species in the presence of saliva. For oromucosal apomorphine, it is hypothesized that degradation into reactive oxygen species in the oropharyngeal space is limited since the apomorphine is administered as a dissolved solution and will be swallowed together with the saliva thereby preventing prolonged presence in the oropharyngeal space. The current study did not show any oropharyngeal AEs, including buccal/sublingual mucosa abnormalities for both oromucosal apomorphine solutions (up to 14 mg/day) during up to three treatment days, nor for a single dose of 30 mg sublingual apomorphine. Further verification is needed to confirm the local tolerability of oromucosal apomorphine HCl during longer exposures.

In summary, the buccal administration of the novel oromucosal apomorphine solution evaluated in this two-part clinical study was generally well tolerated and resulted in clinically relevant plasma concentrations in PD patients. It is expected to offer a promising new administration route for the delivery of apomorphine. Due to the use of dissolved apomorphine, it is hypothesized to result in fewer oropharyngeal side effects than sublingual apomorphine. Moreover, oromucosal apomorphine solution administration will be an easier and more user-friendly way to administer apomorphine than the recently discontinued sublingual film and currently available subcutaneous injections.

TABLE 1 Demographics of participants with Parkinson's disease in study parts A and B.

Demographic variables	PART A (N=12)	PART B (N=13) ^a
Age (years)		
Median (range)	66 (48-79)	67 (55-79)
BMI (kg/m²)		
Median (range)	26 (21-30)	26 (19-32)
Sex (n/n (%/%)		
Female/Male	5/7 (41.7/58.3)	4/9 (30.8/69.2)
Race (n (%))		
White	12 (100)	10 (76.9)
Asian	0 (0)	1 (7.7)
Mixed	0 (0)	2 (15.4) ^b
MMSE		
Median (range)	30 (27-30)	29 (25-30)
Hoehn and Yahr stage		
Stage 1	10 (83.3)	2 (15.4)
Stage 2	1 (8.3)	7 (53.8)
Stage 3	1 (8.3)	4 (30.8)
Stage 4	0 (0)	0 (0)
Concomitant PD medication (n (%))		
Levodopa-containing agents	12 (100.0)	13 (100.0)
Dopamine agonists	6 (50.0)	10 (76.9)
COMT inhibitors	3 (25.0)	1 (7.7)
MAO-B inhibitors	1 (8.3)	1 (7.7)
Amantadine	2 (16.7)	4 (30.8)
Other	2 (16.7) ^c	0 (0)

a. One drop-out after the first dosing (30 mg sublingual apomorphine); refer to Supplemental Figure 3 for a CONSORT flow diagram. / b. Mixed, i.e., White/Asian and White/African. / c. Trihexyfenidyl and glycopyrronium, both N=1.

BMI, body mass index; MMSE, Mini Mental State Examination; PD, Parkinson's disease, COMT, catechol-O-methyltransferase; MAO-B, monoamine oxidase B.

TABLE 2 Pharmacokinetic parameters of apomorphine after 2 mg subcutaneous and 2, 4 and 8 mg oromucosal apomorphine administration (part A), and 30 mg sublingual and 7 and 14 mg oromucosal apomorphine administration (part B) to Parkinson's disease patients.

	PART A				PART B		
	2 mg sc (N=12)	2 mg om (N=12)	4 mg om (N=11)	8 mg om (N=10)	30 mg sl (N=13)	7 mg om (N=12)	14 mg om ^a (N=12)
T_{max} (min)							
Median (range)	19 (8-40)	32 (16-60)	32 (24-90)	32 (20-120)	45 (15-75)	45 (25-77)	53 (29-82)
C_{max} (ng/mL)							
Mean (sd)	10.5 (6.5)	2.0 (1.2)	3.3 (0.9)	4.3 (1.8)	15.5 (5.7)	4.5 (2.4)	8.0 (3.3)
Median (range)	9 (3-24)	2 (1-5)	3 (2-5)	4 (1-7)	15 (9-25)	5 (2-10)	7 (3-14)
Geometric LSM ratio om/sc (part A) or om/sl (part B) (90% ci)		0.20 (0.15-0.26)	0.39 (0.30-0.49)	0.53 (0.42-0.66)		0.28 (0.23-0.34)	0.52 (0.45-0.61)
AUC_{0-inf} (min-ng/mL)							
Mean (sd)	617.8 (182.6)	178.3 (75.5)	303.4 (51.2) ^b	431.6 (116.5) ^b	1524.1 (533.2)	454.4 (174.0)	753.0 (298.6)
Median (range)	572 (296-892)	132 (116-316)	296 (225-390) ^b	384 (294-633) ^b	1546 (773-2573)	497 (177-703)	780 (302-1320)
Geometric LSM ratio om/sc (part A) or om/sl (part B) (90% ci)		0.28 (0.24-0.33)	0.53 (0.44-0.63)	0.75 (0.59-0.94)		0.30 (0.25-0.36)	0.51 (0.45-0.57)
$T_{1/2}$ (min)							
Mean (sd)	48 (7)	44 (6)	45 (4) ^b	47 (6) ^b	54 (8)	51 (7)	54 (7)
Median (range)	46 (39-60)	43 (38-57)	44 (39-54) ^b	48 (37-55) ^b	54 (45-67)	50 (43-63)	51 (45-65)

a. Administered as 2 spray pump actuations at t=0 and another 2 spray pump actuations at t=4 minutes. Calculations are done from t=0. / b. N=9 due to inability to calculate $T_{1/2}$ because of insufficient span ratio (i.e., time interval over which $T_{1/2}$ can be determined) (N=2), and due to one early discontinuation during the visit (N=1)

T_{max} , time to maximum plasma concentration; C_{max} , maximum plasma concentration; sd, standard deviation; LSM, Least Squares Mean; om, oromucosal; sc, subcutaneous; sl, sublingual; ci, confidence interval; AUC_{0-inf} , area under the plasma concentration-time curve from zero to infinity; $T_{1/2}$, apparent terminal elimination half-life.

TABLE 3 Summary of the number of AEs and the number and percentage of participants (n (%)) with any, mild, moderate and severe AE and with a specific AE as indicated per treatment group and study part.

	PART A				PART B		
	2 mg sc (N=12)	2 mg om (N=12)	4 mg om (N=11)	8 mg om (N=10)	30 mg sl (N=13)	7 mg om (N=12)	14 mg om (N=12)
	n (%)	n (%)	n (%)				
#AEs ^a	19	12	14	14	28	27	24
Any AEs	12 (100.0)	8 (66.7)	10 (90.9)	9 (90.0)	11 (84.6)	9 (75.0)	8 (66.7)
Mild AEs	11 (91.7)	8 (66.7)	9 (81.8)	9 (90.0)	11 (84.6)	9 (75.0)	8 (66.7)
Moderate AEs	1 (8.3)	-	1 (9.1)	-	4 (30.8)	-	1 (8.3)
Severe AEs	-	-	-	-	-	-	-
Most common AEs ^b							
Nausea	-	-	-	-	1 (7.7)	1 (8.3)	2 (16.7)
Fatigue	3 (25.0)	1 (8.3)	3 (27.3)	2 (20.0)	4 (30.8)	1 (8.3)	1 (8.3)
Headache	1 (8.3)	1 (8.3)	1 (9.1)	-	2 (15.4)	3 (25.0)	1 (8.3)
Orthostatic hypotension							
· Asymptomatic	7 (58.3)	5 (41.7)	3 (27.3)	3 (30.0)	3 (23.1)	6 (50.0)	3 (25.0)
· Symptomatic	-	1 (8.3)	-	-	-	-	-
Dizziness postural ^c	1 (8.3)	-	-	-	1 (7.7)	1 (8.3)	2 (16.7)
Increased Parkinson's disease symptoms	1 (8.3)	-	-	-	2 (15.4)	1 (8.3)	-
Dyskinesia	-	-	-	-	1 (7.7)	2 (16.7)	-
Somnolence	-	-	-	-	3 (23.1)	4 (33.3)	3 (25.0)
Yawning	4 (33.3)	2 (16.7)	5 (45.5)	8 (80.0)	4 (30.8)	3 (25.0)	3 (25.0)

a. Not expressed as n (%). This parameter describes the total number of AEs reported, and hence is unitless. /

b. AEs reported by ≥ 3 participants in part A or B. / c. Dizziness upon standing but no significant blood pressure drop measured at scheduled standing blood pressure measurement.

AE, adverse event; sc, subcutaneous apomorphine; om, oromucosal apomorphine; sl, sublingual apomorphine.

FIGURE 1 Overview of study designs of part A and B.

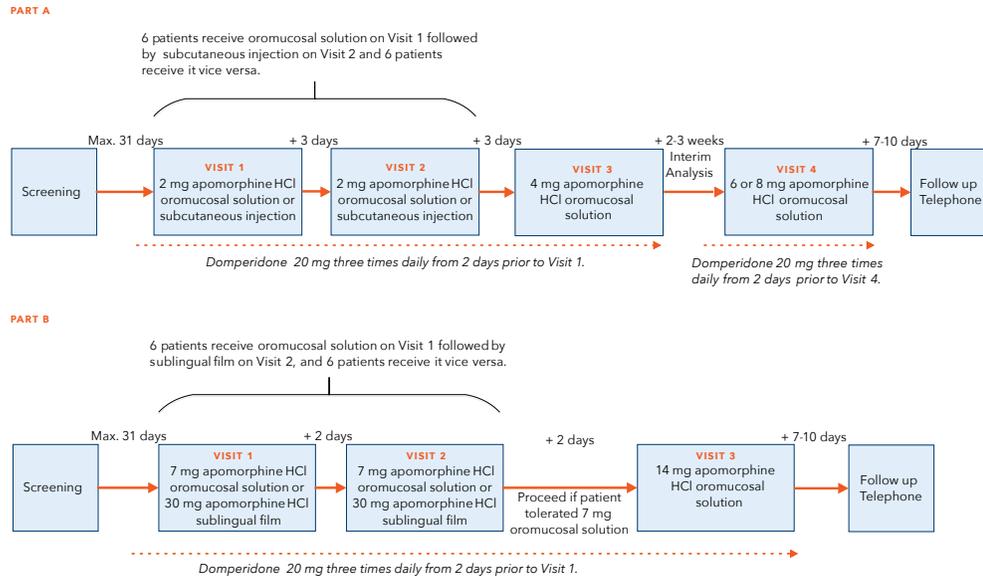
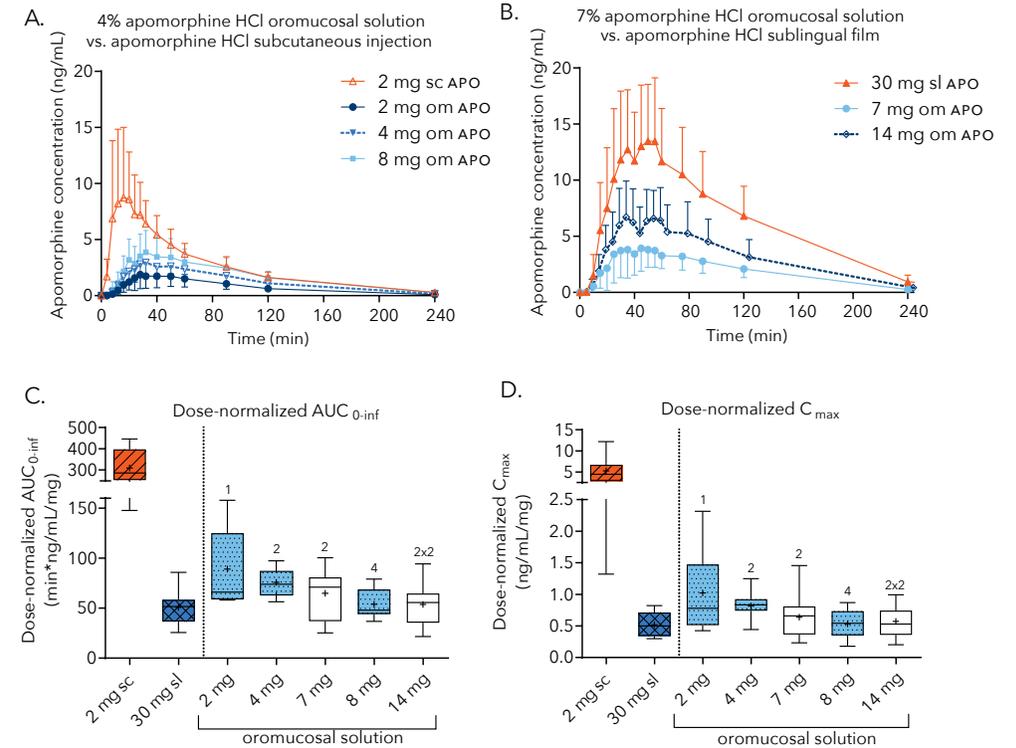


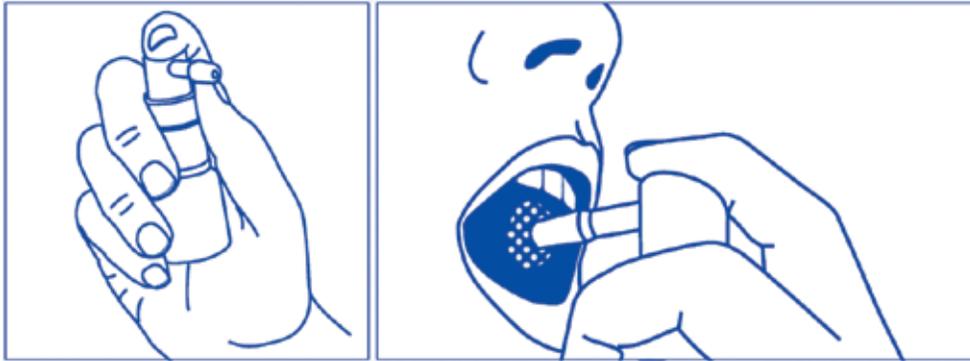
FIGURE 2 Mean (standard deviation) apomorphine concentration time profiles of 2 mg subcutaneous and 2-8 mg oromucosal apomorphine (A), and 30 mg sublingual and 7-14 mg oromucosal apomorphine (B). Dose-normalized AUC_{0-inf} and C_{max} (C-D); number of spray pump actuations indicated above the whiskers.



sc, subcutaneous; om, oromucosal; sl, sublingual.

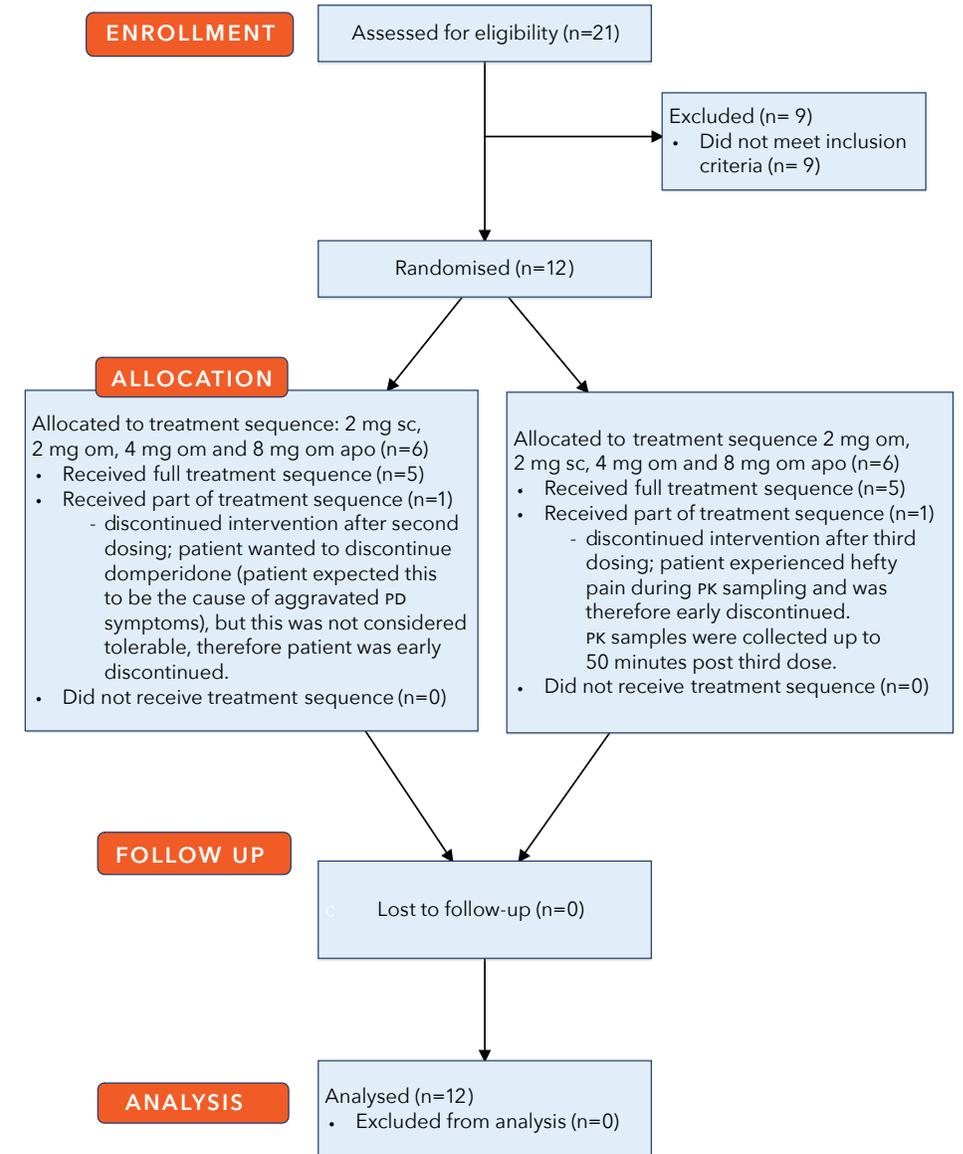
SUPPLEMENTARY MATERIAL

SUPPLEMENTAL FIGURE 1 Buccal administration of apomorphine hydrochloride oromucosal solution, to be administered to alternating cheeks.



SUPPLEMENTAL FIGURE 2 CONSORT flow diagram study part A.

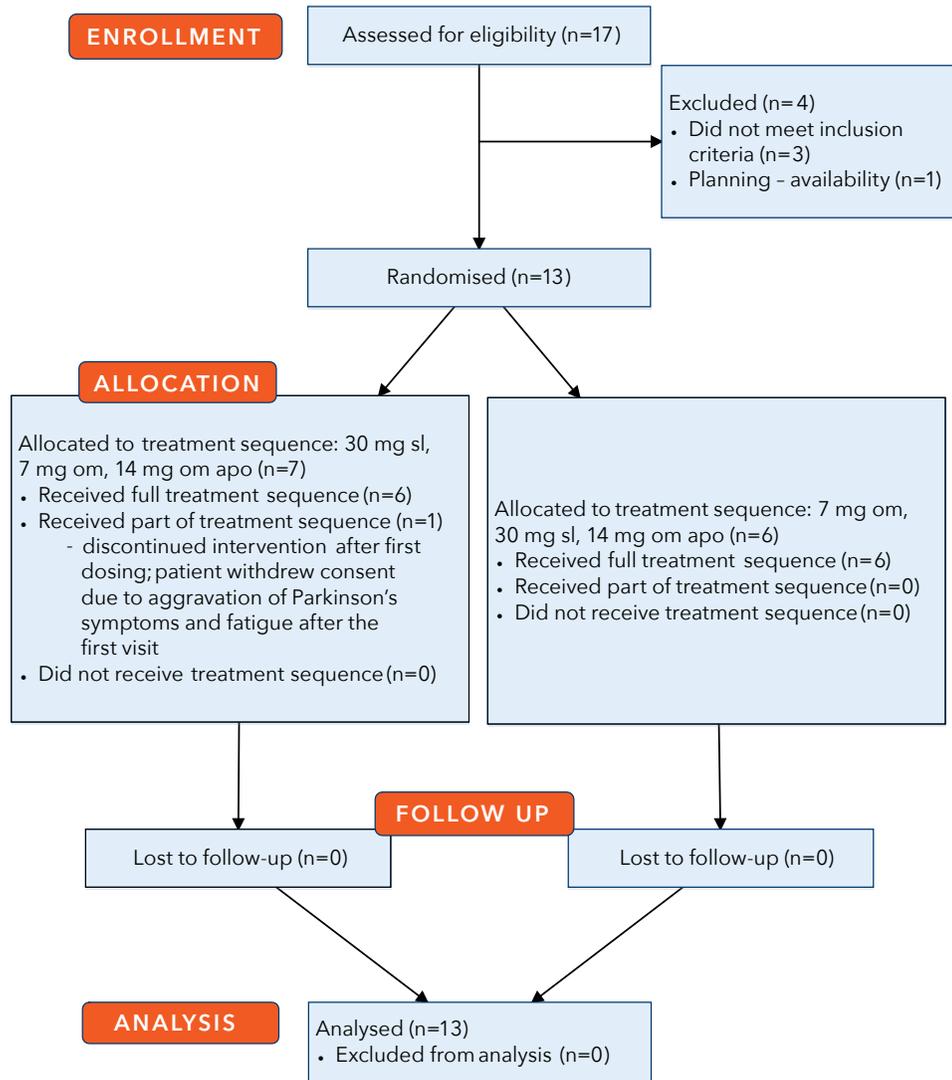
STUDY PART A
APOMORPHINE HCL OROMUCOSAL SOLUTION (4%) AND SUBCUTANEOUS INJECTION



CONSORT, Consolidated Standards of Reporting Trials; sc, subcutaneous; om, oromucosal; PD, Parkinson's disease; PK, pharmacokinetics.

SUPPLEMENTAL FIGURE 3 CONSORT flow diagram study part B.

**STUDY PART B
APOMORPHINE HCL OROMUCOSAL SOLUTION (7%) AND SUBLINGUAL FILM**



CONSORT, Consolidated Standards of Reporting Trials; sl, sublingual; om, oromucosal; PD, Parkinson's disease; PK, pharmacokinetics.

REFERENCES

- Auffret M, Drapier S, Vérin M. The Many Faces of Apomorphine: Lessons from the Past and Challenges for the Future. *Drugs in R&D* 2018 18:2. 2018;18(2):91-107. doi:10.1007/S40268-018-0230-3.
- Chen YL, Shi L, Agbo F, Yong SH, Tan PS, Ngounou Wetie AG. LC-MS/MS simultaneous quantification of apomorphine and its major metabolites in human plasma: Application to clinical comparative bioavailability evaluation for the apomorphine sublingual film and a subcutaneous product. *J Pharm Biomed Anal*. 2020;190:113493. doi:10.1016/J.JPBA.2020.113493.
- Chahine LM, Edison B, Daeschler M, et al. The Most Bothersome Aspects of Off Periods Reported by Individuals with Parkinson's Disease. *Mov Disord Clin Pract*. 2020;7(3):284-292. doi:10.1002/MDC3.12915.
- Grosset KA, Malek N, Morgan F, Grosset DG. Phase IIa randomized double-blind, placebo-controlled study of inhaled apomorphine as acute challenge for rescuing 'off' periods in patients with established Parkinson's disease. *Eur J Neurol*. 2013;20(11):1445-1450. doi:10.1111/ENE.12091.
- Grosset KA, Malek N, Morgan F, Grosset DG. Inhaled dry powder apomorphine (VRO40) for 'off' periods in Parkinson's disease: an in-clinic double-blind dose ranging study. *Acta Neurol Scand*. 2013;128(3):166-171. doi:10.1111/ANE.12107.
- Dewey RB, Maraganore DM, Ahlskog JE, Matsumoto JY. A double-blind, placebo-controlled study of intranasal apomorphine spray as a rescue agent for off-states in Parkinson's disease. *Movement Disorders*. 1998;13(5):782-787. doi:10.1002/MDS.870130505.
- Li GL, De Vries JJ, Van Steeg TJ, et al. Transdermal iontophoretic delivery of apomorphine in patients improved by surfactant formulation pretreatment. *Journal of Controlled Release*. 2005;101(1-3):199-208. doi:10.1016/J.JCONREL.2004.09.011.
- Thijssen E, den Heijer J, Puibert D, et al. A Randomized Trial Assessing the Safety, Pharmacokinetics, and Efficacy During Morning Off of APO-go Pen 10mg/ml Solution for Injection - Summary of Product Characteristics (SmPC) - (emc). *Movement Disorders*. Published online 2022. doi:10.1002/MDS.28926.
- Gancher ST, Nutt JG, Woodward WR. Absorption of apomorphine by various routes in parkinsonism. *Movement Disorders*. 1991;6(3):212-216. doi:10.1002/MDS.870060304.
- Liu KS, Sung KC, Al-Suwayeh SA, et al. Enhancement of transdermal apomorphine delivery with a diester prodrug strategy. *European Journal of Pharmaceutics and Biopharmaceutics*. 2011;78(3):422-431. doi:10.1016/J.EJPB.2011.01.024.
- Gancher ST, Woodward WR, Boucher B, Nutt JG. Peripheral pharmacokinetics of apomorphine in humans. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*. 1989;26(2):232-238.
- Bhidayasiri R, Garcia Ruiz PJ, Henriksen T. Practical management of adverse events related to apomorphine therapy. *Parkinsonism Relat Disord*. 2016;33:S42-S48. doi:10.1016/j.parkrel.2016.11.017.
- Albanese A, Bonuccelli U, Brefel C, et al. Consensus statement on the role of acute dopaminergic challenge in Parkinson's disease. *Movement Disorders*. 2001;16(2):197-201. doi:10.1002/MDS.1069.
- Stacy MA, Murphy JM, Greeley DR, Stewart RM, Murck H, Meng X. The sensitivity and specificity of the 9-item Wearing-off Questionnaire. *Parkinsonism Relat Disord*. 2008;14(3):205-212. doi:10.1016/J.PARKRELDIS.2007.07.013.
- Agbo F, Isaacson SH, Gil R, et al. Pharmacokinetics and Comparative Bioavailability of Apomorphine Sublingual Film and Subcutaneous Apomorphine Formulations in Patients with Parkinson's Disease and "OFF" Episodes: Results of a Randomized, Three-Way Crossover, Open-Label Study. *Neurol Ther*. 2021;10(2):693-709. doi:10.1007/S40120-021-00251-6.
- Agbo F, Crass RL, Chiu YY, et al. Population pharmacokinetic analysis of apomorphine sublingual film or subcutaneous apomorphine in healthy subjects and patients with Parkinson's disease. *Clin Transl Sci*. 2021;14(4):1464-1475. doi:10.1111/CTS.13008.
- Smith BP, Vandenhende FR, Desante KA, et al. Confidence Interval Criteria for Assessment of Dose Proportionality. Published online 2000.
- APO-go Pen 10mg/ml Solution for Injection - Summary of Product Characteristics (SmPC) - (emc). Accessed November 8, 2022. <https://www.medicines.org.uk/emc/product/2232/smpc>.

- 19 Apomorphine-Subcutaneous – Bertek/Britannia. *Drugs in R & D* 2004;5(4):211-212. doi:10.2165/00126839-200405040-00004.
- 20 Rudney JD, Ji Z, Larson CJ. The prediction of saliva swallowing frequency in humans from estimates of salivary flow rate and the volume of saliva swallowed. *Arch Oral Biol.* 1995;40(6):507-512. doi:10.1016/0003-9969(95)00004-9.
- 21 Steele CM, Miller AJ. Sensory Input Pathways and Mechanisms in Swallowing: A Review. *Dysphagia.* 2010;25(4):323. doi:10.1007/S00455-010-9301-5.
- 22 Lagerluf F, Dawes C. The Volume of Saliva in the Mouth Before and After Swallowing. <http://dx.doi.org/10.1177/00220345840630050201>. 2016;63(5):618-621. doi:10.1177/00220345840630050201.
- 23 Van Laar T, Van der Geest R, Danhof M, Boddé HE, Goossens PH, Roos RAC. Stepwise intravenous infusion of apomorphine to determine the therapeutic window in patients with Parkinson's disease. *Clin Neuropharmacol.* 1998;21(3):152-158. Accessed November 16, 2022. <https://pubmed.ncbi.nlm.nih.gov/9617506/>.
- 24 Stocchi F, Peckham EL, De Pandis MF, et al. A Randomized Thorough QT Study of Apomorphine Sublingual Film in Patients With Parkinson's Disease. *Clin Pharmacol Drug Dev.* 2022;11(9):1068-1077. doi:10.1002/CPDD.1147.
- 25 Burkman AM. Some Kinetic and Thermodynamic Characteristics of Apomorphine Degradation. *J Pharm Sci.* 1965;54(2):325-326. doi:10.1002/JPS.2600540242.
- 26 Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinones in toxicology. *Chem Res Toxicol.* 2000;13(3):135-160. doi:10.1021/TX9902082.
- 27 Dos Santos El-Bachá R, Daval JL, Koziel V, Netter P, Minn A. Toxic effects of apomorphine on rat cultured neurons and glial C6 cells, and protection with antioxidants. *Biochem Pharmacol.* 2001;61(1):73-85. doi:10.1016/S0006-2952(00)00524-4.
- 28 Olanow CW, Factor SA, Espay AJ, et al. Apomorphine sublingual film for off episodes in Parkinson's disease: a randomised, double-blind, placebo-controlled phase 3 study. *Lancet Neurol.* 2020;19(2):135-144. doi:10.1016/S1474-4422(19)30396-5.

CHAPTER 5

Touchscreen-based finger tapping: repeatability and configuration effects on tapping performance

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ABSTRACT

Background Parkinson's disease (PD) is a progressive neurodegenerative disease that affects almost 2% of the population above the age of 65. To better quantify the effects of new medications, fast and objective methods are needed. Touchscreen-based tapping tasks are simple yet effective tools for quantifying drug effects on PD-related motor symptoms, especially bradykinesia. However, there is no consensus on the optimal task set-up.

Methods The present study compares four tapping tasks in 14 healthy participants. In the alternate index and middle finger tapping task (IMFT), tapping occurred with the index and middle finger with 2.5 cm between targets. In the alternate index finger tapping task (IFT), tapping occurred with the index finger with 20 cm between targets. Both configurations were tested with or without the presence of a visual cue. Moreover, for each tapping task, within- and between-day repeatability and (potential) sensitivity of the calculated parameters were assessed.

Results Visual cueing reduced tapping speed, impaired rhythm, and improved accuracy. This effect was most pronounced for IFT. On average, IFT had a lower tapping speed with impaired accuracy and improved rhythm compared to IMFT. Of all parameters, the total number of taps and mean spatial error had the highest repeatability and sensitivity.

Conclusions The findings suggest against the use of visual cueing because it is crucial that parameters can vary freely to accurately capture medication effects. The choice for IMFT or IFT depends on the research question, as these tasks assess different aspects of movement. These results encourage further validation of non-cued IMFT and IFT in PD patients.

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease that affects roughly 1 to 2% of the population above the age of 65.^{1,2} The standard treatments remain symptomatic and novel treatments are continuously being investigated.^{3,4} One of the cardinal motor symptoms of PD is bradykinesia, defined as 'slowness of voluntary movement initiation, progressive reduction of speed and amplitude of repetitive movement, and difficulty of task switching'.⁴ Additional motor symptoms include tremor, muscular rigidity, and postural instability.⁴

To assess the effectiveness of new (dopaminergic) medications, the Movement Disorder Society revised - Unified Parkinson's Disease Rating Scale (MDS-UPDRS) serves as the 'gold standard' measurement.⁵ This scale provides a wide range of assessments related to both motor and non-motor symptoms. Part III of the scale assesses motor symptoms, and its administration lasts approximately 15 minutes. However, the clinical rating scale is subject to varying inter-rater reliability, requires training and certification of the assessor, and is time-consuming for both the clinician and patient.⁶⁻⁹ This may hamper the continuous assessment of (motor) symptoms, especially of rapid-acting agents. For instance, it will be difficult to accurately model the pharmacokinetic-pharmacodynamic relationship of a medication with an early T_{max} (e.g., of less than 15-30 minutes) when using the time-consuming MDS-UPDRS part III as a pharmacodynamic measure. Hence, there is a need for short, reliable, and objective motor symptom quantification methods that are easy to implement in clinical research.

The number and variety of technologies aimed at quantifying PD motor symptoms has increased over the last decade.^{8,10} Many focus on finger tapping motions to quantify aspects of tremor, dyskinesia, and bradykinesia.⁸ When quantifying bradykinesia, examples of technologies used vary from more rudimentary to increasingly sophisticated methods. For instance, arcade buttons,¹¹ midi-keyboards,¹² Inertial Measurement Units,¹³⁻¹⁹ and touchscreen devices²⁰⁻²⁸ have all been used in previous studies. Touchscreen-based

tapping tasks have been shown to not only differentiate reliably between PD patients and healthy controls^{12,20,29,21-28} but also to detect medication effects.^{14,20,23,29,30} Despite their potential in clinical research, there is no one standardized touchscreen based tapping task and seemingly minor configuration differences can affect the interpretation of study results.³¹

Two variations of the touchscreen based finger tapping tasks are commonly described in literature: alternate tapping with the index and middle finger of one hand between two closely placed targets (index and middle finger tapping (IMFT)),^{22,25,32} and alternate tapping with the index finger between two targets placed on opposite ends of the screen (alternate index finger tapping (IFT)).^{12,20} Each task assesses a different aspect of movement: whereas IMFT requires fine finger movement, IFT requires upper arm movement. Although studies report whether the IFT and/or IMFT was used, it is often unclear what the precise implementation of the tasks were (Table 1 for a brief overview of studies that used a finger tapping task). Varying target distances have been used both in IMFT and IFT. The inter target distance in IFT studies varies between 1.5 cm to 25 cm. In studies using the IMFT, most set-ups seem to place the targets under the natural position of the fingertips, yet, the precise inter-target distance is not always reported. Furthermore, both visually cued (e.g., by changing target colors)²⁵ and non-cued (e.g., on a keyboard),²⁰ versions of the test have been described. The distinction can be important as it has been shown that aiding PD patients with sensory cues can improve performance in finger tapping rhythm³³ and gait.³⁴ Most importantly, however, most studies do not report all design choices, often omitting details about the inter-target distance, the presence or absence of a cue, or the task duration.

To the best of our knowledge, no study has assessed the effects of cueing and task configuration in a comparative manner in healthy participants. The present study aims to compare four tapping tasks (cued/ non-cued IMFT and IFT) in healthy participants to identify the optimal design choices to be further validated in PD patients. First, the within- and between-day repeatability and (potential) sensitivity of the parameters are evaluated. Subsequently, the effect of the different configurations and cueing on tapping parameters are assessed.

METHODS

Participants

No formal sample size calculations were performed since this was an exploratory, technical validation study. A total of 16 healthy participants were planned for enrolment. The number of participants was chosen to be of similar size as an early phase clinical trial and to achieve a balanced design. Inclusion criteria were self-reported normal or corrected vision and no self-reported significant health problems. Exclusion criteria included the presence of self-reported physical hand/arm impairment, any movement disorder (e.g., PD, essential tremor, dystonia, akinesia) and/or any other neurological condition. Participants were instructed to abstain from caffeine, smoking, and intensive physical exercise starting 12 hours prior to the tasks until the last measurement was completed. Participants gave consent prior to participation and did not receive any form of compensation. All data was collected anonymously (i.e., only age, gender, and handedness were collected). All procedures were approved by the internal Research Committee. The Research Committee considered the study a technical validation study that does not fall under the Dutch Medical Research Involving Human Subjects Act (WMO). Therefore, medical ethical approval from an independent medical-ethics committee was not required.

Study design

All participants visited the Centre for Human Drug Research (CHDR), Leiden, the Netherlands, twice, with a week between visits. To achieve a balanced design, the order of the blocks was counterbalanced using a Latin square method. Tapping tasks were conducted in the morning and their order was identical on both visits. Each task was performed four consecutive times, with 10-minute breaks between sessions. Participants were given a 20-minute break between two tapping tasks (for a schematic overview, see Figure 1). One visit lasted approximately 4 hours.

Finger tapping tasks

All finger tapping tasks were performed with a touchscreen laptop (HP Pavilion x360; resolution=1920 x 1080 pixels; screen width=31 cm; screen height=17.4 cm). The tasks were developed in-house using the Python programming language (version 3.4).³⁶ The PsychoPy library was used for stimulus presentation.³⁷ The visual stimuli were two white circles (radius=1 cm) placed horizontally on the screen on a black background. The two circles were either 2.5 or 20 cm apart, corresponding to the IMFT and IFT task, respectively. Depending on the configuration, targets were presented with or without a visual cue. With visual cueing, one target is visible at a time and only when tapped correctly does this target disappear while the other appears. Hence, a total of four tapping tasks were tested: cued and non-cued IMFT, as well as cued and non-cued IFT (see Figure 2).

Tapping position (X and Y coordinates) and tapping time for each tap were registered. Parameters related to speed, accuracy, fatigue and rhythm were quantified for each of the four tapping tasks.²⁸ We calculated the total number of taps (TNT) as a proxy for tapping speed; the number of tapping errors (NTE), mean spatial error (SEA), and bivariate contour ellipse area (BCA), as variables of accuracy; the inter-tap interval standard deviation (ITS) representing rhythm; and the change in velocity (VEC) to capture fatigue (see Table 2 for an overview of the tapping task parameters and Figure 3 for a visual representation of the data output).

During all tapping tasks, participants were instructed to tap as accurately and fast as possible for 30 seconds. Participants used the index finger of their dominant hand during the IFT tasks, whereas they used the index and middle finger alternately during the IMFT tasks. Additionally, during the IFT tasks, participants were asked to keep their elbow fixed in place on the table to prevent additional movement compensation.

Statistical analysis

All data processing was performed via custom scripts in Python (version 3.8).³⁶ Statistical modeling was performed using custom

scripts as well as the 'lme4'³⁹ and 'emmeans' packages⁴⁰ in the R software package.⁴¹

Repeatability

To assess the repeatability of the parameters, the available dataset was split into two subsets to separately assess the within- and between-day repeatability. For within-day repeatability, only measurements from the first visit were considered. For between-day repeatability, data from both visits was used, but from each visit the four measurements were averaged.

For each parameter and subset, a random intercept Linear Mixed Model (LMM) was fit. For within-day repeatability, both the intercept and measurement number (i.e., 1 to 4) were included as fixed effects. For between-day repeatability, both the intercept and visit number (i.e., 1 and 2) were included as fixed effects. Based on the models, the intraclass correlation coefficient (ICC) was calculated by dividing the between-subject variance by the total variance (i.e., the sum of the between-subject variance and the within-subject error variance).⁴² Excellent degree of repeatability was considered for ICC values above 0.90, good for ICC values between 0.75-0.90, moderate for ICC values between 0.50-0.75, and poor for ICC values below 0.50.⁴²

Minimum detectable effect

To assess potential sensitivity, minimum detectable effect (MDE) values were calculated. First, a random intercept model including measurement number (i.e., 1 to 4) as fixed effect was fitted for each parameter. For each fitted model, fixed intercept, random intercept variance and residual variance were extracted. The MDE was then calculated by multiplying the effect size by the pooled standard deviation (i.e., the square root of the sum of the within- and between-subject variance) and expressed in terms of percentage change relative to the intercept value. The effect size used to calculate the MDE was based on a paired sample t-test with a power of 0.80, a significance level of 5% ($\alpha=0.05$), and a sample size of 20 (a typical sample size for a clinical trial).

Effect of task configuration on performance

To assess the effect of configuration, cueing, measurement number, and visit number, a LMM was fitted for each parameter. For each model, the intercept, configuration (i.e., IMFT or IFT), cueing (i.e., cued or non-cued), measurement number (i.e., 1 to 4), and visit (i.e., 1 or 2) were included as fixed effects. Additionally, a two-way interaction between cueing and configuration was included as fixed effect. Between-subject random effects were included for the intercept. A more elaborate random structure was not possible without running into convergence issues. Type-III F-statistics were used to assess statistical significance of the fixed effects ($\alpha=0.05$). Where the interaction effect between the fixed effects was found to be significant, post-hoc pairwise comparisons with Tukey p-value correction were evaluated using the 'emmeans' package. Degrees of freedom for F-statistic denominators as well as pairwise comparisons were estimated via the Kenward-Roger method.⁴³ For pairwise comparisons, the effect size was estimated by calculating Cohen's d. Effect sizes were considered small, medium, or large for values of d smaller than 0.20, between 0.20 and 0.50, or larger than 0.80, respectively.⁴⁴

RESULTS

Two participants could not be measured due to emerging COVID restrictions, hence data from 14 participants were collected (mean age: $25.6 \pm \text{SD}: 3.1$; 6 females, 13 right-handed). All but one of the participants successfully completed all measurements. For one participant, the first four measurements were not performed due to technical difficulties. A total of 444 tapping experiments were performed, resulting in 61103 recorded taps.

Repeatability

The within-day repeatability of the six parameters in cued/ non-cued IMFT and IFT tasks are presented in Table 3. Excellent to good

repeatability was observed in the speed parameter (i.e., total number of taps) across all tasks ($\text{ICCs} > 0.86$). The number of tapping errors showed good to moderate repeatability in IMFT ($\text{ICC}_{\text{cued}}=0.81$, $\text{ICC}_{\text{non-cued}}=0.69$), but poor repeatability in IFT ($\text{ICC}_{\text{cued}}=0.41$, $\text{ICC}_{\text{non-cued}}=0.08$). The mean spatial error showed good repeatability in IMFT ($\text{ICC}_{\text{cued}}=0.79$, $\text{ICC}_{\text{non-cued}}=0.75$), and good to moderate repeatability in IFT ($\text{ICC}_{\text{cued}}=0.67$, $\text{ICC}_{\text{non-cued}}=0.84$). Good to poor repeatability was observed in the bivariate contour ellipse area in IMFT ($\text{ICC}_{\text{cued}}=0.77$, $\text{ICC}_{\text{non-cued}}=0.05$), and good to moderate repeatability in IFT ($\text{ICC}_{\text{cued}}=0.67$, $\text{ICC}_{\text{non-cued}}=0.84$). The rhythm parameter, inter-tap interval SD, showed good repeatability in both IMFT tasks ($\text{ICC}_{\text{cued}}=0.86$, $\text{ICC}_{\text{non-cued}}=0.84$), while it showed moderate to poor repeatability in IFT ($\text{ICC}_{\text{cued}}=0.20$, $\text{ICC}_{\text{non-cued}}=0.51$). The change in velocity parameter showed moderate repeatability in IMFT ($\text{ICC}_{\text{cued}}=0.56$, $\text{ICC}_{\text{non-cued}}=0.58$) and moderate to poor in IFT ($\text{ICC}_{\text{cued}}=0.25$, $\text{ICC}_{\text{non-cued}}=0.55$).

The between-day repeatability values for the six parameters are presented in Table 4. An excellent to good repeatability was observed in the total number of taps across all tapping tasks ($\text{ICCs}: 0.78\text{-}0.97$). The number of tapping errors showed excellent to good repeatability in IMFT ($\text{ICC}_{\text{cued}}=0.96$, $\text{ICC}_{\text{non-cued}}=0.81$) and moderate to poor repeatability in IFT ($\text{ICC}_{\text{cued}}=0.54$, $\text{ICC}_{\text{non-cued}}=0.06$). Of the accuracy parameters, mean spatial error showed moderate to good repeatability in IMFT ($\text{ICC}_{\text{cued}}=0.80$, $\text{ICC}_{\text{non-cued}}=0.70$), and moderate in IFT ($\text{ICC}_{\text{cued}}=0.53$, $\text{ICC}_{\text{non-cued}}=0.56$). The bivariate contour ellipse area showed moderate to poor repeatability in IMFT ($\text{ICC}_{\text{cued}}=0.60$, $\text{ICC}_{\text{non-cued}}=0.29$), and moderate in IFT ($\text{ICC}_{\text{cued}}=0.73$, $\text{ICC}_{\text{non-cued}}=0.63$). The rhythm parameter, inter-tap interval SD, showed good to moderate repeatability in IMFT ($\text{ICC}_{\text{cued}}=0.85$, $\text{ICC}_{\text{non-cued}}=0.52$), and good to poor repeatability in IFT ($\text{ICC}_{\text{cued}}=0.40$, $\text{ICC}_{\text{non-cued}}=0.75$). The change in velocity showed good to moderate repeatability in IMFT ($\text{ICC}_{\text{cued}}=0.79$, $\text{ICC}_{\text{non-cued}}=0.66$) and good repeatability in non-cued IFT ($\text{ICC}_{\text{non-cued}}=0.85$). For cued IFT, an ICC could not be estimated due to the model not converging.

Minimum detectable effect

The calculated MDE values, expressed in percentages as well as in absolute values, can be found in Table 5. Generally, the MDE values for the IFT configuration were lower than for IMFT. The parameters having the lowest MDE values were the total number of taps, the mean spatial error, and the rhythm parameter (MDE values ranging from 9.5%-23% in IFT, and 19%-71% in IMFT).

Effect of task configuration and cueing on tapping performance

The results of all LMM models are presented in Table 6. The configuration (i.e., IMFT vs IFT) had a significant effect on all parameters. Cueing affected all parameters except the mean spatial error. Lastly, a significant interaction effect between configuration and cueing was found for all parameters except the total number of taps and change in tapping velocity. None of the parameters were affected by the measurement number, see Table 6. However, the total number of taps, mean spatial error, and the inter-tap interval SD were affected by visit. For the pairwise comparisons between testing visits, see Table 7. On the second visit, participants tapped more often than on the first visit ($p < 0.01$). Moreover, the mean spatial error on the second visit was higher than on the first visit ($p < 0.05$). Finally, the inter-tap interval SD was lower on the second visit than on the first visit ($p < 0.01$).

All estimated mean values for the tapping tasks, as well as all pairwise comparisons are presented in Table 8 and Figure 4. Participants tapped more often during IMFT than IFT, and during a non-cued versus a cued task. In addition, more tapping errors were made in IMFT than IFT. In the absence of the visual cue, participants made more tapping errors in the IMFT task and fewer in the IFT task. The mean spatial error was larger in IFT than IMFT. The non-cued task reduced and increased the mean spatial error in the IMFT and IFT configurations, respectively. The bivariate contour ellipse area was significantly larger in IFT than IMFT. The non-cued task increased the bivariate contour ellipse area only in the IFT configuration. The SD of

the inter-tap interval was lower in the IFT configuration than in the IMFT configuration. The absence of the visual cue reduced the SD of the inter-tap interval only in the IFT configuration. The tapping velocity reduced throughout a measurement in both IMFT tasks, with a steeper reduction in the non-cued tapping task. The tapping velocity increased throughout a measurement in cued IFT, but reduced in the non-cued IFT.

DISCUSSION

The current technical validation study provides several key contributions to the growing body of literature on touchscreen-based tapping devices. To the best of our knowledge, this study is the first to assess the effects of cueing and task configuration on tapping performance in a comparative manner. It is also the first study that explicitly assesses the repeatability and MDE of tapping parameters in healthy participants. Based on the results of the current study, recommendations for subsequent studies are discussed.

Repeatability and minimal detectable effect

The first research question assessed the repeatability of tapping parameters across the four tapping tasks. Establishing good within-day repeatability is important as in clinical trials medication effects are often repeatedly assessed in a relatively short period of time.²⁹ Moreover, studies determining the acute pharmacodynamic effects of medication on a symptom, that may vary greatly between patients, (ideally) have a crossover design. Hence, the optimal tapping task must provide repeatable parameters for the same subject both within and between testing visits. The within- and between-day repeatability were comparable for all reported parameters (see Tables 3 and 4). None of the parameters in any task showed significant changes between the four measurements within a day. This indicates the lack of significant learning effects when the measurements are repeated in a relatively short period of time. However, there was a significant effect of testing visit (the second visit occurred one week after the

first) on the total number of taps, spatial error, and the standard deviation of the inter-tap interval. With the increase in number of taps at the second visit, the mean spatial error also increased. One explanation could be that as participants were already familiar with the task on the second visit, their priority might have shifted to speed rather than accuracy. To summarize, the within-day repeatability of the tapping parameters was good, but additional care should be taken when comparing repeated measures between testing visits.

The best repeatability was found in the speed related parameters, followed by accuracy, rhythm, and fatigue parameter. There were two parameters where lower repeatability was observed in IFT compared to IMFT, i.e., the number of tapping errors and the standard deviation of the inter-tap interval (i.e., rhythm parameter). The number of tapping errors showed lower repeatability values, especially in non-cued IFT compared to the other tasks. Since most participants tapped correctly, there was little to no between-subject variation in tapping errors, lowering its ICC value. Additionally, the between-subject variance of the rhythm parameter was lower for IFT compared to IMFT. This finding suggests that it was easier for most people to tap with a steady rhythm during forearm muscle/ elbow joint driven motion than during IMFT. Taken together, the IMFT parameters generally resulted in better within-day repeatability than the IFT ones, mainly driven by the increased between-subject variability in IMFT.

The second research question assessed the parameters' sensitivity to change in all four tapping tasks. Overall, the IFT parameters were more sensitive compared to IMFT parameters. The total number of taps showed moderate sensitivity in IMFT and higher sensitivity in IFT (i.e., MDE values ranging between 9.5%-28%). Previous research indicates that the effect sizes observed on this parameter when comparing PD patients in an *ON* versus an *OFF* state, and when comparing PD patients with healthy controls, range within comparable boundaries.^{20,21,23,25-27} Although less frequently reported in literature, similar effect sizes were found in the mean spatial error and rhythm parameters.^{20,25} Given that PD patients tend to tap more arrhythmically,^{11,14} slowly,^{20,21,28,45} and less accurately,^{20,28} the total number of taps, spatial error and the standard deviation of the inter-tap interval could be valuable parameters in subsequent clinical trials with patients.

The effects of task configuration and cueing on tapping performance

In the IMFT configuration, we found faster tapping, higher accuracy, worse rhythm, and more fatigue than in the IFT configuration. The inter-target distance was 8 times smaller in IMFT than IFT, thereby reducing the travel time between two consecutive taps. IMFT rhythm and fatigue effects, however, could primarily be explained by the increased muscle fatigue during fine, alternating finger movement as opposed to the upper-arm driven IFT motion.^{25,45,46} Why the increased speed was not associated with lower accuracy in IMFT, could be explained by the position of the circles. The targets were placed under the natural position of the fingertips, making deviations from the center of the targets and tapping outside the target areas inherently less likely. Despite these two tasks being interchangeably used in the literature, researchers should be aware that IMFT and IFT are two different tasks, and they assess distinct motor functions.

Understanding the effects of cueing in finger tapping is crucial as cues can significantly improve motor performance in PD.^{34,47} In healthy participants, cueing reduced speed and fatigue for both IMFT and IFT, improved accuracy, and worsened rhythm for IFT. In general, cueing had a larger effect on IFT and seemed to be less relevant for IMFT. The effects of cueing on tapping performance might be explained by the participant hesitating after each tap while waiting for the next circle to appear. More importantly, however, when participants tapped outside the target area, the next circle did not appear. Participants halted their hand movement, returned to correct the erroneous tap, resulting in higher inter-tap intervals, increased variability, lower fatigue, and fewer total taps. Hence cueing, rather than signaling the next target, provided immediate visual feedback. Considering a time-accuracy tradeoff, the immediate feedback and overall lower tapping speed can also account for the improved tapping accuracy in cued conditions. To summarize, cueing seemed to impair speed, rhythm, reduce fatigue, and improve accuracy of healthy participants, and it probably acted as visual feedback as opposed to a visual cue.

Limitations and future research

The most important caveat of the current paper is that we did not assess a PD patient group. Hence, a natural continuation of this work would validate the IMFT and IFT against gold standard clinical scales in a patient population (i.e., the MDS-UPDRS). Whether PD patients perform better on IFT compared to IMFT, and whether IMFT or IFT is more sensitive to detect medication effects will be assessed in a currently ongoing clinical study. Moreover, the current study did not assess the pharmacological sensitivity of the task. The optimal tapping task(s) must also be able to detect medication changes, otherwise, the task(s)' usefulness in clinical studies will be limited. In addition, even though we observed an increase in tapping speed on the second visit, we did not assess the exact nature of this effect. Future research should address the timescale and magnitude of testing visit effects on the tapping performance with respect to tapping style and/or motivation. Lastly, we did not vary the duration of the finger tapping tasks. Previous literature suggests that 30 seconds can be sufficient to detect fatigue effects,²⁰ without overburdening the participants. Hence, the 30 second task length makes the set-up suitable for repeated testing, even when conducting studies with rapid-acting (dopaminergic) agents.

The findings, while preliminary, caution against the use of cueing in studies involving PD patients. Previous literature suggests that tapping speed, fatigue and rhythm are clinically relevant predictors of both PD related bradykinesia, as well as medication effects.^{11,14,48} In healthy participants, cueing appears to impair the speed and rhythm of tapping, while reducing detectable fatigue. Hence, we argue that the tapping task set-up should be kept as simple as possible, to accurately detect potential differences in speed, rhythm, and change parameters, without inducing experimental noise. Additionally, exact comparisons with other studies remains difficult as technical specification on the implementation are not always reported (see Table 1). We encourage researchers to report on the technical implementation details of their tapping tasks (e.g., target distance, cueing, and duration).

Taken together, it seems preferable to use non-cued IFT and IMFT versions for further (validation) studies involving a PD population. The choice for IMFT or IFT should depend on the research question, as these tasks assess different aspects of movement. IMFT appears to be more difficult for most healthy participants, and one could speculate that IMFT would also be more difficult to perform for PD patients. For instance, Agostino showed that it is significantly more difficult for PD patients to perform alternating finger tapping, as opposed to pronation-supination (i.e., forearm, elbow and shoulder driven movement),^{25,45,46} and Lalvay showed that patients with severe parkinsonism have difficulties performing alternate finger tapping as opposed to one finger tapping.²⁵ In addition, bradykinesia appears to worsen increasingly during isolated, sequential finger movements, as opposed to gross hand movements.⁴⁵

CONCLUSION

The current study provides evidence that the custom developed IMFT and IFT tasks are well-functioning and repeatable measurement tools. From a technical point-of-view, they can be used in clinical trials assessing medication effects on bradykinesia. Recommended parameters are total number of taps, mean spatial error, and rhythm as they showed high repeatability and sensitivity. Moreover, the use of cueing in finger tapping tasks is unwarranted as visually cueing the tapping tasks can, in healthy participants, worsen tapping speed and rhythm, while improving accuracy. The choice for IMFT or IFT, should depend on the research question, as these tasks assess different aspects of movement. Concluding the technical validation step with encouraging results, the IMFT and IFT should be further investigated in subsequent studies with PD patients and in response to dopaminergic medication.

TABLE 1 Summary of the various finger tapping tasks found in the literature.

Study	Device	Target distance (cm)	Cueing	Duration	Parameters
Alternate index and middle finger tapping tasks					
Arora ^{30,32}	Phone application	N.A.	N.A.	N.A.	Numerous. e.g. speed, rhythm, accuracy, fatigue
Lalvay ²⁵	Smartphone application ('Momentum')	N.A.	Alternating colors (red vs green)	20s	Regularity, rhythm, and changes in the number of taps
Tian ²²	Phone application	N.A.	N.A.	30s	Average number of buttons pressed between both hands
Alternate index finger tapping tasks					
Giancardo ²⁷	Arcade buttons	25	N.A.	Not clear (possibly 60s)	Average number taps between hands
Lipp ²⁹ ; Nutt ³⁵	Arcade buttons	20	N.A.	60s	Total number of taps
Hasan ²⁰	Keyboard	20	No	30s	Total number of taps, time spent on keyboards, rhythm, and dysmetria score
	iPhone application ('TapPD')	N.A.	Not clear: changing colors	30s	
	Tablet ('TapPD')	N.A.	Not clear: changing colors	30s	
Arroyo-Gallego ²⁶	Keyboard	25	N.A.	N.A.	Not clear (possibly the total number of taps)
Mitsi ²⁴ ; Wissel ²³	Phone app	N.A.	N.A.	30s	Total number of taps, tap interval, tap duration, and tap accuracy
Young-Lee ²¹	Tablet	1.5	N.A.	10s	Numerous. E.g. inter-tap distance, inter-tap interval time, total distance of a finger movement, and tapping speed
Memedi ²⁸	Touch-pad with a pointer	2.7	N.A. (different target colors)	Not clear (possibly 20s)	Numerous. E.g. speed, accuracy, rhythm, and fatigue

N.A., not available.

TABLE 2 Tapping task parameters.

Category	Parameter		Definition
Speed	Total number of taps (#)	TNT	Sum of all taps on the screen
Accuracy	Number of tapping errors (#)	NTE	The number of two (or more) consecutive taps on the same target
	Mean spatial error (mm)	SEA	Average absolute Euclidean distance from the target's center point
	Bivariate contour ellipse area (mm ²)	BCA	Based on Castet & Crossland ³⁸ : A bivariate contour ellipse encompassing a proportion of the highest density of finger taps: $BCA = 2X^2 \pi \sigma_H \sigma_V (1 - \rho^2)$ where, X^2 is a chi-square variable with 2 degrees of freedom; σ_H and σ_V is the SD of the horizontal (X) and vertical (Y) coordinates, respectively; ρ is the product-moment correlation of the two position components
Rhythm	Inter-tap interval SD (ms)	ITS	The SD of the time between two consecutive taps
Fatigue	Velocity: change (cm/min ²)	VEC	A linear slope fitted on all inter-tap velocity values. Velocity was calculated by dividing the inter-tap distance value by the inter-tap interval

SD, standard deviation.

TABLE 3 Within-day repeatability.

Parameter		IMFT	IFT
		ICC (95% CI)	ICC (95% CI)
TNT (#)	Cued	0.94 (0.89, 0.97)	0.86 (0.76, 0.94)
	Non-cued	0.90 (0.82, 0.96)	0.94 (0.89, 0.98)
NTE (#)	Cued	0.81 (0.67, 0.91)	0.41 (0.19, 0.66)
	Non-cued	0.69 (0.5, 0.86)	0.08 (-0.08, 0.37)
SEA (mm)	Cued	0.79 (0.64, 0.90)	0.63 (0.43, 0.82)
	Non-cued	0.75 (0.57, 0.88)	0.76 (0.60, 0.89)
BCA (mm ²)	Cued	0.77 (0.61, 0.89)	0.67 (0.47, 0.83)
	Non-cued	0.05 (-0.12, 0.32)	0.84 (0.71, 0.92)
ITS (ms)	Cued	0.86 (0.76, 0.94)	0.20 (0.00, 0.48)
	Non-cued	0.84 (0.72, 0.93)	0.51 (0.30, 0.74)
VEC (cm/min ²)	Cued	0.56 (0.34, 0.77)	0.25 (0.04, 0.53)
	Non-cued	0.58 (0.34, 0.78)	0.55 (0.34, 0.77)

TNT, total number of taps; NTE, number of tapping errors; SEA, mean spatial error; BCA, bivariate contour ellipse area; ITS, inter-tap interval standard deviation; VEC, velocity: change; IMFT, alternate index and middle finger tapping; ICC, intraclass correlation coefficient; CI, confidence interval; IFT, alternate index finger tapping.

TABLE 4 Between-day repeatability.

Parameter		IMFT	IFT
		icc (95% ci)	icc (95% ci)
TNT (#)	Cued	0.97 (0.93, 0.99)	0.78 (0.51, 0.91)
	Non-cued	0.86 (0.68, 0.94)	0.88 (0.71, 0.95)
NTE (#)	Cued	0.96 (0.89, 0.98)	0.54 (0.13, 0.79)
	Non-cued	0.81 (0.58, 0.92)	0.06 (-0.39, 0.49)
SEA (mm)	Cued	0.80 (0.55, 0.92)	0.53 (0.11, 0.78)
	Non-cued	0.70 (0.38, 0.87)	0.56 (0.15, 0.80)
BCA (mm ²)	Cued	0.60 (0.21, 0.82)	0.73 (0.43, 0.89)
	Non-cued	0.29 (-0.17, 0.65)	0.63 (0.26, 0.84)
ITS (ms)	Cued	0.85 (0.65, 0.94)	0.40 (-0.06, 0.71)
	Non-cued	0.52 (0.01, 0.78)	0.75 (0.47, 0.90)
VEC (cm/min ²)	Cued	0.79 (0.53, 0.91)	-
	Non-cued	0.66 (0.30, 0.85)	0.85 (0.65, 0.94)

TNT, total number of taps; NTE, number of tapping errors; SEA, mean spatial error; BCA, bivariate contour ellipse area; ITS, inter-tap interval standard deviation; VEC, velocity: change; IMFT, alternate index and middle finger tapping; ICC, intraclass correlation coefficient; CI, confidence interval; IFT, alternate index finger tapping; -, value could not be estimated due to the model not converging.

TABLE 5 Sensitivity (MDE) estimates in percentage (%) and absolute values (Abs).

Parameter		IMFT	IFT
		MDE (Abs)	MDE (Abs)
TNT (#)	Cued	28% (45)	9.5% (6.2)
	Non-cued	19% (37)	11% (9.5)
NTE (#)	Cued	98% (6.1)	57% (1.5)
	Non-cued	49% (6.7)	150% (0.54)
SEA (mm)	Cued	24% (0.73)	12% (0.54)
	Non-cued	20% (0.54)	12% (0.56)
BCA (mm ²)	Cued	48% (22)	35% (55)
	Non-cued	88% (29)	26% (55)
ITS (ms)	Cued	32% (31)	23% (19)
	Non-cued	71% (68)	20% (8.4)
VEC (cm/min ²)	Cued	-	90% (400)
	Non-cued	43% (-370)	170% (-460)

MDE, minimum detectable effect; Abs, absolute value; TNT, total number of taps; NTE, number of tapping errors; SEA, mean spatial error; BCA, bivariate contour ellipse area; ITS, inter-tap interval standard deviation; VEC, velocity: change; IMFT, alternate index and middle finger tapping; -, value could not be estimated due to the model not converging; IFT, alternate index finger tapping.

TABLE 6 F-Test results of fixed effects for each parameter.

Category	Speed	Accuracy			Rhythm	Fatigue
	TNT F _(1,423.05)	NTE F _(1,423.07)	SEA F _(1,423.07)	BCA F _(1,423.08)	ITS F _(1,423.14)	VEC F _(1,423.16)
Configuration	1412.11 ***	281.97 ***	593.15 ***	965.02 ***	80.14 ***	98.70 ***
Cueing	36.82 ***	5.61 *	0.01	4.77 *	5.87 *	37.03 ***
Measurement	0.95	0.83	0.13	0.76	0.47	0.21
Visit	10.61 **	0.30	7.08 **	0.72	8.51 **	0.67
Configuration × Cueing	0.33	37.24 ***	16.28 ***	10.15 **	12.78 ***	1.64

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

TNT, total number of taps; NTE, number of tapping errors; SEA, mean spatial error; BCA, bivariate contour ellipse area; ITS, inter-tap interval standard deviation; VEC, velocity: change.

TABLE 7 Occasion effects on tapping performance.

Category	Speed	Accuracy			Rhythm	Fatigue
	Parameter (unit)	TNT (#)	NTE (#)	SEA (mm)	BCA (mm ²)	ITS (ms)
Visit 1- Visit 2 (SE)	-9.86 ** (3.03)	0.29 (0.53)	-0.19 ** (0.07)	-3.99 (4.7)	12.1 ** (4.14)	-46.1 (56.4)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

SE, standard error; TNT, total number of taps; NTE, number of tapping errors; SEA, mean spatial error; BCA, bivariate contour ellipse area; ITS, inter-tap interval standard deviation; VEC, velocity: change.

TABLE 8 Effect of task configuration and cueing on tapping performance.

Parameter	IMFT		IFT		Difference	
	EMMean (SE)	ES	EMMean (SE)	ES	IMFT- IFT (SE)	ES
TNT	Cued	185.0 (8.31)		73.0 (8.30)	112 (4.25)***	3.52
	Non-cued	205.1 (8.34)		89.70 (8.31)	115 (4.31)***	3.62
	Diff (C-NC)	-20.01 (4.31)***	-0.63	-16.70 (4.26)***	-0.52	
NTE	Cued	8.21 (1.19)		2.52 (1.19)	5.7 (0.75)***	1.02
	Non-cued	12.73 (1.20)		0.53 (1.19)	12.2 (0.76)***	2.18
	Diff (C-NC)	-4.52 (0.76)***	-0.81	1.99 (0.75)**	0.36	
SEA	Cued	3.03 (0.16)		4.47 (0.16)	-1.44 (0.1)***	-1.93
	Non-cued	2.74 (0.16)		4.75 (0.16)	-2.01 (0.1)***	-2.70
	Diff (C-NC)	0.29 (0.1)**	0.39	-0.28 (0.01)**	-0.37	
BCA	Cued	41.9 (10.3)		172.9 (10.3)	-131 (6.6)***	-2.65
	Non-cued	37.2 (10.4)		198.2 (10.3)	-161 (6.7)***	-3.25
	Diff (C-NC)	4.71 (6.7)	0.09	25.25 (6.6)***	-0.51	
ITS	Cued	84.5 (7.10)		62.2 (7.09)	22.3 (5.81)***	0.51
	Non-cued	89.2 (7.16)		37.4 (7.10)	51.9 (5.90)***	1.19
	Diff (C-NC)	-4.77 (5.90)	-0.11	24.85 (5.81)***	0.57	
VEC	Cued	-336 (91)		152 (90.9)	-488 (79.2)***	-0.82
	Non-cued	-751 (91.9)		-119 (91)	-633 (40.4)***	-1.07
	Diff (C-NC)	416 (91.0)***	0.70	271 (79.2)***	0.46	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$
 TNT, total number of taps; NTE, number of tapping errors; SEA, mean spatial error; BCA, bivariate contour ellipse area;
 ITS, inter-tap interval standard deviation; VEC, velocity: change; IMFT, alternate index and middle finger tapping;
 EMMean, estimated marginal mean; SE, standard error; ES, effect size Cohen's d; IFT, alternate index finger tapping;
 Diff, difference; c, cued; nc, non-cued.

FIGURE 1 Timing and sequence of tapping tasks during both visits. The order of the experiments was counterbalanced using the Latin square method.

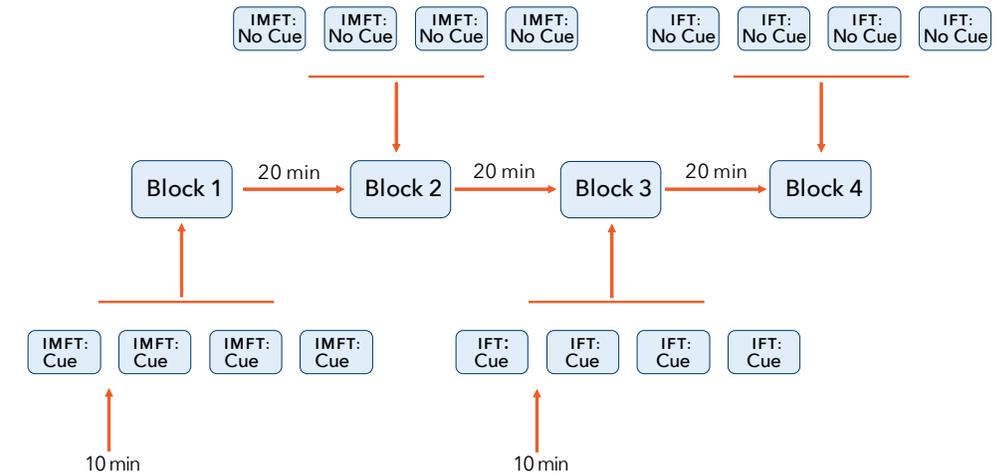


FIGURE 2 Finger tapping tasks. Figures A and B represent alternate index and middle finger tapping (IMFT). Figures C and D represent alternate index finger tapping (IFT). In the cued configurations (A and C), the second circle only appears when a tap inside the target was successfully performed. B and D represent the non-cued tapping tasks.

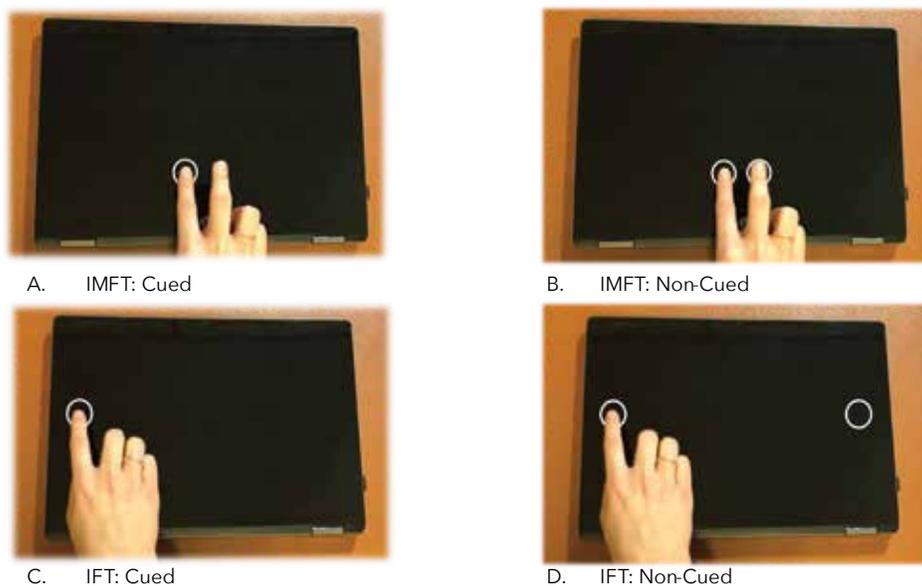
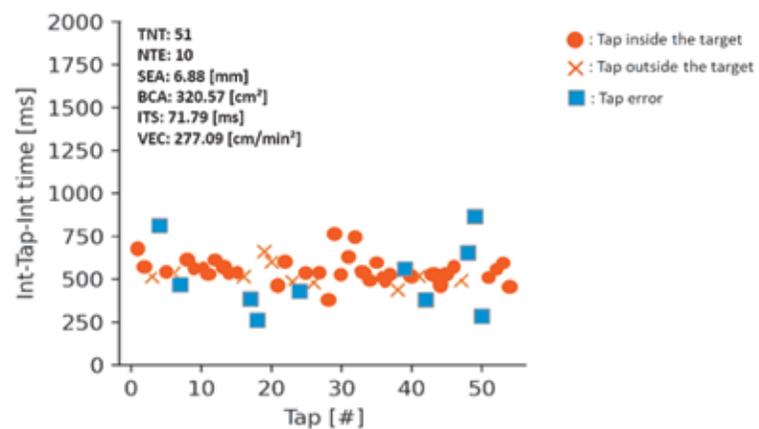
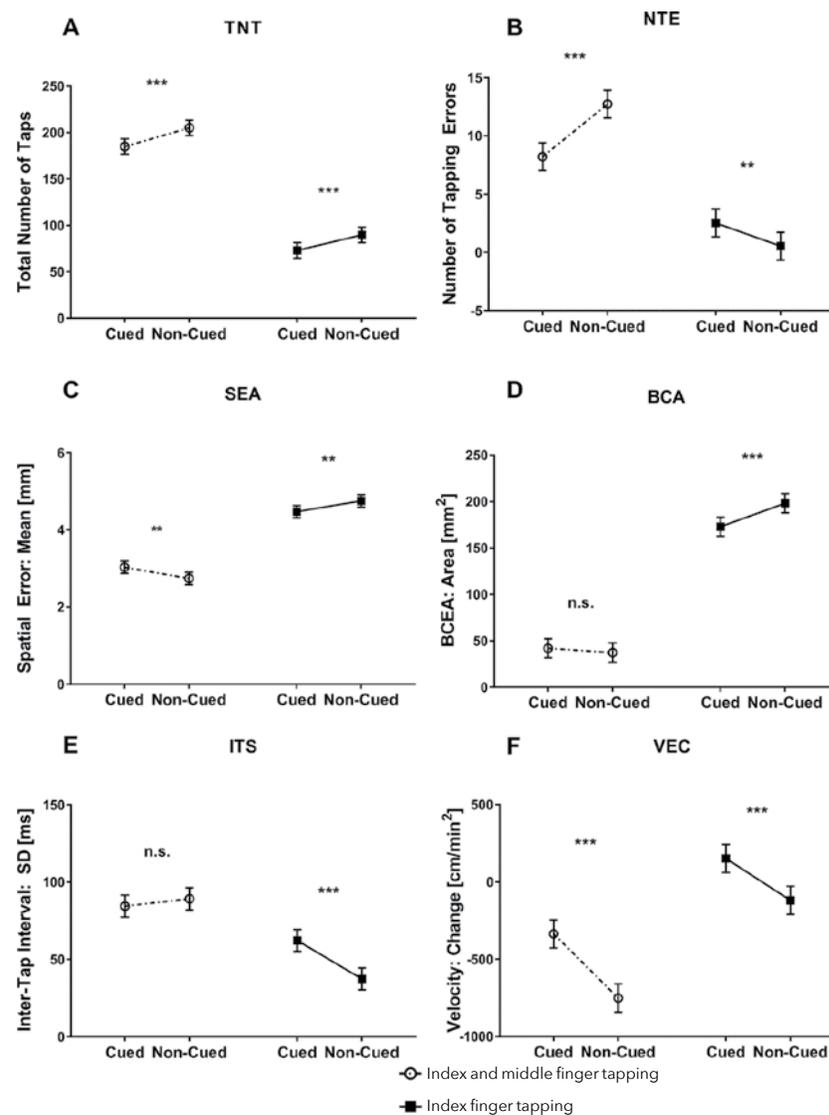


FIGURE 3 Data output example.



TNT, total number of taps; NTE, number of tapping errors; SEA, mean spatial error; BCA, bivariate contour ellipse area; ITS, inter-tap interval standard deviation; VEC, velocity: change.

FIGURE 4 The effects of configuration and cueing on tapping performance. Effects on total number of taps (TNT) (A), number of tapping errors (NTE) (B), mean spatial error (SEA) (C), bivariate contour ellipse area (BCA) (D), inter tap interval standard deviation (ITS) (E), and change in velocity (VEC) (F).



Values are based on estimated marginal means; error bars represent standard error of the marginal mean. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant.

REFERENCES

- 1 Kowal SL, Dall TM, Chakrabarti R, Storm M V., Jain A. The current and projected economic burden of Parkinson's disease in the United States. *Mov Disord.* 2013;28: 311-318. doi:10.1002/mds.25292.
- 2 Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. How common are the "common" neurologic disorders? *Neurology.* 2007;68: 326-337. doi:10.1212/01.wnl.0000252807.38124.a3.
- 3 Titova N, Chaudhuri KR. Apomorphine therapy in Parkinson's and future directions. *Park Relat Disord.* 2016;33: S56-S60. doi:10.1016/j.parkreldis.2016.11.013.
- 4 Kalia L V., Lang AE. Parkinson's disease. *Lancet.* 2015;386: 896-912. doi:10.1016/S0140-6736(14)61393-3.
- 5 Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. *Mov Disord.* 2008;23: 2129-2170. doi:10.1002/mds.22340.
- 6 Haaxma CA, Bloem BR, Borm GF, Horstink MWIM. Comparison of a timed motor test battery to the Unified Parkinson's Disease Rating Scale-III in Parkinson's disease. *Mov Disord.* 2008;23: 1707-1717. doi:10.1002/mds.22197.
- 7 Heldman DA, Espay AJ, LeWitt PA, Giuffrida JP. Clinician versus machine: Reliability and responsiveness of motor endpoints in Parkinson's disease. *Park Relat Disord.* 2014;20: 590-595. doi:10.1016/j.parkreldis.2014.02.022.
- 8 Hasan H, Athauda DS, Foltynie T, Noyce AJ. Technologies Assessing Limb Bradykinesia in Parkinson's Disease. *Journal of Parkinson's Disease.* IOS Press; 2017. pp. 65-77. doi:10.3233/JPD-160878.
- 9 Post B, Merkus MP, de Bie RMA, de Haan RJ, Speelman JD. Unified Parkinson's Disease Rating Scale motor examination: Are ratings of nurses, residents in neurology, and movement disorders specialists interchangeable? *Mov Disord.* 2005;20: 1577-1584. doi:10.1002/mds.20640.
- 10 Espay AJ, Bonato P, Nahab FB, Maetzler W, Dean JM, Klucken J, et al. Technology in Parkinson's disease: Challenges and opportunities. *Mov Disord.* 2016;31: 1272-1282. doi:10.1002/mds.26642.
- 11 Trager MH, Velisar A, Koop MM, Shreve L, Quinn E, Bronte-Stewart H. Arrhythmokinesia is evident during unimanual not bimanual finger tapping in Parkinson's disease. *J Clin Mov Disord.* 2015;2: 1-7. doi:10.1186/s40734-015-0019-2.
- 12 Tavares ALT, Jefferis GSXE, Koop M, Hill BC, Hastie T, Heit G, et al. Quantitative measurements of alternating finger tapping in Parkinson's disease correlate with UPDRS motor disability and reveal the improvement in fine motor control from medication and deep brain stimulation. *Mov Disord.* 2005;20: 1286-1298. doi:10.1002/mds.20556.
- 13 Au WL, Soo I, Seah H, Li W, Chew L, Tan S. Effects of Age and Gender on Hand Motion Tasks. 2015;2015.
- 14 Espay AJ, Giuffrida JP, Chen R, Payne M, Mazzella F, Dunn E, et al. Differential response of speed, amplitude, and rhythm to dopaminergic medications in Parkinson's disease. *Mov Disord.* 2011;26: 2504-2508. doi:10.1002/mds.23893.
- 15 Kim JW, Lee JH, Kwon Y, Kim CS, Eom GM, Koh SB, et al. Quantification of bradykinesia during clinical finger taps using a gyrosensor in patients with Parkinson's disease. *Med Biol Eng Comput.* 2011;49: 365-371. doi:10.1007/s11517-010-0697-8.
- 16 Okuno R, Yokoe M, Akazawa K, Abe K, Sakoda S. Finger taps movement acceleration measurement system for quantitative diagnosis of Parkinson's disease. *Annu Int Conf IEEE Eng Med Biol - Proc.* 2006; 6623-6626. doi:10.1109/IEMBS.2006.260904.
- 17 Summa S, Tosi J, Taffoni F, Biase L Di, Marano M, Rizzo AC, et al. Assessing bradykinesia in Parkinson's disease using gyroscope signals *. 2017; 1556-1561.
- 18 Stamatakis J, Ambroise J, Crémers J, Sharei H, Delvaux V, Macq B, et al. Finger tapping clinimetric score prediction in Parkinson's disease using low-cost accelerometers. *Comput Intell Neurosci.* 2013;2013. doi:10.1155/2013/717853.
- 19 Yokoe M, Okuno R, Hamasaki T, Kurachi Y, Akazawa K, Sakoda S. Opening velocity, a novel parameter, for finger tapping test in patients with Parkinson's disease. *Park Relat Disord.* 2009. doi:10.1016/j.parkreldis.2008.11.003.
- 20 Hasan H, Burrows M, Athauda DS, Hellman B, James B, Warner T, et al. The BRadykinesia Akinesia INcoordination (BRAIN) Tap Test: Capturing the Sequence Effect. *Mov Disord Clin Pract.* 2019;6: 462-469. doi:10.1002/mdc3.12798.
- 21 Lee CY, Kang SJ, Hong SK, Ma H II, Lee U, Kim YJ. A validation study of a smartphone-based finger tapping application for quantitative assessment of bradykinesia in Parkinson's disease. *PLoS One.* 2016;11: 1-11. doi:10.1371/journal.pone.0158852.
- 22 Tian F, Fan X, Fan J, Zhu Y, Gao J, Wang D, et al. What can gestures tell? Detecting motor impairment in early Parkinson's from common touch gestural interactions. *Conf Hum Factors Comput Syst - Proc.* 2019; 1-14. doi:10.1145/3290605.3300313.
- 23 Wissel BD, Mitsi G, Dwivedi AK, Papapetropoulos S, Larkin S, López Castellanos JR, et al. Tablet-Based Application for Objective Measurement of Motor Fluctuations in Parkinson Disease. *Digit Biomarkers.* 2018;1: 126-135. doi:10.1159/000485468.
- 24 Mitsi G, Mendoza EU, Wissel BD, Barbopoulou E, Dwivedi AK, Tsoulos I, et al. Biometric digital health technology for measuring motor function in Parkinson's disease: Results from a feasibility and patient satisfaction study. *Front Neurol.* 2017;8: 1-5. doi:10.3389/fneur.2017.00273.
- 25 Lalvay L, Lara M, Mora A, Alarcón F, Fraga M, Pancorbo J, et al. Quantitative Measurement of Akinesia in Parkinson's Disease. *Mov Disord Clin Pract.* 2017. doi:10.1002/mdc3.12410.
- 26 Arroyo-Gallego T, Ledesma-Carbayo MJ, Sanchez-Ferro A, Butterworth I, Mendoza CS, Matarazzo M, et al. Detection of Motor Impairment in Parkinson's Disease Via Mobile Touchscreen Typing. *IEEE Trans Biomed Eng.* 2017. doi:10.1109/TBME.2017.2664802.
- 27 Giancardo L, Sánchez-Ferro A, Arroyo-Gallego T, Butterworth I, Mendoza CS, Montero P, et al. Computer keyboard interaction as an indicator of early Parkinson's disease. *Sci Rep.* 2016. doi:10.1038/srep34468.
- 28 Memedi M, Khan T, Grenholm P, Nyholm D, Westin J. Automatic and objective assessment of alternating tapping performance in parkinson's disease. *Sensors (Switzerland).* 2013;13: 16965-16984. doi:10.3390/s131216965.
- 29 Lipp MM, Batycky R, Moore J, Leinonen M, Freed MI. Preclinical and clinical assessment of inhaled levodopa for OFF episodes in Parkinson's disease. *Sci Transl Med.* 2016. doi:10.1126/scitranslmed.aad8858.
- 30 Arora S, Venkataraman V, Zhan A, Donohue S, Biglan KM, Dorsey ER, et al. Detecting and monitoring the symptoms of Parkinson's disease using smartphones: A pilot study. *Park Relat Disord.* 2015;21: 650-653. doi:10.1016/j.parkreldis.2015.02.026.
- 31 Wirth R, Foerster A, Kunde W, Pfister R. Design choices: Empirical recommendations for designing two-dimensional finger-tracking experiments. *Behav Res Methods.* 2020; 2394-2416. doi:10.3758/s13428-020-01409-0.
- 32 Arora S, Baig F, Lo C, Barber TR, Lawton MA, Zhan A, et al. Smartphone motor testing to distinguish idiopathic REM sleep behavior disorder, controls, and PD. *Neurology.* 2018;91: E1528-E1538. doi:10.1212/WNL.0000000000006366.
- 33 Vercruyse S, Spildooren J, Heremans E, Wenderoth N, Swinnen SP, Vandenberghe W, et al. The neural correlates of upper limb motor blocks in Parkinson's disease and their relation to freezing of gait. *Cereb Cortex.* 2014. doi:10.1093/cercor/bht170.
- 34 Azulay JP, Mesure S, Blin O. Influence of visual cues on gait in Parkinson's disease: Contribution to attention or sensory dependence? *J Neurol Sci.* 2006. doi:10.1016/j.jns.2006.05.008.
- 35 Nutt JG, Woodward WR, Hammerstad JP, Carter JH, Anderson JL. The On-Off Phenomenon in Parkinson's Disease: Relation to Levodopa Absorption and Transport. *N Engl J Med.* 1984. doi:10.1056/NEJM198402233100802.
- 36 van Rossum G, Drake FL. *Python 3 Reference Manual.* Scotts Valley, CA. 2009.
- 37 Peirce J. Building experiments in Psychopy-good. *Journal of Chemical Information and Modeling.* 2018.
- 38 Castet E, Crossland M. Quantifying eye stability during a fixation task: A review of definitions and methods. *Seeing Perceiving.* 2012;25: 449-469. doi:10.1163/187847611X620955.
- 39 Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. *J Stat Softw.* 2015. doi:10.18637/jss.v067.i01.
- 40 Lenth R, Singmann H, Love J, Buerkner P, Herve M. Package 'emmeans.' *R Packag version 146.* 2020. doi:10.1080/00031305.1980.10483031>.License.
- 41 core Team R. R: A Language and Environment for Statistical Computing. *R Found Stat Comput Vienna, Austria.* 2018.
- 42 Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J Chiropr Med.* 2016. doi:10.1016/j.jcm.2016.02.012.

- 43 Kenward MG, Roger JH. Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood. *Biometrics*. 1997. doi:10.2307/2533558.
- 44 Cohen J. *Statistical power analysis for the behavioral sciences* (revised ed.) Academic Press. New York. 1988.
- 45 Agostino R, Currà A, Giovannelli M, Modugno N, Manfredi M, Berardelli A. Impairment of individual finger movements in Parkinson's disease. *Mov Disord*. 2003;18: 560-565. doi:10.1002/mds.10313.
- 46 Agostino R, Berardelli A, Currà A, Accornero N, Manfredi M. Clinical impairment of sequential finger movements in Parkinson's disease. *Mov Disord*. 1998;13: 418-421. doi:10.1002/mds.870130308.
- 47 Ginis P, Nackaerts E, Nieuwboer A, Heremans E. Cueing for people with Parkinson's disease with freezing of gait: A narrative review of the state-of-the-art and novel perspectives. *Annals of Physical and Rehabilitation Medicine*. 2018. doi:10.1016/j.rehab.2017.08.002.
- 48 Giovannoni G, Van Schalkwyk J, Fritz VU, Lees AJ. Bradykinesia akinesia inco-ordination test (BRAIN TEST): An objective computerised assessment of upper limb motor function. *J Neurol Neurosurg Psychiatry*. 1999;67: 624-629. doi:10.1136/jnnp.67.5.624.

CHAPTER 6

A placebo-controlled study to assess the sensitivity of finger tapping to medication effects in Parkinson's disease

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ABSTRACT

Background Movement Disorder Society–Unified Parkinson’s Rating Scale part III (MDS-UPDRS III) is the gold standard for assessing medication effects in patients with Parkinson’s disease (PD). However, short and rater-independent measurements would be ideal for future trials.

Objectives To assess the ability of three different finger tapping tasks to detect levodopa/carbidopa-induced changes over time, and to determine their correlation and compare their discriminatory power with MDS-UPDRS III.

Methods Randomized, double-blind, crossover study in 20 PD patients receiving levodopa/carbidopa and placebo capsules after overnight medication withdrawal. Pre- and up to 3.5 hours post-dose, MDS-UPDRS III and tapping tasks were performed. Tasks included two touchscreen-based alternate finger tapping tasks (index finger versus index-middle finger tapping) and a thumb-index finger task using a goniometer.

Results In the alternate index finger tapping task, levodopa/carbidopa compared with placebo resulted in significantly faster (total taps: 12.5 (95% confidence interval (CI), 6.7-18.2)) and less accurate tapping (total spatial error: 240 mm (123-357 mm)) with improved rhythm (inter-tap interval standard deviation (SD): -16.3% (-29.9%-0.0%)). In the thumb-index finger task, tapping was significantly faster ((mean opening velocity: 151 degree/s (64-237 degree/s)), with higher mean amplitude ((8.4 degrees (3.7-13.0 degrees)) and improved rhythm ((inter-tap interval SD: -46.4% (-63.7% to -20.9%)). The speed-related endpoints showed a moderate-to-strong correlation with the MDS-UPDRS III ($r=0.45-0.70$). The effect sizes of total taps and spatial error in the alternate index finger tapping task, and opening velocity in the thumb-index finger task were comparable to MDS-UPDRS III. In contrast, the MDS-UPDRS III performed better than the alternate index-middle finger task.

Conclusion The alternate index finger and the thumb-index finger tapping tasks provide short, rater-independent measurements that are sensitive to levodopa/carbidopa effects with a similar effect size as the MDS-UPDRS III.

INTRODUCTION

The Movement Disorder Society - Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) is considered the gold standard for assessing (dopaminergic) medication effects.¹ Part III of the scale is often used in clinical trials to show motor improvements after medication intake. However, part III requires a trained rater who preferably assesses a patient throughout the entire trial to avoid inter-rater variability. Additionally, the assessment takes relatively long (i.e., approximately 15 minutes, but depends on the patient’s clinical state). This makes accurate time-response assessment of fast-acting agents challenging, especially when safety and pharmacokinetic measurements also need to be performed. Hence, a short, rater-independent measurement would be ideal for use in clinical trials. Literature has shown that finger tapping can be used to show differences between healthy controls and PD patients,²⁻⁷ and between medication states (*ON/OFF*).^{3,5,6,8,9} Moreover, various finger tapping configurations have shown correlation with the MDS-UPDRS part III.^{3-6,8,10} However, the set-up and devices used for these tapping tasks vary among studies and it is unclear which is best suitable for determination of medication effects in randomized placebo-controlled trials.

In this randomized, double-blind, placebo-controlled trial, we assessed the response to dopaminergic medication during an induced *OFF* state in PD patients by using the gold standard MDS-UPDRS III as well as three different tapping tasks. For this, two touchscreen-based alternate finger tapping tasks (with 2.5 or 20 cm between targets) and a task using a goniometer that assesses angular movement during thumb-index finger tapping, were developed in-house. The aim was to validate these tapping tasks by demonstrating their ability to detect and quantify acute pharmacodynamic effects over time. Moreover, we evaluated whether the finger tapping endpoints correlated with MDS-UPDRS III.

METHODS

This study is registered in the Netherlands Trial Register (Trial NL8617), and was conducted at the Centre for Human Drug Research (Leiden, the Netherlands) between July and November 2020.

Study design

This was a randomized, double-blind, placebo-controlled, two-way crossover study in 20 PD patients. A sample size of 18 was considered sufficient to show a treatment effect based on a paired t-test with 80% power and a two-sided alpha level of 5%, assuming an expected difference on the best response of 8 total taps ($SD=7$) between placebo and treatment.¹¹ To be conservative, it was decided to include 20 patients. The study consisted of a screening visit followed by two treatment periods of two days each, with a 1-week washout between periods. Patients were randomized 1:1 to one of two treatment sequences (levodopa/carbidopa - placebo, or vice versa). The randomization code was generated using SAS v9.4 by a study-independent statistician. Patients were instructed to withhold their own anti-Parkinson medication in the evening prior to treatment in both treatment periods. Patients were dosed the next morning when in an *OFF* state, as assessed by the physician. Patients were allowed to resume their own medication 110 minutes after dosing, or, if feasible for the patient, after the last efficacy assessments 210 minutes post-dose.

Participants

PD patients with self-described motor fluctuations and recognizable *OFF* periods aged between 20-85 years with Hoehn and Yahr stage I-III were eligible for participation. In addition, patients had to be levodopa responsive as evidenced by current or historical use of levodopa. Reasons to exclude a patient were a previous intolerance, a potentially relevant interaction of co-medication with or a contraindication to levodopa and/or carbidopa. Patients were ineligible when the levodopa equivalent dose (LED) of their morning medication exceeded 500 mg.

Investigational drugs

To ensure blinding, levodopa/carbidopa 100/25 mg (Sinemet) tablets were over-encapsulated in 00 gelatin (Swedish orange) capsules. Similarly, placebo tablets were over-encapsulated. Patients received a semi-individualized dose based on the LED of their morning medication. To calculate the LED, conversion factors as described by Tomlinson et al were used.¹² For long-acting dopamine agonists, only 25% of their LED was included, since only their acute effect was of importance for calculation of the morning LED. Finally, the LED was multiplied by 1.25 to ensure a supramaximal dose was given that was at least 25% higher than the usually administered morning dose (to ensure *OFF-ON* transition). This supramaximal LED was rounded up to a whole number of levodopa/carbidopa 100/25 mg (or placebo) capsules that was required for that patient. Since food, and especially proteins can affect the absorption of levodopa, study drug administration occurred at least 1 hour after finishing a protein-restricted breakfast and food was not allowed until 1 hour after dosing.

Safety

Patients enrolled in this study were already using levodopa or had used it in the past. Therefore, they were expected to tolerate the study treatment well. Nonetheless, subject safety was evaluated by monitoring of adverse events throughout the study, and by examining the patient's vital signs, ECG and physical/neurological examination before discharge. As no notable changes were observed, these data are not shown.

Outcome measures

MDS-UPDRS

MDS-UPDRS part III was used to assess motor function. Physicians administering the scale were trained in its use. To the degree feasible, the same physician evaluated a patient during both treatment periods at Day -1 (day before dosing) and at Day 1 pre-dose and 10, 30, 60, 90 and 210 minutes post-dose. The last measurement was

only performed when the patient had not yet resumed their own medication.

TOUCHSCREEN-BASED TAPPING TASKS¹³

- 1 Alternate index and middle finger tapping: task in which the patient was instructed to alternately tap with the index and middle finger on two circles (radius 1.2 cm) spaced 2.5 cm apart (Figure 1A).
- 2 Alternate index finger tapping: task in which the patient was instructed to alternately tap with the index finger on two circles (radius 1.7 cm) spaced 20 cm apart (Figure 1B).

For both tasks, the instructions were to tap as accurately and as fast as possible for 30 seconds with the hand most affected by PD (or the dominant hand if both sides were equally affected). Calculated endpoints were: total number of taps, total taps inside the target, ratio good: total taps, number of halts, mean inter-tap interval, SD of inter-tap intervals, inter-tap interval change, mean spatial error, SD of spatial error, spatial error change, and total spatial error. Refer to Supplemental Table 1 for a description of each endpoint.

THUMB-INDEX FINGER TAPPING

A goniometer (Biometrics Ltd, UK) placed on the proximal phalanx and metacarpal of the index finger of the most affected (or dominant if both sides were equally affected) hand measured the angle of the index finger (Figure 1C). Patients were instructed to tap the index finger on the thumb as quickly and as wide as possible for 15 seconds. Calculated endpoints included: total number of taps, mean inter-tap interval, SD of inter-tap intervals, inter-tap interval change, mean tapping amplitude, tapping amplitude change, peak frequency area under the curve (AUC), angle frequency change, and mean opening and closing velocity (Supplemental Table 1).

Patients were trained on all three tapping tasks twice on Day -1 and once on Day 1 pre-dose. These measurements were not used in the analysis. Finger tapping tasks included in the analyses were performed on Day 1 pre-dose (double baseline) and approximately 10, 25, 45, 60, 75, 90, 105, and 210 minutes post-dose (if time points coincided with MDS-UPDRS III, then finger tapping tasks were

performed first, followed by MDS-UPDRS III). The last measurement was only performed when the patient had not yet resumed their own medication.

Data exclusion

In case the ratio good: total taps was <0.3 in the alternate index and middle finger tapping task, inter-tap interval parameters (mean, SD, change) and number of halts could not be reliably calculated, so were excluded from analysis. One patient seemed unable to correctly perform and/or the device did not correctly record the alternate index and middle finger tapping, so this task was completely excluded from analysis for this patient.

Statistical analysis

Analyses were performed using SAS v9.4. To detect significant treatment effects on the primary endpoints, each endpoint was analyzed using a mixed model analysis of variance with period, treatment, time, and treatment by time as fixed factors, subject and subject by time as random factors, and the average baseline measurement as covariate. Homoscedasticity assumption of the mixed modelling framework was relaxed by allowing separate variance estimates for each treatment. Within the model, the contrast levodopa/carbidopa versus placebo was calculated based on all post-dose measurements. In case of non-normality, endpoints with positive numerical results were re-analyzed after log-transformation. For ten endpoints, no models could be fitted since they violated the normality assumption, even after log-transformation.

Pearson's or Spearman's (in case of non-normal or log-normal data) correlation was used to evaluate the relationship between finger tapping endpoints and MDS-UPDRS III at a selected time point (90 min for MDS-UPDRS and 105 min (after completion of MDS-UPDRS at 90 min) for tapping). Correlation analysis was performed for placebo and levodopa/carbidopa separately. The strength of the correlation was classified as weak ($r < 0.40$), moderate ($r = 0.40-0.59$), strong ($r = 0.60-0.79$) or very strong ($r = 0.80-1.0$).

For both analyses, a p-value of ≤ 0.05 was used as cut-off for determining significance. No correction for multiple testing was performed due to the exploratory nature of this study.

Standardized effect sizes were calculated by dividing the Least Squares Means (LSMs) difference (levodopa/carbidopa - placebo) by the pooled SD of the treatment effect. The pooled SD was calculated with the formula described by Brown et al.¹⁴ A Hedge's g correction was done to account for small sample size. Effect sizes were calculated for comparison of endpoints and tasks, but are not intended for future power calculations (model-based estimates to be used).

RESULTS

Baseline characteristics

The number of patients screened, randomized, completed and analyzed are summarized in the CONSORT flow diagram in Supplemental Figure 1. Table 1 outlines the demographics and baseline characteristics of the 20 PD patients that completed the study. Most (95%) patients received a levodopa-containing agent as part of their regular medication regimen. Supramaximal morning LED ranged between 47 and 391 mg. Therefore, patients received between 1-4 capsules of levodopa/carbidopa 100/25 mg and placebo in a randomized order.

Overall task performance

For 6 out of 20 PD patients, the alternate tapping task with the index and middle finger was sometimes difficult to correctly perform. Difficulty was being defined as having a ratio of good: total taps less than 0.3 on at least 4 of 22 performed tests (but this reached up to 17 of 22 tests). Difficulties were approximately equally divided over placebo and levodopa/carbidopa tests. One patient seemed unable to correctly perform and/or the device did not correctly record the alternate index and middle finger tapping. This was concluded based on taps only being recorded during the first few seconds or by gaps of >10 seconds where no taps were recorded (in the absence of

freezing). With the alternate index finger tapping and thumb-index finger tapping tasks, the patients usually did not experience any difficulties. However, the goniometer devices used for the thumb-index finger tapping task turned out fragile and broke in a few instances. This led to missing data for one patient after placebo, and two patients after levodopa/carbidopa treatment.

Treatment and treatment by time effects

After placebo treatment, 14 out of 20 patients had to resume their own Parkinson's medication prior to the last assessment planned at 3.5 hours post-dose. After levodopa/carbidopa, this was 6 out of 20. Meaning that the MDS-UPDRS III and finger tapping measurements at 3.5 hours were performed in n=14 levodopa/carbidopa- and n=6 placebo-treated patients.

Table 2 shows treatment and treatment by time effects for the gold standard MDS-UPDRS III and the three tapping tasks. In Figure 2, the LSMs (geometric LSMs for back-transformed data) change from baseline data over time are depicted for MDS-UPDRS III and a subset of three endpoints of each tapping task that showed to be significant in Table 2. For graphs of the other finger tapping endpoints, refer to Supplemental Figure 2.

The MDS-UPDRS III showed a significant treatment effect and treatment by time interaction effect (Table 2), as is also visualized in Figure 2A. For the alternate index and middle finger tapping task, it was shown that levodopa/carbidopa compared to placebo resulted in significantly faster (i.e., lower mean inter-tap interval) and more accurate tapping (i.e., more total taps inside target and higher ratio good: total taps) (Table 2, Figure 2B). No significant treatment effect, but a significant treatment by time interaction effect was found for the total number of taps, indicating that at least at one time point there was a significant difference between placebo and levodopa/carbidopa. Even though a significantly lower inter-tap interval SD, i.e., improved rhythm, was found for levodopa/carbidopa compared to placebo, it did not show a clear time-related response (Figure 2B). Spatial error and number of halts were not significantly different between active and placebo treatment.

Also in the alternate index finger tapping task, significantly faster tapping (increased total number of taps, and as a result, total taps inside the target) was observed after levodopa/carbidopa compared to placebo treatment (Table 2, Figure 2C). In contrast, accuracy was significantly reduced as observed by a higher mean and total spatial error. Lastly, levodopa/carbidopa compared to placebo resulted in a better tapping rhythm as observed by a lower SD of the inter-tap intervals, which showed a clear time-related response.

In the thumb-index finger tapping task, levodopa/carbidopa did not only result in significantly faster tapping (higher mean opening and closing velocities), but also in an increased mean tapping amplitude (Table 2, Figure 2D). Another measure of amplitude, peak frequency area under the curve, was also significantly higher after levodopa/carbidopa than placebo treatment. As in the alternate index and middle finger tapping task, total number of taps did not show a significant overall treatment effect but did show a significant treatment by time interaction effect. SD of the inter-tap intervals was again lower in the levodopa/carbidopa than in the placebo group, indicating improved rhythm. No significant treatment effect on fatigue, i.e., a decrease in tapping amplitude over time, was observed.

To enable the comparison of endpoints within and between tasks, standardized mean differences (Hedge's *g*) between levodopa/carbidopa and placebo treatment were calculated (Supplemental Figure 3). This shows that alternate index finger tapping and thumb-index finger tapping had higher standardized effect sizes than alternate index and middle finger tapping. The endpoint in the alternate index and middle finger tapping task with the highest standardized effect size was the ratio of good: total taps. For alternate index finger tapping, these were total number of taps and total spatial error. For thumb-index finger tapping, the opening and closing velocity had the highest standardized effect sizes, followed by the amplitude endpoints and inter-tap interval SD. Four of these endpoints had a standardized effect size that was similar to that of the MDS-UPDRS III, namely the total number of taps and the total spatial error in the alternate index finger tapping task, and the opening and closing velocity in the thumb-index finger tapping task.

Correlation with MDS-UPDRS III

At 1.5 hours post-dose, none of the alternate index and middle finger tapping endpoints correlated with MDS-UPDRS III total score except for total spatial error after levodopa/carbidopa treatment (Pearson's $r=0.50$, $p=0.0306$) (Table 3).

In the alternate index finger tapping task, the total number of taps showed a significant moderate correlation with MDS-UPDRS III in both the placebo (Pearson's $r=-0.45$, $p=0.0454$) and levodopa/carbidopa (Pearson's $r=-0.45$, $p=0.0457$) group. Similarly, the mean inter-tap interval was significantly correlated with MDS-UPDRS III, but only in the placebo group (Spearman's $r=0.50$, $p=0.0249$). The accuracy parameters, total taps inside the target and ratio good: total taps, significantly correlated with MDS-UPDRS III in the levodopa/carbidopa group (Pearson's $r=-0.55$ and $p=0.0120$; Spearman's $r=-0.45$ and $p=0.0446$ respectively). For the other accuracy and rhythm parameters, no correlation was found.

In the thumb-index finger tapping task, all speed parameters had a strong correlation with MDS-UPDRS III in the placebo group (r ranging between -0.65 and 0.70). Closing velocity also showed a moderate correlation with MDS-UPDRS III in the levodopa/carbidopa group (Pearson's $r=-0.50$, $p=0.0426$). No other significant correlations were found except for a strong correlation of inter-tap interval SD with MDS-UPDRS III in the levodopa/carbidopa group (Spearman's $r=0.66$, $p=0.0037$).

DISCUSSION

In this randomized, placebo-controlled trial, we assessed the ability of three different finger tapping tasks to detect and quantify acute pharmacodynamic effects of dopaminergic medication. Moreover, we investigated whether the finger tapping endpoints correlated with the MDS-UPDRS III score. The advantage of finger tapping over the MDS-UPDRS III is its short duration and rater independence. The short duration allows for frequent assessments and thus for a better detection of the onset of pharmacodynamic effects. Since no trained

rater is required, it is logistically easier to perform the task during a clinical trial, but also allows for testing at home. To our knowledge, this is the first time these tapping tasks have been directly compared to the MDS-UPDRS III in a placebo-controlled study.

Both the alternate index finger tapping and thumb-index finger tapping tasks showed significant differences between levodopa/carbidopa and placebo treatment, with effect sizes comparable to the MDS-UPDRS III. PD patients were able to perform both tasks without difficulties. The goniometer used for the thumb-index finger tapping task was quite fragile and broke several times. In a clinical trial setting where backup devices are available this is not a major problem, but it does make the task unsuitable for at-home testing. In contrast, the alternate index finger tapping only requires a touchscreen tablet and therefore would also be suitable for testing of medication effects or disease progression over time in an at-home setting.

For the alternate index finger tapping task, endpoints relating to speed (i.e., total number of taps) and accuracy (i.e., total spatial error) performed best. An increased speed was associated with reduced accuracy. Such a trade-off between speed and accuracy has previously been described in Parkinson's disease patients,^{6,15} even though not consistently.⁸ Different results between studies might have been obtained due to differences in the test set-up, as well as in how accuracy was calculated (e.g., on a continuous scale vs. inside/outside target). In the alternate index finger tapping task, rhythm was also significantly improved (i.e., lower geometric mean of inter-tap interval SD) after levodopa/carbidopa compared to placebo, albeit with a lower effect size than the speed and accuracy endpoints. The total number of taps correlated moderately with the MDS-UPDRS III. In contrast, the total spatial error and the inter-tap interval SD, which showed significant treatment effects with a time-related response, did not correlate with MDS-UPDRS III. This might be because they quantify aspects of tapping performance that are not captured by (parts of) the MDS-UPDRS III. Therefore, despite the absence of a correlation, they can be valuable additional endpoints in drug efficacy trials. Particularly the total spatial error since it has an effect size comparable to that of the MDS-UPDRS III.

In the thumb-index finger tapping task, levodopa/carbidopa compared to placebo resulted in faster tapping with a bigger amplitude and improved rhythm. This is in line with previously reported results on thumb-index finger tapping when *ON* and *OFF* states were compared.^{5,9} When comparing all endpoints, mean opening and closing velocity had the largest effect sizes, which were comparable to that of the MDS-UPDRS III. In addition, both endpoints showed a moderate-to-strong correlation with the MDS-UPDRS III. The SD of the inter-tap intervals also showed a significant difference between levodopa/carbidopa and placebo, but with a smaller effect size than the opening and closing velocity. Moreover, the inter-tap interval SD showed a strong correlation with the MDS-UPDRS III in the levodopa/carbidopa group and a trend towards a moderate correlation in the placebo group. The mean tapping amplitude and peak frequency AUC, both measures of amplitude, showed a significant treatment effect with a similar effect size. Since they performed equally, but the peak frequency AUC requires a more difficult formula and therefore might be harder to interpret, the mean tapping amplitude is preferred for use in future studies. Mean tapping amplitude did not correlate with MDS-UPDRS III, which was in contrast to the strong correlation ($r=-0.79$) reported by Ling et al. in PD patients when *OFF*.⁵ No medication effects on fatigue, i.e., a change in tapping amplitude over time, were observed. This is in line with what is reported for other thumb-index finger tapping tasks.^{5,9} However, the lack of an effect might be related to the relatively short task duration of 15 seconds in all of these tasks. By increasing the task duration, one might enhance fatigue, and thereby leave more room to show improvement by medication.

Of the three tapping tasks, the alternate index and middle finger tapping task performed worst, i.e., had the lowest effect sizes. Its effect sizes were also below that of the gold standard MDS-UPDRS III. Moreover, the task was sometimes difficult to perform for the PD patients, resulting in a high percentage of same-sided double taps. This is likely the result of the patients not lifting their finger from the touchscreen before tapping with the other finger, resulting in two fingers touching the screen simultaneously. With the used set-up, this was recorded as a single tap. The number of tests with more than

70% of same-sided double taps (i.e., a ratio good: total taps <0.3) was approximately balanced over placebo and levodopa/carbidopa treatment. Nevertheless, the ratio good: total taps on a continuous scale was significantly different between placebo and levodopa/carbidopa treatment and showed a time-related response. The same holds true for the total taps inside the target, albeit with a lower effect size. In contrast, the mean and SD of the inter-tap intervals showed a significant treatment effect, but no clear time-related response, making it possible that these were chance findings due to multiple testing. None of the alternate index and middle finger tapping endpoints with significant treatment or treatment by time interaction effects showed a correlation with the MDS-UPDRS III score. Overall, the problems with correctly performing/recording the alternate index and middle finger tapping task, combined with the relatively small effect sizes, make the task in its current configuration the least suitable for efficacy studies including PD patients.

In conclusion, the alternate index finger tapping and thumb-index finger tapping tasks provide short, rater-independent measurements that are sensitive to dopaminergic medication effects and have a similar effect size as the MDS-UPDRS III. When including these tasks in future trials, at least the following endpoints should be included: total number of taps and total spatial error (for alternate index finger tapping), and opening or closing velocity, mean tapping amplitude and inter-tap interval SD (for thumb-index finger tapping). Even though spatial error and amplitude did not correlate with MDS-UPDRS III, they should be included in future placebo-controlled efficacy trials, since they show a clear difference between active and placebo treatment, as well as a time-related response. Since these measurements only take 15 to 30 seconds, they can be performed repeatedly during clinical trials and are therefore expected to better detect onset of effect and time to reach maximum effect than the MDS-UPDRS III. The alternate index finger tapping task may also be suitable for testing new drugs or monitoring disease progression in an at-home setting.

TABLE 1 Demographics.

All PD patients (N=20)	
Age (years)	
Median (range)	61 (48-70)
Mean (SD)	60.6 (6.0)
BMI (kg/m ²)	
Median (range)	27 (23-30)
Mean (SD)	26.5 (2.5)
Sex (n/n (%/%))	
Female/Male	6/14 (30/70)
Race (n (%))	
White	20 (100)
Hoehn and Yahr stage at screening (n (%))	
Stage 1	7 (35)
Stage 2	7 (35)
Stage 3	6 (30)
MDS-UPDRS III total score on the day prior to dosing (i.e., when using regular medication)	
Median (range), placebo treatment	23 (7-52)
Mean (SD), placebo treatment	24.2 (13.1)
Median (range), active treatment	22 (5-70)
Mean (SD), active treatment	24.6 (14.7)
Concomitant PD medication (n (%))	
Levodopa-containing agents	19 (95)
Dopamine agonists	14 (70)
COMT inhibitors	4 (20)
MAO-B inhibitors	2 (10)
Amantadine	4 (20)
Deep brain stimulation (bilateral subthalamic nucleus)	2 (10)
Levodopa Equivalent Dose (mg) ^a	
Median (range)	275 (47-391)
Mean (SD)	246.9 (112.5)
Number of capsules ^b	
Median (range)	3 (1-4)
Mean (SD)	3 (1)

a. Supramaximal levodopa equivalent dose of the morning medication (for calculation, refer to the Methods). /

b. Number of levodopa/carbidopa 100/25 mg or placebo capsules administered in this study.

SD, standard deviation; BMI, body mass index; MDS-UPDRS III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III; PD, Parkinson's disease; COMT, catechol-O-methyltransferase; MAO-B, monoamine oxidase B.

TABLE 2 Per endpoint, Least Squares Means, Least Squares Means change from baseline, p-values of the treatment and treatment x time effects, and the estimated difference for the levodopa/carbidopa-placebo contrast with its 95% CI are shown.

Category	Parameter (unit) ^a	Least Squares Means		Treatment p-value	Treatment x Time p-value	Contrast levodopa/carbidopa vs placebo (95% CI)	Least Squares Means change from baseline	
		Placebo	Levodopa/carbidopa				Placebo	Levodopa/carbidopa
MDS-UPDRS III total score								
Gold standard	MDS-UPDRS III	34.3	27.0	0.0014	<.0001	-7.3 (-11.6, -3.0)	-0.7	-8.0
Alternate index and middle finger tapping ^b								
Speed	Total number of taps	81.3	87.5	0.2173	0.0052	6.3 (-3.9, 16.4)	-8.9	-2.7
	Mean inter-tap interval (ms)	389.5	317.3	0.0198	0.1106	-18.5% (-31.2%, -3.5%)	15.3%	-6.0%
Accuracy	Total taps inside target	75.0	86.6	0.0308	0.0001	11.6 (1.1, 22.1)	-10.3	1.4
	Ratio good: total taps	0.59	0.72	0.0006	<.0001	0.14 (0.07, 0.21)	-0.1	0.0
	Total spatial error (mm)	470.4	428.5	0.2629	0.1974	-41.9 (-116.9, 33.0)	-6.2	-48.1
	Mean spatial error (mm)	5.6	5.0	0.0950	0.3893	-12.0% (-24.4%, 2.4%)	10.6%	-2.7%
Rhythm	Inter-tap interval SD (ms)	219.7	162.8	0.0304	0.2219	-25.9% (-43.4%, -3.0%)	21.6%	-9.9%
	Spatial error SD (mm)	2.2	2.0	0.4203	0.1024	-8.3% (-26.2%, 13.9%)	1.6%	-6.9%
	Number of halts	3.2	3.4	0.6975	0.2483	0.2 (-0.7, 1.1)	-0.1	0.0
Alternate index finger tapping ^c								
Speed	Total number of taps	66.1	78.6	0.0001	<.0001	12.5 (6.7, 18.2)	-2.4	10.0
Accuracy	Total taps inside target	55.5	63.2	0.0260	<.0001	7.7 (1.0, 14.4)	-2.5	5.1
	Total spatial error (mm)	719.0	959.3	0.0002	<.0001	240.3 (123.3, 357.3)	-29.7	210.6
	Mean spatial error (mm)	10.8	12.0	0.0205	0.6719	1.2 (0.2, 2.2)	0.0	1.2
Rhythm	Inter-tap interval SD (ms)	52.3	43.8	0.0494	0.0307	-16.3% (-29.9%, -0.0%)	8.9%	-8.8%
	Spatial error SD (mm)	4.5	4.9	0.2830	0.1083	7.6% (-6.1%, 23.3%)	3.7%	11.6%

[continuation of Table 2]

Category	Parameter (unit) ^a	Least Squares Means		Treatment p-value	Treatment x Time p-value	Contrast levodopa/carbidopa vs placebo (95% CI)	Least Squares Means change from baseline	
		Placebo	Levodopa/carbidopa				Placebo	Levodopa/carbidopa
Thumb-index finger tapping ^d								
Speed	Total number of taps	46.1	52.6	0.0633	<.0001	6.5 (-0.4, 13.4)	-1.5	5.0
	Mean opening velocity (degree/s)	372.2	522.7	0.0013	<.0001	150.5 (64.2, 236.8)	-62.9	87.6
	Mean closing velocity (degree/s)	479.1	659.0	0.0028	<.0001	180.0 (67.0, 292.8)	-90.4	89.5
Amplitude	Mean tapping amplitude (degree)	27.4	35.7	0.0009	<.0001	8.4 (3.7, 13.0)	-4.9	3.4
	Peak frequency AUC (degree ²)	107.4	187.8	0.0089	0.0034	80.4 (21.8, 138.9)	-44.9	35.5
Rhythm	Inter-tap interval SD (ms)	62.4	33.4	0.0028	0.0004	-46.4% (-63.7%, -20.9%)	24.8%	-33.1%
Fatigue	Tapping amplitude change (degree/s)	-0.34	-0.50	0.1781	0.9049	-0.16 (-0.40, 0.08)	0.0	-0.2

P-values <0.05 are shown in **bold**.

a. For log transformed parameters, Geometric Least Square Means are given, and estimates of the contrast with their 95% confidence intervals are back-transformed and therefore given in percentages. / b. The analysis results of inter-tap interval change (ms/min) and spatial error change (mm/min) have not been reported because they violated the normality assumption. / c. The analysis results of ratio of good: total taps, inter-tap interval change (ms/min), mean inter-tap interval (ms), number of halts, and spatial error change (mm/min) have not been reported because they violated the normality assumption. / d. The analysis results of angle frequency change (Hz/min), inter-tap interval change (ms/min), and mean inter-tap interval (ms) have not been reported because they violated the normality assumption.

CI, confidence interval; MDS-UPDRS III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III; SD, standard deviation; AUC, area under the curve.

TABLE 3 Correlation between each finger tapping endpoint and MDS-UPDRS III total score.

Category	Parameter	Placebo		Levodopa/carbidopa	
		r	P-value	r	P-value
Alternate index and middle finger tapping					
Speed	Total number of taps	0.08	0.7381	0.31	0.1935
	Mean inter-tap interval	-0.11	0.6599	-0.41	0.1001
Accuracy	Total taps inside target	0.06	0.8165	0.28	0.2478
	Ratio good: total taps	-0.17	0.4899	-0.23	0.3379
	Total spatial error	0.30	0.2159	0.50	0.0306
	Mean spatial error	0.35	0.1389	0.32	0.1769
Rhythm	Inter-tap interval sd	-0.06	0.8101	-0.10	0.6889
	Spatial error sd	-0.10	0.6931	0.02	0.9401
	Number of halts	0.22	0.4029	0.22	0.3959
Fatigue	<i>Inter-tap interval change</i>	0.14	0.5928	0.23	0.3758
	<i>Spatial error change</i>	-0.04	0.8635	0.37	0.1189
Alternate index finger tapping					
Speed	Total number of taps	-0.45	0.0454	-0.45	0.0457
	Mean inter-tap interval	0.50	0.0249	0.21	0.3764
Accuracy	Total taps inside target	-0.39	0.0849	-0.55	0.0120
	Ratio good: total taps	-0.24	0.3140	-0.45	0.0446
	Total spatial error	-0.23	0.3365	-0.04	0.8528
	Mean spatial error	0.11	0.6482	0.29	0.2123
Rhythm	Inter-tap interval sd	0.25	0.2822	0.32	0.1733
	Spatial error sd	-0.06	0.7906	0.10	0.6784
	Number of halts	-0.16	0.5022	-0.10	0.6703
Fatigue	<i>Inter-tap interval change</i>	-0.05	0.8397	-0.26	0.2661
	<i>Spatial error change</i>	0.12	0.6143	0.16	0.4984
Thumb-index finger tapping					
Speed	Total number of taps	-0.65	0.0024	-0.21	0.4255
	Mean inter-tap interval	0.70	0.0013	0.17	0.5249
	Mean opening velocity	-0.66	0.0027	-0.24	0.3628
	Mean closing velocity	-0.65	0.0025	-0.50	0.0426
Amplitude	Mean tapping amplitude	-0.27	0.2748	-0.41	0.1021
	Peak frequency AUC	-0.28	0.2376	-0.29	0.2553
Rhythm	Inter-tap interval sd	0.45	0.0586	0.66	0.0037
Fatigue	<i>Inter-tap interval change</i>	-0.09	0.7160	-0.22	0.3886
	Tapping amplitude change	-0.11	0.6577	0.11	0.6732
	Angle frequency change	0.08	0.7418	0.26	0.3201

P-values <0.05 are shown in **bold**. Correlation coefficient r and p-value are given for both the placebo and the levodopa/carbidopa group. For parameters in italics, no model could be fitted. MDS-UPDRS III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III; SD, standard deviation; AUC, area under the curve.

FIGURE 1 Depiction of the 3 finger tapping tasks: alternate index and middle finger tapping (A), alternate index finger tapping (B), and thumb-index finger tapping (C).

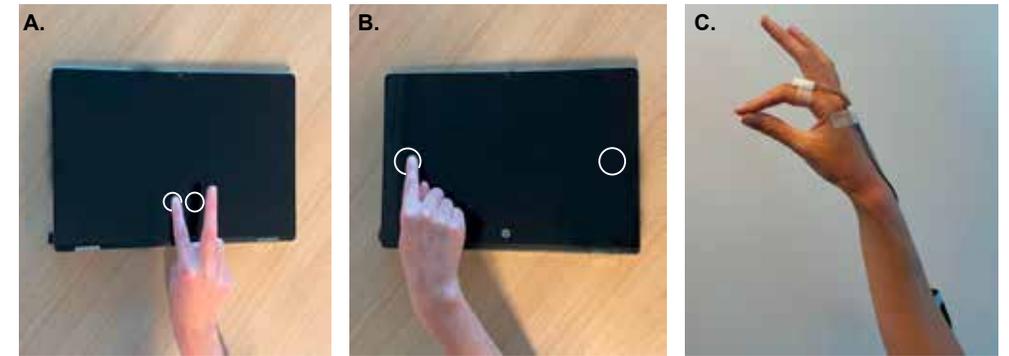
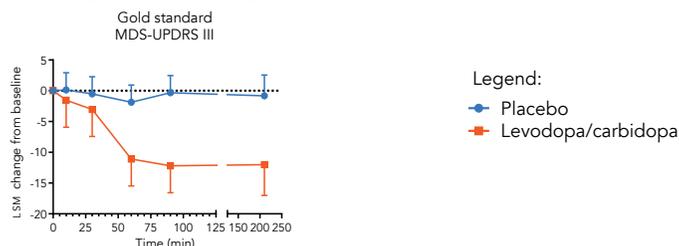
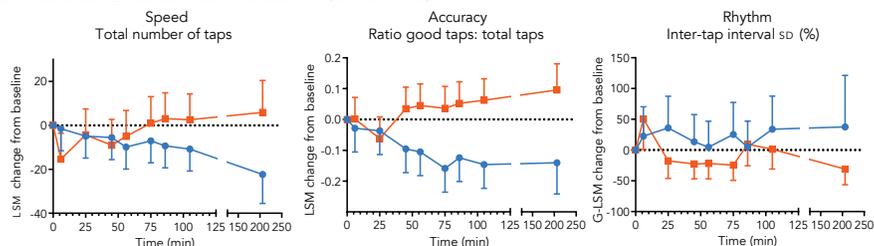


FIGURE 2 (G-)LSM change from baseline with 95% confidence intervals plotted over time for MDS-UPDRS III (A) and for 3 endpoints of the alternate index and middle finger tapping (B), alternate index finger tapping (C), and thumb-index finger task (D).

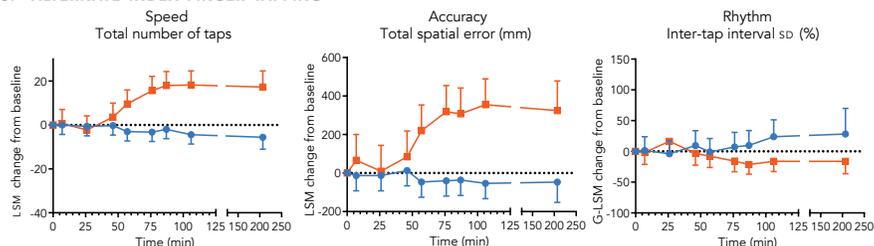
A. MDS-UPDRS III TOTAL SCORE



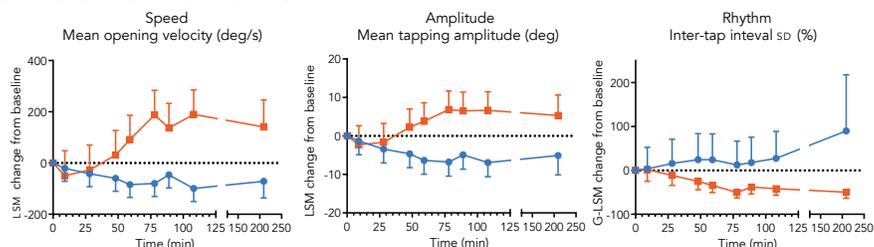
B. ALTERNATE INDEX AND MIDDLE FINGER TAPPING



C. ALTERNATE INDEX FINGER TAPPING



D. THUMB-INDEX FINGER TAPPING



G-LSM, geometric-least square means; LSM, least square means; MDS-UPDRS III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III; SD, standard deviation.

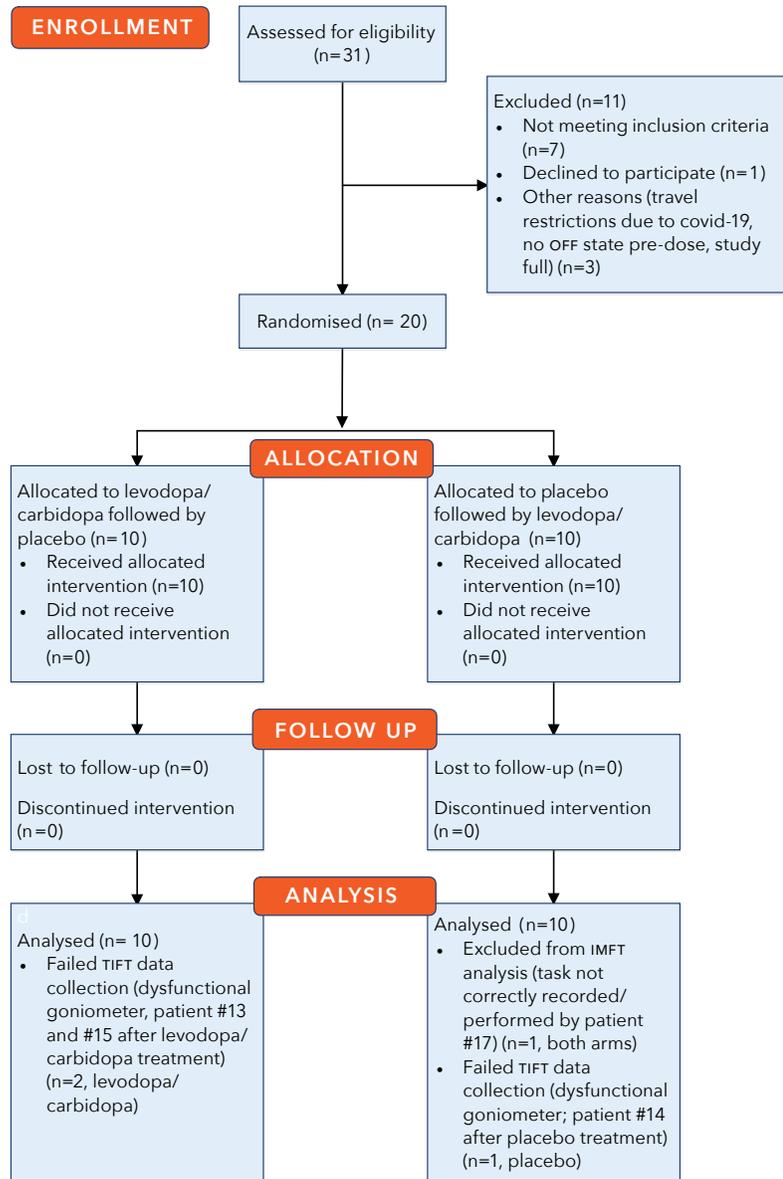
SUPPLEMENTARY MATERIAL

SUPPLEMENTAL TABLE 1 Description of finger tapping endpoints.

Endpoint (unit)	Definition	Calculated for task
Total number of taps	Sum of all taps.	IMFT, IFT, TIFT
Total taps inside target	Taps within the target circle.	IMFT, IFT
Ratio good: total taps	Taps on the correct side (left/right) of the screen divided by total number of taps.	IMFT, IFT
Mean inter-tap interval (ms)	Mean time between two consecutive taps.	IMFT, IFT, TIFT
Inter-tap interval sd (ms)	Standard deviation of all inter-tap intervals.	IMFT, IFT, TIFT
Inter-tap interval change (ms/min)	Change of the inter tap intervals over time.	IMFT, IFT, TIFT
Number of halts	Number of taps where the inter-tap interval is larger than 2 * mean inter-tap interval.	IMFT, IFT
Total spatial error (mm)	Sum of the Euclidean distances between each tap and the center of the target.	IMFT, IFT
Mean spatial error (mm)	Total spatial error divided by total number of taps.	IMFT, IFT
Spatial error sd (mm)	Standard deviation of Euclidean distances of each tap from the targets' center point.	IMFT, IFT
Spatial error change (mm/min)	Slope from linear regression of each tap's spatial error against time.	IMFT, IFT
Mean tapping amplitude (degrees)	Mean of each finger tap's maximum amplitude.	TIFT
Tapping amplitude change (degrees/s)	Change of tapping amplitude over time.	TIFT
Peak frequency area under the curve (degrees ²)	The total power around the peak frequency, i.e., the area under the curve (AUC) in the power spectrum around the peak frequency. Measure of amplitude.	TIFT
Angle frequency change (Hz/min)	Change in peak tapping frequency over time.	TIFT
Mean opening velocity (degrees/s)	Average of the amplitude (i.e., angle) travelled per second for each tap when moving the index finger away from the thumb (opening); velocity extracted from the derivative of the amplitude.	TIFT
Mean closing velocity (degrees/s)	Average of the amplitude (i.e., angle) travelled per second for each tap when moving the index finger towards the thumb (closing); velocity extracted from the derivative of the amplitude.	TIFT

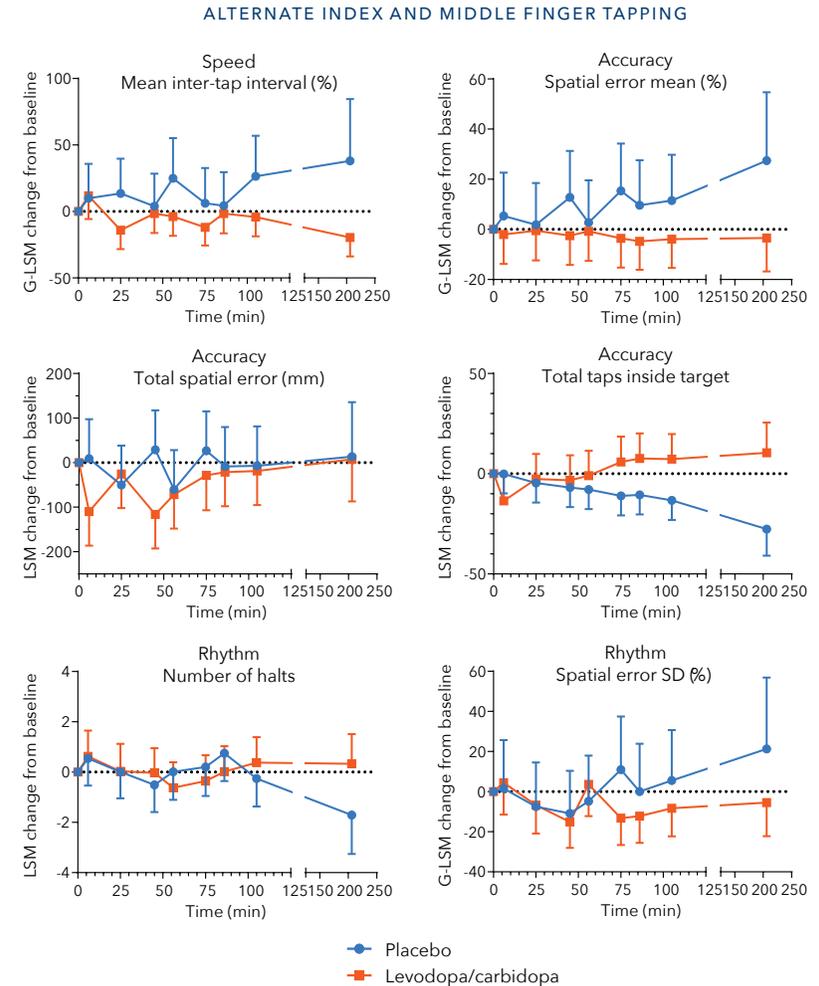
IMFT, alternate index and middle finger tapping; IFT, alternate index finger tapping; TIFT, thumb-index finger tapping.

SUPPLEMENTAL FIGURE 1 CONSORT flow diagram.

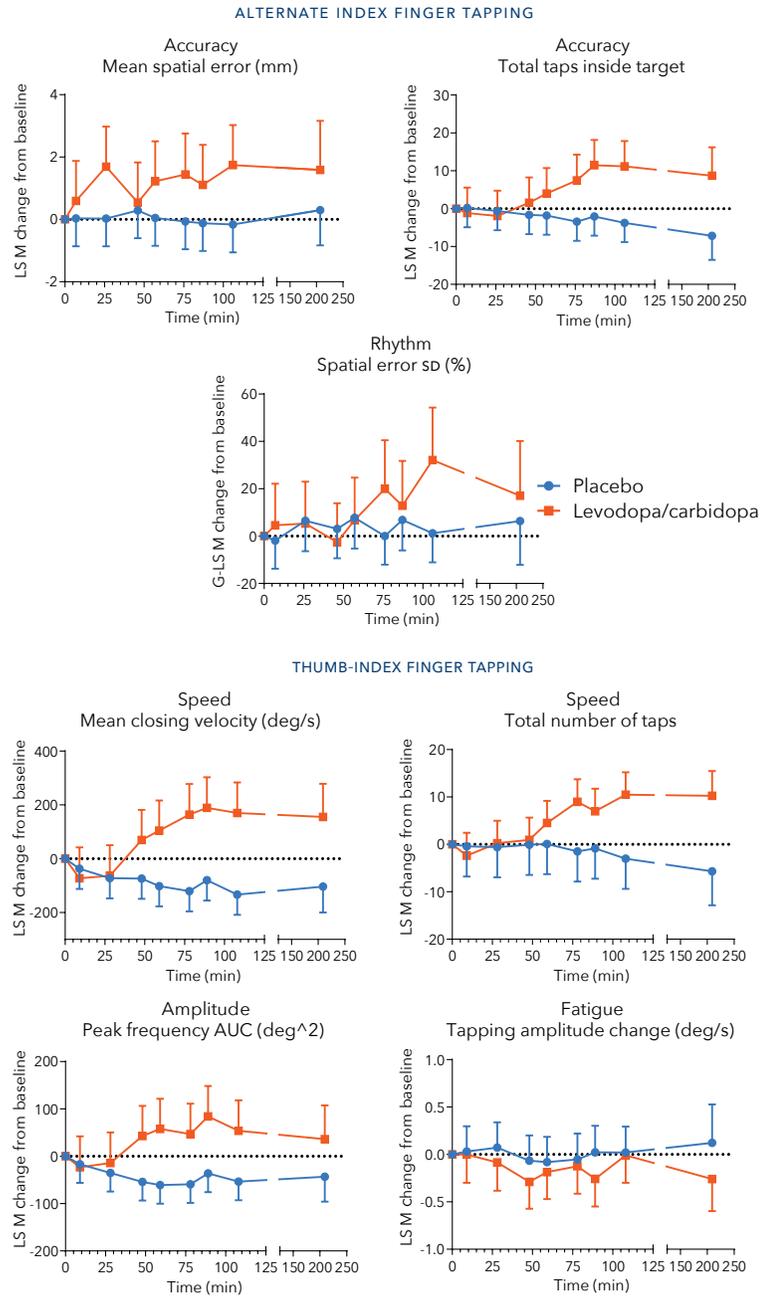


CONSORT, Consolidated Standards of Reporting Trials; TIFT, thumb-index finger tapping; IMFT, alternate index and middle finger tapping.

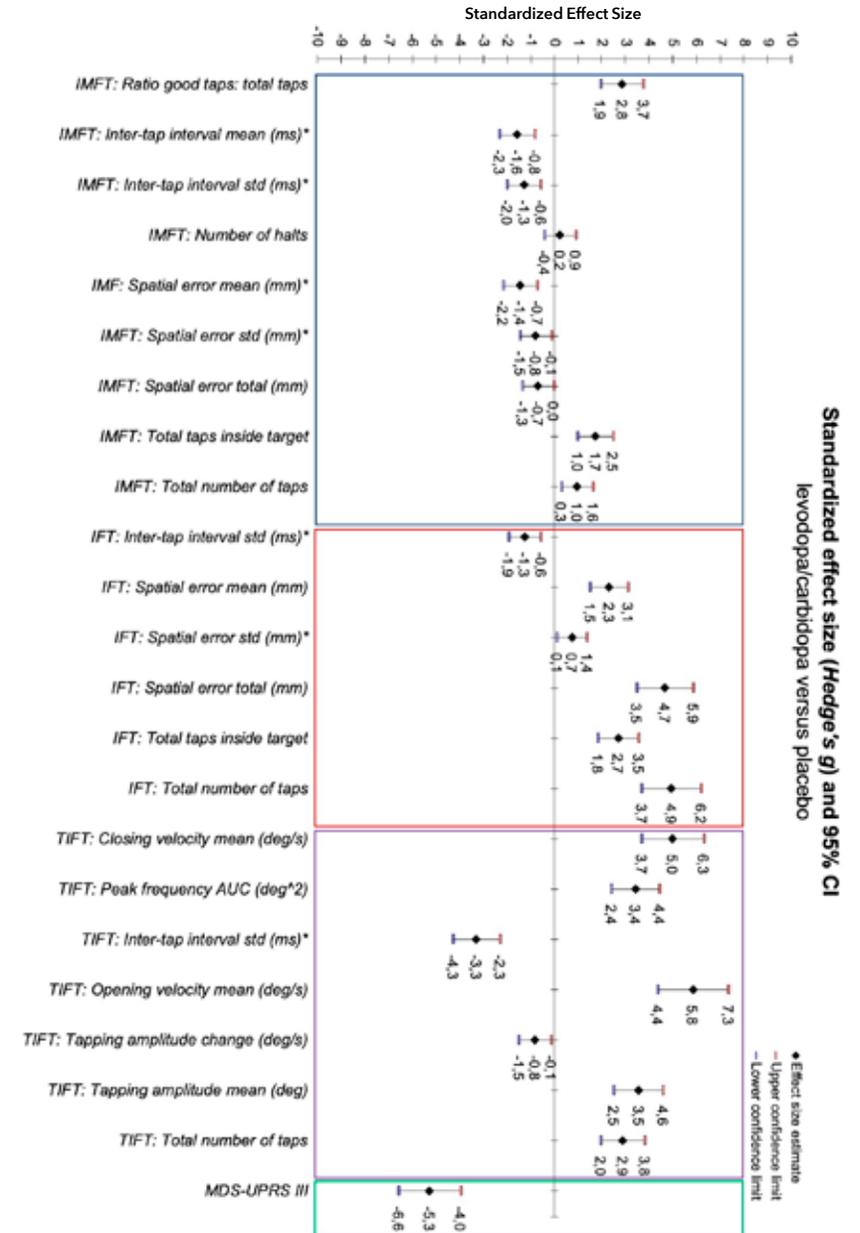
SUPPLEMENTAL FIGURE 2 (Geometric-) Least squares means ((G-)LSM) change from baseline with 95% confidence intervals plotted over time for the endpoints of the alternate index and middle finger tapping, alternate index finger tapping, and thumb-index finger tapping that were not depicted in Figure 2.



[continuation of Supplemental Figure 2]



SUPPLEMENTAL FIGURE 3 Standardized effect sizes.



CI, confidence interval; IMFT, alternate index and middle finger tapping; IFT, alternate index finger tapping; TIFT, thumb-index finger tapping.

REFERENCES

- Goetz CG, Tilley BC, Shaftman SR, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. *Mov Disord.* 2008;23(15):2129-2170. doi:10.1002/MDS.22340.
- Yokoe M, Okuno R, Hamasaki T, Kurachi Y, Akazawa K, Sakoda S. Opening velocity, a novel parameter, for finger tapping test in patients with Parkinson's disease. *Parkinsonism Relat Disord.* 2009;15(6):440-444. doi:10.1016/J.PARKRELDIS.2008.11.003.
- Hasan H, Burrows M, Athauda DS, et al. The BRadykinesia Akinesia INcoordination (BRAIN) Tap Test: Capturing the Sequence Effect. *Mov Disord Clin Pract.* 2019;6(6):462-469. doi:10.1002/MDC3.12798.
- Lee CY, Kang SJ, Hong SK, Ma H II, Lee U, Kim YJ. A Validation Study of a Smartphone-Based Finger Tapping Application for Quantitative Assessment of Bradykinesia in Parkinson's Disease. *PLoS One.* 2016;11(7):e0158852. doi:10.1371/JOURNAL.PONE.0158852.
- Ling H, Massey LA, Lees AJ, Brown P, Day BL. Hypokinesia without decrement distinguishes progressive supranuclear palsy from Parkinson's disease. *Brain.* 2012;135(4):1141-1153. doi:10.1093/BRAIN/AWS038.
- Stavroukoudis A, Larkin S, López Castellanos JR, et al. Tablet-Based Application for Objective Measurement of Motor Fluctuations in Parkinson Disease. *Digit Biomarkers.* 2017;1(2):126-135. doi:10.1159/000485468.
- Akram N, Li H, Ben-Joseph A, et al. Developing and assessing a new web-based tapping test for measuring distal movement in Parkinson's disease: a Distal Finger Tapping test. *Sci Rep.* 2022;12(1). doi:10.1038/S41598-021-03563-7.
- De Vleeschhauwer J, Broeder S, Janssens L, Heremans E, Nieuwboer A, Nackaerts E. Impaired Touchscreen Skills in Parkinson's Disease and Effects of Medication. *Mov Disord Clin Pract.* 2021;8(4):546-554. doi:10.1002/MDC3.13179.
- Espay AJ, Giuffrida JP, Chen R, et al. Differential response of speed, amplitude, and rhythm to dopaminergic medications in Parkinson's disease. *Mov Disord.* 2011;26(14):2504-2508. doi:10.1002/MDS.23893.
- Lee W, Evans A, Williams DR. Validation of a Smartphone Application Measuring Motor Function in Parkinson's Disease. *J Parkinsons Dis.* 2016;6(2):371-382. doi:10.3233/JPD-150708.
- Lipp MM, Batycky R, Moore J, Leinonen M, Freed MI. Preclinical and clinical assessment of inhaled levodopa for OFF episodes in Parkinson's disease. *Sci Transl Med* 2016; 8(360):360ra136. <https://doi.org/10.1126/scitranslmed.aad8858>.
- Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov Disord.* 2010;25(15):2649-2653. doi:10.1002/MDS.23429.
- Makai-Bölöni S, Thijssen E, Van Brummelen EMJ, Groeneveld GJ, Doll RJ. Touchscreen-based finger tapping: Repeatability and configuration effects on tapping performance. *Virmani T, ed. PLoS One.* 2021;16(12):e0260783. doi:10.1371/JOURNAL.PONE.0260783.
- Brown H, Prescott R. Crossover trials. In: *Applied Mixed Models in Medicine.* 2nd ed. John Wiley & Sons, Ltd; 2006:272-274.
- Fernandez L, Huys R, Issartel J, Azulay JP, Eusebio A. Movement speed-accuracy tradeoff in Parkinson's disease. *Front Neurol.* 2018;9(OCT):897. doi:10.3389/FNEUR.2018.00897/BIBTEX.

CHAPTER 7

Treatment detection and Movement Disorder Society-Unified Parkinson's Disease Rating Scale, part III estimation using finger tapping tasks

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ABSTRACT

Background The validation of objective and easy-to-implement biomarkers that can monitor the effects of fast-acting drugs among Parkinson's disease (PD) patients would benefit antiparkinsonian drug development.

Methods We developed composite biomarkers to detect levodopa/carbidopa effects and to estimate PD symptom severity. For this development, we trained machine learning algorithms to select the optimal combination of finger tapping task features to predict treatment effects and disease severity. Data were collected during a placebo-controlled, crossover study with 20 PD patients. The alternate index and middle finger tapping (IMFT), alternative index finger tapping (IFT), and thumb-index finger tapping (TIFT) tasks and the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS III) were performed during treatment. We trained classification algorithms to select features consisting of the MDS-UPDRS III item scores; the individual IMFT, IFT, and TIFT; and all three tapping tasks collectively to classify treatment effects. Furthermore, we trained regression algorithms to estimate the MDS-UPDRS III total score using the tapping task features individually and collectively.

Results The IFT composite biomarker had the best classification performance (83.50% accuracy, 93.95% precision) and outperformed the MDS-UPDRS III composite biomarker (75.75% accuracy, 73.93% precision). It also achieved the best performance when the MDS-UPDRS III total score was estimated (mean absolute error: 7.87, Pearson's correlation: 0.69).

Conclusion We demonstrated that the IFT composite biomarker outperformed the combined tapping tasks and the MDS-UPDRS III composite biomarkers in detecting treatment effects. This provides evidence for adopting the IFT composite biomarker for detecting antiparkinsonian treatment effect in clinical trials.

INTRODUCTION

Parkinson's disease (PD) motor impairments can be characterized as slow and rigid and can lead to a gradual reduction in movement speed over time.¹ The recommended instrument for assessing the severity of PD motor symptoms is the Movement Disorder Society's revised version of the Unified Parkinson's Disease Rating Scale, part III (MDS-UPDRS III).² The MDS-UPDRS III offers a reliable and valid metric for evaluating motor manifestations in each body area affected by PD.³⁻⁵ There are two main limitations of the MDS-UPDRS III. First, the MDS-UPDRS III requires approximately 15 minutes to complete with a trained rater, therefore making it time consuming and labor intensive.⁶ Thus, MDS-UPDRS III is not ideal for demonstrating the time of onset of fast-acting dopaminergic drugs, such as the inhaled forms of levodopa/carbidopa and apomorphine.^{7,8} Second, the MDS-UPDRS III provides only a coarse rating of motor function and therefore cannot identify or differentiate between specific kinematics of finger movements.³ As fine motor control abnormalities are typically the first manifestations of motor impairments in PD patients, it is important to develop composite biomarkers that are sensitive to these changes.⁹ To address these limitations, there is a demand for biomarkers that detect fine-grained changes in motor function and are congruent with the MDS-UPDRS.

Finger tapping tasks provide insights into fine motor activity^{10,11} and have been shown to be quick, effective, and simple assessments for estimating MDS-UPDRS motor disability^{12,13} and assessing antiparkinsonian drug effects.¹⁴⁻¹⁹ These tasks provide insights into finger and forearm movement speed, accuracy, amplitude, frequency, rhythm, and fatigue.^{10,14,20,21} PD patients often experience tremors, stiffness, and difficulty with movement, which can significantly impact their ability to perform daily activities, including buttoning a shirt, typing on a keyboard, or using utensils.^{22,23} As patients want treatments that will improve their ability to carry out daily activities, measuring motor function through tapping biomarkers can provide a more direct and meaningful assessment of the impact of treatments

on patients' lives. Therefore, the tapping tasks could be considered of interest to both clinicians and patients.

The complexity of parkinsonism motor impairment manifestations cannot be captured by a single biomarker. By exploiting machine learning algorithms, we can combine multiple objective biomarkers into a single composite biomarker that would represent a multidimensional characterization of PD.²⁴ Previous studies have demonstrated that composite biomarkers could effectively differentiate between PD and healthy controls and estimate MDS-UPDRS III symptom severity.²⁵⁻²⁷ This study investigates the accuracy and sensitivity of composite tapping biomarkers to detect drug effects and to estimate disease severity among PD patients.

PATIENTS AND METHODS

This is an extension of a previous study that investigated the reliability of tapping tasks to detect the longitudinal effects of levodopa/carbidopa and to determine the correlation of the tapping features with the MDS-UPDRS III.¹⁴ The study was conducted at the Centre for Human Drug Research (CHDR) in Leiden, the Netherlands, between July and November 2020 and is registered in the Netherlands Trial Register (trial NL8617).

Study overview

We conducted a double-blind, placebo-controlled, randomized, two-way crossover study with levodopa/carbidopa in 20 PD patients that had recognizable *OFF* episodes (symptoms not adequately controlled by their medication²⁸). Patients received a semi-individual dose of the investigational drug. To ensure an *OFF-ON* transition, the patients were given a supramaximal dose that was at least 25% higher than their usually administered morning dose.²⁹

Patient criteria

Enrolled patients had a clinical diagnosis of PD, as confirmed by a neurologist, and a classification of a Hoehn-Yahr stages I to III during

their *ON* state by an investigator. Patients were included if they were between ages 20 and 85 years during screening, experienced self-described motor fluctuations, and were taking oral antiparkinsonian medication. Patients were excluded if they had known conditions that would affect levodopa/carbidopa treatment or study compliance, such as previous intolerance, drug dependence, or psychiatric disease.

Assessments

MDS-UPDRS III

We selected the MDS-UPDRS III as the gold standard for the purposes of this study. The MDS-UPDRS III was conducted by trained raters at CHDR. The examination took on average 15 minutes to complete. It was performed pre-dose and at 10, 30, 60, and 90 minutes after dosing.

FINGER TAPPING TASKS

All the tapping tasks were performed twice pre-dose and once at 10, 25, 45, 60, 75, 90, and 105 minutes after dosing. If the tapping tasks and MDS-UPDRS III were planned simultaneously, then tapping tasks were performed first.

ALTERNATE INDEX AND MIDDLE FINGER TAPPING AND ALTERNATE INDEX FINGER TAPPING

Each patient was provided with a touchscreen laptop equipped with the alternate index and middle finger tapping (IMFT) and alternate index finger tapping (IFT) tasks.¹⁰ The patients were instructed to use the hand that was most affected (if both hands were equally affected, to use their dominant hand) and to perform each task as fast and accurately as possible for 30 seconds. For the IMFT, patients were asked to tap between the two targets (2.5 cm apart) with their index and middle fingers. For the IFT, patients were asked to tap the targets (20 cm apart) with their index finger.

The IMFT and IFT require two different movements; the IMFT and IFT are dependent on fine finger and forearm movements, respectively.¹⁰ Each of the two tasks generated 43 features relating to speed (e.g., total number of taps), accuracy (e.g., spatial error),

rhythm (e.g., inter-tap interval SD), and fatigue (e.g., change in velocity) (Supplemental Table 1).^{10,14}

THUMB-INDEX FINGER TAPPING

A wireless goniometer (Biometrics Ltd, Newport, UK) was placed on the metacarpal and proximal phalanx of the index finger of the most affected hand (if both hands were equally affected, to use their dominant hand).^{10,14,30} Each patient was instructed to sit comfortably, hold up the hand, and tap the index finger on the thumb as widely and quickly as possible continuously for 15 seconds. The thumb-index finger tapping (TIFT) assesses unilateral sequential fine finger movements. The 25 features of the TIFT include progressive changes in amplitude, hesitations, and tapping speed during the task (Supplemental Table 1).¹⁴

Statistical analysis

All data preprocessing and statistical analyses were conducted using Python (version 3.8.0)³¹ and the Scikit-Learn library (version 1.0.1).³²

DATA PREPROCESSING

All features were visually and statistically inspected for normality using histograms and Shapiro-Wilk tests, respectively. Log or square root transformations were applied when the features were not normally distributed. Only features that were normally distributed were included in the analysis. Missing values were not imputed, and only complete cases were considered.

As the tapping composite biomarker is designed to be a proxy for overall motor function, we did not account for laterality of the tapping task in the biomarkers. The need for assessing the tapping tasks with both hands is therefore avoided, which could streamline the assessment process and reduce the burden on patients.

COMPOSITE BIOMARKERS

We developed 10 composite biomarkers. The composite biomarkers represented the baseline-uncorrected or baseline-corrected MDS-UPDRS III 18-item scores; all three tapping tasks combined; and the

IFT, IMFT, and TIFT tasks individually. From a statistical viewpoint, we corrected for baseline to remove any concomitant variability in the treatment response, which would therefore improve the precision of the treatment detection.³³ From a practical viewpoint, we considered using the baseline-uncorrected values to reduce the number of measurements needed for treatment classification. The baseline-uncorrected model would require only a single tapping assessment, whereas the baseline-corrected model would require two.

CROSS-VALIDATION

We applied a nested k-fold cross-validation strategy to assess the performance and the generalizability of the composite biomarkers.³⁴ In nested cross-validation, the outer fold assesses the performance of the model, whereas the inner fold performs the model and hyperparameter selection. In our study, the outer-fold step was repeated 100 times, with each iteration containing a different combination of training (80% of the data) and test sets (20%). Each outer training set was further split into an inner training (80% of the data) and validation sets (20%). The inner-fold step was repeated 50 times, and the best-performing inner model would be evaluated in the outer fold. The final results would be represented as the averaged and standard deviation of the models selected by each outer fold.³⁴

For the classification and regression models, we applied a group-shuffle split (same distribution of placebo and active treatments in each split) and a stratified-shuffle split (same distribution of MDS-UPDRS III scores in each split), respectively. To stratify the MDS-UPDRS III scores, we assigned each score to one of three binned ranges (e.g., the baseline-corrected MDS-UPDRS III binned ranges were [-13, -8.76], [-8.76, -4.53], and [-4.53, 0.3]). Each outer fold had the same distribution of binned ranges. Stratification was not applied to the inner fold, as the small sample size would limit the number of samples available per bin. Within each inner fold, all features were standardized by subtracting the mean and scaling to the unit variance. To identify the features that were predictive of the outcomes, we identified features that were selected at least once by all outer-fold models.³⁴

CLASSIFICATION OF ACTIVE OR PLACEBO TREATMENTS

Classification models were trained to classify the active or placebo treatments. As we intended to predict the probability of treatment at all time points, we chose the last measurements to train the models. The MDS-UPDRS III classification model was trained on the 90-minute MDS-UPDRS III item scores.¹⁴ The tapping classification models were trained on measurements taken immediately after the MDS-UPDRS III starting at 105 minutes.

To identify the optimal classification model, we compared three classification models: support vector machines, logistic regression, and linear discriminant analysis (LDA). These classification models were selected as they are easy to implement and to interpret.³⁵⁻³⁷ Previous studies have also used these algorithms to classify PD diagnosis or estimate MDS-UPDRS III.³⁸⁻⁴¹ Models were compared based on their mean accuracy, precision, and F1 scores.⁴⁰

In addition, each model selected by the outer folds was used to predict the treatment at the other time points, with the 20% of patients who were not used for training. This would allow researchers to identify at which time point treatment effects are detected. For each time point, the mean and standard deviation of the class probabilities were based on the predicted log-odd ratios from each fold. Additionally, these probabilities were used to estimate the repeatability and effect size. The repeatability was assessed by calculating the intraclass correlation coefficients (ICC) using the placebo results only. Using a random intercept model with the intercept and time point as fixed effects, the ICC was calculated by dividing the between-subject variance by the sum of the between-subject and within-subject variances. The effect size was calculated using all available data and a random intercept model with intercept, time point, treatment, and interaction between time point and treatment as fixed effects. In addition, the effect size was calculated as the contrast between the probabilities after treatment and the averaged baseline probabilities divided by the square root of the sum of the between-subject and within-subject variations.

ESTIMATION OF THE MDS-UPDRS III TOTAL SCORE

To assess if the tapping composite biomarkers (baseline uncorrected and baseline corrected) could estimate the MDS-UPDRS III total score, linear regression with elastic-net regularization (optimized for α and the l1 ratio) was used to predict the MDS-UPDRS III total score at 90 minutes using the 105-minute tapping biomarkers. These two time points were compared, as it was previously shown that the IFT and TIFT showed significant and moderate-to-strong correlations with the MDS-UPDRS III.¹⁴ Further, the 90- and 105-minute tapping tasks were equally as close to the 90-minute MDS-UPDRS III in timing and therefore we assumed would perform equally well.

To assess the performance of the models, we estimated the mean absolute error (MAE) of the outer-fold models. We evaluated the correlation between the predicted and true MDS-UPDRS III scores at all time points for each outer-fold model. Like the classification models, the MDS-UPDRS III scores were estimated at other time points with the 20% patients who were not used for training. Additionally, as for the classification models, those data were also used to estimate the repeatability and effect size.

RESULTS

Data collected

Twenty PD patients participated in this study. An overview of the demographic and disease characteristics of the patients was published previously;¹⁴ 14 patients were male, and their ages ranged from 48 to 70 years. Patients received one to four capsules of 100/25 mg levodopa/carbidopa as they had a supramaximal morning levodopa equivalent dose (LED) ranging from 47 to 391 milligrams. The median MDS-UPDRS III score (when using regular medication) was 23 and 22 on their placebo and active treatment days, respectively.¹⁴

We analyzed 31 IMFT, 31 IFT, and 25 TIFT features. No features were excluded due to nonnormal distribution. Due to goniometer

damage, we had missing data for 1 patient in the placebo condition and 2 patients in the active condition. As 6 patients had difficulties performing the IMFT, this led to missing data. However, the missing data were equally distributed across the treatment conditions and therefore deemed missing at random.

Classification of placebo and active treatments

We found that the LDA classifier consistently yielded the highest accuracy for all models (for both baseline uncorrected and baseline corrected); thus, we reported only the LDA results.

CLASSIFICATION OF TREATMENT EFFECTS

The best-performing baseline-uncorrected composite biomarker, the IFT, yielded an accuracy, precision, F1 score, and large effect size of 68.50%, 70.23%, 68.93%, and 1.60 respectively (Table 1). The best-performing baseline-corrected composite biomarker, the TIFT, achieved a higher average accuracy, precision, F1 score, and large effect size of 83.50%, 93.95%, 80.09%, and 2.58. Both models outperformed the MDS-UPDRS III classification models across all metrics. The IFT features that were mutually identified as important features for the baseline-uncorrected and baseline-corrected classification models were related to accuracy (e.g., spatial errors and the bivariate contour ellipse area), fatigue (e.g., velocity changes), and velocity (e.g., inter-tap intervals) (Figure 1).

CLASSIFICATION OF TREATMENT EFFECTS AT ALL TIME POINTS

In Figure 2, the classification models were applied to all time points, showing the mean predicted probability of an active (>0.5) or placebo treatment (<0.5). In the baseline-corrected IFT, TIFT, and MDS-UPDRS III models, the mean predicted probability of a patient receiving a placebo treatment was consistently less than 0.5. In contrast, when active treatment was administered, the baseline-corrected IFT and MDS-UPDRS III model had a mean predicted probability above 0.5 from 60 minutes onward. The baseline-corrected IMFT and TIFT models crossed the 0.5 thresholds after 45 minutes. We found that the baseline-corrected IFT biomarker determined a large effect size

(0.81) at 30 minutes, whereas the baseline-uncorrected IFT biomarker reached a large effect size of 0.84 at 60 minutes. The MDS-UPDRS III achieved a large effect size at 60 minutes (1.69 and 1.04 for baseline corrected and baseline uncorrected, respectively) (Supplemental Figure 2). The MDS-UPDRS III demonstrated higher repeatability than the tapping tasks. Whereas the baseline-uncorrected MDS-UPDRS III biomarker obtained an excellent ICC, the IFT and TIFT both achieved good ICCs (0.78, 0.80).⁴² However, the ICCs of the baseline-corrected MDS-UPDRS III and the IFT, IMFT, and TIFT biomarkers decreased to a moderate ICC range between 0.52 and 0.66.⁴²

Estimation of MDS-UPDRS III

The mean MDS-UPDRS III total scores at 90 minutes for the placebo and active treatments were 33.5 and 22.0, respectively. When baseline-corrected, the mean MDS-UPDRS III scores for the placebo and active treatments were 0.3 and -13.0, respectively (Figure 3).

ESTIMATION OF MDS-UPDRS III

The best-performing baseline-uncorrected regression models were the TIFT and IFT composite biomarkers, which achieved the lowest average MAE of 10.31 and 10.36, respectively. In addition, the TIFT and IFT showed large effect sizes of 1.47 and 2.23, respectively, when estimating the MDS-UPDRS III. The best-performing baseline-corrected model was the IFT composite biomarker, which yielded the lowest average MAE of 7.87. For both the baseline-uncorrected and baseline-corrected models, the best-performing composite biomarkers outperformed that of the composite biomarkers of the three tasks. For the IFT features, the features that were mutually selected by both models were similar to that of the IFT classification features (Figure 2; Supplemental Figure 1).

ESTIMATION OF MDS-UPDRS III AT ALL TIME POINTS

The predicted and true MDS-UPDRS III scores were significantly correlated for the baseline-corrected and baseline-uncorrected models (Table 2). Once again, the best positive correlations were achieved by the TIFT baseline-uncorrected composite biomarker

($r=0.58$, $p<0.01$) and the IFT baseline-corrected composite biomarker ($r=0.69$, $p<0.01$). The greatest difference in the true MDS-UPDRS III scores between the placebo and active treatment interventions was at 90 minutes (Figure 3). The tapping tasks achieved a moderate to good ICC (Table 2).

DISCUSSION

Detection of treatment effects

The IFT biomarker (baseline corrected and baseline uncorrected) was, on average, more predictive of and more sensitive to treatment effects than the MDS-UPDRS III biomarker in terms of accuracy, precision, and clinical significance (as supported by the effect-size performances) (Table 1). This is significant as the ability to detect changes in aspects of motor function that may be missed by traditional assessments allows for a more sensitive measure of treatment efficacy. This can be valuable for detecting small and early changes in motor function that are indicative of a treatment response. The most important IFT features used to classify treatment effects are in concert with previous studies (Figure 1) that also identified that forearm movements relating to velocity, amplitude, and rhythm are sensitive to antiparkinsonian drug effects.^{10,15,43,44}

We demonstrated that treatment effects were detected at 45 and 60 minutes for the TIFT and IFT composite biomarkers, respectively (Figure 2). This finding is notable as the mean onset of levodopa/carbidopa action is about 50 minutes.⁴⁵ This suggests that tapping tasks can detect the onset of oral levodopa/carbidopa. The MDS-UPDRS III was not performed at 45 minutes, so it could not be determined whether the MDS-UPDRS III biomarker could detect treatment effects at 45 minutes. These findings further propound that the tapping tasks are practical and sensitive composite biomarkers for detecting motor response changes induced by antiparkinsonian drugs.⁴⁶ Further, the large effect sizes can potentially reduce sample size requirements and enhance power for future tapping task trials that assess treatment effects.

The performance of the classification models (except for the ICC) improved when the features were baseline corrected. Despite this, both models provide practical and clinical value. The baseline-uncorrected models required only a single measurement and represent the current motor function status. The baseline-corrected models require two measurements and represent the changes in motor function over time. The increased performance suggests that treatment response is dependent on the patient's tapping profile during their *OFF* state and adjusting for baseline removes variation in the levodopa/carbidopa response.

Estimation of MDS-UPDRS III

We found that the baseline-corrected IFT biomarker, despite yielding the best performance among all the biomarkers, achieved a prediction error of approximately eight points and was significantly moderately correlated using the MDS-UPDRS III. The prediction error is comparable to existing sensor-based composite biomarkers used to estimate the MDS-UPDRS III. Studies using data sourced from an Axitvity AX3 (placed on the wrist and back or only the wrist) to estimate the gold standard achieved an MAE ranging from 4.29 to 6.29 points.^{47,48}

The tapping biomarkers predicted a smaller range of MDS-UPDRS III scores compared to that of the true MDS-UPDRS III scores (Figure 3). It is likely due to using only hand and forearm motor function assessments to predict the MDS-UPDRS III total scores, which includes motor assessments of other regions affected by PD, such as gait, facial expression, and speech.⁴ As the correlations of the true and predicted MDS-UPDRS III scores were moderate (Table 2), the tapping biomarkers still showed concurrent validity with the gold standard. This suggests that the tapping biomarkers could provide clinicians with an understanding of the acute effects of drugs on motor fluctuations within a short monitoring period.

Despite the discrepancies between the true and predicted MDS-UPDRS III total scores, with the advancements in technology, it is not unusual for the performance of new clinical assessments to

outperform the current gold standard. However, the discrepancy between the two assessments influences the accuracy estimates of the new clinical assessments, and as it would be interpreted as a prediction error.⁴⁹ Therefore, we argue that accurate estimation of the MDS-UPDRS III score is not essential for the adoption of the composite biomarker as a new complementary assessment for estimating symptom severity. Rather, the consequences resulting from the disagreement between the gold standard and the tapping composite biomarkers should be investigated.

Future work

We demonstrated that the tapping composite biomarkers could detect the onset of oral levodopa/carbidopa at 45 minutes. A follow-up study could investigate if the tapping composite biomarkers could detect an earlier onset of an even faster-acting antiparkinsonian drug, such as inhaled apomorphine that has an onset as early as 8 minutes.⁸ This would further validate the sensitivity of the tapping composite biomarker to detect fast-acting dopaminergic drug effects.

Our sample size may limit the generalizability of this study's findings as a small sample size may not be representative of the broader population of patients with PD, making it difficult to generalize its results to a larger population.⁵⁰ This is particularly relevant for PD studies, where the disease can manifest in different ways and progress at different rates in different patients. To mitigate the effect of the small sample sizes, we employed cross-validation to bootstrap and validate the models against different groups of patients. We propose conducting a follow-up trial to implement the tapping tasks among more PD patients with more diverse MDS-UPDRS III profiles. The data collected from the trial can be used as an independent data set to assess the validity, reliability, and generalizability of our current methods.

Although composite biomarkers have the advantage of capturing multiple aspects of motor function, the effects of individual components within the composite biomarker must be carefully examined to avoid misleading interpretations of the results. For example, a treatment that improves tapping speed but worsens

tapping rhythm may result in an overall neutral effect, making it difficult to interpret the treatment's efficacy. Like other composite measures, such as the MDS-UPDRS III total score, it is crucial to examine the effects of each feature of the composite biomarker separately, as well as in conjunction with the overall composite score, to better understand the treatment's impact on finger motor function.

CONCLUSION

In conclusion, the IFT biomarker was more predictive of and sensitive to the detection of treatment effects than the MDS-UPDRS III biomarker; therefore, the tapping biomarkers appear to hold promise for evaluating the early and rapid effects of antiparkinsonian drugs. Moreover, the tapping task is easy to perform and can be done in clinical settings as well as at home by patients themselves, making it a practical and convenient method for monitoring disease progression and treatment response. Using tapping biomarkers, clinicians can obtain accurate and reliable data that can inform treatment decisions in real time.

TABLE 1 The mean and standard deviations of the accuracy, precision, F1 score, and effect size for each biomarker (at 90 minutes for MDS-UPDRS III and 105 minutes for the tapping task) are based on the 100 outer folds of the nested cross-validation.

	Tasks	Accuracy	Precision	F1 score	ICC	Effect size
Baseline uncorrected	IMFT	56.90% (15.09%)	61.67% (22.53%)	56.56% (18.07%)	0.60 (0.25)	0.64 (0.57)
	IFT	68.50% (12.56%)	70.23% (16.31%)	68.93% (14.9%)	0.78 (0.21)	1.60 (0.82)
	TIFT	67.72% (15.84%)	65.55% (21.03%)	67.51% (18.22%)	0.78 (0.22)	1.14 (0.80)
	All three tasks	63.0% (16.91%)	64.35% (27.32%)	59.82% (23.16%)	0.68 (0.29)	0.91 (0.68)
	MDS-UPDRS III item scores	63.75% (11.25%)	61.20% (10.9%)	68.90% (11.52%)	0.92 (0.10)	1.03 (0.60)
Baseline corrected	IMFT	66.86% (15.23%)	70.83% (17.25%)	69.01% (15.04%)	0.57 (0.17)	1.44 (0.98)
	IFT	83.50% (10.74%)	93.95% (11.25%)	80.09% (14.92%)	0.53 (0.16)	2.58 (0.90)
	TIFT	77.86% (14.97%)	82.32% (21.43%)	74.72% (18.44%)	0.52 (0.17)	1.14 (0.80)
	All three tasks	77.98% (13.26%)	81.85% (21.15%)	74.66% (19.17%)	0.48 (0.18)	0.91 (0.61)
	MDS-UPDRS III item scores	75.75% (14.45%)	79.95% (17.64%)	73.93% (16.42%)	0.66 (0.11)	2.12 (1.25)

The mean ICC and standard deviation are based on all time points for the placebo condition only. The numbers in **bold** font represent the highest mean performance per model per column.

IMFT, alternate index and middle finger tapping; IFT, alternate index finger tapping; TIFT, thumb-index finger tapping; MDS-UPDRS III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III; ICC, intraclass correlation coefficient.

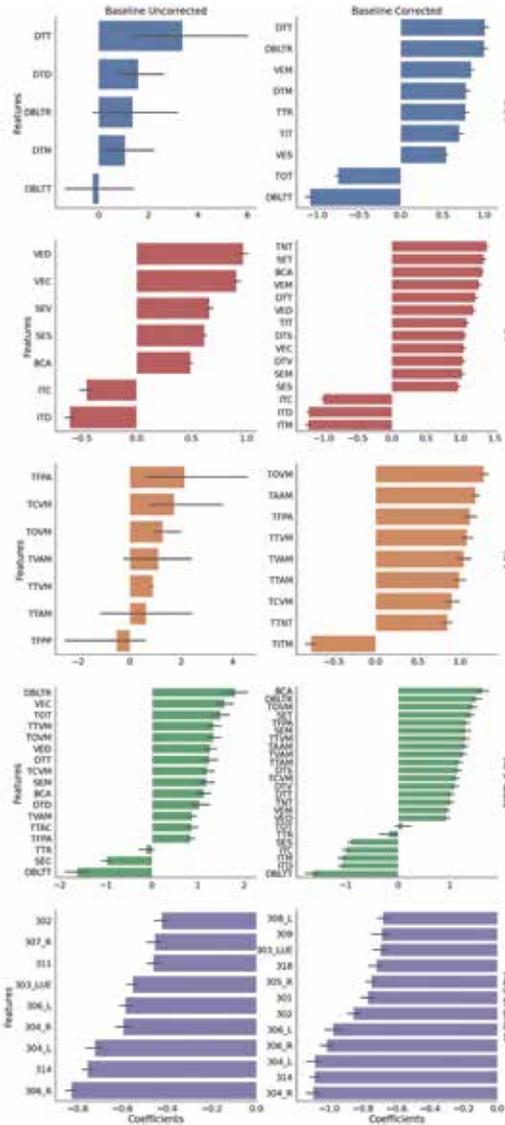
TABLE 2 Average correlation and ICC (95% CI) between the true and predicted MDS-UPDRS scores across all time points for the repeated nested cross-validation 100 outer-fold predictions.

	Tasks	Correlation coefficient (r)	P-value	ICC	Effect size
Baseline uncorrected	IMFT	0.10 (0.03, 0.16)	p<0.05 (<0.05, 0.05)	0.69 (0.65, 0.73)	0.67 (0.53, 0.81)
	IFT	0.52 (0.45, 0.59)	p<0.01 (<0.01, <0.01)	0.80 (0.76, 0.83)	1.02 (0.91, 1.14)
	TIFT	0.58 (0.53, 0.63)	p<0.05 (<0.01, <0.05)	0.78 (0.74, 0.82)	1.47 (1.27, 1.67)
	All three tasks	0.11 (0.04, 0.18)	p<0.05 (<0.05, 0.05)	0.66 (0.61, 0.71)	0.75 (0.62, 0.88)
	IMFT	0.34 (0.27, 0.40)	p<0.05 (<0.01, 0.06)	0.48 (0.44, 0.52)	1.10 (0.92, 1.28)
Baseline corrected	IFT	0.69 (0.65, 0.73)	p<0.001 (<0.001, <0.005)	0.45 (0.42, 0.48)	2.23 (2.01, 2.45)
	TIFT	0.65 (0.60, 0.69)	p<0.001 (<0.001, <0.001)	0.50 (0.46, 0.54)	1.37 (1.20, 1.54)
	All three tasks	0.56 (0.52, 0.61)	p<0.05 (<0.001, <0.05)	0.43 (0.39, 0.47)	1.06 (0.91, 1.21)

The average effect size (95% CI) between the baseline and 90 minutes for MDS-UPDRS III and 105 minutes for the tapping tasks was also included. The numbers in **bold** font represent the highest correlation coefficient (r), ICC, and effect size for each treatment and task.

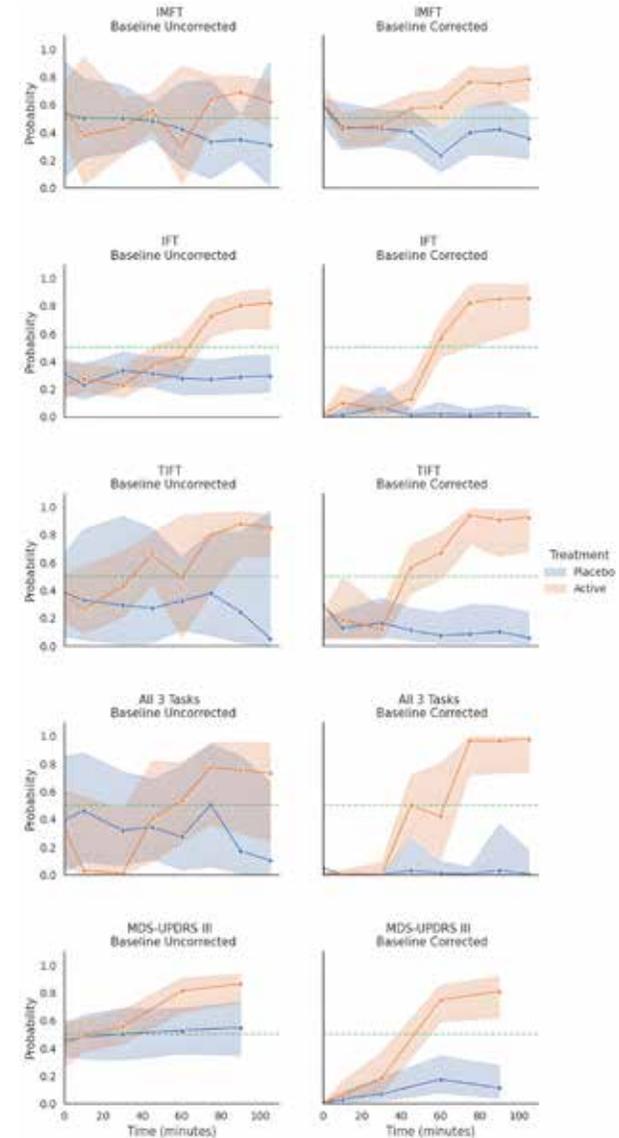
ICC, intraclass correlation coefficient; CI, confidence interval; MDS-UPDRS, Movement Disorder Society-Unified Parkinson's Disease Rating Scale; IMFT, alternate index and middle finger tapping; IFT, alternate index finger tapping; TIFT, thumb-index finger tapping.

FIGURE 1 The average feature coefficients of the respective features selected by the linear discriminant analysis (LDA) classifier for each finger tapping task feature and the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III item score features (baseline-uncorrected and baseline-corrected models). The error bars represent the 95% confidence interval.



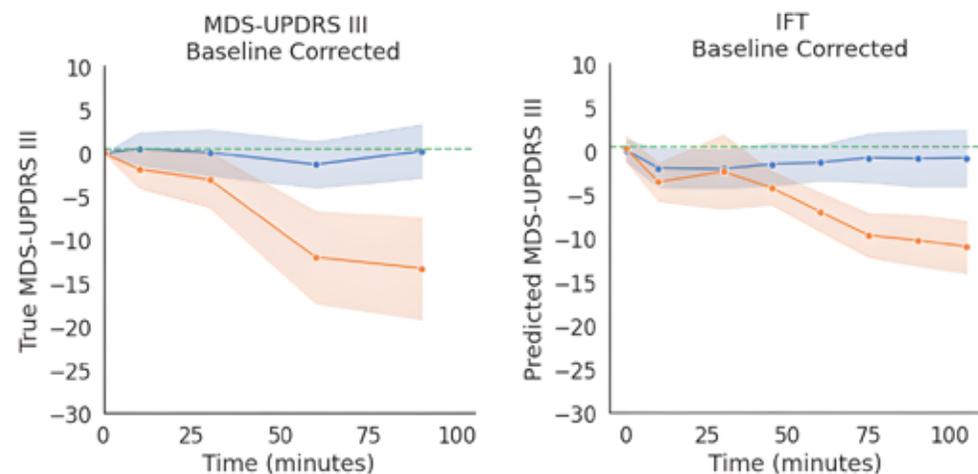
An overview of finger tapping task features is provided in Supplemental Table 1. IMFT, alternate index and middle finger tapping; IFT, alternate index finger tapping; TIFT, thumb-index finger tapping.

FIGURE 2 The mean predicted probability that active treatment was administered in the placebo (blue) and active (orange) treatment groups. The green dotted line represents the 0.5 decision boundary. The bands represent the 95% confidence interval.



IMFT, alternate index and middle finger tapping; IFT, alternate index finger tapping; TIFT, thumb-index finger tapping, MDS-UPDRS III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III.

FIGURE 3 Average true and predicted Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III scores with standard deviation from 0 to 105 minutes post dose for the placebo (blue) and active (orange) treatment interventions when corrected for baseline.



MDS-UPDRS III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III; IFT, alternate index finger tapping.

SUPPLEMENTARY MATERIAL

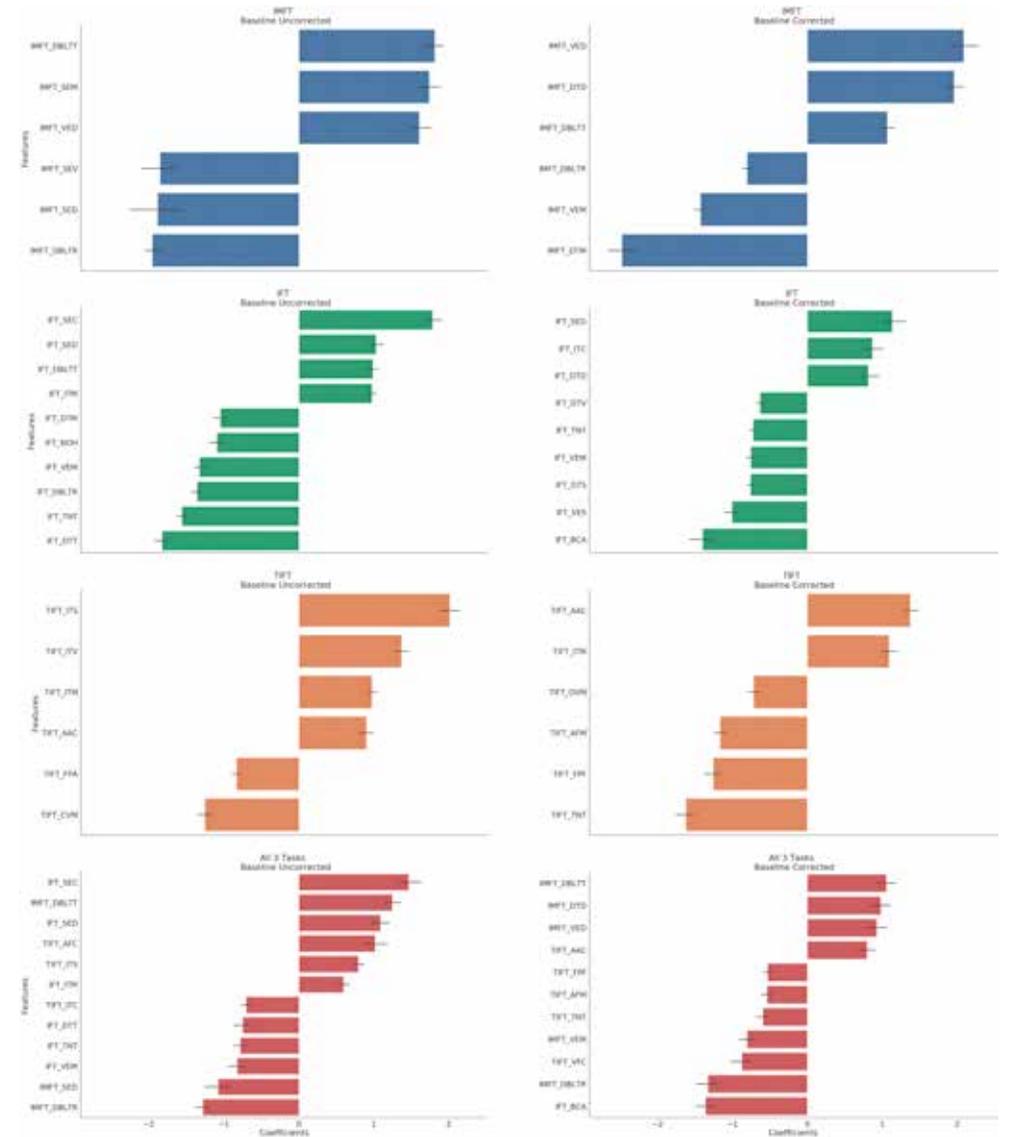
SUPPLEMENTAL TABLE 1 Overview of features for the alternate index and middle finger tapping (IMFT), alternate index finger tapping (IFT), and thumb-index finger tapping (TIFT) tasks.

Acronym	Description (unit)	Task
BCA	Bivariate contour ellipse area (represents the area of an ellipse which encompasses the fixation points) (mm ²)	IMFT, IFT
BCT	Angle of the bivariate contour ellipse's major axis (degree)	IMFT, IFT
DBLTR	Ratio good taps:total taps (a good tap is defined here as a tap on the correct side (left/right) of the touchscreen)	IMFT, IFT
DBLTT	Total number of double/missed taps	IMFT, IFT
DTC	Change in distance travelled over time, i.e., linear slope over all inter-tap distances (mm/min)	IMFT, IFT
DTD	Difference in mean distance travelled between the first 10 taps and the last 10 taps (cm)	IMFT, IFT
DTM	Mean distance travelled between two consecutive taps (cm)	IMFT, IFT
DTS	Standard deviation of all distances between consecutive taps (cm)	IMFT, IFT
DTT	Total distance travelled between consecutive taps (cm)	IMFT, IFT
DTV	Distance traveled: coefficient of variation (DTS/DTM * 100) (%)	IMFT, IFT
ITC	Change in inter-tap interval over time, i.e., linear slope over all inter-tap intervals (ms/min)	IMFT, IFT, TIFT
ITD	Difference in mean inter-tap interval between the first 10 taps and the last 10 taps (ms)	IMFT, IFT
ITM	Mean inter-tap interval (ms)	IMFT, IFT, TIFT
ITS	Standard deviation of all inter-tap intervals (ms)	IMFT, IFT, TIFT
ITV	Inter-tap interval: coefficient of variation (ITS/ITM * 100) (%)	IMFT, IFT, TIFT
NOH	Number of halts (taps where the inter-tap interval is larger than 2 * ITS)	IMFT, IFT
SEC	Change in spatial error over time, i.e., linear slope over all taps' spatial errors (mm/min). (Spatial error is the Euclidean distance of a tap from the targets' center point)	IMFT, IFT
SED	Difference in mean spatial error between the first 10 taps and the last 10 taps (mm)	IMFT, IFT
SEM	Mean spatial error (mm)	IMFT, IFT
SES	Standard deviation of the spatial errors (mm)	IMFT, IFT
SET	Total spatial error (mm)	IMFT, IFT
SEV	Spatial error: coefficient of variation (SES/SEM * 100) (%)	IMFT, IFT
TIT	Taps inside the target circle	IMFT, IFT

[continuation of Supplemental Table 1]

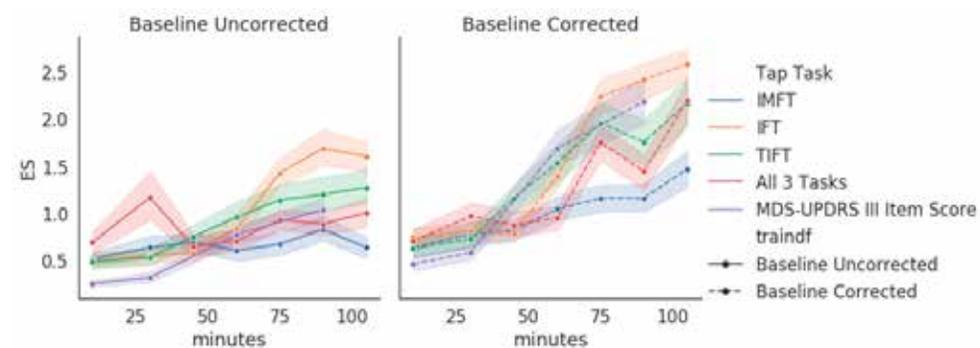
Acronym	Description (unit)	Task
TNT	Total number of taps	IMFT, IFT
TOT	Taps outside the target circle	IMFT, IFT
TTR	Ratio taps inside:total taps	IMFT, IFT
VEC	Change in velocity over time, i.e., linear slope over all inter- tap velocities (cm/min ²)	IMFT, IFT
VED	Difference in mean velocity between the first 10 taps and the last 10 taps (cm/min)	IMFT, IFT
VEM	Mean velocity (cm/min)	IMFT, IFT
VES	Standard deviation of the velocities (cm/min)	IMFT, IFT
VEV	Velocity: coefficient of variation (VES/VEM * 100) (%)	IMFT, IFT
AAC	Change in maximum angle amplitude over time (degree ² /s)	TIFT
AAM	Mean of the tapping angle amplitude (degree ²)	TIFT
AFC	Change in peak tapping frequency over time (Hz/min)	TIFT
AFM	Mean peak tapping frequency (Hz)	TIFT
CVM	Mean closing velocity: mean of the amplitude (i.e., angle) travelled per second for each tap when moving the index finger towards the thumb (closing); velocity extracted from the derivative of the amplitude (degree/s)	TIFT
FPA	Amplitude at peak frequency (degree ² /Hz)	TIFT
FPF	Peak frequency (Hz)	TIFT
FPP	The total power around the peak frequency, i.e., the area under the curve in the power spectrum around the peak frequency (measure of amplitude) (degree ²)	TIFT
OVM	Mean opening velocity: mean of the amplitude (i.e., angle) travelled per second for each tap when moving the index finger away from the thumb (opening); velocity extracted from the derivative of the amplitude (degree/s)	TIFT
TAC	Change in tapping amplitude over time, i.e., linear slope over all tapping amplitudes (degree/s)	TIFT
TAM	Mean tapping amplitude (degree)	TIFT
TVM	Mean angular tapping velocity (degree/s)	TIFT
VAM	Mean tapping angle velocity ((degree/s) ²)	TIFT
VFC	Change in tapping angle velocity frequency over time (Hz/min)	TIFT

SUPPLEMENTAL FIGURE 1 The average feature coefficients selected by the elastic-net linear regression models for each of the composite biomarkers under baseline-uncorrected and baseline-corrected conditions. The errors represent the 95% confidence intervals.



IMFT, alternate index and middle finger tapping; IFT, alternate index finger tapping; TIFT, thumb-index finger tapping.

SUPPLEMENTAL FIGURE 2 Effect sizes of each of the tapping tasks and the Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III composite biomarkers at each time point.

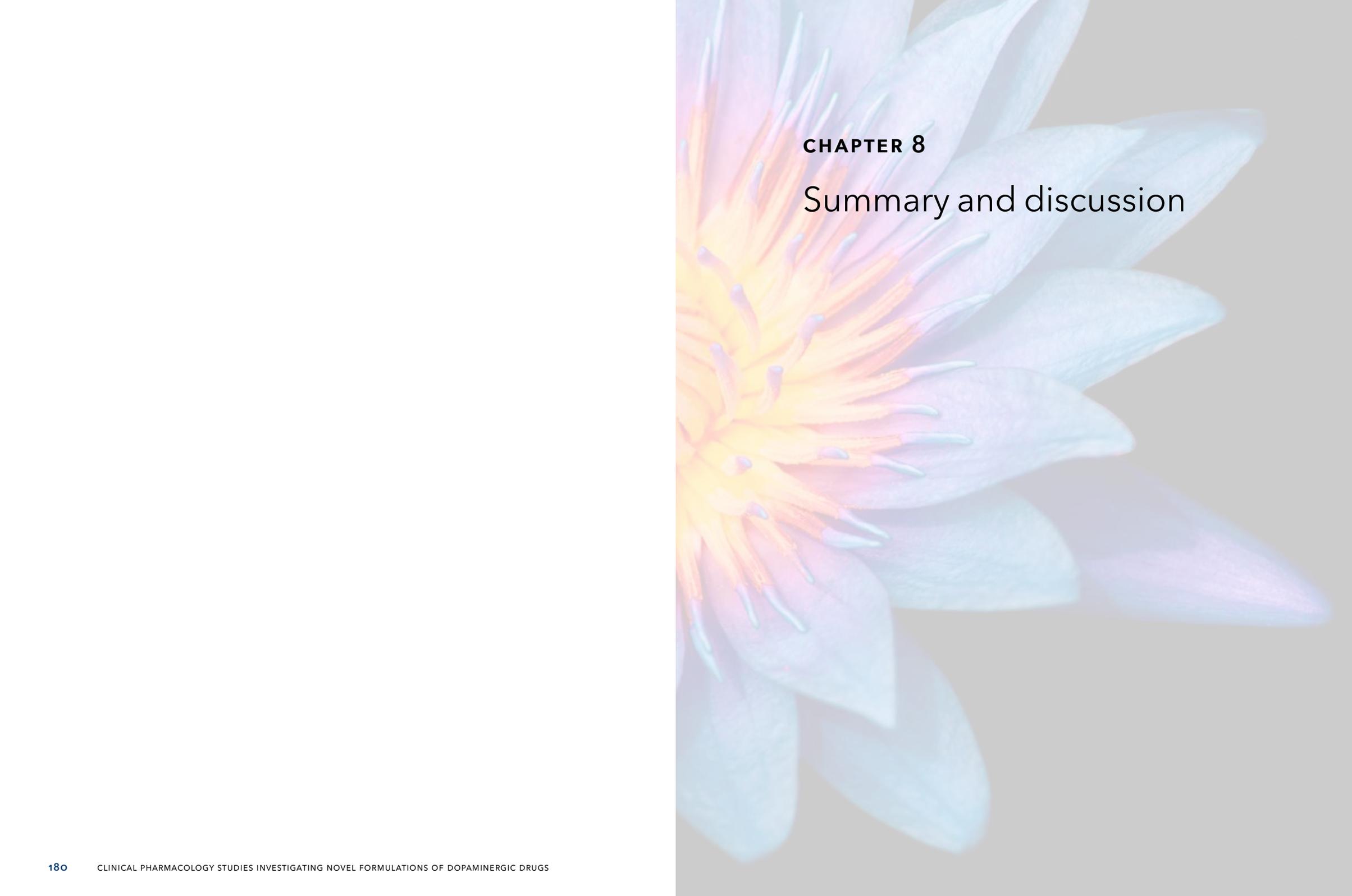


IMFT, alternate index and middle finger tapping; IFT, alternate index finger tapping; TIFT, thumb-index finger tapping; MDS-UPDRS III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale part III.

REFERENCES

- 1 Davie CA. A review of Parkinson's disease. *Br Med Bull* 2008; 86(1): 109-127. <https://doi.org/10.1093/bmb/ldn013>.
- 2 Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry* 2008; 79(4): 368-376. <https://doi.org/10.1136/jnnp.2007.131045>.
- 3 Regnault A, Boroojerdi B, Meunier J, Bani M, Morel T, Cano S. Does the MDS-UPDRS provide the precision to assess progression in early Parkinson's disease? Learnings from the Parkinson's progression marker initiative cohort. *J Neurol* 2019; 266: 1927-1936. <https://doi.org/10.1007/s00415-019-09348-3>.
- 4 Goetz CG et al. Movement Disorder Society-sponsored revision of the unified Parkinson's disease rating scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov Disord* 2008; 23(15): 2129-2170. <https://doi.org/10.1002/mds.22340>.
- 5 Martinez-Martin P, Rodriguez-Blazquez C, Alvarez-Sanchez M, et al. Expanded and independent validation of the Movement Disorder Society-unified Parkinson's disease rating scale (MDS-UPDRS). *J Neurol* 2013; 260(1): 228-236. <https://doi.org/10.1007/s00415-012-6624-1>.
- 6 Ramsay N, Macleod AD, Alves G, et al. Validation of a UPDRS-/MDS-UPDRS-based definition of functional dependency for Parkinson's disease. *Parkinsonism Relat Disord* 2020; 76: 49-53. <https://doi.org/10.1016/j.parkreldis.2020.05.034>.
- 7 Patel AB, Jimenez-Shahed J. Profile of inhaled levodopa and its potential in the treatment of Parkinson's disease: evidence to date. *Neuropsychiatric Disease and Treatment* 2018; 14: 2955-2964. <https://doi.org/10.2147/NDT.S147633>.
- 8 Grosset KA, Malek N, Morgan F, Grosset DG. Inhaled apomorphine in patients with 'on-off' fluctuations: a randomized, double-blind, placebo-controlled, clinic and home based, parallel-group study. *J Parkinsons Dis* 2013; 3(1): 31-37. <https://doi.org/10.3233/JPD-120142>.
- 9 Koop MM, Shivitz N, Brontë-Stewart H. Quantitative measures of fine motor, limb, and postural bradykinesia in very early stage, untreated Parkinson's disease. *Mov Disord* 2008; 23(9): 1262-1268. <https://doi.org/10.1002/mds.22077>.
- 10 Makai-Böölöni S, Thijssen E, van Brummelen EMJJ, Groeneveld GJ, Doll RJ. Touchscreen-based finger tapping: repeatability and configuration effects on tapping performance. *PLoS One* 2021; 16(12): e0260783. <https://doi.org/10.1371/journal.pone.0260783>.
- 11 Nalçacı E, Kalaycıoğlu C, Çiçek M, Genç Y. The relationship between handedness and fine motor performance. *Cortex* 2001; 37(4): 493-500. [https://doi.org/10.1016/S0010-9452\(08\)70589-6](https://doi.org/10.1016/S0010-9452(08)70589-6).
- 12 Taylor Tavares AL, Jefferis GSXE, Koop M, Hill BC, Hastie T, Heit G, Bronte-Stewart HM. Quantitative measurements of alternating finger tapping in Parkinson's disease correlate with UPDRS motor disability and reveal the improvement in fine motor control from medication and deep brain stimulation. *Mov Disord* 2005; 20(10): 1286-1298. <https://doi.org/10.1002/mds.20556>.
- 13 Fukawa K, Okuno R, Yokoe M, Sakoda S, Akazawa K. Estimation of UPDRS finger tapping score by using artificial neural network for quantitative diagnosis of Parkinson's disease. *Proceedings of the IEEE/EMBS Region 8 International Conference on Information Technology Applications in Biomedicine, ITAB; IEEE, New York City; 2007: 259-260.* <https://doi.org/10.1109/ITAB.2007.4407396>.
- 14 Thijssen E, Makai-Böölöni S, van Brummelen E, den Heijer J, Yavuz Y, Doll RJ, Groeneveld GJ. A placebo-controlled study to assess the sensitivity of finger tapping to medication effects in PD. *Mov Disord Clin Pract* 2022; 9: 1074-1084. <https://doi.org/10.1002/mdc3.13563>.
- 15 Espay AJ, Giuffrida JP, Chen R, et al. Differential response of speed, amplitude, and rhythm to dopaminergic medications in Parkinson's disease. *Mov Disord* 2011; 26(14): 2504-2508. <https://doi.org/10.1002/mds.23893>.
- 16 Hasan H, Burrows M, Athauda DS, et al. The BRadykinesia akinesia INcoordination (BRAIN) tap test: capturing the sequence effect. *Mov Disord Clin Pract* 2019; 6(6): 462-469. <https://doi.org/10.1002/mdc3.12798>.
- 17 Wissel BD, Mitsi G, Dwivedi AK, et al. Tablet-based application for objective measurement of motor fluctuations in Parkinson disease. *Digit Biomark* 2018; 1(2): 126-135. <https://doi.org/10.1159/000485468>.
- 18 Lipp MM, Batycky R, Moore J, Leinonen M, Freed MI. Preclinical and clinical assessment of inhaled

- levodopa for OFF episodes in Parkinson's disease. *Sci Transl Med* 2016; 8(360):360ra136–360ra136. <https://doi.org/10.1126/scitranslmed.aad8858>.
- 19 Arora S et al. Smartphone motor testing to distinguish idiopathic REM sleep behavior disorder, controls, and PD. *Neurology* 2018; 91(16): E1528–E1538. <https://doi.org/10.1212/WNL.0000000000006366>.
- 20 Kimber TE, Tsai CS, Semmler J, Brophy BP, Thompson PD. Voluntary movement after pallidotomy in severe Parkinson's disease. *Brain* 1999; 122(5): 895–906. <https://doi.org/10.1093/brain/122.5.895>.
- 21 Yokoe M, Okuno R, Hamasaki T, Kurachi Y, Akazawa K, Sakoda S. Opening velocity, a novel parameter, for finger tapping test in patients with Parkinson's disease. *Parkinsonism Relat Disord* 2009; 15(6): 440–444. <https://doi.org/10.1016/j.parkrelidis.2008.11.003>.
- 22 Espay AJ, Hausdorff JM, Sánchez-Ferro Á, et al. A roadmap for implementation of patient-centered digital outcome measures in Parkinson's disease obtained using mobile health technologies. *Mov Disord* 2019; 34(5): 657–663. <https://doi.org/10.1002/mds.27671>.
- 23 Nisenzon AN, Robinson ME, Bowers D, Banou E, Malaty I, Okun MS. Measurement of patient-centered outcomes in Parkinson's disease: what do patients really want from their treatment? *Parkinsonism Relat Disord* 2011; 17(2): 89–94. <https://doi.org/10.1016/j.parkrelidis.2010.09.005>.
- 24 Sikap P et al. Perancangan Prototipe Sistem Pemesanan Makanan dan Minuman Menggunakan Mobile Device. *Indonesia Jurnal on Networking and Security* 2015; 1(2): 1–10. <https://doi.org/10.1145/242224.242229>.
- 25 Zhan A, Mohan S, Tarolli C, et al. Using smartphones and machine learning to quantify Parkinson disease severity the mobile Parkinson disease score. *JAMA Neurol* 2018; 75(7): 876–880. <https://doi.org/10.1001/jamaneurol.2018.0809>.
- 26 Mei J, Desrosiers C, Frasnelli J. Machine learning for the diagnosis of Parkinson's disease: a review of literature. *Frontiers in Aging Neuroscience* 2021; 13: 184. <https://doi.org/10.3389/fnagi.2021.633752>.
- 27 Yang N, Liu DF, Liu T, et al. Automatic detection pipeline for accessing the motor severity of Parkinson's disease in finger tapping and postural stability. *IEEE Access* 2022;10: 66961–66973. <https://doi.org/10.1109/access.2022.3183232>.
- 28 Kalia LV, Lang AE. Parkinson's disease. *The Lancet* 2015; 386(9996): 896–912. [https://doi.org/10.1016/S0140-6736\(14\)61393-3](https://doi.org/10.1016/S0140-6736(14)61393-3).
- 29 Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov Disord* 2010; 25(15): 2649–2653. <https://doi.org/10.1002/mds.23429>.
- 30 Biometrics Ltd. Twin-Axis goniometers for dynamic joint movement analysis; 2020.
- 31 Van Rossum G, Drake FL Jr. Python 3 Reference Manual, Version 3.7.3. Scotts Valley, CA: CreateSpace; 2009.
- 32 Pedregosa F. Scikit-learn: machine learning in Python. *Journal of Machine Learning Research* 2011; 12: 2825–2830.
- 33 Kaiser L. Adjusting for baseline: change or percentage change? *Stat Med* 1989; 8(10): 1183–1190. <https://doi.org/10.1002/sim.4780081002>.
- 34 Parvande S, Yeh HW, Paulus MP, McKinney BA. Consensus features nested cross-validation. *Bioinformatics* 2020; 36(10): 3093–3098. <https://doi.org/10.1093/bioinformatics/btaa046>.
- 35 Navia-Vázquez A, Parrado-Hernández E. Support vector machine interpretation. *Neurocomputing* 2006; 69(13–15): 1754–1759. <https://doi.org/10.1016/j.neucom.2005.12.118>.
- 36 Deng Y, Liu X, Xin C, Jia W. An interpretable classifier with linear discriminant analysis based on AFS theory. 2019 Chinese Control Conference (CCC). IEEE, New York City; 2019: 7583–7588. <https://doi.org/10.23919/ChiCC.2019.8866096>.
- 37 Stiglic G, Kocbek P, Fijacko N, Zitnik M, Verbert K, Cilar L. Interpretability of machine learning-based prediction models in healthcare. *WIREs Data Mining and Knowledge Discovery* 2020; 10(5): e1379. <https://doi.org/10.1002/widm.1379>.
- 38 Moon S, Song HJ, Sharma VD, Lyons KE, Pahwa R, Akinwuntan AE, Devos H. Classification of Parkinson's disease and essential tremor based on balance and gait characteristics from wearable motion sensors via machine learning techniques: a data-driven approach. *J Neuroeng Rehabil* 2020; 17(1): 125. <https://doi.org/10.1186/s12984-020-00756-5>.
- 39 Wu Y, Krishnan S. Statistical analysis of gait rhythm in patients with Parkinson's disease. *IEEE Trans Neural Syst Rehabil Eng* 2010; 18(2): 150–158. <https://doi.org/10.1109/TNSRE.2009.2033062>.
- 40 Geetha R, Sivagami G. Parkinson Disease Classification using Data Mining Algorithms; 2011.
- 41 Yadav G, Kumar Y, Sahoo G. Prediction of Parkinson's disease using data mining methods: a comparative analysis of tree, statistical and support vector machine classifiers. 2012 National Conference on Computing and Communication Systems. IEEE, New York City; 2012: 1–8. <https://doi.org/10.1109/NCCCS.2012.6413034>.
- 42 Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med* 2016; 15(2): 155–163. <https://doi.org/10.1016/j.jcm.2016.02.012>.
- 43 Trager MH, Velisar A, Koop MM, Shreve L, Quinn E, Bronte-Stewart H. Arrhythmokinesis is evident during unimanual not bimanual finger tapping in Parkinson's disease. *J Clin Mov Disord* 2015; 2(1): 8. <https://doi.org/10.1186/s40734-015-0019-2>.
- 44 Giovannoni G, Van Schalkwyk J, Fritz VU, Lees AJ. Bradykinesia akinesia inco-ordination test (BRAIN TEST): an objective computerised assessment of upper limb motor function. *J Neurol Neurosurg Psychiatry* 1999; 67(5): 624–629. <https://doi.org/10.1136/jnnp.67.5.624>.
- 45 Hauser RA, Ellenbogen A, Khanna S, Gupta S, Modi NB. Onset and duration of effect of extended-release carbidopa-levodopa in advanced Parkinson's disease. *Neuropsychiatr Dis Treat* 2018; 14: 839–845. <https://doi.org/10.2147/NDT.S153321>.
- 46 Contin M, Riva R, Martinelli P, Albani F, Avoni P, Baruzzi A. Levodopa therapy monitoring in patients with Parkinson disease: a kinetic-dynamic approach. *Ther Drug Monit* 2001; 23(6): 621–629. <https://doi.org/10.1097/00007691-200112000-00005>.
- 47 Lobo V, Branco D, Guerreiro T, Bouça-Machado R, Ferreira J. Machine-learning models for MDS-UPDRS III prediction: a comparative study of features, models, and data sources. *Information Society* 2022.
- 48 Ur Rehman RZ, Rochester L, Yarnall AJ, Del Din S. Predicting the progression of Parkinson's disease MDS-UPDRS-III motor severity score from gait data using deep learning. Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society. EMBS, Institute of Electrical and Electronics Engineers Inc., New York City; 2021: 249–252. <https://doi.org/10.1109/EMBC46164.2021.9630769>.
- 49 Walsh T. Fuzzy gold standards: approaches to handling an imperfect reference standard. *J Dent* 2018; 74: S47–S49. <https://doi.org/10.1016/j.jdent.2018.04.022>.
- 50 Berisha V, Krantsevich C, Hahn PR, Hahn S, Dasarathy G, Turaga P, Liss J. Digital medicine and the curse of dimensionality. *npj Digital Medicine* 2018; 4(1): 1–8. <https://doi.org/10.1038/s41746-021-00521-5>.



CHAPTER 8

Summary and discussion

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects millions of people worldwide. Despite all research efforts, there is currently no disease-modifying treatment available and the main treatment avenue remains symptomatic treatment. As the disease progresses, patients develop motor and non-motor fluctuations that significantly impact activities of daily living and quality of life. Patients alternate between periods of favorable response to medication (*ON*) and periods of inadequate response (*OFF*). Apomorphine is a dopamine agonist that has been used to treat *OFF* episodes for three decades. It is available both as subcutaneous intermittent injections and subcutaneous continuous infusion. The intermittent injections are especially suited for patients with only a few *OFF* episodes per day, which is the focus of this thesis. Despite the well-known efficacy of apomorphine in treating *OFF* episodes, its administration route has several impracticalities. For instance, patients must self-administer injections during their *OFF* periods. This can be inconvenient, especially since the injection site is often covered by clothing. Additionally, the occurrence of injection site reactions and the fear of needles may serve as further limitations to its use.

APOMORPHINE

In this thesis, we evaluated two new administration routes of apomorphine that are expected to be more user-friendly. In [chapter 2 and 3](#), a breath-actuated, oral inhalation device was investigated in healthy volunteers and PD patients. Staccato apomorphine (AZ-009) reached maximum plasma concentrations 1-2 minutes after inhalation and improved mean motor function (MDS-UPDRS III) in PD patients during an induced morning *OFF* state from the first measurement at 10 minutes post-dose. Its systemic absorption was significantly faster than that of the subcutaneous apomorphine injection for which maximum plasma concentrations were reached only after 30 minutes. This suggests the potential for a quicker transition from *OFF* to *ON* following treatment. To establish this conclusively, a direct head-to-head comparison is necessary. Additionally, it is important to assess and compare the duration of effect of both formulations in this study.

In healthy volunteers, apomorphine inhalation was not well tolerated with nausea, vomiting and hypotension being the most troublesome AEs. In contrast, PD patients tolerated apomorphine inhalation up to 4 mg reasonably well with AEs most often related to the inhalation: coughing and throat irritation. These AEs were mild and transient, usually resolving within minutes. No apparent accumulation and changes in safety profile were observed when AZ-009 was dosed three times daily with 2 hours between doses. In clinical practice, subcutaneous apomorphine is initiated at a low dose (1 or 2 mg) and titrated up until an optimal balance between side effects and efficacy is reached. In contrast, in the studies described in [chapter 2 and 3](#), patients received a fixed dose. This likely led to suboptimal dosing, where for some the dose was too high and therefore resulted in AEs preventing the conduct of MDS-UPDRS III, and for others might have been too low to reach optimal efficacy. While these initial studies show that AZ-009 improves motor function, the described effects are likely an underestimation. Hence, future studies should investigate AZ-009's efficacy when administered at a patient's individually optimized dose. Currently, a phase 2 study is ongoing including an open-label titration phase followed by a double-blind at-home treatment period with an in-clinic visit.¹ Therefore, this study will provide more information on the efficacy of a titrated dose, as well as the usability of the device by patients during an *OFF* state in an at-home setting. Future studies should also investigate the long-term (pulmonary) safety and tolerability.

Overall, the data provided in [chapter 2 and 3](#) provide confidence for the further development of Staccato apomorphine (AZ-009) in larger scale trials.

In [chapter 4](#), buccal administration of an oromucosal apomorphine solution was evaluated. Its safety, tolerability and PK were compared to a subcutaneous apomorphine injection and a sublingual apomorphine film. Both comparator formulations were marketed at the time of study execution. However, in 2023, the sublingual apomorphine film was retracted from the market by the company (Sunovion) due to limited utilization. The company gave no further information on the reason for retracting, but it might have been related to the relatively high incidence of oropharyngeal side

effects upon repeated exposure.² The side effects are likely the result of apomorphine undergoing autooxidation in the saliva,³ resulting in the formation of quinone derivatives and reactive oxygen species which have been associated with cytotoxicity.^{4,5} It is hypothesized that this will occur less when apomorphine is administered as a solution, but this requires confirmation in future studies. Nonetheless, we showed that short-term treatment with oromucosal apomorphine was generally well-tolerated without oropharyngeal side effects and buccal mucosa abnormalities. Although relevant and reproducible plasma concentrations were reached, the exposures are not expected to be sufficient to treat all PD patients. Currently, the maximum dose that can be administered is 14 mg (0.2 mL). Administering a higher volume is not recommended so as to prevent saliva production and the induction of a swallowing reflex. Separating the administration of sprays by a few minutes instead of administering them sequentially was shown to not increase dose-normalized exposure. Therefore, future administrations are suggested to be administered as consecutive sprays. This is also more user-friendly. To make the oromucosal solution useful for the entire PD population, it is recommended to investigate other options to increase its exposure. Increasing the apomorphine concentration is unfortunately not possible due to apomorphine's limited solubility. However, an option could be to increase the surface area over which apomorphine solution is dispersed. This could be facilitated by using a different spray nozzle capable of dispersing the solution across a wider buccal area. Another potential avenue is to change the solvent composition. Adding/increasing for example ethanol might improve apomorphine's solubility, increase buccal absorption and enhance the dispersibility of the solution (thereby again increasing the surface area).^{6,7} Lastly, the addition of a permeation enhancer to the formulation can theoretically increase buccal absorption.^{8,9} However, due to the risk of local tolerability issues during prolonged daily use, this is considered a less suitable option.

Median T_{\max} of oromucosal apomorphine ranged between 32 and 53 minutes over different dose groups with an overall range between 15 and 120 minutes. Therefore, absorption was slower than for subcutaneous injection in the abdomen (19 minutes (range:

8-40 minutes), and more comparable (although on the low end) to subcutaneous injection in the thigh described in chapter 2 (30 min (20-60 minutes)). Moreover, it was also considerably slower than apomorphine inhalation described in chapter 2. Future studies should therefore assess how this T_{\max} relates to onset of effect of the oromucosal apomorphine formulation in order to confirm its usefulness as a rescue medication for *OFF* episodes.

A limitation of the apomorphine studies outlined in this thesis is the lack of an investigation into the usability of the devices. Given that the development of new apomorphine formulations is aimed at delivering a less invasive and easier to use formulation for PD patients, it is imperative that future studies verify that PD patients can independently use the breath-actuated inhaler and the spray pump device during an *OFF* state. Encouraging results have been published though on the use of dry powder inhalers by PD patients. Others have shown that most PD patients could handle a dry powder inhaler, had sufficiently high inspiratory flow rates and were able to hold their breath for up to 5 seconds after inhalation.¹⁰ Moreover, a breath-actuated inhaler of levodopa dry powder has been approved for the treatment of *OFF* episodes. In a phase 2b study with this inhaler, patients were able to prepare and self-administer the treatment, even though some indicated concerns about inhaler system use during telephone contact (7% placebo, 14% levodopa).¹¹ Overall, this provides evidence that a breath-actuated inhaler can be used by PD patients.

ACUTE DOPAMINERGIC TREATMENT EFFECTS

To assess the effects of new fast-acting compounds, objective, quantitative and fast measurements are ideal. Especially for the treatment of *OFF* episodes, the onset of effect is crucial to evaluate whether the drug is suitable for this indication. Currently, the rather extensive MDS-UPDRS part III scale is often used to evaluate drug efficacy. Even though it is useful, it requires a trained rater, takes relatively long to complete (approximately 15 minutes) and is subject

to inter- and intra-rater variability. In [chapter 5, 6 and 7](#) of this thesis, multiple finger tapping tasks were evaluated for their use as objective, quantitative and fast pharmacodynamic measurements.

In [chapter 5](#), four different touchscreen-based finger tapping tasks were evaluated in a technical validation study in healthy volunteers. Configurations included alternate index and middle finger tapping (IMFT) with 2.5 cm between targets and repetitive alternate index finger tapping (IFT) with 20 cm between targets. Both tasks were assessed with and without a visual cue. The results indicated that the visual cue, rather than signaling the next target, provided immediate visual feedback. When participants tapped outside the target area, the next circle did not appear, prompting participants to pause and correct the error. This resulted in a reduced tapping speed and lower fatigue in both tasks. If and how these data would translate to a PD population was uncertain and would have required further validation in a PD population. This uncertainty combined with the good performance of the uncued tasks, led to the decision to only validate the uncued tasks further in [chapter 6](#).

No significant differences were observed in tapping measurements within a day, but these were observed between days. It appeared that participants changed their tapping strategy during the second visit, prioritizing speed over accuracy, possibly due to familiarity with the task. The absence of a learning effect within a day supported the further evaluation of these tasks in response to fast-acting medication, without the need for extensive training sessions. Considering the observed changes between days, the next study was conducted using a balanced crossover design ([chapter 6](#)). Overall, this technical validation study provided evidence that the uncued IMFT and IFT tasks functioned well and were repeatable, and that speed, accuracy and rhythm parameters showed good potential sensitivity in healthy volunteers.

Hence, in [chapter 6](#), these two touchscreen-based finger tapping tasks, together with a thumb-index finger tapping (TIFT) task, were further evaluated in a follow up study in PD patients during an induced OFF state. This randomized, double-blind, placebo-controlled crossover study assessed their ability to detect and quantify dopaminergic medication effects.

Of the three tapping tasks, the alternate IMFT task performed the worst, that is, had the lowest effect sizes. Its effect sizes were also below that of the gold standard MDS-UPDRS III. Moreover, the task was sometimes difficult to perform for the patients with PD, resulting in a high percentage of same-sided double taps. These problems with correctly performing/recording the IMFT task, combined with the relatively small effect sizes, make the task in its current configuration the least suitable for efficacy studies in PD patients. In contrast, PD patients were able to perform the IFT and TIFT tasks without difficulties. The IFT task showed significantly faster tapping (total taps), improved rhythm (inter-tap interval SD), and decreased accuracy (total spatial error) in response to levodopa/carbidopa compared to placebo. Total number of taps and total spatial error had the largest standardized effect sizes, and these were comparable to MDS-UPDRS III. That speed- and accuracy-related parameters had the largest effect sizes was consistent with expectations based on the potential sensitivities calculated in [chapter 5](#). In the TIFT task, levodopa/carbidopa compared with placebo resulted in faster tapping (opening and closing velocity) with a bigger amplitude and improved rhythm (inter-tap interval SD). Mean opening and closing velocity had the largest effect sizes, and were comparable to the effect size of the MDS-UPDRS III. The speed-related parameters in both tasks showed a moderate-to-strong correlation with the MDS-UPDRS III ($r = 0.45-0.70$). Moreover, the inter-tap interval SD showed a strong correlation with the MDS-UPDRS III in the levodopa/carbidopa group ($r = 0.66$) and a trend toward a moderate correlation ($r = 0.45$) in the placebo group. In conclusion, the alternate IFT and TIFT tasks provided short, rater-independent measurements sensitive to dopaminergic medication effects with similar effect sizes as the MDS-UPDRS III.

In [chapter 7](#), the data from the clinical study in [chapter 6](#) were used to train machine learning algorithms to select the optimal combination of finger tapping task parameters ('composite biomarker') to predict the treatment effect (i.e., did the patient receive active or placebo treatment?) and estimate the disease severity (i.e., MDS-UPDRS III score). A composite biomarker was created for each tapping task individually, for the three tapping tasks combined

and for the MDS-UPDRS III. Overall, the baseline corrected models performed better than the uncorrected models. The baseline-corrected IFT composite biomarker had the best classification performance (83.50% accuracy, 93.95% precision, effect size 2.58 ± 0.90) and outperformed the MDS-UPDRS III composite biomarker (75.75% accuracy, 73.93% precision, effect size 2.12 ± 1.25). The IFT composite biomarker included total number of taps and total spatial error, which was in line with expectations based on the effect sizes reported in chapter 6. The baseline-corrected IFT composite biomarker also achieved the best performance when the MDS-UPDRS III total score was estimated (mean absolute error: 7.87, Pearson's correlation: 0.69).

Overall, we demonstrated that the IFT composite biomarker outperformed the combined tapping tasks and the MDS-UPDRS III composite biomarkers in detecting treatment effects. Combining the most relevant parameters instead of using a single parameter, improves the ability to detect medication effects. Therefore, this provides evidence to include the IFT composite biomarker in future clinical trials for the detection of medication effects. Despite these positive outcomes, it is essential to note that these conclusions are based on a relatively small sample size. To address this limitation, chapter 7 employed nested cross-validation. Nevertheless, the generalizability of the findings from this specific group of PD patients to the broader and heterogeneous PD population remains uncertain. While finger tapping tasks are good at detecting bradykinesia in forearm and fine finger movements, they may not provide a comprehensive measure of overall motor function. Consequently, certain subsets of PD patients might not show improvement in finger tapping, even if their overall motor function has improved. Therefore, it is imperative to confirm the validity, reliability, and generalizability of our methods using an independent dataset. Therefore, we propose to conduct a follow-up study with a larger cohort of PD patients with diverse MDS-UPDRS III scores, in which both akinetic-rigid dominant and tremor-dominant PD subtypes are represented.

While completion of the MDS-UPDRS III scale typically requires about 15 minutes, the finger tapping tasks take only 15 to 30 seconds. This makes the finger tapping tasks less burdensome for patients

but also allows for more frequent and closely spaced assessments compared to the MDS-UPDRS III. This enables a better detection of the onset of effect and the time to reach maximum effect. This is especially useful for drugs with an anticipated fast onset of effect, like apomorphine for the treatment of *OFF* episodes. Hence, it is advised to include (at a minimum) the IFT task in future trials with inhaled apomorphine and apomorphine oromucosal solution to determine their precise onset of efficacy.

Since the IFT task is a touchscreen tapping task and does not require a trained rater like the MDS-UPDRS III, it could also be suitable for testing medication effects or monitoring disease progression in a home setting. However, this would require further validation of the tapping tasks' variability over a longer time period when performed without study staff supervision. The advantage of performing the IFT task at home would be the ability of the investigator to monitor the patient in their real-life environment and reduce the number of in-clinic visits required, thereby reducing patient burden.

LOOKING TOWARDS THE FUTURE

The Parkinson 'pandemic' and the search for a disease-modifying therapy

PD is the fastest growing neurological disorder.¹² Whereas in 1990, 2.5 million people were affected by PD worldwide, this number had increased to 6.1 million in 2016.¹² Projections estimate that this will increase further to 13-14 million people by 2040.¹³ This substantial rise has led some to call Parkinson's disease a pandemic. The increase in incidence can be attributed to the aging of the worldwide population. Environmental factors linked to industrialization are thought to contribute as well.¹² Population-based incident PD cohorts have shown that motor fluctuations manifest in 22.8-54.3% of patients within 5 years after diagnosis, and increase to 100% 10 years after diagnosis (Table 1).¹⁴⁻¹⁶ For levodopa-induced dyskinesia this was 14.5-29.6% within 5 years, and 55.7% within 10 years. This means that within 5-10 years after disease onset, the majority of PD patients suffer from motor complications. With the aging of the worldwide population, more

patients will live long enough to fall victim to motor complications. This adds significant disease burden but also economic costs. Hence, the need for disease-modifying therapies is high.

Our knowledge about the pathology of PD is expanding and shows that it is a complex interplay of alpha-synuclein aggregation and spreading, mitochondrial dysfunction, oxidative stress, lysosomal dysfunction, and neuroinflammation.¹⁷ Therapies targeting these dysfunctional processes are currently undergoing extensive research.¹⁸ For alpha synuclein, multiple options are being investigated, aiming either for the inhibition of its aggregation (stabilizing small molecule blockers, autophagy induction with ABL1 inhibitors), reducing its synthesis (antisense oligonucleotides, small interfering RNAs), preventing its cell-to-cell transmission (monoclonal antibodies, active immunization), or reducing its gene transcription (beta2 adrenergic receptor agonists).¹⁸ Improving mitochondrial function has shown promise in preclinical models by preventing neurodegeneration. Thus far, however, these results have not translated into a slower disease progression in PD patients in clinical trials. To improve lysosomal function, various strategies targeting the beta glucocerebrosidase (GCase) enzyme have been investigated.¹⁸ These include increasing GCase activity (GCase modulators), reducing accumulated GCase substrate (glucosylceramide synthase inhibitors) and GBA1 gene therapy. LRRK2 inhibitors and LRRK2 antisense oligonucleotides are in clinical development aiming to decrease LRRK2 activity in PD patients with a LRRK2 mutation, but might also be useful for patients without a mutation but with elevated LRRK2 activity.¹⁸ Targeting neuroinflammation is another strategy that is being investigated, for example by inhibiting the NLRP3 inflammasome.¹⁸ Lastly, stem cell-based therapies are in early phase clinical development. Studies investigate transplantation of dopamine neurons derived from embryonic stem cells or induced pluripotent stem cells to replace lost dopaminergic cells.¹⁹ In addition, mesenchymal stem cells are investigated for their neuroprotective and immunomodulatory effects.^{20,21}

Despite all efforts, no disease-modifying drug has reached the market yet. When it does, it is expected to slow disease progression

but not cure the disease. Therefore, the need for symptomatic treatment of response fluctuations in Parkinson's disease remains high. In the next section, an overview of drugs that are currently in clinical development is provided.

Symptomatic treatments in clinical development for response fluctuations

INTERMITTENT OFF

For sudden unpredictable *OFF* periods, there are currently only two treatment options available, i.e., subcutaneous apomorphine injection (APO-go, APOKYN) and levodopa dry powder inhalation (Inbrija). Between 2020 and 2023, apomorphine sublingual film (KYNMOBI) was shortly available in the US and Canada, but it was discontinued, again reducing the number of treatment options. Currently, there are only a few alternatives in development, of which two are described in this thesis: 1) Staccato apomorphine inhalation, 2) oromucosal apomorphine solution for buccal delivery, and 3) levodopa dry powder inhalation (Cyclops). Staccato apomorphine is further investigated in an ongoing phase 2 clinical trial expected to complete in March 2024.²² The Cyclops dry powder inhaler has completed a phase 2 clinical trial,²³ and recently in 2023, a pilot comparative bioavailability study investigating levodopa Cyclops and Inbrija.²⁴ Between November 2023 and December 2024, a study will be conducted to investigate and compare the usability of both inhalation devices.²⁵ According to pureIMS, the developer of levodopa Cyclops, these studies are undertaken to support a marketing authorization in the US.²⁶ Although both Inbrija and Cyclops deliver levodopa as a dry powder for inhalation, differences between the devices exist. For Inbrija, patients must complete multiple steps to inhale a full dose (2 capsules). This involves removing a capsule from its blister immediately before use, loading it into the inhaler, inhaling and holding the breath for 5 seconds, removing the capsule, loading a second one, and repeating the process. Following the second inhalation, the inhaler's mouthpiece must be cleaned.²⁷ So even though the administration route is more user-friendly than a subcutaneous injection, it does require patients

to complete multiple fine finger movements during an *OFF* state. On the other hand, the levodopa Cyclops inhaler requires fewer steps. Opening the pouch containing the inhaler and pulling out a cover foil readies it for use.²⁸ These steps are fewer than those for Inbrija because the Cyclops inhaler comes prefilled with levodopa. Additionally, being a single-use inhaler eliminates the need for cleaning. These differences suggest that the levodopa Cyclops inhaler may offer an even more straightforward administration route, although this hypothesis requires confirmation in the planned study comparing the usability of both devices.

CONTINUOUS DELIVERY

Another avenue to decrease symptom fluctuations (*OFF* episodes and dyskinesia) is to provide a more continuous stimulation of the (dopaminergic) neuronal system. A few treatment options are already available, namely DBS, continuous subcutaneous apomorphine infusion and continuous levodopa-carbidopa intestinal infusion.²⁹ However, research is ongoing to develop other drugs/formulations. Currently, there are three subcutaneous formulations in development for continuous infusion of (fos)levodopa/(fos)carbidopa. Two are in late stage development (NDO612, ABBV-951), and one is in early stage development (DIZ102).^{30,31} The advantage of continuous subcutaneous levodopa/carbidopa administration, as opposed to intestinal administration, is that no surgery to insert a permanent percutaneous endoscopic gastro-jejunal (PEG-J) tube is needed. This is considered an invasive procedure and there is a considerable risk for device complications.²⁹ Continuous subcutaneous levodopa/carbidopa infusion has been shown to result in stable drug plasma concentrations.^{30,32,33} For ABBV-951, the product that is furthest in its development, *ON* time without troublesome dyskinesia has been shown to increase with 2.72 ± 0.52 hours/day, compared to 0.97 ± 0.50 hours for oral immediate-release levodopa/carbidopa.³⁴ ABBV-951 can be used as a monotherapy, but NDO612 should be combined with oral levodopa/carbidopa to reach therapeutic concentrations, which might be a disadvantage of the latter pump.³¹ Moreover, continuous subcutaneous therapies can result in infusion site reactions.³¹ Another continuous therapy being investigated is the

DopaFuse, which provides continuous oral delivery of levodopa/carbidopa to the back of the mouth via a specialized mouthpiece. It is a non-invasive delivery system, but it does not bypass the gastric dysmotility or the challenges related to erratic gastric emptying prevalent in PD patients. However, phase 2 results published in the EU Clinical Trials Register do show that it leads to less fluctuating plasma levels than oral immediate-release levodopa/carbidopa tablets.³⁵ In addition, *OFF* time was 1.51 ± 1.44 hours/day when DopaFuse was combined with a morning oral levodopa/carbidopa dose, compared to 3.23 ± 2.18 hours/day for oral levodopa/carbidopa tablets alone. One should note that this was a single arm non-randomized study, so future studies should address its efficacy further. Complications associated with the oral device occurred in 31.25% of the patients.

EXTENDED RELEASE

Another way to achieve more stable levodopa plasma concentrations, is the use of extended release formulations. Three extended-release levodopa/carbidopa capsules are in clinical development: IPX203, DM-1992 and the Accordion Pill. IPX203 is a capsule containing immediate-release levodopa/carbidopa granules and extended-release levodopa beads with an enteric coating to prevent early disintegration in the stomach. In a phase 3 trial, IPX203 resulted in 0.53 more hours of *ON* time without troublesome dyskinesia per day compared to immediate-release levodopa/carbidopa, while it had to be dosed less often (3 versus 5 times/day).³⁶ The New Drug Application (NDA) that was submitted to the FDA was rejected in July 2023. The FDA requested additional safety information on carbidopa in the formulation. The company will resubmit the NDA with additional information when available.³⁶ The other two formulations, DM-1992 and the Accordion Pill, are both gastric retentive formulations including immediate and extended release components. DM-1992 swells when exposed to gastric fluid and the Accordion Pill consists of folded sheets in a capsule that extend while in the stomach. As a result, both formulations remain in the stomach longer, where they dissolve slowly and provide controlled release of levodopa to the small intestine.³⁷ Phase 2 results comparing DM-1992 with immediate-release levodopa/carbidopa were positive for DM-1992,

demonstrating steadier levodopa plasma concentrations and reduced *OFF* time despite a lower dosing frequency.³⁶ However, no follow up studies with DM-1992 have been reported in study registries after this publication in 2015. The same is observed for the Accordion Pill. This formulation was investigated in a phase 3 clinical trial that completed in 2019. Topline results shared in July 2019 indicated that the Accordion Pill was not superior to immediate-release levodopa/carbidopa in reducing daily *OFF* time.³⁸ Nevertheless, in 2020, the company suggested that this lack of superiority might be attributed to the administration of doses that were too low to reach optimal efficacy. This idea was supported by the fact that patients who did not reach the maximum dose during the dose titration phase did show a relevant reduction in *OFF* time. Consequently, the pharmaceutical company expressed the intention to seek a strategic partner capable of advancing the levodopa Accordion Pill through a final phase 3 pivotal trial and progressing it towards marketing authorization.³⁹ However, results of this trial have not been published to date and no follow up studies have been registered in the registries. Given the lack of recent updates on the clinical development progress of DM-1992 and the Accordion Pill, the main hope is for IPX203 to reach the market.

LEVODOPA-INDUCED DYSKINESIA

The focus of this thesis was the treatment of sudden intermittent *OFF* periods. However, most patients with advanced PD also experience dyskinesia which has a major impact on their quality of life. To reduce dyskinesia, one can change the treatment regimen (timing, dose), have DBS surgery, or use the abovementioned continuous therapies that provide lower peak-trough oscillations. Moreover, amantadine, a non-selective N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, has shown benefit in reducing dyskinesia by reducing glutamatergic activation. However, it can result in neuropsychological side effects which limit its use. Several other more selective glutamatergic drugs have been evaluated but clinical development has been stopped due to insufficient anti-dyskinetic effect.³⁵ Another glutamatergic drug, dipraglurant, an mGluR5-negative allosteric modulator, was already in phase 2b/3 clinical trials, when the study was terminated in mid-2022.^{40,41} According to

Addex Therapeutics this was due to the slow recruitment of patients. Only AV-101, a selective NMDA receptor antagonist, seems to be still in development targeting the glutamatergic system.⁴² Not only the glutamatergic system is expected to play a role in dyskinesia, but also the serotonergic system. It is known that serotonergic neurons can take over the function of striatal dopaminergic neurons, but since they lack autoreceptors and dopamine reuptake abilities, they release dopamine in an uncontrolled manner.⁴³ Therefore, inhibition of serotonin neurons (via G_i-coupled 5-HT₁ receptors) might prove beneficial in reducing dyskinesia.³⁷ Various drugs are in development to test this hypothesis and initial results are positive. Drugs in clinical development include 5-hydroxytryptophan (5-HTP), buspirone/zolmitriptan (JM-010), eltoprazine, and befiradol.^{37,44} In addition, the D₃ antagonist, mesdopetam has shown benefit in phase 2 clinical trials and is therefore expected to progress to phase 3 clinical trials.^{45,46} Lastly, two phosphodiesterase inhibitors are in development. CPL500036, a phosphodiesterase 10A (PDE10A) inhibitor, has shown anti-dyskinetic effects without reducing the effect of levodopa in an animal PD model,⁴⁷ and is expected to complete its phase 2 trial in PD patients with levodopa-induced dyskinesia at the end of 2023.⁴⁸ Lenrisopodun, a PDE1 inhibitor, is currently also in phase 2 clinical trials, where it is investigated as an adjunctive therapy for PD patients with wearing *OFF* symptoms and levodopa-induced dyskinesia.⁴⁹

INITIAL TREATMENT WITH NOVEL (MORE PHYSIOLOGICAL) DOPAMINE AGONISTS

The abovementioned treatments in development are all focused on treating patients that already experience motor fluctuations and/or dyskinesia. However, ideally, we are able to at least delay the onset of these complications. Previously, it was thought that delaying the initiation of levodopa might help delay the onset of motor fluctuations and dyskinesia. The LEAP study has shown that this is not the case, and that starting levodopa later in early PD patients does not reduce or delay response fluctuations. In contrast, the group that started levodopa earlier had fewer patients experiencing motor response fluctuations after 80 weeks, underscoring the importance of timely intervention.⁵⁰

Another option that has been contemplated is to start treatment in early PD patients with a levodopa-sparing therapy instead of levodopa. The PD-MED study has shown that patients that started on levodopa compared to a levodopa-sparing therapy (dopamine agonist or MAO-B inhibitor) were more likely to develop dyskinesia, but showed no differences in motor fluctuations. Despite the higher likelihood of developing dyskinesia, patients initially treated with levodopa had small but persistent higher patient-rated mobility scores and had less side effects (mainly psychological, sleep disturbance, and gastrointestinal). Hence, levodopa was, and still is, considered the preferred initial treatment in most patients.⁵¹ However, if a dopamine agonist with a better risk-benefit profile would become available, this preference might shift. Tavapadon might be such a dopamine agonist. It is a novel selective D1/D5 partial agonist.⁵² Preclinical studies have shown that it improves motor function as effectively as levodopa but with a longer duration of effect. Moreover, animal studies have shown that D1/5- but not D2/3-selective dopamine agonists can improve motor symptoms in animals with progressive neurodegeneration that are unresponsive to levodopa.⁵² This indicates that D1/5 agonism could be useful both in early as well as advanced PD. Tavapadon is currently indeed investigated in multiple phase 3 clinical trials as a monotherapy in treatment-naïve early PD patients, as well as an adjunctive treatment in levodopa-treated patients with motor fluctuations.⁵³⁻⁵⁶ Due to its partial D1/5 agonism, tavapadon is hoped to result in fewer D2/3-associated side effects (e.g. impulse control disorder, sleep disturbance) and less D1/5 full agonism-associated side effects (e.g. cardiovascular and dyskinesic side effects). Its partial agonism is also expected to provide a more physiological stimulation, since the likelihood of receptor overstimulation and hence desensitization and tolerance is lower. A final added benefit of D1 stimulation is that D1 is not only involved in motor control but also in cognition, and hence might have a beneficial effect on cognition. Results from phase 1 and phase 2 clinical trials are encouraging with significant improvements in motor function and MDS-UPDRS I-III combined scores, a substantially longer half-life (~24 hours) than levodopa and available D2/3 agonists, and only mild cardiovascular changes (e.g.

decreases in blood pressure, increases in heart rate).⁵² The phase 3 clinical trials will have to confirm whether tavapadon will live up to its potential.

The development of response fluctuations is largely due to the progressive degeneration of dopaminergic neurons, but also partly due to non-physiological pulsatile stimulation by dopaminergic drugs. By developing drugs that provide a more physiological stimulation, we may be able to delay the development of response fluctuations and dyskinesia. Positive allosteric modulators (PAMs) have been implicated to provide such a more physiological stimulation. They do not directly activate the dopamine receptor, but enhance the effects of endogenously available dopamine. Therefore, they have the potential to prevent excessive stimulation and resulting receptor desensitization and the development of tolerance, as well as contribute to a better tolerability profile. D1 PAMs are already in development for PD. A phase 1b study in PD patients has been completed with mevidalen (LY3154207) showing improved motor function in all patients receiving mevidalen and in some receiving placebo.⁵⁷ However, the compound is further being developed for symptomatic Lewy body dementia.⁵⁸ Another D1 PAM, UCBO022, has shown preclinically to improve motor function similar to levodopa but with less dyskinesia.⁵⁸ It will be investigated in a phase 2 clinical trial starting at the end of 2023. The trial will evaluate the effect on OFF time when UCBO022 is given as an adjunctive therapy to advanced PD patients.⁵⁹ UCBO022 has not yet been investigated as a monotherapy and compared to the efficacy of levodopa in PD patients, but this will be an interesting next step. Similarly, D2 PAMs hold promise for progressing the treatment of PD by offering a more physiological stimulation compared to existing dopamine agonists. However, its development is still in the preclinical stage.⁶⁰

Other applications of Staccato inhalation and buccal drug delivery

The Staccato technology is designed to administer drug aerosol particles into the deep lung with a single breath. The Staccato device holds a distinct advantage over other inhalation devices due to its

excipient-free composition. Unlike pressurized metered dose inhalers (pMDIs), it does not require coordination between device actuation and inhalation.⁶² Moreover, unlike most dry powder inhalers that require a moderate-to-high inspiratory flow rate (usually at least 30 L/min) to separate the drug from the carrier particles and aerosolize it, the Staccato device requires only a low inspiratory flow rate of about 15 L/min for device actuation.^{62,63} But above all, inhalation of a drug with the Staccato device results in rapid systemic absorption, mimicking that of an intravenous administration. In this thesis, we indeed showed that maximum apomorphine plasma concentrations were reached within 1-2 minutes after inhalation and that motor symptoms in PD patients were improved at the first measurement time point 10 minutes post administration. This rapid absorption, and consequently, quick onset of action, provides opportunities to use the Staccato device in other indications that require quick resolution of complaints. Indeed, Staccato loxapine has already been approved for the acute treatment of agitation associated with schizophrenia or bipolar I disorder.⁶⁴⁻⁶⁶ The phase 3 trials showed improvements in agitation at the first assessment at 10 minutes post administration, which is significantly earlier than for oral or intramuscular loxapine.⁶⁷ Moreover, ongoing research is exploring other therapeutic indications. A currently ongoing phase 3 trial in epilepsy patients investigates the safety and tolerability of Staccato alprazolam which is intended for the rapid termination of epileptic seizures.^{68,69} The phase 2b study showed promising results, i.e., Staccato alprazolam resulted in a significantly greater proportion of patients with seizure cessation within 2 minutes and no recurrence within 2 hours, compared to placebo.⁷⁰ In addition, Staccato granisetron is being investigated for the acute treatment of sudden, repeated episodes of severe nausea and vomiting (cyclic vomiting syndrome). The phase 2 study has been completed in 2022, but results are pending.⁷¹ In addition to these therapeutic indications, many others that require acute treatment can be explored, for example acute allergic reactions. Buccal drug administration can be used both for local and systemic treatment. The focus of this thesis was on achieving systemic exposure through buccal drug delivery. Small lipophilic drugs can be easily absorbed through the buccal mucosa while avoiding first-pass

hepatic metabolism and enzymatic degradation in the gastrointestinal tract. Moreover, buccal drug administration is considered more user-friendly than e.g. intravenous, subcutaneous or rectal administration. Hence, it provides an administration route that is not only interesting for the treatment of *OFF* episodes (this thesis), but also for various other indications. However, despite this potential, only a limited number of buccal formulations are on the market. Available formulations include oromucosal solutions (e.g. midazolam),⁷² buccal films (e.g. buprenorphine/naloxone, and fentanyl),^{73,74} and buccal tablets (e.g. fentanyl, prochlorperazine, and testosterone).⁷⁵⁻⁷⁷ The development of buccal formulations has many challenges, including the residence time of the drug in the buccal cavity. This time is usually limited due to insufficient adhesion of the formulation to the wet buccal mucosa and swallowing of the drug with the saliva. Another challenge, especially for larger and hydrophilic drugs, is crossing the mucosal barrier that consists of multiple epithelial cell layers and a mucus layer. To overcome these challenges, current research is focused on the development of mucoadhesive films and patches that strongly adhere to the buccal cavity.^{78,79} This increases the drug's residence time and hence the time available for drug absorption. Also significant research efforts are targeted at using nanoparticles as drug carriers in buccal formulations.⁸⁰ These nanocarriers can increase the permeability of the drug through the mucus layer and protect the drug from enzymatic degradation. Moreover, the nanoparticles can be adjusted to have controlled or sustained release characteristics. To increase bioavailability, researchers are also exploring permeation enhancers that can effectively increase the permeability of the buccal mucosa without toxicity.⁷⁸ For protein and peptide delivery, protease inhibitors may also be added to buccal formulations to protect the drug from degradation.

Taken together, extensive research is being conducted on buccal drug delivery systems to enhance systemic exposure and to expand the range of drugs that can be delivered via the buccal route beyond that of small lipophilic compounds. In this pursuit, mucoadhesive buccal films and patches containing drug-loaded nanoparticles, possibly with permeation enhancers and protease inhibitors, hold promise for the future.

OVERALL CONCLUSION

With the aging of the worldwide population, more PD patients will live long enough to fall victim to response fluctuations. This has a significant impact on their quality of life. Although there is hope that disease-modifying drugs will enter the market, these drugs are expected to slow disease progression rather than cure the disease. Consequently, the need for symptomatic treatment is expected to remain in the future. The goal is to develop user-friendly symptomatic drugs with fewer side effects to improve patient's quality of life. Fortunately, there are several drugs in clinical development that target response fluctuations. These include novel apomorphine formulations, as discussed in this thesis, which show promise in treating sudden *OFF* episodes. To accurately assess the (onset of) efficacy of fast-acting dopaminergic drugs, future clinical trials could be improved by adding finger tapping tasks as a pharmacodynamic measurement.

TABLE 1 Cumulative incidence of motor fluctuations and levodopa-induced dyskinesia in incident population-based Parkinson's disease cohorts from the time of diagnosis.

Reference	Cohort size	Motor fluctuations	Levodopa-induced dyskinesia
Scott et al. 2016 ¹⁴	N=189	22.8% at 5 years	29.6% at 5 years
Bjornestad et al. 2016 ¹⁵	N=189	42.9% at 5 years	24.3% at 5 years
Kim et al. 2020 ¹⁶	N=141	54.3% at 5 years 100% at 10 years	14.5% at 5 years 55.7% at 10 years

FIGURE 1 Symptomatic treatments in development for response fluctuations.

DRUG	MECHANISM OF ACTION	PHASE 1	PHASE 2	PHASE 3	NDA
Intermittent OFF					
Staccato apomorphine inhalation (AZ-009)	D1 and D2 agonist	→	→		
Oromucosal apomorphine solution for buccal delivery (APORON)	D1 and D2 agonist	→			
Levodopa dry powder inhalation (Cyclops)	Dopamine precursor	→	→		
Continuous delivery					
Continuous subcutaneous levodopa/carbidopa infusion (ND0612)	Dopamine precursor	→	→	→	
Continuous subcutaneous foslevodopa/foscarbidopa infusion (ABBV-951)	Prodrug of levodopa/carbidopa	→	→	→	
Continuous subcutaneous levodopa/carbidopa infusion (D12102)	Dopamine precursor	→			
Continuous oral levodopa/carbidopa (DopaFuse)	Dopamine precursor	→	→		
Extended release					
Levodopa/carbidopa oral capsules (IPX203)	IR LD/CD granules and ER LD beads with enteric coating. Dopamine precursor.	→	→	→	
Levodopa/carbidopa oral tablets (DM -1992)	IR LD/CD layer and ER LD/CD gastro-retentive core. Dopamine precursor.	→	→		
Levodopa/carbidopa accordion pill	Gastro-retentive multilayer sheets with IR CD and IR/ER LD. Dopamine precursor.	→	→		
Levodopa-induced dyskinesia					
AV-101 (L-4-chlorokynurenine)	NMDA glycine site antagonist	→			
5-hydroxytryptophan (5-HTP)	Serotonin precursor	→	→		
Buspiron/zolmitriptan (JM-010)	5-HT1A agonist and 5-HT1B/5-HT1D agonist combination	→	→		
Etopirazine	5-HT1A and 5-HT1B agonist	→	→		
Befiradol (NLX-112)	Selective 5-HT1A agonist	→	→		
Mesdopetam (IRL790)	D3 antagonist	→	→		
CPL500036	PDE10A inhibitor	→	→		
Lenrisopodun (ITI-214)	PDE1 inhibitor	→	→		
Partial agonism and positive allosteric modulation					
Tavapadon	Partial D1/D5 receptor agonist	→	→		
UCB0022	D1 positive allosteric modulator	→			

Based on literature reviews,^{37,61} trial registries (clinicaltrialsregister.eu, clinicaltrials.gov) and press releases. CD, carbidopa; ER, extended-release; IR, immediate-release; LD, levodopa; NDA, New Drug Application.

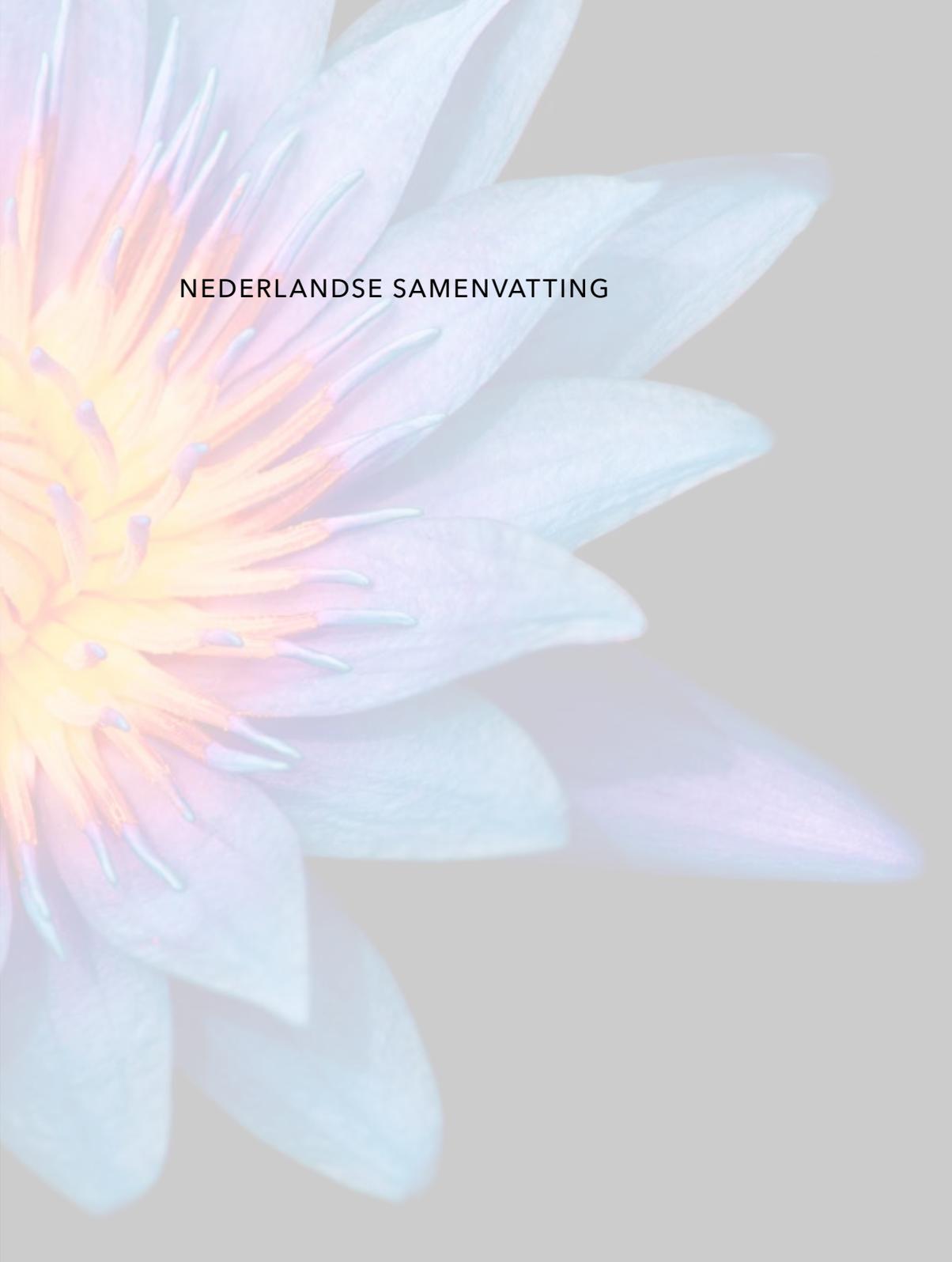
REFERENCES

- Study Details | Study to Evaluate the Efficacy and Safety of Staccato Apomorphine (AZ-009) in Patients With Parkinson's Disease Experiencing OFF Episodes | ClinicalTrials.gov. Accessed November 15, 2023. <https://clinicaltrials.gov/study/nct05979415?aggFilters=phase:2&intr=apomorphine&rank=7>.
- Olanow CW, Factor SA, Espay AJ, et al. Apomorphine sublingual film for off episodes in Parkinson's disease: a randomised, double-blind, placebo-controlled phase 3 study. *Lancet Neurol.* 2020;19(2):135-144. doi:10.1016/S1474-4422(19)30396-5.
- Burkman AM. Some Kinetic and Thermodynamic Characteristics of Apomorphine Degradation. *J Pharm Sci.* 1965;54(2):325-326. doi:10.1002/JPS.2600540242.
- Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinones in toxicology. *Chem Res Toxicol.* 2000;13(3):135-160. doi:10.1021/TX9902082.
- Dos Santos El-Bachá R, Daval JL, Koziel V, Netter P, Minn A. Toxic effects of apomorphine on rat cultured neurons and glial C6 cells, and protection with antioxidants. *Biochem Pharmacol.* 2001;61(1):73-85. doi:10.1016/S0006-2952(00)00524-4.
- Watkinson RM, Herkenne C, Guy RH, Hadgraft J, Oliveira G, Lane ME. Influence of Ethanol on the Solubility, Ionization and Permeation Characteristics of Ibuprofen in Silicone and Human Skin. *Skin Pharmacol Physiol.* 2009;22(1):15-21. doi:10.1159/000183922.
- Levang AK, Zhao K, Singh J. Effect of ethanol/propylene glycol on the in vitro percutaneous absorption of aspirin, biophysical changes and macroscopic barrier properties of the skin. *Int J Pharm.* 1999;181(2):255-263. doi:10.1016/S0378-5173(99)00055-1.
- Peterson B, Weyers M, Steenekamp JH, Steyn JD, Gouws C, Hamman JH. Drug Bioavailability Enhancing Agents of Natural Origin (Bioenhancers) that Modulate Drug Membrane Permeation and Pre-Systemic Metabolism. *Pharmaceutics.* 2019;11(1). doi:10.3390/PHARMACEUTICS11010033.
- Maher S, Casettari L, Illum L. Transmucosal Absorption Enhancers in the Drug Delivery Field. *Pharmaceutics* 2019, Vol 11, Page 339. 2019;11(7):339. doi:10.3390/PHARMACEUTICS11070339.
- Luinstra M, Rutgers AWF, Dijkstra H, et al. Can Patients with Parkinson's Disease Use Dry Powder Inhalers during Off Periods? *PLoS One.* 2015;10(7):e0132714. doi:10.1371/JOURNAL.PONE.0132714.
- LeWitt PA, Hauser RA, Grosset DG, et al. A randomized trial of inhaled levodopa (CVT-301) for motor fluctuations in Parkinson's disease. *Movement Disorders.* 2016;31(9):1356-1365. doi:10.1002/MDS.26611.
- Ray Dorsey E, Elbaz A, Nichols E, et al. Global, regional, and national burden of Parkinson's disease, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 2018;17(11):939-953. doi:10.1016/S1474-4422(18)30295-3.
- Dorsey ER, Bloem BR. The Parkinson Pandemic—A Call to Action. *JAMA Neurol.* 2018;75(1):9-10. doi:10.1001/JAMANEUROL.2017.3299.
- Scott NW, Macleod AD, Counsell CE. Motor complications in an incident Parkinson's disease cohort. *Eur J Neurol.* 2016;23(2):304-312. doi:10.1111/ENE.12751.
- Bjornestad A, Forsaa EB, Pedersen KF, Tysnes OB, Larsen JP, Alves G. Risk and course of motor complications in a population-based incident Parkinson's disease cohort. *Parkinsonism Relat Disord.* 2016;22:48-53. doi:10.1016/J.PARKRELDIS.2015.11.007.
- Kim HJ, Mason S, Foltynie T, Winder-Rhodes S, Barker RA, Williams-Gray CH. Motor Complications in Parkinson's Disease: 13-Year Follow-up of the CamPaGN Cohort. *Movement Disorders.* 2020;35(1):185-190. doi:10.1002/MDS.27882.
- Poewe W, Seppi K, Tanner CM, et al. Parkinson disease. *Nat Rev Dis Primers.* 2017;3:1-21. doi:10.1038/NRDP.2017.13.
- Vijiaratnam N, Simuni T, Bandmann O, Morris HR, Foltynie T. Progress towards therapies for disease modification in Parkinson's disease. *Lancet Neurol.* 2021;20(7):559-572. doi:10.1016/S1474-4422(21)00061-2.
- Barbuti PA, Barker RA, Brundin P, et al. Recent Advances in the Development of Stem-Cell-Derived Dopaminergic Neuronal Transplant Therapies for Parkinson's Disease. *Movement Disorders.* 2021;36(8):1772-1780. doi:10.1002/MDS.28628.
- Study Details | Evaluating Safety, Tolerability, and

- Efficacy of Autologous MitoCell Transplantation in Subjects With Idiopathic Parkinson's Disease | ClinicalTrials.gov. Accessed December 3, 2023. https://clinicaltrials.gov/study/nct05094011?distance=50&start=2021-11-04_&cond=Parkinson&term=OFF&page=3&rank=67.
- 21 Heris RM, Shirvaliloo M, Abbaspour-Aghdam S, et al. The potential use of mesenchymal stem cells and their exosomes in Parkinson's disease treatment. *Stem Cell Research & Therapy* 2022;13(1):1-14. doi:10.1186/S13287-022-03050-4.
- 22 Study Details | Study to Evaluate the Efficacy and Safety of Staccato Apomorphine (AZ-009) in Patients With Parkinson's Disease Experiencing OFF Episodes | ClinicalTrials.gov. Accessed November 22, 2023. <https://clinicaltrials.gov/study/nct05979415?intr=az-009&aggFilters=phase:2&rank=1>.
- 23 Study Details | Therapeutic effects of an inhaled levodopa dry powder formulation on the recovery from off periods in patients with Parkinson's disease | Clinical Trials Register. Accessed November 22, 2023. <https://www.clinicaltrialsregister.eu/ctr-search/trial/2017-004006-18/NL>.
- 24 Study Details | A Pilot Comparative Bioavailability Study of Levodopa Administered Via Levodopa Cyclops™ Relative to INBRIJA® | ClinicalTrials.gov. Accessed November 22, 2023. https://clinicaltrials.gov/study/nct06037590?cond=MotorFluctuations&distance=50&start=2021-11-02_&rank=6.
- 25 Study Details | Usability of Levodopa Cyclops™ vs INBRIJA® in Parkinson's Patients | ClinicalTrials.gov. Accessed November 22, 2023. https://clinicaltrials.gov/study/nct05499572?cond=Parkinson&distance=50&start=2017-11-02_&intr=cyclops&rank=2.
- 26 Cyclops platform inhalation technology - PureIMS. Accessed November 22, 2023. <https://pureims.com/cyclops/>.
- 27 Inbrija Product Information | European Medicines Agency. Accessed November 22, 2023. <https://www.ema.europa.eu/en/medicines/human/EPAR/inbrija#product-information-section>.
- 28 Luinstra M, Isufi V, de Jong L, et al. Learning from Parkinson's patients: Usability of the Cyclops dry powder inhaler. *Int J Pharm*. 2019;567:118493. doi:10.1016/J.IJPHARM.2019.118493.
- 29 Deuschl G, Antonini A, Costa J, et al. European Academy of Neurology/Movement Disorder Society-European Section Guideline on the Treatment of Parkinson's Disease: I. Invasive Therapies. *Movement Disorders*. 2022;37(7):1360-1374. doi:10.1002/MDS.29066.
- 30 Bergquist F, Ehrnebo M, Nyholm D, et al. Pharmacokinetics of Intravenously (DIZ101), Subcutaneously (DIZ102), and Intestinally (LCIG) Infused Levodopa in Advanced Parkinson Disease. *Neurology*. 2022;99(10):E965-E976. doi:10.1212/WNL.000000000000200804.
- 31 Doggrell SA. Continuous subcutaneous levodopa-carbidopa for the treatment of advanced Parkinson's disease: is it an improvement on other delivery? *Expert Opin Drug Deliv*. Published online September 2, 2023. doi:10.1080/17425247.2023.2253146.
- 32 LeWitt PA, Stocchi F, Arkadir D, et al. The pharmacokinetics of continuous subcutaneous levodopa/carbidopa infusion: Findings from the NDO612 clinical development program. *Front Neurol*. 2022;13:1036068. doi:10.3389/FNEUR.2022.1036068/BIBTEX.
- 33 Rosebraugh M, Liu W, Neenan M, Facheris MF. Foslevodopa/Foscarbidopa Is Well Tolerated and Maintains Stable Levodopa and Carbidopa Exposure Following Subcutaneous Infusion. *J Parkinsons Dis*. 2021;11(4):1695-1702. doi:10.3233/JPD-212813.
- 34 Soileau MJ, Aldred J, Budur K, et al. Safety and efficacy of continuous subcutaneous foslevodopa-foscarbidopa in patients with advanced Parkinson's disease: a randomised, double-blind, active-controlled, phase 3 trial. *Lancet Neurol*. 2022;21(12):1099-1109. doi:10.1016/S1474-4422(22)00400-8.
- 35 EudraCT Number 2020-003372-41 - Clinical trial results DopaFuse - EU Clinical Trials Register. Accessed November 23, 2023. <https://www.clinicaltrialsregister.eu/ctr-search/trial/2020-003372-41/results>.
- 36 Hauser RA, Espay AJ, Ellenbogen AL, et al. IPX203 vs Immediate-Release Carbidopa-Levodopa for the Treatment of Motor Fluctuations in Parkinson Disease: The RISE-PD Randomized Clinical Trial. *JAMA Neurol*. 2023;80(10):1062-1069. doi:10.1001/JAMANEUROL.2023.2679.
- 37 Di Luca DG, Reyes NGD, Fox SH. Newly Approved and Investigational Drugs for Motor Symptom Control in Parkinson's Disease. *Drugs* 2022;82(10):1027-1053. doi:10.1007/S40265-022-01747-7.
- 38 Accordion Pill Carbidopa/Levodopa Not Superior to Immediate Release Formulation. Published July 23, 2019. Accessed November 24, 2023. <https://www.neurologylive.com/view/accordion-pill-carbidopalevodopa-not-superior-immediate-release-formulation>.
- 39 Intec Ready for New Phase 3 Study of Parkinson's Accordion Pill. Published May 18, 2020. Accessed November 24, 2023. <https://parkinsonsnewstoday.com/2020/05/18/intec-readying-new-phase-3-accordion-pill-study-parkinsons>.
- 40 Study Details | Dipraglurant (ADX48621) for the Treatment of Patients With Parkinson's Disease Receiving Levodopa-based Therapy | ClinicalTrials.gov. Accessed November 23, 2023. https://clinicaltrials.gov/study/nct04857359?cond=dyskinesia&start=2019-09-29_&rank=2.
- 41 Addex Terminates Dipraglurant Phase 2b/3 Study in Patients with Dyskinesia Associated with Parkinson's Disease due to Slow Recruitment Rate. Accessed November 23, 2023. <https://www.addextherapeutics.com/en/investors/press-releases/addex-terminates-dipraglurant-phase-2b3-study-patients-dyskinesia-associated-parkinsons-disease-due-slow-recruitment-rate/>.
- 42 Study Details | AV-101 (L-4-chlorokynurenine) in Parkinson's Disease Subjects With Levodopa-Induced Dyskinesia | ClinicalTrials.gov. Accessed November 23, 2023. https://clinicaltrials.gov/study/nct04147949?start=2019-09-29_&intr=AV-101&rank=4.
- 43 Cenci MA, Skovgård K, Odin P. Non-dopaminergic approaches to the treatment of motor complications in Parkinson's disease. *Neuropharmacology*. 2022;210:109027. doi:10.1016/J.NEUROPHARM.2022.109027.
- 44 Meloni M, Puligheddu M, Sanna F, et al. Efficacy and safety of 5-Hydroxytryptophan on levodopa-induced motor complications in Parkinson's disease: A preliminary finding. *J Neurol Sci*. 2020;415:116869. doi:10.1016/J.JNS.2020.116869.
- 45 Study Details | A Clinical Study of Mesdopetam in Patients With Parkinson's Disease Experiencing Levodopa Induced Dyskinesia | ClinicalTrials.gov. Accessed November 23, 2023. [https://clinicaltrials.gov/study/nct04435431?intr=Mesdopetam%5C\(IRL790%5C\)&rank=2](https://clinicaltrials.gov/study/nct04435431?intr=Mesdopetam%5C(IRL790%5C)&rank=2).
- 46 Study Details | Efficacy and Tolerability of IRL790 in Parkinson's Disease Dyskinesia | ClinicalTrials.gov. Accessed November 23, 2023. [https://clinicaltrials.gov/study/nct03368170?intr=Mesdopetam%5C\(IRL790%5C\)&rank=1](https://clinicaltrials.gov/study/nct03368170?intr=Mesdopetam%5C(IRL790%5C)&rank=1).
- 47 Lenda T, Ossowska K, Berghauzen-Maciejewska K, et al. Antiparkinsonian-like effects of CPL500036, a novel selective inhibitor of phosphodiesterase 10A, in the unilateral rat model of Parkinson's disease. *Eur J Pharmacol*. 2021;910:174460. doi:10.1016/J.EJPHAR.2021.174460.
- 48 Study Details | Efficacy, Safety and Pharmacokinetics Study of CPL500036 (PDE10A Inhibitor) in Patients With Schizophrenia | ClinicalTrials.gov. Accessed November 24, 2023. <https://clinicaltrials.gov/study/nct05278156?intr=CPL500036&rank=2>.
- 49 Study Details | Lenrispodun as Adjunctive Therapy in the Treatment of Patients With Motor Fluctuations Due to Parkinson's Disease | ClinicalTrials.gov. Accessed November 24, 2023. <https://clinicaltrials.gov/study/nct05766813?intr=lenrispodun&rank=1>.
- 50 Frequin HL, Schouten J, Verschuur CVM, et al. Levodopa Response in Patients With Early Parkinson Disease. *Neurology*. 2023;100(4):e367-e376. doi:10.1212/WNL.00000000000201448.
- 51 de Bie RMA, Clarke CE, Espay AJ, Fox SH, Lang AE. Initiation of pharmacological therapy in Parkinson's disease: when, why, and how. *Lancet Neurol*. 2020;19(5):452-461. doi:10.1016/S1474-4422(20)30036-3.
- 52 Bezard E, Gray D, Kozak R, Leoni M, Combs C, Duvvuri S. Rationale and Development of Tavapadon, a D1/D5-Selective Partial Dopamine Agonist for the Treatment of Parkinson's Disease. *CNS Neurol Disord Drug Targets*. 2023;23(4):476-487. doi:10.2174/1871527322662230331121028.
- 53 Study Details | Fixed-Dose Trial in Early Parkinson's Disease (PD) | ClinicalTrials.gov. Accessed November 24, 2023. <https://clinicaltrials.gov/study/nct04201093?intr=tavapadon&aggFilters=phase:3&rank=4>.
- 54 Study Details | Flexible-Dose Trial in Early Parkinson's Disease (PD) | ClinicalTrials.gov. Accessed November 24, 2023. <https://clinicaltrials.gov/>

- study/nct04223193?intr=tavapadon&aggFilters=phase:3&rank=3.
- 55 Study Details | Flexible-Dose, Adjunctive Therapy Trial in Adults With Parkinson's Disease With Motor Fluctuations | ClinicalTrials.gov. Accessed November 24, 2023. <https://clinicaltrials.gov/study/nct04542499?intr=tavapadon&aggFilters=phase:3&rank=2>.
- 56 Study Details | Open-label Trial in Parkinson's Disease (PD) | ClinicalTrials.gov. Accessed November 24, 2023. <https://clinicaltrials.gov/study/nct04760769?intr=tavapadon&aggFilters=phase:3&rank=1>.
- 57 Wilbraham D, Biglan KM, Svensson KA, et al. Safety, Tolerability, and Pharmacokinetics of Mevidalen (LY3154207), a Centrally Acting Dopamine D1 Receptor-Positive Allosteric Modulator, in Patients With Parkinson Disease. *Clin Pharmacol Drug Dev*. 2022;11(3):324-332. doi:10.1002/CPDD.1039.
- 58 Study Details | A Study of LY3154207 in Participants With Dementia Due to Lewy Body Dementia (LBD) Associated With Idiopathic Parkinson's Disease (PD) or Dementia With Lewy Bodies (DLB) | ClinicalTrials.gov. Accessed November 24, 2023. <https://clinicaltrials.gov/study/nct03305809?intr=LY3154207&aggFilters=phase:2&rank=1>.
- 59 Study Details | A Study to Evaluate the Efficacy, Safety, Tolerability, and Pharmacokinetics of UCBO022 in Study Participants With Advanced Parkinson's Disease | ClinicalTrials.gov. Accessed November 27, 2023. [https://clinicaltrials.gov/study/nct06055985?cond=Motor Fluctuations&distance=50&start=2021-11-02_&rank=5](https://clinicaltrials.gov/study/nct06055985?cond=Motor%20Fluctuations&distance=50&start=2021-11-02_&rank=5).
- 60 Editors A, Wei Lim L, Aquili L, Kaczor AA, Wróbel TM, Bartuzi D. Allosteric Modulators of Dopamine D2 Receptors for Fine-Tuning of Dopaminergic Neurotransmission in CNS Diseases: Overview, Pharmacology, Structural Aspects and Synthesis. *Molecules* 2023, Vol 28, Page 178. 2022;28(1):178. doi:10.3390/MOLECULES28010178.
- 61 AlShimemeri S, Fox SH, Visanji NP, Visanji Edmond J, Safra Program in Parkinson Disease NP, Shulman G. Emerging drugs for the treatment of L-DOPA-induced dyskinesia: an update. *Expert Opin Emerg Drugs*. 2020;25(2):131-144. doi:10.1080/14728214.2020.1763954.
- 62 Dinh KV., Myers DJ, Noymer PD, Cassella J V. In vitro aerosol deposition in the oropharyngeal region for staccato® loxapine. *J Aerosol Med Pulm Drug Deliv*. 2010;23(4):253-260. doi:10.1089/JAMP.2009.0814/ASSET/IMAGES/LARGE/FIGURE9.JPG.
- 63 Ghosh S, Ohar JA, Drummond MB. Peak Inspiratory Flow Rate in Chronic Obstructive Pulmonary Disease: Implications for Dry Powder Inhalers. <https://home.liebertpub.com/jamp>. 2017;30(6):381-387. doi:10.1089/JAMP.2017.1416.
- 64 Drug Approval Package: Adasuve (loxapine) NDA #022549. Accessed December 14, 2021. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/022549_adasuve_toc.cfm.
- 65 Adasuve | European Medicines Agency. Accessed December 14, 2021. <https://www.ema.europa.eu/en/medicines/human/EPAR/adasuve#authorisation-details-section>.
- 66 Allen MH, Feifel D, Lesem MD, et al. Efficacy and Safety of Loxapine for Inhalation in the Treatment of Agitation in Patients With Schizophrenia: A Randomized, Double-Blind, Placebo-Controlled Trial. *J Clin Psychiatry*. 2011;72(10):0-0. doi:10.4088/JCP.10Mo6011YEL.
- 67 Zeller S, Zun L, Cassella J V., Spyker DA, Yeung PP. Response to inhaled loxapine in patients with schizophrenia or bipolar I disorder: PANSS-EC responder analyses. *BJPsych Open*. 2017;3(6):285-290. doi:10.1192/BJPO.BP.117.005363.
- 68 Study Details | A Study to Test the Safety and Tolerability of Staccato Alprazolam in Study Participants 12 Years of Age and Older With Stereotypical Prolonged Seizures | ClinicalTrials.gov. Accessed November 15, 2023. <https://clinicaltrials.gov/study/nct05076617?aggFilters=phase:3&intr=alprazolam&rank=1>.
- 69 Study Details | A Study to Test the Efficacy and Safety of Staccato Alprazolam in Study Participants 12 Years of Age and Older With Stereotypical Prolonged Seizures | ClinicalTrials.gov. Accessed November 15, 2023. <https://clinicaltrials.gov/study/nct05077904?aggFilters=phase:3&intr=alprazolam&rank=2>.
- 70 French J, Biton V, Dave H, et al. A randomized phase 2b efficacy study in patients with seizure episodes with a predictable pattern using Staccato® alprazolam for rapid seizure termination. *Epilepsia*. 2023;64(2):374-385. doi:10.1111/EPI.17441.
- 71 Study Details | Staccato Granisetron® (AZ 010) for the Treatment of Cyclic Vomiting Syndrome | ClinicalTrials.gov. Accessed November 15, 2023. [https://clinicaltrials.gov/study/nct04645953?intr=staccato granisetron&rank=3](https://clinicaltrials.gov/study/nct04645953?intr=staccato%20granisetron&rank=3).
- 72 Buccolam | European Medicines Agency. Accessed November 30, 2023. <https://www.ema.europa.eu/en/medicines/human/EPAR/buccolam>.
- 73 Suboxone | European Medicines Agency. Accessed November 30, 2023. <https://www.ema.europa.eu/en/medicines/human/EPAR/suboxone>.
- 74 Onsolis (fentanyl buccal soluble film) Information | FDA. Accessed November 30, 2023. <https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/onsolis-fentanyl-buccal-soluble-film-information>.
- 75 Fentanyl Buccal Tablets (marketed as Fentora) Information | FDA. Accessed November 30, 2023. <https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/fentanyl-buccal-tablets-marketed-fentora-information>.
- 76 Drug Approval Package: Striant (Testosterone Buccal System) NDA #021543. Accessed November 30, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-543_Striant.cfm.
- 77 Prochlorperazine 3 mg Buccal Tablets - Summary of Product Characteristics (SmPC) - (emc). Accessed November 30, 2023. <https://www.medicines.org.uk/emc/product/5227/smpc#gref>.
- 78 Jacob S, Nair AB, Boddu SHS, Gorain B, Sreeharsha N, Shah J. An Updated Overview of the Emerging Role of Patch and Film-Based Buccal Delivery Systems. *Pharmaceutics* 2021, Vol 13, Page 1206. 2021;13(8):1206. doi:10.3390/PHARMACEUTICS13081206.
- 79 Hu S, Pei X, Duan L, et al. A mussel-inspired film for adhesion to wet buccal tissue and efficient buccal drug delivery. *Nature Communications* 2021 12:1. 2021;12(1):1-17. doi:10.1038/s41467-021-21989-5.
- 80 Macedo AS, Castro PM, Roque L, et al. Novel and revisited approaches in nanoparticle systems for buccal drug delivery. *Journal of Controlled Release*. 2020;320:125-141. doi:10.1016/J.JCONREL.2020.01.006.

APPENDICES



NEDERLANDSE SAMENVATTING

De ziekte van Parkinson is een progressieve neurodegeneratieve aandoening die wereldwijd miljoenen mensen treft. Ondanks alle onderzoeksinspanningen is er momenteel geen ziekte-modificerende behandeling beschikbaar en blijft symptomatische behandeling de enige behandelingsmethode.

Naarmate de ziekte vordert, ervaren patiënten motorische en niet-motorische symptoomschommelingen die aanzienlijke invloed hebben op hun dagelijkse activiteiten en kwaliteit van leven. Ze wisselen tussen perioden van een goede respons op medicatie (*ON*) en perioden van onvoldoende respons (*OFF*). Apomorfine is een dopamineagonist die al drie decennia lang wordt gebruikt om *OFF*-perioden te behandelen. Het is beschikbaar als subcutane intermitterende injecties en als subcutane continue infusie. De intermitterende injecties zijn met name geschikt voor patiënten met slechts enkele *OFF*-perioden per dag (de focus van dit proefschrift). Ondanks de effectiviteit van apomorfine, wordt het onvoldoende gebruikt omdat de beschikbare toedieningsroutes niet erg gebruiksvriendelijk zijn. Zo moeten patiënten in staat zijn om zichzelf te injecteren tijdens hun *OFF*-perioden en is de plaats waar ze moeten injecteren vaak bedekt met kleding. Ook reacties op de injectieplaats en angst voor naalden kunnen ervoor zorgen dat patiënten apomorfine liever niet gebruiken. Er is dus behoefte aan een gebruiksvriendelijkere manier van toedienen.

APOMORFINE

In dit proefschrift onderzochten we twee nieuwe apomorfine formuleringen die naar verwachting gebruiksvriendelijker zijn. In [hoofdstuk 2 en 3](#) werd de absorptie, veiligheid en effectiviteit van apomorfine orale inhalatie onderzocht in gezonde vrijwilligers en Parkinson patiënten. Staccato apomorfine (AZ-009) bereikte maximale plasmaconcentraties 1-2 minuten na inhalatie en verbeterde de motorische functie (gemeten met MDS-UPDRS III) bij Parkinson patiënten tijdens een geïnduceerde *OFF*-periode vanaf de eerste meting 10 minuten na toediening. In vergelijking met de subcutane apomorfine injectie, waarbij het ongeveer 30 minuten duurde om maximale

plasmaconcentraties te bereiken, was de systemische absorptie bij inhalatie dus aanzienlijk sneller. Dit betekent dat apomorfine inhalatie mogelijk voor een snellere overgang van *OFF* naar *ON* kan zorgen dan subcutane injectie. Om dit met zekerheid vast te stellen, is een directe vergelijking van beide formuleringen in een volgend onderzoek nodig.

In gezonde vrijwilligers werd apomorfine inhalatie slecht verdragen met misselijkheid, braken en lage bloeddruk als de meest problematische bijwerkingen. Parkinson patiënten daarentegen verdroegen dosissen tot en met 4 mg goed in zowel enkele als meervoudige doseringen. De meest voorkomende bijwerkingen waren gerelateerd aan de inhalatie, namelijk hoesten en keelirritatie. Deze bijwerkingen waren mild en van voorbijgaande aard, en verdwenen meestal binnen enkele minuten. Bij driemaal daags doseren met 2 uur tussen opeenvolgende doseringen werden geen duidelijke plasma accumulatie en veranderingen in het veiligheidsprofiel waargenomen. In de klinische praktijk wordt subcutaan apomorfine gestart met een lage dosis (1 of 2 mg) en stapsgewijs verhoogd tot een dosis is bereikt met een optimale balans tussen bijwerkingen en effectiviteit. In de studies beschreven in hoofdstuk 2 en 3 kregen patiënten een vooraf bepaalde dosis. Dit heeft waarschijnlijk geleid tot een suboptimale dosering, waarbij de dosis voor sommige patiënten te hoog was en daardoor bijwerkingen veroorzaakte, en voor anderen mogelijk te laag was om optimale effectiviteit te bereiken. Hoewel deze eerste studies aantonen dat AZ-009 de motorische functie verbetert, zijn de beschreven effecten waarschijnlijk een onderschatting vanwege deze suboptimale dosering. Toekomstige studies moeten daarom het effect van AZ-009 onderzoeken wanneer het wordt toegediend in de optimale dosis voor de patiënt. Momenteel loopt er een fase 2 studie met een open-label titratiefase gevolgd door een dubbelblinde thuisbehandelingsperiode. Dit onderzoek zal dus meer informatie geven over de effectiviteit van een getitreerde dosis en over het gebruiksgemak van de inhalator door patiënten tijdens een *OFF*-periode in de thuissituatie. Toekomstige studies moeten ook de (pulmonale) veiligheid en verdraagbaarheid op lange termijn onderzoeken.

Samengevat geven hoofdstuk 2 en 3 vertrouwen voor de verdere ontwikkeling van Staccato apomorfine (AZ-009) in grootschaliger studies.

In **hoofdstuk 4** werd een oromucosale apomorfine oplossing voor buccale toediening onderzocht. De veiligheid, verdraagbaarheid en farmacokinetiek van de oromucosale oplossing werden vergeleken met een subcutane apomorfine injectie en een sublinguale apomorfine film. Zowel de subcutane injectie als de sublinguale film waren op de markt toen de studie uitgevoerd werd. Echter, in 2023 werd de sublinguale apomorfine film door Sunovion van de markt gehaald vanwege 'beperkt gebruik'. Het bedrijf gaf geen verdere informatie over de reden van terugtrekking, maar het zou verband kunnen houden met de relatief hoge incidentie van orofaryngeale bijwerkingen bij herhaalde blootstelling. De bijwerkingen zijn waarschijnlijk het gevolg van auto-oxidatie van apomorfine in het speeksel, wat leidt tot de vorming van quinone derivaten en reactieve zuurstofverbindingen die zijn geassocieerd met cytotoxiciteit. Er wordt gedacht dat dit minder zal gebeuren wanneer apomorfine als oplossing wordt toegediend, maar dit zal in toekomstige studies bevestigd moeten worden. We toonden in hoofdstuk 4 al wel aan dat kortdurende behandeling met oromucosale apomorfine over het algemeen goed werd verdragen zonder orofaryngeale bijwerkingen en afwijkingen aan de buccale mucosa. Hoewel er relevante en reproduceerbare plasmaconcentraties werden bereikt, is de blootstelling naar verwachting niet voldoende om alle Parkinson patiënten te behandelen. Momenteel is de maximale dosis die kan worden toegediend 14 mg (0,2 mL). Het toedienen van een groter volume wordt afgeraden om speekselproductie en het opwekken van een slikreflex te voorkomen. Om de oromucosale oplossing bruikbaar te maken voor de hele Parkinson populatie, wordt aanbevolen andere opties te onderzoeken om de blootstelling te verhogen. Een optie die onderzocht kan worden, is het gebruik van een andere spraykop die in staat is de oplossing over een groter buccaal gebied te verspreiden. Dit vergroot het oppervlak waarover absorptie kan plaatsvinden. Ook het veranderen van de samenstelling van het oplosmiddel is iets dat onderzocht kan worden. Zo zou het toevoegen/verhogen van ethanol bijvoorbeeld de oplosbaarheid van apomorfine kunnen

verbeteren, de buccale absorptie verhogen en de dispergeerbaarheid van de oplossing verbeteren (waardoor het oppervlak opnieuw toeneemt). Tot slot zou de toevoeging van een permeatieverbeteraar aan de formulering de buccale absorptie kunnen verhogen. Vanwege het risico op lokale irritatie bij langdurig meermaal daags gebruik wordt dit echter als een minder geschikte optie gezien.

De mediane tijd waarop de maximale plasmaconcentraties van de oromucosale apomorfine oplossing werden bereikt, varieerde tussen de 32 en 53 minuten in de verschillende dosisgroepen. De volledige range besloeg 15-120 minuten. De absorptie was dus trager dan van subcutane injecties in de buik (19 minuten, range: 8-40 minuten (hoofdstuk 4)) en vergelijkbaar, zij het aan de lagere kant, met subcutane injecties in het dijbeen, zoals beschreven in hoofdstuk 2 (30 minuten, range: 20-60 minuten). Daarnaast was het ook aanzienlijk langzamer dan apomorfine inhalatie zoals beschreven in de hoofdstukken 2 en 3. Toekomstige studies moeten daarom de aanvang van het effect onderzoeken om te bevestigen dat deze formulering daadwerkelijk geschikt is als noodmedicatie voor *OFF*-perioden.

Een beperking van de apomorfine studies die we in dit proefschrift beschrijven, is het ontbreken van onderzoek naar het gebruiksgemak van de inhalator en de spray. Aangezien de ontwikkeling van nieuwe apomorfine formuleringen gericht is op het vinden van een minder invasieve en gemakkelijker te gebruiken formulering, is het van cruciaal belang dat toekomstige studies verifiëren dat Parkinson patiënten de inhalator en de spray zelfstandig kunnen gebruiken tijdens een *OFF*-periode. Er zijn echter al bemoedigende resultaten gepubliceerd over het gebruik van poederinhalatoren door Parkinson patiënten. Luinstra en collega's toonden aan dat de meeste Parkinson patiënten een poederinhalator konden gebruiken, een voldoende hoge inademingssnelheid hadden en in staat waren hun adem tot 5 seconden in te houden na inhalatie. Bovendien is een levodopa poederinhalator reeds goedgekeurd voor de behandeling van *OFF*-perioden. In een fase 2b-studie met deze inhalator waren patiënten in staat om de inhalator voor te bereiden voor gebruik en zelf de medicatie toe te dienen. Wel uitten sommige patiënten bezorgdheid over het gebruik van de inhalator tijdens tussentijds

telefonisch contact (7% placebo, 14% levodopa). Over het algemeen bevestigt dit dat een inhalator kan worden gebruikt door (de meeste) Parkinson patiënten.

ACUTE EFFECTEN VAN DOPAMINERGE MEDICATIE

Om het effect van nieuwe snelwerkende medicatie te onderzoeken, zijn objectieve, kwantitatieve en snelle metingen ideaal. Vooral voor de behandeling van *OFF*-perioden is het bepalen van de aanvang van het effect essentieel om te kijken of een medicijn geschikt is voor deze indicatie. Momenteel wordt vaak de vrij uitgebreide MDS-UPDRS deel III-schaal gebruikt om de effectiviteit van medicijnen te beoordelen. Hoewel deze schaal goed werkt, vereist het een getrainde beoordelaar, duurt het relatief lang om uit te voeren (ongeveer 15 minuten) en is het onderhevig aan variatie tussen en binnen beoordelaars. In de hoofdstukken 5 tot en met 7 van dit proefschrift hebben we meerdere vingertaptaken geëvalueerd op hun geschiktheid als objectieve, kwantitatieve en snelle farmacodynamische metingen.

In **hoofdstuk 5** werden vier verschillende touchscreen vingertaptaken onderzocht in een technische validatiestudie in gezonde vrijwilligers. Er werden twee soorten taken getest, namelijk afwisselend wijs- en middelvinger tappen met 2,5 cm tussen cirkels ('IMFT'), en afwisselend wijsvinger tappen met 20 cm tussen cirkels ('IFT'). Beide taken werden onderzocht met en zonder een visuele aanwijzing ('cue'). De resultaten gaven aan dat de visuele aanwijzing, in plaats van het signaleren van de volgende cirkel, onmiddellijke visuele feedback gaf. Wanneer deelnemers buiten de cirkel taptten, verscheen de volgende cirkel niet, waardoor de deelnemers pauzeerden en de fout corrigeerden. Dit resulteerde in een verminderde tap snelheid en minder vermoeidheid in beide taken. Of en hoe deze resultaten zich zouden vertalen naar een Parkinson populatie was onzeker en zou verdere validatie in een Parkinson populatie hebben vereist. Deze onzekerheid, gecombineerd met de goede prestaties van de taken zonder aanwijzing, leidde tot de beslissing om alleen de taken zonder aanwijzing verder te valideren in hoofdstuk 6.

Er werden geen significante verschillen waargenomen in vingertap metingen binnen een dag, maar wel tussen dagen. Het leek erop dat deelnemers hun tapstrategie tijdens het tweede bezoek veranderden, waarbij ze snelheid prioriteerden boven nauwkeurigheid, mogelijk als gevolg van bekendheid met de taak. Het ontbreken van een leereffect binnen een dag ondersteunde de verdere evaluatie van deze touchscreen taken als reactie op snelwerkende medicatie, zonder de noodzaak van uitgebreide trainingssessies. Vanwege de waargenomen veranderingen tussen dagen werd de vervolgstudie uitgevoerd met een gebalanceerd crossover design (hoofdstuk 6).

Samengevat, liet deze technische validatiestudie zien dat de IMFT en IFT taken goed functioneerden en herhaalbaar waren, en dat snelheids-, nauwkeurigheds- en ritme-gerelateerde parameters een goede potentiële gevoeligheid hadden in gezonde vrijwilligers.

Daarom werden in **hoofdstuk 6** deze twee touchscreen vingertaptaken, samen met een duim-wijsvinger-taptaak (TIFT), verder geëvalueerd in een vervolgstudie in Parkinson patiënten tijdens een geïnduceerde *OFF*-periode. Deze gerandomiseerde, dubbelblinde, placebo-gecontroleerde crossover studie beoordeelde hun vermogen om dopaminerge medicatie effecten te detecteren en kwantificeren.

Van de drie taptaken presteerde de IMFT taak het slechtst, dat wil zeggen, had de laagste effectgroottes. De effectgroottes waren ook lager dan die van de gouden standaard MDS-UPDRS III. Bovendien was de taak soms moeilijk uit te voeren voor Parkinson patiënten, wat resulteerde in een hoog percentage dubbele taps. Deze problemen met het correct uitvoeren en/of registreren van de IMFT taak, gecombineerd met de relatief kleine effectgroottes, maken de taak in de huidige configuratie het minst geschikt voor gebruik in medicatie effect studies in Parkinson patiënten. In tegenstelling tot de IMFT taak, konden patiënten de IFT en TIFT taken zonder problemen uitvoeren. Levodopa/carbidopa zorgde voor significant sneller tappen (totaal aantal taps), een verbeterd ritme (standaarddeviatie van tussen-tap intervals) en verminderde nauwkeurigheid (totale ruimtelijke fout) in vergelijking met placebo in de IFT taak. Het totaal aantal taps en de totale ruimtelijke fout hadden de grootste gestandaardiseerde effectgroottes en hun grootte was vergelijkbaar met die van de

MDS-UPDRS III. In de TIFT taak resulteerde levodopa/carbidopa vergeleken met placebo in sneller tappen (open- en sluitsnelheid) met een grotere amplitude en verbeterd ritme (standaarddeviatie van tussen-tap intervals). Gemiddelde open- en sluitsnelheid hadden de grootste effectgroottes en waren vergelijkbaar met de effectgrootte van MDS-UPDRS III. De snelheids-gerelateerde parameters in beide taptaken vertoonden een matige tot sterke correlatie met de MDS-UPDRS III ($r=0,45-0,70$). Bovendien vertoonde de standaarddeviatie van de tussen-tap intervals een sterke correlatie met de MDS-UPDRS III in de levodopa/carbidopa groep ($r=0,66$) en een trend naar een matige correlatie ($r=0,45$) in de placebo groep.

Samenvattend boden de IFT en TIFT taken korte, beoordelaar-onafhankelijke metingen die gevoelig waren voor dopaminerge medicatie effecten met vergelijkbare effectgroottes als de MDS-UPDRS III.

In **hoofdstuk 7** werden de resultaten uit de klinische studie in hoofdstuk 6 gebruikt om machine learning algoritmen te trainen voor het selecteren van de optimale combinatie van vingertap parameters ('samengestelde biomarker') om de behandeling te voorspellen (d.w.z. ontving de patiënt actieve behandeling of een placebo?) en de ernst van de ziekte te schatten (d.w.z. de MDS-UPDRS III score). Er werd een samengestelde biomarker gecreëerd voor elke tap taak afzonderlijk, voor de drie taptaken gecombineerd en voor de MDS-UPDRS III. Over het algemeen presteerden de modellen na correctie voor de baseline beter dan de ongecorrigeerde modellen. De baseline-gecorrigeerde IFT samengestelde biomarker had de beste classificatieprestatie (83,50% nauwkeurigheid, 93,95% precisie, effectgrootte $2,58 \pm 0,90$) en overtrof de MDS-UPDRS III samengestelde biomarker (75,75% nauwkeurigheid, 73,93% precisie, effectgrootte $2,12 \pm 1,25$). De IFT samengestelde biomarker includeerde het totale aantal taps en de totale ruimtelijke fout, wat in lijn was met de verwachtingen op basis van de effectgroottes die gezien werden in hoofdstuk 6. De baseline-gecorrigeerde IFT samengestelde biomarker behaalde ook de beste prestaties bij het schatten van de MDS-UPDRS III score.

Samengevat hebben we aangetoond dat de IFT samengestelde biomarker beter in staat was om medicatie effecten aan te tonen dan

de MDS-UPDRS III samengestelde biomarker en de samengestelde biomarker op basis van de drie taptaken gecombineerd. Het combineren van de meest relevante parameters in plaats van het gebruik van één enkele parameter verbetert het vermogen om medicatie effecten aan te tonen. Dit levert bewijs om de IFT samengestelde biomarker te includeren in toekomstige klinische studies voor de detectie van medicatie effecten. Ondanks deze positieve resultaten is het essentieel om op te merken dat deze conclusies gebaseerd zijn op een relatief klein aantal patiënten. Om deze beperking aan te pakken, maakte hoofdstuk 7 gebruik van nested cross-validatie. Desondanks blijft de generaliseerbaarheid van de bevindingen van deze groep Parkinson patiënten naar de bredere en heterogene Parkinson populatie onzeker. Hoewel vingertaptaken goed zijn in het detecteren van bradykinesie in onderarm- en fijne vingerbewegingen, bieden ze mogelijk geen alomvattende meting van de motorische functie. Het zou dus zo kunnen zijn dat bepaalde subsets van Parkinson patiënten geen verbetering laten zien in vingertappen, terwijl ze wel een verbeterde algehele motorische functie hebben. Daarom is het essentieel om de geldigheid, betrouwbaarheid en generaliseerbaarheid van onze methoden te bevestigen met een onafhankelijke dataset. Daarom stellen we voor om een vervolgstudie uit te voeren met een groter cohort Parkinson patiënten met uiteenlopende MDS-UPDRS III-scores, waarin zowel akinetisch-rigide dominante als tremor-dominante Parkinson subtypen zijn vertegenwoordigd.

Omdat de vingertaptaken slechts 15 tot 30 seconden duren, kunnen ze herhaaldelijk worden uitgevoerd tijdens klinische studies. Om deze reden kunnen ze de aanvang van effect en de tijd tot het maximale effect beter detecteren dan de MDS-UPDRS III. Een goede volgende stap is om (tenminste) de IFT taak te includeren in aankomende studies met de in dit proefschrift beschreven nieuwe apomorfine formuleringen. De IFT taak zou mogelijk ook geschikt kunnen zijn voor het testen van medicatie effecten of het monitoren van ziekteprogressie in een thuisomgeving, maar dit zou verdere validatie vereisen van de variabiliteit over een langere periode wanneer de taak uitgevoerd wordt zonder toezicht van onderzoekspersoneel.

CURRICULUM VITAE

Eva Thijssen was born in Zevenaar, the Netherlands on November 23th, 1990. She graduated from secondary school (gymnasium) Liemers College in Zevenaar in 2009, after which she commenced her Bachelor study Biology at Wageningen University. She completed this cum laude in 2012 and continued her education at the Radboud University in Nijmegen, where she graduated cum laude in 2014 in Medical Biology. During her master's program, she performed an internship at the research group of Experimental Rheumatology at the Radboud Institute for Molecular Life Sciences. After her graduation, she started her first job here doing fundamental research in the field of osteoarthritis. In 2016, she changed jobs to work for a CRO where she was involved in monitoring phase 3 clinical trials. After these two jobs, it was clear that she was passionate about clinical research and she was therefore content to start as a Clinical Scientist at the Centre for Human Drug Research (CHDR) in 2018. Here she began her PhD trajectory as described in this thesis under supervision of prof. dr. G.J. Groeneveld, prof. dr. T. van Laar and dr. P.H.C. Kremer. Since 2023, Eva works as an Experienced Clinical Scientist at CHDR supervising Clinical Scientists. She has completed her training to become a board-certified Clinical Pharmacologist in 2024.

LIST OF PUBLICATIONS

- Thijssen E**, Tuk B, Cakici M, van Velze V, Klaassen E, Merkus F, van Laar T, Kremer PHC, Groeneveld GJ. Clinical trial evaluating apomorphine oromucosal solution in Parkinson's disease patients. *Clin Transl Sci.* (submitted Dec 2023).
- ZhuParris A, **Thijssen E**, Elzinga WO, Makai-Bölöni S, Kraaij W, Groeneveld GJ, Doll RJ. Treatment detection and Movement Disorder Society-Unified Parkinson's Disease Rating Scale, part III estimation using finger tapping tasks. *Mov Disord.* 2023;38(10):1795-1805. doi:10.1002/mds.29520.
- Thijssen E**, Makai-Bölöni S, van Brummelen E, den Heijer J, Yavuz Y, Doll RJ, Groeneveld GJ. A placebo-controlled study to assess the sensitivity of finger tapping to medication effects in Parkinson's disease. *Mov Disord Clin Pract.* 2022 Sep 27;9(8):1074-1084. doi:10.1002/mdc3.13563.
- Thijssen E**, den Heijer JM, Puibert D, van Brummelen EMJ, Naranda T, Groeneveld GJ. Safety and pharmacokinetics of multiple dosing with inhalable apomorphine (AZ-009), and its efficacy in a randomized crossover study in Parkinson's disease patients. *Parkinsonism Relat Disord.* 2022;97:84-90. doi:10.1016/J.Parkreldis.2022.02.014.
- Thijssen E**, den Heijer JM, Puibert D, Moss L, Lei M, Hasegawa D, Keum K, Mochel K, Ezzeldin Sharaf M, Alfredson T, Zeng W, van Brummelen EMJ, Naranda T, Groeneveld GJ. A randomized trial assessing the safety, pharmacokinetics, and efficacy during morning OFF of AZ-009. *Mov Disord.* 2022;37(4):790-798. doi:10.1002/MDS.28926.
- Makai-Bölöni S, **Thijssen E**, van Brummelen EMJ, Groeneveld GJ, Doll RJ. Touchscreen-based finger tapping: Repeatability and configuration effects on tapping performance. *PLoS One.* 2021;16(12):e0260783. doi:10.1371/Journal.PONE.0260783.
- Vissers MFJM, Troyer MD, **Thijssen E**, Heuberger JAAC, Groeneveld GJ, Huntwork-Rodriguez S. A leucine-rich repeat kinase 2 (LRRK2) pathway biomarker characterization study in patients with Parkinson's disease with and without LRRK2 mutations and healthy controls. *Clin Transl Sci.* 2023;00(1):1-13. doi:10.1111/CTS.13541.
- Yu HJ, **Thijssen E**, van Brummelen E, van der Plas JL, Radanovic I, Moerland M, Hsieh E, Groeneveld GJ, Dodart JC. A randomized first-in-human study with UB-312, a UB1Th® α -synuclein peptide vaccine. *Mov Disord.* 2022 Jul;37(7):1416-1424. doi:10.1002/mds.29016.
- den Heijer JM, Kruithof AC, van Amerongen G, de Kam ML, **Thijssen E**, Grievink HW, Moerland M, Walker M, Been K, Skerlj R, Justman C, Dudgeon L, Lansbury P, Cullen VC, Hilt DC, Groeneveld GJ. A randomized single and multiple ascending dose study in healthy volunteers of LT1-291, a centrally penetrant glucocerebrosidase activator. *Br J Clin Pharmacol.* 2021;87(9):3561-3573. doi:10.1111/bcp.14772.
- van Caam A, Madej W, **Thijssen E**, Garcia de Vinuesa A, van den Berg W, Goumans MJ, ten Dijke P, Blaney Davidson EN, van der Kraan PM. Expression of TGF β -family signalling components in ageing cartilage: age-related loss of TGF β and BMP receptors. *Osteoarthr Cartil.* 2016;24(7):1235-1245. doi:10.1016/J.Joca.2016.02.008.
- Thijssen E**, Van Caam A, Van Der Kraan PM. Obesity and osteoarthritis, more than just wear and tear: pivotal roles for inflamed adipose tissue and dyslipidaemia in obesity-induced osteoarthritis. *Rheumatology.* 2015;54(4):588-600. doi:10.1093/Rheumatology/Keu464.
- Blaney Davidson EN, van Caam APM, Vitters EL, Bennink MB, **Thijssen E**, van den Berg WB, Koenders MI, van Lent PLEM, van de Loo FAJ, van der Kraan PM. TGF- β is a potent inducer of Nerve Growth Factor in articular cartilage via the ALK5-Smad2/3 pathway. Potential role in OA related pain? *Osteoarthr Cartil.* 2015;23(3):478-486. doi:10.1016/J.Joca.2014.12.005.

