

Exenatide as a potential treatment for patients with Parkinson's disease: First steps into the clinic

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Abstract

Background: There is an increasing number of approaches to try and relieve the motor symptoms of Parkinson's disease (PD) that focus predominantly on strategies of dopaminergic replacement or deep brain stimulation. There remains, however, a major need to slow down or reverse the relentless progression of the disease to prevent the evolution of disabling motor and nonmotor features that continue to cause disability despite the existing symptomatic approaches. Data emerging from the laboratory suggest that agonists for the glucagonlike peptide 1 (GLP-1) receptor may have biological properties relevant to PD pathogenesis and progression.

Methods: Future progress in the evaluation of GLP-1 agonists such as exenatide as potential disease-modifying treatments in PD can be facilitated by collection of proof-of-concept data to mitigate against the risk associated with major financial investments into these agents. There are, nevertheless, multiple issues that must be considered in the planning, setup, and conduct of pilot trials of potential disease-modifying drugs.

Results: Open-label proof-of-concept data have been collected in a small cohort of patients with moderate severity PD that suggest that this agent is well tolerated. Patients randomized to receive exenatide showed advantages on validated motor and nonmotor scales of PD that persisted after a 2-month drug washout period.

Conclusions: Although data must be interpreted with caution, given the strong possibility of placebo effects, the clinical evaluation of these patients supports additional investment into double-blind trials of the GLP-1 agonists in PD.

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Keywords:

Exenatide; Exendin 4; GLP-1; Parkinson's disease; Clinical trial design

1. Introduction

In the early stages of Parkinson's disease (PD), many symptoms can be relieved effectively with the use of dopamine replacement therapy. However, some symptoms can cause persistent disability despite even high doses of levodopa (L-dopa), likely related to degeneration of nondopaminergic cell types [1]. Additional troublesome problems emerge later in the disease because of complications resulting from chronic L-dopa use—fluctuations in motor control accompanied by involuntary movements known as dyskinesias [2]. The need for a neuroprotective agent therefore arises because of those symptoms that do not respond to

L-dopa or that emerge later despite the beneficial effects of L-dopa. Specifically, these include dopa-refractory tremor, postural instability, gait freezing, psychiatric disturbance, and cognitive dysfunction/dementia.

Great effort and resources have been invested into a search for a neuroprotective drug for PD. Nevertheless, there are still no agents that have been licensed for this use. The reasons behind this failure to date include inappropriate selection of potential candidate agents, poor animal models of PD relied on to provide the basis on which agents are selected, difficulty in distinguishing symptomatic from neuroprotective effects, and the consequent difficulty in convincing regulatory authorities that any candidate drug may indeed fulfill such a neuroprotective role, acting as a deterrent for major commercial investment [3].

The difficulties experienced in this field are most recently highlighted with the failure of Cogane (PYM50028), an

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orally administered agent that crosses the blood–brain barrier and protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-associated neurodegeneration in the nonhuman primate model of PD, through induction of endogenous neurotrophic factor release. The promising laboratory data prompted a double-blind, randomized trial among more than 400 patients with PD. Initial press releases from Phytopharm (the commercial company sponsoring the trial) have suggested that there was no signal of biological effect after 12 months of exposure to the drug.

This disappointing news confirms that, unless and until we have improved laboratory models that mimic the neurodegenerative processes of PD accurately, the data emerging from the laboratory cannot be solely relied on when deciding on making major financial investments in the development of a potential neuroprotective agent. Nevertheless, this does not diminish the urgency of the need to test the most encouraging candidate neuroprotective therapies to avoid adding to the already long process of drug development and licensing.

To this end, the strategy that we have adopted for further exploration of exenatide is to collect proof-of-concept data, in a timely and highly cost-efficient fashion, from patients with PD in the hope of gaining preliminary insights into whether the data emerging from the laboratory may be, to some extent, replicated in patients with the disease, while ensuring that major resources are not unnecessarily devoted toward agents that ultimately do not fulfill their potential indicated from the laboratory.

2. Exenatide as a neuroprotective candidate: Scientific rationale

Exenatide is the synthetic version of exendin 4, confirmed to be an agonist for the glucagonlike peptide 1 (GLP-1) receptor, and resistant to the normal GLP-1 enzymatic degradation processes [4]. Evaluation of its possible role as a potential neuroprotective/disease modifying agent in PD needs careful consideration whether (i) the effects of exenatide in the laboratory are of relevance to PD pathogenesis; (ii) the biological effects are accompanied by behavioral improvements in a range of animal models of neurodegeneration not limited to the simple dopamine toxin animal model system; (iii) it has sufficient safety in humans based on its license for use in patients with type 2 diabetes; (iv) it can cross the blood–brain barrier in animals, indicating that peripheral administration may be possible in humans; (v) doses administered peripherally, in theory, may reach the brain concentrations required to show efficacy; and (vi) proof-of-concept data can be collected quickly and efficiently from patients with PD to provide preliminary support for its further study.

2.1. The effects of exenatide in the laboratory are of relevance to PD pathogenesis

Neurodegeneration in PD appears to have cell autonomous and noncell autonomous components [5]. Cell

autonomous processes include (i) production of excessive levels or abnormal forms of α synuclein, (ii) dysfunction of the normal autophagy/lysosomal processes required to clear excess or abnormally folded proteins, and (iii) mitochondrial dysfunction. These processes can also interfere ultimately with microtubule function, and lead to abnormalities of vesicle storage at the synapse and elevation of intracellular calcium ions. Additional noncell autonomous processes include possible cell-to-cell transmission of abnormal or excessive levels of α synuclein, local inflammation and generation of toxic reactive oxygen species, and loss of neurotrophic support [6]. Some or all of these processes contribute to the neurodegenerative process of PD and may be manipulated potentially by exenatide or other GLP-1 agonists.

2.1.1. Neurotrophic properties

There is evidence to suggest that the progressive nature of PD may relate to loss of trophic support. Lower levels of brain-derived neurotrophic factor and nerve growth factor are seen in the substantia nigra of patients with PD, whereas levels of glial cell-derived neurotrophic factor are seemingly better maintained [7,8]. Neurotrophic factors are known to upregulate calcium buffering proteins, antioxidant enzymes, and antiapoptotic factors, and can protect against neurotoxicity in animal models [9].

Laboratory work has showed that exenatide has beneficial effects on neurons in vitro. In rat pheochromocytoma cells, exenatide induced neurite outgrowth, promoted neuronal differentiation, and rescued degenerating neuronal cells [10]. The effects of endogenous GLP-1 and exenatide were likened to the trophic effects of nerve growth factor, although it is clear that its effects are mediated through the GLP-1 receptor rather than the TrkB (tyrosine receptor kinase B) receptor. Exenatide was also shown to protect against excitotoxic damage and also to reverse the damage provoked by glutamate or ibotenic acid in in vitro or in vivo animal models [11].

2.1.2. Anti-inflammatory properties

The possible role of neuroinflammation in the pathogenesis of PD disease is gaining increasing evidence (reviewed in [12]), based broadly on epidemiologic data hinting at lower rates of PD among patients using nonsteroidal anti-inflammatory drugs [13], a consistent association between the human leukocyte antigen locus and PD risk from the meta-analyses of genomewide association studies [14], the presence of activated microglia seen in patients with PD using the PK11195 positron emission tomographic ligand [15], and the presence of proinflammatory mediators seen in the postmortem tissue of patients with PD [16].

Exenatide has been shown to attenuate toxicity in the MPTP mouse model, sparing neurons in the substantia nigra pars compacta and their striatal dopaminergic projections in association with reduced activation of microglia, and reduced expression of proinflammatory molecules: matrix metalloproteinase-3, tumor necrosis factor- α , and

interleukin 1B [17]. In parallel with the reduction of proinflammatory molecules, the effects of exenatide on macrophages have also been evaluated. GLP-1 receptors are expressed on macrophages, and in the presence of exenatide, human monocyte-derived macrophages develop an M2 phenotype through activation of signal transducer and activator of transcription 3 (STAT3), leading to upregulation of anti-inflammatory molecules such as interleukin 10 and transforming growth factor- β [18].

2.1.3. *Effects on mitochondrial number and mitochondrial gene expression*

Mitochondrial dysfunction is also clearly related to PD pathogenesis. This is most evident based on the observation that mitochondrial toxins can cause parkinsonism [19], together with the study of the autosomal recessive juvenile-onset forms of the disease (mutated forms of parkin, DJ-1, and pten-induced kinase 1 (PINK-1), all of which have toxic effects on normal mitochondrial function) [20–22]. The impact of exenatide on mitochondrial number and function has been evaluated by colleagues researching its mechanisms of action in type 2 diabetes. In vitro work performed using human amyloid polypeptide as a toxin for insulinoma cells showed that exenatide increased cell survival through a reduction in apoptosis. This was then shown to be mediated through activation of the AKT pathway, known to be a critical step in normal mitochondrial function. Furthermore, it was shown that exenatide induced mitochondrial gene expression and led to recovery of mitochondrial enzyme activity and mitochondrial number [23].

2.1.4. *Exenatide promotes neurogenesis*

There has been speculation that the neurodegeneration of PD represents a failure of intrinsic neuroregeneration [24]. Exenatide (and related GLP-1 agonists liraglutide and lixisenatide) have been shown to increase the number of neural stem/progenitor cells in the subventricular zone in animal models. GLP-1 receptor messenger RNA has been identified in the subventricular zone, and the co-administration of exenatide with BrDU (a marker for actively dividing cells) revealed a doubling of dividing cells in the subventricular zone in response to peripheral exenatide administration to adult animals. Additional staining with doublecortin confirmed that these cells were neuroblasts.

2.1.5. *Exenatide impacts synaptic function*

There is mounting evidence that synaptic dysfunction plays a critical role in PD pathogenesis. α Synuclein plays a pivotal role in presynaptic neurotransmitter vesicle pools [25]; leucine-rich repeat kinase 2 (LRRK2) impacts synaptic vesicle motility and recycling [26], whereas DJ-1, parkin, and PINK-1 knockout mice all exhibit presynaptic deficits [27,28]. It has been shown recently that the administration of GLP-1 agonists into the cerebral ventricles enhances synaptic function (long-term potentiation) blocked by antago-

nists of the GLP-1 receptor [29]. These data confirm the effects of GLP-1 receptor agonists on neurotransmission in the brain and synaptic plasticity.

2.2. *The biological effects are accompanied by behavioral improvements in a range of animal models of neurodegeneration not limited to the simple dopamine toxin model system*

Given that one of the weaknesses in PD research is the lack of a reliable and valid animal model of the disease that reflects the chronic, progressive nature of the disease, evidence emerging from these models must be considered very carefully. Therapeutic effects in *multiple* animal models including nondopaminergic lesions and also other neurodegenerative disease models can add additional evidence from the laboratory of relevant biological effects. Independent groups have investigated and confirmed beneficial effects of exenatide administration in multiple rodent models of PD as well as models of other neurodegenerative processes.

In London, intraperitoneal injections of 0.1 microgram/kg exenatide twice daily (bd) or 0.5 microgram/kg exenatide bd administered to rats 7 days after either unilateral lipopolysaccharide or unilateral 6-hydroxydopamine (6-OH DA) toxins decreased markedly abnormal amphetamine-induced circling, increased striatal dopamine levels to near normal, and increased both striatal and nigral tyrosine hydroxylase (TH) activity compared with vehicle injections [30]. This group (acknowledging that PD degeneration is not limited to the dopaminergic system) then went further and created another animal model with partial noradrenergic deficits and serotonergic deficits created by the administration of N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) and parachloroamphetamine (pCA), in addition to the 6-OH DA-provoked dopaminergic lesion. These animals had evidence of memory deficits and depression. Subsequent administration of exenatide led to normalization of levels of all three neurotransmitters accompanied by normalization of behavior [31].

In Sweden, administration of exenatide at a dose of 0.1 $\mu\text{g}/\text{kg}$ bd to rats after 6-OH DA toxin again led to near complete normalization of amphetamine-induced rotations that persisted for several weeks after the administration of the drug was terminated. Histologic examination of the substantia nigra revealed a doubling of TH and vesicular monoamine transporter 2-positive neurons (responsible for the proper storage and handling of dopamine) among the animals treated with exenatide compared with those treated with vehicle [32].

In the United States, mice pretreated with exenatide had complete protection against the toxicity of MPTP. Although untreated mice had a 71% loss of dopaminergic neurons in the substantia nigra, TH neurons in exenatide-treated mice were no different from control mice not given MPTP. Furthermore, the exenatide-treated mice had normal

dopamine levels and motor activity after MPTP—in stark contrast to untreated mice given MPTP [33].

The neurotrophic properties of exenatide are of interest not only in PD [34] but also are of interest to clinicians and scientists working on Alzheimer's disease [35]. GLP-1-deficient mice have been shown to have learning deficits that can be restored by GLP-1 receptor gene transfer, whereas rats overexpressing GLP-1 receptors have improved learning and memory ability [36]. Furthermore, administration of exenatide has been shown to lower amyloid- β and amyloid precursor protein levels in vitro and in vivo [37].

Beneficial effects on learning and memory have provoked speculation about the relevance of the GLP-1 receptor from an evolutionary perspective. By enhancing learning and memory, GLP-1 agonists may improve the acquisition of nutrition in the face of competition. By enhancing insulin production, GLP-1 stimulation would also enhance storage of nutrition and, via both means, improve the survival of the organism [38].

To summarize, extensive laboratory data indicate beneficial effects in animal models of PD, and mechanistic actions of relevance to our current understanding of PD pathogenesis. Although the exact mechanisms underpinning the neurodegenerative processes of PD are still not elucidated completely, there is emerging evidence to indicate overlap between these processes and the biological effects of exenatide.

2.3. It has sufficient safety in humans based on its licensed for use in patients with type 2 diabetes

Exenatide is licensed as a short-acting formulation (Byetta; administered 5 μ g or 10 μ g subcutaneously bd) or as a long-acting formulation (Bydureon; 2 mg administered subcutaneously once weekly) in which exenatide is released from microspheres. Both formulations of exenatide are licensed for the treatment of type 2 diabetes mellitus. It is thought that exenatide improves glucose control in patients with type 2 diabetes by its action on the GLP-1 receptor, which leads to stimulation of glucose-level-dependent insulin release from the beta islet cells of the pancreas, induction of insulin biosynthesis and beta islet cell proliferation, resistance to beta islet cell apoptosis, glucose-level-dependent inhibition of glucagon secretion, and decreased food ingestion [4].

Exenatide received its license for the treatment of type 2 diabetes in 2005 in the United States and in 2006 in Europe. Gastrointestinal side effects of exenatide such as nausea and vomiting are common, and weight loss (frequently considered an additional benefit in patients with type 2 diabetes) is also seen frequently. Exenatide slows gastric emptying and therefore may reduce the extent and rate of absorption of orally administered medicinal products. A meta-analysis of published papers of exenatide use in 58,290 patients with diabetes found 82 cases of acute pancreatitis (0.1%), with an odds ratio of 0.84 compared with patients with diabetes in control arms of these trials [39]. In a health insurance database, 24,237 users of exenatide were identified

and compared with 457,797 patients initiated on other anti-diabetic drugs (initiators of exenatide had more severe diabetes than initiators of other antidiabetic therapy). The authors found an odds ratio of pancreatitis of 0.95 in patients treated with exenatide [40]. An additional study by Elashoff and colleagues [41] examined the Food and Drug Administration Medwatch database to quantify retrospectively reports of acute pancreatitis in patients exposed to GLP-1 agonists. They found a sixfold increased risk in patients with diabetes using GLP-1 agents (this included 971 pancreatitis events among exenatide users; however, the denominator of individuals at risk was not stated). To try and gauge the absolute size of this risk, another study looked at a registry of 1.1 million patients with type 2 diabetes and found 1269 hospitalized cases of acute pancreatitis (0.1%), and again there was an adjusted odds ratio of 2.24 for acute pancreatitis in users of GLP-1 agonists [42]. Although the percentages are very small, the concern remains whether acute pancreatitis is the tip of the iceberg, and greater numbers of patients may have subclinical chronic pancreatitis, which in turn may be a risk factor for pancreatic cancer. Type 2 diabetes and obesity are known risk factors for pancreatitis and pancreatic cancer, and it has been speculated that such individuals have an increased incidence of premalignant lesions in the pancreas. These lesions might be targets for GLP-1-induced proliferation.

Debate about the possible association between GLP-1 agonist use and the small increased risk of pancreatitis (in patients with type 2 diabetes mellitus) continues [43,44]. Nevertheless, in the absence of definitive data, vigilance for possible adverse events resulting from the use of GLP-1 agonists must be paramount, and patients must be made aware of this potential adverse event.

Reports of renal failure occurring among patients with diabetes using exenatide has led to the advice that the drug is avoided in patients with end-stage renal disease (creatinine clearance less than 30 mL/minute).

In rats given exenatide for 2 years, an increased incidence of benign thyroid C-cell adenomas was observed at a dose of 250 μ g/kg/day (exenatide plasma exposure was 130-fold the human clinical exposure). In a 2-year study with Bydureon, a statistically significant increase in thyroid C-cell tumor incidence (adenomas and/or carcinomas) was again observed in rats at all doses (1.4- to 26-fold the human clinical exposure with Bydureon). The human relevance of these findings is currently unknown. In view of these data, exenatide should not be used in patients at risk of thyroid cancer.

2.4. It can cross the blood–brain barrier in animals, indicating that peripheral administration may be possible in humans

It is highly likely that any beneficial effects of exenatide in patients with PD would be mediated through direct receptor stimulation in the central nervous system. Exenatide

cannot be administered orally; however, after subcutaneous administration of Byetta to patients with type 2 diabetes, exenatide reaches median peak plasma concentrations in 2 hours. Exenatide shares more than 50% sequence homology with endogenous GLP-1; however, its resistance to degradation by dipeptidyl peptidase gives it a far longer half-life of 60 to 120 minutes, with biological effects lasting as long as 6 hours after a single subcutaneous injection [4]. Bioavailability of exenatide is comparable after subcutaneous injection into the abdomen, thigh, or arm [33,45]. The mean apparent volume of distribution of exenatide after subcutaneous administration of a single dose of exenatide is 28 L. Bydureon has advantages in comparison to Byetta with respect to frequency of administration, no requirement for incremental dose introduction, and an improved side effect profile with respect to gastrointestinal effects.

There is evidence that exenatide can cross the blood–brain barrier in rodents [46]. Although the ratio of distribution of exenatide between serum and brain is uncertain, data using radiolabeled exendin 9–39 suggests a ratio of 5:1 (i.e. ~20%) (see Fig. 3 in Banks and colleagues [47]). Although exenatide (and other GLP-1 agonists) cross the blood–brain barrier when administered peripherally in animal models [36,46–48], there are, however, no human data yet available to confirm the relationship between plasma levels and cerebrospinal fluid (CSF)/brain concentrations. CSF levels of exenatide are being measured in an ongoing phase 2 trial assessing the efficacy of exenatide as a treatment for Alzheimer's disease.

2.5. Peripheral administered doses may, in theory, reach brain concentrations required to show efficacy

In neuronal cultures, 50% GLP-1 receptor occupancy is achieved with 14 nm exendin 4 [11], whereas complete protection against toxicity in vitro is achieved with 100 nm exendin 4 [33]. In vivo effects are seen at 20 nm exendin 4 with little variation between 10 nm and 100 nm during intracerebroventricular infusion [33]. (These experiments were performed to confirm that beneficial effects of exendin 4 are indeed mediated centrally rather than peripherally.)

Based on estimates of the ratio of distribution between serum and brain, the theoretical target concentrations in serum would need to be approximately fivefold that shown to be efficacious in brain (i.e., 50–500 nm). It has been shown that administration of exenatide (as 10 µg bd Byetta, known to be tolerated in humans with diabetes) leads to plasma levels of ~200 pg/mL [45] (200 pg/mL is equivalent to 48 nm [33]).

After weekly administration of 2 mg Bydureon (the licensed dose for the treatment of diabetes), mean exenatide concentrations increase gradually over 6 to 7 weeks. Approximately 10 weeks after discontinuation of Bydureon therapy, mean plasma exenatide concentrations decreased to less than minimal detectable concentrations. Plasma

levels of exenatide (as Bydureon) have been investigated in patients with diabetes. The 0.8-mg dose leads to plasma concentrations of 81.2 pg/mL (19 nm), whereas the 2-mg dose led to levels of 344.5 pg/mL (82 nm) [49]. The 2-mg dose therefore compares with the efficacious dose seen in rodent models.

2.6. Proof-of-concept data can be collected quickly and efficiently from patients with PD to provide preliminary support for its further study

The results of a proof-of-concept, open-label trial evaluating exenatide as a possible disease-modifying treatment for PD have been published [50]. The following text describes some of the issues that have arisen and the decisions made during the setup and design of this trial, and an additionally planned double-blind trial.

2.6.1. Issues arising in clinical trial design

2.6.1.1. Funding

Exenatide was developed at the National Institutes of Health, then the intellectual property was sold to Amylin Pharmaceuticals, which negotiated successfully its subsequent manufacture by Eli Lilly for patients with type 2 diabetes. Exenatide (as Byetta) is manufactured as a “pen device” that can be used to dispense repeated doses of the drug sufficient for one patient for 1 month. A double-blind, placebo-controlled trial evaluation therefore requires the sourcing/manufacture of a matched placebo. In 2010, estimates of the cost of manufacture of a good manufacturing practice-quality placebo were considered to be too high on the basis that there was not yet sufficient human, open-label data of beneficial effects in patients with PD to lend reassuring support to the data emerging from the laboratory. To enable forward progress to be made in this context, the Cure Parkinson's Trust agreed to support a small proof-of-concept trial of exenatide to provide an additional indication of whether the biological effects in the laboratory might be reproducible in patients with PD. Given the restriction of funds preventing manufacture of a matched placebo version of the exenatide pens, this trial was configured necessarily to be open label. Open-label results cannot, however, be interpreted as proof of efficacy, given the substantial placebo responses that are known to occur in the motor evaluation of patients with PD. Small-scale, double-blind replication of open-label data is therefore vital before deciding whether phase 3 evaluation of this agent is warranted, and the Michael J. Fox Foundation has provided support for this next step.

2.6.1.2. Proof-of-concept trial design

A variety of different trial designs have been used in the evaluation of possible neuroprotective agents. Parallel group designs with a washout period have been used previously to allow comparisons between patients exposed or not exposed to the drug, including a prolonged period of drug withdrawal

to allow any symptomatic effects to lessen or disappear [51,52]. Nevertheless, this design is subject to possible long-duration symptomatic effects, and even a lengthy washout period cannot necessarily distinguish a true neuroprotective effect from a symptomatic effect that might have led to the preservation of healthy behaviors with long-term impacts such as exercise [53].

This is particularly problematic for agents that act on the dopaminergic system and that are known to have additional symptomatic effects. For drugs such as rasagiline and pramipexole, more complex trial designs, such as the delayed start design [54–56], have been used to compare outcomes between patients with shorter or longer exposure to the same drug dose, and have incorporated several outcome variables to try and distinguish symptomatic from disease-modifying effects. Even with this level of complex design, the possibility exists that “cumulative” symptomatic effects may be interpreted erroneously as evidence of neuroprotection [3].

An alternative approach is to adopt a “long-term simple” design using a composite outcome measure with long-term follow-up to look for responses to potential disease-modifying therapies being used in the -NET-PD LS-1 Creatine in Parkinson’s Disease trial [57]. This is perhaps the most scientifically robust approach, but requires long-term follow-up and substantial investment therefore needs robust pilot data to indicate potential efficacy in advance of using this approach.

Our initial proof-of-concept trials of exenatide are adopting a simple parallel group design with a 12-month exposure period and a subsequent 2- to 3-month washout period to provide preliminary data regarding safety and tolerability, and to gather initial evidence of whether the impressive effects of exenatide in laboratory animals might be measured objectively in patients with PD. We have considered that an exposure period of 12 months is likely to be the minimum period necessary to allow clinically detectable differences to emerge between the exenatide and placebo groups.

2.6.1.3. Patient inclusion criteria

Trial design and inclusion criteria are complicated further by the effectiveness of dopaminergic replacement therapy to relieve the symptoms of PD. A relatively small “signal” of a neuroprotective effect may be easily lost in the “noise” associated with introduction of symptomatic treatment. Many previous investigators have restricted recruitment to untreated patients with PD only, and used the duration of time before dopaminergic replacement needed as the primary outcome measure. Using this as the main or only primary outcome has been criticized because (i) time point at which dopaminergic replacement is needed is highly subjective, with high inter- and intra-rater variability; (ii) untreated patients with greater baseline severity may reach the threshold for dopaminergic treatment earlier than patients with lower baseline severity, and such baseline differences must also be incorporated

in the measurement of the primary outcome; (iii) patients with very early disease may progress at profoundly differing rates according to treatment allocation but still not reach the threshold for dopaminergic treatment initiation, and again this must be measurable as part of the primary outcome measure; and (iv) patients with intrinsically slower progressive disease become progressively overrepresented during the course of the trial, and conclusions regarding efficacy or lack of efficacy may be biased toward this subgroup of individuals.

Although it can be argued that neuroprotective treatment will be of greater use in the earlier stages of PD, when a greater number of dopaminergic cells are still salvageable, patients with PD have already lost the majority of dopaminergic neurons even at the time of presentation. Given that the likely beneficial impact of any neuroprotective PD agent will be in the prevention of degeneration of both dopaminergic and nondopaminergic neurons, and thus prevent the more troublesome later features of the disease—namely, postural instability, gait freezing, and cognitive decline—the use of patients with moderately advanced disease allows a greater likelihood that any impact on these features will be detectable during the trial follow-up period.

Proof-of-concept trials are inevitably relatively small; therefore, patients with established PD in whom there is a confirmed response to L-dopa allow greater confidence in the diagnosis and minimize the possible “noise” introduced by inclusion of patients without PD. In this group, the severity of PD can be evaluated in the OFF dopaminergic medication state. Other investigators have used the Unified Parkinson’s Disease Rating Scale (UPDRS) score OFF and ON dopaminergic replacement to assess changes in underlying PD severity (e.g., the glial cell-derived neurotrophic factor trials) [58,59]. This is readily achievable in patients with moderately advanced PD who fluctuate in response to dopaminergic replacement therapy. Change in UPDRS scores among dopamine-treated patients with PD have also been used as a secondary outcome measure in the preliminary safety study of isradipine as a potential disease-modifying drug in PD [60]. This approach also allows the ability to evaluate further the interaction of exenatide with conventional PD medication.

Other inclusion and exclusion criteria must be chosen carefully to ensure patients can adhere to trial protocols and that they have reduced risks from the known adverse effects of exenatide. There are insufficient safety data to allow inclusion of patients above the age of 75 years, or who are pregnant or breastfeeding.

2.6.2. Outcome measures

The Movement Disorder Society (MDS)-UPDRS, part 3, in the practically defined OFF state has been chosen as the primary end point for our exenatide trials. This scale is a clinically relevant end point that has widespread acceptance, has objective instructions for use and a certification process

Table 1
Inclusion and exclusion criteria for completed and planned proof-of-concept exenatide trials

Inclusion criteria	Exclusion criteria
Patient has a diagnosis of idiopathic Parkinson's disease of moderate severity equivalent to Hoehn/Yahr stage 2 to 2.5 (bilateral symptoms but still physically independent). Patient is 45–75 years old. Patient is on L-dopa treatment. Patient demonstrates L-dopa responsiveness. Patient is able to give informed consent Patient is able to comply with trial protocol and willing to attend clinic necessary visits.	Patient has a diagnosis or suspicion of other cause for parkinsonism. Patient has a known abnormality on computed tomographic or magnetic resonance imaging brain images likely to compromise compliance with trial protocol. Patient has concurrent dementia. Patient has concurrent severe depression. Patient has prior intracerebral surgical intervention for Parkinson's disease. Patient is already participating actively in a trial of a device, drug, or surgical treatment for Parkinson's disease. Patient has diabetes mellitus. Patient has end-stage renal disease. Patient has a history of pancreatitis. Patient has severe gastrointestinal disease. Patient is pregnant or breastfeeding.

to ensure interrater consistency, and has been validated and calibrated against the original UPDRS scale [61,62].

This primary outcome is supported by a wide range of secondary end points and objective measures that together allow detection of effects on dopaminergic and nondopaminergic systems, as well as motor and nonmotor symptoms. Benefits extending through a range of circuits using different neurotransmitter systems may be more convincing of true biological effects on neuronal function.

Secondary outcomes chosen include the MDS UPDRS, part 3, ON dopaminergic replacement; The MDS UPDRS, parts 1, 2, and 4; the Unified Dyskinesia Rating scale; the Mattis Dementia Rating Scale; the Montgomery and Asberg Depression Rating Scale PDQ39; the Nonmotor Symptoms Severity scale; L-dopa equivalent doses; a quantitative change in DATSCAN uptake between baseline and 12-month evaluations; and vital signs, weight, and adverse events.

One of the objective measures is the use of serial evaluations of dopamine transporter availability (DATSCAN), performed at baseline and study end. Previous trials have observed “neuroprotection” based on imaging [63,64], but not accompanied by clinical advantage in the comparison of dopamine agonist treatment with L-dopa. Nevertheless DATSCAN represents the most cost-efficient technique to quantify dopaminergic degeneration currently available.

2.6.3. Results

The detailed results of our open-label, proof-of-concept trial have been reported separately [50]. In summary, after 12 months of follow-up, a mean advantage of 7.0 points on the MDS UPDRS, part 3, in the practically defined OFF medication state was seen in patients randomized to self-administer exenatide in comparison with control subjects. This persisted even after a 2-month washout period of exenatide. Among the secondary outcome measures, there were also more favorable scores in the other MDS UPDRS subdo-

main (parts 1, 2, and 4) in the ON medication state, improvements in the Mattis Dementia Rating Scale, as well as in timed motor tests. Notably, there was also a worsening of dyskinesia severity in patients randomized to exenatide that resolved with lowering of their L-dopa medication. Nevertheless, although consistent differences were seen favoring the group of patients randomized to exenatide, the small sample size and the open-label design (which is therefore vulnerable to placebo effects), do not allow the data to be interpreted as evidence of efficacy.

3. The next steps

As mentioned, the potential of exenatide as a disease-modifying treatment in patients with PD is supported by *in vitro* and *in vivo* data, and in a small proof-of-concept, open-label trial in patients. Together with increasing insights regarding both the pathogenesis of PD and the potential biological effects of GLP-1 agonists, there is now strong support for further evaluation of this group of drugs in larger, double-blind clinical trials, including our own currently in setup. The issues discussed continue to be relevant in planning future trial designs; choosing patient inclusion criteria, outcome measures, the need for dose ranging studies and assays to confirm drug levels in plasma, urine, and CSF; and the development of possible antibodies to exenatide.

Optimizing this “investigator-initiated” drug development pathway nevertheless requires close discussions with the commercial manufacturers of exenatide and charitable funding agencies to devise the most effective strategy to proceed with the clinical investigation of the drug. It remains to be seen whether exenatide will be confirmed as a useful disease-modifying agent in PD or other neurodegenerative diseases. Similar issues will emerge for the related GLP-1 agonist drugs liraglutide and lixisenatide. Appropriate ongoing investment in trials of the promising disease-modifying agents needs to continue in a timely and cost-efficient basis, and must also be accompanied by parallel

investment into developing the biomarkers and laboratory models to improve on the selection process.

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