New perspectives on study designs for evaluating neuroprotection in Parkinson's disease

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1. Title Page

New perspectives on study designs for evaluating neuroprotection in Parkinson's disease

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Supplementary Material:

- S-Previous designs
- S-Figure 1
- S-Table 1
- S-Table 2

2. Abstract (optional)

None of the currently available pharmacologic treatments for Parkinson's disease (PD) has demonstrated neuroprotective effects in clinical trials. Of the many factors that might account for this failure (including complex disease mechanisms and the medications' pharmacodynamic limitations), some may be related to the choice of the trial design. Here, we review clinical trial designs and make a number of recommendations concerning the choice of the trial design as a function of the clinical development phase. We searched the PubMed and ClinicalTrials.gov databases for previous and ongoing clinical trials related to neuroprotection and/or a disease-modifying effect in PD. We highlight adaptations of these designs and suggest several potentially valuable new designs from outside the field of PD. A total of 38 completed studies and 9 ongoing studies in PD were analyzed, along with 3 completed studies outside the field of PD. We suggest that Phase II should start with a futility design or a double (or single-blind with blind rating) parallel-group trial; depending on the drug's action, it could be a molecularly targeted therapy. Next, a simple withdrawal design could be used to assess dose ranging and symptomatic effects. A drug with a known symptomatic effect should be studied in a relatively long-term, randomized withdrawal trial and not in a delayed-start paradigm. A Phase III randomized withdrawal trial with three groups and two 6-month periods may be a judicious choice. The final step would require a specific long-term design in which the time to achievement of various PD milestones is analyzed.

3. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disease that affects millions of people around the world. PD is a neuropsychiatric disease characterized by both motor and non-motor symptoms.[1, 2, 3] The pathological hallmarks are the loss of dopaminergic neurons in the substantia nigra pars compacta, iron overload and alpha synuclein aggregates in the remaining dopaminergic cells.[4] By the time symptoms of PD appear, patients have lost 80 percent of their striatal or putaminal dopamine.[5] At present, there is no cure for PD; the available treatments are primarily symptomatic, and are essentially based on the restoration of dopaminergic transmission.[6] The concept of neuroprotection has been defined as the capacity to slow down, stop or reverse the course of the disease by protecting neurons against degeneration. Since there is no means of counting the number of remaining neurons in live patients, the concept of disease modification has been introduced. Although some encouraging preclinical data suggest that neuronal loss can be slowed, the clinical trial data have been largely disappointing.[7,8]

Many factors could account for this failure (such as poorly understood disease mechanisms, and a medication's pharmacokinetic and pharmacodynamics limitations) but some may be related to the choice of the clinical trial design. Another factor is the placebo effect observed in clinical studies; consequently, a large sample size is required.[9] Moreover, slow disease progression means that long-term, expensive studies are needed to detect a significant effect. Furthermore, it has already been pointed out that a drug's symptomatic effect might mask a putative disease-modifying effect.[10,11] Lastly, the development of neuroprotective drugs is restricted by the lack of reliable biomarkers.

In view of this lack of success, greater attention must be paid to selecting the most appropriate clinical trial design. This Viewpoint highlights adaptations of designs previously used in PD and also suggests the novel application of trial designs taken from outside the field of PD. Lastly, we make several recommendations for the assessment of disease-modifying drugs at each phase of clinical development.

4. NEW DESIGNS

Trial designs previously used in PD (i.e. the futility design, the simple withdrawal design, the delayed-start design, and the simple long-term study) are presented in the **online Supplementary Material and S-Figure 1**. Previous and ongoing clinical trials intended to evidence neuroprotection are listed in **S-Table 1**.

Randomized withdrawal designs

Randomized withdrawal designs with three groups

This design has already been described [12] but has never been used in PD (**Figure 1a**). Given that its two control groups (placebo/placebo and treatment/treatment) can be compared with the treatment/placebo group, this design is better that the simple withdrawal or delayed-start designs for differentiating between symptomatic effects and disease-modifying effects. However, the presence of an additional (third) group in this promising design decreases the power of the statistical analysis and thus requires a larger total sample size (i.e. by about one third). Thus, recruitment may be more challenging and more expensive.

Randomized withdrawal of all the patients

This constitutes a novel adaptation of the simple withdrawal design but has never been used to study neuroprotection. All patients having received active treatment during period 1 are then randomized to further active treatment or to a placebo for period 2 (**Figure 1b**). Treating all the patients in period 1 has two advantages: (i) there is no concern about loss of blinding, and (ii) the patients spend less time on placebo (less ethical concerns). Lastly, this design might be a good option if the treatment is thought to have a large symptomatic effect as well as a disease-modifying effect.

Randomized withdrawal of the responders

In this variation of design, only patients classified as responders during period 1 are randomized to further active treatment or placebo during period 2 (**Figure 1c**). This design has been used in Phase III trials on patients with orthostatic hypotension [13], schizophrenia [14] and pediatric bipolar I disorder [15] but has never been applied to neuroprotection. This would be an interesting way of assessing a drug with a large symptomatic effect or with a specific, known mechanism of neuroprotection linked to reliable biomarkers. The *a priori* definition of responders can be based on clinical criteria and/or a particular genetic or pharmacogenetic profile. Thus, the drug's effect could be studied in a specific group of PD patients in which it is expected to have the greatest possible effect. However, this design is associated with several limitations in PD, such as the lack of an established definition of a responder, and the risk of overestimating a drug's effect in a subgroup relative to the effect in the patient population as a whole. Furthermore, a large total number of patients may have to be recruited to obtain a sufficient subpopulation of responders.

Specific long-term designs

The demonstration of a long-term effect requires a high level of efficacy and a very large study population, which may only be achievable in Phase III studies. A simple, long-term design has nevertheless been used to demonstrate neuroprotection; the study endpoints were a change in the UPDRS score and the need for symptomatic treatment. [16,17] However, these endpoints were not standardized because the investigators themselves decided when the participants required symptomatic treatment. In the future, these problems could be circumvented by defining the point at which symptomatic treatment is initiated in *de novo* patients.

A specific long-term design in a strict protocol

Patients having been randomized to placebo or active treatment during period 1 are not allowed to take symptomatic medications for a predetermined length of time (**Figure 1f**). The length of period 1 is the same for all patients. During period 2, the patients stay in their study groups but are allowed to take symptomatic medications. Patients who need rescue medications during period 1 are withdrawn from the study.

A specific long-term design in an open protocol

Patients receive the symptomatic drug only when they have reached a specific milestone; the length of each period is not predefined. Hence, a specific "time to event" (i.e. the time needed to reach the milestone) is the primary criterion. (**Figure 1 d,e**). The time to event can be calculated for each patient, and then the two groups can be compared. A longer time interval in the group having received the investigational drug is suggestive of a long-term effect (**Figure 1g**).

Strict or open protocols?

These long-term designs might be more valuable than the delayed-start design or withdrawal designs because patients are treated with a symptomatic medication during the second period, and therefore avoid being undertreated for too long. Thus, the study itself can be planned over a longer period of time. Moreover, these designs differ from the simple long-term study in that the recruited patients are still assessed in a double-blind manner when they receive symptomatic treatment during the second period.

The statistical analysis is easier with a strict protocol than an open protocol because the length of period 1 is the same for all patients. However, there are some sources of potential bias; in particular, some patients may not require symptomatic treatment at the start of period 2 or may have to withdraw when they need symptomatic treatment during period 1. Moreover, the length of period 1 is difficult to determine. Conversely, the fact that the open protocol is better suited to each patient's needs should reduce the drop-out rate. However, the specific "time to event" that triggers the initiation of symptomatic medication must be carefully determined. Moreover, the average intergroup differences in an open design will be much smaller than in a strict protocol because changes in symptomatic treatment will diminish the ability to observe a treatment effect. Thus, the sample size would have to be larger and the trial period would have to be longer.

5. RECOMMENDATIONS

The designs' respective strengths and limitations are summarized in the online Supplementary Material (S-Table 2).

Choice of the design as a function of the clinical development phase (Table 1)

Design

A Phase I safety trial can be followed by a *Phase II non-superiority trial*, in order to test a drug over a short period in a small number of patients. This futility design could be considered for a pilot trial. Alternatively, the first Phase II trial could adopt a conventional, double- or single-blind (e.g. with blind video rating) parallel-group design. The novel concept of molecularly targeted therapies might also be of value if the drug is known to act preferentially in a particular pharmacogenetic context. This would reveal the drug's maximum efficacy in the corresponding subpopulation of patients.

Next, a straightforward *simple withdrawal* design would be useful for dose-ranging studies. To evidence different dose-effects and determine its nature (i.e. symptomatic vs. disease-modifying), the primary endpoint should be analyzed at the end of period 1. The withdrawal period provides initial evidence of a disease-modifying effect, without taking a risk on the whole protocol. Alternatively, the *randomized withdrawal* of patients who respond to a known symptomatic stimulus might be an interesting way of assessing a concomitant disease-modifying effect.

The detection of a disease-modifying effect requires large, long, proof-of-concept studies in *Phase II or at the start of Phase III*. A drug with a known symptomatic effect should be

studied in a relatively long, randomized withdrawal trial, rather than in a delayed-start paradigm. A drug lacking an observed symptomatic effect in initial studies would not require a two-period trial design. A randomized withdrawal trial with three groups and two 6-month periods might evidence a disease-modifying effect more effectively but would constitute a large Phase III trial.

Ideally, the clinical development program should end with the demonstration of a disease-modifying effect (i.e. the time to various PD milestones). This would require a specific long-term design of 24-36 months.

Target population

De novo patients constitute the best population, so that the remaining dopaminergic neurons (often 50% of the normal number at diagnosis and less than 30% loss three years later) can be saved.[18, 19] Furthermore, there is no bias caused by the effects of dopaminergic treatment. However, this population is difficult to recruit and thus requires a long enrollment period. Moreover, there is a risk of including patients with atypical forms of parkinsonism. [20, 21]

Conversely, patients treated in the early stages of the disease (i.e. 30% of remaining neurons at 3 years of disease progression) are easier to recruit than *de novo* patients and thus could be considered in the very first Phase II trial. [22] This population must be receiving a stable, moderate-dose regimen with a dopamine agonist and/or L-dopa.

Duration of the trial

The ADAGIO trial [23] provided valuable information on the duration of the treatment-free period: a period of 9 months in *de novo* patients was associated with a drop-out rate of 10-15%. A shorter period (e.g. 6 months) would reduce the drop-out rate but would also decrease the study's ability to detect an effect. A shorter period might be suitable for early clinical development. Conversely, a larger trial would have a greater chance of success with a 9-month period. The length of period 2 represents a compromise between the minimum drug wash-out time and the duration of period 1, since the patients would have to go for a long time without symptomatic treatment.

The primary criterion for evaluation

Radiologic and biological endpoints (iron overload [24, 25, 26], atrophy [25], and loss of presynaptic nigrostriatal projections [27, 28]) might be options for early development but they are not yet validated biomarkers.

The *total MDS-UPDRS score* is a more suitable primary endpoint because it accurately measures motor and non-motor symptoms. It has already been used in a few neuroprotection trials[29]. However, the *MDS-UPDRS part III score* might be a good primary endpoint for an early Phase II trial because the motor handicap is less variable than the total handicap, which would reduce the number of patients required.

Although *quality of life* (as rated with the specific Parkinson's Disease Questionnaire-39) is strongly correlated with the MDS-UPDRS score and symptomatic benefit, it is unlikely to be a suitable primary endpoint when seeking a disease-modifying effect - at least in early clinical development.

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7. Authors' roles

1) Research project: A. Conception, B. Organization, C. Execution;

2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique;

3) Manuscript: A. Writing of the first draft, B. Review and Critique.

LT: 1ABC, 3A

OR: 1A, 3B

JCC: 1A, 3B

JF: 3A

LD: 3A

DDep: 3A

RB: 3A

CM: 3A

DDev: 1A,BC, 3A,B

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Dr. Moreau has received a grant from France Parkinson. She has also received honoraria for lectures at symposia from Abbvie and Medtronic.

Prof. Defebvre served on several Scientific Advisory Boards for Abbvie and Zambon. He has received various honoraria from pharmaceutical companies for consultancy and lectures on Parkinson's disease at symposia.

Prof. Devos served on several Scientific Advisory Boards for Novartis, Aguettant, Orkyn, and has received PHRC grants from the French Ministry of Health and research funding from the France Parkinson and ARSLA charities. He has received various honoraria from pharmaceutical companies for consultancy and lectures on Parkinson's disease at symposia.

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1. Figures

Figure 1. A randomized withdrawal study with three groups. There are two arms (placebo or treatment) during period 1 and three arms during period 2. The green arrows indicate the mean change from baseline in the total MDS-UPDRS score during periods 1 and/or 2. In theory, the design can differentiate between overall, symptomatic and disease-modifying effects. B. Randomized withdrawal with all patients. At the end of period 1, all patients are randomized to placebo or active treatment. C. Randomized withdrawal with responders only. Patients who respond to the treatment during period 1 are randomized to placebo or active treatment at the start of period 2.

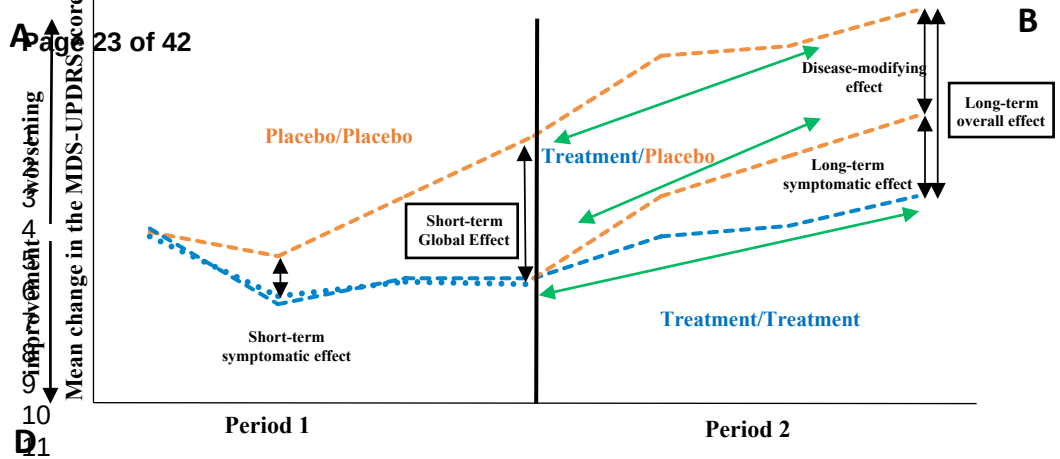
D. A table showing examples of milestones in PD that can be used as the "time to event" and thus might be delayed by a disease-modifying drug. The milestones can be assessed accurately using a single MDS-UPDRS subscore or a combination of MDS-UPDRS subscores with a threshold of 2 or 3. E. A schematic illustration showing the progression of the functional state in the absence of treatment (grey line), in the presence of a symptomatic drug effect (blue line), and in the presence of a disease-modifying effect (green line). A symptomatic drug improves the functional state at the beginning of the treatment but the benefit is lost later on, and the disease progression is the same as it would be in untreated patients (the natural course of the disease). However, a disease-modifying drug can delay the time to each PD milestone (pink to red lines) and the time to event (black arrows). F. A long-term study with a strict protocol. *De novo* patients are randomized into the placebo group (in orange) or the active treatment group (in blue). The green arrows indicate the mean change in the MDS-UPDRS score over the two periods for each group. G. A long-term study with an open protocol. *De novo* patients are randomized into the placebo group (in orange) or the active treatment group (in blue). The trigger for symptomatic treatment (milestone 1, the

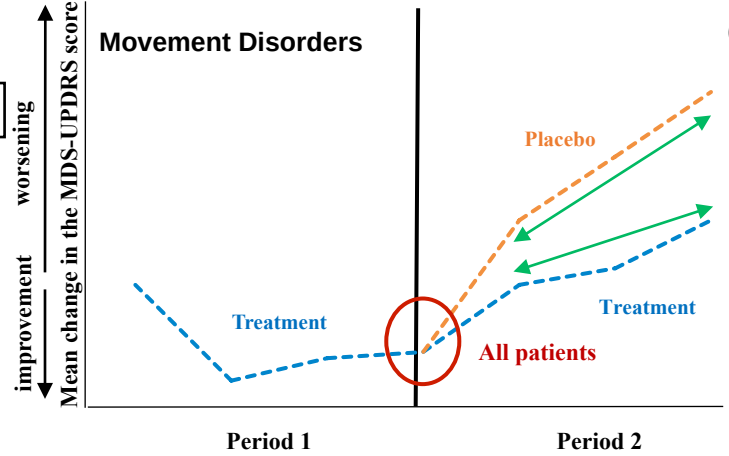
dotted dark line) is a defined increase in handicap (e.g. by 15-20%). For example, a patient in the placebo group receives symptomatic treatment at T1, whereas a patient in the active treatment group receives the symptomatic treatment at T2. If T2 is longer than T1, the disease progression is slower and the drug might have a disease-modifying effect. The histogram represents the mean time to reach the milestone 1 in the active treatment group or the placebo group. Patients in the active treatment group reach M1 less rapidly than the placebo group, so the treatment slows the disease progression and may thus have a disease-modifying effect.

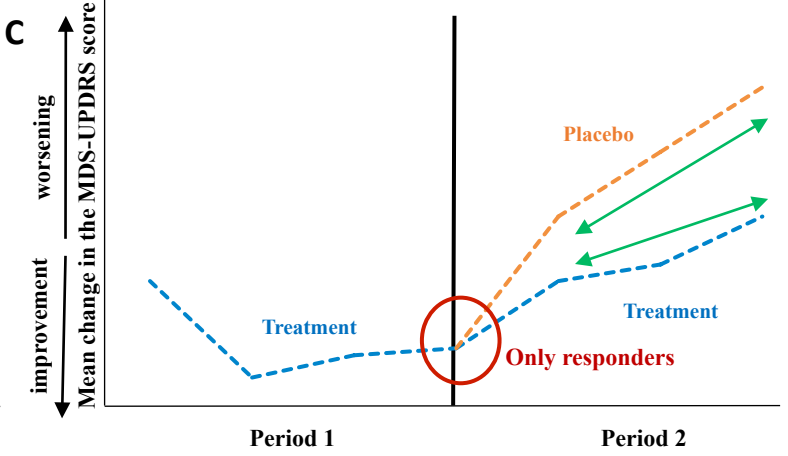
Table 1. Suggested trial designs as a function of the clinical development phase.

Table. 1 Suggested trial designs, as a function of the clinical development phase.

Development phase	Design	Population	Primary criterion	Question
Initial Phase II - Pilot trial - Dose-ranging trial	***-Futility***: for a pilot trial ***- Simple withdrawal*** (6-9 months followed by 15-30 days) for a pilot or dose-ranging trial	- 40-200 early-stage, treated PD patients (<3 years since diagnosis) - Subpopulation selected with a molecular target	Depending on the drug's action, primary or secondary criteria can be: ***- A radiologic or wet biomarker*** (e.g. an MRI R2* sequence for iron, a DaT scan, a PET scan, or cerebrospinal fluid parameters) ***- A molecular target (e.g. gene mutation)*** ***- MDS UPDRS part III score***	- Is there a treatment ***effect*** vs. placebo? - What is the magnitude of a symptomatic effect? - Is there initial evidence of a disease-modifying effect?
Large Phase II/III trials - Efficacy (proof of concept) - Initial Phase III trial	- For drugs with a symptomatic effect: → ***Randomized withdrawal with 3 groups*** (two periods of 6-9 months) → ***Randomized withdrawal of all the patients (or of the responders for a particular drug)*** - For drugs with weak or no symptomatic effects: → ***Delayed start*** (two periods of 6-9 months) → ***Randomized withdrawal with 2 groups*** (two periods of 6 months) - For drugs with a short half-life: → ***Simple withdrawal*** (9 months, followed by 1-2 months)	200-500 *de novo* patients	***Total MDS UPDRS score***	Is there a ***disease-modifying effect***?
Phases III trials	***A long term study with a fixed or open protocol*** (24-36 months in the protocol, and then open-label for up to 5 years)	400-1000 *de novo* patients	***PD milestones*** with assessment of the time to event at 24-36 months (primary endpoint)	Is there a ***long-term disease-modifying effect***?

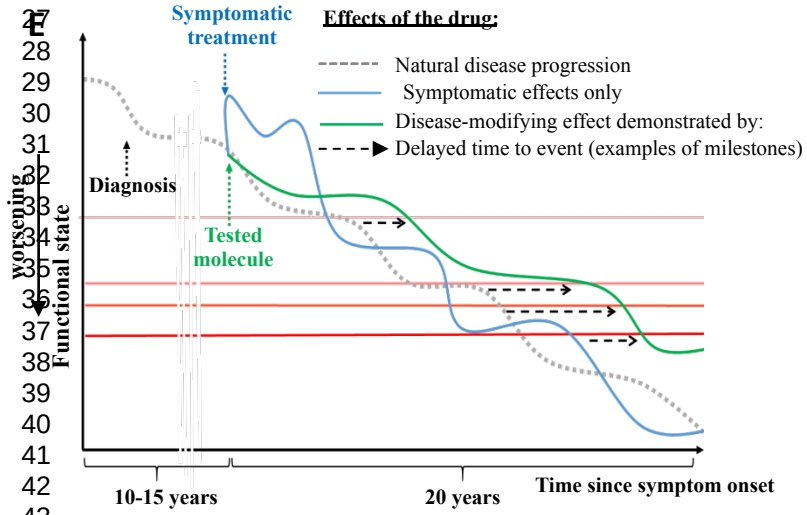


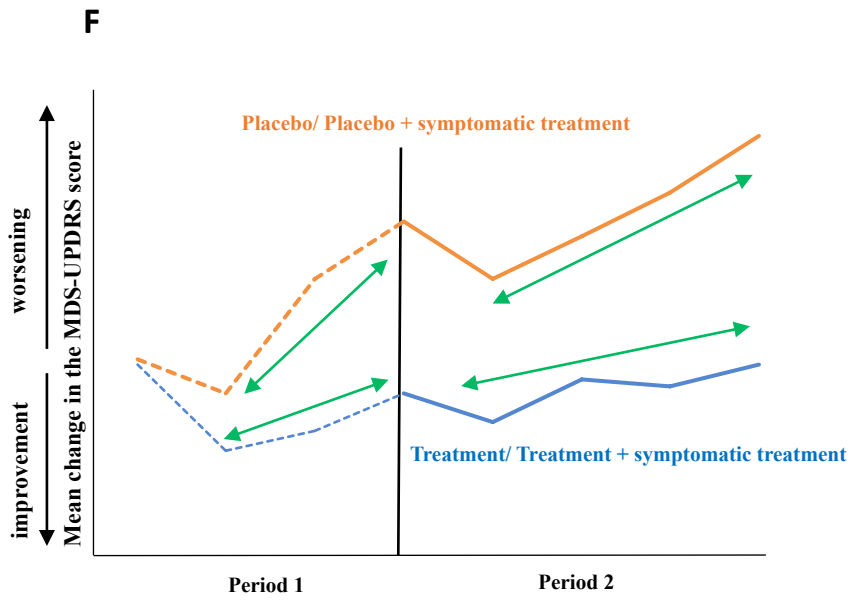


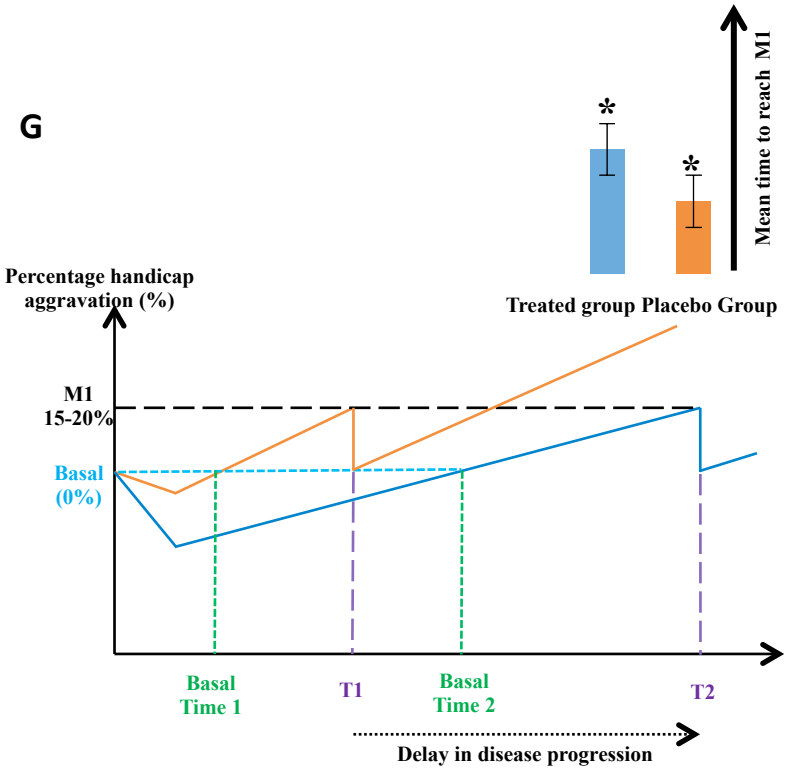


D

	Examples of milestones in PD
A	Need for dopaminergic treatments
	Bilateral symptoms
	Axial symptoms: slight impact on activities of daily living
	Slight impact on cognitive function
	Slight impact on central nervous system
	Slight impact on sleep
B	End-of-dose motor fluctuations
	Dyskinesia
C	Severe impact on axial involvement: dysphagia, dysarthria, gait disorders (freezing postural instability)
	Severe impact on cognitive or on behavior: dementia, hallucination, psychosis
	Severe impact on central nervous system
	Severe sleep disorders
D	Institutionalization (dependent state)







Supplementary Material

Previous designs:

MATERIALS AND METHODS

The present review was conducted by searching online databases, including Medline (via PubMed) and the ClinicalTrials.gov database. PubMed was searched using different logical combinations of keywords: “Parkinson's disease neuroprotection designs”, “Parkinson's disease trial neuroprotection design”, “Parkinson's disease modifier effect”, and “Parkinson's disease clinical trials designs disease modification”.

In an attempt to identify (i) randomized, controlled trials (RCTs) that had completed but had not been published at the time of this review and (ii) RCTs underway or being planned, we also searched the ClinicalTrials.gov online database. The following keywords were used: “Parkinson’s disease modification”, “Parkinson’s disease slow disease course” and “Parkinson’s disease”, in combination with the names of drugs having already been used in other neuroprotection trials.

In order to check whether adaptions of the designs had already been used in other pathologies, we analyzed the following key words on PubMed: “randomized withdrawal study placebo-controlled double-blind patients”, “long-term, two-period”, “long-term study two-period design patients placebo-controlled”, “long-term study two-period design patients time to event”, and “long-term study two-period time to event”.

There were no limitations on the publication date. However, only publications in English were considered.

RESULTS

The PubMed search identified a total of 908 articles, of which 87 concerned PD: “Parkinson's Disease neuroprotection designs” (18 records), “Parkinson's Disease trial neuroprotection design” (35 records), “Parkinson's Disease modifier effect” (29 records), “Parkinson's Disease clinical trials designs disease modification” (5 records).

After the exclusion of duplicates, preclinical studies and reviews, 44 publications (from 32 different clinical studies of neuroprotection) were analyzed. The ClinicalTrials.gov search (up until September 2016) identified 9 ongoing clinical trials studying possible disease modification in PD.

Adaptations of the trial designs outside the field of PD were found in 832 records: "randomized withdrawal study placebo-controlled double blind patients" (782 records), "long-term, two-period" (38 records), and "long-term study two-period design patients placebo-controlled" (1 records). The searches containing the term "time to event" did not yield any hits.

Previous designs (Table 2)

The futility design: the non-superiority study

Randomized, controlled trials are costly and difficult to set up. A Phase I safety and tolerability trial can be followed by a non-superiority Phase II study in which the investigational drug is administered to a small number of patients over a short period of time. This design does not provide information on the treatment's putative efficacy and cannot differentiate between purely symptomatic and disease-modifying effects. However, it can indicate whether the treatment is worth assessing in an expensive RCT [30]. In a non-blinded futility study with a single treatment arm, the observed disease progression is compared with a predetermined lower limit of success (or an upper limit of worsening) in a single-sample test. The use of historical controls can give rise to bias. Firstly, the disease has to remain stable over the time and across the various trials and populations; this may not always be the case, especially since new diagnostic criteria (e.g. the Movement Disorders Society (MDS) criteria and the exclusion of DaT scans without evidence of dopaminergic deficit) and new therapeutic strategies (e.g. rasagiline in *de novo* PD patients) have been introduced. These modifications might influence the outcomes of future studies, relative to historical populations. Moreover, the rating scale used must be exactly the same, which will not be the case for PD (following the recent replacement of the UPDRS [31] by the MDS-UPDRS [32]). The non-superiority design was used by the National Institute of Neurological Disorders and Stroke to identify twelve possible neuroprotective agents, [33] and has been applied in many other trials in PD; however, these positive results have never been confirmed in subsequent randomized trials (S-Table 1).

To differentiate between purely symptomatic effects and a disease-modifying effect, a number of specific designs have been created.

The simple withdrawal design (see Figure 1a and its detailed legend)

This design was first introduced in the field of Alzheimer's disease [34]. In a first (fixed) period (period 1), participants are randomly assigned to receive either active treatment or placebo. In a second period (period 2), all participants receive placebo. Period 1 should be long enough to observe the appearance of a change in disease progression but not long enough to create ethical problems and prompt a high drop-out rate. Period 2 should be long enough to eliminate (or "wash out") any symptomatic effects of the treatment administered during period 1. Active vs. placebo differences at the end of period 1 may be related to a symptomatic effect, a disease-modifying effect, or both. A better score at the end of period 2 in the group having received active treatment during period 1 can be attributed to a longer-term symptomatic effect or to a disease-modifying effect. This simple withdrawal design has been widely used (S-Table 1). In the ELLDOPA trial, participants were randomized to receive one of three dose levels of levodopa or a matching placebo for a 40-week period, after which time the study medication was withdrawn for two weeks [35]. After this withdrawal period, participants receiving levodopa continued to have substantially better mean total UPDRS scores than those receiving placebo. However, the study's imaging data showed a lack of protection that was possibly related to levodopa's impact on the DaT scan. Moreover, the 2-week withdrawal period was probably too short to totally wash out a residual effect of levodopa. Consequently, the disease-modifying effect of levodopa has not been demonstrated. The withdrawal design is simple, relatively inexpensive and non-biased. However, it requires knowledge of the investigational drug's pharmacological properties in general and its pharmacokinetic and pharmacodynamic half-lives in particular. For pharmacokinetic effects, the usual rule is that the withdrawal period (period 2) should last for at least five half-lives. The pharmacodynamic effect is more complex because it cannot be easily measured without a clinical assessment. Moreover, the dopaminergic effect of antiparkinsonian drugs can persist for several days to weeks after withdrawal, especially in early-stage disease [36]. It is always challenging to set the length of period 2, which is always a compromise between scientific and ethical considerations. Another limitation relates to the risk of not seeing a drug effect if period 1 is too short. Lastly, blinding is lost during period 2, which can induce a "nocebo" effect.

In view of the ethical limitations associated with period 2 in the simple withdrawal design, another design has been developed.

The delayed-start design (see Figure 1e and its detailed legend)

In period 1, participants are randomized to placebo or active treatment. In period 2, all participants are given active treatment [37]. Again, active vs. placebo differences at the end of period 1 may be related to an effect on symptoms, a disease-modifying effect, or both. However, an intergroup difference at the end of period 2 argues in favor of a disease-modifying effect. Indeed, effects on symptoms alone cannot readily explain persistent differences between the two groups at the end of period 2. This approach is based on the assumption that in trials that last for a year or more, symptomatic effects should be stable and similar in both groups at the end of the study. However, this assumption is not necessarily true. If there is a symptomatic effect in the long term, the group having received placebo in period 1 might never catch up with the other group. There are a number of other potential problems with this design. Indeed, the drop-out rate may differ in the two groups (e.g. with a higher rate in the placebo group) and thus impede the analysis [38]. The delayed-start design has been used in several trials (S-Table 1). The ADAGIO trial tested rasagiline's putative disease-modifying effect [39]. To obtain a positive result, the early-start treatment group had to meet three hierarchical endpoints in a primary analysis based on the UPDRS score. The study's results showed that early treatment with 1 mg per day rasagiline provided benefits that were consistent with a possible disease-modifying effect but that early treatment with a higher dose (2 mg per day) did not. Consequently, the presence of a disease-modifying effect remains subject to debate [37]. The ADAGIO results highlighted an important limitation of the delayed-start design (notably relative to the withdrawal design); at a high dose level, a drug's symptomatic effect may mask its disease-modifying effect. Moreover, this design (like the simple withdrawal design) suffers from a loss of blinding during period 1 for the early start group and during period 2 for the delayed-start group, which may lead to assessment bias. Since clinical scoring at the end of the study is crucial, these two unblinding periods might compromise the findings. Lastly, this study is expensive; the high potential drop-out rate over the long term means that a large number of patients must be included.

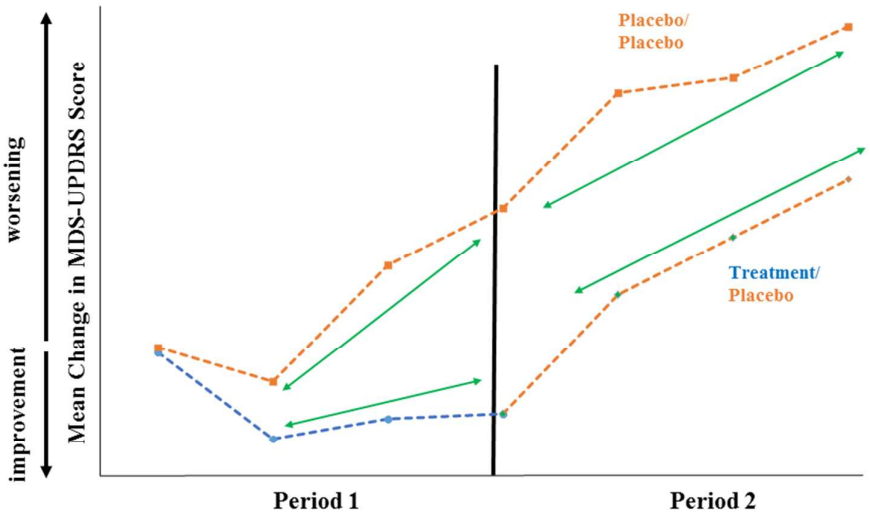
A third trial design has been used to study neuroprotection but cannot differentiate between symptomatic and disease-modifying effects.

The simple long-term study

Patients are randomized into parallel active versus placebo groups for a long period of time (usually several years, if the goal is to confirm a positive risk/benefit ratio). Long-term studies generally use global outcome measures, such as the overall level of handicap (motor and non-motor) and quality of life. Positive effects on long-term, cumulative disability can still provide very useful evidence of disease modification. The long-term design has been widely used in clinical trials of early-stage and/or untreated patients with PD (S-Table 1) [40, 41, 42, 43, 44, 45] and in late-stage, treated patients with PD [46, 47]. However, it has not been possible to ascribe a positive outcome to a disease modifying effect alone.

S-Figure 1. A. The simple withdrawal design. Patients are randomized into two groups. The first group receives placebo during periods 1 and 2, whereas the second group receives the active treatment in period 1 and then placebo in period 2. **B. The delayed-start design.** Patients are randomized into two groups. The first group (the early-start group) receives the active treatment during period 1 and period 2. The second group (the delayed-start group) receives placebo during period 1 and switches to active treatment during period 2. The ADAGIO trial's three primary end points are shown. To determine a positive result with either dose, the early-start treatment group had to meet three hierarchical endpoints in a primary analysis based on the 176-point UPDRS (with a higher score indicating more severe disease): superiority over placebo in the rate of change in the UPDRS score between weeks 12 and 36 (green arrows), superiority over the delayed-start treatment in the change in the score between baseline and week 72 (red arrows), and non-inferiority with regard to the delayed-start treatment in the rate of change in the score between weeks 48 and 72 (purple arrows).

A



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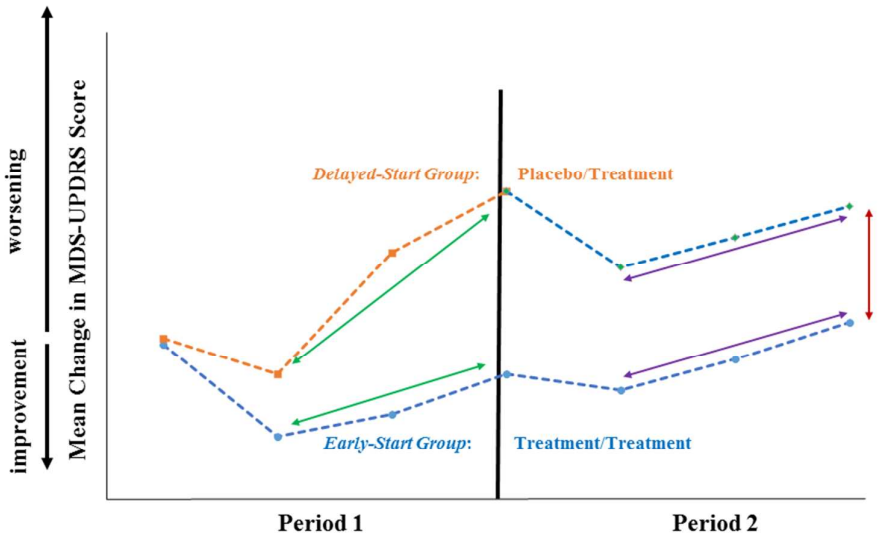

S-Table 1. Previous and ongoing clinical trials of putative disease-modifying treatments in PD.

Drug	Study authors	Design (length)	PD population	Primary criterion for evaluation	Symptomatic effect?	DM effect?	Comments/ Limitations
Pharmacological studies							
Selegiline	Tetrud JW, Langston JW [40]	LT (3 years)	54 early-stage, untreated patients	The need for symptomatic treatment	Positive	Inconclusive	The confounding symptomatic effects of selegiline were not considered
	Myllylä *et al*., 1992 [48]	LT (not specified)	52 early-stage, untreated patients	The change in UPDRS	Positive	Inconclusive	It is not clear whether the benefit of treatment was due to a symptomatic effect alone or to a combination of symptomatic and DM effects
	DATATOP PSG. 1989, [49]	LT (2 years)	800 early-stage, untreated patients	The need for symptomatic treatment	Positive	Inconclusive	It is not clear whether the benefit of treatment was due to a symptomatic effect alone or to a combination of symptomatic and DM effects
	PSG 1996 [50]	Extension of the DATATOP trial (18 months with 2 months of WO before the open-label administration of deprenyl)	310 early-stage, untreated patients (DATATOP subjects not requiring levodopa).	Disability requiring levodopa	Negative	Negative	Subjects who received deprenyl in DATATOP trial did not have sustained benefits after re-initiation of this therapy. The investigators stated that this result may have been due (in part) to the more severe impairment of deprenyl-assigned subjects at baseline, who originally received deprenyl in the DATATOP trial but were more likely to require levodopa during this extended period of observation. The second limitation is that the second period was an open-label period.
	SINDEPAR	SWD (14 months, with	101 early-stage,	The change in	Positive	Inconclusive	The two-month period 2 was considered too short to

	Olanow *et al*.,1995 [51]	2 months of WO)	untreated patients	UPDRS			eliminate symptomatic effects
	Pålhagen *et al*., 1998 [52]	SWD (2 months of WO after levodopa therapy became necessary)	157 early-stage, untreated patients	The change in UPDRS	Positive	Inconclusive	Many consider the washout period to have been too short to provide conclusive evidence of neuroprotection, as the observed benefits could also have been explained by a long-term symptomatic effect (Shoulson et al, 2002)
	Pålhagen *et al*., 2006 [53]	LT (7 years)	157 early-stage, untreated patients	The change in UPDRS	Inconclusive	Negative	UPDRS scores were significantly lower in the selegiline group after 48 months but not after 60 months. Data on UPDRS scores were only available for 19 patients in the selegiline arm and 28 on placebo; thus, the results were hard to interpret
Tocopherol (vitamin E)	DATATOP (PSG), 1989 [49]	LT (2 years)	800 early-stage, untreated patients	The need for symptomatic treatment	Negative	Negative	It is not clear whether the benefit of treatment was due to a symptomatic effect alone or to a combination of symptomatic and DM effects
Rasagiline	TEMPO (PSG), 2004 [35]	DS (26 weeks)	404 early-stage, untreated patients	The change in UPDRS	Positive	Inconclusive	Between 61% and 65% of the patients completed the full follow-up period, and LOCF was used to compute data for the remainder. LOCF may not have been appropriate for all the patients who end-pointed along the way
	ADAGIO, Olanow et al., 2009 [39]	DS (72 weeks)	1176 early-stage, untreated patients (less than 18 months after a documented diagnosis)	The change in UPDRS	Positive	Inconclusive	Rasagiline 1 mg reached significance in all primary end points but rasagiline 2 mg did not. The small overall change in UPDRS and the lack of a dose response complicated the interpretation of clinical relevance
Co-Q10	Shults et al., 2002 [41]	LT (16 months)	80 early-stage, untreated patients	The change in UPDRS	Positive	Inconclusive	It is not clear whether the benefit of treatment was due to a symptomatic effect alone or to a combination of symptomatic and DM effects. This was a small study, and the effect size was not determined
	NINDS NETPD investigators, 2007 [54]	FS (12 months)	213 early-stage, untreated patients (less than 5 years after diagnosis)	The change in UPDRS	Non-futile	Negative	

	NCT00740714 [55]	LT (16 months)	600 early stage, untreated patients (less than 5 years after diagnosis)	The change in UPDRS	Negative	Negative	Early termination. Results not yet published
Ropinirole	REAL-PET, Whone et al., 2003 [42]	LT (2 years)	186 early-stage, untreated patients	Surrogate imaging marker	Less change in striatal F-dopa with ropinirole versus L-dopa	Negative	Absence of a placebo arm, possible use of L-dopa or ropinirole influenced the striatal decarboxylase activity
	NCT01485172 [56]	LT	186 early-stage, treated PD (limited prior exposure to low or moderate doses of L-DOPA: up to 3 months in total)	The change in UPDRS motor score	Results not published		
Pramipexole	CALM-PD (PSG), 2000 & 2002 [57, 58]	LT (23.5 months)	301 early PD patients who require dopaminergic therapy for emerging disability	Surrogate imaging marker	Less change in striatal β-CIT with pramipexole versus L-dopa	Negative	Firm conclusions were precluded by the absence of a placebo arm, the lack of a clinical correlate, and the potential pharmacodynamic impact of chronic dopamine treatment on the primary outcome measure.
	PROUD, Schapira *et al*., 2009 & 2010 & 2013 [59, 60, 61]	DS (15 months)	535 early-stage, untreated patients (diagnosed within the previous 3 years)	The change in UPDRS	Negative	Negative	The DAT results also failed to evidence a neuroprotective effect of pramipexole
L-dopa	Dopa ELLDOPA, Fahn *et al*., 2004 [10]	SWD (42 weeks)	361 early-stage, untreated patients	The change in UPDRS	Positive for UPDRS	Inconclusive	The short WO period and the well-established "long duration L-dopa effect" prevent any firm conclusions from being drawn
Riluzole	Jankovic *et al.,* 2002 [62]	SWD (6 months)	20 early-stage, untreated patients	The change in UPDRS	Negative	Negative	Given the exploratory nature of the design and the small sample size, it was not possible to determine whether riluzole affected the natural history of PD

CEP-1347	[44]	LT (21.4 months)	806 early-stage, untreated patients	The need for symptomatic treatment	Negative	Negative	Early termination (futile)
TCH346	Olanow *et al*., 2006 [63]	SWD (18 months)	301 early-stage, untreated patients	The need for symptomatic treatment	Negative	Negative	The doses of TCH346 selected for testing might be a problem, as many neuroprotective drugs exhibit inverted U-shaped curves in which higher- or lower-than-optimum concentrations are ineffective
GDNF	PSG PRECEPT investigators, 2007 [46]	LT open-label trial	Treated patients with advanced idiopathic PD	The change in UPDRS	Negative	Negative	The patients in the active arm had more severe disease and received lower doses than in the positive open trials. The infusion methods and catheter diameter could have potentially given different physiological results
Minocycline	NINDS NETPD investigators, 2006 [64]	FS (12 months)	200 early-stage, untreated patients (less than 5 years after diagnosis)	The change in UPDRS from baseline to either the time when there was sufficient disability to warrant symptomatic therapy for PD or 12 months, whichever came first.	Non-futile	Negative	Phase II futility trials are designed to determine whether the agent is actually effective in slowing the clinical progression of PD and hence cannot be used clinically for treatment in PD, based on the results of this study. Tooth discoloration was frequent in the minocycline arm and hence this was added to the informed consent form during the trial. It is possible that tooth discoloration could have unblinded the trial, with the potential over-estimation of the positive results for minocycline
Creatine	NINDS NETPD investigators, 2006 [64]	FS (12 months)	200 early-stage patients	The change in UPDRS from baseline up until either sufficient disability to warrant symptomatic therapy for PD or 12 months, whichever came first.	Positive	Inconclusive	Additional factors must be weighed up before performing Phase III trials for creatine, including safety, tolerability, activity, cost, and availability of these two agents relative to others in clinical development for PD

Creatine monohydrate	NINDS NETPD investigators, 2015 [45]	FS + LT (6 years)	1741 early-stage patients (within 5 years of diagnosis) treated for less than 2 years.	Overall statistical test (Modified Rankin Scale, Symbol Digit Modalities Test, PDQ-39 Summary Index, Schwab and England Activities of Daily Living scale, and ambulatory capacity)	Negative	Negative	The trial was terminated early for futility, based on the results of a planned interim analysis of participants enrolled at least 5 years prior to the date of the analysis (n=955).
PYM50028 (Cogane)	NCT01060878 [65]	LT (28 weeks)	425 early stage, untreated patients (diagnosed in the 2 years prior to screening)	The change in UPDRS	Detailed results not yet published		
Immunophilin	NINDS NETPD Investigators 2006 [66]	FS (12 months)	213 early-stage, untreated patients (less than 5 years after diagnosis)	The change in UPDRS	Positive for a further phase III trial	Inconclusive	Phase II futility trials are designed to determine whether the agent is actually effective in slowing the clinical progression of PD and hence cannot be used clinically for treatment in PD, based on the results of this study
Paliroden	NCT00220272 [67]	LT (2 years)	183 early-stage, treated (less than 3 years after diagnosis.)	Change in average (left and right) putamen 18F-Dopa influx constant (Ki) from baseline to two-year 18F-Dopa PET	Results not published		
AAV2-neurturin	Marks et al., 2010 [47]	LT (12 months)	58 late-stage, treated patients	The change in UPDRS	Negative	Negative	A subsequent trial administered AAV2-neurturin to the putamen plus SNc.

GM1 ganglioside	Schneider *et al*.,2010 [68]	Simple short-term 16 weeks, open-label 5 years	26 moderate-stage, treated patients	Changes in UPDRS	Practically defined OFF scores at 5 years same or better than at baseline	Inconclusive	The randomized period was only 16 weeks long, and the rest of the study was open-label
	Schneider *et al*., 2013 [69]	DS (120 weeks)	177 early-stage, treated patients (6 months after diagnosis)	The change in UPDRS	Positive for symptomatic effects	Inconclusive	A small study that provided little evidence of DM effects
Mitoquinone (MitoQ)	Snow et al. 2010 [43]	LT (12 months)	128 early-stage, untreated patients	The change in UPDRS	Negative	Negative	Methodological problems (including inadequate sample size) and the underlying severity of DA deficiency could potentially counter any benefit from a neuroprotective agent. Possibly insufficient brain penetration of MitoQ
Pioglitazone	NINDS NETPD investigators, 2015 [70]	LT and FS (44 weeks)	210 early-stage, treated patients (on a stable regimen of 1 mg/day rasagiline or 10 mg/day selegiline)	The change in UPDRS	Negative	Negative	The findings suggest that the studied doses of pioglitazone were unlikely to modify progression in early-stage PD. Further study of pioglitazone in a larger trial is not recommended
N-Acetylcysteine	NCT01470027 [71]	LT (30 days, 3 groups)	50 patients (PD duration less than 15 years), untreated except for anticholinergic agents	Change in brain cerebral glutathione levels on proton MRS	Results not yet published		
Exenatide	Aviles-Olmos *et al*., 2013 [72]	LT (60 weeks)	45 moderate severity, treated patients (more than 5 years since the diagnosis)	Change in MDS-UPDRS	Positive for symptomatic effects	Inconclusive	A single-blind, proof-of-concept trial
	Aviles-Olmos *et al*., 2014	SWD (24 months)		Change in MDS-UPDRS	Positive	Inconclusive	
	NCT01971242 [73]	LT (60 weeks)	60 early-stage, treated patients	Change in MDS-UPDRS	Results not yet published		

Deferiprone	Devos et al. [26]	DS (12 months)	40 early-stage, treated patients (mainly less than 2 to 3 years since disease onset and never more than 4 years)	The change in UPDRS	Positive	Initial evidence to be confirmed	
	NCT02655315 FAIRPARK-II [74]	SWD (40 weeks)	338 early-stage, untreated patients (disease duration less than 18 months)	Change in MDS-UPDRS	Results not yet published		
Transdermal nicotine	NCT01560754 [75]	SWD (14months)	160 early-stage untreated patients (disease duration less than 18 months)	The change in the total UPDRS (part I-III) score	Results not yet published		
Isradipine	NCT02168842 [76]	LT (36 months)	336 early-stage treated	The change in the total UPDRS (part I-III) score	Results not yet published		
Non-pharmacological studies							
Aerobic walking	Uc *et al*., 2014 [77]	Two-cluster RCT (6 months)	60 early-stage, treated patients	Trails A and B task	Positive on safety and tolerability	Inconclusive	Although physical exercise improves motor aspects of Parkinson's disease (PD), it is not clear whether it may also have a neuroprotective effect.
Physical exercise	Frazzitta *et al*., 2015 [78]	LT (2 years)	40 early-stage, untreated with anti-parkinsonian medication or having received medication, for less than two years, medication-responsive without fluctuations.	The change in UPDRS II, UPDRS III, TUG, and PDDS L-dopa equivalent	Positive	Inconclusive	It is not clear whether the benefit of treatment was due to a symptomatic effect alone or to a combination of symptomatic and DM effects

DM: disease-modifying effect; GDNF: glial-derived neurotrophic factor; Co-Q10: co-enzyme Q10**;** DA: dopaminergic; OFF: off-state; PSG: Parkinson Study Group; LT: long-term; SWD: simple withdrawal; WO: washout; DS: delayed start; FS: futility study; UPDRS: Unified Parkinson's Disease Rating; MDS: Movement Disorders Society; MRS: magnetic resonance spectroscopy; MRI: magnetic resonance imaging; SN: substantia nigra; LOCF: last observation carried forward; TUG: Timed Up-and-Go test; PDDS: PD Disability Scale; RCT: randomized, controlled trial. Treated PD: dopaminergic therapy. Early PD: Hoehn and Yahr Scale stage I or II. All the cited clinical trials are listed at http://www.ncbi.nlm.nih.gov/ and/or https://clinicaltrials.gov.

S-Table 2. Strengths and limitations of clinical trial designs that can be used to assess neuroprotection in PD. WD: withdrawal; RWD: randomized withdrawal; DS: delayed-start. Designs in bold type have already been used to assess neuroprotection in PD. Designs in white type are new designs not previously applied to PD and that may be of value for studying disease modification in this field.

Clinical trial design	Futility design: non-superiority study	Simple withdrawal (SWD)	Randomized withdrawal (RWD)			Delayed-start (DS)	Long-term study		
			Randomization of responders for period 2	Randomization of all patients for period 2	Randomization for period 1:3 groups		Strict protocol	Open protocol	Simple long-term study
Patients	Treated or *de novo* patients	*De novo*: treatment-naïve and newly diagnosed							Treated patients at any stage of the disease
Strengths	- More rapid identification of agents that should not be candidates for larger, expensive Phase III trials - Minimizes costs and sample size -Historical controls can be used	Ability to differentiate between short-term and long-term effects					-Long-term side effects of a treatment on various and variable disease parameter. -Assessment of safety		
		-Simple design with little bias - Less expensive than RWD and DS designs -No confounding effects of symptomatic medication, compared with the DS design.	- Shorter time on placebo, compared with the simple withdrawal design. - No unblinding concerns during period 1		- Evidence of a treatment effect will be more reliable than in the two other randomized withdrawal designs because of the two "control" groups	- Fewer ethical constraints on the length of the placebo period than the WD design	- Easier to set up and analyze than an open protocol	- Fewer drop outs in period 1 than in a strict protocol -The length of period 1 is adapted to suit each patient	- Easier to recruit patients - Simple trial -Broader endpoints because all disease stages are represented
			-More selective than the randomization of all patients for period 2	- Evidence of a treatment effect will be more reliable than for the randomization of responders only.			- Period 2 can be long because all patients are receiving symptomatic treatment (no ethical concerns) -Allows assessment of disease progression from an early stage in the disease. - Double-blind in period 2 (in contrast to the simple long-term study followed by an open-label extension)		
Weaknesses	-Not possible to evaluate treatment efficacy -Historical controls can induce bias in comparisons. - One might conclude that an	-This design cannot accurately track slow overall disease progression. -One cannot be sure that a treatment benefit is present several years later - A long-term study is required to characterize any side effects -Difficult to recruit only early-stage, untreated patients					More drop-outs, due to the length of period 2		
		-Difficulty in finding the ideal length of the periods because of the antiparkinsonian drugs' pharmacokinetic and pharmacodynamic effects. -Risk of period 1 being too short to see an effect on disease progression	-The treatment effect is overestimated - The need for a large population, since only responders	-All responders and non-responders are confounded: possible masked effects of treatment.	-Expensive and complex trial - Risk of drop-outs	-Symptomatic effects can mask the long-term effect of the drug at the end of the study (with a high dosage) - Complex and expensive to set up and analyze	-More drop outs in this strict protocol because the length of period 1 is predetermined and some patients may need levodopa before period 2 -During period 1,	-Very complicated to analyze, with a lot of study variables -Complex to set up - The need for a large population, in order to compare the two groups at the same time (when	-An intervention associated with benefits may not reflect a truly disease-modifying effect, although positive effects can evidence slower disease progression because all patients

	effective drug is ineffective (in a short study) - One cannot rule out confounding symptomatic effects	-Balance between a long period without symptomatic treatment (to see long-term effects) vs. ethical constraints - Loss of blinding during period 1 and period 2 (possible nocebo effects)	continue the trial -Complex design - No current clinically established definition of a responder in PD		-Loss of blinding during period 2	- Long study : high risk of drop-out in the delayed-start group -Loss of blinding during period 1 and 2 (an assessment bias)	some patients may need rescue medication at different moments -Difficulty in determining the length of period 1	symptomatic treatment is required).	are receiving symptomatic treatment