BMJ Neurology Open

Ambroxol as a disease-modifying treatment to reduce the risk of cognitive impairment in *GBA*-associated Parkinson's disease: a multicentre, randomised, double-blind, placebo-controlled, phase II trial. The AMBITIOUS study protocol

Fabiana Colucci,[1,2] Micol Avenali,[3,4] Rosita De Micco,[5] Marco Fusar Poli,[6] Silvia Cerri,[4] Mario Stanziano,[7] Ana Bacila,[4] Giada Cuconato,[8] Valentina Franco,[4,9] Diego Franciotta,[4] Cristina Ghezzi,[4] Matteo Gastaldi,[4] Antonio Emanuele Elia,[1] Luigi Romito,[1] Grazia Devigili,[1] Valentina Leta,[1,10] Barbara Garavaglia,[11] Nico Golfrè Andreasi,[1] Federico Cazzaniga,[12] Chiara Reale,[11] Caterina Galandra,[8] Giancarlo Germani,[4] Pierfrancesco Mitrotti,[3] Gerardo Ongari,[4] Ilaria Palmieri,[4] Marta Picascia,[4] Anna Pichiecchio,[3,4] Mattia Verri,[7] Fabrizio Esposito,[5] Mario Cirillo,[5] Federica Di Nardo,[5] Simone Aloisio,[5] Mattia Siciliano,[5,13] Sara Prioni,[6] Paolo Amami,[6] Sylvie Piacentini,[6] Maria Grazia Bruzzone,[7] Marina Grisoli,[7] Fabio Moda,[12] Roberto Eleopra,[1] Alessandro Tessitore,[5] Enza Maria Valente,[4,8] Roberto Cilia [1]

To cite: Colucci F, Avenali M, De Micco R, *et al*. Ambroxol as a disease-modifying treatment to reduce the risk of cognitive impairment in *GBA*-associated Parkinson's disease: a multicentre, randomised, double-blind, placebo-controlled, phase II trial. The AMBITIOUS study protocol. *BMJ Neurology Open* 2023;**5**:e000535. doi:10.1136/bmjno-2023-000535

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bmjno-2023-000535).

FC, MA and RDM contributed equally.

Received 12 September 2023
Accepted 10 October 2023

For numbered affiliations see end of article.

Correspondence to
Dr Roberto Cilia;
roberto.cilia@istituto-besta.it

ABSTRACT

Background Heterozygous mutations in the *GBA* gene, encoding the lysosomal enzyme β-glucocerebrosidase (GCase), are the most frequent genetic risk factor for Parkinson's disease (PD). *GBA*-related PD (GBA-PD) patients have higher risk of dementia and reduced survival than non-carriers. Preclinical studies and one open-label trial in humans demonstrated that the chaperone ambroxol (ABX) increases GCase levels and modulates α-synuclein levels in the blood and cerebrospinal fluid (CSF).

Methods and analysis In this multicentre, double-blind, placebo-controlled, phase II clinical trial, we randomise patients with GBA-PD in a 1:1 ratio to either oral ABX 1.2 g/day or placebo. The duration of treatment is 52 weeks. Each participant is assessed at baseline and weeks 12, 26, 38, 52 and 78. Changes in the Montreal Cognitive Assessment score and the frequency of mild cognitive impairment and dementia between baseline and weeks 52 are the primary outcome measures. Secondary outcome measures include changes in validated scales/questionnaires assessing motor and non-motor symptoms. Neuroimaging features and CSF neurodegeneration markers are used as surrogate markers of disease progression. GCase activity, ABX and α-synuclein levels are also analysed in blood and CSF. A repeated-measures analysis of variance will be used for elaborating results. The primary analysis will be by intention to treat.

Ethics and dissemination The study and protocols have been approved by the ethics committee of centres. The study is conducted according to good clinical practice and the

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ To date, clinical trials failed to demonstrate the efficacy of disease-modifying interventions in Parkinson's disease (PD). Heterozygous beta-glucosylceramidase gene (GBA) mutations are currently recognised as the most frequent genetic risk factor for PD. Globally, GBA-related patients with PD have a more aggressive disease course.

WHAT THIS STUDY ADDS

⇒ This trial aims to study the role of ambroxol (ABX) as a potential disease-modifying therapy for GBA-PD. We thought that precision-medicine approaches targeting smaller cohorts with homogeneous molecular characteristics have a more robust rationale and chances of success. This trial reflects this approach, and the blindness can reduce bias and confirm the role of ABX. Given the prominent impact of GBA mutations on the risk of incident dementia, the primary objective of the AMBITIOUS trial is to demonstrate a reduced progression of cognitive dysfunction in GBA-PD after 12 months on ABX. In addition, ABX is manufactured in galenic form to obtain 1200 mg daily in only six tablets, increasing patient compliance.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results of this study will hopefully provide further evidence to support the growing idea of using ABX as a disease-modifying treatment in PD. ABX might slow the progression of the neurodegenerative process in PD. We believe that the ABX safety profile and 'repositioning of an over-the-counter drug' would make this treatment more readily and rapidly available on a large scale.

Declaration of Helsinki. The trial findings will be published in peer-reviewed journals and presented at conferences.

Trial registration numbers NCT05287503, EudraCT 2021-004565-13.

INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder worldwide and about 46% of patients with PD progress to PD dementia (PDD) within 10 years of diagnosis.[1 2] Heterozygous beta-glucosylceramidase gene (*GBA*) mutations are currently recognised as the most frequent genetic risk factor for PD with a threefold increased risk of developing PDD and Lewy body dementia than non-carriers.[3–11] In addition, a linear decrease in the Montreal Cognitive Assessment (MoCA) score is observed in patients with PD, which is greater in GBA-PDs than in non-carriers.[12 13]

An estimated 5%–14% of patients with PD worldwide carry *GBA* mutations with even higher prevalence rates in specific ancestries: from 3% to 15% in Caucasian patients with PD (2.8%–4.5% in Italy) and from 10% to 31% in Ashkenazi Jewish.[5 6 9–11 14–19]

The *GBA* gene (OMIN *606463) encodes for the 497-amino acid lysosomal beta-glucocerebrosidase (GCase) hydrolase that catalyses the breakdown of the glycolipid glucosylceramide to ceramide and glucose.[20] Homozygous and heterozygous compound *GBA* mutations cause the autosomal recessive lysosomal disorder Gaucher disease (GD), classified into three clinical subtypes according to the absence/presence of neurological involvement.[21] To date, more than 500 *GBA* mutations and rearrangements have been reported (http://www.hgmd.cf.ac.uk/ac/index.php), of which approximately 130 mutations occur in PD.[22] Almost 82% of all mutant alleles in PD include four missense *GBA* variants (p.E326K, p.T369M, p.N370S and p.L444P), without a direct genotype/phenotype relation due to the high interindividual clinical variability.[4] However, *GBA* mutations are classified as '*mild*' (eg, p.N370S) and '*severe*' mutations (eg, p.L444P), according to the residual GCase activity of 13%–24% and 32%–38%, respectively.[23 24]

Globally, patients with GBA-PD have an early age at disease onset, asymmetric akinetic-rigid phenotype and excellent response to levodopa, with a high risk of early motor fluctuations and dyskinesias. Patients with GBA-PD have higher non-motor symptoms burden (hyposmia, autonomic dysfunction, sleep disturbances, visual hallucinations, cognitive decline) and have more rapid disease progression with greater axial involvement, frequent falls, higher hospitalisation rates and reduced survival than non-carriers.[10 17 25–28]

On the pathogenic level, a reduction in GCase enzymatic activity increases non-soluble toxic α-Synuclein (α-Syn) species, promoting its aggregation. On the other hand, α-Syn aggregates colocalise with GCase, altering the latter's transport to the endoplasmatic reticulum, which is essential for its maturation and function.[29 30] Indeed, overexpression of GCase activity in the brain of animal models with Gaucher-related synucleinopathy has demonstrated to reduce α-Syn aggregates in the hippocampus and improve memory function.[29–31]

These observations suggest that a therapeutic intervention that effectively restores GCase activity in the central nervous system (CNS) is likely expected to modify the course of PD progression in patients carrying *GBA* mutations independently from the residual GCase activity between carriers of severe or mild *GBA* mutations.[10 23 24 32–36]

Ambroxol (ABX) is a metabolite of bromhexine, commonly used as an oral mucolytic agent, with an excellent safety profile.[37] ABX easily enters the CNS, given its lipophilicity (cLogP=2.8) and low polar surface area (PSA 58 Å2), and preclinical studies demonstrated that ABX acts as the chaperone of GCase.[37 38] Indeed, ABX increases lysosomal GCase activity and protein levels, promoting proper GCase folding within the endoplasmatic reticulum and its shuffling into the lysosome.[38 39] Moreover, ABX improves α-Syn clearance at the cellular level.[38–41]

In mouse models, ABX induces higher GCase concentrations in the spleen, heart, and cerebellum. In mice (wild-type, L444P-mutated and overexpressing human α-Syn) and non-human primates, ABX therapy increases GCase brain levels and reduces the amount of α-Syn.[42 43]

In humans affected by GD, pilot studies explored the treatment with ABX resulting in increased GCase function, reduced cerebrospinal fluid (CSF) glucosylsphingosine levels, and improved clinical manifestations, with good safety and tolerability profile.[43 44] Narita *et al* administrated ABX for up to 48 months in five patients with neuronopathic GD at a dose of 1.2–1.3 g/day (up to 25 mg/kg/day), detecting increased GCase activity in lymphocytes and a concentration of ABX in CSF of about 10%–20% of that in the serum.[44] Importantly, ABX proved safe and well tolerated even at the highest dose (1.3 g/day).[44]

In PD, two phase II trials that studied the effects of ABX have been completed. Silveira *et al* concluded a single-centre, double-blind, randomised placebo-controlled trial of 75 patients with PD with mild to moderate dementia, regardless of the presence or absence of *GBA* mutations. Patients were randomised to receive 1050 mg/day ABX, 525 mg/day ABX or placebo and were assessed at 6 months and 12 months from enrollment (ClinicalTrials.gov ID Identifier: NCT02914366), whose results are expected.[45] Mullin *et al* designed a single-centre,

open-label, non-controlled clinical trial to study the safety, tolerability, CSF penetration and target (GCase) engagement of ABX in eight GBA-PD and nine non-mutated PD (ClinicalTrials.gov ID NCT02941822;239).[46] Patients received an escalating daily dose of oral ABX up to 1260 mg for 6 months (up to 21 daily tablets). The results showed increased levels of GCase and α-Syn in CSF and motor improvements measured by Unified PD Rating Scale (UPDRS)-III scores.[46] However, the single-site study design, the small cohort of patients, the challenging compliance to the treatment (due to the intake of 21 tablets/day in addition to usual pharmacological therapy), and the lack of blindness and placebo-control group represent limitations for study validation.

To address these gaps, we designed a multi-centre, double-blind, randomised, placebo-controlled clinical trial to test the safety and clinical effects of ABX on the progression of cognitive impairment and other motor-related and nonmotor-related disabilities in a large and well-characterised cohort of patients with GBA-PD. At a subclinical level, we will investigate changes in neuronal activity by using brain functional MRI (fMRI) and CSF markers of neurodegeneration. Pharmacokinetics and pharmacodynamics of ABX at 52 weeks and after 26 weeks of ABX washout will be additionally assessed.

We hypothesised that ABX increases GCase enzymatic activity and reduces the rate of cognitive dysfunction compared with placebo, modifying the course of cognitive decline not only at the clinical level but also at biochemical, molecular and subclinical neuronal network levels.[47]

Our study was designed to overcome all the limitations of previously mentioned clinical trials. Indeed, we chose a 1200 mg/day dose of ABX to ensure efficacy in detecting adequate amounts of ABX in CSF and restoring the GCase activity levels in the CNS. Data available from animal models and patients indicate that an oral dose of ABX less than 1200 mg/day may not penetrate the CNS and result in insufficient enhancement of GCase activity to act on α-Syn levels and, thus, disease progression. Moreover, ABX is commercially available only at low dose (75 mg/capsule), and patients should take 16 tablets per day to reach the maintenance dose (1.2 g/day; NCT02941822). In this study, we have reduced the risk of a high dropout rate due to the low intervention compliance because of the high number of daily tablets by administering galenic formulations of 200 mg/tablet (six tablets/day to reach the 1.2 g/daily dose).

METHODS AND ANALYSIS

Objectives

Main objective

To evaluate the clinical efficacy of high-dose oral ABX in reducing the progression of cognitive dysfunction.

Secondary objective

1. To evaluate the safety and tolerability of high-dose oral ABX.
2. To evaluate the clinical efficacy of ABX in reducing the progression of motor, non-motor symptoms and worsening of quality of life.
3. To detect subclinical neuroprotective effects of ABX using brain MRI scans.
4. To evaluate the association between motor and non-motor symptom progression with changes in GCase activity, ABX levels and CSF neurodegeneration markers.
1. To evaluate the safety of ABX 26 weeks after the end of the study.
2. To evaluate the efficacy of ABX on clinical outcome and biomarkers measures 26 weeks after the end of the study.

Study design

This is a phase II, multicentre, double-blind, randomised 1:1, placebo-controlled study aiming to evaluate the safety and efficacy of ABX in patients with PD carrying *GBA* mutations.

Patients diagnosed with PD and carriers of *GBA* mutations are randomly allocated to either ABX 1.2 g/day or placebo. Galenic formulation of ABX 200 mg per tablet and similar placebo tablets have been manufactured ad hoc, and their use in this study has been authorised by the Regulatory Medicines Agency (AIFA).

Patients are evaluated in six visits: baseline (V1), at week 12±7 days (V2), 26±7 days (V3), 38±7 days (V4), 52±7 days (V5-end of IMP/placebo treatment), and after 26 weeks from the end of the treatment. The study protocol is summarised in figure 1.

Setting

The study sites include Fondazione IRCCS Istituto Neurologico Carlo Besta (Milan), IRCCS Mondino Foundation (Pavia) and Luigi Vanvitelli University Hospital (Naples). Participants are enrolled after obtaining and signing an informed consent.

All study visits are conducted by expert neurologists and neuropsychologists (when required by protocol). Blood collection, lumbar puncture, structural and fMRI are performed at each of the three recruiting centres. Secondarily, blood and CSF are centralised for biochemical, pharmacokinetic and pharmacodynamic analysis at the laboratory of the IRCCS Mondino Foundation in Pavia, while imaging data are centralised for processing and analysis through Brain Voyager QX and SPM V.12 software at the Laboratory of Neuroimaging at Luigi Vanvitelli University in Naples.

Recruitment and participants

Patients with PD carrying *GBA* mutations are screened by a neurologist expert in movement disorders. The screening visit reviews demographic information, concomitant medication, and medical and neurological history to check eligibility for the study.

Subjects included meet the following criteria: (1) age between 21 and 80 years; (2) Diagnosis of PD according to the International Parkinson and Movement Disorders

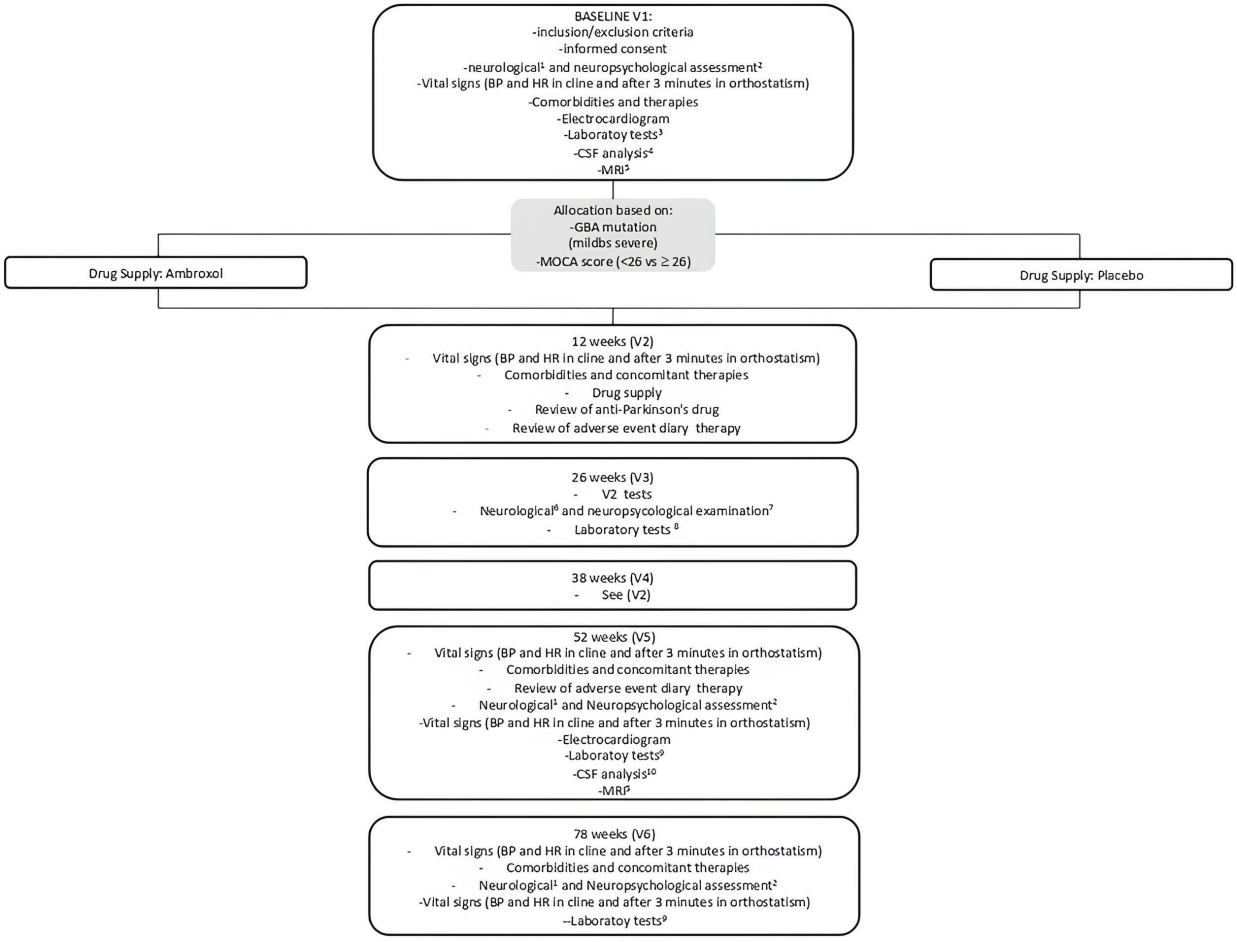

Figure 1 CONSORT flow chart of the trial. ADL, Activities of Daily Living; AES, Apathy Evaluation Scale; BDI, Back Depression Index; Benton JoLO, Benton's Judgment of Line Orientation test; CDT, Clock drawing test, COMPASS 31, Composite Autonomic Symptom Score 31; CSF, cerebrospinal fluid; FAB, Frontal Assessment Battery; FAS, Phonemic fluency test; FCSRT, Free and Cued Selective Reminding test; FOG-Q, Freezing of Gait Questionnaire; H&Y, Hoehn and Yahr Stage; IADL, Instrumental Activities of Daily Living; MDS-UPDRS, Movement disorder society-Unifed Parkinson's Disease Rating Scale; MoCA, Montreal Cognitive Assessment Scale; NMSS, Non-Motor Symptoms Scale; PD-CFRS, Parkinson's Disease Cognitive Functional Rating Scale; PDQ-39, 39-item Parkinson's disease Questionnaire; RAVLT, Rey's Auditory Verbal Learning Test; RBD-Q, REM Sleep Behavior Disorder Screening Questionnaire; TMT, Trail Making Test.

Society (MDS) Criteria[48]; (3) disease duration ≥5 years from motor symptoms onset; (4) Hoehn and Yahr stage ≤4 in ON-phase; (5) absence of contraindications to study procedures (eg, lumbar puncture); (6) compliance and adherence to double-barrier contraception. Potential enrolling patients are excluded if one of the following exclusion criteria is present: (1) atypical parkinsonism; (2) PDD according to the MDS criteria[49 50]; (3) deep brain stimulation; (4) relevant or unstable comorbidities that could represent a risk for the subject (ie, previous gastric/duodenal peptic ulcer, chronic obstructive pulmonary disease (COPD), severe hepatic or renal insufficiency, major cardiovascular event, neoplastic diseases); (5) bronchial asthma; (6) medical conditions that preclude a safe execution of lumbar puncture (ie, treatment with anticoagulants, significant bleeding diathesis, coagulopathy or thrombocytopaenia, severe abnormalities or malformations of the lower spine or other spinal pathology, hypersensitivity to lidocaine); (7) known hypersensitivity to the active ingredient ABX or any of its excipients (online supplemental file 1).

Patients who meet the eligibility criteria are enrolled within 28 days.

Enrolled patients are informed by the investigator on the aim of the study and related procedures. After understanding and agreeing with all procedures, patients sign a written informed consent for the study prior to any procedure. Completed consent forms are retained by each investigator centre (with a copy to the sponsor). During the trial, it is possible to change medications acting on motor symptoms (eg, levodopa, dopamine agonists, MAO-B and COMT enzyme inhibitors, amantadine) and non-motor symptoms (eg, antidepressants, antipsychotics), excepted for acetylcholinesterase inhibitors and other cognitive function enhancers, which are not permitted. At the time of the manuscript submission, the study is active, data collection is ongoing. The primary

completion date (anticipated) will be on 15 December 2024.

Randomisation

Enrolled patients are randomised 1:1 to receive either ABX hydrochloride (1200 mg daily) or placebo for 52 weeks in addition to the usual antiparkinsonian therapy. Each patient self-administers the IMP/placebo at five titration doses, reaching the maintenance dose of 200 mg six tablets daily in 25 days (see online supplemental file 2).

The study randomisation list is generated by the randomisation module of the Research Electronic Data Capture (REDCap) software. A computer-generated randomisation method, considering an 'a priori stratification' based on MoCA score at baseline (<26 vs ≥26), and type of *GBA* mutations ('mild' vs 'severe'), randomly 1:1 assigns the participants in one of the two treatment arms (ABX/placebo).[51 52]

The assessors and patients are blinded to group assignments. For this blindness assumption, the pharmacy labelled the investigational box with an identification number (ID) in a double-blind manner. Each participant receives an ID by the computer randomisation corresponding to a box. The shape of the box, the number and shape of the bottle containing the tablets, and the size, shape, colour and number of tablets per bottle are the same for each participant.

In case of serious adverse events (SAEs) or suspected unexpected serious adverse reactions, patient blinding status will be broken after consultation with the principal investigator (PI).

Intervention

Active and placebo formulations are used in the trial. Galenic ABX hydrochloride 200 mg (investigational medical product, IMP) and placebo tablets are packaged in a bottle (1 start bottle of 75 tablets and the remaining bottles of 60 tablets each). Each patient self-administers IMP or placebo at five titration doses, reaching the maintenance dose of two pills of 200 mg three times a day (1200 mg/day) within 25 days (see online supplemental file 2).

Manufacturing, packaging and labelling of IMP are done in accordance with current regulations.

ABX hydrochloride and placebo tablets are manufactured in accordance with current good manufacturing practices by an AIFA-approved manufacturing laboratory. The label production, application to the drug box and shipment to the centre at a controlled temperature are provided by a third facility. Investigators and/or other authorised persons store the IMP/placebo safely and securely.

All IMP/placebo bottles (whether empty or unused) are returned by the patient at each visit, along with a patient diary in which participants record any AEs, doses of medication not taken or changes in the treatment regimen.

Outcome measurements

Primary outcome

The primary objective aims to detect the clinical efficacy of high-dose oral ABX in reducing the progression of cognitive dysfunction by assessing changes in the MoCA scale score. In addition, total score changes will be used as coprimary measures to determine the frequency of conversion from a normal cognitive function (PD-N) to mild cognitive impairment (PD-MCI) and from PD-N or PD-MCI to PD-D.[53] The MoCA accurately and rapidly evaluates: (1) short-term memory, (2) visuospatial abilities, (3) executive functions, (4) attention, (5) concentration and working memory, (6) language and (7) orientation to time and place.

Secondary outcomes

1. Safety and tolerability of the IMP will be evaluated by systematic collection of all AEs, frequency of discontinuation and blood laboratory test [complete blood count (CBC), aspartate amino transferase (AST-GOT), alanine amino transferase (ALT-GPT), Gamma-glutamyl transferase (GGT), indirect and total bilirubin, prothrombin time (PT), activated partial thromboplastin time (aPTT), International Normalized Ratio (INR), creatinine, azotemia, sodium, potassium, uricaemia].
2. Cognitive and psychiatric function (2 a), Motor signs and symptoms (2b), other non-motor symptoms and quality of life (2c) changes will be calculated by score changes on neuropsychological scales and questionnaires (2a); the International PD and MDS version of the Unified PD Rating Scale (MDS-UPDRS) parts II-III, and the Hoehn and Yahr Stage scores (2b); the 39-items version of Parkinson's Disease Questionnaire (2c); the 31-item Composite Autonomic Rating Scale (2c); the Non-Motor Symptoms Scale (2 c); the Freezing of Gait Questionnaire (2c); the REM Sleep Behaviour Disorder Questionnaire (2c).
3. The subclinical neuroprotective effects of ABX will be detected using brain MRI scan as a surrogate biomarker of neuronal activity and network dysfunction by assessing changes in cortical thickness, cortical and subcortical volumes (3D T1-weighted images), white matter tract damage (diffusion tensor images) and resting-state functional connectivity.
4. The association between the progression of motor and non-motor symptoms and changes in GCase activity (in peripheral blood mononuclear cells, PBMCs and CSF), ABX levels (in plasma and CSF) and neurodegeneration markers levels (α-Syn in PBMCs and CSF; total tau and phospho-tau in CSF; beta-amyloid-42 in CSF) will be assessed after 52 weeks of ABX therapy.[54–56]
5. The safety of ABX will also be evaluated at 26 weeks after the end of the study by blood laboratory test (CBC, GOT, GPT, GGT, indirect and total bilirubin, PT, aPTT, INR, creatinine, azotemia, sodium, potassium, uricaemia).
6. The efficacy of ABX on clinical outcome measures, α-Syn levels in PBMC, plasma pharmacokinetics and

pharmacodynamics will also be evaluated 26 weeks after the end of the study.

Safety/AEs/SAEs assessment and management

A review of the studies shows a high safety profile related to the use of high-dose oral ABX hydrochloride (up to 1200 mg/day). There are no reports of additional, more frequent or more SAEs than reported in the summary of product characteristics (SmPC) for ABX hydrochloride at the doses authorised for marketing authorisation (EMA) EMA/PRAC/800767/2015.[37]

AE monitoring

An AE is a harmful clinical event or unintended reaction that occurs in a clinical trial participant taking the investigational medicinal product (ABX or placebo) and does not necessarily have a causal relationship with the treatment.

An SAE or serious adverse reaction is related to (1) a fatal outcome, (2) life-threatening to the subject, (3) hospitalisation or prolongation of ongoing hospitalisation, (4) severe or prolonged disability or incapacit and (5) congenital anomaly or malformation or congenital disability.

AEs and SAEs are collected, properly investigated, and documented in the original documents. SAEs must be reported no later than 24 hours after the study team becomes aware. SAEs reports are handled, managed and ensured by an external provider specialising in pharmacovigilance (https://www.pharmades.it/pharmacovigilance/#research). If significant new information becomes available, a follow-up file report must be prepared within 5 days of the initial notification.

A causal relationship of AE/SAE is generally assessed by the Investigator and is classified as

- Unrelated: the event is definitely not associated with the device or study procedures; a relationship can be ruled out.
- Possibly related: the relationship between device use or study procedures and the event is possible, but other causes cannot be ruled out with certainty.
- Related: the event is definitely associated with the device or study procedures.

Open of blindness

Blinding should be opened only in cases of confirmed pregnancy in the enrolled patient, for unexpected SAEs related to the investigational drug (ABX or placebo), and only for the patient in question or when required by regulatory authorities.

The PI and the coordinating investigator identified two people from the company ensuring packaging and shipping as qualified people outside the research group who must necessarily be aware of the randomisation list as they prepare and label the drug and placebo packs. If there is a need to open the blind, the investigator centre will contact the qualified people identified to receive the participant's details. The patient should discontinue the study drug but should be encouraged to continue the study by completing all evaluations included in the study design. The investigator must collect information about the date, hour and reason for treatment discontinuation and blind opening.

Criteria for drug discontinuation and subject withdrawal

ABX has a good safety profile, and no severe adverse reactions are expected. However, participants may be excluded from treatment (alternatively, the study may be terminated prematurely) if unexpected, significant or unacceptable AEs jeopardise their safety (or that of other study participants), according to the investigators' opinion.

Study discontinuation will also occur if (a) the participant withdraws the consent, (b) the enrolled subject fails to comply (noncompliance) with the study protocol, (c) the investigator opens the blind, (d) AE or change in medical or laboratory conditions, that may jeopardise participant's safety, occur, (e) the patient becomes pregnant during the study and (f) the patient is unable to continue (lost to follow-up).

Withdrawn participants could still be assessed at weeks 52 (V5), and safety data (eg, monitoring of AEs) are collected up to 30 days after the last IMP intake.

Pregnant women should be followed throughout the pregnancy until about 6–8 weeks after delivery to collect information on the pregnancy outcome.

If enrolled participants discontinue the study prematurely, additional participants may be included and assigned to the same treatment sequence at the discretion of the study coordinator.

Analysis plan

Sample size calculation

The primary endpoint is the change in total MoCA score. Considering (1) an average decrease in 24 months in the total MoCA score of 1±2 points in the general PD population (0.5±2 points/year),[57] (2) a more rapid decline of MoCA score even in asymptomatic carriers of *GBA* gene mutations compared with non-carriers,[58] (3) expecting a threefold greater risk for dementia in GBA-PD than non-carriers underlain by a with faster rate of MoCA score decrease (3±3 points in 24 months) with a linear pattern of decline[6–13] and (4) assuming that ABX treatment will reduce this increase by 50% (1.5±2 points in 24 months), the sample size sufficient to have 80% power with a type I error of 20% is 54 patients (27 ABX, 27 placebo). Considering an expected dropout rate of 10%, it is necessary to enrol at least 60 patients (30 in each arm).

Statistical analysis

Patients with at least one planned follow-up visit will be considered for analysis. Mean and SD, range, median and quartiles will be used to analyse continuous variables; frequency and percentage will be used for categorical ones. The primary analysis will be by intention-to-treat (ITT) principle as far as is practically possible. Per-protocol

(PP) analysis will be conducted after excluding patients with major protocol deviations. Interim analyses are not planned.

A generalised linear regression model will be used to analyse the continuous variables, and the difference between the means and the corresponding 95% CI will be calculated.

Binary outcomes will be analysed using logistic regression analysis, and differences between the proportions and relative 95% CI will be calculated. If necessary, a normalising transformation will be applied before fitting the model.

MRI data preparation, normalisation, preprocessing, statistical analyses and visualisation will be performed through Brain Voyager QX and SPM V.12 software. A generalised linear model for repeated measures, applying scanner type as a covariate to minimise the effect on the results by this variable, will be used. Major resting-state functional connectivity networks, local grey matter volume and cortical thickness will be extracted in all patients. Data from the 52 weeks will be compared between the two treatment groups (IMP vs placebo), and for each subject, data will be analysed longitudinally at two time points (V1 and V5). Homogeneity between centres will also be tested by calculating Kendall's W concordance coefficient for all I voxels in the mask extracted from the mean structural and functional connectivity in all enrolled patients.

Data management and monitoring

All information is collected on source documents in each study centre during each study visit. Site data managers transcribe these source documents into the electronic clinical database. The PI reviews each source document from all centres and confirms its correct transcription. The REDCap software prevents double data entry and checks for data value range.

Data are processed in accordance with the General Data Protection Regulation of the European Union (EU) and in accordance with GCP.

On enrolment, each participant is identified by an alphanumeric code to preserve anonymity, generated in a consecutive manner.

Consent forms and data collection forms are storage safety. The PI is responsible for the secure storage of the study documents, which will be archived for a minimum of 25 years after completion of the study.

Intermitted meetings among study sites are held every 3 months to ensure quality and consistency. Auditing is conducted annually on-site by the data monitoring committee (clinical research centre).

DISSEMINATION

Trial registration

Protocol modifications/deviations will be communicated to the PI centre.

The trial was registered on ClinicalTrials.gov on 28 January 2022 (ID: NCT05287503) and EudraCT (ID: 2021-004565-13) and it was authorised by the Italian Regulatory Medicine Agency (Agenzia Italiana del Farmaco, AIFA; Provvedimento 2021-004565-13 SC23119).

Principal investigator: RC, ORCID ID: 0000-0002-1990-1939.

Consent to participate

All participants signed written informed consent prior to being enrolled by study team members. Withdraw from the study may occur at any time.

Dissemination plan

The trial results will be published in an international peer-reviewed journal and presented at national and international conferences.

DISCUSSION

Current treatments available for PD are symptomatic. Several large and controlled trials have failed to demonstrate the efficacy of disease-modifying therapies in PD over the last 20 years, likely due to a lack of adequate stratification according to the underlying pathophysiological mechanisms and/or pathways. Drugs targeting GCase activity, reduced in individuals with mutations in the *GBA* gene, might represent a disease-modifying approach in PD. ABX is a drug that can increase the GCase enzymatic levels and promote the clearance of α-Syn in vitro and in vivo, in animal models and humans.[35–38] By acting on this pathogenic loop, ABX could delay the neurodegeneration progress in patients with PD carrying *GBA* mutations and slow the disease course. This could impact the progression of cognitive decline, a feature responsible for the high psychological and social burden on patients with PD, families and the healthcare system.

Indeed, the first strength of the present disease-modifying trial is the primary endpoint that measures the evolution of cognitive dysfunction rather than the MDS-UPDRS III score. This choice aims to eliminate the possible confounding effects of concomitant therapy on motor aspects:

a. The long-duration response of levodopa is present in all stages of the disease and counts up to 60%;[59] its mechanisms are not yet known, and it could not be ruled out that some with possible disease-modifying action (such as ABX) may act on these mechanisms rather than on neurodegeneration.
b. ABX, as a mucolytic, could influence the pattern of response to drugs (mainly levodopa, but also oral DA agonists) by altering pharmacokinetics at the gastrointestinal level.[60]

Therefore, using motor overnight-OFF as a primary outcome measure could be susceptible to bias in studies of drugs with potential disease-modifying action.

Second, the novel ABX formulation: ABX was manufactured ad hoc for the AMBITIOUS trial to allow a gradual increase in dosage up to the standard treatment dose of only 6 tablets (as compared with previous studies including up to 21 tablets).[46] This formulation might increase the compliance of

patients enrolled. In addition, ABX safety is strictly controlled by an external pharmacovigilance.

The limitation of the present trial is the duration of the IMP treatment. Indeed, 12 months of therapy may not be sufficient to detect cognitive changes from baseline to 12 months. This treatment period was defined on the basis of the 36-month application timeline. To address this shortcoming, we included surrogate laboratory and imaging biomarkers (CSF markers of neurodegeneration and brain MRI), which may result in more sensitive and detectable measures of disease progression.

However, the results of this study will hopefully provide further evidence to support the growing idea of using ABX as a disease-modifying treatment in PD. Therefore, although no immediate benefits are expected for study participants, a safe and effective 'precision' neuroprotective therapy may finally be available to slow the progression of the neurodegenerative process in PD. If the results are positive, we plan to extend this trial as a multicentric phase III, lasting at least 24 months. We believe that the ABX safety profile and 'repositioning of an over-the-counter drug' would make this treatment more readily and rapidly available on a large scale.

STUDY STATUS

Recruitment and data collection began in March 2022. At the time of the manuscript submission (8 September 2023) the trial status is active and ongoing. The primary completion date of the trial (anticipated) is expected on December 2024.

Author affiliations
[1]Department of Clinical Neurosciences, Parkinson and Movement Disorders Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
[2]Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Italy
[3]Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy
[4]IRCCS Mondino Foundation, Pavia, Italy
[5]Department of Advanced Medical and Surgical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy
[6]Neuropsychology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
[7]Neuroradiology Unit, Foundation IRCCS Carlo Besta Neurological Institute, Milano, Italy
[8]Department of Molecular Medicine, University of Pavia, Pavia, Italy
[9]Division of Clinical and Experimental Pharmacology, Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy
[10]Parkinson's Centre of Excellence, King's College London, London, UK
[11]Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
[12]Unit of Neurology 5 and Neuropathology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
[13]Department of Psychology, University of Campania Luigi Vanvitelli, Caserta, Italy

Acknowledgements The authors would like to thank patients and caregivers who gave content to participate in the trial. In addition, we would like to thank the Clinical Trial Research Centre of the three study sites for help in the organisation and data collection. Finally, a special thanks to Teresa Bordoni for helpful comments on the study protocol and to Irene Tramacere and Teresa Del Santo for their collaboration in study procedures and medication management.

Contributors RC led the acquisition of funding from the Italian Ministry of Health. RC, MA and RDM made substantial contributions to conception, design and data acquisition. FColucci, MA and RC drafted the manuscript. MFP, MStanziano, AB, SC, GC, VF, DF, CGhezzi, MGastaldi, AEE, NGA, LR, GD, VL, CGalandra, GG, PM, GO, IP, MP, AP, MV, FE, MC, FDN, SA, MSiciliano, BG, FCazzaniga, CR, IT, SPrioni, PA, SPiacentini, MGB, MGrisoli, FM, RE, AT and EMV revised it critically for important intellectual content. All authors have read and approved the final manuscript.

Funding This work was supported by the Italian Ministry of Health (GR-2018-12366771; RRC 2023).

Competing interests RC has received speaking honoraria from Zambon Italia; Zambon SAU; Bial Italia Srl; advisory board fees from Bial; research support from the Italian Ministry of Health; he is Editor-in-Chief of the neuromuscular and movement disorders section of Brain Sciences (MDPI); Associate Editor of Parkinsonism and Related Disorders (Elsevier) and Frontiers in Ageing Neuroscience.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and the study has been approved by the Ethics Committee of the coordinating center (Fondazione IRCCS Istituto Neurologico Carlo Besta; Protocol ID GR-2018-12366771, original Version 1.0, 2 July 2021; latest amended Version 2.0, 30 March 2023; reference number CE n.90/2021) and partner centers (IRCCS Mondino Foundation and University of Campania 'Luigi Vanvitelli'). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; internally peer reviewed.

ORCID iD
Roberto Cilia http://orcid.org/0000-0002-1990-1939

REFERENCES

1 GBD 2016 Parkinson's disease collaborators. Global, regional, and national burden of Parkinson's disease, 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet Neurol* 2018;17:939–53.
2 Williams-Gray CH, Mason SL, Evans JR, *et al*. The campaign study of Parkinson's disease: 10-year outlook in an incident population-based cohort. *J Neurol Neurosurg Psychiatry* 2013;84:1258–64.
3 Gan-Or Z, Liong C, Alcalay RN. GBA-associated Parkinson's disease and other synucleinopathies. *Curr Neurol Neurosci Rep* 2018;18:44.
4 Lesage S, Anheim M, Condroyer C, *et al*. French Parkinson's disease genetics study group. large-scale screening of the Gaucher's disease-related glucocerebrosidase gene in Europeans with Parkinson's disease. *Hum Mol Genet* 2011;20:202–10.
5 Sidransky E, Nalls MA, Aasly JO, *et al*. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med* 2009;361:1651–61.
6 Zhang Y, Shu L, Zhou X, *et al*. A meta-analysis of *GBA*-related clinical symptoms in Parkinson's disease. *Parkinsons Dis* 2018;2018:3136415.
7 Winder-Rhodes SE, Evans JR, Ban M, *et al*. Glucocerebrosidase mutations influence the natural history of Parkinson's disease in a community-based incident cohort. *Brain* 2013;136(Pt 2):392–9.
8 Blauwendraat C, Reed X, Krohn L, *et al*. Genetic modifiers of risk and age at onset in GBA associated Parkinson's disease and lewy body dementia. *Brain* 2020;143:234–48.
9 Sidransky E, Lopez G. The link between the GBA gene and parkinsonism. *Lancet Neurol* 2012;11:986–98.
10 Cilia R, Tunesi S, Marotta G, *et al*. Survival and dementia in GBA-associated Parkinson's disease: the mutation matters. *Ann Neurol* 2016;80:662–73.
11 Creese B, Bell E, Johar I, *et al*. Glucocerebrosidase mutations and neuropsychiatric phenotypes in Parkinson's disease and lewy

body dementias: review and meta-analyses. *Am J Med Genet B Neuropsychiatr Genet* 2018;177:232–41.
12 Ortega RA, Wang C, Raymond D, *et al*. Association of dual Lrrk2 G2019S and GBA variations with Parkinson disease progression. *JAMA Netw Open* 2021;4:e215845.
13 Ren J, Zhan X, Zhou H, *et al*. Comparing the effects of GBA variants and onset age on clinical features and progression in Parkinson's disease. *CNS Neurosci Ther* August 10, 2023.
14 Anheim M, Elbaz A, Lesage S, *et al*. Penetrance of Parkinson disease in glucocerebrosidase gene mutation carriers. *Neurology* 2012;78:417–20.
15 Ren J, Zhang R, Pan C, *et al*. Prevalence and genotype-phenotype correlations of GBA-related Parkinson disease in a large Chinese cohort. *Eur J Neurol* 2022;29:1017–24.
16 Zuckerman S, Lahad A, Shmueli A, *et al*. Carrier screening for Gaucher disease: lessons for low-penetrance, treatable diseases. *JAMA* 2007;298:1281–90.
17 Gan-Or Z, Giladi N, Rozovski U, *et al*. Genotype-phenotype correlations between GBA mutations and Parkinson disease risk and onset. *Neurology* 2008;70:2277–83.
18 Neumann J, Bras J, Deas E, *et al*. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain* 2009;132(Pt 7):1783–94.
19 De Marco EV, Annesi G, Tarantino P, *et al*. Glucocerebrosidase gene mutations are associated with Parkinson's disease in southern Italy. *Mov Disord* 2008;23:460–3.
20 Beutler E. Gaucher disease: new molecular approaches to diagnosis and treatment. *Science* 1992;256:794–9.
21 Mignot C, Gelot A, De Villemeur TB. Gaucher disease. *Handb Clin Neurol* 2013;113:1709–15.
22 Hruska KS, LaMarca ME, Scott CR, *et al*. Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). *Hum Mutat* 2008;29:567–83.
23 Greuel A, Trezzi J-P, Glaab E, *et al*. GBA variants in Parkinson's disease: clinical, metabolomic, and multimodal neuroimaging phenotypes. *Mov Disord* 2020;35:2201–10.
24 Beutler E, Gelbart T, Scott CR. Hematologically important mutations: Gaucher disease. *Blood Cells Mol Dis* 2005;35:355–64.
25 Gan-Or Z, Amshalom I, Kilarski LL, *et al*. Differential effects of severe vs mild GBA mutations on Parkinson disease. *Neurology* 2015;84:880–7.
26 Malek N, Weil RS, Bresner C, *et al*. Features of GBA-associated Parkinson's disease at presentation in the UK tracking Parkinson's study. *J Neurol Neurosurg Psychiatry* 2018;89:702–9.
27 Lanore A, Casse F, Tesson C, *et al*. Differences in survival across monogenic forms of Parkinson's disease. *Ann Neurol* 2023;94:123–32.
28 Oftedal L, Maple-Grødem J, Dalen I, *et al*. Association of CSF Glucocerebrosidase activity with the risk of incident dementia in patients with Parkinson disease. *Neurology* 2023;100:e388–95.
29 Sardi SP, Cheng SH, Shihabuddin LS. Gaucher-related synucleinopathies: the examination of sporadic neurodegeneration from a rare (disease) angle. *Prog Neurobiol* 2015;125:47–62.
30 Alcalay RN, Levy OA, Waters CC, *et al*. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. *Brain* 2015;138(Pt 9):2648–58.
31 Sardi SP, Clarke J, Kinnecom C, *et al*. CNS expression of glucocerebrosidase corrects alpha-synuclein pathology and memory in a mouse model of Gaucher-related synucleinopathy. *Proc Natl Acad Sci U S A* 2011;108:12101–6.
32 Sardi SP, Clarke J, Viel C, *et al*. As a therapeutic strategy for parkinsonism and other Gaucher-related synucleinopathies, augmenting CNS glucocerebrosidase activity. *Proc Natl Acad Sci U S A* 2013;110:3537–42.
33 Sardi SP, Cedarbaum JM, Brundin P. Targeted therapies for Parkinson's disease: from genetics to the clinic. *Mov Disord* 2018;33:684–96.
34 Behl T, Kaur G, Fratila O, *et al*. Cross-talks among GBA mutations, glucocerebrosidase, and A-synuclein in GBA-associated Parkinson's disease and their targeted therapeutic approaches: a comprehensive review. *Transl Neurodegener* 2021;10:4.
35 Blandini F, Cilia R, Cerri S, *et al*. Glucocerebrosidase mutations and synucleinopathies: toward a model of precision medicine. *Mov Disord* 2019;34:9–21.
36 Peterschmitt MJ, Crawford NPS, Gaemers SJM, *et al*. Pharmacokinetics, pharmacodynamics, safety, and tolerability of oral venglustat in healthy volunteers. *Clin Pharmacol Drug Dev* 2021;10:86–98.
37 Malerba M, Ragnoli B. Ambroxol in the 21st century: pharmacological and clinical update. *Expert Opin Drug Metab Toxicol* 2008;4:1119–29.
38 McNeill A, Magalhaes J, Shen C, *et al*. Ambroxol improves lysosomal biochemistry in glucocerebrosidase mutation-linked Parkinson disease cells. *Brain* 2014;137(Pt 5):1481–95.
39 Maegawa GHB, Tropak MB, Buttner JD, *et al*. Identification and characterization of ambroxol as an enzyme enhancement agent for Gaucher disease. *J Biol Chem* 2009;284:23502–16.
40 Luan Z, Li L, Higaki K, *et al*. The chaperone activity and toxicity of ambroxol on Gaucher cells and normal mice. *Brain Dev* 2013;35:317–22.
41 Yang S-Y, Beavan M, Chau K-Y, *et al*. A human neural crest stem cell-derived dopaminergic neuronal model recapitulates biochemical abnormalities in Gba1 mutation carriers. *Stem Cell Reports* 2017;8:728–42.
42 Migdalska-Richards A, Daly L, Bezard E, *et al*. Ambroxol effects in glucocerebrosidase and A-synuclein transgenic mice. *Ann Neurol* 2016;80:766–75.
43 Zimran A, Altarescu G, Elstein D. Pilot study using ambroxol as a pharmacological chaperone in type 1 Gaucher disease. *Blood Cells Mol Dis* 2013;50:134–7.
44 Narita A, Shirai K, Itamura S, *et al*. Ambroxol chaperone therapy for neuronopathic Gaucher disease: a pilot study. *Ann Clin Transl Neurol* 2016;3:200–15.
45 Silveira CRA, MacKinley J, Coleman K, *et al*. Ambroxol as a novel disease-modifying treatment for Parkinson's disease dementia: protocol for a single-centre, randomized, double-blind, placebo-controlled trial. *BMC Neurol* 2019;19:20.
46 Mullin S, Smith L, Lee K, *et al*. Ambroxol for the treatment of patients with Parkinson disease with and without glucocerebrosidase gene mutations: a nonrandomized, noncontrolled trial. *JAMA Neurol* 2020;77:427–34.
47 Shinotoh H, Tessitore A. Resting-state fMRI sheds light on neural substrates of cognitive decline in Parkinson disease. *Neurology* 2014;83:2000–1.
48 Postuma RB, Berg D, Stern M, *et al*. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* 2015;30:1591–601.
49 Emre M, Aarsland D, Brown R, *et al*. Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Mov Disord* 2007;22:1689–707.
50 Barton B, Grabli D, Bernard B, *et al*. Clinical validation of movement disorder society-recommended diagnostic criteria for Parkinson's disease with dementia. *Mov Disord* 2012;27:248–53.
51 Dalrymple-Alford JC, MacAskill MR, Nakas CT, *et al*. The moca: well-suited screen for cognitive impairment in Parkinson disease. *Neurology* 2010;75:1717–25.
52 Hoops S, Nazem S, Siderowf AD, *et al*. Validity of the moca and MMSE in the detection of MCI and dementia in Parkinson disease. *Neurology* 2009;73:1738–45.
53 Litvan I, Goldman JG, Tröster AI, *et al*. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: movement disorder society task force guidelines. *Mov Disord* 2012;27:349–56.
54 Avenali M, Cerri S, Ongari G, *et al*. Profiling the biochemical signature of GBA-related Parkinson's disease in peripheral blood mononuclear cells. *Mov Disord* 2021;36:1267–72.
55 Kim H-J, Jeon B, Song J, *et al*. Leukocyte glucocerebrosidase and B-hexosaminidase activity in sporadic and genetic Parkinson disease. *Parkinsonism Relat Disord* 2016;23:99–101.
56 Wen A, Hang T, Chen S, *et al*. Simultaneous determination of amoxicillin and ambroxol in human plasma by LC-MS/MS: validation and application to pharmacokinetic study. *J Pharm Biomed Anal* 2008;48:829–34.
57 Hu MTM, Szewczyk-Królikowski K, Tomlinson P, *et al*. Predictors of cognitive impairment in an early stage Parkinson's disease cohort. *Mov Disord* 2014;29:351–9.
58 Beavan M, McNeill A, Proukakis C, *et al*. Evolution of Prodromal clinical markers of Parkinson disease in a GBA mutation-positive cohort. *JAMA Neurol* 2015;72:201–8.
59 Cilia R, Cereda E, Akpalu A, *et al*. Natural history of motor symptoms in Parkinson's disease and the long-duration response to levodopa. *Brain* 2020;143:2490–501.
60 Leta V, Klingelhoefer L, Longardner K, *et al*. Gastrointestinal barriers to levodopa transport and absorption in Parkinson's disease. *Eur J Neurol* 2023;30:1465–80.