Ambroxol in Parkinson disease patients with and without glucocerebrosidase mutations

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Abstract

Background: Alpha-synuclein accumulation is an important factor in Parkinson disease (PD) pathogenesis. I*n vitro* and *in vivo* models indicate that ambroxol may modulate glucocerebrosidase activity and reduce alpha-synuclein levels in PD brain.

Methods: In this single-centre, non-placebo-controlled trial, patients with moderate PD received an escalating dose of oral ambroxol to 1.26g/day. Primary outcomes at 180 days were 1) safety and tolerability 2) central nervous system (CNS) penetration 3) a change in cerebrospinal fluid (CSF) glucocerebrosidase enzyme (GCase) activity. The study is completed (NCT02941822).

Results: Primary analysis included 17 patients (8 *GBA1*+, 9 *GBA1*-). Ambroxol was well tolerated with no serious adverse events. Between day 0 and 180 days there was a 156ng/ml increase in CSF ambroxol (lower 95%CI 129, $p<0.0001$). CSF GCase activity reduced by 19% (0.059nmol/hr/ml, 95%CI CI -0.115 to -0.002, $p=0.043$). There was a 13% increase in CSF alpha synuclein concentration (50pg/ml, SE 17, 95% CI 14 to 87) and a 35% increase in CSF GCase protein levels (88ng/mol SE 22, 95%CI 40 to 137). Movement Disorders Society unified Parkinson disease rating scale part III fell (improved) by 6.8 (SE 1.7, 95%CI -10.4 to -3.1).

Conclusion: We demonstrate safety, tolerability, CNS penetrance and target engagement of ambroxol; CSF alpha synuclein was increased. Larger placebo-controlled trials are required to determine if ambroxol exerts an effect on the natural progression of PD.

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Introduction

Glucocerebrosidase (GCase) is a pH dependent hydrolase enzyme that catalyses the breakdown of the sphingolipid glucosylceramide to ceramide and glucose within the acidic environment of the lysosome. Mutations in the glucocerebrosidase gene (*GBA1*) cause the autosomal recessive lysosomal storage disorder Gaucher disease (GD) (1). They are numerically the most significant genetic risk factors for Parkinson disease (PD) (2) and exhibit a penetrance of 10-30% (3, 4). They are present in 5-15% of Caucasian PD, up to 25% of Ashkenazi cases and 1% of controls (5).

In cell and animal models of *GBA1* mutations, there is increased alpha synuclein (A-SYN) accumulation and a reciprocal relationship between A-SYN levels and GCase activity has been demonstrated (6-11). GCase activity is reduced in the brains of PD patients both with and without *GBA1* mutations, but is lower in the former (12). Reduced brain GCase activity is associated with increased levels of A-SYN (13). GCase enzyme activity is decreased in CSF of *GBA1* and non *GBA1* PD cases compared to controls (14). It has been postulated that upregulation of brain cytosolic/lysosomal GCase activity may reduce A-SYN levels, mediating a neuroprotective effect in both *GBA1* and non *GBA1* PD cases (15-17).

Ambroxol has been safely used as a cough linctus since the 1970s (see summary of product characteristics - section 1, supplementary materials). Its principle side effects are gastrointestinal (GI) disturbance and a small risk of anaphylaxis. In a high throughput screen of compounds it was found to increase GCase activity in a pH dependent manner (18). An increase in GCase activity and a reduction in A-SYN levels in cell lysates following ambroxol administration has been demonstrated in a number of *in vitro* and *in vivo* models

(6-11). Ambroxol is thought to mobilise sequestered mutant GCase enzyme from the endoplasmic reticulum by binding to and inhibiting the enzyme active site, inducing conformational change and facilitating transport to the lysosome (19, 20). Once in the acidic environment of the lysosome, it is eluted, allowing normal catalysis to resume, restoring lysosomal function. A-SYN is predominantly degraded via chaperone-mediated autophagy, and so it would be anticipated that enhanced lysosomal function will increase A-SYN degradation (21).

Ambroxol may modulate A-SYN levels through several mechanisms. GCase may have a direct role in A-SYN protein disposal (22) and ambroxol has been shown to upregulate GCase expression through the transcription factor E-beta (TFEB) pathway and stimulation of lysosomal exocytosis (9, 23). Alternatively, *GBA1* mutations may interrupt physiological post-translational folding, preventing trafficking of the enzyme to the lysosome (19, 20, 24, 25). This appears to result in sequestration in the endoplasmic reticulum and an unfolded protein response (UPR) which may induce A-SYN aggregation (24). There is evidence that ambroxol corrects post-translational folding, mitigating UPR (19).

We have investigated the biological effect of ambroxol in human subjects and its impact on biochemical and clinical markers of PD. The primary endpoints were safety and tolerability of ambroxol, CNS penetration and change in cerebrospinal fluid (CSF) GCase activity between baseline and 180 days.

Results

Between 11th January 2017, and 27th July 2017, 24 patients were screened for eligibility, 23 of whom entered the study 'Ambroxol in the Modification of Parkinson Disease (AiM-PD,

for flow diagram see Figure 1). The study was carried out between 11th January 2017 and 25th April 2018. The *GBA1* mutations of participants were p.E326K/wt (n=3), p.N370S/wt (n=1), p.R463C/wt* (n=2), p.T369M/p.W393X* (n=1), RecNcil (p.L444P + p.A456P + p.V460V) */wt (n=1). An asterix adjacent to the mutation indicates a 'severe' GD causing phenotype. One participant was excluded from the study following unsuccessful lumbar puncture attempts by two experienced operators. Two withdrew due to post lumbar puncture headache prior to commencement of the ambroxol. One participant withdrew at day 1 post commencement of ambroxol, citing the high tablet count (21 tablets per day). One participant withdrew at 90 days post ambroxol commencement citing personal reasons (family illness) as the main rationale for withdrawal, although the high tablet load and constipation were also deemed contributory. One *GBA1*- participant was excluded from CSF analyses (including the primary analysis) on account of a red cell count in excess of 500 cells/cm^3 in their baseline CSF sample, indicative of blood contamination. Seven participants (3 *GBA1*+, 4 *GBA1*-) undertook a third lumbar puncture at 270 days. This included the above (excluded) participant found to have blood contaminated baseline CSF. Accordingly, 18 participants (8 *GBA1*+ and 10 *GBA1*-) were included in blood analyses and clinical analyses at 180 and 270 days. Seventeen (8 *GBA1*+, 9 *GBA1*-) were included in the CSF analyses at 180 days. Six (3 *GBA1*+, 3 *GBA1*-) were included in the CSF analyses at 180 days.

Based on empty collected blister pack we estimated a mean compliance of 89% (SD 13). Blood and CSF ambroxol levels at baseline confirmed no participants had taken the drug prior to the start date. The drug was well tolerated. There were no serious adverse events (AEs) reported by participants. There were 176 AEs of which 121 were deemed not related, 32 unlikely to be related, 15 possibly related, 5 probably related and 3 definitely related to the IMP. The AEs probably related to the IMP were nausea, vomiting (x2), a burning sensation

after swallowing the IMP and loose stools. Definitely related AEs were acid reflux, nausea and a transitory rash on the chest, back and arms. A full list of patient reported AEs is available in Table S5. There was a mean 1.3kg (SD 2.5) loss of weight between baseline and 180 days (SE 0.6, 95%CI -2.59 to 0.01).

Table 1 shows a summary of results for those participants who completed the study. Results of the *GBA1* and non *GBA1* subgroups are available in Tables S3 and S4.

Primary outcomes

Ambroxol was undetectable in serum and CSF at baseline. At day 180 CSF ambroxol was 156ng/ml (SE 12.9, 95% lower confidence limit 129ng/ml, one-sided paired t test $p<0.0001$). At 180 days mean CSF ambroxol levels were 11% of mean blood levels. Mean CSF GCase activity fell by 0.059nmol/hr/ml (SE 0.026, 95%CI CI -0.115 to -0.002 two-sided paired t-test $p=0.043$ – Figure 2A), a 19% reduction from mean baseline GCase activity.

Secondary outcomes

Between baseline and 180 days there was a mean 50pg/ml (13%) increase in total CSF A-SYN concentration (SE 17, 95%CI 14 to 87 – Figure 2B). Between baseline and 180 days there was an 88pmol/L mean increase in CSF GCase protein level (SE 22, 95%CI 40 to 137; Figure 2C), a mean increase of 35% from baseline protein levels. Effect sizes did not indicate a significant change in CSF tau (mean change 5pg/ml, SE 6, 95%CI -7 to 17) or glucosylceramide levels (mean change 14pmol/L, SE 10, 95%CI -6 to 35). The correlation

between ambroxol dose and the change in CSF GCase activity was not significant (Pearson correlation coefficient, r=-0.161, p= 0.524).

Between baseline and 180 days, mean blood leucocyte GCase activity increased by 1.0 nmol/mg/hr (SE 1.4, 95%CI -2.0 to 4.0), a nine percent increase, although it appeared the change in blood leucocyte GCase activity peaked at 90 days (mean change 2.1, SD 5.1) a mean 19% increase from baseline – table 1 and figure 2D). There was wide variation in the recorded change in serum alpha synuclein concentration (mean change 2602 pg/ml, SE 3455, 95%CI -4689 to 9893) although there was a mean 0.20 pg/ml decrease in serum tau levels (SE 0.08, 95%CI -0.37 to -0.05).

Between baseline and 180 days, the mean total MDS UPDRS score reduced (improved) by 8.7 (SE 3.0, 95%CI -15.3 to -2.2 - Figure 3A) with a rebound by 7.2 (SD 9.8) between 180 days and 270 days. This change appeared to be driven primarily by the MDS UPDRS part III motor score which showed a mean 6.8 point (SD 7.1) fall between baseline and 180 days (Figure 3B - SE 1.7, 95%CI -10.4 to -3.1) and a mean rise of 7.6 (SD 7.0) between 180 days and 270 days. Only 7 of 18 patients increased their dopaminergic therapy during the course of the study, and the same deflection of MDS UPDRS changes was seen in those on stable therapy as well as those who increased medication (see supplementary materials section 2, figures S1 and S2). As anticipated, the change in MoCa scores were skewed and bound by zero due to the preponderance of participants who maintained their scores from baseline. There was a rise (improvement) in the mean MoCa scores between baseline and 180 days of 1.7 points (SD 1.3). Between baseline and 180 days mean NMSS score rose (worsened) by 11.5 points (SE 4.4, 95% CI 2.4 to 20.8), but the mean change of NMSQuest score was only 0.2 points (SD 2.6) between baseline and 180 days.

CSF analysis of the 6 participants in whom CSF was collected at baseline, 180 and 270 days is presented in suppl table 4.

In vitro assays to characterise interaction of ambroxol and GCase in CSF

Prior to the trial we carried out *in vitro* assays to predict the effect of penetrance of ambroxol, an inhibitory chaperone, on GCase activity. We added 500nM (189 ng/ml - chosen on basis of CSF ambroxol level in a previous clinical trial (26)) of ambroxol to human CSF taken from healthy subjects (diagnostic lumbar punctures for suspected idiopathic intracranial hypertension). Additionally we carried out a positive control by thermodynamically denaturing and chemically inhibiting the GCase enzyme with conduritol B epoxide (CBE) (27). The experiment comprised 5 technical repeats for CSF derived from 5 subjects for each condition. Compared to controls there was a mean 42% (SD 12%) reduction in CSF GCase activity (mean change in activity -0.093, 95% CI 0.046 to 0.140 nmol/hr/ml) upon addition of ambroxol. Denatured and CBE inhibited samples registered no residual activity.

Discussion

This study represents the first clinical trial of personalised therapy for a stratified (genetically defined) subtype of PD and the first use of ambroxol in PD. It has met its primary outcomes; to confirm the safety and tolerability of ambroxol in this population, to prove CNS penetration of ambroxol and to confirm a modulatory effect on CSF GCase.

Both *in vitro* and *in vivo,* we demonstrate an inhibitory effect of ambroxol on GCase activity within human CSF, consistent with its known inhibitory chaperone activity. Within largely acellular CSF, GCase protein is free, in contrast to its normal intracellular lysosomal location. Ambroxol is a pH-dependent inhibitory small molecular chaperone which binds to the active site of the GCase protein and reduces activity. Binding enables transport to the lysosome where it is eluted under acidic conditions, releasing free active enzyme. Therefore in the acellular CSF, ambroxol will bind to and inhibit free GCase, but will increase brain intracellular GCase activity as demonstrated in rodent and primate models (7,8).

The sustained upregulation of expression of GCase protein levels within the brain and the concomitant increase in total CSF A-SYN concentration indicate target engagement of the ambroxol molecule with GCase. Moreover these results imply an increase in GCase activity within the brain itself. There is no clear consensus on the effect of PD on total CSF A-SYN, but reduced levels of total CSF A-SYN have been described , while oligomeric and phosphorylated A-SYN are increased (28, 29). Ambroxol upregulates expression of GCase, probably by way of the TFEB pathway, and increases vesicular export of cytosolic products (9, 23). The increase in CSF total A-SYN in ambroxol treated PD subjects could be interpreted as an increase of extracellular export of the protein from the brain parenchyma to the CSF.

Interpretation of the changes in MDS UPDRS and MoCA is difficult in the context of a non-placebo controlled study. However, they support the clinical impression that there was no substantial deleterious effect of ambroxol on subjects taking ambroxol, including on the motor features of their PD.

Our study has limitations. Numbers are relatively small, although the complexity of the study and its nature as a proof of principle/concept trial influenced its design and numbers recruited. There is no placebo arm, so the clinical outcomes in particular should be interpreted with this in mind. We could have elected to have a more genetically homogenous *GBA1* study group, but this may have limited the interpretation of the data and whether the observed findings were mutation-dependent.

In conclusion, our study confirms that ambroxol has potential as a drug to target the glucocerebrosidase pathway in PD and increase brain GCase activity. It concurs with cell and animal modelling which indicate that it modulates A-SYN levels. We believe it has promise for further investigation as a drug to improve outcome, particularly in *GBA1* positive but potentially in *GBA1* negative PD patients as well. Further larger placebo controlled studies are warranted.

Materials and Methods

Study design and participants

We performed an open label, single-centre trial of oral ambroxol 1.26g/day (420 mg TID) in PD patients of moderate severity. The trial was undertaken at the Leonard Wolfson Experimental Neuroscience Centre (LWENC, London, UK), a dedicated National Institute for Health Research (NIHR) Clinical Research Facility and part of the University College London (UCL) Queen Square Institute of Neurology and the National Hospital for Neurology & Neurosurgery (NHNN) at University College London Hospital (UCLH). Clinical oversight was provided by a trial steering committee. Statistical support was provided by Peninsula Clinical Trials Unit (PenCTU). Trial operations were supported by the LWENC and the NIHR Biomedical Research Centre at the UCL Institute of Neurology and the NHNN.

Eligible subjects were aged 40–80 years, had idiopathic PD as defined by Queen Square Brain Bank criteria (30) were judged able to administer the trial drug, and were at Hoehn and Yahr stage 3 or less. A portion were pre-selected on the basis of their previously ascertained *GBA1* mutation carrier status. We pre-screened subjects over the phone against these criteria before formal in-person screening. Key exclusion criteria (see supplementary materials section 3 for full list) included use of an interventional medicinal product (IMP) within the last 30 days or exposure to 3 or more IMPs within the last 12 months. All participants underwent confirmatory sequencing of exons 1-11 of the *GBA1* gene.

Recruitment

Patients were recruited from established research databases held at the Royal Free London Hospital, and the NHNN, London, UK.

Procedures

A detailed summary of study visits is available in table S1. At screening for trial entry, each patient underwent a physical and neurological examination and blood sampling for clinical laboratory tests. Women of childbearing potential also had a pregnancy test. An electrocardiograph (ECG) was also performed.

Our trial had a washout design. There was a 180 day exposure period comprising 30 days of dose escalation: 60 mg TID (day 1-7), 120 mg TID (day 8-14), 180 mg TID (day 15-21), 300 mg TID (day 22-28). This was followed by 150 days of administration of ambroxol 1.26g/day (420 mg TID).

After confirmation of patient eligibility, subsequent visits were held at baseline (day 0), day 10, day 90, day 180 and day 270 with telephone contacts at predetermined intervals (see supplementary materials Table S1 for full details of study visits). In addition to routine clinical bloods, blood samples were taken at each assessment. CSF examination was performed at baseline and 180 days with a third optional lumbar puncture performed at 270 days.

Patients were issued ambroxol in 2 batches; at baseline and 90 days. They were asked to attend each visit in an off-medication state, which was defined as a period of withdrawal of levodopa for 8 h (ie. overnight) or 24 h in the case of long-acting drugs such as once-daily ropinirole, pramipexole, and rotigotine.

All assessments were carried out between 8am-9am by a single assessor (SM). We carried out Movement Disorders Society unified Parkinson disease rating scale (MDS-UPDRS – day 0, 90, 180, 270), Montreal cognitive assessment (MoCa – day 0 and 180), non-motor symptoms scale (NMSS - day 0 and 180) and non-motor symptoms questionnaire (NMSQuest - day 0 and 180).

Empty blister packs were collected at 90 days and 180 days to assess compliance.
All adverse events, biochemical results, blood pressure, heart rate, and weight were recorded.

Outcomes

The primary outcomes, all assessed at 180 days, were safety and tolerability of ambroxol in this population (measured by frequency and severity of adverse events, abnormal findings on

clinical examination, blood tests or ECG), change in CSF ambroxol levels and change in CSF GCase activity.

Predefined secondary outcomes all assessed at 180 days, were change in blood leucocyte GCase activity, change in CSF GCase enzyme protein levels, change in CSF total glucosylceramide levels, change in CSF and serum alpha synuclein levels, change in CSF and serum tau levels.

We also reported on the MoCa, NMSS, NMSQuest assessments conducted at 180 days and at baseline.

A full list of study outcomes, exclusion and inclusion criteria can be found in the supplementary materials section 3.

Sample collection

CSF was collected before 10am from subjects fasted overnight. Samples were processed were frozen at -80 degrees within 60 minutes of collection in the case of CSF and 90 minutes in the case of leucocyte pellets. Samples were defrosted only immediately prior to performance of assays. CSF GCase assays were all carried out within 7-14 days of sample collection. Leucocyte GCase assays were carried out within 7-28 days of sample collection.

CSF GCase activity

Cerebrospinal fluid was collected in 15ml polypropylene tubes (Starstedt 62.554.002) centrifuged at 2200G for 10min and frozen at -80 degrees within an hour of collection in 200ul aliquots in polypropylene 2ml microtubes (Starstedt 72.694.217). CSF was vortexed and centrifuged at 8000rpm for 10 minutes. Ten repeats of 20ul of CSF were added to a 96 well plate. The reaction was commenced by adding 40ul of 5uM 4-MU-β-D-glucopyranoside solution dissolved in McIlvaine citrate buffer (0.15M, pH 5.9) with 28nM sodium taurochlorate. The plate was covered and incubated at 38 degrees for 3 hours whereupon the reaction was stopped using 240ul glycine stopping buffer (1M, pH 10.4). A standard was prepared by 5 repeats of 200ul 1mM 4-methylumbelliferone in distilled water and adding 100ul glycine buffer. The plate was measured at excitation wavelength of 365nm, emission of 450nm with a PerkinElmer (Waltham, MA) fluorescence spectrometer. GCase assays were expressed as nanomoles of substrate catalysed per millilitre of CSF per hour using the standard and a blank in which the CSF was replaced distilled water. Our optimisation experiments have indicated an *inter* and *intra* assay coefficient of variance of 3% and 2% respectively

Leucocyte pellet GCase activity assay

Leucocyte pellets were collected in BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube – Sodium Heparin (catalogue number 362753). Tubes were spun at 3000rpm for 25 minutes. Leucocytes were collected from above the intermediate density liquid and aliquoted into 1.5ml microtubes (Starlabs cat. E1415-2230). Samples were spun at 6000rpm for 6 minutes. If no pellet was visible at this point a further spin at 6000rpm for 6 minutes was carried out. The supernatant was drained and the sample was re-suspended in 1ml red cell lysis buffer (155 mM NH_4Cl 12 mM $NaHCO_3$ 0.1 mM EDTA) for 10 minutes at room temperature. The sample was spun again, the supernatant drained and re-suspended in

PBS. One final spin was carried out and the supernatant was drained prior to being assayed or frozen at -80 degrees. In the case of the latter this was always within 1 hour of collection.

Leucocyte pellets were lysed in Fermantas ProteoJET Mammalian Cell Lysis Reagent and diluted 8X in McIlvaine citrate buffer (0.15M, pH 5.9). The reaction was commenced by adding 40ul of 5uM 4-MU-β-D-glucopyranoside solution dissolved in McIlvaine citrate buffer (0.15M, pH 5.9). The plate was covered and incubated at 38 degrees for 3 hours whereupon the reaction was stopped using 240ul glycine stopping buffer (1M, pH 10.4). A standard was prepared by preparing 5 repeats of 200ul 1mM 4-methylumbelliferone in distilled water and adding 100ul glycine buffer. The plate was measured at excitation of 365nm, emission of 450nm with a PerkinElmer (Waltham, MA) fluorescence spectrometer. Protein concentration was determined using a Pierce BCA Protein Assay. GCase assays were expressed as nanomoles of substrate catalysed per milligram of protein per hour.

Liquid chromatography mass spectrometry of GCase and glucosylceramide

To 600 µL CSF, 3 pmol heavy labelled GCase peptide, NFVDSPII**V**DITK (Genscript, USA), were added as internal standard. The samples were freeze dried and trypsin digested as previously described (31). Sample clean-up was performed using C_{18} cartridges (Biotage, Sweden) which were washed with two 1 mL aliquots of 70% acetonitrile, 0.1% trifluoroacetic acid (TFA) and primed with two 1 mL aliquots of 0.1% TFA before the sample was loaded. The flow-through was re-applied and the bound peptides washed with one 1 mL aliquot of 0.1% TFA. The peptides were eluted with 500 µL 70% acetonitrile, 0.1% TFA and solvents were evaporated using a SpeedVac. Before analysis, the peptides were re-constituted in 120 µL 3% acetonitrile, 0.1% TFA.

Mass spectral analysis was performed as previously described (32). 5 µL of digest were injected and peptides separated on a Waters Acquity UPLC system coupled to a Xevo TQ-S mass spectrometer. The monitored peptides were quantifier NFVDSPIIVDITK (m/z 730.9>1100.6), qualifier SYFSEEGIGYNIIR (m/z 824.7>905.5) and internal standard (m/z 733.9>1106.6). The data were integrated using an in-house script written in Python. Analyte responses were normalised to internal standard response before concentrations were calculated in the average of three technical replicates using a calibration curve constructed from synthetic peptides (GenScript, USA) ranging from 0 to 1 pmol μL^{-1}. Quality control samples composed of pooled digest were run at least every seventh sample and showed a coefficient of variance of 5.6%.

Glucosylceramides were extracted using a modified Bligh and Dyer procedure. Briefly, to 200 µL CSF, 400 µL methanol containing the deuterated internal standard glucosylceramide C16:0-D_3 (#1533, Matreya, USA) were added. Samples were vortexed and stored on dry ice followed by sonication. 200 µL chloroform were added and the samples were again vortexed and stored on dry ice followed by sonication. Finally, 200 µL water and 200 µL chloroform were added. The samples were incubated in room temperature for 60 minutes before centrifugation at +4 °C, 5000*g* for 10 minutes. 300 µL of the organic phase were transferred to a glass vial and solvents were evaporated under nitrogen. Before analysis, the samples were re-constituted in 50 µL methanol.

Analysis was performed using a Waters Acquity Liquid Chromatography Quaternary Solvent Manager system coupled to a Waters Xevo TQ-S mass spectrometer. 5 µL of sample were injected and separated on a Waters Acquity UPLC BEH C8 column, 1.7 µm, 2.1 x 50 mm

with a VanGuard pre-column of the same chemistry. The mobile phase consisted of A: water, 0.1% formic acid and B: methanol, 0.1% formic acid. The gradient profile lasted for 6.5 minutes and was initially set to 50% B for 0.2 minutes, then linearly increased to 100% B over 1.8 minutes. The column was washed with 100% B for 1 minute, then returning to equilibrate at initial conditions before the next injection. The flow rate was 0.5 mL min^{-1}. Glucosylceramides were detected using multiple reaction monitoring in positive mode, see below for transitions. The data were integrated using an in-house script written in Python. Analyte responses were normalised to internal standard response before concentrations were calculated in the average of three technical replicates using a calibration curve created from glucosylceramide standard (#1522, Matreya, USA) ranging from 0 to 0.5 ng μL^{-1}. Quality control samples consisting of pooled extract were run at least every seventh sample and showed a coefficient of variance of 11%.

ISOFORMS	MONITORED TRANSITIONS
C16:0-d3 glucosylceramide (internal standard)	725.7 > 545.7/536.7
C16:1 glucosylceramide	720.7 > 558.7
C16:0 glucosylceramide	722.7 > 542.6/560.6
C18:1 glucosylceramide	748.6 > 586.6
C18:0 glucosylceramide	750.6 > 588.6
C20:1 glucosylceramide	776.6 > 614.6
C20:0 glucosylceramide	778.6 > 616.7
C22:1 glucosylceramide	804.7 > 642.7
C22:0 glucosylceramide	806.7 > 644.7
C22:0-OH glucosylceramide	820.7 > 658.8
C24:2 glucosylceramide	830.7 > 668.7
C24:1 glucosylceramide	832.7 > 670.7
C24:0 glucosylceramide	834.7 > 672.7
C24:2-OH glucosylceramide	846.7 > 684.8
C24:1-OH glucosylceramide	848.7 > 686.8
C24:0-OH glucosylceramide	850.7 > 688.8
C26:1 glucosylceramide	860.7 > 698.8

C26:0 glucosylceramide	862.7 > 700.8
C26:1-OH glucosylceramide	876.7 > 714.8
C26:0-OH glucosylceramide	878.7 > 716.8

Alpha synuclein and tau measurement

Total A-SYN concentration was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) from Covance (Covance, Dedham, MA, USA). Tau concentration was measured using a commercially available INNOTEST ELISA (Fujirebio, Ghent, Belgium).

In vitro assays estimating effect of ambroxol of CSF GCase activity

For each condition fluorescence was measured with five technical repeats (5 wells) per subject, per condition all measured and performed on a single 96 well plate. For each subject CSF was pooled from the same multiple aliquots to prevent variability due to variation between aliquots (i.e CSF was not pooled from different subjects). CSF GCase activity was measure on control CSF, CSF with 500nM ambroxol added and two negative controls: CSF denatured at 80 degrees and CSF with 1mM Conditurol B epoxide (CBE), an irreversible inhibitor of GCase enzyme, added to it.

Ambroxol powder (Sigma – A0363700) was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of 10mM. An intermediate stock of 100μM was made, aliquoted and stored at -20°C. For the assay ambroxol was added in a 1:200 dilution directly to CSF to obtain a working concentration of 500nM and incubated at 37°C for 15min (to ensure the solution was dissolved) before adding to the plate.

A 10mM stock solution of Conditurol B epoxide (CBE), an irreversible inhibitor of GCase enzyme, was made by dissolving the solute in dH_2O and was subsequently aliquoted and stored at -20°C. For the assay, the CBE was added in a 1:10 dilution directly to CSF to obtain a working concentration of 1mM and incubated at 37°C for 15min before adding to the plate. As a second negative control, some of the CSF sample was denatured prior to performing the assay. Denaturation was achieved by heating the CSF to 80°C for a minimum of 15min in a dry bath incubator.

Statistics

Analyses were carried according to a predefined statistical analysis plan written by the trial statisticians (AS and JoH). Analyses were carried on STATA version 14.2. The distribution of the outcomes was assessed through inspection of the plotted data (see supplementary materials section 4, Figures S3-5). For the primary analysis, 95% confidence intervals (CIs) were presented alongside the result from two-sided t-test of the change in CSF GCase activity. The lower CI and one-sided t-test presented for the change in CSF ambroxol reflected the change being lower-bounded by zero because of anticipated zero concentrations at baseline. Ambroxol concentrations were required at 180 days to be greater than the assay limit of sensitivity at 1.0ng/ml to register as a change. A descriptive analysis correlating the change in ambroxol and change in CSF GCase activity (Pearson's coefficient) was also carried out. The change in secondary outcomes between baseline and 180 days were presented with 95% confidence intervals.

Role of the funding source

The study funder and drug provider had no role in study design, data interpretation, data collection, analysis, or writing of the Article. All authors had full access to all study data, and

the corresponding author had responsibility for the final decision to submit the article for publication.

Study approval

The study was approved by the Institutional Joint Research Office (JRO) of UCL, the UK Medicines and Healthcare products Regulatory Agency (MHRA - EudraCT number 2015-002571-24) and the Research Ethics Committee for London – Bloomsbury (16/LO1341). Ambroxol was supplied on a complimentary basis by PRO.MED.CS Praha a.s, Telčská 377/1, Michle, 140 00 Prague 4, Czech Republic.

The study is registered on the public database ClinicalTrials.gov (NCT02941822) and was conducted in compliance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonisation and Good Clinical Practice guidelines. Written informed consent was obtained from all participating patients before initiating any study-related procedures. The study protocol is available in the supplementary materials, section 5.

Author contributions

Design: SM AHVS AS JoH GD RK VL PW HZ TF
Recruitment: SM SC TF PL MT
Assay optimisation: SM KL LS KM JH
Data Collection: SM LS PC MT
Biochemical and molecular assays SM KL LS JH KM WH
Data Analysis: SM AS AHVS
Manuscript preparation: SM AHVS GD RK VL PW HZ TF SC LS KL JH PL PC MT AS

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help and assistance with the study. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health

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Competing interests

TF has received honoraria for speaking at meetings supported by Profile Pharma, BIAL, AbbVie, served on advisory boards for BIAL and Oxford Biomedica. HZ has served at scientific advisory boards for Eli Lilly, Roche Diagnostics, Wave, Samumed and CogRx, has received travel support from Teva and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. PL has received travel support and honoraria from Boston scientific and Medtronic. WH has received funding and travel support from Shire pharmaceuticals. WH and KM have received honoraria from Freeline therapeutics. KM has received travel support from Actelion and Genzyme Sanofi.

Data and materials availability

Study data is deposited in the EudraCT depository (2015-002571-24). It available upon reasonable request.

Supplementary materials

References

1. Grabowski GA, Zimran A, Ida H. Gaucher disease types 1 and 3: Phenotypic characterization of large populations from the ICGG Gaucher Registry. *Am J Hematol* 2015;90:S12–S18.

2. Sidransky E et al. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med* 2009;361(17):1651–1661.

3. Rana HQ, Balwani M, Bier L, Alcalay RN. Age-specific Parkinson disease risk in GBA mutation carriers: information for genetic counseling. *Genet. Med.* 2013;15(2):146–149.

4. Anheim M et al. Penetrance of Parkinson disease in glucocerebrosidase gene mutation carriers. *Neurology* 2012;78(6):417–420.

5. Zhang Y et al. Integrated Genetic Analysis of Racial Differences of Common GBAVariants in Parkinson's Disease: A Meta-Analysis. *Front Mol Neurosci* 2018;11:43.

6. Yang S-Y, Beavan M, Chau K-Y, Taanman J-W, Schapira AHV. A Human Neural Crest Stem Cell-Derived Dopaminergic Neuronal Model Recapitulates Biochemical Abnormalities in GBA1 Mutation Carriers. *Stem Cell Reports* 2017;8(3):728–742.

7. Migdalska-Richards A, Daly L, Bezard E, Schapira AHV. Ambroxol effects in glucocerebrosidase and α-synuclein transgenic mice. *Ann. Neurol.* 2016;80(5):766–775.

8. Migdalska-Richards A, Ko WKD, Li Q, Bezard E, Schapira AHV. Oral ambroxol increases brain glucocerebrosidase activity in a nonhuman primate. *Synapse* 2017;256(5):e21967.

9. Magalhaes J, Gegg ME, Migdalska-Richards A, Schapira AH. Effects of ambroxol on the autophagy-lysosome pathway and mitochondria in primary cortical neurons. *Sci Rep* 2018;8(1):1651.

10. Mazzulli JR et al. Activation of -Glucocerebrosidase Reduces Pathological -Synuclein and Restores Lysosomal Function in Parkinson's Patient Midbrain Neurons. *Journal of Neuroscience* 2016;36(29):7693–7706.

11. McNeill A et al. Ambroxol improves lysosomal biochemistry in glucocerebrosidase mutation-linked Parkinson disease cells. *Brain* 2014;137(Pt 5):1481–1495.

12. Gegg ME et al. Glucocerebrosidase deficiency in substantia nigra of Parkinson disease brains. *Ann. Neurol.* 2012;72(3):455–463.

13. Murphy KE et al. Reduced glucocerebrosidase is associated with increased α-synuclein in sporadic Parkinson's disease. *Brain* 2014;137(Pt 3):834–848.

14. Parnetti L et al. Cerebrospinal fluid lysosomal enzymes and alpha-synuclein in Parkinson's disease. *Movement Disorders* 2014;29(8):1019–1027.

15. Sardi SP et al. Augmenting CNS glucocerebrosidase activity as a therapeutic strategy for parkinsonism and other Gaucher-related synucleinopathies. *Proceedings of the National Academy of Sciences* 2013;110(9):3537–3542.

16. Stojkovska I, Krainc D, Mazzulli JR. Molecular mechanisms of α-synuclein and GBA1 in Parkinson's disease. *Cell Tissue Res*. 2018; 373(1):51-60

17. Schapira AH, Olanow CW, Greenamyre JT, Bezard E. Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: future therapeutic perspectives. *Lancet*. 2014; 384(9942):545-55

18. Maegawa GHB et al. Identification and characterization of ambroxol as an enzyme enhancement agent for Gaucher disease. *J. Biol. Chem.* 2009;284(35):23502–23516.

19. Bendikov-Bar I, Maor G, Filocamo M, Horowitz M. Ambroxol as a pharmacological chaperone for mutant glucocerebrosidase. *Blood Cells Mol. Dis.* 2013;50(2):141–145.

20. Luan Z et al. The chaperone activity and toxicity of ambroxol on Gaucher cells and normal mice. *Brain Dev.* 2013;35(4):317–322.

21. Xilouri M, Brekk OR, Stefanis L. α-Synuclein and protein degradation systems: a reciprocal relationship.2013;47(2):537–551.

22. Yap TL et al. α-Synuclein Interacts with Glucocerebrosidase Providing a Molecular Link between Parkinson and Gaucher Diseases. *Journal of Biological Chemistry* 2011;286(32):28080–28088.

23. Fois G et al. A new role for an old drug: Ambroxol triggers lysosomal exocytosis via pH-dependent Ca2+ release from acidic Ca2+ stores. *Cell Calcium* 2015;58(6):628–637.

24. Maor G et al. Unfolded protein response in Gaucher disease: from human to Drosophila. *Orphanet J Rare Dis* 2013;8:140.

25. Babajani G, Tropak MB, Mahuran DJ, Kermode AR. Pharmacological chaperones facilitate the post-ER transport of recombinant N370S mutant β-glucocerebrosidase in plant cells: evidence that N370S is a folding mutant. *Mol. Genet. Metab.* 2012;106(3):323–329.

26. Narita A et al. Ambroxol chaperone therapy for neuronopathic Gaucher disease: A pilot study. Ann Clin Transl Neurol. 2016; 3(3):200-15.

27. Rocha EM et al. Sustained Systemic Glucocerebrosidase Inhibition Induces Brain α-Synuclein Aggregation, Microglia and Complement C1q Activation in Mice. *Antioxid. Redox Signal.* 2015;23(6):550–564.

28. Eusebi P et al. Diagnostic utility of cerebrospinal fluid alpha-synuclein in Parkinson's disease: A systematic review and meta-analysis. *Movement Disorders* 2017; 32(10):1389–1400.

29. Schirinzi T et al. CSF α-synuclein inversely correlates with non-motor symptoms in a cohort of PD patients. *Parkinsonism Relat. Disord.* 2019;61:203–206.

30. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *Journal of Neurology, Neurosurgery & Psychiatry* 1992;55(3):181–184.

31. Heywood W et al. The development of a peptide SRM-based tandem mass spectrometry assay for prenatal screening of Down syndrome. *J Proteomics* 2012;75(11):3248–3257.

32. Heywood WE et al. Identification of novel CSF biomarkers for neurodegeneration and their validation by a high-throughput multiplexed targeted proteomic assay. *Mol Neurodegener* 2015;10:64.

Table 1. Summary table of participant characteristics and results (Participants who completed study).

Data are mean (SD), apart from % male (percentage), Hoehn and Yahr stage (median). Clinical markers are recorded in the off state. SD – Standard deviation, *GBA1* – Glucocerebrosidase gene, . CSF – cerebrospinal fluid, GCase – glucocerebrosidase enzyme.

Participant characteristics				
	Age, yr	**male, %**	**Hoehn & Yahr stage (on)**	**Age at onset, yr**
All (n=18)	60.2 (9.7)	83.3	2	51.7 (11.5)
***GBA1* (n=8)**	56.1 (9.2)	87.5	2	44.5 (7.9)
Non *GBA1* (n=10)	63.4 (9.2)	80.0	2	58.9 (10.2)

Summary of results				
	Baseline	**Day 10**	**Day 90**	**Day 180**
Number of participants (n)	**Blood 18, CSF 17**	**Blood 18, CSF 17**	**Blood 18, CSF 17**	**Blood 18, CSF 17**
Ambroxol, ng/ml				
CSF	0 (0)	-	-	156 (53)
Blood (serum)	0 (0)	316 (196)	1084 (396)	1432 (570)
Glucocerebrosidase (GCase) enzyme activity, nmol/ml/hr (CSF) or nmol/mg/hr (blood)				
CSF	0.309 (0.153)	-	-	0.250 (0.142)
Blood (leucocytes)	11.0 (5.2)	12.8 (4.9)	13.1 (4.8)	12.0 (5.2)
Glucocerebrosidase (GCase) protein levels, pmol/L				
CSF	250 (47)	-	-	338 (104)
Alpha synuclein (A-SYN) , pg/ml				
CSF	383 (103)	-	-	433 (117)
Blood (serum)	20793 (9418)	19991 (7380)	24964 (9391)	23395 (9998)
Tau, pg/ml				
CSF	206 (59)			211 (63)
Blood (serum)	1.00 (0.25)	0.84 (0.24)	0.88 (0.22)	0.80 (0.24)
Glucosylceramide, pg/ml				
CSF	246 (83)	-	-	260 (80)
Movement disorders Society Unified Parkinson disease rating scale (MDS UPDRS)				
Part III	31.1 (14.5)		27.2 (10.7)	24.3 (12.1)
Total	62.6 (32.2)		57.7 (27.6)	53.9 (30.3)
Montreal Cognitive assessment (MoCa)				
	25.0 (4.8)	-	-	26.7 (4.0)
Non Motor symptoms scale (NMSS)				
	49.3 (36.1)	-	-	60.8 (38.6)
Non motor symptoms questionnaire (NMSQuest)				
	10.6 (6.0)	-	-	10.8 (6.0)
Mean Weight, kg				
	83 (17)	83 (17)	82 (17)	82 (17)
Mean arterial blood pressure, mmHg				
	90 (8)	88 (9)	90 (10)	90 (11)

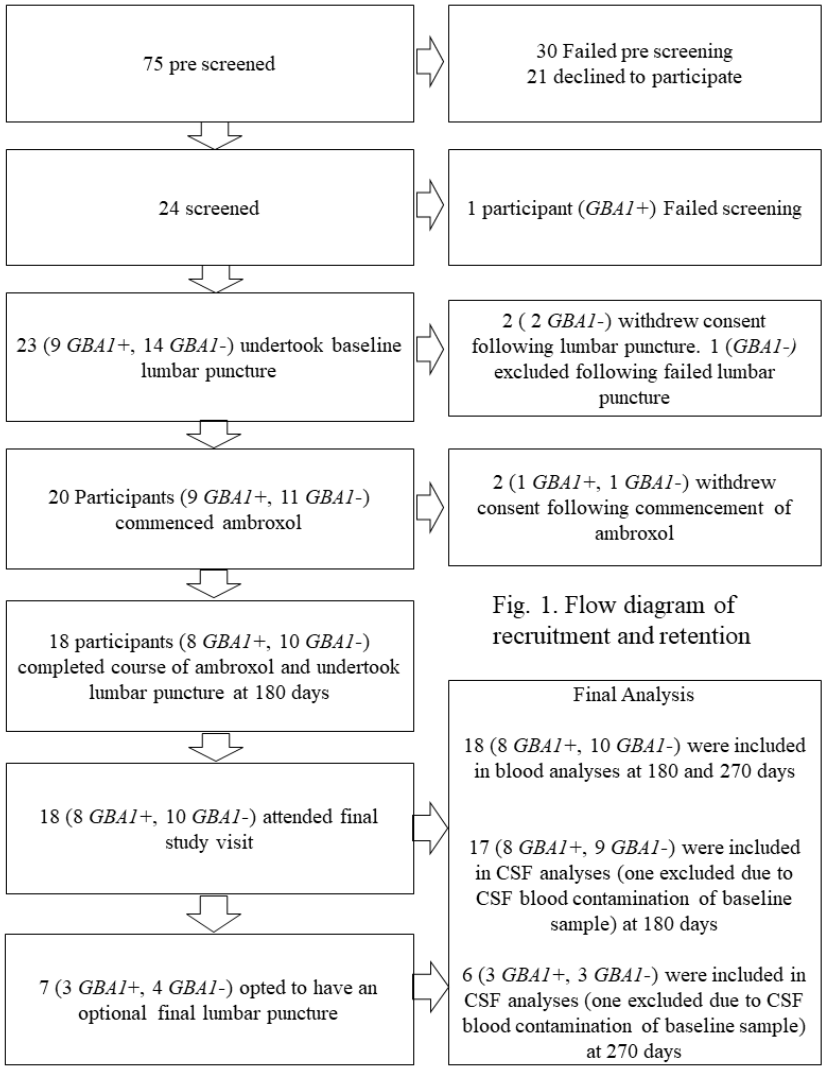

Fig. 1. Flow diagram of recruitment and retention

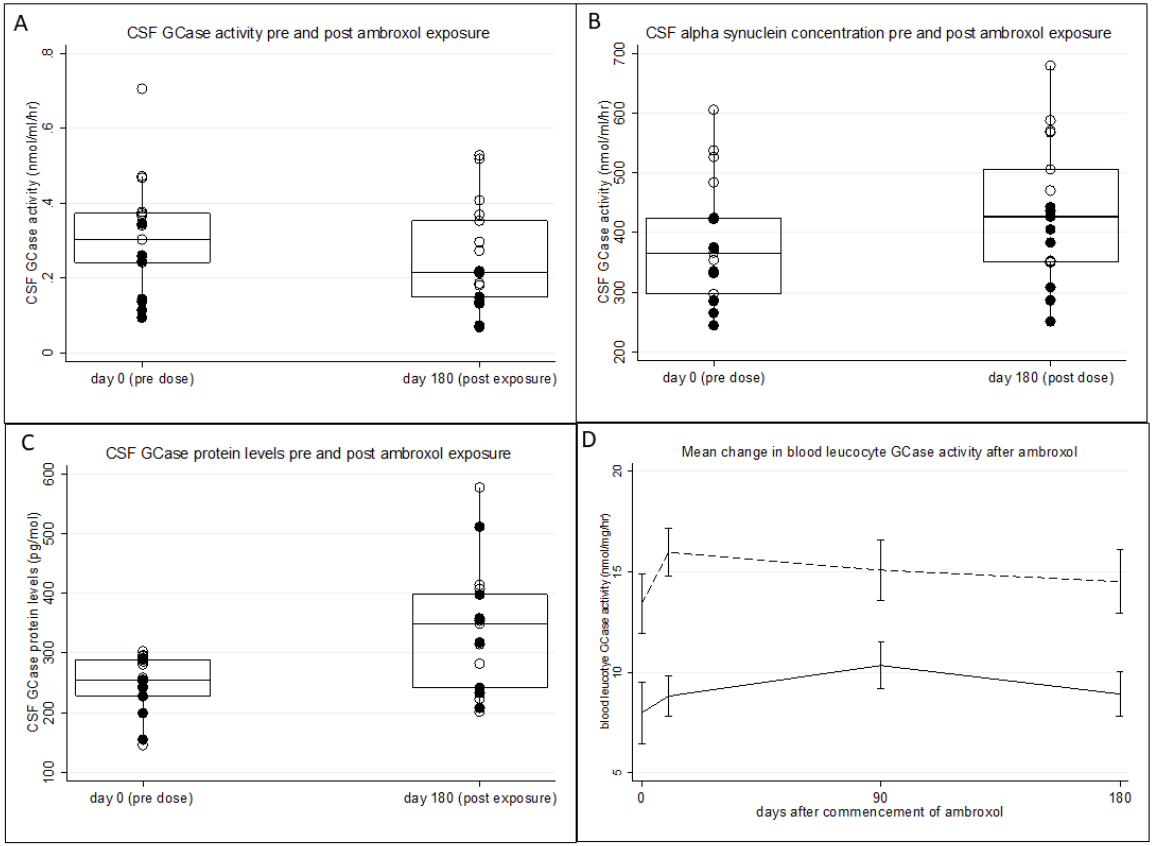

Fig. 2. Box plot (median and IQR) with superimposed data points at baseline and 180 days. *GBA1+* black circles. *GBA1-* white circles of (A) CSF GCase activity. Mean change 0.059 nmol/hr/ml (19% reduction), SE 0.026, 95%CI -0.115 to -0.002 two-sided paired t-test p=0.043) (B) CSF alpha synuclein concentration. Mean 50pg/ml change (13% increase), SE 17, 95%CI 14 to 87 (C) CSF GCase protein levels. Mean change 88 pmol/L mean (35% increase), SE 22 95%CI 40 to 137 (D) Mean change in blood leucocyte GCase activity following administration of ambroxol with error bars (standard error of the mean). *GBA1-* dashed line, *GBA1+* solid line. Mean change between baseline and 180 days 1.0 nmol/mg/hr (9% increase), SE 1.4, 95%CI -2.0 to 4.0. For CSF studies n=17 (8 *GBA1+*, 9 *GBA1-*). For blood studies n=18 (8 *GBA1+*, 10 *GBA1-*).

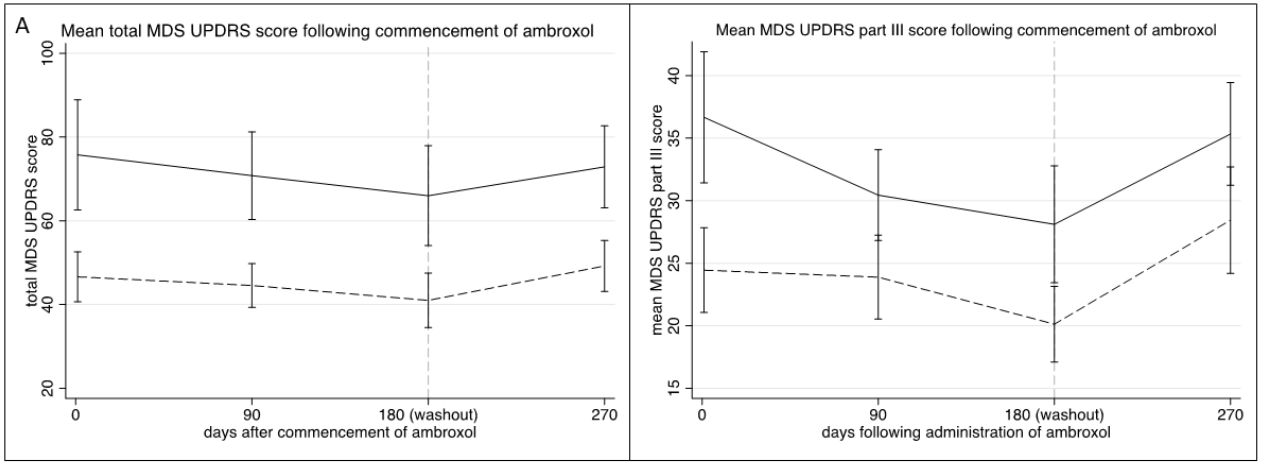

Fig. 3. (A) Mean total MDS UPDRS total score following ambroxol administration and washout. Between baseline and 180 days a mean fall of 8.7 (SE 3.0, 95%CI -15.3 to -2.2) (B) Mean MDS UPDRS part III score following ambroxol administration and washout a mean fall of 6.8 (SE 1.7, 95%CI -10.4 to -3.1). In both cases GBA1- dashed line, GBA+ solid line. Error bars standard error of the mean. n=18 (8 *GBA1+,* 10 *GBA1-*).

Supplementary section 1: Summary of product characteristics of ambroxol

SUMMARY OF PRODUCT CHARACTERISTICS

1. NAME OF THE MEDICINAL PRODUCT

Ambrosan 60 mg tablets

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

Each tablet contains 60 mg of ambroxol hydrochloride. Excipients with known effect: 109 mg of lactose monohydrate. For the full list of excipients, see section 6.1.

3. PHARMACEUTICAL FORM

T ablet

Almost white round cross-scored tablets, diameter 9.5 mm. The tablet can be divided into 4 equal doses.

4. CLINICAL PARTICULARS

4.1 Therapeutic indications

Mucolytic therapy in acute and chronic bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport (inflammations of upper and lower respiratory airways, infection diseases of respiratory airways), inflammatory rhinopharyngeal diseases.
The product can be used in children from 5 years, adolescents and adults.

4.2 Posology and method of administration

Adults and adolescents over 12 years: 1 tablet 2 times a day. This dosing regimen is suitable for the treatment of acute respiratory disease and initial treatment of chronic conditions for up to 14 days.
Children 5–12 years: 1⁄4 of tablet 2–3 times a day.

The tablets should be taken after meal and rinsed down with sufficient amount of liquid. Liquid consumption enhances the mucolytic effect of ambroxol.

Treatment duration with Ambrosan 60 mg tablets is given individually depending on the concrete indication and type of disease.

4.3 Contraindications

Hypersensitivity to the active substance or to any of the excipients listed in section 6.1.

4.4 Special warnings and precautions for use

In the presence of impaired renal function or severe hepathopathy ambroxol should be administered with precaution. Accumulation of the metabolites of ambroxol generated in the liver can be expected in the presence of severe renal insufficiency.
There have been very few reports of severe skin lesions such as Stevens-Johnson syndrome and Lyell's syndrome in temporal association with the administration of expectorants such as ambroxol. Mostly these could be explained by the severity of the patient's underlying disease and/or concomitant medication. In addition during the early phase of a Stevens-Johnson syndrome or Lyell's syndrome a patient can first experience non-specific influenza-like prodromes like e.g. fever, aching body, rhinitis, cough and sore throat. Misled by these non-specific influenza-like prodromes it is possible that a

symptomatic treatment is started with a cough and cold medication. Therefore, if new skin or mucosal lesions occur, medical advice should be sought immediately and treatment with ambroxol discontinued as a precaution.
This medicinal product contains lactose. Patients with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose malabsorption should not take this medicine.

4.5 Interaction with other medicinal products and other forms of interaction

Concomitant administration of ambroxol and antibiotics (amoxicillin, cefuroxime, erythromycin) results in increased antibiotic concentrations in bronchopulmonary secretion and sputum. This effect could be used therapeutically.

No clinically relevant unfavourable interaction with other medications has been reported.

4.6 Pregnancy and lactation

Ambroxol crosses the placental barrier. Animal studies do not indicate direct or indirect harmful effects with respect to pregnancy, embryonal/foetal development, parturition or postnatal development. Extensive clinical experience after the 28th week of pregnancy has shown no evidence of harmful effects on the foetus. Nonetheless, the usual precautions regarding the use of drugs during pregnancy should be observed. Especially during the first trimester, the use of medicines containing ambroxol is not recommended.

Ambroxol is excreted in breast milk. Although unfavourable effects on breastfed infants would not be expected, Ambrosan 60 mg tablets is not recommended for use in nursing mothers.

4.7 Effects on ability to drive and use machines

There is no evidence for an effect on the ability to drive and use machines.

Studies on the effects on the ability to drive and use machines have not been performed.

4.8 Undesirable effects

Frequency of undesirable effects is defined using the following convention: Very common (≥1/10)

Common (≥1/100 to <1/10)

Uncommon (≥1/1,000 to <1/100)

Rare (≥1/10,000 to <1/1,000)

Very rare (<1/10,000)

Not known (cannot be estimated from the available data)

Ambrosan 60 mg tablets is usually well tolerated. During treatment, it can occur:

Immune system disorders, Skin and subcutaneous tissue disorders:

Rare: Rash, urticaria.

Not known: Anaphylactic reactions including anaphylactic shock, angioedema, pruritus and other hypersensitivity.

Gastrointestinal disorders:

Common: Nausea.

Uncommon: Dyspepsia, vomiting, diarrhoea and abdominal pain.

4.9 Overdose

No specific overdose symptoms have been reported in man to date.
Based on accidental overdose and/or medication error reports the observed symptoms are consistent with the known side effects of ambroxol at recommended doses and may need symptomatic treatment.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: expectorants, mucolytics. ATC code: R05CB06.

Preclinically ambroxol, the active substance of Ambrosan 60 mg tablets, has been shown to increase secretion of mucus in respiratory system and modify its viscosity. It enhances pulmonary surfactant production and stimulates ciliary activity. These actions result in improved mucus flow and transport (mucociliary clearance). Enhancement of mucous secretion and mucociliary clearance facilitates expectoration and eases cough. Ambroxol decreases bronchial hyperreactivity, increases secretion of IgA in bronchial mucus and shows antioxidant activity.

5.2 Pharmacokinetic properties

Absorption of ambroxol after oral use is rapid and nearly complete, with dose linearity in the therapeutic range. Maximum plasma levels are reached within 0.5 to 3 hours.
In the therapeutic range plasma protein binding is approximately 90%. Free fraction of ambroxol is distributed from blood to tissues relatively well, with the highest concentration found in the lungs. Plasma half-life is 7 to 12 hours, accumulation has not been shown.

About 30% of the administered oral dose is eliminated via first pass. Ambroxol is metabolised primarily in the liver by conjugation. About 90% of the dose is eliminated by kidneys.

5.3 Preclinical safety data

Ambroxol has a low index for acute toxicity. In repeat-dose studies, oral doses of 150 mg/kg/day (mouse, 4 weeks), 50 mg/kg/day (rat, 52 and 78 weeks), 40 mg/kg/day (rabbit, 26 weeks) and 10 mg/kg/day (dog, 52 weeks) were the no-observed adverse effect level (NOAEL). No toxicological target organs were detected. Four week intravenous toxicity studies with ambroxol in rats (4, 16 and 64 mg/kg/day) and in dogs (45, 90 and 120 mg/kg/day (infusion 3 h/day)) showed no severe local and systemic toxicity including histopathology. All adverse effects were reversible.

Ambroxol was neither embryotoxic nor teratogenic when tested at oral doses up to 3000 mg/kg/day in rats and up to 200 mg/kg/day in rabbits. The fertility of male and female rats was not affected up to 500 mg/kg/day. The NOAEL in the peri- and post-natal development study was 50 mg/kg/day.
At 500 mg/kg/day, ambroxol was slightly toxic for dams and pups, as shown by a retarded body-weight development and reduced litter size.

Genotoxicity studies *in vitro* (Ames and chromosome aberration test) and *in vivo* (mouse micronucleus test) did not reveal any mutagenic potential of ambroxol.
Ambroxol did not show any tumorigenic potential in carcinogenicity studies in mice (50, 200 and 800 mg/kg/day) and rats (65, 250 and 1000 mg/kg/day) when treated with a dietary admixture for 105 and 116 weeks, respectively.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Lactose monohydrate, granulated microcrystalline cellulose, copovidone, magnesium stearate. **6.2**

Incompatibilities

Not applicable.

6.3 Shelf life

4 years

6.4 Special precautions for storage

This medicinal product does not require any special storage conditions.

6.5 Nature and contents of container

Transparent PVC/PVdC/Al blister, carton. Pack size: 20, 30, 60, 100 or 500 tablets. Not all pack sizes may be marketed.

6.6 Special precautions for disposal and other handling

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

7. MARKETING AUTHORISATION HOLDER

PRO.MED.CS Praha a.s., Telčská 1, 140 00 Praha 4, Czech Republic

8. MARKETING AUTHORISATION NUMBER(S)

52/093/13-C

Supplementary materials section 2: Influence of change in PD meds on MDS UPDRS scores

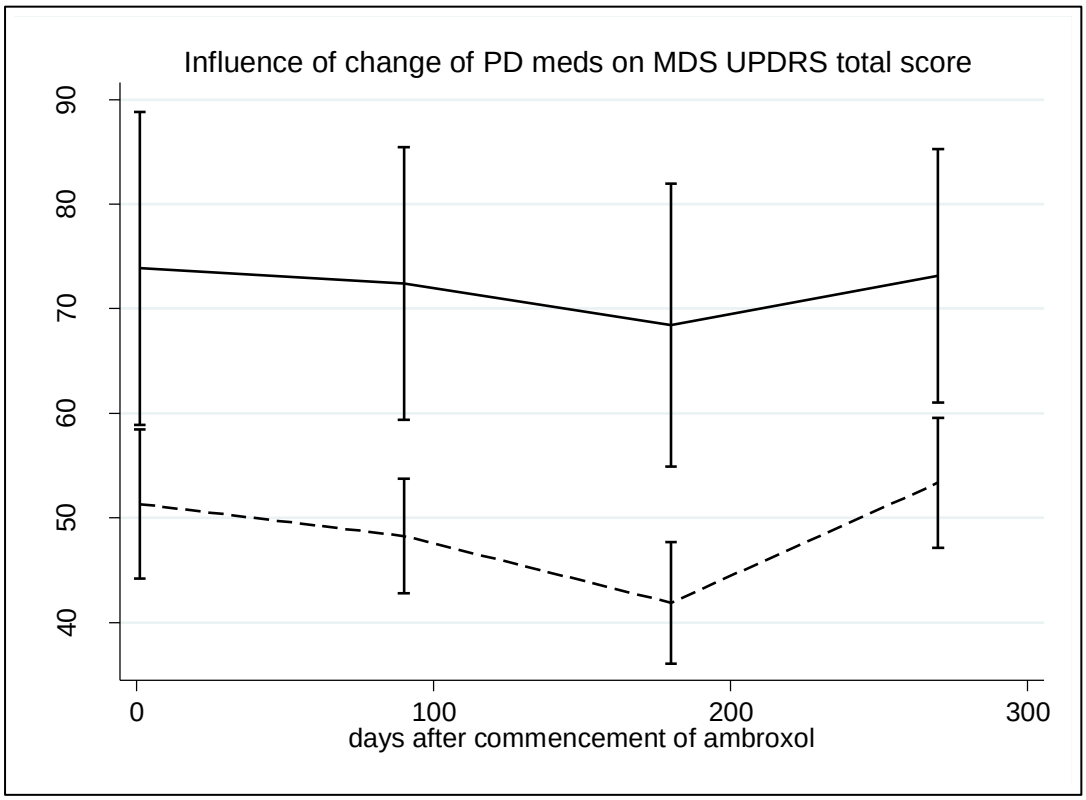

Figure S4. MDS UPDRS total scores of those who did (solid) or did not (dashed) change PD meds during the study

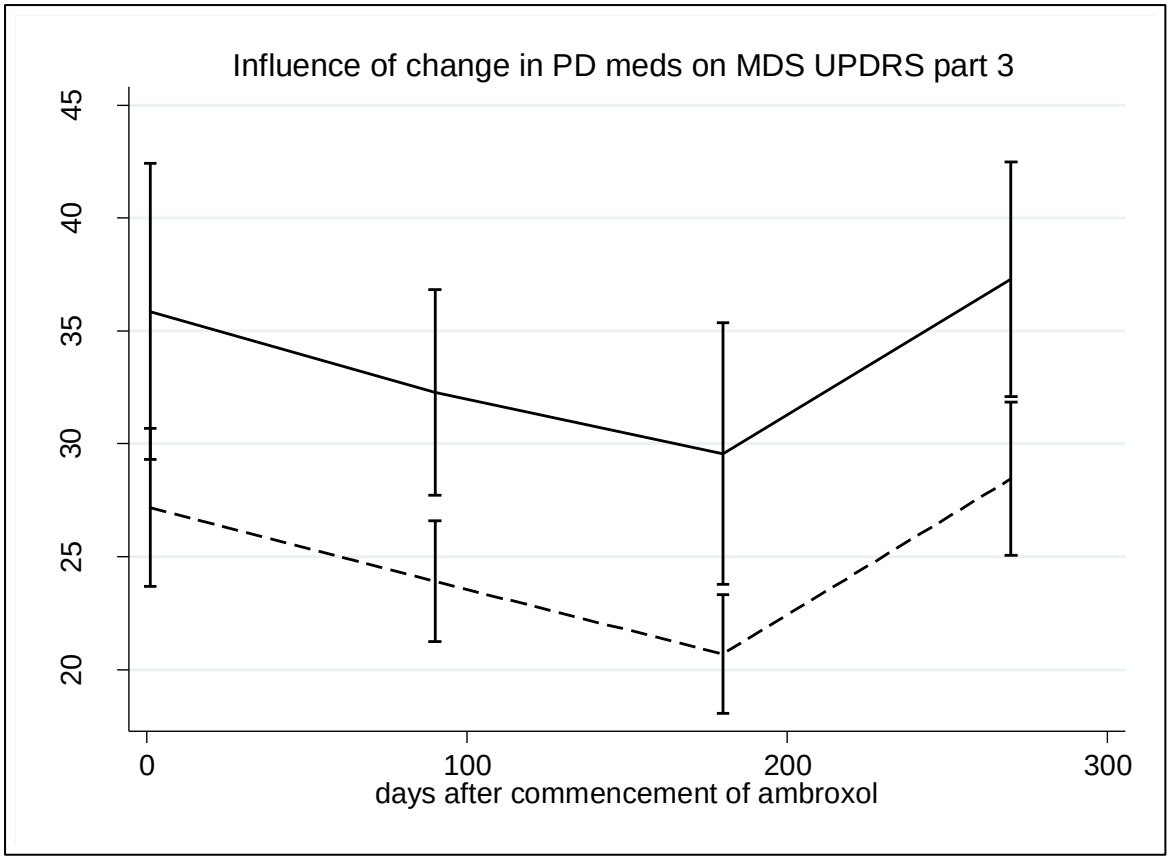

Figure S5. MDS UPDRS part 3 scores of those who did (solid) or did not (dashed) change PD meds during study

Supplementary section 3: Inclusion and exclusion criteria

1. Male or female;
2. Age ≥ 40 and ≤ 80 years of age;
3. Confirmed diagnosis of Parkinson disease at any time; and Hoehn and Yahr criteria, confirmed staged between I – III, inclusive;
4. Able and willing to provide informed consent prior to any study related assessments and procedures at screening visit 1;
5. Capable of complying with all study procedures, including fasting lumbar puncture;
6. Willing to provide a blood sample for screening genomic for Parkinson Disease related DNA analysis and/or consent to Investigators obtaining and using participants previous DNA results if applicable;
7. Willing and able to self-administer oral ambroxol medication, from day 1 to 186 (at 60 mg TID (day 1-7), 120 mg TID (day 8-14), 180 mg TID (day 15-21), 300 mg TID (day 22-28) and 420 mg TID (day 29-186));
8. Able to travel to the participating study site;
9. A female participant is eligible to participate if she is of:

- Non-childbearing potential defined as pre-menopausal females with a documented tubal ligation or hysterectomy; or postmenopausal defined as 12 consecutive months of spontaneous amenorrhea, at least 6 weeks post-surgical bilateral oophorectomy (with or without hysterectomy) or post tubal ligation. In questionable cases, menopausal status will be confirmed by demonstrating levels of follicle stimulating hormone (FSH) 25.8 – 134.8 IU/L and oestradiol < 201 pmol/l at entry.
- Women of child-bearing potential must use accepted contraceptive methods (listed below), and must have a negative serum at screening visit 1 and urine pregnancy tests

at subsequent visits if applicable. An additional pregnancy test will be performed, and results obtained, prior to administration of the first dose of ambroxol.

Accepted contraception methods:

• True abstinence: When this is in line with the preferred and usual lifestyle of the participant. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception).

Contraceptive Methods with a Failure Rate of < 1%:

- Oral contraceptive, either combined or progestogen alone;
- Injectable progestogen;
- Implants of levonorgestrel;
- Estrogenic vaginal ring;
- Percutaneous contraceptive patches;
- Intrauterine device (IUD) or intrauterine system (IUS) that meets the <1% failure rate as stated in the product label;

Please note:

- All male and female participants of child bearing potential must agree with their partners to use double-barrier birth control or abstinence while participating in the study and for 2 weeks following the last dose of the study drug.
- Participants may continue to take PD medications including glutamate antagonists, anticholinergics, dopamine agonists, Levodopa (L-DOPA and decarboxylase (DDC) inhibitor), Monoamine oxidase B (MAO-B) inhibitors catechol-O-methyltransferase (COMT) inhibitors, beta blockers, selective serotonin uptake inhibitors (SSRIS), tricyclic antidepressants (TCAs) and indomethacin.

Exclusion Criteria:

Participants are excluded from participating in this study if 1 or more of the following criteria are met:

1. Current treatment with anticoagulants (e.g. warfarin) that might preclude safe completion of the lumbar puncture and in the opinion of the Investigator;
2. Current use of investigational medicinal product or participation in another interventional clinical trial or who have done so within 30 days prior to the first dose in the current study;
3. Exposure to more than three investigational medicinal products within 12 months prior to the first dose in the current study;
4. Confirmed dysphagia that would preclude self-administration of ambroxol up to 7 tablets TID for the duration of day 1 to day 186);
5. Significant known lower spinal malformations or other spinal abnormalities that would preclude lumbar puncture;
6. History of known sensitivity to the study medication,ambroxol or its excipients (lactose monohydrate, granulated microcrystalline cellulose, copovidone and magnesium stearate) in the opinion of the investigator that contraindicates their participation;
7. History of known rare hereditary disorders of galactose intolerance, Lapp lactase deficiency or glucose-galactose malabsorption;
8. Evidence or history of hypersensitivity to lidocaine or its derivatives;
9. History of drug abuse or alcoholism in the opinion of the Investigator that would preclude participation in the study;

10. Donation of blood (one unit or 350 ml) within three months prior to receiving the first dose of the study drug;
11. Pregnant or breastfeeding;
12. All participants of child bearing potential in the opinion of the Investigator that would preclude participation in the study and who do not agree to use double-barrier birth control or abstinence while participating in the study and for two weeks following the last dose of study drug;
13. Any clinically significant or unstable medical or surgical condition that in the opinion of the PI or PI-delegated clinician may put the participant at risk when participating in the study or may influence the results of the study or affect the participant's ability to take part in the study, as determined by medical history, physical examinations, electrocardiogram (ECG), or laboratory tests. Such conditions may include:
    1. Impaired renal function
    2. Moderate/Severe hepatic impairment
    3. A major cardiovascular event (e.g. myocardial infarction, acute coronary syndrome, decompensated congestive heart failure, pulmonary embolism, coronary revascularisation that occurred within 6 months prior to the screening visit.

Supplementary section 4: Distributions of primary outcome

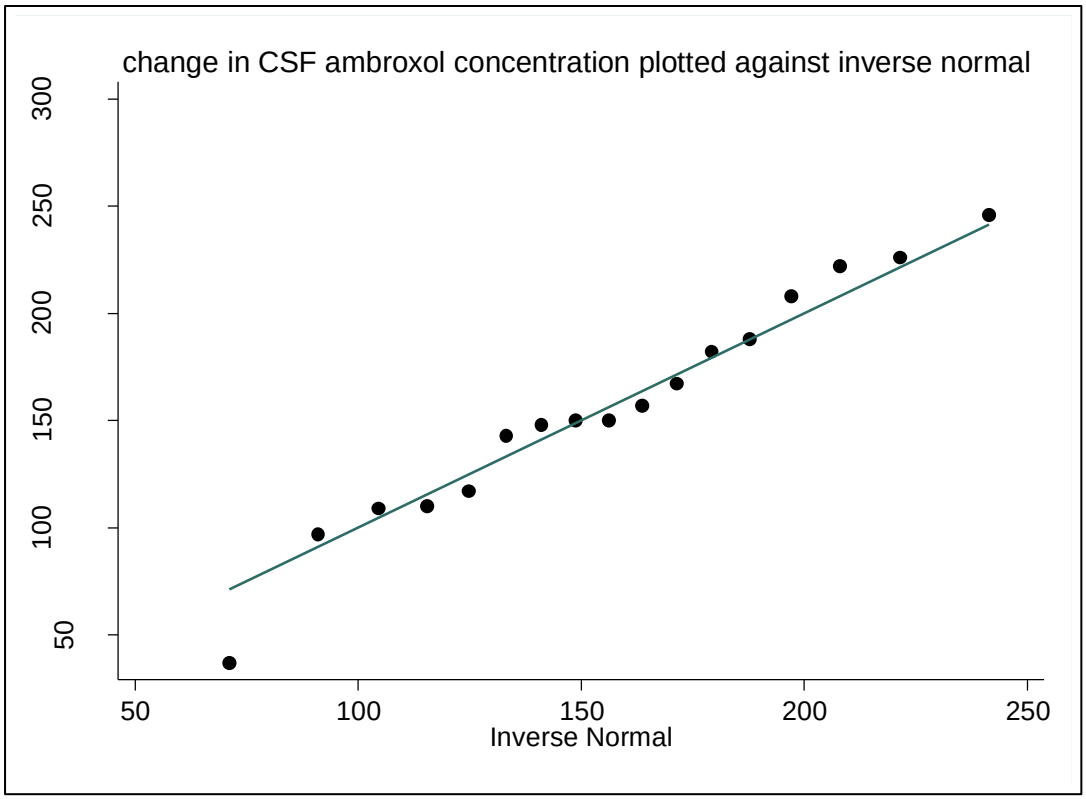

Figure S1. Change in CSF ambroxol concentration plotted against inverse of normal.

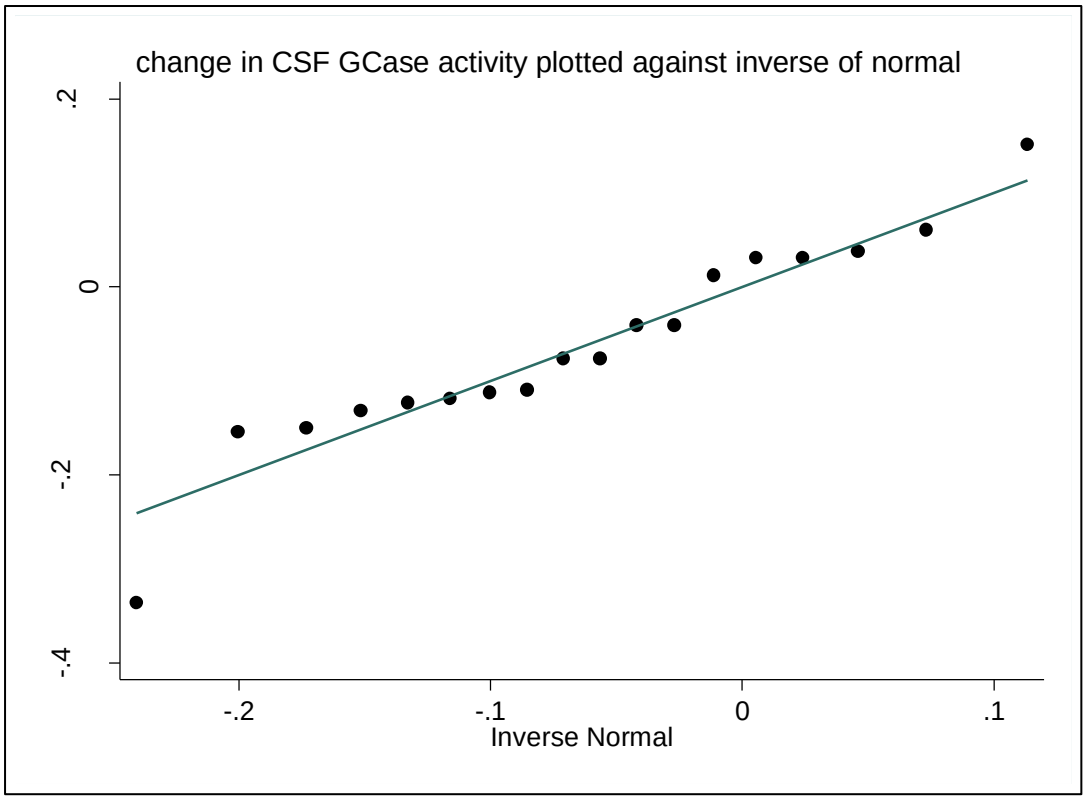

Figure S2. Change in CSF GCase activity plotted against inverse of normal.

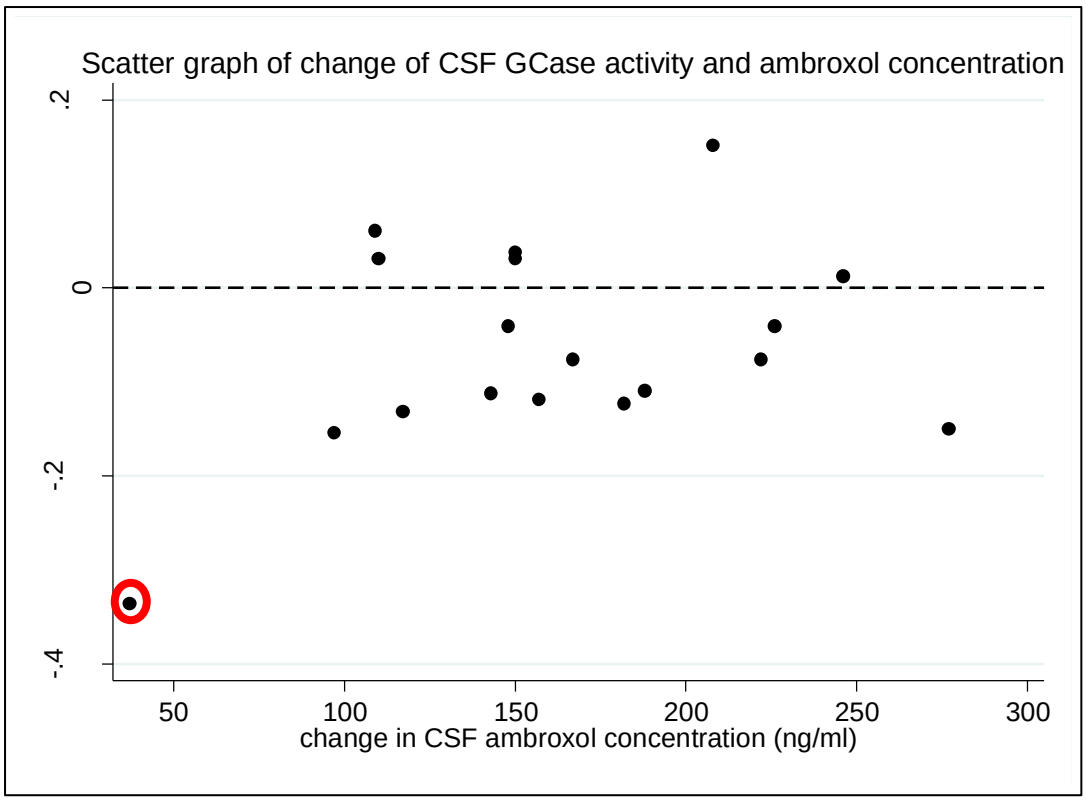

Figure S3. Scatter plot of change in CSF GCase activity against change in CSF ambroxol. Dashed line represents no change in CSF GCase activity.

We considered the distribution of samples for the primary outcomes. In the case of both CSF GCase and CSF ambroxol the data appeared to be normally distributed (see supplementary figures 1 and 2). We identified one outlier who had marked fall in CSF GCase activity with a minimal rise in CSF ambroxol concentration (circled in red in supplementary figure 3). We considered whether this could be due to blood contamination, however at both timepoints no red blood cells were seen on CSF microscopy. In the absence of a convincing scientific rationale to exclude the subject, they were included in both primary and secondary analyses.

Supplementary section 5 – study protocol

Please see attached file.

Table S1. Summary of study visits

Study Period	**Screening Phase (Pre-Treatment Phase)**		**Treatment Phase**								**Follow Up Assessment and washout/Final visit (End of Study/Early Termination)**
Visit	**Visit 1 (at hospital)**	**Visit 2[t] (at hospital)**	**Visit 3 (at hospital) Dose Escalation 1[t]**	**Dose Escalation 2[t]**	**Visit 4[t] (at hospital)**	**Dose Escalation 3[t]**	**Dose Escalation 4[t]**	**Dose Escalation 5[t]**	**Visit 5 (at hospital) Month 3**	**Visit 6 (at hospital) Month 6**	**Visit 7 (at hospital) Month 9**
Dose Escalation Day			**1**	**8**		**15**	**22**	**29**	**93**	**186**	**279**
Visit Window			(within 60 days of screening Visit 1 & 2)		*(within 3 days after dose escalation 2)*				(+/- 14 days)	(+/- 14 days)	**Early termination visit (+/- 30 days)
Day	-60	-60	1	8-14		15-21	22-28	29-186	93	186	279
Informed consent	X										
Medical History	X										
Physical and neurological examinations	X	X	X		X				X	X	X
MDS-UPDRS[c]		X	X[a]						X	X	X
Screening Inclusion/Exclusion criteria		X									
Screening Genotyping (*GBA* & *LRRK2*) if applicable	X										
Vital Signs (HR, BP RR & Temperature)	X	X	X[b,b*,d*]		X				X	X	X
Height and Weight[3,c]	X	X	X		X				X	X	X
ECG	X	X	X[b]							X	X
Lumbar Puncture[c] (Up to 20 mL)		X								X	X*
Pregnancy Test[1***] (if applicable)	X	X	X[a]		X				X	X	X
Routine blood collection/panel[2,***]	X	X			X				X	X	X

Study Period	**Screening Phase (Pre-Treatment Phase)**		**Treatment Phase**								**Follow Up Assessment and washout/Final visit (End of Study/Early Termination)**
Visit	**Visit 1 (at hospital)**	**Visit 2[t] (at hospital)**	**Visit 3 (at hospital) Dose Escalation 1[t]**	**Dose Escalation 2[t]**	**Visit 4[t] (at hospital)**	**Dose Escalation 3[t]**	**Dose Escalation 4[t]**	**Dose Escalation 5[t]**	**Visit 5 (at hospital) Month 3**	**Visit 6 (at hospital) Month 6**	**Visit 7 (at hospital) Month 9**
Dose Escalation Day			**1**	**8**		**15**	**22**	**29**	**93**	**186**	**279**
Visit Window			(within 60 days of screening Visit 1 & 2)		*(within 3 days after dose escalation 2)*				(+/- 14 days)	(+/- 14 days)	**Early termination visit (+/- 30 days)
Day	-60	-60	1	8-14		15-21	22-28	29-186	93	186	279
Blood enzyme activity & ELISA antibody panels***	X				X				X	X	X

CSF enzyme activity & ELISA antibody panels***		X								X	X
Blood ambroxol collection***	X				X				X	X	
CSF ambroxol collection***		X								X	X
Urine Collection[c] (Up to 50 mL)***			X		X				X	X	X
Dosing, day 1 to 186 (inclusive)d (dosing, on-site, if applicable)			X		X				X	X	
Dispensing 3 months' supply IMP			X						X		
Collecting IMP packaging (pill count)									X	X	X
Cognitive/Questionnaire – MoCA[c], NMSS & NMS			X							X	
IMP compliance and dosing[d] instructions			X	X	X	X	X	X			
Adverse Events Review	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medication Review	X	X	X	X	X	X	X	X	X	X	X

(table continued)

Table S2. Summary table of biochemical results including *GBA1* and non *GBA1* subgroups. (Participants who completed study).
Data are mean (SD). CSF – cerebrospinal fluid, SD – standard deviation, GCase – glucocerebrosidase enzyme

		Baseline	Day 30	Day 90	Day 180	Day 270 (washout)
Number of participants (n)		**Blood 18, CSF 17**	**Blood 18, CSF 17**	**Blood 18, CSF 17**	**Blood 18, CSF 17**	**Blood 18, CSF 6**
Ambroxol, ng/ml						
CSF	**All**	0 (0)	-	-	156 (53)	0(0)
	GBA1	0 (0)	-	-	173 (44)	0(0)
	non GBA1	0 (0)	-	-	141 (59)	0(0)
Blood (serum)	**All**	0 (0)	316 (196)	1084 (396)	1432 (570)	0(0)
	GBA1	0 (0)	348 (100)	1090 (223)	1568 (557)	0(0)
	non GBA1	0 (0)	299 (200)	1080 (507)	1324 (586)	0(0)
Glucocerebrosidase (GCase) enzyme activity, nmol/ml/hr (CSF) or nmol/mg/hr (blood)						
CSF	**All**	0.309 (0.153)	-	-	0.250 (0.142)	0.223 (0.131)
	GBA1	0.200 (0.090)	-	-	0.142 (0.055)	0.128 (0.044)
	non GBA1	0.405 (0.133)	-	-	0.345 (0.126)	0.318 (0.118)
Blood (leucocytes)	**All**	11.0 (5.2)	12.8 (4.9)	13.1 (4.8)	12.0 (5.2)	9.8 (4.5)
	GBA1	8.0 (4.3)	8.8 (2.9)	10.3 (3.2)	8.9 (3.1)	9.0 (3.1)
	non GBA1	13.4 (4.8)	16.0 (3.8)	15.6 (4.8)	14.5 (5.0)	10.6 (5.6)
Glucocerebrosidase (GCase) protein levels, pmol/L						
CSF	**All**	250 (47)	-	-	338 (104)	394 (66)
	GBA1	233 (41)	-	-	328 (100)	388 (101)
	non GBA1	264 (50)	-	-	347 (113)	399 (21)
Alpha synuclein (A-SYN) , pg/ml						
CSF	**All**	383 (103)	-	-	433 (117)	372 (46)
	GBA1	336 (68)	-	-	367 (74)	401 (23)
	non GBA1	425 (115)	-	-	492 (120)	342 (46)
Blood (serum)	**All**	20793 (9418)	19991 (7380)	24964 (9391)	23395 (9998)	22266 (12423)
	GBA1	22209 (8373)	19638 (8374)	26867 (12002)	23253 (10689)	21623 (10295)
	non GBA1	19660 (10472)	20273 (6940)	23441(6984)	23509 (9997)	22779 (14437)
Tau, pg/ml						
CSF	**All**	206 (59)	-	-	211 (63)	207 (53)
	GBA1	179 (43)	-	-	186 (51)	227 (59)
	non GBA1	230 (62)	-	-	233 (66)	188 (49)
Blood (serum)	**All**	1.00 (0.25)	0.84 (0.24)	0.88 (0.22)	0.80 (0.24)	0.66 (0.23)
	GBA1	0.96 (0.24)	0.93 (0.29)	0.88 (0.21)	0.79 (0.26)	0.64 (0.28)
	non GBA1	1.04 (0.26)	0.76 (0.17)	0.88 (0.24)	0.81 (0.24)	0.68 (0.20)
Glucosylceramide, pg/ml						
CSF	**All**	246 (83)	-	-	260 (80)	256 (103)
	GBA1	276 (65)	-	-	274 (53)	333 (49)
	non GBA1	219 (91)	-	-	247 (99)	178 (79)

Table S3. Summary table of clinical results including *GBA1* and non *GBA1* subgroups. (Participants who completed study).
Data are mean (SD). Clinical markers markers are recorded in the off state. MDS UPDRS – Movement disorders society unified Parkinson disease ratings scale, MoCa – Montreal cognitive assessment, NMSS – Non motor symptoms scale, NMSQuest – Non motor symptoms questionnaire, SD – Standard deviation

	Baseline	**Day 10**	**Day 90**	**Day 180**	**Day 270 (washout)**
Number of participants (n)	18	18	18	18	18
Movement disorders Society Unified Parkinson disease rating scale (MDS UPDRS) part I					
All	10.3 (8.1)		10.5 (7.8)	10.1 (7.1)	9.4 (6.8)
GBA1	15.2 (9.7)		16.0 (8.6)	14.8 (7.0)	13.6 (7.8)
non *GBA1*	6.3 (4.6)		6.1 (3.2)	6.4 (4.9)	6.1 (3.5)
Movement disorders Society Unified Parkinson disease rating scale (MDS UPDRS) part II					
All	15.2 (10.2)		14.9 (9.8)	15.6 (10.6)	14.4 (10.1)
GBA1	22.5 (10.5)		22.1 (10.0)	23.5 (9.1)	21.6 (10.3)
non *GBA1*	9.4 (5.1)		9.1 (4.7)	8.6 (5.7)	8.6 (5.2)
Movement disorders Society Unified Parkinson disease rating scale (MDS UPDRS) part III					
All	31.1 (14.5)		27.2 (10.7)	24.3 (12.1)	31.9 (12.7)
GBA1	38.4 (15.9)		31.5 (11.1)	30.3 (13.1)	37.4 (11.4)
non *GBA1*	24.3 (9.6)		23.7 (9.5)	19.0 (8.7)	27.5 (12.4)_
Movement disorders Society Unified Parkinson disease rating scale (MDS UPDRS) part IV					
All	4.9 (4.0)		5.1 (4.5)	4.3 (3.6)	5.3 (3.2)
GBA1	5.6 (5.7)		6.4 (5.8)	6.0 (4.2)	6.1 (3.6)
non *GBA1*	4.4 (2.4)		4.1 (3.2)	3.1 (2.7)	4.7 (2.9)
Movement disorders Society Unified Parkinson disease rating scale (MDS UPDRS) total					
All	62.6 (32.2)		57.7 (27.6)	53.9 (30.3)	61.1 (26.7)
GBA1	82.4 (34.6)		76.0 (29.0)	73.8 (27.4)	78.8 (25.1)
non *GBA1*	44.6 (16.8)		43.0 (15.5)	37.6 (19.9)	46.9 (18.7)
Montreal Cognitive assessment (MoCa)					
All	25.0 (4.8)	-	-	26.7 (4.0)	-
GBA1	23.6 (6.2)	-	-	25.6 (5.2)	-
non *GBA1*	26.1 (3.2)	-	-	27.5 (2.7)	-
Non Motor symptoms scale (NMSS)					
All	49.3 (36.1)			60.8 (38.6)	-
GBA1	77.1 (38.0)			85.8 (37.9)	-
non *GBA1*	27.0 (10.4)			40.8 (26.2)	-
Non motor symptoms questionnaire (NMSQuest)					
All	10.6 (6.0)	-	-	10.8 (6.0)	-
GBA1	14.2 95.8)	-	-	14.3 (5.0)	-
non *GBA1*	7.6 (4.5)	-	-	7.9 (5.4)	-
Safety information					
Weight, kg (all)	83 (17)	83 (17)	82 (17)	82 (17)	83 (15)
Mean arterial blood pressure, mmHg (all)	90 (8)	88 (9)	90 (10)	90 (11)	95 (9)

Table S4. Summary table of CSF biochemical results including *GBA1* and non *GBA1* subgroups in those who underwent 3 lumbar punctures.
Data are mean (SD). CSF – cerebrospinal fluid, SD – standard deviation, GCase – glucocerebrosidase enzyme

		Baseline	Day 180	Day 270 (washout)
Number of participants (n)		**6**	**6**	**6**
Ambroxol, ng/ml				
CSF	**All**	0 (0)	191 (39)	0 (0)
	GBA1	0 (0)	187 (52)	0 (0)
	non GBA1	0 (0)	196 (34)	0 (0)
Glucocerebrosidase (GCase) enzyme activity, nmol/ml/hr (CSF) or nmol/mg/hr (blood)				
CSF	**All**	0.292 (0.135)	0.268 (0.159)	0.223 (0.130)
	GBA1	0.180 (0.068)	0.146 (0.075)	0.128 (0.044)
	non GBA1	0.403 (0.059)	0.389 (0.114)	0.318 (0.118)
Glucocerebrosidase (GCase) protein levels, pmol/L				
CSF	**All**	259 (50)	337 (102)	382 (67)
	GBA1	234 (38)	328 (101)	388 (102)
	non GBA1	277 (51)	345 (107)	378 (46)
Alpha synuclein (A-SYN) , pg/ml				
CSF	**All**	351 (73)	378 (65)	372 (46)
	GBA1	364 (103)	365 (72)	401 (23)
	non GBA1	338 (46)	391 (69)	342 (46)
Tau, pg/ml				
CSF	**All**	184 (50)	190 (37)	207 (53)
	GBA1	192 (49)	204 (34)	227 (59)
	non GBA1	176 (39)	175 (41)	188 (48)
Glucosylceramide, pg/ml				
CSF	**All**	224 (76)	243 (71)	255 (103)
	GBA1	283 (43)	287 (21)	333 (50)
	non GBA1	166 (48)	199 (78)	179 (78)

Table S5 – List of recorded adverse events

Adverse event description	serious?	Related to ambroxol?	severity	Expected?	resolved?	occurred whilst taking ambroxol?
Slightly raised serum urea	no	not related	mild	no	resolved	no
Slightly raised serum urate	no	not related	mild	no	resolved	no
Mild lymphopaenia	no	not related	mild	no	resolved	no
Ejection systolic murmur	no	not related	mild	no	resolved	no
Headache	no	not related	mild	no	resolved	no
Muscle stiffness in back	no	not related	mild	no	resolved	no
Fall out of bed	no	unlikely	mild	no	resolved	yes
Fall forward on to abdomen	no	unlikely	mild	no	resolved	yes
Bruise on left rib	no	unlikely	mild	no	resolved	yes
Unsteady on feet	no	unlikely	mild	no	unresolved	yes
Fall	no	unlikely	mild	no	resolved	yes
Cut on left thigh	no	unlikely	mild	no	resolved	yes
Productive cough	no	not related	mild	no	resolved	yes
Arguing with wife in sleep	no	not related	mild	no	resolved	yes
Fall	no	unlikely	mild	no	resolved	yes
Bruise Right arm	no	unlikely	mild	no	resolved	yes
Slurred speech	no	unlikely	mild	no	resolved	yes
Memory loss	no	unlikely	mild	no	resolved	yes
Fall	no	unlikely	mild	no	resolved	yes
Urinary incontinence	no	unlikely	mild	no	resolved	yes
Injury when paving fell on leg	no	not related	mild	no	resolved	yes
Chest infection	no	not related	mild	no	resolved	no
Headache post-LP	no	not related	severe	no	resolved	no
Nausea	no	not related	mild	no	resolved	no
Vomiting x1	no	not related	mild	no	resolved	no
Urinary Frequency	no	not related	mild	no	unresolved	no
Pain in Back	no	not related	mild	yes	resolved	no
Mitral valve leak	no	not related	mild	no	unresolved	yes
Shortness of breath	no	not related	mild	no	unresolved	yes
Neck Stiffness	no	not related	mild	no	resolved	yes

Worsening Dysarthria	no	not related	mild	no	resolved	yes
Worsening Dysphonia	no	not related	mild	no	resolved	yes
Fatigue	no	not related	mild	no	unresolved	yes
Fall	no	not related	mild	no	resolved	yes
Post LP headache	no	not related	mild	no	resolved	no
Backache LP Site	no	not related	mild	no	resolved	no
Headache post lumbar puncture	no	not related	mild	no	resolved	no
Diarrhoea	no	unlikely	mild	no	resolved	yes
Kidney stone pain	no	not related	mild	no	resolved	yes
Dry Mouth	no	unlikely	mild	no	resolved	yes
Constipation	no	unlikely	mild	no	resolved	yes
Dry eyes	no	unlikely	mild	no	resolved	yes
Slight kidney infection	no	not related	mild	no	resolved	yes
Pain in ribcage	no	not related	mild	no	resolved	yes
Aches and pains in ribcage	no	not related	mild	no	unresolved	yes
Pain in back	no	not related	mild	no	resolved	yes
Left arm muscle cramps	no	not related	mild	no	resolved	yes
Erythema at LP site	no	not related	mild	no	resolved	yes
Headache	no	not related	mild	no	resolved	no
Nausea	no	not related	mild	no	resolved	no
Headache	no	not related	mild	no	resolved	no
Mild Back Pain (Post LP)	no	not related	mild	no	resolved	no
Vomiting post LP	no	not related	mild	no	resolved	no
Headache post LP	no	not related	mild	no	resolved	no
Right arm muscular pain	no	not related	mild	no	resolved	yes
Rashes on chest,back and arms	no	probably	mild	yes	resolved	yes
memory loss	no	possibly	mild	no	resolved	yes
Weight loss	no	possibly	mild	no	unresolved	yes
Fall	no	unlikely	mild	no	resolved	yes
Fall	no	unlikely	mild	no	resolved	yes
Back pain around lumbar puncture site	no	not related	mild	no	resolved	yes
Slight Dryness to Face and Hands	no	not related	mild	no	resolved	yes
Slight Redness to Face and Hands	no	not related	mild	no	resolved	yes

Post LP headache	no	not related	mild	no	resolved	yes
Pneumonia	no	not related	mild	no	resolved	yes
Pain at LP Site	no	not related	mild	no	resolved	no
Anxiety pre LP	no	not related	mild	no	resolved	no
Bruise at LP Site (~2 inches)	no	not related	mild	no	resolved	no
Area of LP Erythema Site	no	not related	mild	no	resolved	yes
Acid Reflux	no	probably	mild	yes	resolved	yes
Loose Stools	no	probably	mild	yes	resolved	yes
Burning sensation to back of throat when taking IMP	no	probably	mild	yes	resolved	yes
Constipation	no	unlikely	mild	no	resolved	yes
Nausea	no	probably	mild	yes	resolved	yes
Anxiety	no	unlikely	mild	no	resolved	yes
Spasm in left lower back at night at LP site	no	not related	mild	no	unresolved	yes
Walking and balance problems	no	possibly	mild	no	resolved	yes
Mild discomfort/pain on LP site	no	not related	mild	no	resolved	no
Cut on finger	no	not related	mild	no	resolved	yes
Prolonged bleeding from finger cut	no	not related	mild	no	resolved	yes
Soreness around LP site	no	not related	mild	no	resolved	no
Bruising around LP site	no	not related	mild	no	resolved	no
Tendonitis	no	not related	mild	no	resolved	yes
Diarrhoea	no	unlikely	mild	no	resolved	yes
Rash at top of thighs	no	unlikely	mild	no	resolved	yes
Constipation	no	possibly	mild	no	resolved	yes
Nausea	no	possibly	mild	yes	resolved	yes
Cough	no	not related	mild	no	resolved	no
Sore throat	no	not related	mild	no	resolved	no
Back Pain (LP)	no	not related	mild	no	resolved	no
Itchy Red Area on Abdomen	no	possibly	mild	yes	resolved	yes
Headache	no	not related	mild	no	resolved	yes
Calcified area on left breast	no	not related	mild	no	unresolved	yes
Breast biopsy	no	not related	mild	no	resolved	yes
Breast biopsy	no	not related	mild	no	resolved	yes

Nausea	no	possibly	mild	yes	resolved	yes
Back Pain	no	not related	mild	no	resolved	yes
Knee pain	no	not related	mild	no	unresolved	yes
Fall	no	not related	mild	no	resolved	no
Coccyx pain	no	not related	mild	no	resolved	no
Muscular pain in neck	no	not related	mild	no	resolved	yes
Runny nose	no	possibly	moderate	no	resolved	yes
Headache	no	not related	mild	no	resolved	yes
Deterioration of gait	no	not related	mild	no	unresolved	no
Back ache at the site of LP	no	not related	mild	no	resolved	no
Fall	no	not related	mild	no	resolved	no
Trochanteric bursitis	no	not related	mild	no	unresolved	yes
Pain LP Site	no	not related	mild	no	resolved	yes
Lumbar Pain	no	not related	mild	no	unresolved	yes
Floating feeling/light headedness	no	possibly	mild	no	resolved	yes
Floating feeling/light headedness	no	possibly	mild	no	resolved	yes
Afternoon sweats and dizziness	no	possibly	mild	no	resolved	yes
Impaired swallow	no	unlikely	mild	no	unresolved	yes
Chest infection	no	unlikely	mild	no	resolved	yes
UTI	no	not related	mild	no	resolved	yes
Transient postural dizziness	no	not related	mild	no	resolved	yes
Transient postural dizziness	no	not related	mild	no	resolved	no
20 min period of increased shaking	no	not related	mild	no	resolved	no
Rapid eye movement sleep disorder	no	not related	mild	no	unresolved	yes
Feels 'spaced out' after first dosing. Lasts from 5 to 30 minutes.	no	possibly	mild	no	resolved	yes
Fall	no	unlikely	mild	no	resolved	yes
Cellulitis on left arm at biopsy site	no	not related	mild	no	resolved	yes
Vomiting (after 3 pints of beer)	no	definitely	mild	yes	resolved	yes
Nausea	no	definitely	mild	yes	resolved	yes
Vomiting	no	definitely	mild	yes	resolved	yes
Diarrhoea	no	possibly	mild	no	resolved	yes
Fall	no	unlikely	mild	no	resolved	yes

Tingling in left side of neck	no	not related	mild	no	resolved	yes
Vomiting	no	unlikely	mild	yes	resolved	yes
Falls	no	not related	mild	no	resolved	yes
Fall	no	not related	mild	no	resolved	yes
Slight headache	no	not related	mild	no	resolved	no
Soreness and mild pain at LP site	no	not related	mild	no	resolved	no
Whooshing in ears post Lp	no	not related	moderate	no	resolved	no
Severe headache post Lp	no	not related	severe	no	resolved	no
Nausea	no	not related	moderate	no	resolved	no
Non malignant skin growth	no	not related	mild	no	resolved	yes
Tender sacrum post LP	no	not related	mild	no	resolved	no
Headache post LP	no	not related	mild	no	resolved	no
Fatigue	no	not related	mild	no	resolved	no
UTI	no	not related	mild	no	resolved	yes
Dizziness	no	possibly	mild	no	resolved	yes
UTI, recurrent	no	not related	moderate	no	unresolved	yes
Back pain post LP	no	not related	mild	no	resolved	yes
Bruise post LP	no	not related	mild	no	resolved	yes
Increased coldness/freezing	no	unlikely	mild	no	resolved	yes
Headache post LP	no	not related	moderate	no	resolved	yes
Nausea post LP	no	not related	moderate	no	resolved	yes
Whooshing sound in ears post LP	no	not related	mild	no	resolved	yes
Headache post LP	no	not related	mild	no	resolved	no
Decreased hearing post LP	no	not related	mild	no	resolved	no
Nausea post LP	no	not related	mild	no	resolved	no
Headache, stated as 3/10 pain score	no	not related	mild	no	resolved	yes
Decreased hearing	no	not related	mild	no	resolved	yes
Decreased volume of voice	no	not related	mild	no	resolved	yes
Increased sleep	no	not related	mild	no	resolved	yes
Itching left forearm	no	not related	mild	no	resolved	yes
Erectile dysfunction	no	unlikely	mild	no	unresolved	yes
Viral illness	no	possibly	mild	no	resolved	yes
Headache post LP	no	not related	moderate	no	resolved	no

Back pain post LP	no	not related	mild	no	resolved	no
Nausea	no	not related	mild	no	resolved	no
Vomiting	no	not related	mild	no	resolved	no
Grinding of teeth	no	unlikely	mild	no	resolved	yes
Upper respiratory tract infection	no	not related	mild	no	resolved	yes
Umbilical hernia	no	not related	mild	no	unresolved	yes
Mild pain/ discomfort and soreness at the LP site	no	not related	mild	no	resolved	yes
Vomiting	no	unlikely	mild	no	resolved	yes
Increased tremors	no	unlikely	mild	no	resolved	yes
Vomiting	no	not related	mild	no	resolved	no
Post LP headache	no	not related	mild	no	resolved	no
Anaemia	no	not related	mild	no	unresolved	no
Increased tremors	no	not related	mild	no	resolved	no
Neck stiffness	no	not related	severe	no	resolved	yes
Headache	no	not related	severe	no	resolved	yes
Back pain	no	not related	mild	no	resolved	yes