Genetic and clinical pharmacology studies in GBA1-associated Parkinson's disease

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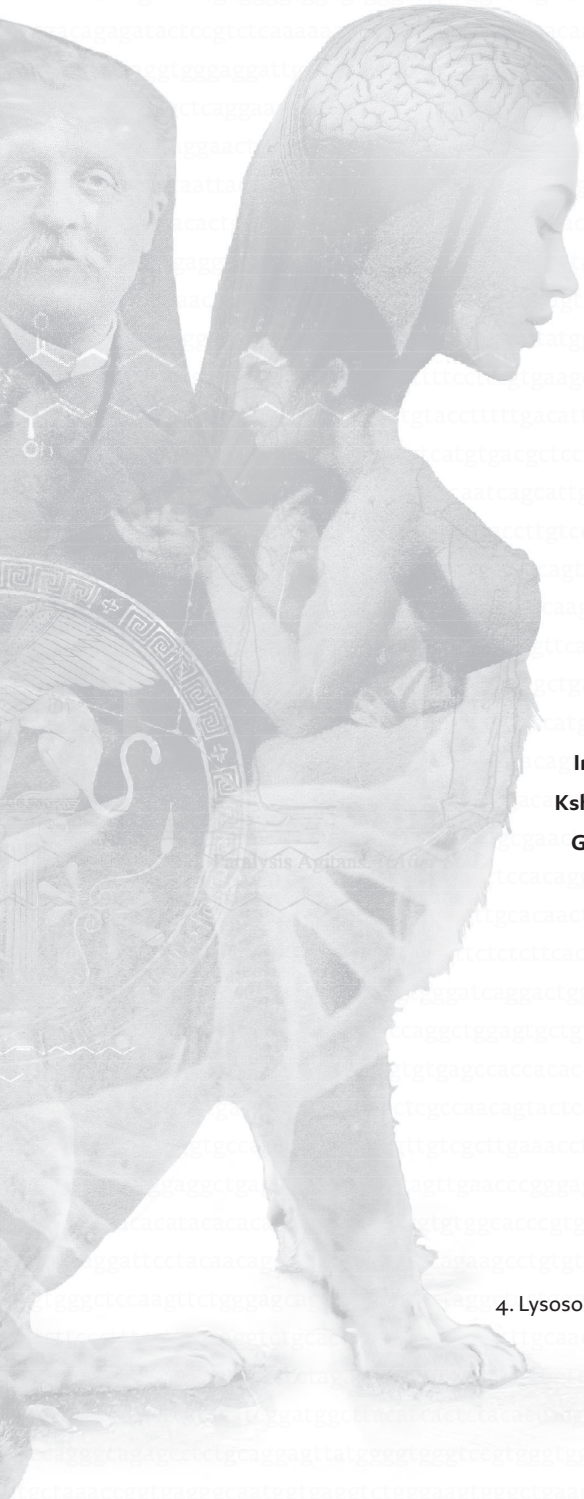

CHAPTER 8

A phase 1b trial in *GBA1*-associated Parkinson's disease of LTI-291, a glucocerebrosidase activator

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Abstract

Background Loss-of-function mutations in the *GBA1* gene are one of the most common genetic risk factors for onset of Parkinson's disease and subsequent progression (GBA-PD). *GBA1* encodes the lysosomal enzyme glucocerebrosidase (GCase); a promising target for a possible first disease-modifying therapy. LTI-291 is an allosteric activator of GCase, which increases the activity of normal and mutant forms of GCase.

Objectives This first-in-patient study evaluated the safety, tolerability, pharmacokinetics and pharmacodynamics of 28 daily doses of LTI-291 in GBA-PD.

Methods This was a randomized, double-blind, placebo-controlled trial in 40 GBA-PD participants. 28 consecutive daily doses of 10, 30 or 60mg LTI-291 or placebo were administered (n=10 per treatment allocation). Glycosphingolipid (GluCer and LacCer) levels were measured in peripheral blood mononuclear cells, plasma, and CSF, and a test battery of neurocognitive tasks, the MDS-UPDRS and the MMSE were performed.

Results LTI-291 was generally well tolerated and no deaths or treatment-related SAEs occurred, and no participants withdrew due to AEs. $C_{max,}$ and AUC_{0-6} of LTI-291 increased in a dose proportional manner, with free CSF concentrations equal to the free fraction in plasma. A treatment-related transient increase of intracellular glucosylceramide (GluCer) in peripheral blood mononuclear cells was measured.

Conclusion These first-in-patient studies demonstrated that LTI-291 was well-tolerated when given orally for 28 consecutive days to patients with GBA-PD. Plasma and CSF concentrations were reached that are considered pharmacologically active (*i. e.,* sufficient to at least double GCase activity). Intracellular GluCer changes were detected that suggest target engagement. Clinical benefit will be assessed in a larger long-term trial in GBA-PD.

Introduction

Parkinson's disease (PD; MIM: 168600) is the second most common neurodegenerative disorder and has a likely multifactorial disease etiology, consisting of both environmental as well as genetic risk factors (Kalia and Lang 2015). A disease-modifying treatment is lacking. Mutations in the *GBA1* gene are one of the most common genetic risk factors for Parkinson's disease (GBA-PD)(Gasser 2015; Schapira 2015; Gan-Or et al. 2015; Ruskey et al. 2019; den Heijer, Cullen, et al. 2020). GBA-PD presents at a slightly younger age than idiopathic PD, with a greater prevalence of non-motor symptoms (Petrucci et al. 2020; Mata et al. 2016; den Heijer, van Hilten, et al. 2020). *GBA1* encodes the lysosomal enzyme glucocerebrosidase (GCase; EC 3.2.1.45) and risk-associated *GBA1* mutations cause a loss of enzymatic activity. Greater relative risk is associated with a decreased activity of the mutant enzyme; the greatest relative risk is associated with a mutant allele that is not translated (hence residual activity is *ca* 50% of normal). In addition to their role in PD risk, *GBA1* mutations have also been linked to more rapid progression of motor (Davis et al. 2016; Ortega et al. 2021) and cognitive (Cilia et al. 2016; Liu et al. 2021) symptoms of PD. Activation of GCase is therefore a promising strategy for a possible first disease-modifying therapy in PD.

GCase functions at the luminal face of the lysosomal membrane and catalyzes one step in the multi-step hydrolytic degradation of glycosphingolipids (GSLs), leading ultimately to sphingosine, the building block for the synthesis of new GSLs (Kitatani, Idkowiak-Baldys, and Hannun 2008; Boer et al. 2020). GSLs are essential for maintenance of membrane properties that play a role in many diverse cellular functions (Merrill 2011). GCase hydrolyzes glucosylceramide (GluCer), producing glucose and ceramide. Ceramide is subsequently deacylated to produce sphingosine, which is transported to the cytosol for elaboration. Ceramide and all of its conjugates, including GluCer, comprise a group of acyl chain isomers that are produced from sphingosine, by acylation with activated fatty acids of diverse chain lengths by the ceramide synthases (Tidhar et al. 2018). The acyl chain isomers of ceramide do *not* interconvert (*e.g.*, by addition or removal of carbons from

the acyl chain). All ceramide isomers can be glucosylated by glucosylceramide synthase (GCS) to produce the GluCer isomers, the starting points for ganglioside synthesis in the Golgi.

It is not possible to measure lysosomal activation of GCase by LTI-291 with a fluorogenic probe, since the leaving group of the available probe occupies the allosteric binding site of LTI-291. As an alternative, time-dependent changes in the levels of substrate GluCer can be used to infer enzyme activity/activation. It is important to note that, as predicted by Michaelis-Menten kinetic theory (Conzelmann and Sandhoff 1983), GluCer levels are not sensitive to GCase activity when GCase activity exceeds *ca.* 30% of the normal, or average, level (Gegg et al. 2015), as is the case in GBA-PD (*in contrast*, Gaucher disease, which is characterized by very low GCase activity, is characterized by accumulation of GluCer isomers in peripheral cells and tissue).

LTI-291 (now designated BIA-28) is a small-molecule GCase allosteric activator that increases V_{max} and decreases K_m of wild-type and at least some mutant enzymes, such that *in vitro* activity is increased by up to 3-fold (LTI, unpublished). When administered to healthy volunteers for fourteen days, with a maximal single dose of 90 mg and multiple daily doses of 60 mg, LTI-291 was generally well-tolerated, without any treatment emergent serious adverse events (SAEs) or any AEs that led to discontinuation (den Heijer, Kruithof, et al. 2021). No AEs were attributed as being related to the administration of LTI-291/BIA-28. CSF unbound drug concentrations were estimated to be in an approximate 1:1 ratio with the unbound plasma drug concentration, across all doses, indicating excellent central penetrance. Based on *in vitro* studies, the central exposures reached by multiple LTI-291 doses of 10 mg to 60 mg were sufficient to *at least* double *in vitro* GCase activity (LTI, unpublished). Doubling of GCase activity is expected to restore 100% of average non-GBA-PD activity in most, if not all, GBA-PD patients. In an earlier study of LTI-291/BIA-28 in healthy elderly (den Heijer, Kruithof, et al. 2021), intracellular GluCer isomers (in PBMCs) did not change significantly over 14 days of dosing. This may be attributable to the possibility that these healthy volunteers had 'normal' GSL flux, which cannot be increased by further GCase activation. The same GluCer isomers were again measured as exploratory biomarkers for the current 28-day study in GBA-PD patients, with

significantly different results. This paper describes these studies, assessing safety, tolerability, pharmacokinetics (PK) and pharmacodynamics, in GBA-PD in a 28-day-treatment trial of LTI-291.

Methods

This was a randomized, double-blind and placebo-controlled trial. The study was approved by the Independent Ethics Committee of the Foundation 'Evaluation of Ethics in Biomedical Research' (Stichting Beoordeling Ethiek Biomedisch Onderzoek), Assen, The Netherlands. The trial is registered in the Dutch Trial Registry (Nederlands Trial Register, NTR) under study number NTR6960. The trial took place between January and June 2018 at the Centre for Human Drug Research, Leiden, the Netherlands. All participants signed an informed consent form prior to any study-related activity, in accordance with the Declaration of Helsinki.

Participants

GBA-PD patients (minimum age of 18 years), with Hoehn and Yahr (H&Y) stage 1-4 and a mini mental state exam (MMSE) score ≥18, male and female of non-childbearing potential were enrolled for 28 consecutive daily oral doses of LTI-291 or placebo. Stable treatment with antiparkinsonian treatments from 1 month prior to the screening (2 months for monoamine oxidase B inhibitors) was allowed. Other prior concomitant medication was only allowed at the discretion of the investigator. The following dose levels were investigated: 10 mg, 30 mg and 60 mg LTI-291. Treatment was administered as powder in a capsule. Each treatment arm consisted of 10 patients. Patients were randomized in 10 blocks of 4 to receive one of the three dose levels of LTI-291 or placebo in a 1:1:1:1 ratio. The randomization code was generated using SAS version 9. 4 by a study-independent statistician. Patients visited the clinical research unit at start of dosing, after one week, two weeks and four weeks. A safety call was performed after three weeks. Between visits, patients self-administered LTI-291 daily. A safety follow-up visit was performed 7-14 days after last dose.

Safety

A medical screening (medical history, record of prior concomitant medication, participant demographics, height and weight, 12-lead electrocardiography (ECG), vital signs, routine hematology, biochemistry/electrolytes and urinalysis, urine pregnancy test (for females), virology, urine drug screen, ethanol breath test, physical examination, MMSE and H&Y staging) was performed to assess a participant's eligibility. During study periods, safety was assessed using monitoring of adverse events (AEs), concomitant medication, vital signs, ECG, physical examination and safety chemistry and hematology blood sampling.

Pharmacokinetics (PK)

LTI-291/BIA-28 levels were measured in K_2EDTA plasma and in cerebrospinal fluid (CSF). Plasma PK samples were taken predose, 2-, 4- and 6-hours after first and last dose and a single sample after seventh (±2) and fourteenth (±2) dose. CSF was taken predose and 4-hours after the last (28^{th}) dose. Non-compartmental analysis was performed on the plasma data from each participant as data permitted.

Pharmacodynamics

GLYCOSPHINGOLIPID LEVELS Biochemical pharmacodynamic markers were measured in K_2EDTA plasma, PBMCs and CSF, as described previously (den Heijer, Kruithof, et al. 2021). GluCer and LacCer were measured in plasma, PBMCs and CSF. GluSph was measured in plasma and PBMCs. The acyl chain of the ceramide group in GluCer and LacCer can be of varying length and saturation. Both in plasma and PBMCs, concentrations were measured of GluCer C16:0, C18:0, C22:0, C24:0 and C24:1 and of LacCer C16:0, C18:0, C20:0, C22:0, C22:1, C24:0 and C24:1. In CSF, GluCer C16:0, C18:0, C22:0, C24:0 and C24:1 were measured and LacCer C16:0, C18:0, C20:0, C22:0, C22:1, C24:0 and C24:1 were measured but are not reported here. In the first-in-human multiple dose studies, GluCer (five isomers) and GluSph were investigated as potential biomarkers in PBMCs, plasma, and CSF in healthy

elderly (55+) volunteers. No significant changes were detected. Biological (intra-individual) variability of all GluCer isomers was determined to be < 13. 3%, except for GluCer C18:0 (17. 2%) (in draft: den Heijer, Pereira, et al. 2022). Inter-individual variability is much greater (see below).

MDS-UPDRS PART III, MMSE AND NEUROCOGNITIVE BIOMARKERS

No clinical effect was expected after 28 days of LTI-291/BIA-28 dosing, but the Movement Disorder Society – Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (Goetz et al. 2008) part III (motor assessment) in ON state and the MMSE were performed at baseline and at end of dosing as pharmacodynamic parameters for safety.

The NeuroCart®(Groeneveld, Hay, and Van Gerven 2016), a CNS test battery, was used to exclude any adverse effects of LTI-291/BIA-28 on CNS function. This was performed at baseline and after two weeks of dosing, to spread the burden of different measurements over different visits. Steady state exposures of LTI-291/BIA-28 were expected to be achieved after seven days of dosing. The test battery consists of neurophysiological, psychomotor and cognitive tests and has been extensively used previously in clinical drug development. (Muehlan et al. 2019; Baakman et al. 2019; Groeneveld, Hay, and Van Gerven 2016; Van Steveninck et al. 1991; Chen et al. 2012)

In short, measurements consist of saccadic and smooth pursuit eye movements, the adaptive tracking test (a visuo-motor task sensitive to disturbances in vigilance and attention), the body sway (a test of postural stability), the Bond and Lader test (visual analogue scale (VAS) of alertness, calmness and mood), the Visual Verbal Learning Test (VVLT) (a test of immediate and delayed memory), and pharmaco-EEG (measured separately after last dose instead of after two weeks). Tests were performed in a quiet room with ambient illumination with only one participant in the same room (and a research assistant) per session.

GBA1 genotyping

GBA1 genotype was determined in a previous large-scale *GBA1* screening in the Netherlands. (den Heijer, Cullen, et al. 2020) In short, full gene sequencing was performed on saliva-derived DNA, using next generation sequencing

and a primer set unique for the functional gene, thereby preventing amplification of the nearby pseudogene. For this trial, *GBA1* genotypes were confirmed by repeating sequencing in a whole blood sample (Table 1).
GBA1 genotypes were categorized into two categories:

1. carriers of one allele that has been reported in at least a single Gaucher's disease (GD), or
2. carriers of a non-GD *GBA1* allele linked to PD-risk, for alleles associated with PD or reported in PD patient(s), but never GD. It is important to emphasize that, although *GBA1* genotype is related to *average* residual GCase activity, there is considerable inter-individual variation and overlap between genotypes.

Statistical analysis

Neurocognitive pharmacodynamic data were analyzed with an analysis of covariance with fixed factor treatment and average predose value as covariate.

All safety and neurocognitive pharmacodynamic statistical programming were conducted with SAS 9. 4 for Windows (SAS Institute Inc., Cary, NC, USA). All PK analyses were performed in Phoenix 64 build 8. 0. 0. 3176 using WinNonlin 8. 0 (Certara L.P.). Statistical analysis of PK was performed using R version 3. 3. 1 ((2016-06-21).

Biochemical pharmacodynamic data were analyzed with a linear mixed model, with fixed factors treatment, time and treatment by time, random factor participant, and covariates average baseline value, sex, age and *GBA1* type (GD or non-GD carriers). An overall treatment effect was assessed and an effect over time, both for all active dose levels combined and per dose level compared to placebo. Statistical programming was conducted with R version 3.6.2 (2019-12-12).

This was an exploratory study; therefore, the sample size was not based on statistical considerations. 10 patients per dose level and 10 placebo patients were considered adequate to define initial safety and tolerability and to explore pharmacodynamics in the target patient population over 28 days of dosing.

Biomarkers were measured in an exploratory hypothesis-generating setting and were therefore not corrected for multiple testing.

Data availability

Data are available upon reasonable request.

Results

Forty-nine participants signed the informed consent form and underwent a medical screening. Seven participants were not enrolled because they were excluded based on the inclusion and exclusion criteria or withdrew consent to participate. A total number of 42 participants were enrolled. Two participants were withdrawn prior to the first dose based on physician decision (significant ECG abnormalities, not visible at screening). In total 40 participants were treated in the study, and all completed the study including the follow-up visit (Supplementary Figure 1).

Demographics and baseline characteristics

In total 20 males and 20 females were included in the study. The mean weight ranged from 69.0 kg (10 mg LTI-291) to 81. 0 kg (30 mg LTI-291/BIA-28). Participants in the different dose levels were comparable regarding mean age, mean height, mean MMSE and mean MDS-UPDRS part III score. Demography data are summarized in Table 1.

Safety and tolerability

28 consecutive daily administrations of LTI-291/BIA-28 up to the highest dose of 60 mg were generally well tolerated in people with GBA-PD. No serious AEs (SAEs) occurred after dosing and no AEs led to discontinuation. No clinically relevant changes in blood chemistry, hematology (Supplementary table 1), urinalysis, vital signs, ECG or CNS tests were identified. See Table 2 for a full listing of all AEs after dosing. Back pain was only reported in LTI-291/BIA-28

dose groups (N=4) and not in placebo, however there is no clear rationale for this and there was no dose-dependent increase in frequency, therefore this was considered unlikely related to administration of LTI-291/BIA-28. Other frequently reported AEs like fatigue and headache occurred in a similar or higher frequency in the placebo group and no dose-dependent increase was observed, therefore these are also considered unlikely related to administration of LTI-291/BIA-28. Five participants (LTI-291 n=4; placebo n=1) reported a mild subjective worsening of Parkinson's disease symptoms. Three participants related this to a stressful period. In four out of five participants this subjective worsening of symptoms resolved prior to the last dose of LTI-291/BIA-28 or placebo. Most AEs were mild in severity. Only 3 moderate AEs were reported, namely urinary tract infection (10 mg LTI-291), tendonitis (placebo) and paronychia (30 mg LTI-291). Both infections were successfully treated with antibiotics and the participant with tendonitis was referred for physiotherapy. These three AEs were all considered to be unlikely related to LTI-291/BIA-28 treatment.

Pharmacokinetics

Pharmacokinetic analysis of LTI-291/BIA-28 showed a maximum plasma concentration (T_{max}) ranging from 2 to 6 hours. $C_{max,}$ and AUC_{0-6} increased in a dose proportional manner. Half-life could not be determined due to limited sampling, but the pharmacokinetic profile otherwise was similar to results from previous studies in healthy volunteers (den Heijer, Kruithof, et al. 2021). The CSF:plasma concentration ratios range from 0.00634 to 0.0187 and were similar at all the dose levels. See Supplementary Table 2 and Supplementary Table 3 for details.

Pharmacodynamics

GROUP AVERAGE LEVELS OF INTRACELLULAR GLUCER ISOMERS IN PBMCS SIGNIFICANTLY INCREASE 14 DAYS AFTER DOSING WITH LTI-291/BIA-28, THEN PARTIALLY RETURN TO THE PRE-DOSE LEVEL

In PBMCs, GluCer C16:0, C22:0, C24:0 and C24:1 showed a statistically significant overall treatment-associated *increase* in all active treatment groups

(doses were combined since all doses were expected to at least double activity based on estimated brain exposure) at all times combined, compared to placebo (Table 3). The effect was significant in the 10 mg LTI-291/BIA-28 and the 60 mg LTI-291/BIA-28 dose groups, but not in the 30 mg treated group (Table 3). The effect was largest on Day 14 (Table 4, Figure 1) (Supplementary Table 4). Age, sex and *GBA1* genotype were not significant covariates. LacCer and GluSph in PBMCs were omitted from analysis, because of influence of leukocyte subtype ratios (including granulocyte contamination), which vary between blood draws. No significant changes were detected in extracellular GluCer levels at any time (plasma or CSF; data not shown).

MDS-UPDRS PART III, MMSE AND NEUROCOGNITIVE BIOMARKERS WERE UNCHANGED BY 28 DAYS OF DOSING No clinically significant changes were seen in MDS-UPDRS-Part III (ON state) or MMSE total score in any dosing group compared to placebo (Table 5). See Supplementary Table 5 for details.

There were no dose-dependent effects of LTI-291 on any of the neurocognitive biomarkers, indicating that 28 consecutive oral doses in participants with GBA-PD were not observed to cause any effects on CNS functioning. Some isolated differences from placebo were seen in single, mostly submaximal, dose levels, but due to the lack of dose dependency these were considered chance findings due to multiple testing (Supplementary Table 6).

Discussion

Here we report the first administration of LTI-291 (now designated BIA-28), a centrally penetrant small molecule, aimed at increasing glucocerebrosidase activity in patients with GBA-PD. Safety, tolerability, PK and pharmacodynamics of LTI-291/BIA-28 were evaluated. LTI-291/BIA-28 was administered in 28 consecutive daily doses at 10, 30 or 60 mg. This was generally well tolerated, no treatment-related SAEs or deaths occurred, and no participants withdrew due to AEs.

A significant and transient increase in 4/5 *intracellular* GluCer isomers was detected in PBMCs in dosed participants as compared to placebo (the fifth, which is also the lowest in abundance, was also increased, but not

statistically significantly so). No change of *extracellular* GluCer was observed in plasma at any time or in CSF at 28 days (not shown). Intracellular GluCer levels and plasma GluCer levels do not correlate (in draft: den Heijer, Pereira, et al. 2022). Drug-associated elevation of intracellular PBMC GluCer isomers also occurred at the lowest dose of 10mg, which may be expected, since measured exposures at the lowest dose were sufficient to double GCase activity *in vitro* (den Heijer, Kruithof, et al. 2021). The observed increase in intracellular GluCer was slow, with no change at 6h, a mild increase after seven days of dosing and a significant increase after 14 days of dosing. A second phase of the response was suggested by the fact that GluCer levels seemed to return towards pre-dose levels by day 28 (Table 4). Two unpublished observations from previous trials are pertinent to the analysis of the response. First, the initial increase in intracellular GluCer was *not* observed in a previous 14 day phase 1 trial in healthy elderly (den Heijer, Kruithof, et al. 2021). Second, all analyzed clinical data demonstrated that *intracellular* GluCer levels in GBA-PD, non-GBA-PD patients and healthy controls are comparable, with possibly a trend for slightly *lower intracellular* GluCer levels in GBA-PD compared to healthy controls (in draft: den Heijer, Pereira, et al. 2022). This temporary elevation may therefore be a response selective to individuals with a chronic suboptimally functioning GluCer recycling, like in GBA-PD.

The observed response to BIA-28 constitutes two phases; a slow (7-14 days) increase in intracellular GluCer, followed by an even slower decrease/return to pre-dose levels. It should be noted that intracellular GluCer measures include lysosomal GluCer (the *GBA1* substrate), as well as non-lysosomal or cytoplasmic GluCer (Fuller et al. 2008). Since the majority of GluCer is non-lysosomal, we propose that the activation of GCase activity by dosing with LTI-291/BIA-28 may cause a transient increase of salvaged ceramide available for GluCer synthase in the cytosol (Kitatani, Idkowiak-Baldys, and Hannun 2008; Boer et al. 2020). As the systemic ceramide (and GluCer) levels increase, de novo synthesis, which is known to be sensitive to ceramide (Wattenberg 2021), is down-regulated. This effect subsides as the system returns to a new homeostasis.

GluCer transient elevation was seen for the 10mg and 60mg treated groups, but small increases in the 30mg group did not reach statistical

significance. Based on preclinical experiments, an effect was expected with a C_{max} of ~360 ng/mL, with similar responses for higher dose levels, indicating a flattening of the dose response. In the 10mg group, the mean C_{max} was 554 ng/mL, showing all dose levels reached expected active concentrations. Lack of a clear signal in the 30mg group could be explained by the inherent variability of the biomarker, combined with a small sample size of subgroups.

Variability of GluCer in PBMCs as a biomarker can also be seen in placebo data. No change over 28 days is expected in placebo treated participants, so fluctuations in the placebo group likely reflect natural variability. The strongest signal at day 14 seems driven by both a GluCer increase in LTI-291 treated participants and a random trough in the placebo-treated group (Figure 1). Nevertheless, the overall treatment effect is still statistically significant different in LTI-291 treated participants compared to placebo, accounting for this variability over time (Table 2). Considering the exploratory setting of these pharmacodynamic measurements, without correction for multiple testing, these effects require validation in a larger cohort.

Measurements in PBMCs of GluSph and various LacCer isoforms were heavily influenced by the cell subtype composition of the PBMC isolate (in draft: den Heijer, Pereira, et al. 2022). This composition also varied within-individual between samples. Because this variation could not be distinguished from a potential treatment effect, these were omitted from analysis.

GBA1 genotype category (GD-risk (n=25) vs PD-risk (n=15)) was investigated as covariate. GD-risk showed a trend for a stronger effect in all GluCer isoforms in PBMCs, but did not reach statistical significance (data not shown). It can be speculated that patients with a larger GCase deficiency, may have more benefit of treatment. Subgroups were small however, and GCase activity is known to vary between individuals with the same mutation. Whether this translates to a clinical effect will be determined in an upcoming trial.

Pharmacokinetic sampling was limited with three plasma samples up to six hours post-dose on day 1 and day 28, showing a dose-proportional increase in C_{max} and AUC_{0-6}. The mean CSF:plasma concentration ratios ranged from 0. 0113 to 0. 0122 at 4 hours after the 28^{th} dose and were similar

at all dose levels (Supplementary Table 3). This ratio corresponds with a free distribution of unbound LTI-291 between plasma and CSF, which again is in distribution equilibrium with brain tissue, as was shown in preclinical rat neuro PK experiments. This PK profile is similar to what was determined in healthy volunteers (den Heijer, Kruithof, et al. 2021), which also showed a median half-life of 28. 0 hours, favoring daily single dosing.

A neurocognitive test-battery showed no adverse effect on CNS functioning, performed after the 14^{th} dose, during which steady state LTI-291 plasma concentration was already achieved. No clinical improvement was expected after 28 days of dosing and no deterioration was observed, as confirmed by MDS-UPDRS part III (motor assessment) and MMSE testing. The MDS-UPDRS was performed in ON state, since the burden of testing in OFF state was not considered justified, as no clinical change was expected. In a long-term study to assess clinical improvement, OFF state measures will be appropriate.

In five participants, a mild subjective worsening of PD symptoms was reported, which resolved before end of dosing in four participants (three active treatment, one placebo). Considering the natural variation in Parkinson's disease symptom severity and the progressive disease course, these complaints were considered unlikely to be caused by administration of LTI-291.

In conclusion, LTI-291 was observed to be well tolerated when given orally once daily for 28 consecutive days at all dose levels tested in this GBA-PD population. Plasma concentrations were reached that are expected to be active in at least doubling glucocerebrosidase activity. Exploratory pharmacodynamic markers suggest peripheral target engagement. A long-term (one year) dosing study is being planned to assess clinical benefit.

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Table 1 Overview of demographic variables. The *GBA1* allelic names are given, excluding the 39-amino acid signaling peptide. In case of two mutations, variants within the staple signs [ ] are on the same allele, and variants in separate staple signs are on separate alleles. A semicolon in parentheses indicates it is uncertain how these mutations are distributed over alleles. GD mutations are designated (1) and non-GD mutations (2).

Demographics and baseline characteristics					
Demographic variables	**All participant (N=40)**	**10 mg LTI-291 (N=10)**	**30 mg LTI-291 (N=10)**	**60 mg LTI-291 (N=10)**	**Placebo (N=10)**
AGE (YEARS)					
Mean (SD)	61.1 (9.3)	59.9 (11.5)	62.6 (8.2)	59.1 (9.1)	62.8 (8.9)
Min, Max	40, 80	40, 79	51, 80	46, 80	47, 73
HEIGHT (CM)					
Mean (SD)	172.2 (8.9)	170.1 (9.7)	172.0 (7.1)	174.8 (8.8)	172.1 (10.5)
Min, Max	156.1, 189.2	156.1, 189.0	161.6, 184.1	162.4, 189.2	157.3, 183.5
WEIGHT (KG)					
Mean (SD)	73.9 (13.4)	69.0 (15.2)	81.0 (14.0)	74.0 (13.1)	71.6 (9.5)
Min, Max	46.25, 110.05	46.25, 88.2	63.9, 110.05	55.5, 92.85	60.2, 87.3
BMI (KG/M^2)					
Mean (SD)	24.8 (3.6)	23.6 (3.1)	27.3 (3.9)	24.1 (3.5)	24.3 (3.4)
Min, Max	17.9, 35.1	19, 27.9	22.6, 35.1	19.4, 29	17.9, 28.9
MMSE Total					
Mean (SD)	27.9 (2.6)	27.7 (4.1)	27.1 (1.9)	29.0 (1.3)	27.7 (2.3)
Median	29.0	30.0	27.0	29.5	28.5
Min, Max	19, 30	19, 30	25, 30	26, 30	24, 30
MDS-UPDRS part III Total (ON STATE)					
Mean (SD)	31.4 (14.0)	31.1 (17.4)	28.4 (12.6)	32.8 (14.3)	33.4 (12.7)
Median	27.5	27.5	22.5	26.5	31.0
Min, Max	14, 72	14, 72	17, 54	20, 65	17, 52
SEX					
Female	20 (50.0%)	6 (60.0%)	4 (40.0%)	5 (50.0%)	5 (50.0%)
Male	20 (50.0%)	4 (40.0%)	6 (60.0%)	5 (50.0%)	5 (50.0%)
RACE					
White	40 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)

Table 1 *(Continuation of previous page)*

Demographics and baseline characteristics					
Demographic variables	**All participant (N=40)**	**10 mg LTI-291 (N=10)**	**30 mg LTI-291 (N=10)**	**60 mg LTI-291 (N=10)**	**Placebo (N=10)**
GBA1 mutation (allelic name)[disease association]					
P.[D140H;E326K][1]	6 (15.0%)	1 (10.0%)	3 (30.0%)	2 (20.0%)	0 (0%)
P.E326K[2]	9 (22.5%)	4 (40.0%)	2 (20.0%)	2 (20.0%)	1 (10.0%)
P.[E326K];[E326K][2]	1 (2.5%)	0 (0%)	0 (0%)	0 (0%)	1 (10.0%)
P.G202R[1]	1 (2.5%)	0 (0%)	1 (10.0%)	0 (0%)	0 (0%)
P.G325R[1]	1 (2.5%)	0 (0%)	1 (10.0%)	0 (0%)	0 (0%)
P.L444P[1]	5 (12.5%)	2 (20.0%)	1 (10.0%)	1 (10.0%)	1 (10.0%)
P.T369M(;)L444P[1]	2 (5.0%)	0 (0%)	0 (0%)	1 (10.0%)	1 (10.0%)
P.N370S[1]	7 (17.5%)	2 (20.0%)	1 (10.0%)	1 (10.0%)	3 (30.0%)
P.R120W[1]	2 (5.0%)	0 (0%)	0 (0%)	1 (10.0%)	1 (10.0%)
P.R329C[1]	1 (2.5%)	0 (0%)	0 (0%)	1 (10.0%)	0 (0%)
P.T369M[2]	4 (10.0%)	1 (10.0%)	1 (10.0%)	1 (10.0%)	1 (10.0%)
P.[E326K];[T369M][2]	1 (2.5%)	0 (0%)	0 (0%)	0 (0%)	1 (10.0%)

BMI=body mass index; min=minimum; max=maximum; SD=Standard deviation, MMSE=Mini Mental State Examination, MDS-UPDRS=Movement Disorder Society-Unified Parkinson's Disease Rating Scale.

Table 2 All treatment emergent adverse events by treatment. All AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.0. Greyed rows depict system organ classes and numbers are a summation of all preferred terms in that class. Multiple AEs could be reported by the same participant. *Preferred term Parkinson's disease was used for a participant-reported worsening of Parkinson's disease related symptoms.

	10mg LTI-291		30mg LTI-291		60mg LTI-291		Placebo	
	N=10		N=10		N=10		N=10	
System Organ Class/ Preferred Term	Events (N)	Subjects (N (%))	Events (N)	Subjects (N (%))	Events (N)	Subjects (N (%))	Events (N)	Subjects (N (%))
ANY EVENTS	17	9 (90.0)	20	7 (70.0)	9	8 (80.0)	12	7 (70.0)
EAR AND LABYRINTH DISORDERS	3	2 (20.0)	-	-	-	-	-	-
Tinnitus	2	2 (20.0)	-	-	-	-	-	-
Vertigo	1	1 (10.0)	-	-	-	-	-	-
GASTROINTESTINAL DISORDERS	2	2 (20.0)	2	1 (10.0)	-	-	-	-
Abdominal pain	-	-	1	1 (10.0)	-	-	-	-
Diarrhoea	-	-	1	1 (10.0)	-	-	-	-
Gastroenteritis viral	2	2 (20.0)	-	-	-	-	-	-
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	2	2 (20.0)	5	3 (30.0)	1	1 (10.0)	2	2 (20.0)
Fatigue	1	1 (10.0)	4	3 (30.0)	1	1 (10.0)	2	2 (20.0)
Feeling hot	-	-	1	1 (10.0)	-	-	-	-
Oedema	1	1 (10.0)	-	-	-	-	-	-
INFECTIONS AND INFESTATIONS	1	1 (10.0)	1	1 (10.0)	1	1 (10.0)	1	1 (10.0)
Influenza	-	-	-	-	1	1 (10.0)	-	-
Paronychia	-	-	1	1 (10.0)	-	-	-	-
Urinary tract infection	1	1 (10.0)	-	-	-	-	1	1 (10.0)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	1	1 (10.0)	-	-	-	-	-	-
Vessel puncture site phlebitis	1	1 (10.0)	-	-	-	-	-	-
Soft tissue injury	-	-	1	1 (10.0)	2	2 (20.0)	-	-

Table 2 *(Continuation of previous page)*

	10mg LTI-291		30mg LTI-291		60mg LTI-291		Placebo	
	N=10		N=10		N=10		N=10	
System Organ Class/ Preferred Term	Events (N)	Subjects (N (%))	Events (N)	Subjects (N (%))	Events (N)	Subjects (N (%))	Events (N)	Subjects (N (%))
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	-	-	6	5 (50.0)	4	4 (40.0)	3	3 (30.0)
Back pain	-	-	3	3 (30.0)	1	1 (10.0)	-	-
Muscle spasms	-	-	1	1 (10.0)	-	-	-	-
Muscle strain	-	-	-	-	1	1 (10.0)	-	-
Musculoskeletal pain	-	-	-	-	-	-	1	1 (10.0)
Myalgia	-	-	1	1 (10.0)	-	-	1	1 (10.0)
Tendonitis	-	-	-	-	-	-	1	1 (10.0)
NERVOUS SYSTEM DISORDERS	5	3 (30.0)	4	3 (30.0)	2	2 (20.0)	5	3 (30.0)
Headache	5	3 (30.0)	1	1 (10.0)	-	-	4	3 (30.0)
Parkinson's disease*	-	-	2	2 (20.0)	2	2 (20.0)	1	1 (10.0)
Restless legs syndrome	-	-	1	1 (10.0)	-	-	-	-
PSYCHIATRIC DISORDERS	-	-	-	-	-	-	1	1 (10.0)
Stress	-	-	-	-	-	-	1	1 (10.0)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	2	1 (10.0)	2	2 (20.0)	1	1 (10.0)	-	-
Cough	-	-	2	2 (20.0)	-	-	-	-
Sinusitis	-	-	-	-	1	1 (10.0)	-	-
Viral upper respiratory tract Infection	2	1 (10.0)	-	-	-	-	-	-
VASCULAR DISORDERS	1	1 (10.0)	-	-	-	-	-	-
Epistaxis	1	1 (10.0)	-	-	-	-	-	-

Table 3 Summary of linear mixed model analysis results per GluCer isoform. The table shows the overall treatment effect (all dose groups (n=30) compared to placebo (n=10)) and treatment effect per dose level (n=10 per level). Estimates of the difference with 95% confidence intervals and p-values are shown per comparison. Log-transformed parameters were back-transformed after analysis and therefore these parameters are shown as percentage change.

Overall treatment effect on GluCer isoforms of LTI-291 vs placebo								
	LTI-291 overall vs placebo		LTI-291 10mg vs placebo		LTI-291 30mg vs placebo		LTI-291 60mg vs placebo	
Isoform	Contrast (95%CI)	p	Contrast (95%CI)	p	Contrast (95%CI)	p	Contrast (95%CI)	p
GluCer C16:0	6.7% (1.2%-12.6%)	0.018	8.3% (1.4%-15.7%)	0.019	4.6% (-2.0%-11.7%)	0.170	7.3% (0.1%-15.0%)	0.047
GluCer C18:0	3.6% (-3.0%-10.7%)	0.284	4.8% (-3.2%-13.4%)	0.237	0.4% (-7.2%-8.6%)	0.925	7.2% (-1.9%-17.2%)	0.121
GluCer C22:0	6.4% (0.1%-13.0%)	0.046	9.8% (2.6%-17.5%)	0.008	0.3% (-6.3%-7.4%)	0.923	9.7% (2.0%-17.9%)	0.014
GluCer C24:0	8.8% (1.5%-16.7%)	0.018	12.5% (3.8%-21.8%)	0.005	2.4% (-5.5%-11.0%)	0.549	12.5% (3.4%-22.3%)	0.007
GluCer C24:1	0.197 (0.065-0.329)	0.004	0.238 (0.084-0.391)	0.003	0.102 (-0.052-0.257)	0.188	0.266 (0.103-0.428)	0.002

Table 4 Summary of analysis over time results per GluCer isoform. The table shows the overall treatment effect over time (all dose groups compared to placebo). Estimates of the difference with 95% confidence intervals and p-values are shown per comparison. Log-transformed parameters were back-transformed after analysis and therefore these parameters are shown as percentage change.

Treatment effect over time on GluCer isoforms of all LTI-291 dosed vs placebo								
	Day 1		Day 7		Day 14		Day 28	
Isoform	Contrast (95%CI)	p-value	Contrast (95%CI)	p-value	Contrast (95%CI)	p-value	Contrast (95%CI)	p-value
GluCer C16:0	6.3% (-3.7%–17.2%)	0.224	7.1% (-2.9%–18.1%)	0.170	15.6% (4.8%–27.5%)	0.004	7.2% (-2.8%–18.2%)	0.166
GluCer C18:0	6.4% (-4.8%–18.9%)	0.273	0.2% (-10.3%–11.9%)	0.974	10.5% (-1.1%–23.5%)	0.078	3.3% (-7.6%–15.4%)	0.567
GluCer C22:0	5.2% (-5.5%–17.1%)	0.354	6.4% (-4.4%–18.5%)	0.252	17.5% (5.5%–30.8%)	0.003	5.2% (-5.5%–17.1%)	0.353
GluCer C24:0	5.5% (-6.4%–18.9%)	0.376	12.4% (-0.3%–26.6%)	0.056	22.6% (8.8%–38.1%)	0.001	5.4% (-6.5%–18.8%)	0.388
GluCer C24:1	0.099 (-0.168–0.367)	0.293	0.570 (0.303–0.838)	0.153	0.143 (-0.124–0.411)	<0.001	0.195 (-0.073–0.462)	0.465

Table 5 Summary of MDS-UPDRS part III (motor assessment, ON state) and MMSE total scores.

MDS-UPDRS part III and MMSE scores at baseline and after 28th dose				
	10 mg LTI-291 (N=10)	30 mg LTI-291 (N=10)	60 mg LTI-291 (N=10)	Placebo (N=10)
MDS-UPDRS PART III (ON STATE) (MEAN (SD))				
Predose	31.1 (17.4)	28.4 (12.6)	32.8 (14.3)	33.4 (12.7)
EOT	31 (13)	30 (9)	33 (12)	35 (15)
Change from baseline	-0.1 (7.6)	1.2 (7.1)	0.3 (8.9)	1.6 (6)
MMSE (MEAN (SD))				
Predose	27.3 (3.2)	27.4 (2.5)	28.7 (2.4)	27.7 (1.8)
EOT	27.3 (4.0)	28.0 (1.8)	29.0 (1.3)	27.5 (1.9)
Change from baseline	0.0 (2.0)	0.6 (2.3)	0.3 (2.0)	-0.2 (1.3)

MDS-UPDRS=Movement Disorder Society-Unified Parkinson's disease rating scale, MMSE=mini mental state examination, SD=standard deviation, EOT=end of treatment.

Figure 1 (A) The GCase reaction targeted by LTI-291, part of the sphingolipid (SL) recycling pathway. The SL pathway is a closed system, with de novo synthesis as input and sphingosine lyase as output. The former pathway is endogenously inhibited, but may play a role in maintaining GSL flux when GCase activity is low. Two pools of GluCer (lysosomal and non-lysosomal) exist, which are not distinguishable in our measures. (B) GBA-PD patients recycle ceramide slowly, so de novo synthesis of ceramide is increased and GluCer levels are maintained (left panel). Treatment with LTI-291 increases availability of cytosolic ceramide, resulting in a transient increase in GluCer synthesis (middle panel). However, increased ceramide levels are known to result in decreased de novo synthesis, bringing steady-state levels of GluCer and Cer back to the pre-dose levels at 28 days. However, the pre-dose and day 28 pathways differ in that the day 28 pathway has greater GSL flux (comparable amount, but faster rate) and reduced de novo synthesis. GCase = glucocerebrosidase, Cer = ceramide.

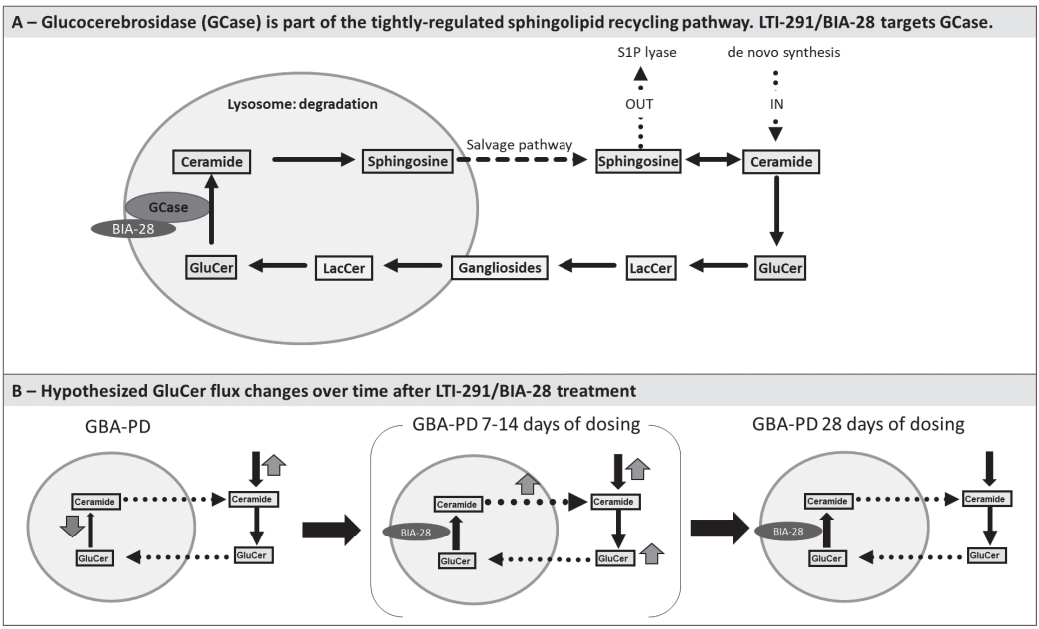
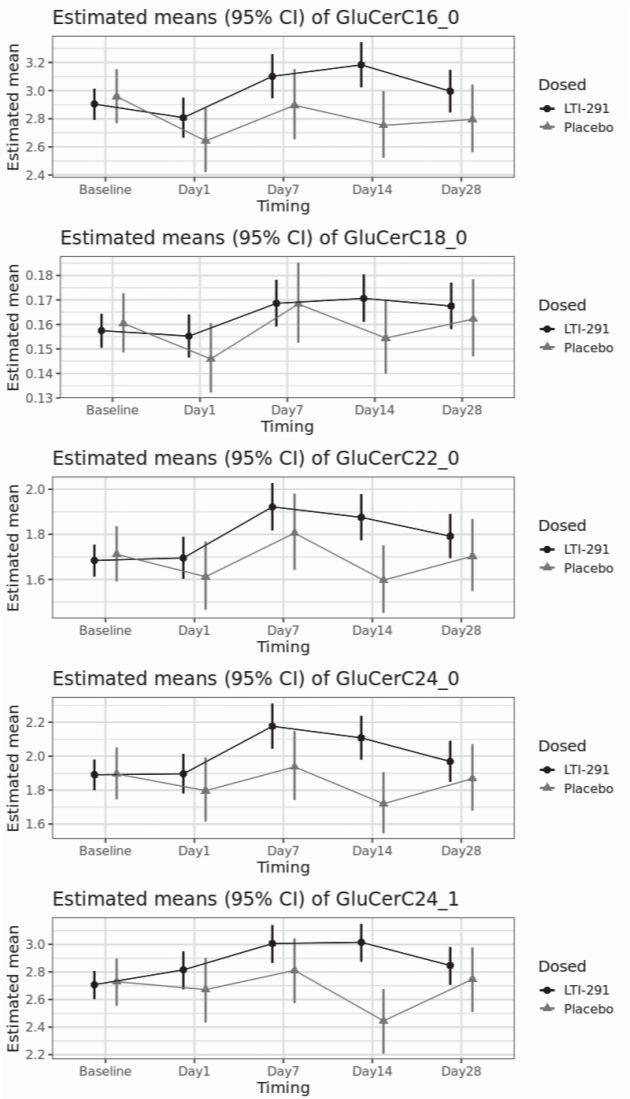


Figure 2 Graphs depicting the estimated means (95% confidence interval) of different GluCer isoforms over time, separate for participants treated with LTI-291/BIA-28 (all dose levels combined, n=30, dark bars) or placebo (n=10, light bars). The Day1 sample was taken 6 hours after dosing. Sample timing was the same for all participants, offset of the means and bars is for readability.



SUPPLEMENTARY MATERIAL

H8SF1 /H8ST1 / H8ST2 / H8ST3 / H8ST4 / H8ST5 / H8ST6

SCAN ME