Safety, tolerability, and efficacy of NLY01 in early untreated Parkinson's disease: a randomised, double-blind, placebo-controlled trial

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Summary

Background Converging lines of evidence suggest that microglia are relevant to Parkinson's disease pathogenesis, justifying exploration of therapeutic agents thought to attenuate pathogenic microglial function. We sought to test the safety and efficacy of NLY01—a brain-penetrant, pegylated, longer-lasting version of exenatide (a glucagon-like peptide-1 receptor agonist) that is believed to be anti-inflammatory via reduction of microglia activation—in Parkinson's disease.

Methods We report a 36-week, randomised, double-blind, placebo-controlled study of NLY01 in participants with early untreated Parkinson's disease conducted at 58 movement disorder clinics in the USA. Participants meeting UK Brain Bank or Movement Disorder Society research criteria for Parkinson's disease were randomly allocated (1:1:1) to one of two active treatment groups (2·5 mg or 5·0 mg NLY01) or matching placebo, based on a central computer-generated randomisation scheme using permuted block randomisation with varying block sizes. All participants, investigators, coordinators, study staff, and sponsor personnel were masked to treatment assignments throughout the study. The primary efficacy endpoint for the primary analysis population (defined as all randomly assigned participants who received at least one dose of study drug) was change from baseline to week 36 in the sum of Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) parts II and III. Safety was assessed in the safety population (all randomly allocated participants who received at least one dose of the study drug) with documentation of adverse events, vital signs, electrocardiograms, clinical laboratory assessments, physical examination, and scales for suicidality, sleepiness, impulsivity, and depression. This trial is complete and registered at ClinicalTrials.gov, NCT04154072.

Findings The study took place between Jan 28, 2020, and Feb 16, 2023. 447 individuals were screened, of whom 255 eligible participants were randomly assigned (85 to each study group). One patient assigned to placebo did not receive study treatment and was not included in the primary analysis. At 36 weeks, 2·5 mg and 5·0 mg NLY01 did not differ from placebo with respect to change in sum scores on MDS-UPDRS parts II and III: difference versus placebo –0·39 (95% CI –2·96 to 2·18; p=0·77) for 2·5 mg and 0·36 (–2·28 to 3·00; p=0·79) for 5·0 mg. Treatment-emergent adverse events were similar across groups (reported in 71 [84%] of 85 patients on 2·5 mg NLY01, 79 [93%] of 85 on 5·0 mg, and 73 [87%] of 84 on placebo), with gastrointestinal disorders the most commonly observed class in active groups (52 [61%] for 2·5 mg, 64 [75%] for 5·0 mg, and 30 [36%] for placebo) and nausea the most common event overall (33 [39%] for 2·5 mg, 49 [58%] for 5·0 mg, and 16 [19%] for placebo). No deaths occurred during the study.

Interpretation NLY01 at 2·5 and 5·0 mg was not associated with any improvement in Parkinson's disease motor or non-motor features compared with placebo. A subgroup analysis raised the possibility of motor benefit in younger participants. Further study is needed to determine whether these exploratory observations are replicable.

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Introduction

Parkinson's disease is a common progressive disorder involving dysfunction of numerous neurotransmitter networks, notably degeneration of the dopaminergic nigrostriatal pathway that facilitates voluntary movement. Symptomatic treatments can alleviate the symptoms of Parkinson's disease with variable success over time, although functional disability gradually accrues.[1] New therapies that are aimed at the underlying biology of disease and that can forestall functional disability are needed.

Parkinson's disease pathogenesis is linked to the accumulating effects of toxic α-synuclein species,[2] which are believed to misfold, aggregate, and provoke a variety of pathological responses, including inflammation and microglial activation.[3,4] This activation is thought to

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See **Comment** page 2

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Research in context

Evidence before this study

We searched PubMed on July 26, 2023, using the terms "Parkinson's disease", "exenatide", "GLP-1R agonist", and "clinical trials" for English-language papers published between May 20, 2013, and July 26, 2023. This search identified a single-blind study of exenatide—a glucagon-like peptide-1 receptor agonist—in 45 participants treated for 12 months, and a randomised, double-blind clinical trial of 62 participants treated with exenatide for 48 weeks; both studies suggested motor benefit from exenatide. In the double-blind exenatide study, findings also indicated relevant target engagement based on analysis of serum exosomes from participants, and there was a suggestion in a post-hoc analysis that older participants with a longer duration of Parkinson's disease might not respond as well to treatment. Single-blind studies and post-hoc analyses carry particular risk for bias and should be interpreted with appropriate caution.

Added value of this study

This was a larger study than any prior exenatide trial (255 randomly allocated participants) and used a different form of exenatide expected to maximise exposure (via PEGylation).

Implications of all the available evidence

No differences between groups in MDS-UPDRS parts II and III sum scores were noted in our study. Our findings contradict those from previous studies that suggest motor benefit with exenatide in Parkinson's disease, and so it remains uncertain whether exenatide can have a beneficial effect on motor function in Parkinson's disease. In our study, a prespecified analysis by age suggested that younger participants (<60 years) preferentially improved compared with older individuals, a finding that could be driven in part by a greater decline in the younger placebo group. Further studies should look to test a younger population for potentially better treatment outcomes. A phase 3, 96-week study of exenatide in 200 participants with mild to moderate Parkinson's disease on standard treatment closed recruitment in May 2022 and results are anticipated in 2024 (NCT04232969).

promote continued misfolding, neuronal dysfunction, and ultimately neurodegeneration.[5] Post-mortem analysis of brain tissue from people with Parkinson's disease revealed activated microglia and accumulation of inflammatory mediators in the substantia nigra.[6] In individuals with Parkinson's disease who received transplanted embryonic dopaminergic neurons, activated microglia preceded the appearance of α-synuclein inclusions in the transplanted tissue, suggesting that the inflammatory process might trigger the accumulation of aggregates.[7] PET also shows an increase in microglial activation in the early stages of Parkinson's disease.[8] Microglial activation in early untreated Parkinson's disease correlates inversely with dopaminergic terminal density and directly with motor impairment.[9] In addition to producing neurotoxic cytokines, activated microglia also induce differentiation of astroglial cells into neurotoxic reactive astrocytes, which are direct mediators of neuronal cell death.[10] These converging lines of evidence suggest that microglia are relevant to Parkinson's disease pathogenesis, justifying the exploration of therapeutic agents thought to attenuate pathogenic microglial function.

The glucagon-like peptide-1 receptor (GLP-1R) is present in the brain, and agonist activity is believed to be anti-inflammatory via reduction of microglia activation.[11] In both prospective and retrospective studies, data have suggested that GLP-1R agonists could hold promise as treatments for Parkinson's disease. Exenatide is a GLP-1R agonist approved for the treatment of diabetes.[12] In a database of more than 100 000 individuals with diabetes, those using GLP-1R agonists had a 62% lower risk of developing Parkinson's disease.[13] A double-blind, placebo-controlled study of exenatide in people with Parkinson's disease receiving dopaminergic treatment and experiencing wearing off demonstrated improvement in Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III (motor examination) scores in the practically defined off state at 60 weeks (–3·5 point difference; p=0·0318).[14] Active treatment ended at 48 weeks, suggesting that exenatide could have lasting effects beyond the duration of exposure.

NLY01 is a brain-penetrant pegylated analogue of exenatide. Preclinical work in mice has shown that NLY01 protects against dopaminergic neuronal loss and motor dysfunction and prolongs survival, effects that were predominantly achieved through microglial inhibition. NLY01 has shown an ability to attenuate preformed fibril-induced mRNA induction for *Il1a*, *Il1b*, *Tnfα*, *C1qa*, and *Il6*, corresponding protein levels for IL-1α, TNFα, IL-1ß, and C1q, and blockage of downstream astrocytic conversion to the neurotoxic reactive astrocyte phenotype.[15] We sought to extend the observations from the studies with exenatide and the preclinical work with NLY01. Therefore, we aimed to investigate the safety and efficacy of NLY01 in people with early untreated Parkinson's disease with respect to change in MDS-UPDRS parts II and III sum scores at week 36.

Methods

Study design

We conducted a 36-week, randomised, double-blind, placebo-controlled study at 58 movement disorder clinics

in the USA. Institutional review boards at all sites provided ethics approval for study activity. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice Guidelines.

Participants

All participants provided written informed consent. Key inclusion criteria were: diagnosis of Parkinson's disease consistent with UK Brain Bank or Movement Disorder Society research criteria for Parkinson's disease; dopamine transporter (DaT) imaging consistent with Parkinson's disease; age 30–80 years; Hoehn and Yahr no greater than 2·5 at screening; and a Montreal Cognitive Assessment (MoCA) score of at least 24. The MoCA cutoff was originally set at 26, but it was adjusted through a protocol amendment to 24 after study team discussion to facilitate recruitment. Key exclusion criteria were: diagnoses of secondary or atypical parkinsonism; onset of parkinsonism more than 5 years before screening; previous treatment with Parkinson's disease medications for more than 28 days, or within 14 days of screening (irreversible type-B monoamine oxidase inhibitors were discontinued at least 90 days before screening); and a current diagnosis of diabetes. Complete inclusion and exclusion criteria are available in the appendix (pp 77–79).

Randomisation and masking

Participants were randomly allocated (1:1:1) to one of two active treatment groups (2·5 mg or 5·0 mg NLY01) or placebo, using a central computer-generated randomisation scheme with permuted block randomisation and varying block sizes. The production randomisation schedule was generated by the Rho Unblinded Interactive Web Response System team and was uploaded into Medidata Randomization and Trial Supply Management. Site staff used this software to randomly allocate participants and receive kit numbers to dispense to them. Randomisation was not stratified. Placebo was matched to the study drug for physical characteristics and packaging. All participants, investigators, coordinators, study staff, and sponsor personnel were masked to treatment assignments throughout the study.

Procedures

During the screening visit (V1), all candidates underwent DaT imaging. Participants were further evaluated by an enrolment authorisation committee for suitability and eligibility to participate. After approval by the enrolment authorisation committee, participants were randomly allocated to either 2·5 mg NLY01, 5·0 mg NLY01, or placebo and proceeded to the baseline visit (V2) within 60 days of screening. After pre-dose baseline assessments, participants self-administered the study drug subcutaneously while under supervision and were monitored for adverse events for at least 1 h before discharge. Participants were then instructed to administer the study drug once a week for the next 36 weeks. Supervised study drug administration and surveillance took place at weeks 2 (V3) and 3 (V4). Thereafter, in-person visits for clinical assessments occurred at weeks 4 (V5), 12 (V6), 24 (V7), and a final visit while on the study drug at week 36 (V8). Safety follow-up visits took place at weeks 40 (V9) and 44 (V10), with V10 including clinical assessments. Telephone contact took place between in-person visits throughout the study.

DaT imaging was repeated at week 36. Blood draws for anti-NLY01 antibodies, population pharmacokinetic assessments, and future exploratory analyses not specified in the protocol were drawn throughout the study. Protocol adherence and study drug accountability were monitored throughout. If felt medically necessary, initiation of anti-Parkinson's disease medication was allowed during the study.

Outcomes

The primary efficacy endpoint was the change from baseline to week 36 in the sum of MDS-UPDRS scores for parts II and III. The MDS-UPDRS is a well established and widely used assessment to quantify the signs and symptoms of Parkinson's disease. The MDS-UPDRS has four parts: part I (non-motor aspects of experiences of daily living), part II (motor aspects of experiences of daily living), part III (motor examination), and part IV (motor complications). Each subscale has a rating from 0 (normal) to 4 (severe). Because our study participants were untreated individuals with early Parkinson's disease without motor complications, part IV was not used. For parts I, II, and III, lower numerical scores indicate less impairment of performance or function, whereas higher scores indicate greater impairment.

See Online for appendix

Secondary endpoints were changes from baseline to week 36 in MDS-UPDRS part I, the Clinical Global Impression of Severity (CGI-S), the Patient Global Impression of Severity (PGI-S), individual scores for MDS-UPDRS parts II and III, the Schwab and England Activities of Daily Living Scale (SE-ADL), the Parkinson's Disease Questionnaire 39 (PDQ-39), MoCA, Scales for Outcomes of Parkinson's Disease Cognition (SCOPA-Cog), the Non-Motor Symptoms Scale (NMSS), and DaT imaging parameters. Safety was assessed with documentation of adverse events collected at in-person and telephone visits, vital signs, electrocardiograms, clinical laboratory assessments, physical examination, and scales for suicidality (Columbia Suicide Severity Rating Scale), sleepiness (Epworth Sleepiness Scale), impulsivity (Questionnaire for Impulsive–Compulsive Disorders in Parkinson's Disease—Rating Scale), and depression (Beck Depression Inventory II). Adverse events were graded as mild, moderate, or severe and judged for relatedness to the study drug.

Statistical analysis

We calculated that a sample size of approximately 80 participants per group (2·5 mg, 5·0 mg, and matching placebo) would provide more than 80% power to detect a 4-point difference between either treatment group and placebo in mean change from baseline in the sum of MDS-UPDRS scores for parts II and III, with an SD of 8, two-sided α of 0·05, and 15% dropout assumption. The 4-point difference was based on the previous performance of exenatide in a 48-week study (3·5 point difference in MDS-UPDRS part III) and expectation that part II would decline by about 1 point over 1 year in a population with early Parkinson's disease;[14,16] the SD (twice the effect size) was felt to be appropriately conservative and give realistic power to the study.

The primary analysis of the primary endpoint (treatment difference of least squares mean change from baseline to week 36 in the sum of MDS-UPDRS parts II and III for 2·5 and 5·0 mg NLY01 *vs* placebo) was conducted in a modified intention-to-treat (mITT) population using a mixed model for repeated measures. mITT was defined as participants who were randomly allocated and received at least one dose of the study drug. Data collected after initiation of anti-parkinsonian medication were not included in the primary analysis if such medication was initiated after randomisation. Statistics were calculated using a restricted maximum-likelihood mixed model for repeated measures analysis, with fixed effects for treatment group, week, and treatment-by-week interaction, and associated baseline sum score as a continuous covariate. Within-subject variability was modelled using an unstructured covariance matrix and denominator degrees of freedom calculated using Kenward-Roger approximation. Type I error was maintained at an overall α of 0·05 using a Hochberg step-up procedure to analyse both dose levels, with no predefined order for their analysis. If both doses had p values below 0·05, both would be declared significant; if one p value was greater than 0·05, the other would need to be below 0·025 to be significant. Secondary endpoints were not alpha-protected and were treated as exploratory. The secondary endpoints MDS-UPDRS parts I–III, SE-ADL, PDQ-39, and NMSS were analysed using a mixed model for repeated measures. CGI-S, PGI-S, MoCA, and SCOPA-Cog were analysed using an ANCOVA model with baseline values as covariates and treatment group as a fixed effect.

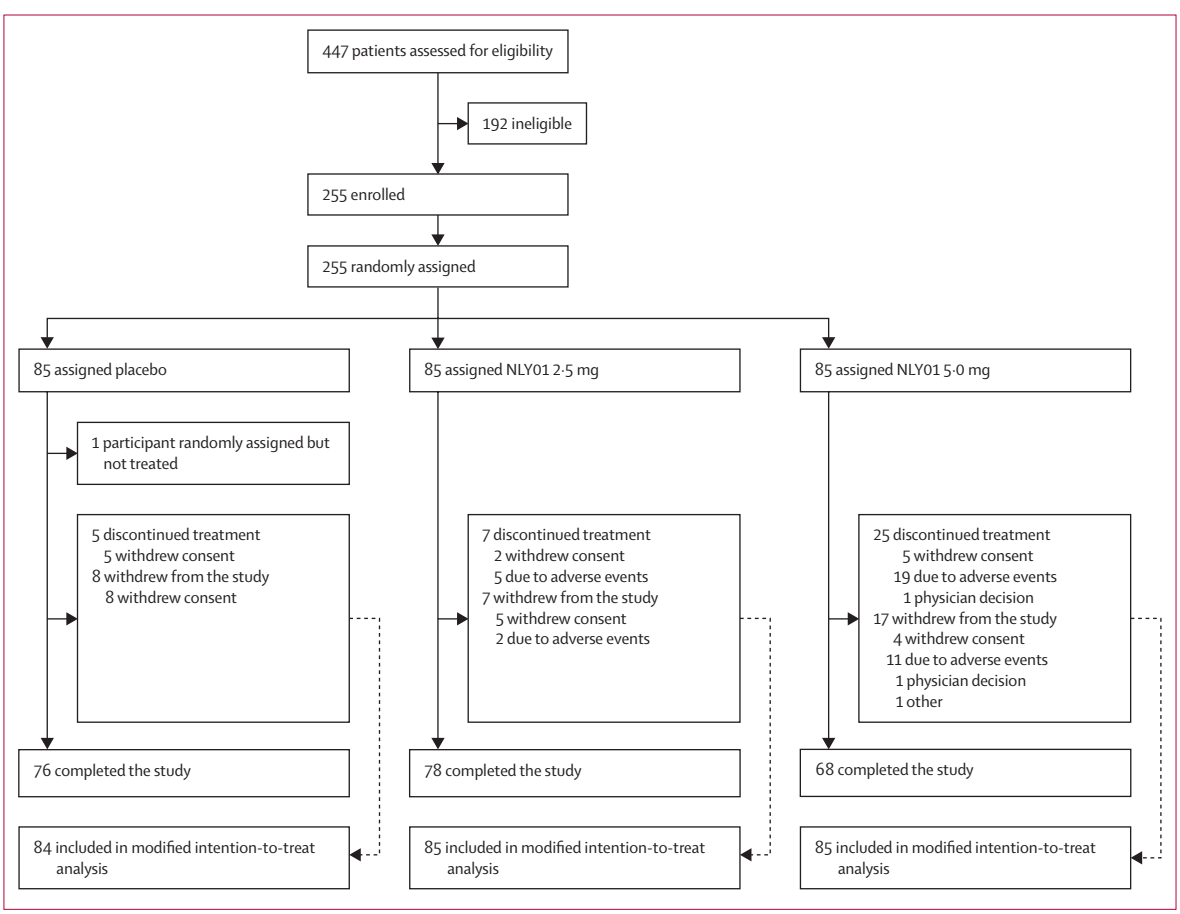

Figure 1: **Trial profile**

	Placebo (n=84)	NLY01 2·5 mg (n=85)	NLY01 5·0 mg (n=85)
Age, years	61·8 (8·1)	62·1 (9·0)	60·6 (10·0)
Sex			
Male	52 (62%)	60 (71%)	54 (64%)
Female	32 (38%)	25 (29%)	31 (36%)
Race			
White	81 (96%)	82 (96%)	80 (94%)
Asian	2 (2%)	2 (2%)	4 (5%)
Not reported	1 (1%)	1 (1%)	1 (1%)
Weight, kg	77·8 (16·2)	80·8 (16·6)	79·1 (17·4)
BMI, kg/m^2	26·03 (4·66)	26·42 (4·14)	25·81 (4·58)
Hoehn and Yahr stage			
0	0	0	0
1	14 (17%)	22 (26%)	15 (18%)
1·5	8 (10%)	3 (4%)	7 (8%)
2	58 (69%)	57 (67%)	60 (71%)
2·5	4 (5%)	3 (4%)	3 (4%)
Duration of Parkinson's disease, days	328·0 (363·7)	370·9 (378·8)	351·6 (341·7)
MDS-UPDRS score			
Part I	4·7 (4·2)	4·2 (3·1)	4·0 (3·7)
Part II	4·9 (3·6)	4·8 (3·6)	5·0 (4·1)
Part III	22·3 (9·1)	22·7 (8·1)	22·0 (8·2)
Sum of parts II and III	27·2 (10·3)	27·5 (10·0)	27·0 (10·3)

Data are mean (SD) or n (%). MDS-UPDRS=Movement Disorder Society Unified Parkinson's Disease Rating Scale.

Table 1: **Demographics and disease characteristics at baseline in the modified intention-to-treat population**

	Placebo (n=84)	NLY01 2·5 mg (n=85)	NLY01 5·0 mg (n=85)
Any treatment-emergent adverse event	73 (87%)	71 (84%)	79 (93%)
Gastrointestinal disorders	30 (36%)	52 (61%)	64 (75%)
Nausea	16 (19%)	33 (39%)	49 (58%)
Constipation	6 (7%)	10 (12%)	14 (16%)
Vomiting	1 (1%)	4 (5%)	22 (26%)
Diarrhoea	7 (8%)	7 (8%)	11 (13%)
Dyspepsia	2 (2%)	8 (9%)	14 (16%)
Gastro-oesophageal reflux	2 (2%)	9 (11%)	13 (15%)
Abdominal discomfort	4 (5%)	5 (6%)	4 (5%)
Eructation	1 (1%)	4 (5%)	8 (9%)
Abdominal distention	1 (1%)	4 (5%)	5 (6%)
Nervous system disorders	34 (40%)	25 (29%)	39 (46%)
Headache	14 (17%)	14 (16%)	20 (24%)
Dizziness	7 (8%)	3 (3·5)	7 (8·2)
Worsening of parkinsonism	7 (8%)	3 (4%)	6 (7%)
General and administration site disorders	31 (37%)	32 (38%)	34 (40%)
Fatigue	11 (13%)	10 (12%)	12 (14%)
Injection site bruising	13 (15%)	9 (11%)	7 (8%)
Injection site erythema	1 (1%)	7 (8%)	8 (9%)
Infections	28 (33%)	14 (16%)	23 (27%)
COVID-19	11 (13%)	7 (8%)	13 (15%)
Urinary tract infection	6 (7%)	3 (4%)	2 (2%)
Musculoskeletal disorders	20 (24%)	19 (22%)	16 (19%)
Arthralgia	2 (2%)	5 (6%)	3 (4%)
Injury and procedural complications	16 (19%)	10 (12%)	10 (12%)
Skin and subcutaneous skin disorders	11 (13%)	9 (11%)	13 (15%)
Investigations	6 (7%)	11 (13%)	12 (14%)
Weight decreased	2 (2%)	5 (6%)	5 (6%)
Metabolism and nutrition disorders	7 (8%)	8 (9%)	13 (15%)
Decreased appetite	5 (6%)	7 (8%)	13 (15%)
Psychiatric disorders	7 (8%)	9 (11%)	12 (14%)
Anxiety	1 (1%)	3 (4%)	5 (6%)
Vascular disorders	5 (6%)	8 (9%)	7 (8%)
Renal and urinary disorders	9 (11%)	6 (7%)	4 (5%)
Respiratory and thoracic disorders	5 (6%)	2 (2%)	9 (11%)

Data are n (%). Treatment-emergent adverse events occurring with a frequency of 5% or greater in any group are shown.

Table 2: **Treatment-emergent adverse events**

Analyses of the primary endpoint were also conducted on per-protocol, completer, and intention-to-treat populations and prespecified subgroups (age, sex, and race). The per-protocol population was all mITT participants who had no major protocol deviations with respect to the primary outcome measure. Completers were all mITT participants who completed all protocol-specified tests and observations and completed treatment as per the protocol. A sensitivity analysis for the primary endpoint using the missing-at-random and missing-not-at-random assumptions was done with a placebo-based multiple-imputation pattern-mixture model. The safety analysis was conducted on all randomly allocated participants who received at least one dose of the study drug. Analyses were conducted using the SAS System, version 9.4.

This trial is registered at ClinicalTrials.gov, NCT04154072.

Role of the funding source

The funder participated in the design of this study, analysis and interpretation of data, and considerations on submission for publication. All sponsor authors had complete access to study data.

Results

This study was conducted from Jan 28, 2020, to Feb 16, 2023, at 58 sites in the USA. 447 individuals were screened, of whom 192 were deemed ineligible. 255 eligible participants were randomly allocated to study groups, with 85 people allocated per group (figure 1). One participant who was randomly assigned to placebo did not receive study treatment and was not included in the mITT analysis. Baseline characteristics were similar across treatment groups (table 1).

217 (85%) participants completed the 36-week treatment period. 37 (15%) people discontinued treatment (25 [29%] in the 5·0 mg group, seven [8%] in the 2·5 mg group, and five [6%] in the placebo group). The most common reason

	NLY01 2·5 mg dose				NLY01 5·0 mg dose		
	NLY01 2·5 mg (n=85)	Placebo (n=84)	Difference from placebo at week 36	p value	NLY01 5·0 mg (n=85)	Difference from placebo at week 36	p value
Primary outcome							
MDS-UPDRS parts II and III	5·2 (0·9)	5·6 (0·9)	−0·39 (−2·96 to 2·18)	0·77	5·9 (1·0)	0·36 (−2·28 to 3·00)	0·79
Secondary outcomes							
MDS-UPDRS part I	0·5 (0·4)	0·5 (0·4)	0·04 (−0·98 to 1·06)	0·94*	0·5 (0·4)	0·01 (−1·03 to 1·06)	0·98*
MDS-UPDRS part II	2·0 (0·4)	1·7 (0·4)	0·29 (−0·84 to 1·42)	0·61*	1·8 (0·4)	0·14 (−1·02 to 1·29)	0·82*
MDS-UPDRS part III	3·2 (0·8)	3·9 (0·8)	−0·70 (−2·88 to 1·49)	0·53*	4·1 (0·8)	0·24 (−2·01 to 2·49)	0·84*
CGI-S	0·3 (0·1)	0·2 (0·1)	0·10 (−0·12 to 0·31)	0·38*	0·3 (0·1)	0·08 (−0·15 to 0·30)	0·50*
PGI-S	0·3 (0·1)	0·3 (0·1)	−0·01 (−0·31 to 0·28)	0·93*	0·2 (0·1)	−0·05 (−0·35 to 0·24)	0·72*
SE-ADL	−2·2 (0·7)	−2·1 (0·7)	−0·11 (−2·07 to 1·85)	0·91*	−1·4 (0·7)	0·76 (−1·25 to 2·76)	0·46*
PDQ-39	1·7 (0·7)	2·5 (0·7)	−0·73 (−2·63 to 1·16)	0·45*	1·9 (0·7)	−0·57 (−2·44 to 1·31)	0·55*
MoCA	−0·8 (0·2)	−1·0 (0·3)	0·20 (−0·49 to 0·90)	0·56*	−0·9 (0·3)	0·14 (−0·59 to 0·86)	0·71*
SCOPA-Cog	0·1 (0·4)	−0·4 (0·4)	0·45 (−0·59 to 1·49)	0·39*	0·4 (0·4)	0·73 (−0·35 to 1·81)	0·18*
NMSS	4·1 (1·4)	1·9 (1·5)	2·19 (−1·82 to 6·21)	0·28*	1·3 (1·5)	−0·58 (−4·67 to 3·50)	0·78*

Data are mean (SE), least squares mean (95% CI), or p value. MDS-UPDRS=Movement Disorder Society Unified Parkinson's Disease Rating Scale. CGI-S=Clinical Global Impression of Severity. PGI-S=Patient Global Impression of Severity. SE-ADL=Schwab and England Activities of Daily Living Scale. PDQ-39=Parkinson's Disease Questionnaire 39. MoCA=Montreal Cognitive Assessment. SCOPA-Cog=Scales for Outcomes of Parkinson's Disease Cognition. NMSS=Non-Motor Symptoms Scale. *Indicates value is nominal and exploratory only.

Table 3: **Changes from baseline in primary and secondary outcome measures at week 36 in the modified intention-to-treat population**

for discontinuation was adverse events (five [6%] in the 2·5 mg group and 19 [22%] in the 5·0 mg group). For 32 (13%) participants, the study was terminated early (17 [20%] in the 5·0 mg group, seven [8%] in the 2·5 mg group, and eight [10%] in the placebo group). Early terminations were most commonly from withdrawal of consent (n=17, 7%) and adverse events (n=13, 5%). 29 (11%) participants started Parkinson's disease treatment before week 36 (eight [9%] in the 5·0 mg group, nine [11%] in the 2·5 mg group, and 12 [14%] in the placebo group). 12 (5%) participants who started Parkinson's disease treatment subsequently withdrew from the study before the end of the treatment phase (six [7%] in the 5·0 mg group, three [4%] in the 2·5 mg group, and three [4%] in the placebo group). Compliance with study treatment, as determined by drug accountability, was generally high (85% for placebo, 82% for 2·5 mg, and 77% for 5·0 mg).

NLY01 was generally safe and well tolerated, with most adverse events of mild or moderate severity (table 2). Injection-related adverse events were similar across groups, with bruising more common in placebo (13 [15%] *vs* nine [11%] for 2·5 mg and seven [8%] for 5·0 mg) and injection site erythema more common in active groups (seven [8%] for 2·5 mg and eight [9%] for 5·0 mg *vs* one [1%] for placebo). Gastrointestinal disorders were the most common system organ class for adverse events. This class of adverse events was most prevalent in the 5·0 mg group, particularly nausea (49 [58%] *vs* 33 [39%] in 2·5 mg and 16 [19%] in placebo) and vomiting (22 [26%] *vs* four [5%] in the 2·5 mg group and one [1%] in placebo). Gastrointestinal side effects were primarily temporary with onset after administration of the study drug, resolution within 1–2 days, and mild to moderate severity. Weight loss was slightly more common in active groups (five [6%] for both 2·5 mg and 5·0 mg *vs* two [2%] in placebo). Other safety-related outcomes (Epworth Sleepiness Scale, Columbia Suicide Severity Rating Scale, and Beck Depression Inventory II) did not differ between either active group compared with placebo at 36 weeks (appendix pp 18–29).

At 36 weeks, treatment with 2·5 mg or 5·0 mg NLY01 did not differ from placebo for the primary endpoint of change from baseline in sum scores for MDS UPDRS parts II and III (table 3, figure 2). Differences from baseline at week 36 compared with placebo were –0·39 (95% CI –2·96 to 2·18, p=0·77) for 2·5 mg and 0·36 (–2·28 to 3·00, p=0·79) for 5·0 mg. Observed exploratory values at week 44 did not reflect improvement compared with placebo (treatment difference 0·1 [3·83 to 7·97] for 2·5 mg and 1·0 [4·68 to 8·92] for 5·0 mg; appendix p 1). Sensitivity analyses of the primary endpoint (placebo-based imputation for missing data, and inclusion and exclusion of data after start of anti-parkinsonian medication) and primary endpoint analyses of per-protocol (n=224) and completer (n=222) populations did not differ from placebo for either dose.

None of the secondary endpoints differed from placebo for either dose (table 3). Prespecified CGI-S responder analyses at 36 weeks did not reflect improvement compared with placebo (18% placebo *vs* 6% for 2·5 mg, p=0·047; and 8% for 5·0 mg, p=0·16). Specific DaT data are available in the appendix (pp 4–17).

A prespecified subgroup analysis of participants younger than 60 years of age versus those aged 60 years

or older was performed. 31 participants in the 2·5 mg group, 34 in the 5·0 mg group, and 30 in the placebo group were younger than 60 years. Nominally significant reductions in the change from baseline in the sum of scores on MDS-UPDRS parts II and III at 36 weeks were observed compared with placebo (difference –5·11, nominal p=0·006 for 2·5 mg; and –5·01, nominal p=0·007 for 5·0 mg; appendix pp 2–3). This effect was not observed in participants aged 60 years or older, nor in subanalyses by gender or race. Repeat post-hoc analyses performed in the same age groups confirmed these findings, and they suggested the observation was primarily driven by MDS-UPDRS part III score (–4·3 points for both 2·5 mg and 5·0 mg, nominal p=0·008; appendix p 1).

Discussion

In the present study, NLY01 at 2·5 mg and 5·0 mg delivered subcutaneously for 36 weeks was not associated with an improvement in Parkinson's disease motor or non-motor features. Results for global measures of improvement (CGI-C and PGI-C) were also consistent with absence of treatment effect. NLY01 was generally safe and well tolerated. The adverse effect profile was as expected with this class of compound, with gastrointestinal symptoms (nausea and vomiting) being prevalent in a dose-dependent fashion. More participants dropped out in the 5·0 mg group due to adverse events, including gastrointestinal intolerance; future work could look to mitigate tolerability issues at higher doses, potentially with different titration schedules at the time of initiation. Relatively few notable injection-site reactions were recorded, which was a prespecified event of interest. Most participants were able to complete the 9-month study period, with a relatively low number starting anti-Parkinson's disease medications during the study. Plasma NLY01 levels remained consistent throughout the study, suggesting sufficient exposure to test for treatment effect (appendix p 2).

Considering the promising preclinical data and suggestion of motor benefit for exenatide that was observed previously, including evidence of target engagement in exosomes, it is not clear why NLY01 did not have a beneficial effect on clinical outcome measures in this large, double-blind study.[15,17] Although NLY01 was designed with a polyethylene glycol configuration to allow for blood–brain barrier penetration, and CNS penetration has been demonstrated in mice,[15] dogs (unpublished data), and non-human primates (unpublished data), it is possible that NLY01 was unable to achieve sufficient concentration in the brain for an unknown reason. Human equivalent doses of 1·6–16·0 mg/week were associated with benefit in preclinical models; given the approximately threefold accumulation expected when administered once weekly in people, the 2·5 mg and 5·0 mg doses used in the study were expected to correspond to single doses of 7·5 mg and 15·0 mg, respectively, well within the expected efficacious range. It could be that a longer duration of treatment or observation is required for this particular molecule; in the previous exenatide clinical trial, a 48-week treatment period was used. Alternatively, participants at an even earlier, potentially presymptomatic, stage of Parkinson's disease might be more likely to show beneficial change. These considerations face the practical challenges of measuring meaningful clinical change in individuals with very early Parkinson's disease, as well as the realistic duration of clinical trials recruiting untreated participants. Assessment of treatment effects might be limited in participants with mild symptoms and variable disease progression. Reduction in microglial activation and astrocytic conversion alone might not be sufficient to alter pathology, and clinical characteristics might need longer to exert noticeable clinical effects or might require a combination of interventions to achieve benefit.

Participants in our study were generally young (mean age 61·5 years), with an average time since diagnosis of 350 days and a baseline MDS-UPDRS part III score of 22·4, reflecting participants at an early stage of Parkinson's disease and with mild motor signs and symptoms. Further subanalysis by age (<60 years *vs* ≥60 years) suggested a beneficial effect on the primary endpoint for the youngest participants. It is unclear whether this finding represents a preferential treatment effect, since the placebo deterioration in the younger subgroup (9·21 [1·3]) was substantially different compared to that for those aged 60 years and

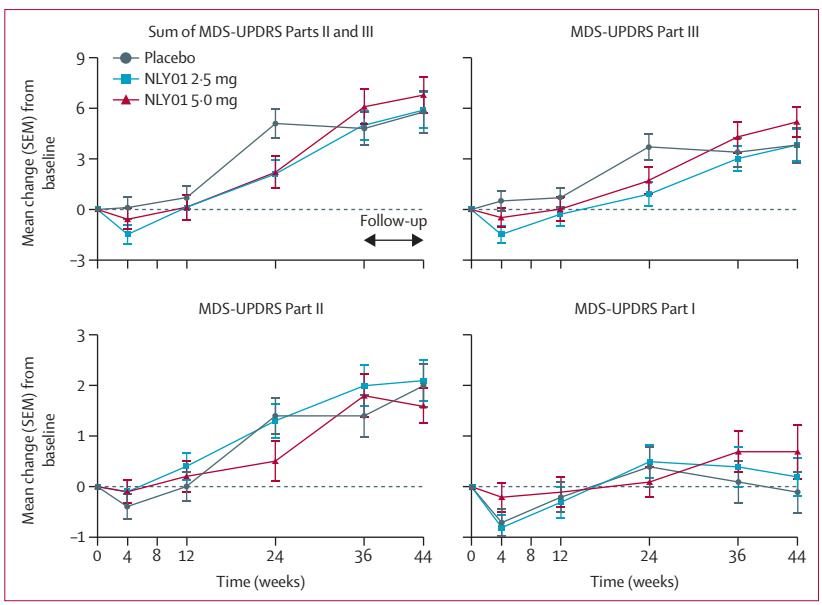

Figure 2: **Changes from baseline in primary and secondary MDS-UPDRS outcome measures during the treatment period and at post-treatment follow-up in the mITT population**

Primary and secondary outcome measures were assessed at week 36. Datapoints represent absolute mean changes; error bars represent SEM. MDS-UPDRS=Movement Disorder Society Unified Parkinson's Disease Rating Scale. mITT=modified intention-to-treat population.

older (3·42 [1·2]), whereas the magnitude of change from baseline for the group aged under 60 years (4·09 [1·2] for 2·5 mg and 4·20 [1·3] for 5·0 mg) was more modestly different compared with the older group (6·02 [1·2] for 2·5 mg and 7·0 [1·3] for 5·0 mg). In a post-hoc analysis of the Exenatide-PD study, younger participants in the placebo group appeared to deteriorate on MDS-UPDRS part III more than the older participants did, but the differences were smaller than in our study (age <50 years: n=4, 1·28 points; age 50–64 years: n=19, 2·12 points; age >65 years: N=6, 0·67 points).[18] Younger participants receiving the active drug also appeared to experience greater improvement from baseline in MDS-UPDRS part III (age <50 years: –4·71 points [95% CI –28·5 to 16·5], p=0·50; age 50–64 years: –3·38 points [–9·4 to –1·5], p=0·008; age >65 years: –0·93 points [–5·8 to 2·6], p=0·44). It is not clear why the younger placebo group would deteriorate more quickly than the older comparator; conversely, it might be expected that younger people could have better compensatory resiliency and perhaps decline more slowly than older people, a hypothesis for which there is support in the literature. Several studies suggest motor progression is faster in older people.[19–24] In a study of 129 patients with Parkinson's disease across a wide range of ages, younger participants also took longer to reach non-motor progression milestones of frequent falling, cognitive impairment, or hallucinations, suggesting a longer disease course in early-onset cases.[25]

An alternative explanation for this placebo observation is regression towards the mean, such that the change seen in younger participants does not accurately reflect the true average change for this subgroup. These data should be interpreted with caution given the small numbers and questionable plausibility of the placebo finding. Apart from this issue, although the magnitude of decline is numerically less in the younger group, it is not obvious why this should be biologically plausible. Whether or not microglial activation and astrocytic conversion are more relevant or robust a target in younger participants, or reduction thereof is more therapeutically efficient in that subgroup, is unknown but deserving of further exploration.

Overall, NLY01 was generally safe and tolerable, with a side-effect profile consistent with expectations. Efficacy endpoints were not met, but a subgroup analysis raised the possibility of motor benefit in younger participants. Further study, possibly including exploration of target engagement, is needed to determine whether these exploratory observations are replicable.

Contributors

AM was involved in study design, data collection, analysis, interpretation, and writing the first draft of the manuscript. SR performed the main statistical analysis for the study. ML performed post-hoc exploratory statistical analyses. KKi, CWO, DT, AB, DL, and VR were involved in study design, data collection, analysis, interpretation, and reviewing the manuscript. AM and DT verified the data used for analysis and interpretation. AM, DT, CWO, AB, DL, VR, and KKi had final responsibility for the decision to submit for publication. JD, SL, and TMD reviewed the manuscript. JC was the medical monitor for the study. RD, DB, SP, JF, JQ, RP, MA, AR-Z, KC, AT, CL, KKl, YB, M-HSH, and DS were investigators who collected study data and reviewed the manuscript. All authors had full access to the data.

Declaration of interests

AM, KKi, CWO, ML, and JD report receiving salary support from Neuraly for their work in the study. SR and JC report being employees of the contract research organisation (Rho) and receiving support from Neuraly for their work. DT, AB, DL, SL, and VR report being employees of Neuraly. RD, DB, SP, JF, JQ, RP, MA, AR-Z, KC, AT, CL, KKl, YB, M-HSH, and DS report being investigators who received research support from Neuraly. TMD reports receiving compensation for consulting or advising services in the provision of stock and equity in D&D Pharmatech.

Data sharing

All study data, including de-identified participant data, data dictionaries used, and study documents (protocol, statistical analysis plan, and informed consent), will be made available upon request to DT (dto@neuralymed.com) from the time of publication onwards.

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