First-in-Man Clinical Trial of Intraputamenal CDNF in Parkinson's Disease Finds a Consorted Biomarker Response in a Subgroup of Subjects

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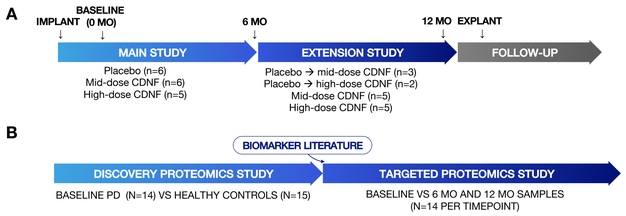
BACKGROUND

Cerebral Dopamine Neurotrophic Factor (CDNF) is a member of a novel family of unconventional neurotrophic factors which is structurally and mechanistically very distinct from all known neurotrophic factors. CDNF protects midbrain dopaminergic neurons and improves both motor and non-motor functions in rodent and primate models of PD (Lindholm et al, 2007) via a multimodal mechanism that involves modulation of endoplasmic reticulum (ER) stress, α-synuclein aggregation and toxicity, and neuroinflammation (Huttunen & Saarma, 2019).

A Phase I first-in-human clinical study in moderately advanced Parkinson's disease (PD) patients with monthly intraputamenal infusions of recombinant human CDNF protein (using the Renishaw Neuro Solutions drug delivery system) was conducted at three study centers in Sweden and Finland. Secondary endpoints included clinical measures including UPDRS. Exploratory endpoints included dopamine transporter PET imaging and a targeted proteomics study of CSF samples collected at baseline, and after 6 and 12 months of treatment with CDNF (or placebo during the first six months). A panel of 50 CSF biomarkers revealed changing profiles, particularly in the mid-dose CDNF group indicative of biological response to the treatment. In 3 of 17 subjects a clear CSF biomarker profile change was associated with an improvement in UPDRS part III (off) or dopamine transporter PET signal.

Figure 1. Study design of the CDNF Phase I study and the CSF proteomics studies.

(**A**) All subjects started with either placebo or low-dose CDNF for the two first infusions, and then continued on placebo or mid or high-dose CDNF in the Main study. In the Extension study, all patients received six additional monthly infusions of CDNF. Vehicle group received ascending low-mid-high doses while Mid-dose group remained on mid dose and High-dose group remained on high dose. Arrows indicate PET imaging timepoints. IMPL = device implantation; EXPL = device explantation. All patients were enrolled to a follow-up study without study treatment for up to four years. The results presented in this poster are from the randomized, placebo-controlled Main study part highlighted in red. (**B**) Discovery proteomics study was conducted with baseline CSF samples from study participants and age-matched healthy controls. A targeted proteomics study was conducted with CSF samples collected at baseline, 6-month and 12-month timepoints.



METHODS

Clinical study design

A phase I-II clinical trial with 17 subjects with idiopathic PD of moderate severity enrolled in a placebo-controlled, double-blind six-month main study (the main study) followed by a six-month active treatment extension study. Patients randomized to placebo (n=6) or incremental CDNF dosage (n=6 for low-mid dose and n=5 for low-mid-high dose) groups received doses every four weeks via an intraputamenal drug delivery device (Renishaw Plc). The clinical results of the study have been presented before (MDS 2020; manuscript in review).

Investigational medicinal product (IMP)

- Recombinant human CDNF (rhCDNF), 1.0 mg/ml concentrate for solution for infusion
- Placebo / Vehicle: artificial cerebrospinal fluid without glucose (aCSF)

Investigational medicinal device (IMD)

The Drug Delivery System (DDS) and the Drug delivery module of neuroinspire™ surgical planning software were designed and manufactured by Renishaw Neuro Solutions Ltd. The system is intended to be implanted to specific anatomical targets in the deep brain and has transcutaneous access port to facilitate chronic intermittent infusion to specific intraparenchymal targets via 4 implanted catheters.

Study endpoints

- Primary endpoint: safety and tolerability of the IMP (rhCDNF)
- Co-primary endpoint: safety and tolerability of the Investigational Medicinal Device, and accuracy of device implantation
- Secondary endpoints included: Unified Parkinson's Disease Rating Scale (UPDRS III-OFF and UPDRS I-IV), patient home diary, timed up and go (TUG), PDQ-39 and clinical global impression (CGI).
- Exploratory endpoints included: dopamine transporter (DAT) PET, actigraphy (PKG®), α-synuclein (total, phospho-Ser129, oligomeric, aggregation propensity) in CSF and serum, levels of infused rhCDNF in CSF and serum, and, proteomic biomarker screen in CSF.

Regulatory approvals

The Swedish regulatory authority, MPA (drug and device units), the regional ethics committee in Stockholm, the biobank and radiation committee have approved the studies. In Finland, the approvals have been received from the regulatory authorities Fimea (drug) and Valvira (device), as well as from the ethics committee I in Helsinki. The studies are registered at ClinicalTrials.gov database with numbers NCT03295786, NCT03295786, and NCT04228653 and EudraCT database with numbers 2015-004175-73, 2018-000346-19, and 2017-005170-19.

Dopamine transporter (DAT) PET

PET imaging was performed at baseline, at 6 months (end of Main study), at 12 months (end of Extension study) and at 19 months (Follow-up study) using the DAT radioligand [^{18}F]FE-PE2I (Fazio et al, 2018). PET scans were performed using high-resolution research tomography. Parametric images of binding potential (BP_{ND}) were generated and coregistered to the structural MRI.

Discovery proteomics study

Equal amounts of total protein from 29 CSF samples were digested with trypsin and injected on a nano-LC-MS/MS system for label-free discovery proteomics. The data was processed in Perseus to identify differentially abundant proteins.

Targeted proteomics study

Equal volume of CSF samples were digested with trypsin and injected onto a triple quadrupole LC-MS/MS system, operated in timed multiple reaction monitoring mode (MRM). Unique surrogate peptides for each of the 50 pre-selected proteins were monitored simultaneously. Cassetted calibration lines were constructed with synthetic reference peptides and heavy isotope-labelled internal standards to achieve absolute quantification.

RESULTS

Clinical and genetic findings

The study was conducted at three academic medical centers and two PET-centers in Sweden and Finland between September 2017 and July 2020.

A total of seventeen subjects were enrolled in the study and underwent implantation of the DDS. Altogether fifteen subjects completed the placebo-controlled main study, and fourteen subjects completed the active treatment extension study. Demographic data is shown in Table 1. 13 of 17 patients consented for genotyping. Four subjects (31%) were found to carry a missense mutation potentially linked to increased risk of PD (Table 2).

The primary endpoint on safety and tolerability of CDNF was met. There were two serious adverse events (SAE) in the study that were related to the drug delivery system and infusion procedures. Both occurred after the patients had started receiving infusions (not in the healing period after surgery). After procedural improvements, no further SAEs were observed.

At the group level, there were no significant differences between treatment groups in clinical measures, including UPDRS part III in OFF-state. However, some subjects showed clinical improvement during the treatment period, including >10 point improvements from baseline in UPDRS part III in OFF-state.

Table 1. Demographic and PD characteristic data at screening.

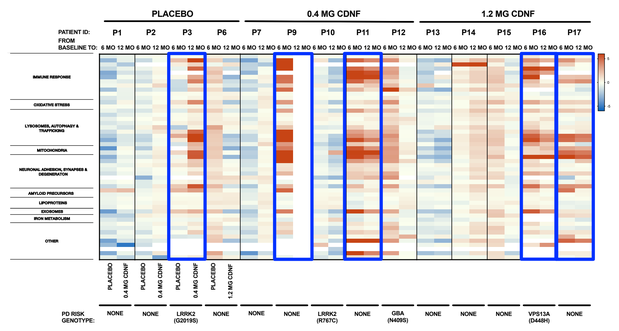
Characteristic	Placebo (n=6)	CDNF (mid-dose, n=6)	CDNF (high-dose, (n=5)
Age (years)	63.8 ± 6.4	63.2 ± 8.9	57.8 ± 6.7
Male sex, n (%)	5 (83.3%)	3 (50%)	4 (80%)
White race, n (%)	6 (100%)	6 (100%)	5 (100%)
Hoehn & Yahr (OFF), n (%)			
Stage 2	4 (66.7%)	3 (50%)	2 (40%)
Stage 2.5	1 (16.7%)	1 (16.7%)	2 (40%)
Stage 3	1 (16.7%)	2 (33.3%)	1 (20%)
Disease duration since diagnosis (years)	10.5 ± 2.7	10.7 ± 3.1	10.8 ± 2.3
UPDRS III, OFF	33.3 ± 7.6	34.7 ± 7.3	31.0 ± 6.8
UPDRS III, ON	14.8 ± 6.9	14.3 ± 4.5	11.8 ± 7.1
Levodopa response, %	60.7 ± 13.9	57.6 ± 11.8	57.0 ± 20.4
OFF-time per day, h	4.7 ± 0.7	6.1 ± 1.5	4.4 ± 1.5

Table 2. Genetic analysis of the study cohort.

ID	Genotype
P3	**LRRK2 G2019S** • Autosomal dominant, pathogenic variant (not fully penetrant) • At baseline: 62 y, disease duration 10 y, H&Y 2, levodopa response 74%
P10	**LRRK2 R767C** • Autosomal dominant, variant of unknown significance • At baseline: 75 y, disease duration 14 y, H&Y 2.5, levodopa response 66%
P12	**GBA N409S** • Autosomal recessive, pathogenic variant • At baseline: 59 y, disease duration 14 y, H&Y 3, levodopa response 66%
P16	**VPS13A D448H** • Autosomal recessive, variant of uncertain significance (mutations in VPS13 isoforms can cause various CNS disorders including parkinsonism) • At baseline: 69 y, disease duration 14 y, H&Y 2, levodopa response 46%

Figure 2. CSF biomarker signatures of individual patients.

Heatmaps show fold change in biomarker expression from baseline to 6 months (*left lane*) and from baseline to 12 months (*right lane*). For patients in the placebo group, the left lane shows the change during placebo period (Main study) and the right lane shows the change after crossing over to CDNF treatment (Extension study). Blue rectangles show the biological responders who shared a similar change in their CSF biomarker profile.



Discovery proteomics study

In the Discovery proteomics study, we identified 977 proteins in the CSF of PD subjects and healthy controls. 327 proteins were significantly differentially abundant between the two cohorts using thresholds: q-value <0.05 and fold change ≥ 2 for upregulated proteins and ≤ 0.5 for downregulated proteins.

Targeted proteomics study

For the targeted CSF proteomics study, a preselected CSF biomarker panel was designed based on the Discovery study data, published PD biomarker literature and our previous unpublished findings. The 50 biomarkers in the panel belong to the following functional categories:

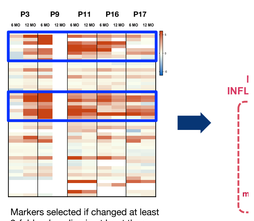
- Immune response and inflammation (7)
- Oxidative stress (2)
- Mitochondrial function (3)
- Autophagy and lysosomal function (8)
- Ubiquitin-proteasome system (2)
- Amyloid proteins (2)
- Axonal and synaptic adhesion and function (8)
- Lipid/lipoprotein metabolism (3)
- Tissue injury and remodeling (5)
- Exosomes (1)
- Other CDNF or PD-related markers (8)
- Hemoglobin as a control (1)

In the Targeted proteomics study, se
treatment in a subset of patients [highlig
the proteomics study (35.7%)], in the sc
and P17). Interestingly, in one patient,
profile did not show change during the
months, the patient showed similar patt
receiving CDNF throughout the study. T
highly similar pattern in the five biologica

Pathway analysis of the 20 CSF prote

The 20 CSF proteins that change in
associations: 30% markers were associ
response and neuroinflammation, and
(Figure 3).

Figure 3. Gene ontology (GO) term enric in biological responders.



Markers selected if changed at least 2-fold vs baseline in at least three patients in at least one timepoint selected for pathway analysis

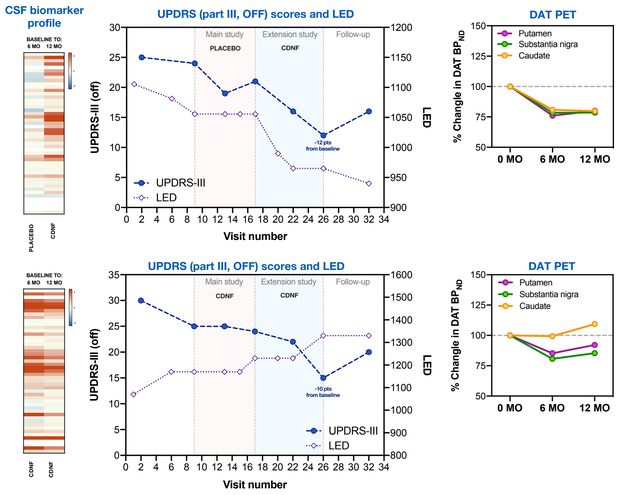
Pathway an

Biological responders show improvement in UPDRS and DAT PET

Analysis of the clinical and DAT PET imaging data from the five biological responders showed that those three patients who had received 0.4 mg CDNF there were clear correlations between CSF biomarker profile change and clinical and imaging improvements. In two patients, the CSF biomarker pattern correlated with improvement in motor function (≥10 point decrease in UPDRS part III in OFF-state) and stabilization of the DAT PET signal (Figure 4). In one patient with the CSF biomarker pattern, there was a robust increase in dopamine transporter DAT PET signal (≥50%) in basal ganglia structures but no significant change in UPDRS.

Figure 4. Correlation of CSF biomarker change with clinical improvement in patients P3 and P11.

CSF biomarker profile, UPDRS part in OFF-state and DAT PET data for basal ganglia structures are shown for patients P3 (top) and P11 (bottom).



CONCLUSIONS

- **In the targeted CSF proteomics study, several CSF biomarkers changed in response to CDNF treatment in a subset of patients (5 of 14 patients; 35.7%). These changes correlated with improved motor function (≥10 point improvement in off-state UPDRS-III) in two patients and dopamine transporter DAT PET signal (≥50% increase in DAT signal in putamen and substantia nigra) in one patient.**
- **A patient with LRRK2 G2019S mutation showed changes in multiple readouts (CSF biomarker profile, UPDRS) after crossing over from placebo to CDNF treatment but not during the placebo treatment. This suggests that LRRK2 G2019S mutation carriers may represent a specific CDNF-responsive subgroup of PD.**
- **CSF biomarker profiling suggests that biological response to intraputamenal CDNF treatment (with 100% bioavailability in the target area of the brain) in PD patients may be associated with modulation of proteostasis (lysosomes and autophagy), neuroinflammation and synaptic remodeling.**

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TreatER CONSORTIUM

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Co-funded by the Horizon 2020 programme of the European Union
Grant agreement No 732386

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