A long-term follow-up of safety and clinical efficacy of NTCELL® [Immunoprotected (Alginate-Encapsulated) Porcine Choroid Plexus Cells for Xenotransplantation] in Patients with Parkinson's Disease

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Abstract

Introduction

In 2019, we published the results of a Phase IIb randomized controlled trial of putaminal encapsulated porcine choroid plexus cell (termed NTCELL®) administration in patients with Parkinson's disease. This study failed to meet its primary efficacy end-point of a change in UPDRS part III score in the 'off' state at 26-weeks post-implant. However, a number of secondary end-points reached statistical significance. We questioned whether with longer follow-up, clinically significant improvements would be observed. For this reason, we decided to follow-up all patients periodically to week 104. Herein, we report the results of this long-term follow-up.

Methods

All 18 patients included in the original study were periodically re-assessed at weeks 52, 78 and 104 post-implant. At each time-point, motor and non-motor function, quality of life and levodopa equivalent daily dose was assessed using a standardized testing battery.

Results

At week 104, no significant differences in UPDRS part III scores in the 'off' state were observed in any of the treatment groups compared to baseline. Only a single serious adverse event - hospitalisation due to Parkinson's disease rigidity not responding to changes in medications – was considered potentially related to the implant procedure. There was no evidence of xenogeneic viral transmission.

Conclusion

Un-blinded, long-duration follow-up to week 104 post-implantation showed no evidence that putaminal NTCELL® administration produces significant clinical benefit in patients with moderately advanced Parkinson's disease.

Parkinson's disease (PD) is the second commonest neurodegenerative disease worldwide(1). It is characterized neuropathologically by nigral dopaminergic neuron depletion, an estimated 50-80% of which have already been lost before the appearance of clinically manifest disease(2). Symptomatic therapies provide modest benefit in early PD, but with time, most patients develop complications related to non-physiologic striatal dopamine delivery and off-target effects. Inevitably, patients experience progressive loss of function and disability, with resultant impaired quality of life and significant social and healthcare burden(3,4). Disease-modifying treatments capable of slowing, stopping or reversing neurodegeneration in PD remain elusive.

Neuroregenerative treatment approaches in PD aim to re-establish damaged neuronal circuitry, either through cell replacement strategies or through the delivery of neurotrophic factors. The latter approach has primarily focused on glial cell-line derived neurotrophic factor (GDNF) or its analogue, neurturin, owing to their propensity for promoting survival and sprouting of dopaminergic neurons(5,6). Despite promising open-label studies, GDNF and neurturin have failed to demonstrate efficacy in the controlled trial setting(7). However, observations of persistent benefit on an individual patient level and post-mortem evidence of dopaminergic fibre sprouting indicate that in some cases, disease modification could be achieved using these approaches(8,9).

Neurotrophin delivery is technically complex. These molecules are largely blood-brain-barrier impermeable and have short in-vivo half lives, thus requiring continuous delivery to target tissues(10). This has generally been achieved through the surgical implantation of pump devices for direct intra-striatal infusion, or more recently through the use of adenoviral vectors(7,11–13).

In 2019, we reported the results of a phase IIb, randomised, double-blind, placebo-controlled, dose-ranging investigation of the safety and efficacy of immunoprotected (alginate-encapsulated) porcine choroid plexus cells for xenotransplantation (NTCELL®) in patients with PD (Clinicaltrials.gov: NCT02683629)(14). This study employed a novel neurotrophin delivery method

(NTCELL®), which obviated the need for mechanical infusion systems(and their associated hardware complications)(12,14). Moreover, in contrast to previous studies employing single neurotrophins, NTCELL® produced a range of neurotrophins, antioxidants and molecular chaperones (see supplementary table 1)(15), possibly providing more 'physiologic' support to ailing striatal neurons.

Building upon promising data from rodent and non-human primate models of neurodegenerative disease(16–18), we first conducted an open-label safety study examining unilateral putaminal NTCELL implantation in 4 patients with PD(19). This confirmed both tolerability and feasibility of the procedure, with a small efficacy signal. We then proceeded to conduct the above randomized controlled trial, the results of which have been reported previously(14). In summary, at 26-weeks follow-up, the primary efficacy end-point of improvement in UPDRS part III score in the 'OFF' state was not met.

However, some secondary end-points did reach statistical significance, leading us to question whether, with longer duration follow-up, significant clinical benefit would have been observed. For this reason, and to ensure continued freedom from implant-related adverse events, a decision was made to follow-up all patients periodically to 104 weeks. Herein, we report the results of this long-term follow-up.

Methods

Patient selection

Between April 2016 and April 2017, 18 patients aged between 40 and 65 years with a clinical diagnosis of idiopathic Parkinson's disease (Queen Square Brain Bank Criteria) of at least 5 years duration (median 8.5 years, minimum 5

years, maximum 18 years) were enrolled to the Phase IIb randomised, double-blind, placebo-controlled trial. The 26-weeks follow-up was completed in October 2017 and results published(14). Unblinded prospective follow-up of all patients continued to 104-weeks post-implant. Ethical approval was granted by Medsafe, and by the Northern A Regional Ethics Committee branch of the New Zealand Health and Disability ethics committee. The clinical trial was registered with Clinicaltrials.gov: NCT02683629.

Randomisation procedure and NTCELL implantation

The 18 participants were divided into three groups of 6 patients. Within each group, patients were randomised to bilateral putaminal NTCELL implantation or sham surgery in a 4:2 ratio. There were no significant differences in disease duration between groups. Doses administered to each group were as follows:

-Group 1 :40 NTCELL® microcapsules (± 5%) bilaterally (total 80 microcapsules)
-Group 2 :80 NTCELL® microcapsules (± 5%) bilaterally (total 160 microcapsules)
-Group 3 :120 NTCELL® microcapsules (± 5%) bilaterally (total of 240 microcapsules)

Each group implantation was followed by an 8-week evaluative period following which the data safety monitoring board (DSMB) determined the safety of enrolling the next group of patients.

Follow-up assessments

The initial 26-week follow-up schedule has been detailed previously(14). During the follow-up extension, all patients were reviewed at 52, 78 and 104 weeks post-implant.
The following assessments were recorded at each follow-up time point: Unified

Parkinson's disease rating scale (UPDRS in 'on' and 'off' state), Modified Hoehn and Yahr Staging, Unified Dyskinesia Rating Scale (UDysRS), modified walking test in accordance with the CAPSIT-PD protocol(20), Parkinson's Disease Questionnaire (PDQ-39), and levodopa equivalent daily dose (LEDD). The 'off' state was defined as being free of anti-parkinsonian medication for >12 hours(20).

At 1 and 2 years (52 weeks and 104 weeks respectively) after receiving NTCELL® implants, recipients were monitored for xenogeneic organisms by testing of blood samples. Lifelong monitoring of recipients for xenogeneic organisms will continue on a 10-yearly basis.

Outcomes

The pre-specified primary outcomes for the original 26-week study have been detailed previously(14). In summary, the primary efficacy outcome was defined as a change in UPDRS Part III in the 'off' state at 26 weeks post-intervention compared with baseline. The primary safety outcomes was the occurrence of adverse events, serious adverse events or evidence of xenogeneic infection in transplant recipients or their spouses during the follow-up period.

The following additional secondary efficacy analyses were conducted on data to Week 104:

- Change in UPDRS Part III in the 'off' and 'on' state
- Change in total UPDRS score in the 'off' and 'on' state
- Change in Quality of life as assessed by Parkinson's Disease Questionnaire (PDQ-39)
- Change in L-dopa dosage
- Change in UDysRS scores
- Change in scores measured by the modified walking test
- Change in Modified Hoehn and Yahr stage

Statistical analysis

Changes in outcome measures from baseline at week 26, 52, 78 and 104 in each dose group and in the sham procedure group were calculated using pairwise comparisons generated from a 1-way ANOVA which compared the four groups. A two-tailed p-value <0.05 without correction for multiple comparison was used to indicate statistical significance.

Results

Demographic and clinical data

Demographic and clinical data pertaining to the 18 patients (10 men, 8 women) who were recruited to the original study is summarized in Table 1. All patients underwent follow-up at each of the specified time points in the unblinded extension phase. No patients were lost to follow-up.

Clinical efficacy outcomes

During the unblinded follow-up study extension to week 104, no significant differences in UPDRS part III scores in the 'off' state were observed in any of the treatment groups compared to baseline (see figure 1 and supplementary figure 1). Aside from patients in Groups 1 and 2 who did show statistically significant improvements in total and part III UPDRS 'on' scores, none of the other clinical effectiveness parameters (UPDRS score, UDysRS score, PDQ scales and subscales, Hoehn &Yahr stage, walking test) demonstrated a significant trend towards improvement at week 104. At week 104, no significant differences in LEDD was

observed between the groups. Data relating to efficacy outcomes is provided in supplementary table 2.

Safety outcomes

In total, 196 treatment emergent adverse events (AEs) were reported. Of these, 26 were considered to be possibly related to NTCELL® and 4 possibly related to the implant procedure. The remainder were considered to be unrelated to either NTCELL® or the implant procedure (supplementary table 3). The majority of AEs were mild (53.1%) or moderate (42.4%) in intensity. Of the 9 severe AEs, 6 were reported by placebo participants. One adverse event in an implanted patient from Group 3 met criteria for a serious adverse event - hospitalisation due to Parkinson's disease rigidity that did not respond to changes in medications. Three patients in Group 3 had >10mm of putaminal haemosiderin staining on postoperative MRI scanning, suggesting a dose-related microlesioning effect.

Discussion

This regenerative treatment trial employed a novel striatal neurotrophin delivery system (NTCELL®) in an attempt to effect disease modification in people with PD. The technique had shown significant benefit in animal models of neurodegenerative disease(16–18,21). Our first in-human open label study confirmed safety and tolerability, and showed an efficacy signal. However, the Phase IIb randomized, double-blind, placebo-controlled study failed to demonstrate clinical efficacy at 26 weeks, as did this follow-up unblinded assessments to 104 weeks. No significant safety concerns were raised from the perspective either of the implantation procedure, or of xenogeneic viral transmission. We established that 120 NTCELL® microcapsules per putamen is likely beyond the maximum tolerated dose, given the haemosiderin staining observed on MRI scanning.

The apparent disconnect between promising animal/open-label studies and lack of efficacy in the controlled trial setting is a common observation in neurotrophin treatment trials, and has been a focus of intense scrutiny(2,12,22). Previous trials of GDNF and neurturin have posited insufficient follow-up duration as one possible reason, the argument holding that post-treatment evaluation periods (which generally ranged from 26 to 80 weeks), though at times sufficient to produce detectable changes on F-Dopa PET scanning, were insufficient to demonstrate detectable clinical improvement (6,7,11,12,23). Our failure to detect clinical improvement at 104 weeks likely excludes short follow-up times as a possible contributing factor.

We considered whether sufficient putaminal coverage with neurotrophins was achieved using outward diffusion from NTCELL® microcapsules. In previous trials administering GDNF using pump-driven catheter delivery systems, limited diffusivity around the catheter tip meant that potentially, less than 2% of the putamen achieved coverage(24). In this study, we planned to escalate NTCELL® dose as tolerated up to a maximum of 240 microcapsules per side. This would have been the human equivalent dose of the 40 microcapsules which had shown beneficial effect in chemically lesioned non-human primates(16). As detailed above however, 120 NTCELL® per putamen was likely beyond the maximum tolerated dose, and the detrimental micro-lesion effect may have masked any therapeutic benefit derived from NTCELL® in this group. We believe that further increases would have involved unacceptable risks of adverse events. It remains possible that NTCELL® doses used in our study were insufficient.

The long-term viability of NTCELL® in humans is also uncertain. In a rodent model, 67.5% of NTCELL® remained viable at 6 months(15). One could postulate that the improvements in motor scores observed in groups 1 and 2 at 52 and 78 weeks might have represented a true therapeutic benefit beyond placebo and iatrogenically micro-lesioned patients in group 3, which failed to persist to week 104 due to lack of long-term NTCELL® viability. This possibility

could be evaluated using repeated yearly re-dosing with 40 or 80 NTCELL® microcapsules.

Difficulties in measuring PD progression may also have limited our ability to detect clinically significant improvements. Indeed, though widely employed in the clinical trial setting, the UPDRS is a crude measurement tool whose ability to detect within-subject changes in disease severity over time may be limited(25). Moreover, natural symptom fluctuations in people with PD render current intermittent assessment models sub-optimal; observed changes may reflect disease fluctuations rather than treatment effects. This is especially so if, as in our study, sample size is small- we were therefore particularly cautious not to over-interpret isolated findings, such as the apparent improvement in some 'ON' UPDRS parameters. Novel health technologies such as wearable sensors, which provide continuous monitoring in the patient's natural environment may in the future help address some of these limitations(26,27).

The significant and prolonged placebo responses observed in PD patients (sometimes sustained for a number of months) could also have masked treatment effects(28). We believe this to be unlikely however, both due to our long-duration follow-up and because the patients were unblinded as to their treatment allocation at 26 weeks, and informed of the lack of clinical efficacy.

Questions have also been raised as to whether α-synuclein mediated alterations in neurotrophin signalling pathways may render such treatments ineffective(29). However, F-Dopa PET evidence of dopaminergic re-innervation in previous studies combined with marked clinical improvements in some patients make this questionable as an explanation of failed neurotrophin trials. Our approach delivered a range of trophic and supportive factors, likely further limiting possible contributions from pathway dysfunction.

Finally, it is possible that neurodegeneration in our studied population might have already progressed beyond the point where neuroregeneration was

achievable. Viable nigrostriatal neurons available to re-establish new connections may have been limited.

Despite failing to demonstrate clinical efficacy, this study was instructive on many levels. We confirmed that stereotactic NTCELL® administration can be conducted safely without significant side effects. The absence of porcine retrovirus transmission also confirms the suitability of Auckland Island pigs as a source of xenotransplant material. Furthermore, the study highlighted some well recognized issues in the sphere of Parkinson's disease research, such as the pressing need for the development of a high-fidelity animal model of PD, disease progression biomarkers and more holistic clinical severity assessment tools.

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Authors' contributions:

1A, conception and design of the study; 1B: acquisition of data; 1C, analysis and interpretation of data.
2A, drafting the article; 2B, revising it critically for important intellectual content
3, Final approval of the version to be submitted.

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