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## Review

# GDNF and Parkinson's Disease: Where Next? A Summary from a Recent Workshop

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**Abstract.** The concept of repairing the brain with growth factors has been pursued for many years in a variety of neurodegenerative diseases including primarily Parkinson's disease (PD) using glial cell line-derived neurotrophic factor (GDNF). This neurotrophic factor was discovered in 1993 and shown to have selective effects on promoting survival and regeneration of certain populations of neurons including the dopaminergic nigrostriatal pathway. These observations led to a series of clinical trials in PD patients including using infusions or gene delivery of GDNF or the related growth factor, neurturin (NRTN). Initial studies, some of which were open label, suggested that this approach could be of value in PD when the agent was injected into the putamen rather than the cerebral ventricles. In subsequent double-blind, placebo-controlled trials, the most recent reporting in 2019, treatment with GDNF did not achieve its primary end point. As a result, there has been uncertainty as to whether GDNF (and by extrapolation, related GDNF family neurotrophic factors) has merit in the future treatment of PD. To critically appraise the existing work and its future, a special workshop was held to discuss and debate this issue. This paper is a summary of that meeting with recommendations on whether there is a future for this therapeutic approach and also what any future PD trial involving GDNF and other GDNF family neurotrophic factors should consider in its design.

Keywords: GDNF, dopaminergic neurons, NRTN, Parkinson's disease, clinical trials

## INTRODUCTION

The discovery and characterisation of specific neurotrophic factors in the context of neuronal development and synapseformation in the last half of the 20th century, led to the hypothesis that certain adult neuronal populations lost to chronic disease processes might be rescued and potentially regenerated by the administration of these agents [1]. This has been extensively explored in Parkinson's disease (PD) with the use of GDNF and related factor NRTN and the dopaminergic (DA) nigrostriatal pathway - the loss of which is known to be central and critical to the development and clinical expression of this condition [2].

In this short paper, we critically appraise the pre-clinical and clinical trial work with GDNF and NRTN in patients with PD. This appraisal is based on a meeting held over 2 days in August 2019 that brought together experts who had direct and practical experience in this field. The timing of this meeting was linked to the recent publication a UK-based clinical trial and parallel airing on the

BBC of the two-part documentary "The Parkinson's Drug Trial: A Miracle Cure? [<https://www.bbc.co.uk/mediacentre/proginfo/2019/09/parkinsons-drug-trial-a-miracle-cure>]? The meeting was organised and funded by The Cure Parkinson's Trust and supported by The Michael J Fox Foundation and Van Andel Institute.

## THE PRECLINICAL EVIDENCE THAT GDNF CAN RESCUE THE NIGROSTRIATAL PATHWAY

The discovery of GDNF in 1993 was made at a time of great interest in the therapeutic development of neurotrophic factors which offered potential for treating a number of disease states. The search for a survival factor with high selectivity for midbrain DA neurons had already been going on for some time. As such, when Lin et al. reported the cloning and bioactivity of this new trophic factor in 1993 there was great excitement [3]. Indeed, this in part helps explain why there was such a short time span between

76 the first pre-clinical *in vivo* studies (performed and  
77 published in 1994-95) and the first clinical trial with  
78 this agent [4] which started recruiting patients in July  
79 1996.

80 The second member of the GDNF family of lig-  
81 ands, NRTN, was discovered in 1996 [5] along with  
82 the receptor signaling pathways for these 2 factors  
83 [6]. This work revealed that while GDNF and NRTN  
84 are members of the transforming growth factor beta  
85 (TGF- $\beta$ ) family, they signal through a completely  
86 different receptor system compared to other TGF- $\beta$   
87 family members. GDNF first binds to the Glyco-  
88 sylphosphatidylinositol (GPI)-anchored co-receptor  
89 GDNF family receptor alpha-1 (GFR $\alpha$ 1) and then  
90 the GDNF-GFR $\alpha$ 1 complex binds to, and activates  
91 the transmembrane receptor tyrosine kinase RET.  
92 NRTN likewise signals to the cells via the RET  
93 receptor, but its binding to RET is mediated through  
94 the GFR $\alpha$ 2 co-receptor although when delivered at  
95 high levels it also can bind to GFR $\alpha$ 1[6].RET then  
96 activates the intracellular mitogen-activated protein  
97 kinase (MAPK), Akt (protein kinase B) and Src sig-  
98 naling cascades that are responsible for the survival  
99 and regeneration of DA neurons. It is important to  
100 stress that GDNF and NRTN trigger rapid responses  
101 in DA neurons through protein phosphorylation, but  
102 in addition to that they activate a number of tran-  
103 scription factors that have longer-lasting effects on  
104 DA neurons

105 The initial work with GDNF was made possi-  
106 ble through having access to a recombinant human  
107 form of protein from Synergen and Genentech. This  
108 enabled the generation of preliminary *in vivo* data  
109 on DA neuroprotection in the three rodent PD mod-  
110 els available at the time: the rat 6 hydroxydopamine  
111 (6-OHDA) model [7, 8], the mouse 1-methyl-  
112 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model  
113 [9] and the knife-transection model [10]. In addi-  
114 tion, a study performed in intact rats showed that  
115 GDNF, administered into the substantia nigra, could  
116 stimulate DA neuronal function [11]. The fact that  
117 the findings from all of these studies provided posi-  
118 tive evidence in the same direction was re-assuring –  
119 namely a growth factor that seemed to work on DA  
120 neuronal rescue and regeneration.

121 These initial preclinical studies used intracere-  
122 bral administration of GDNF, (single or repeated  
123 injections of microgram amounts over the substan-  
124 tia nigra). Nevertheless, Amgen, the company that  
125 had acquired the rights to GDNF, opted for an  
126 intraventricular delivery approach in its first clin-  
127 ical trial [4] (see below). A critical factor in this

128 decision were the results of a study, sponsored by  
129 Amgen, reporting significant, dose-dependent ben-  
130 efiticial effects obtained by monthly intraventricular  
131 injections of GDNF in MPTP-treated rhesus mon-  
132 keys [12]. The results again looked promising: all the  
133 major motor features (bradykinesia, rigidity, posture  
134 and balance) were improved when assessed 4 weeks  
135 after the last (fourth) monthly injection. Doubts on  
136 this mode of administration, however, soon arose:  
137 studies on the distribution of GDNF after intraven-  
138 tricular delivery indicated very limited diffusion into  
139 the brain parenchyma given its strong binding to  
140 extracellular matrix and cell surface heparin sulphate  
141 proteoglycans [13, 14]. Furthermore, a follow-up  
142 study by another team, performed in the same mon-  
143 key model, failed to show any protective effect on the  
144 MPTP-lesioned DA neurons [15].

145 While this trial was ongoing, experimental work  
146 performed in rodent and primate PD models pro-  
147 vided further and more compelling evidence that  
148 GDNF must be administered directly into the brain  
149 parenchyma to exert its actions, and that the site, dose  
150 and timing of GDNF delivery are important. Experi-  
151 ments in 6-OHDA lesioned rats showed that delivery  
152 into the substantia nigra could rescue DA neuronal  
153 cell bodies against toxic damage but failed to protect  
154 their axonal projections in the striatum [16]. Res-  
155 cue of both DA neuronal cell bodies and their axons  
156 projecting to the striatum required that the factor be  
157 delivered into the striatum, or into both the striatum  
158 and substantia nigra, provided that it is given before  
159 or soon after the toxin treatment [17]. Furthermore,  
160 the timing of the delivery of GDNF was also found  
161 to be important. In both rodents and primates there  
162 was evidence that delayed intrastriatal GDNF deliv-  
163 ery, starting weeks or months after 6-OHDA or MPTP  
164 treatment, could still protect surviving DA neurons  
165 and stimulate regenerative sprouting from spared  
166 axons in the partially denervated striatum [18–21].

167 Based on all this experimental data it was con-  
168 cluded that the therapeutic potential of GDNF is  
169 due to a combination of three interacting mecha-  
170 nisms: 1) Protection of midbrain DA neurons against  
171 toxin-induced cell death; 2) stimulation of axonal  
172 regeneration in the area reached by GDNF; and 3)  
173 recovery of function through up regulation of DA  
174 turnover and release.

175 These experimental studies, performed over the  
176 first decade after GDNF's discovery, were very  
177 encouraging and stimulated a series of clinical trials,  
178 including some where the agent was given intra-  
179 putaminally (see below). At that time, the preclinical

180 data seemed to support such a move to the clinic  
 181 based on all the studies performed in mice, rats and  
 182 non-human primates. There were, however, obvious  
 183 weaknesses in the pre-clinical data: Firstly, GDNF  
 184 was relatively ineffective in the face of severe lesions  
 185 giving >80% loss of DA neurons which more closely  
 186 mimics the human condition. Secondly, the data  
 187 showing efficacy was obtained in toxin-based models  
 188 where 6-OHDA or MPTP are administered acutely  
 189 which results in a degenerative process that is pri-  
 190 marily driven by oxidative damage or mitochondrial  
 191 dysfunction not protein aggregation. Further, these  
 192 models do not replicate the late stages of PD nor  
 193 the progressive, alpha-synuclein related pathophys-  
 194 iology that is characteristic of the human disease.  
 195 Given this difference, there was a concern that the  
 196 results obtained in toxin-based models may not be  
 197 predictive for human PD patients receiving this exper-  
 198 imental therapy. In addition, there were also concerns  
 199 about whether the GDNF made at that time using *E.*  
 200 *coli* (as opposed to human recombinant protein made  
 201 in mammalian cells) would work less well in human  
 202 patients.

203 Some of these anxieties have been borne out in  
 204 more recent experiments. Over the last few years  
 205 the access to alpha-synuclein-based PD models has  
 206 allowed this first question to be further explored [22].  
 207 These models not only offer better opportunities to  
 208 more faithfully replicate the alpha-synuclein related  
 209 pathology seen in people with PD, but the lesions so  
 210 induced evolve slowly over time in contrast to the  
 211 far more rapid time course of acute toxin models of  
 212 PD. Using such alpha-synucleinopathy models, stud-  
 213 ies designed to reproduce the type of neuroprotective  
 214 and restorative effects of GDNF, consistently seen in  
 215 MPTP and 6-OHDA models, have so far failed [23,  
 216 24].

217 In a follow-up study performed in the AAV-  
 218 alpha-synuclein model [25], Decressac et al. (2012)  
 219 suggested that this could be due to a failure of alpha-  
 220 synuclein overexpressing nigral neurons to respond  
 221 to GDNF. This, the authors postulated, could be  
 222 due to down regulation of the GDNF receptor RET,  
 223 mediated by a reduced expression of the DA related  
 224 transcription factor, nuclear receptor related 1 protein  
 225 (Nurr1). Nurr1 is known to regulate RET expres-  
 226 sion in DA neurons [26] and the two are thus closely  
 227 related.

228 In human PD postmortem material, Nurr1 has been  
 229 shown to be down-regulated in DA neurons that  
 230 over express alpha-synuclein [27] and a similar down  
 231 regulation of Nurr1 is also seen in alpha-synuclein

232 over expressing neurons in the AAV model of PD.  
 233 This, in turn, is accompanied by a marked reduc-  
 234 tion in the expression of RET [25]. The dependence  
 235 on Nurr1/RET expression is further supported by an  
 236 experiment performed in Nurr1 deleted mice show-  
 237 ing that the ability to respond to GDNF is abolished  
 238 in DA neurons lacking Nurr1 [25].

239 The findings obtained in the AAV-alpha-synuclein  
 240 model should however be interpreted with caution.  
 241 The cellular levels of alpha-synuclein obtained in this  
 242 model are artificially high (4-5-fold above normal)  
 243 and thus may not reflect the milieu in the affected DA  
 244 neurons in the PD brain. Furthermore, showing that  
 245 RET expression is reduced in the human PD brain  
 246 has proven inconclusive (see Su et al. 2017 for a  
 247 dissenting view [28]).

248 In summary, the preclinical data suggests that  
 249 GDNF can rescue DA neurons and their projections  
 250 in the nigrostriatal pathway in a range of toxin animal  
 251 models but the ability to rescue may be different in the  
 252 context of the alpha synuclein pathology that is seen  
 253 in the brain of people with PD.

## 254 THE EARLY CLINICAL TRIALS

255 The move from the lab to the clinic is always chal-  
 256 lenging and in order to assess progress and success,  
 257 new agents are often evaluated against four key ele-  
 258 ments. These include whether the drug (in this case  
 259 GDNF for PD):

- 260 ● Reaches its proposed site of action at sufficient  
 261 concentrations (namely the DA nigrostriatal  
 262 pathway);
- 263 ● Shows target engagement at that site in a mea-  
 264 surable way (GFRa1/RET signalling leading to  
 265 positive changes in this DA system);
- 266 ● Displays functional downstream pharmacolog-  
 267 ical effects (shows sprouting, growth and /or  
 268 survival of DA fibres/synapses in the presence  
 269 of an ongoing degenerative disease process);
- 270 ● Exhibits improvement in the relevant phenotyp-  
 271 e of the treated individuals (better motor perfor-  
 272 mance around measures known to be sensitive  
 273 to this DA network).

274 The first of these criteria falls under the umbrella of  
 275 delivery, the latter three provide a basis for potential  
 276 efficacy, if delivery sufficient to cover the putamen  
 277 can be achieved. In the sections below, we consider  
 278 the open-label and double-blind, placebo-controlled  
 279 clinical trials to-date in which recombinant human

GDNF has been directly administered to people with PD. This will be followed by a description and discussion of the clinical studies where a related trophic factor NRTN was administered as a viral vector injection to the basal ganglia as well as an ongoing GDNF gene therapy trial.

*The initial double-blind randomised control trial of intracerebroventricular Injections of GDNF protein*

The first clinical trial of GDNF was conducted by Amgen and was a multicentre, randomized, double-blind, placebo-controlled study of intracerebroventricular (ICV) administration of GDNF [4]. Monthly ICV injections were given via an intraventricular cannula inserted in the right frontal horn using standard stereotactic techniques. This was a dose escalation study with five dosage arms (25 µg, 75 µg, 150 µg, 300 µg, and 4000 µg) with 7-8 patients receiving active drug and 2-3 patients receiving placebo for a period of 8 months followed by an open-label extension period of up to an additional 20 months giving maximum single doses of up to 4,000 µg in 16 subjects. The primary outcome variables, the change in “practically defined OFF” and ON motor Unified Parkinson’s Disease Rating Scale (UPDRS) scores, were not significantly different from placebo in any of the active treatment groups apart from a mild but significant worsening in OFF scores in the 75 µg group and ON scores in the 300 µg group. Adverse effects were more common in the active treatment groups and included anorexia, weight loss (>5% body weight), hyponatremia and the unexpected finding (at the time) of sensory symptoms such as paraesthesia and Lhermitte’s phenomenon.

Although the adverse effect profile indicated that GDNF administered by ICV injection was having biological effects (the anorexia and weight loss were thought to be due to its action in the hypothalamus), this approach did not improve the clinical state of the patient.

It was postulated that this lack of benefit could relate to a failure of GDNF to reach and mediate effects in the target tissue (putamen and indirectly the substantia nigra) [4]. Supporting this hypothesis was a report of the postmortem assessment of a single patient from this trial [29]. In contrast to experiments in monkeys, where GDNF immunoreactivity was observed within the caudate nucleus ipsilateral to the infused ventricular frontal-horn and in

the septum bilaterally (although whether this was sufficient to activate RET signalling was not assessed), the human postmortem evaluation demonstrated no intra-parenchymal diffusion of GDNF across the cerebrospinal fluid: brain barrier from the ventricular cavity to the relevant basal ganglia structures. As such, it was to be expected that the autopsied tissue failed to demonstrate evidence of significant regeneration of nigral neurons and their fibres [29].

While this first in-human trial failed to hit its primary end point, the above results showed that monthly infusions of a biologic-agent unable to penetrate the blood-brain-barrier was well tolerated and “relatively” safe even when high doses of GDNF were given (4000 µg) [4]. However, the lack of parenchymal penetration coupled with an absence of motor benefit led on to further trials with infusions directly to the putamen.

*The initial open label trials of intraputaminial Injections of GDNF protein*

To ensure that GDNF reached the DA terminal plexus within the posterior-dorsal striatum, two small open label studies evaluated direct-to-putamen continuous (rather than bolus) catheter infusions of GDNF [30, 31]. To effect continuous infusions, GDNF was administered from subcutaneous pumps placed in the abdomen connected to a single catheter to each putamen in a 5-patient cohort in Bristol, UK and to a single-sided unilateral catheter only in a 10-patient cohort studied in Kentucky, USA. The Bristol group initially reported after 6 and 12 months as did the Kentucky group [30, 31]. Doses in the Kentucky study were escalated to 30 µg per day and in the Bristol trial, patient’s doses were on average 30 µg per day. Some patients did receive even higher doses (>30 µg GDNF per day) but this produced high signal changes on MRI in the putamen—changes which resolved with dose reduction.

Both of these small open label studies reported marked benefits in UPDRS motor (part III) scores in the practically defined OFF state with a mean reported improvement of approximately 30%–40%. Changes in diary fluctuations were equally encouraging at this open-label stage [30, 31] although it should be noted that the changes were bilateral even in patients who had been in receipt of unilateral infusions for reasons that were not clear.

In the Bristol study, 18-fluorine-dihydroxyphenylalanine ([<sup>18</sup>F]DOPA) positron emission tomography (PET) scans showed an

380 increase in tracer uptake mainly around the catheter  
381 tip, which potentially represented sprouting of  
382 remaining terminals, supported in part by a  
383 subsequent single case postmortem study [32].

384 In the Kentucky study, there was one serious  
385 adverse event (SAE) when the catheter became  
386 exposed which was associated with oedema around  
387 the catheter track in the putamen of this same patient.  
388 Three patients reported mild tingling sensations in the  
389 forehead, neck and lower back and two patients expe-  
390 rienced transient Lhermitte's phenomenon. Seven  
391 patients developed antibodies to GDNF without clini-  
392 cal sequelae. High resolution MRI scans revealed that  
393 there was no evidence of GDNF-induced cerebellar  
394 toxicity, which became more of a concern in some  
395 of the later preclinical non-human primate studies  
396 with GDNF (see below). Finally, all improvements in  
397 UPDRS scores were lost within 9-months of stopping  
398 the GDNF infusions.

399 Based on these encouraging open label observa-  
400 tions, a prospective, double-blind, placebo-controlled  
401 trial of continuously infusing GDNF to the putamen  
402 was initiated.

## 403 **DOUBLE-BLIND,** 404 **PLACEBO-CONTROLLED TRIALS**

### 405 *The Amgen trial*

406 Amgen sponsored the first double-blind trial  
407 involving direct intra-putaminal delivery of  
408 GDNF. In this multicentre trial, patients were  
409 randomized 1:1 to receive bilateral continuous  
410 intra-putaminal infusions of either GDNF at a dose  
411 of 15 µg/putamen/day or placebo [33]. One catheter  
412 was stereotactically placed on each side with its tip  
413 targeted to the posterior-dorsal putamen and attached  
414 to a separate SynchroMed pump (Medtronic),  
415 implanted subcutaneously over the patient's  
416 abdomen. The primary end point was the change  
417 in UPDRS motor score in the practically defined  
418 OFF condition at 6 months. Secondary end points  
419 included other UPDRS scores, motor tests, dyskine-  
420 sia ratings, patient diaries, and [<sup>18</sup>F]-DOPA uptake  
421 on positron emission tomography (PET) imaging.  
422 Patients were stratified by baseline UPDRS OFF  
423 motor score (<44, >45) and 30 subjects (15 in each  
424 group) were calculated to be needed to give a 90%  
425 power to detect a between group difference of 25%  
426 in the percent change in UPDRS OFF motor score.

427 34 patients were implanted and randomized; 17  
428 received GDNF (all completed the trial), and 17  
429 received placebo (with 16 completing the trial, and

430 one discontinuing due to pump site infection). At 6  
431 months, the mean percentage change in OFF UPDRS  
432 motor score was -10% in the GDNF group compar-  
433 ed to -4.3% in the placebo group which was not  
434 statistically significant. Secondary end point results  
435 were also similar between the groups. There was no  
436 significant relationship between the change in motor  
437 scores and the catheter tip location. In the two thirds  
438 of paired evaluable PET scans (1/3 of paired scans  
439 were excluded due to head movement artefact) there  
440 was a 32.5% treatment difference favouring GDNF in  
441 mean [<sup>18</sup>F]DOPA influx constant ( $p=0.019$ ) but this  
442 did not correlate with changes in the OFF UPDRS  
443 motor scores. Procedure- and device-related compli-  
444 cations were not uncommon while treatment related  
445 complications were infrequent. The marked anorexia  
446 and weight loss observed in the higher dose ICV study  
447 were not seen. Serious, device-related adverse events  
448 required surgical repositioning of catheters in two  
449 patients and removal of devices in another [33].

450 Three patients, one in the double-blind phase and  
451 two in the open label extension, developed neu-  
452 tralizing anti-GDNF antibodies—again without any  
453 obvious clinical sequelae- which may relate to the  
454 way the GDNF was delivered with leakage to the  
455 periphery, and activation of the immune system.  
456 Furthermore, contemporaneously, new toxicologi-  
457 cal studies in non-human primates (NHPs) found  
458 focal limited loss of Purkinje cells and near com-  
459 plete loss of molecular and granule cell layers in  
460 3/5 monkeys rapidly withdrawn from 3 months of  
461 unilateral infusions of much higher doses of GDNF  
462 (100 µg/putamen/day) while one monkey continuing  
463 on treatment was found to have milder cerebellar  
464 cortical pathology [34].

465 As a result of this combination of a negative clin-  
466 ical double-blind placebo-controlled trial result, the  
467 finding of neutralizing antibodies in a small number  
468 of patients and concerns about the NHP toxicological  
469 findings, Amgen chose to terminate their GDNF pro-  
470 gram for PD. This led to a vigorous debate between  
471 various researchers and patient groups as to why  
472 the double-blind trial and the open-label studies had  
473 come to different conclusions. These included:

- 474 1. The potential for a major placebo effect in open-  
475 label trials given it involved an invasive surgical  
476 approach and problems in maintaining true clinical  
477 equipoise. However, it should be noted that  
478 in the double-blind study there was no major  
479 placebo effect, but rather an absence of a positive  
480 clinical effect in either group.

- 481 2. Differences in dosages given, in particular  
 482 higher doses were generally used in the open  
 483 label studies, although benefit had also been  
 484 claimed with lower doses in these early studies;  
 485 3. Differences in delivery including catheter  
 486 dimension and design. In general, catheter  
 487 dimensions including its external diameter,  
 488 design (no step, stepped or recessed stepped)  
 489 and number of catheters inserted along with  
 490 implantation technique could all have an effect  
 491 on the extent to which the agent was delivered  
 492 and remained at the target site. In addition, dif-  
 493 ferences in the diffusion of the agent across the  
 494 target structure could also have impacted on  
 495 the total volume of putaminal tissue exposed to  
 496 study drug (see below) [35] and thus its poten-  
 497 tial therapeutic effectiveness. All of this has led  
 498 to the development of new convection enhanced  
 499 delivery systems (see below).  
 500 4. Differences in the patients selected for trials,  
 501 in particular whether more advanced patients  
 502 with more severe DA losses were recruited to  
 503 the double-blind study.

504 Given this uncertainty, there was a feeling in some  
 505 quarters that this therapy should not be abandoned  
 506 at this stage, a position reinforced by further obser-  
 507 vations from the original open-label Bristol cohort  
 508 [32, 36, 37]. This included the fact that the origi-  
 509 nal five subjects who continued to receive continuous  
 510 infusions from 12 to 24 months and beyond, all main-  
 511 tained their improved UPDRS part II and part III  
 512 OFF scores compared to baseline, consistent with  
 513 their improved [<sup>18</sup>F]DOPA PET data [36]. Finally,  
 514 one subject who had been infused continuously for 39  
 515 months and then reviewed at 36 months after GDNF  
 516 cessation, continued to experience a major clinical  
 517 benefit. This benefit was accompanied by [<sup>18</sup>F]DOPA  
 518 PET putaminal uptake that continued to show an  
 519 improvement compared to pre-treatment scans [37].  
 520 Although a single case, this did support the concept  
 521 that GDNF might still work if methodological aspects  
 522 of its administration were improved. Thus, a new  
 523 GDNF trial was proposed.

#### 524 *The recent Bristol study*

525 This new double-blind investigation of directly  
 526 administered GDNF took the form of a randomised,  
 527 placebo-controlled, single-centre trial sponsored by  
 528 the UK National Health Service (and funded by  
 529 Parkinson's UK and The Cure Parkinson's Trust)

530 which started in 2012[38] (NCT03652363). Patients  
 531 selected were 35–75 years old, had motor symp-  
 532 toms for 5 or more years, with moderate disease  
 533 severity in the OFF state (Hoehn and Yahr stage  
 534 2–3 and a UPDRS motor score between 25–45) and  
 535 motor fluctuations (average of at least 2.5 hours of  
 536 OFF time per day on 3-day fluctuation diaries). They  
 537 all had marked levodopa responsiveness as defined  
 538 by  $a \geq 40\%$  improvement in UPDRS motor score  
 539 following a levodopa challenge after a practically  
 540 defined OFF period. Importantly, the major differ-  
 541 ence with this trial with what had gone before was  
 542 the use of a new delivery device designed to establish  
 543 excellent coverage of the putamen.

544 Once implanted with this new intermittent  
 545 enhanced drug administration system that enabled  
 546 convection enhanced delivery (CED), patients were  
 547 randomised. Post-randomisation, patients received  
 548 a total of 10 study treatments at 4-weekly inter-  
 549 vals (Weeks 0 to 36). At each treatment, 400  $\mu\text{L}$   
 550 of infusate (300  $\mu\text{L}$  GDNF or placebo, followed by  
 551 100  $\mu\text{L}$  aCSF) was delivered per catheter into the  
 552 post-commissural putamen at a GDNF concentra-  
 553 tion of 0.2  $\mu\text{g}/\mu\text{L}$ . Thus, the total GDNF dose given  
 554 every 4 weeks was 240  $\mu\text{g}$  (120  $\mu\text{g}$ /putamen given as  
 555 60  $\mu\text{g}$ /catheter).

556 The results of this trial were published in February  
 557 2019 [38] and it revealed that the trial did not reach  
 558 its prespecified primary endpoint; the mean OFF state  
 559 UPDRS motor score decreased by  $17.3 \pm 17.6\%$  in  
 560 the active group and  $11.8 \pm 15.8\%$  in the placebo  
 561 group. A range of secondary and supplementary  
 562 efficacy endpoints also failed to show significant dif-  
 563 ferences between the groups as well. In contrast to the  
 564 non-significant clinical results, the [<sup>18</sup>F]DOPA PET  
 565 findings were positive. Between baseline and Week  
 566 40 there was no change in the placebo group, whereas  
 567 in the GDNF group there were significant changes  
 568 across the putamen (in a graded fashion ranging from  
 569 25% anteriorly to 100% in the posterior putamen) but  
 570 not in the caudate (which acted as an internal control).  
 571 These marked relative percentage increases, while  
 572 statistically significant, still meant that the absolute  
 573 improvement was only a level that was 50–60%  
 574 of that seen in the normal intact posterior putamen  
 575 which may explain why the treatment did not result  
 576 in significant clinical changes.

577 At the conclusion of this double-blind study, all  
 578 patients had the chance to enrol into an open label  
 579 extension trial that used the same GDNF dose regi-  
 580 men and intermittent infusion parameters as for the  
 581 initial double-blind study. This open label exten-



582 sion trial, also lasted 40 weeks, and was initiated  
583 before the results from the double-blind parent inves-  
584 tigation were known. It was undertaken primarily to  
585 gain longer term safety data and to gather further  
586 exploratory information on GDNF clinical effects  
587 over a more prolonged period of repeated tissue expo-  
588 sures [39].

589 The primary endpoint of this extension study  
590 was the percentage change from baseline (Week 0)  
591 to Week 80 in the practically defined OFF state  
592 UPDRS motor score, comparing the group that had  
593 received GDNF in the initial trial followed by open-  
594 label GDNF (GDNF/GDNF) versus the group that  
595 received placebo in the parent investigation followed  
596 by open-label GDNF (placebo/GDNF) (in effect a  
597 delayed-start design). Secondary endpoints included  
598 absolute change from baseline in OFF and ON state  
599 UPDRS part II and part III scores and change from  
600 baseline in diary ratings. A further pre-specified  
601 secondary endpoint included comparing Week 80  
602 UPDRS scores in the GDNF/GDNF group against  
603 Week 40 scores in the placebo/GDNF group (i.e., at  
604 the end of the placebo treatment).

605 All 41 parent study participants were enrolled into  
606 the extension study, and all were included in the anal-  
607 yses. Again, there were no significant differences.  
608 Comparing baseline (Week 0) to the end of treat-  
609 ment (Week 80), the OFF state UPDRS motor score  
610 improved by  $26.7 \pm 20.7\%$  (mean  $\pm$  standard devia-  
611 tion) in the GDNF/GDNF group and by  $27.6 \pm 23.6\%$   
612 in the placebo/GDNF group. Likewise, none of the  
613 secondary or supplementary outcomes spanning the  
614 entire 80-week period were significantly different  
615 outside changes in L-DOPA equivalent dose (the  
616 increase in the daily L-DOPA equivalent dose from  
617 baseline to Week 80 was smaller in the GDNF/GDNF  
618 group ( $59 \pm 194$  mg) than in the placebo/GDNF  
619 group ( $289 \pm 365$  mg) [35].

620 Treatment emergent adverse events (TEAEs,  
621 events commencing post initiation of GDNF or  
622 placebo infusions) were reported for all 41 patients.  
623 No patient had a TEAE that led to discontinuation of  
624 study medication. Of the eight serious TEAEs, three  
625 were considered to be device related and included  
626 two occurrences of a hypertrophic skin reaction  
627 around the port site that required surgical skin thin-  
628 ning and a possible port site infection that occurred  
629 approximately 15 weeks into the treatment phase and  
630 required inpatient treatment with oral antibiotics.

631 Two patients enrolled into the double-blind study  
632 did not proceed to randomisation and were withdrawn  
633 prior to the start of treatment because they failed

634 the post-surgery eligibility criteria. One patient expe-  
635 rienced a mildly symptomatic putaminal ischemic  
636 stroke coincident with the initial test infusion. The  
637 patient recovered completely but was withdrawn to  
638 avoid unnecessary risks. The second patient suffered  
639 a small asymptomatic haemorrhage in both putamina  
640 during the initial test infusion.

641 Blood sample analyses showed no measurable  
642 GDNF plasma concentrations and no GDNF-binding  
643 serum antibodies in GDNF-treated patients at any  
644 point. This contrasts with the double-blind Amgen  
645 study and the earlier open label studies and may  
646 relate to the different delivery devices and delivery  
647 regimens that were used in each trial.

648 In summary, these two studies have shown that  
649 direct infusions of GDNF administered in a man-  
650 ner to achieve CED can be given every 4-Weeks  
651 over 18 months in a fashion that patients found  
652 tolerable. Employing this approach, as evidenced  
653 by a combination of direct Gadolinium infusion  
654 through the delivery system and improvement in  
655 [ $^{18}$ F]DOPA PET uptake, appeared to achieve accu-  
656 rate and whole putamen-wide target tissue delivery  
657 with some evidence of target receptor engagement  
658 using PET imaging. Despite this apparent optimisa-  
659 tion of delivery, however, the clinical primary and  
660 secondary endpoints in both trials were negative.  
661 Whilst the partial restoration in PET signal may alle-  
662 viate some of the concerns around insensitivity to  
663 GDNF in the face of an alpha-synucleinopathy or that  
664 patients more than 5 years from the point of diagno-  
665 sis have no terminals left to restore, the fact remains  
666 that improvement in [ $^{18}$ F]DOPA PET signal cannot  
667 be used as evidence for improvement in functional  
668 pharmacology, especially as this tracer has also been  
669 said to label inflammation [40]. Questions therefore  
670 remain over whether the lack of significant benefit  
671 in placebo-controlled trials to date reflects therapeu-  
672 tic ineffectiveness or whether this would be resolved  
673 with an increased dose and exposure of mammalian  
674 cell made GDNF coupled totreating patients with ear-  
675 lier stage disease.

## 676 GENE THERAPY TRIALS WITH NRTN 677 AND GDNF

678 In contrast to the immense logistical challenges  
679 and potential safety concerns associated with contin-  
680 uous or repeated long-term delivery of recombinant  
681 GDNF protein, gene therapy promises sustained,  
682 durable and localized production of properly folded

683 biologically active GDNF following a one-time dos-  
684 ing procedure. Several clinical studies have now been  
685 conducted in PD, including a multi-phase program of  
686 NRTN gene transfer, a homolog of GDNF, and more  
687 recently a Phase 1 clinical safety trial of GDNF gene  
688 transfer. Both the NRTN and GDNF gene therapy  
689 programs utilized gene transfer vectors derived from  
690 the non-pathogenic adeno-associated virus serotype  
691 2 (AAV2) with a constitutive CMV promoter. These  
692 vectors appear to have a favourable safety profile for  
693 neurotrophic factor gene delivery in PD, in addition  
694 to which AAV2 has an exclusive neuronal tropism  
695 and restricted distribution when directly delivered to  
696 the brain [41], thus minimising off target side effects.

697 The initial preclinical studies exploring this  
698 approach demonstrated that GDNF and NRTN  
699 gene delivery conveyed efficient protection against  
700 MPTP/6-OHDA lesions when the gene transfer was  
701 performed prior to, or shortly after, neurotoxin expo-  
702 sure. However, in an attempt to more closely mimic  
703 both early and later stages of PD a more refined MPTP  
704 model was developed in non-human primates (NHPs  
705 [21, 42]). Using this model, animals with established  
706 parkinsonian signs were randomized to receive either  
707 1) AAV2-GDNF ( $9.9 \times 10^{11}$  vector genomes, vg;  
708  $n = 8$ ), or 2) sham PBS ( $n = 7$ ) intraputamina  
709 l infusions via CED [21], and were followed for 1, 6, 14,  
710 or 24 months.

711 In one of these NHP studies, it was demon-  
712 strated that there were marked functional motor  
713 improvements following AAV2-GDNF (mean 56%  
714 reduction of motor rating scores) in both the moder-  
715 ately and severely lesioned MPTP monkeys. This  
716 motor recovery directly correlated with increased 6-  
717 [ $^{18}\text{F}$ ]Fluoro-L-M-tyrosine (FMT) PET uptake that  
718 remained stable throughout the 24-month time point  
719 and which also correlated with enhanced dopamine  
720 and dopamine metabolites when assayed from tis-  
721 sue homogenates from these same animals. Increased  
722 tyrosine hydroxylase-immunoreactive (TH-IR) fibre  
723 density was also seen in the partially lesioned hemi-  
724 sphere (equivalent to "early" PD) receiving the  
725 AAV2-GDNF but was much less prominent in the  
726 severely lesioned side (comparable to advanced PD).  
727 Together these findings suggested that intraputamina  
728 l infusions of AAV2-GDNF were safe and that greater  
729 parenchymal GDNF levels ( $\sim 24$  ng/mg protein) were  
730 well-tolerated, without the adverse effects seen with  
731 protein infusions of GDNF (e.g., weight loss) [4,  
732 33]. This also indicates that GDNF is capable of  
733 restoring dopaminergic terminals with an associated  
734 significant recovery of motor function, particularly

735 in the partially lesioned conditions. In addition, there  
736 was strong evidence that GDNF delivery provided  
737 greater potential for intrinsic TH-IR positive sprout-  
738 ing in earlier rather than later stages of nigrostriatal  
739 degeneration.

740 These and related studies [43] also found that  
741 there was anterograde transport of AAV2-GDNF, via  
742 direct and indirect connections, which was independ-  
743 ent of the degenerating nigrostriatal dopaminergic  
744 (DA) neurons [43, 44] and their capacity to retro-  
745 gradely transport GDNF protein. This mechanism  
746 resulted in the broad expression of GDNF from the  
747 putamen to the substantia nigra (SN) pars reticu-  
748 lata, despite varying degrees of nigrostriatal DA  
749 neuro degeneration and raised the potential that this  
750 therapeutic may provide distinct advantages through  
751 rebuilding DA nigrostriatal networks within the PD  
752 brain.

753 This NRTN preclinical work led to clinical tri-  
754 als that were performed by Ceregene Inc. using  
755 the AAV-2 serotype and the NRTN transgene, the  
756 first of which was an open label clinical trial [45]  
757 (NCT00252850). Twelve patients aged 35–75 years  
758 with a diagnosis of PD for at least 5 years, in  
759 accordance with the Parkinson's UK Brain Bank  
760 Criteria, received bilateral, stereotactic, intraputamina  
761 l injections of AAV2-neurturin (CERE-120). The  
762 first six patients received doses of  $1.3 \times 10^{11}$  vec-  
763 tor genomes (vg)/patient, and the next six patients  
764 received  $5.4 \times 10^{11}$  vg/patient. The treatment was  
765 well tolerated with no side effects and a number of  
766 clinical endpoints suggested improvement. However,  
767 disappointingly there was no increase in  $^{18}\text{F}$ -DOPA  
768 uptake on PET imaging.

769 This initial trial was followed by a multi-centre  
770 randomized (2 : 1) double-blind trial comparing intra-  
771 putamina l injections of AAV2-neurturin to sham  
772 surgery in 58 PD patients [46]. An infusion volume  
773 of 40  $\mu\text{l}$  of vector was injected into each putamen  
774 with subjects in the active treatment arm receiving a  
775 dose of  $5.4 \times 10^{11}$  vg/patient. Disappointingly, there  
776 was no significant difference between the two groups  
777 based on UPDRS Part III motor scores in the OFF  
778 state at 12 months post-transduction, the primary  
779 endpoint. However, a significant placebo effect was  
780 noted, with a 6-point reduction in UPDRS seen at 3  
781 months in the sham group, which persisted for the  
782 duration of the study. However, a pre-specified *post-*  
783 *hoc* analysis suggested that those patients blindly  
784 assessed at the 15–18 months post-treatment time  
785 point may have had some benefit, although there was  
786 no controlling for multiple comparisons. In addition,

787 it should be noted that the whole cohort could not be  
788 followed blindly out to these time points due to the  
789 ending of the trial and the blind being broken at 12  
790 months. This may have created a bias in the effect  
791 seen.

792 Histological data from patients who died from  
793 events unrelated to the procedure, indicated that  
794 NRTN was being expressed within the vicinity of the  
795 injection sites, and that this resulted in focal upregulation  
796 of TH, but to an extent that was probably  
797 insufficient to provide any clinical benefit. Additionally,  
798 there was very limited NRTN seen in nigral  
799 neurons, suggesting that severity of the nigrostriatal  
800 axonopathy in these advanced PD patients did not  
801 allow sufficient retrograde transport of NRTN to the  
802 nigral perikarya to provide neurorestorative effects  
803 [47].

804 Based on the small area of transduction, the lack  
805 of NRTN expression in nigral neurons, the perceived  
806 defect in retrograde transport and the potential for  
807 changes to occur at a longer time-point, a second  
808 randomized double-blind trial comparing higher putaminal  
809 volumes plus a direct injection into the nigra  
810 was undertaken [48]. Fifty-one patients were enrolled  
811 in this multi-centre trial and randomly assigned  
812 (1 : 1) to receive either bilateral AAV2-NRTN (180  $\mu$ L  
813 injection volume per hemisphere) into the substantia  
814 nigra ( $2.0 \times 10^{11}$  vg/patient, 15  $\mu$ L  $\times$  2 infusions)  
815 and putamen ( $1.0 \times 10^{12}$  vg/patient, 50  $\mu$ L  $\times$  3  
816 infusions), or sham surgery. Again, no statistically  
817 significant clinical differences were seen in UPDRS  
818 Part III motor OFF scores at 15-months (primary  
819 endpoint) between the active treatment and sham  
820 operated arms.

821 Following the NRTN studies, an open-label, dose-  
822 escalation Phase 1 study of AAV2-GDNF was  
823 initiated in 2013 (NCT01621581) [49]. In this GDNF  
824 gene therapy study, 13 (of an intended 24) partici-  
825 pants with advanced PD received bilateral magnetic  
826 resonance imaging (MRI)-guided, CED intraputaminal  
827 infusions of AAV2-GDNF ( $9 \times 10^{10}$  ( $n=6$ ),  
828  $3 \times 10^{11}$  ( $n=6$ ),  $9 \times 10^{11}$  vg ( $n=1$ ); delivered in  
829 a 450  $\mu$ L volume per putamen. Safety and tolerability  
830 of AAV2-GDNF intraputaminal delivery by  
831 CED was confirmed by real-time MRI and postoperative  
832 monitoring, with no serious adverse events  
833 (SAEs) attributed either to the procedure, or to the  
834 investigational product. Increased PET uptake values  
835 of  $^{18}$ F-DOPA were noted at the documented infusion  
836 sites at the 6-month time point as compared  
837 to baseline values, with further enhanced uptake  
838 observed at 18-months post-treatment time point.

839 A trend was noted for earlier and more marked  
840 increases in patients with shorter disease duration.  
841 No significant differences were seen at 18-months  
842 between the three treated cohorts in terms of their  
843 UPDRS Part III motor scores or total levodopa equivalent  
844 doses. The unchanged PD motor scores and  
845 stabilisation of their anti-parkinsonian medications  
846 following putaminal AAV2-GDNF delivery might  
847 support possible biological effects of this therapy  
848 in participants with advanced PD but this remains  
849 unproven.

850 Several key changes were made as part of the  
851 AAV2-GDNF Phase 1 study design compared to prior  
852 direct infusion studies in PD conducted in the early  
853 2000s, including:

- 854 a) the use of intraoperative MRI-guidance, and  
855 gadolinium co-infusion with AAV-GDNF
- 856 b) using a reflux-resistant delivery cannula with a  
857 stepped design to increase distribution within  
858 the target putamen while reducing off-target  
859 leakage;
- 860 c) allowing the visualisation and monitoring of  
861 CED infusions in real-time with an ability to  
862 surgically modify the cannula position and infusion  
863 parameters to maximise the putaminal  
864 coverage, and
- 865 d) increasing the infusion volumes up to 450  $\mu$ L  
866 per putamen, 3 times greater than the volume  
867 delivered in the Phase II AAV2-NRTN study  
868 [46, 48].

869 Despite these key modifications in methods, the  
870 average putaminal volumetric coverage documented  
871 by retrospective interim analysis of MRIs was only  
872 26%, much lower than that anticipated to be required  
873 for a meaningful clinical benefit. In part, this limitation  
874 in putaminal coverage may have been due to the  
875 transfrontal surgical approach to the putamen, where  
876 the trajectories are perpendicular to the long axis of  
877 the target volume. This inability to broadly cover  
878 the putamen with this standard surgical approach  
879 may have also been a relevant disadvantage in the  
880 AAV2-NRTN and earlier recombinant GDNF protein  
881 infusion studies, where there was minimal putaminal  
882 transgene expression or effects with small localised  
883 changes relative to the radiographic improvement  
884 displayed via  $^{18}$ F-DOPA PET imaging.

885 Although long term follow-up for the Phase 1  
886 adeno associated virus (AAV) 2-GDNF cohorts is  
887 ongoing, enrolment was closed following the interim  
888 analysis, due to the insufficient putaminal coverage  
889 (mean of 26%). Other studies of a AAV2-L-aromatic

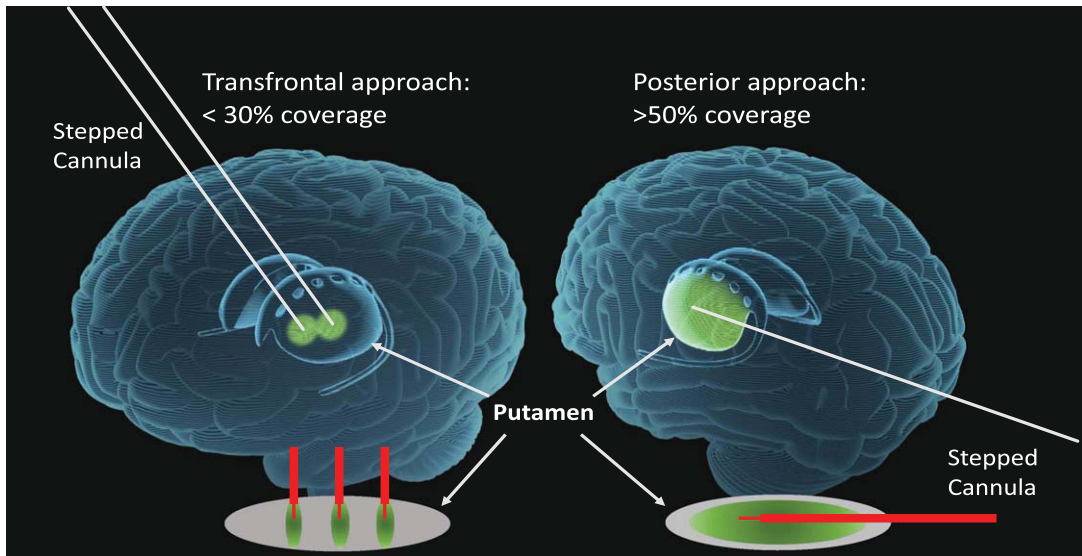


Fig. 1. Transfrontal versus Posterior (occipital) trajectories utilizing CED. MRI-guided stereotactic approaches for vector delivery to the putamen utilizing convection enhanced delivery (CED) and stepped cannulae. This approach allows for precise targeting, shape-fitting infusions, and larger delivery volumes to improve the extent of transduced putaminal tissue, thereby increasing transgene production capacity. With the transfrontal approach, the cannulae are oriented to the short axis of the putamen limiting vector coverage to  $< 30\%$ , often requiring 2-3 tracts. The posterior (occipital) approach maximizes the delivery by paralleling the putaminal long-axis, requiring only 1 tract, which achieves  $> 50\%$  putaminal vector coverage with larger infusion volumes.

amino acid decarboxylase (AADC) gene therapy for PD [50], however have shown that delivering volumes up to  $1800 \mu\text{L}$  per putamen using a CED approach is feasible with a good safety profile and providing putaminal volumetric coverage of  $> 50\%$ . More importantly, this AAV2-AADC PD gene therapy investigation has provided convincing evidence that the clinical benefit improved concurrently with increases in volume of vector delivered and thereby the extent of putaminal coverage. These findings underscore the importance of optimising the transduced tissue volume and putaminal infusion coverage as factors correlating directly with clinical efficacy in PD [49].

These latter efforts have prompted the design of a new Phase 1b trial to assess a higher dose of the AAV2-GDNF therapeutic in moderately advanced PD patients, (similar to those in Phase 1), as well as in subjects with early disease (namely within 5 years of PD onset). Furthermore, this new study will be using a posterior (occipital) trajectory to each putamen, (paralleling the long axis), that allows shape-fitting CED of higher infusion volumes, thereby improving putaminal coverage and GDNF production levels more uniformly throughout the putamen [51]. This posterior putaminal approach is similar to that recently reported in the GDNF protein infusion study

[38] and has also been safely performed using MRI-guidance and CED, in the ongoing AAV2-AADC study (NCT03562494) (see Fig. 1).

#### WHERE NEXT?

The question as to whether GDNF has a competitive future in the treatment of PD is still unclear. A number of conclusions can be drawn from the studies undertaken to date with GDNF and related factors (see Table 1) along with a number of recommendations about what another trial with GDNF should consider and thus might look like (see Table 2).

Table 1

Summary of main findings on the effects of GDNF and related factors in models and clinical trials in PD

- Studies have shown a statistically significant response in some patients, but these are not consistent, and the majority of studies have been negative in terms of reaching their primary outcome;
- Striatal dopamine has increased in most patients in receipt of GDNF as evidenced using  $^{18}\text{F}$ -dopa PET imaging;
- There is little evidence of sufficient retrograde transport of GDNF/NRTN to the substantia nigra in patients when the agent is delivered into the striatum;
- Postmortem studies show that where there is expression of GDNF/NRTN there is some upregulation of TH.

Table 2  
Factors to consider in future clinical trials with GDNF and related factors in PD

---

**What form should the GDNF be given in?**

- A gene therapy approach was favoured over protein infusions given the complexity of the neurosurgical intervention required for the former and the burden this places on the patients with Parkinson's disease.
- Consider using mammalian cell produced GDNF and NRTN proteins.

**Patient type**

- Younger patients with marked L-dopa response and no major ventral striatal dopamine loss on dopamine imaging.
- Avoid certain genetic forms/variants associated with Parkinsonism (Parkin; GBA).

**Disease stage**

- Avoid late stage disease.

**Dose given**

- Depends on the neurotrophic factor that is being delivered, but probably need higher doses than have been trialed to date (with the exception of the first ICV trial of GDNF).

**Volume given**

- Depends on delivery system but need to give up to ~1 ml per striatum treated.

**Delivery device**

- Several now in existence, e.g., Renishaw, Clear point.

**Need for adjunct therapies**

- Not proven to be needed, although preclinical data suggests that Nurr 1 agonists may enhance the efficacy of GDNF—so perhaps this should be included as part of further trials.

**Need for imaging? If so with what?**

- F-dopa PET imaging seems to have provided useful information in trials to date, but need for other PET markers looking DA turnover/release as well as network reconstruction.

**Trial end points: What and when?**

- Standard measures UPDRS part 2 ± PDCore scores at 18–24 month as the primary end point.
- Sample size currently undecided given lack of major effects seen to date which would allow one to power such a study.

**Trial design**

- Consider a delayed start design to the trial or arandomized double-blind placebo-controlled study.
- Keep the trial outcomes and measures simple.
- In postmortem samples, it is important to show that GDNF and NRTN have activated RET-dependent signalling pathways or to show direct RET receptor activation.

**Health economics for this agent?**

- Depends on where it is positioned

BUAlthough it works uniquely to restore the dopaminergic nigrostriatal, it will nevertheless have to compete with other “DA” therapies/interventions- new dopamine drugs; DuoDopa®; Deep Brain Stimulation etc and the newer dopamine gene or cell-based therapies should they be shown to work.

- Currently it would not be competitive given the size of effects seen to date, but this may relate to suboptimal delivery, etc.
- 

928 In particular, it was felt that a viral delivery sys- 943  
 929 tem using some of the newer modified approaches 944  
 930 would be advantageous given the one-off nature of 945  
 931 the surgery compared to the relative complexity of 946  
 932 the neurosurgery needed to implant the infusion delivery 947  
 933 systems used in the recent GDNF trial and conse- 948  
 934 quent requirement for on-going infusions. That said, 949  
 935 the efficacy of GDNF may require intermittent rather 950  
 936 than continuous RET receptor stimulation and, whilst 951  
 937 in the development phase, understanding the exact 952  
 938 dose administered and retaining the ability to reduce 953  
 939 and stop dosing may have utility. In addition, it 954  
 940 seems logical to assume that the individuals most 955  
 941 likely to benefit from such a treatment would be 956  
 942 those individuals with most neurons and fibres left

to rescue, namely patients with early stage PDwith 943  
 evidence of fibre loss restricted to the dorsal striatum 944  
 [52]—where the therapeutic agent would be targeted. 945  
 If such an approach were recommended, then ensur- 946  
 ing the patient actually has Dopa-responsive PD will 947  
 be critical, and the use of imaging to help support 948  
 such a diagnosis would be essential, including both 949  
 DA imaging as well possibly fluorodeoxyglucose 950  
 (FDG) PET (for both diagnostic stratification and cor- 951  
 roboratorion of functional target engagement) [53]. In 952  
 addition, the exclusion of certain genetic forms of 953  
 parkinsonism may be wise, for example GBA het- 954  
 erozygote patients, given that they progress more 955  
 quickly especially with pathology outside of the DA 956  
 nigrostriatal pathway [54, 55]. 957

958 Whether an adjunct therapy to up regulate Nurr1  
959 would be required is still unclear given the clinical  
960 data to date, but the preclinical data would suggest  
961 this. However, there is no such agent that has yet  
962 been identified with a safety profile that is accept-  
963 able and even if one existed, questions still arise as  
964 to how long such a therapy should be given. Coupled  
965 to this is a need to better understand the optimal dose  
966 of GDNF and volume of its distribution, to ensure  
967 that the treatment has the best chance of showing a  
968 clinical effect, and that this effect is the maximal one  
969 that one could expect for that agent. Finally, since  
970 *E. coli* cannot form seven disulphide bonds correctly  
971 and glycosylate GDNF, the use of mammalian cells  
972 to make GDNF should be considered if protein infu-  
973 sions are being considered although this brings with  
974 it major cost implications.

975 As to what any trial should look like, there is still  
976 much debate as to what primary end-point should be  
977 used and at what time point, and input from the patient  
978 community on this will be vital going forward. How-  
979 ever, this end point should reflect changes in those  
980 clinical aspects of PD that respond to dopaminergic  
981 interventions given this is the pathway being targeted  
982 by these treatments. A double-blind sham surgery  
983 trial would be the preferred design for future studies,  
984 although whether more optimisation of the delivery  
985 of GDNF should be carried out before such a trial is  
986 undertaken is debatable. Overall there was a consen-  
987 sus from the workshop, that longer trials may be better  
988 for fully exploring whether this agent can mediate  
989 neurorestoration and thus waiting at least 18 months  
990 from the start of any therapeutic intervention would  
991 increase the chances of seeing any such effects. In  
992 addition, using composite end points may also have  
993 some merit given that the use of any single one, such  
994 as the UPDRS part III score, has limitations. As to  
995 what that composite clinical end point should look  
996 like is unclear as regulatory agencies are currently  
997 not accepting these for licensing purposes. However,  
998 one that has recently been proposed relating to the  
999 recent Bristol GDNF trial, PDCORE, embraces good  
1000 quality on-time; activities of daily living and reflects  
1001 previous participant feedback [56].

1002 In addition to the use of wild type GDNF and  
1003 NRTN given as protein deliveries or a gene ther-  
1004 apy, other similar approaches for treating PD were  
1005 also discussed. In pre-clinical studies, new GDNF  
1006 and NRTN mutants with improved diffusion and  
1007 stability have shown beneficial effects [57, 58]. Fur-  
1008 thermore, to overcome the limitations of some of  
1009 the pharmacokinetic properties of the GDNF and

1010 NRTN proteins, a blood-brain-barrier penetrating  
1011 small molecule GDNF receptor agonist has recently  
1012 been developed. This compound activates RET-  
1013 dependent intracellular signaling cascades in DA  
1014 neurons both *in vitro* and *in vivo* and also stimulates  
1015 the release of dopamine in the mouse striatum—all of  
1016 which suggests that this agent could be a novel future  
1017 treatment of PD [59].

1018 In this respect, cerebral dopamine neurotrophic  
1019 factor (CDNF) is a relatively recently discov-  
1020 ered endoplasmic reticulum (ER) located, but also  
1021 secreted, protein that protects and restores the func-  
1022 tion of DA neurons in rodent and non-human primate  
1023 models of PD and does so more effectively than  
1024 GDNF [60]. CDNF is very different from other  
1025 known trophic factors—it has a unique structure  
1026 and mode of action protecting neurons by inhibit-  
1027 ing cell death, regulating ER stress, the unfolded  
1028 protein response (UPR) and reducing inflammation  
1029 [61]. In addition, CDNF rescues only ER-stressed or  
1030 degenerating neurons and does not influence naïve  
1031 healthy neurons. This agent is now the subject on  
1032 an EUH2020 funded phase I–II clinical trial in PD  
1033 patients (NCT03295786) [61].

## 1034 CONCLUSIONS

1035 This special workshop comprehensively covered  
1036 the studies evaluating GDNF and the related trophic  
1037 factor NRTN in PD both preclinically and clinically.  
1038 It critically appraised the work so that conclusions  
1039 could be drawn as to **what has been shown and what  
1040 has not been shown** with these agents. It was gen-  
1041 erally agreed that GDNF and NRTN have worked  
1042 relatively well in neurotoxic animal models of PD  
1043 but that their translation to the clinic has so far failed  
1044 to show a major impact—perhaps highlighting the  
1045 predictive limitations of toxin animal models being  
1046 commonly used in the preclinical space in PD and the  
1047 way we plan clinical trials.

1048 Clinically, there is evidence that these neurotrophic  
1049 factors are able to rescue the expression of TH in the  
1050 human PD brain with some suggestion of a clinical  
1051 correlate. Nevertheless, the current size of any such  
1052 effect is not competitive compared to what is already  
1053 clinically available for the DA-related features of PD  
1054 (DuoDopa<sup>®</sup>; deep brain stimulation; lesion surgery  
1055 such as pallidotomy; apomorphine pumps, etc.) and  
1056 this may also be the case with new agents that are cur-  
1057 rently being trialled in PD around dopamine rescue  
1058 (stem cell derived DA neurons; CDNF, “dopamine”  
1059

gene therapies). However, it must be realised that these agents are uniquely designed to restore and regenerate the dopaminergic pathway which is very different from these other symptomatic therapies.

In conclusion, further work is needed to understand better what can be achieved with GDNF and related factors in the clinic to improve the lives of patients with PD, although ultimately whether it will ever have a competitive place for treating people with PD remains unclear.

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## CONFLICTS OF INTEREST

Roger Barker is a member of the advisory board for Novo Nordisk; Living Cell Technologies; FCDI; BlueRock Therapeutics, Aspen Neuroscience and UCB. He receives royalties from Springer-Nature and Wiley. He has received grant support from the EU; NIHR; MRC; Wellcome Trust; HDA; Rosetrees Trust; Cure Parkinson's Trust; Parkinson's UK and Evelyn Trust. He is Co-Editor in Chief of the Journal of Neurology and an Associate Editor for the Journal of Parkinson's Disease.

Don Gash is a founding Partner in Avast Therapeutics Inc. with patents on drug candidates for treating neurological disorders.

Alan Whone was a PI on the recent UK GDNF trial that received support from Parkinson's UK and the Cure Parkinson's Trust

Tony Lang reports consultancy support from Abbvie, Acorda, AFFiRis, Biogen, Denali, Janssen, Intracellular, Kallyope, Lundbeck, Paladin, Retrophin, Roche, Sun Pharma, Theravance, and Corticobasal Degeneration Solutions; advisory board support from Jazz Pharma, PhotoPharmics, Sunovion; other honoraria from Sun Pharma, AbbVie, Sunovion, American Academy of Neurology and the International Parkinson and Movement Disorder Society; grants from Brain Canada, Canadian Institutes of Health Research, Corticobasal Degeneration Solutions, Edmond J Safra Philanthropic Foundation, Michael J. Fox Foundation, the Ontario Brain Institute, Parkinson Foundation, Parkinson Canada, and W. Garfield Weston Foundation and royalties from

Elsevier, Saunders, Wiley-Blackwell, Johns Hopkins Press, and Cambridge University Press.

Amber van Laar is an employee of Brain Neurotherapy Bio, Inc.

Adrian Kells is an employee and stock holder of Brain Neurotherapy Bio, Inc.

Massimo Fiandaca is Vice President, Clinical Affairs, at Brain Neurotherapy Bio, Inc, a company developing AAV2-GDNF gene therapy for Parkinson's disease. I also have an equity interest in this company.

Jeff Kordower serves as a consultant and scientific advisor to Fuji-Cellular dynamics Inc., a company performing preclinical, and is in the planning stages of clinical IPSC dopamine grafts in patients with PD. Dr. Kordower is also a consultant for Seelos-Inc., NsGene Inc., Brainstorm Inc., Abbvie, Inc., Biogen Inc., and Inhibikase Inc. Dr. Kordower is also an Associate Editor for Journal of Comparative Neurology, Neurobiology of Aging, and Journal of Parkinson's Disease.

Kris Bankiewicz is the Founder and CEO of Brain Neurotherapy Bio that is developing AAV2-GDNF for PD, co-founder of Voyager Therapeutics that is developing AAV2AADC for PD, co-founder of Med-Genesis Therapeutics that is developing rec. GDNF protein for PD. I have financial interest in all the companies.

Karl Kieburtz is a consultant for Clintrex Research Corp, Roche/Genentech, Novartis, Blackfynn LLC. He has grant support from NIH (NINDS, NCATS), Michael J Fox Foundation and has ownership in Clintrex Research Corp, Hoover Brown LLC, Blackfynn LLC, Safe Therapeutics LLC.

Sigrid Booms is an employee of Herantis Pharma Plc.

Henri Huttunen is co-founder, shareholder and an employee of Herantis Pharma Plc.

A. Jon Stoessl reports personal fees from AbbVie, personal fees from Voyager/Neurocrine Biosciences, and is Editor-in-Chief, Movement Disorders (stipend).

Howard Feder off is a co-founder of Med Genesis Therapetix and Brain Neurotherapy Bio. He also serves as the CEO of Aspen Neuroscience. He is a consultant for Juvenescence, a scientific advisory board member for Ovid Therapeutics, chair of the board for Souvien and director for Perthera. He holds equity interests in all of the above.

Mart Saarma is founder and shareholder in the Herantis Pharma Plc. This company is testing CDNF in clinical trials.

Merja H. Voutilainen is an inventor in the CDNF-patent, which is owned by Herantis Pharma Plc.

Codrin Lungu has received honoraria from Elsevier, Inc. for editorial work. The work has been conducted as part of official duty in the course of employment with the NIH, and agency of the US Federal Government.

Anders Bjorklund, David Eidelberg, David Dexter, Jamie Eberling, Richard Wyse, Simon Stott, Eros Bresolin, Lyndsey Issacs, Patrik Brundin, Brain Fiske, Camille Carroll, Alasdair Coles, Leah Mursaleen all have no disclosures.

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