

Title	Neurotrophic factors for the treatment of Parkinson's disease
Authors	Sullivan, Aideen M.;Toulouse, André
Publication date	2011-06
Original Citation	Sullivan A.M., Toulouse A.; (2011) 'Neurotrophic factors for the treatment of Parkinson's disease'. Cytokine & Growth Factor Reviews, 22 (3):157-165. doi: <a href="http://dx.doi.org/10.1016/j.cytogfr.2011.05.001">http://dx.doi.org/10.1016/j.cytogfr.2011.05.001</a>
Type of publication	Article (peer-reviewed)
Link to publisher's version	<a href="http://dx.doi.org/10.1016/j.cytogfr.2011.05.001">10.1016/j.cytogfr.2011.05.001</a>
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Download date	2024-04-20 00:59:09
Item downloaded from	<a href="https://hdl.handle.net/10468/759">https://hdl.handle.net/10468/759</a>



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## **Neurotrophic factors for the treatment of Parkinson's disease**

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## **Abstract**

Parkinson's disease (PD) is a common neurodegenerative disorder caused by the progressive degeneration of the nigrostriatal dopaminergic pathway. The resulting loss of dopamine neurotransmission is responsible for the symptoms of the disease. Available treatments are initially successful in treating PD symptoms; however, their long-term use is associated with complications and they cannot stop the neurodegeneration. Current research aims at developing new therapies to halt/reverse the neurodegenerative process, rather than treating symptoms. Neurotrophic factors are proteins critical for maintenance and protection of neurones in the developing and adult brain. Several neurotrophic factors have been investigated for their protective effects on dopaminergic neurones. Here we review some of the most promising factors and provide an update on their status in clinical trials.

**Keywords:** Glial cell line-derived neurotrophic factor; Growth/differentiation factor 5; Neurturin; Mesencephalic astrocyte-derived neurotrophic factor; Cerebral dopaminergic neurotrophic factor

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## **1. Parkinson's disease**

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with an incidence of 1.5-2% in the population over 60 years of age, which increases significantly with advancing age. As life expectancy is significantly increasing in the Western world, the incidence of PD is steadily escalating. Consequently, the financial and economical burden of the treatment and care of PD patients is substantial and increasing [1]. Thus, research on the causes of this debilitating disease is critical, as is the development of new treatments.

PD is caused by the progressive degeneration of the nigrostriatal (A9) dopaminergic pathway, which projects from the substantia nigra in the midbrain to the caudate-putamen (striatum) in the forebrain [2, 3]. The resulting loss of dopamine neurotransmission in the striatum causes the cardinal symptoms of the disease: tremor at rest, rigidity and bradykinesia. Approximately 5% of PD cases are caused by heritable genetic mutations. The remaining cases are sporadic and of unknown origin, although many theories have been proposed to explain the cause of dopaminergic neuronal death which occurs in PD, such as environmental toxins, mitochondrial dysfunction with resulting oxidative stress, and inflammatory mechanisms [4, 5].

The therapies presently available for PD are not effective in the long-term and cannot stop the ongoing neurodegeneration. The most commonly-used treatment is the dopamine precursor, levodopa, which replaces lost dopamine in the denervated striatum and relieves motor symptoms. Levodopa is generally administered in conjunction with an inhibitor of peripheral decarboxylase (carbidopa, benserazide), which has the effect of enhancing the central activity of levodopa. Levodopa is initially successful, however about 50% of patients develop

complications within the first five years of treatment, primarily severe motor fluctuations and dyskinesias. Other drug treatments include inhibitors of the dopamine breakdown enzymes catechol-O-methyl-transferase (tolcapone, entacapone) or monoamine oxidase-B (selegiline, rasagiline), and dopamine receptor agonists (bromocriptine, pergolide, pramipexole, ropinirole). Surgical methods involving ablation of deep brain structures or deep brain stimulation have also been used with good success, but these procedures are not widely-available or applicable for all patients. In summary, none of the current treatments provide safe and long-lasting relief from the symptoms and have little or no effect on the progression of the disease [1]. Current research is aimed at developing therapies that will halt the neurodegenerative process, rather than simply treat the symptoms. These include the use of antioxidants, anti-apoptotic agents, cell-based therapies and neuroprotective agents such as neurotrophic factors (NTF).

## **2. Neurotrophic factors for dopaminergic neurones**

NTF are secreted proteins that play critical roles in the induction, specification, survival and maturation of developing neurones. Certain NTF also act in the adult brain, to support and protect mature neuronal populations. As PD is primarily caused by the degeneration of a single neuronal population, several factors have been investigated for their neurotrophic and protective effects on dopaminergic neurones. The goal of this therapeutic approach is to apply a factor(s) which can halt or reverse the progressive degeneration of nigrostriatal dopaminergic neurones, and which can be administered to patients in a safe, targeted and long-lasting manner. NTF that have selective effects on dopaminergic neurones represent good targets for this approach. These include glial cell line-derived neurotrophic factor

(GDNF), neurturin, growth/differentiation factor (GDF) 5, mesencephalic astrocyte-derived neurotrophic factor (MANF) and cerebral dopaminergic neurotrophic factor (CDNF).

## 2.1 GDNF family of ligands (GFL)

### 2.1.1 Effects of GDNF *in vitro*

The GFL family is composed of four factors - GDNF, neurturin, persephin and artemin. GDNF, its prototypical member, was isolated from a glial cell line due to its neurotrophic effects on cultured dopaminergic neurones [6]. Subsequent studies have shown that it can also act on other neuronal types (see [7]). GDNF has been shown to induce the dopamine synthetic enzyme, tyrosine hydroxylase (TH), in fetal human and rat cortical cultures (Table 1) [8]. GDNF has been consistently shown to promote the survival and differentiation of dopaminergic neurones *in vitro* [6, 9, 10] and to protect these cells from the dopaminergic toxins, 1-methyl-4-phenylpyridinium ion (MPP+) and 6-hydroxydopamine (6-OHDA), [11-13]. GDNF treatment has also been reported to reduce apoptosis in dopaminergic neurones cultured from embryonic rat [14, 15] and human [16] midbrain. GDNF can also protect cultured dopaminergic neurones from lipopolysaccharide-induced degeneration, a model of neuroinflammation [17]. Most of the above studies were conducted on embryonic day 14 (E14) rat midbrain, the time point at which dopaminergic neurones are undergoing their terminal mitotic divisions and are beginning to differentiate. An *in vitro* study showed that GDNF can also support these neurones during their postnatal period of natural developmental death [18]. Midbrain cultures may contain dopaminergic neurones of two origins, the nigrostriatal pathway (A9), which degenerates in PD and the mesolimbic pathway (A10), which is largely spared in this disease. Differential effects of GDNF treatment on A9 and A10 dopaminergic neurones *in vitro* have been reported, whereby a single dose of GDNF

selectively enhanced the survival of A9 cells, while repeated exposure to this factor only increased the survival of A10 cells [19].

### *2.1.2 Effects of GDNF in vivo*

In normal adult rats, a single injection of GDNF into either the substantia nigra or striatum significantly increased the levels of dopamine and its metabolites in the striatum and nigra [20]. Several studies have reported neuroprotective and functional effects of GDNF in adult animal models of PD (see [21]). In one early study, repeated injections of recombinant rat GDNF protected against dopaminergic cell loss induced by transection of the adult rat medial forebrain bundle (MFB), the fibre bundle containing the dopaminergic projections from the substantia nigra to the striatum [22].

The most widely-used laboratory model of PD involves unilateral injection of the selective dopaminergic toxin, 6-OHDA in the adult rat. This results in the degeneration of nigrostriatal dopaminergic neurones and consequent depletion of striatal dopamine transmission on one side of the brain. Stereotaxic injection of 6-OHDA into the MFB or substantia nigra induces a complete lesion of the nigrostriatal pathway, while intrastriatal injection induces progressive neurodegeneration. Several groups have examined the effects of intracerebral injection of recombinant GDNF in rats with 6-OHDA-lesions of the MFB. Injection of GDNF in or near the substantia nigra at four weeks after or just before a 6-OHDA lesion resulted in reduction of motor deficits, and preservation of nigral dopaminergic neurones and striatal dopamine release and uptake [23-25]. In adult rats with bilateral 6-OHDA lesions of the MFB, injection of high doses of GDNF into the lateral ventricles resulted in improved motor function and sparing of nigral dopaminergic neurones [26]. GDNF's effects may be dependent on host age,

as one study found that young rats displayed significantly higher levels of neuroprotection than aged rats [27]. This may be relevant to clinical trials, where the age of the patient may determine the extent of neuroprotection that is achievable with GDNF treatment.

The intrastriatal lesion model has been used extensively since it is possible to administer the NTF while neurodegeneration is progressing. Administration of single or multiple doses of recombinant human GDNF near or in the substantia nigra starting at the day of, or the day before, a 6-OHDA-induced lesion, had protective effects on nigral dopaminergic cell bodies [28, 29] A series of four intrastriatal injections of GDNF was found to decrease drug-induced rotations and preserve nigrostriatal dopaminergic neurones in adult rats with 6-OHDA-induced lesions [30]. Long-term rescue of nigrostriatal dopaminergic neurones from 6-OHDA lesions was reported after short-term GDNF treatment [31]. Long-term protection against rotational asymmetry, reductions in striatal dopamine levels and uptake, and death of nigral dopaminergic cell bodies induced by 6-OHDA lesions of the MFB was conferred by a single dose of GDNF, divided between the lateral ventricle and substantia nigra [32]. GDNF injections into the striatum one week after an intrastriatal 6-OHDA lesion resulted in re-innervation of the striatum as well as recovery of motor function [33], indicating that the ability of intrastriatal GDNF injection to confer behavioural improvements may be due to its effects on the remaining striatal afferents in the partially-denervated striatum.

For application to clinical studies, the optimal injection site for production of safe and effective results is obviously an important consideration. Some studies have directly compared the sites of administration of GDNF in 6-OHDA-lesioned rats. Kirik et al. found that intrastriatal GDNF delivery had protective effects on motor function and the integrity of

the nigrostriatal pathway, intranigral GDNF protected nigral cell bodies but not striatal innervation or motor function, while intraventricular GDNF had no significant effects [34]. Another study found that intraventricular infusion of GDNF starting two weeks after an intrastriatal lesion had protective effects on the integrity and function of the nigrostriatal pathway, which lasted for six weeks after cessation of GDNF infusion, whereas the effects of intrastriatal infusion stopped upon withdrawal of GDNF [35]. Another group found that intrastriatal infusion of a high dose of GDNF four weeks after an intrastriatal lesion induced restorative effects on motor behaviour and the integrity of dopaminergic neurones and their terminals [36]. Thus, the intrastriatal route of administration appears to be the most efficacious in this progressive model of PD. Sequential application of GDNF over the nigra for two weeks, followed by injections of GDNF into the striatum for three weeks, in rats with intrastriatal 6-OHDA lesions, protected nigral dopaminergic cell bodies but did not prevent striatal denervation or improve motor function [37]. This suggests that the motor improvements observed in the other studies were dependent on an ability of GDNF to induce reinnervation of the lesioned striatum, perhaps by stimulating axonal sprouting from the remaining dopaminergic neurones. Thus, once the axonal retraction to the level of the nigra has occurred, application of GDNF to the striatum appears to be ineffective. This is an important consideration for clinical studies, as it suggests that there is a window of opportunity in which GDNF application may be therapeutically effective, but that this factor may not be useful at advanced stages of the disease.

In another commonly-used animal model of PD, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated adult mice, GDNF injected either before or one week after MPTP treatment, conferred significant protective or restorative effects, respectively [38].

Gash and colleagues showed that administration of recombinant GDNF in MPTP-treated rhesus monkeys induced motor recovery and protection of nigral dopaminergic neurones and their striatal projections [39]. Combined application of oral levodopa and intracerebroventricular GDNF resulted in significant behavioural improvements with reduced levodopa-induced side-effects, in MPTP-treated monkeys [40]. Another study found that intraventricular GDNF improved motor function and reduced levodopa-induced dyskinesias in this model [41]. Significant recovery of motor function for at least four months was reported in MPTP-treated monkeys that had received GDNF into the lateral ventricles [42], while intraventricular injections of GDNF were found to increase intrastriatal but not intranigral dopamine levels [43]. Infusion of GDNF into the putamen of MPTP-treated monkeys induced a gradual and significant reduction in parkinsonian symptoms [44]. This appeared to be a regenerative action, since GDNF was injected at three months after the MPTP lesion, when the nigrostriatal pathway had presumably undergone significant degeneration. Chronic intraputaminal administration of GDNF in aged monkeys had a long-lasting protective action on nigrostriatal dopaminergic neurones and on motor function, without any adverse side-effects [45].

Neurotrophic proteins like GDNF are metabolised rapidly in the brain and thus single injections of this factor cannot confer permanent effects. Gene therapy approaches have been applied to achieve long-term and targeted delivery of GDNF to the injured nigrostriatal pathway. Adenoviral vector delivery of GDNF into or close to the substantia nigra [46, 47] or into the striatum [48, 49] of rats with intrastriatal 6-OHDA lesions resulted in significant motor improvements and protection of nigral dopaminergic neurones. Adenoviral-delivered GDNF induced behavioural and neuroprotective effects when injected into the substantia nigra, but not into the striatum, in rats that had intrastriatal 6-OHDA lesions [50]. In MPTP-

treated mice, adenoviral vector-mediated GDNF delivery to the striatum prevented depletion of striatal dopamine levels [51]. A study which compared the effects of intrastriatal and perinigral injection of an adenoviral vector encoding GDNF found that, while both injection routes conferred protective effects on dopaminergic cell bodies in the nigra, only the intrastriatal route reduced motor deficits, in rats with intrastriatal 6-OHDA lesions [52].

Another vector system, based on adeno-associated virus 2 (AAV2), has also shown efficacy in animal models of PD. Mandel and co-workers reported significant protective effects on the nigrostriatal pathway and its functioning, in adult rats with intrastriatal 6-OHDA lesions, following intranigral injection of AAV2-GDNF either three weeks before [47] or just after the lesion [53]. AAV2-mediated delivery of GDNF to the striatum, but not to the substantia nigra, induced gradual behavioural recovery and regeneration of the 6-OHDA-lesioned nigrostriatal system in adult rats [34]. AAV2 vectors have the advantage over adenoviral vectors in that they can integrate and stably express their transgene product in non-dividing cells such as neurons. Also, they are relatively safe as there is little or no host immune response due to the absence of viral genes in these vectors. Their disadvantage is that they can only deliver gene constructs of relatively small size compared to adenoviral vectors. Furthermore, there is a delay before the transgene is expressed following intracerebral injection of an AAV2 vector.

A third type of vector system, based on lentiviruses, has also been used to deliver GDNF in PD animal models, with promising results. Lentiviral vectors have the capacity to deliver large transgenes and they can integrate efficiently into non-dividing cells. Delivery of the human GDNF gene using lentiviral vectors in MPTP-lesioned and aged rhesus monkeys achieved long-term gene expression and significant functional benefits [54]. Kordower and

colleagues administered lentiviral-GDNF to the striatum and substantia nigra of nonlesioned aged monkeys and MPTP-treated young monkeys and found extensive expression of GDNF in all of the brains. Lentiviral-delivered GDNF reversed motor deficits in the aged monkeys and prevented nigrostriatal degeneration and the development of functional deficits in the MPTP-lesioned animals. Another study used a lentiviral vector to achieve long-term delivery of GDNF to the striatum and substantia nigra of aged rhesus monkeys, and found that this treatment conferred significant protective effects on the functioning and integrity of the nigrostriatal pathway [55]. Lentiviral delivery of GDNF was also found to increase the number of intrinsic dopaminergic neurones in the primate striatum [56]. Lentiviral-mediated delivery is very effective but there are concerns about its safety and these will have to be addressed before clinical application of this system is feasible.

Another avenue of exploration is the co-administration of NTF with neuronal transplants in cell replacement therapy approaches to PD. Transplantation of embryonic midbrain tissue is a promising and successful therapy for PD, but is limited by the poor survival of the transplanted dopaminergic neurones (see [57]). GDNF has been shown to improve the survival and integration of grafted embryonic dopaminergic neurones in animal models of PD. Rosenblad and colleagues reported that repeated injections of this factor adjacent to embryonic rat ventral midbrain grafts in the 6-OHDA-lesioned rat striatum improved the survival of the grafted dopaminergic cells and induced earlier recovery of motor function than untreated grafts [58]. Improvements in the survival of grafted dopaminergic neurones and their integration into the host striatum were also reported after pre-incubation of the grafts with GDNF [59-62]. Injection of GDNF along the nigrostriatal tract stimulated the outgrowth of dopaminergic fibres from intranigral grafts towards the striatum [63-65]. Enhancement of complex motor functions, as well as improved graft survival, were found in 6-OHDA-

lesioned rats that had received GDNF-pretreated grafts [66]. GDNF pre-treatment has also been used to promote the survival of human fetal midbrain tissue, when grafted into two PD patients [67]. These patients displayed a large increase in fluorodopa uptake after one year, an index of striatal dopaminergic transmission, as measured by positron emission tomography (PET).

Ex vivo gene therapy approaches have been applied in attempts to extend the effects of exogenous GDNF, which is rapidly metabolised *in vivo*. Genetically-modified embryonic rat midbrain cells which over-express GDNF have been found to induce earlier functional recovery in 6-OHDA-lesioned rats than control grafts [68]. GDNF-overexpressing rat neural precursor cells also significantly increased the survival of co-grafted embryonic dopaminergic neurones [69]. Human neural progenitor cells have been used to deliver GDNF, which conferred protective effects on the lesioned nigrostriatal pathway in adult rats [70]. This cellular delivery system, which allows the release of GDNF under an inducible promoter, has also been found to provide GDNF to the aged monkey brain for at least three months [70]. Encapsulation technology involves enclosing cells within a semi-permeable membrane composed of polymer fibres, which allows outward diffusion of any proteins secreted by the cells, while preventing the cells from proliferating extensively and forming tumours. Intrastratial grafting of an encapsulated GDNF-expressing human (BHK) cell line has been shown to confer neuroprotective and restorative effects in 6-OHDA-lesioned rats [71, 72], particularly when the GDNF-expressing cells are implanted at an early stage of the disease progression [73]. Encapsulated human fibroblasts genetically engineered to overexpress GDNF were found to exert regenerative effects when implanted into the rat striatum one week after an intrastratial 6-OHDA lesion [74]. Although the cells were removed after six weeks, the regenerative effects on motor function and on nigral dopaminergic neurones were evident

for a further seven weeks, indicating that transient delivery of GDNF was sufficient to confer sustained effects. In MPTP-treated primates, encapsulated GDNF-expressing cells induced transient motor improvements and increases in striatal dopamine uptake, without any adverse side-effects [75]. Encapsulated cells expressing GDNF have also been applied in combination with embryonic rat brain grafts in 6-OHDA-lesioned rats, and were found to improve the survival of the grafted dopaminergic neurones and their functional effects [76]. Encapsulated cell technology may have great potential for future clinical studies, should the promising effects found in these animal studies be extended to show long-term and safe delivery of appropriate doses of neurotrophic proteins (see [77]).

### *2.1.3 Effects of neurturin in vitro*

A second member of the GFL family, neurturin, was identified for its survival-promoting effects on sympathetic neurones [78]. It was found to promote the survival of developing and mature dopaminergic neurones *in vitro*. These effects were similar in strength to those of GDNF [79, 80]. Neurturin is expressed in the ventral midbrain and striatum during development [80].

### *2.1.4 Effects of neurturin in vivo*

Neurturin has been found to exert protective and functional effects on dopaminergic nigrostriatal neurones after 6-OHDA lesions of the adult rat MFB [79, 80] or striatum [81, 82] and after axotomy of the adult rat MFB [83]. The study by Rosenblad and colleagues directly compared the effects of neurturin with those of GDNF in the striatal 6-OHDA lesion model. They found that neurturin was less efficacious than GDNF after intrastriatal and especially after intraventricular delivery, which may reflect poor solubility of neurturin *in vivo* [82].

Another study showed that delivery of neurturin into the cerebral ventricles of adult rats using mini-pumps resulted in an increase in striatal dopamine levels [84]. A recent study reported that intranigral injection of recombinant neurturin induced increases in striatal dopamine release, which were similar in magnitude to those induced by intranigral injection of recombinant GDNF [85]. Intracerebral delivery of recombinant neurturin has also been found to protect nigrostriatal dopaminergic neurones and induce improvements in motor function in MPTP-treated monkeys [86, 87]. Co-administration with recombinant neurturin protein has been reported to improve the survival of fetal rat dopaminergic neurones after intrastriatal grafting into 6-OHDA-lesioned adult rats [88].

Lentiviral gene delivery to the striatum of 6-OHDA-lesioned adult rats of a modified neurturin construct, which had the pro-region deleted and replaced with an immunoglobulin heavy-chain signal peptide, had protective effects on the nigrostriatal pathway [89]. Sustained functional recovery, with minimal side-effects, was achieved following stereotaxic injection of an AAV2-based vector encoding the human neurturin gene, in MPTP-treated monkeys [90] and 6-OHDA-lesioned rats [91]. Stable expression of neurturin using this AAV2 delivery system was achieved for at least a year in rats [92]. Kordower and colleagues showed that, ten months after injection of AAV2-neurturin into the striatum and substantia nigra, MPTP-treated parkinsonian monkeys displayed a large reduction in the intensity of their motor symptoms compared to buffer-injected animals. This functional recovery was accompanied by significant preservation of dopaminergic neurones [90]. A study using the same expression system in 6-OHDA-lesioned adult rats found long-term neurturin expression in the striatum and dose-dependant protective effects on the nigrostriatal dopaminergic neurones for at least ten months [91]. Similar results were found after application of this system in aged monkeys and no adverse effects were recorded in this study after thorough toxicological testing [93]. A

further study by this group found that the expression of neurturin using this system could be sustained for a year in rhesus monkeys, as could its therapeutic effects [94]. Unlike the case with GDNF, no antibodies to neurturin or no pathological abnormalities were detected after AAV2-delivery in primates [95].

#### *2.1.5 Effects of persephin and artemin in vitro and in vivo*

Persephin, a third member of the GFL family, has also been found to exert neurotrophic effects on midbrain dopaminergic neurones *in vitro* [96] and *in vivo* [97]. This factor also has trophic effects on motor neurones [96] and has not been extensively investigated for its clinical potential in PD. The fourth member of the GFL family, artemin, has survival-promoting effects on dopaminergic neurones in culture [98] and *in vivo* [99], and also has potent actions on sensory neurones of the dorsal root ganglia [98]. Like persephin, artemin has not progressed into clinical trials for PD; however it has been tested as a therapeutic for neuropathy [100].

#### *2.2 GDF5*

GDF5, a member of the Transforming Growth Factor  $\beta$  superfamily of proteins, is also being investigated for its therapeutic potential in PD. GDF5 is related to the Bone Morphogenetic Proteins, which are involved in diverse physiological functions, including the development of the nervous system, where they play roles in early CNS patterning as well as in neural cell fate determination, differentiation, and survival (see [101]).

GDF5 mRNA and protein expression have been found in the embryonic, neonatal and adult rat brain including the striatum and midbrain [102-104]. GDF5 protein expression in the rat brain peaks at E14, the time at which dopaminergic neurones in the developing midbrain are undergoing terminal differentiation [103].

### 2.2.1 Effects of GDF5 *in vitro*

On embryonic rat dopaminergic neurones *in vitro*, GDF5 has selective trophic actions which are comparable to those of GDNF. Application of GDF5 promotes the survival of dopaminergic neurones in embryonic rat midbrain cultures and protects them against the dopaminergic neurotoxin MPP<sup>+</sup> [102] and against free radical-induced damage [105]. Application of recombinant human GDF5 induced a dramatic increase in the number of dopaminergic neurones in cultures of embryonic rat midbrain [106]. This study found that the effects of GDF5 may be dependent on BMPR1b, since application of GDF5 at the time of plating, when BMPR1b is expressed, increases dopaminergic neuronal number, but application after six days *in vitro*, when this receptor is no longer expressed, had no effect. GDF5 treatment also induced morphological changes in cultured embryonic rat dopaminergic neurones, stimulating neurite outgrowth and branching [106, 107]. Clayton and Sullivan found that the effects of GDF5 were much greater when cultures were prepared from the lateral part of the developing midbrain. Furthermore, the BMPR1b receptor was expressed at higher levels in the lateral than in the medial region, suggesting that GDF5 acts through this receptor to increase dopaminergic neuronal number. Combined application of GDF5 and GDNF have been shown to have additive neurotrophic effects on cultured embryonic rat dopaminergic neurones, indicating that these two factors may act on separate subpopulations of cells [108].

### 2.2.2 Effects of GDF5 *in vivo*

Studies have shown that GDF5 can protect and restore adult rat nigrostriatal dopaminergic neurones in 6-OHDA-lesioned animals. The first such study reported that intracerebral injection of recombinant human GDF5 just above the substantia nigra and into the lateral ventricles produced improvements in motor function, protected nigral dopaminergic neurones and their striatal terminals, and preserved striatal levels of dopamine, its metabolites and its uptake, in rats with 6-OHDA lesions of the MFB [109]. A follow-up study compared three injection sites and found that application of GDF5 into either the striatum or substantia nigra, but not into the lateral ventricle, produced optimal neuroprotective effects [110]. Delayed administration of recombinant human GDF5 at one or two weeks after an intrastriatal 6-OHDA lesion resulted in significant improvements in motor behaviour, but only the one-week injection regimen induced protection of nigral dopaminergic cell bodies, whereas there was no significant rescue of striatal dopaminergic terminals after either time-point of treatment [111]. This indicates that there is a window of time at which the degenerating nigrostriatal pathway can be rescued by exogenous trophic factors, which has relevance to clinical studies, as such therapies may only be effective at earlier stages of the disease. GDF5 has also been reported to improve the survival and function of grafted dopaminergic neurones to the same extent as GDNF [61]. This study found that pre-incubation of embryonic rat midbrain tissue in GDF5 or GDNF produced significant improvements in cell survival after intrastriatal grafting in 6-OHDA-lesioned rats. Furthermore, GDF5-treated grafts conferred significant motor improvements and preservation of nigral dopaminergic neurones and their striatal terminals in 6-OHDA-lesioned rats, to at least the same extent as GDNF-treated grafts [61]. Each of these *in vivo* studies involved infusion of recombinant GDF5 protein, which can only be effective for a limited time due to its being metabolised in the brain. Future studies will

examine alternative administration methods, such as viral vector-mediated delivery or the use of encapsulated cell technology. One recent study reported that GDF5-over-expressing embryonic rat midbrain transplants survived well in the 6-OHDA-lesioned adult rat striatum and had significant effects to improve motor behaviour in these animals [112].

## 2.3 *MANF and CDFN*

### 2.3.1 *Effects of MANF and CDFN in vitro*

MANF and CDFN are members of a recently-described family of evolutionarily-conserved proteins which are secreted from glial cells and have potent effects on dopaminergic neurones (see [113]). CDFN mRNA and protein have been found in both developing and adult mouse striatum and substantia nigra, suggesting that this factor may provide trophic support to mature dopaminergic neuronal cell bodies and their terminals, as well play a role in the development of these cells [114]. CDFN is a paralogue of MANF, which was originally isolated from a rat mesencephalic astrocyte cell line and found to have selective trophic effects on dopaminergic neurones *in vitro* [115]. Polymorphisms in CDFN have recently been linked to an early-onset form of PD [116]. Like CDFN, MANF is expressed in the rodent nigrostriatal system during the early postnatal period, as well as in the adult [117]. The *Drosophila* homologue of MANF, DmMANF, is essential for the maintenance and function of dopaminergic neurones [118]. Although the receptors for MANF and CDFN have yet to be identified, it appears that they may act by a different mechanism to the GFL family members to exert their neurotrophic effects on dopaminergic neurones. It is possible that at least part of the action of MANF is via inhibition of endoplasmic reticulum (ER) stress-induced neuronal cell death [113, 119]. Overexpression of MANF has recently been shown to block apoptotic

cell death in sympathetic neurones cultured from the neonatal mouse superior cervical ganglion [120], supporting an intracellular mechanism of action.

### *2.3.2 Effects of MANF / CDNF in vivo*

Intracerebral injection of recombinant human CDNF or MANF has been reported to have potent protective and restorative effects on the 6-OHDA-lesioned adult rat nigrostriatal pathway [114]. Each of these studies reported motor recovery and preservation of dopaminergic nigral cell bodies and their terminals in the striatum, conferred by intrastriatal injection of the NTF six hours before or four weeks after intrastriatal injection of 6-OHDA. The same group recently reported that intrastriatal infusion of CDNF via mini-pumps for two weeks, beginning at two weeks after an intrastriatal 6-OHDA lesion, was able to confer motor improvements and partially protect dopaminergic nigral cell bodies and their striatal terminals [121]. It is interesting that MANF appears to be transported through the brain in a different manner to that of GDNF, as radiolabelled MANF is transported to the cortex after intrastriatal injection [119], while labelled GDNF is retrogradely transported to the substantia nigra [122, 123], as is labelled CDNF [121].

## **3. Dopaminergic neurotrophic factors in clinical trials**

### *3.1 GDNF in clinical trials*

The potent and reproducible effects of GDNF in animal models led to the initiation of clinical trials in PD patients. The clinical application of NTF is hampered by the fact that these proteins do not cross the blood-brain barrier, and are rapidly degraded *in vivo*. The need for direct intracerebral delivery of NTF may increase the level of complications in patients. The

first clinical trial was a randomised controlled trial involving 50 patients, and used intraventricular delivery of recombinant human GDNF (r-metHuGDNF, liatermin®, manufactured by Amgen) or placebo [124]. This study reported no significant benefits of GDNF treatment over the placebo, probably because GDNF did not reach the striatum in sufficient amounts. In addition, troublesome side-effects were observed, including pain, depression, appetite loss and l'Hermitte's sign [124, 125], and these may have been due to the intraventricular delivery (Table 2).

Since intraventricular delivery did not achieve GDNF delivery to the striatum, subsequent trials used direct administration to brain parenchyma using a catheter system. Promising results emerged from two open-label trials, which used intraputaminal infusion of recombinant GDNF [126-129]. These trials both reported improvements in the patients' motor symptoms and in activities of daily living, without any serious side-effects. Gill and colleagues demonstrated that direct uni- or bilateral intraputaminal infusion of GDNF had long-lasting benefits in five patients suffering from advanced PD. After 24 months of treatment, all patients reported complete absence of akinesia and a significant reduction in the duration of dyskinetic episodes. Motor dysfunction was significantly reduced in both on- and off-medication phases compared to pre-treatment levels [128]. The second study, by Slevin and co-workers, used unilateral intraputaminal infusion of escalating doses of GDNF in ten patients with advanced PD and reported similar results, showing bilateral motor improvements after 24 weeks [129]. In both studies, the only consistent adverse effect was a mild l'Hermitte's sign. Improvements in dopamine storage were detected in the regions surrounding the catheter which was used for infusion of GDNF [126, 127].

These two studies demonstrated the feasibility and sustainability of GDNF treatment by intraputamina l infusion. However, a subsequent randomised placebo-controlled trial, involving 34 patients (17 received GDNF and 17 received placebo), reported no significant motor improvements in PD patients [124, 130]. After six months of bilateral intraputamina l infusion of recombinant GDNF, there were no significant differences between the motor scores of the unified Parkinson's disease rating scale (UPDRS) in patients that had received GDNF and in those that had received a placebo. Furthermore, safety concerns were raised, since approximately 10% of the GDNF-treated patients developed antibodies against the peptide, which could potentially counteract the therapeutic benefits [131]. A similar proportion of patients who had participated in the two open-label trials also developed antibodies to GDNF [129, 130]. PET studies showed a significant increase in 18F-dopa intake in the GDNF-infused patients compared to the placebo group, demonstrating a functional effect of GDNF infusion, although this did not translate into significant motor improvements [130]. The discrepancies between the open-label and placebo-controlled trials may have been due to variations in patient selection, as well as to a placebo effect. Optimisation of surgical methodologies, catheter design and positioning, drug dosage and diffusion, and patient selection will be necessary for any future GDNF clinical studies. The development of inexplicable cerebellar pathology in a primate model of PD after administration of a high dose of GDNF raised a further safety issue [132]. This resulted in a controversial decision by Amgen to cease all clinical trials using GDNF (see [133-135]). A recent study paved the way for future clinical trials by investigating GDNF distribution in the non-human primate brain following AAV2-mediated intraputamina l delivery [136]. It appears to be generally accepted that GDNF therapy for PD requires further development in pre-clinical trials and it is probable that alternative methodologies, such as viral vector-mediated expression, may prove to be more effective for achieving long-term and targeted GDNF delivery (see [137]).

### *3.2 Neurturin in clinical trials*

Based on the promising results found in animal studies using AAV2-mediated gene transfer of neurturin (CERE-120®), an open-label phase 1 clinical trial was initiated in PD patients by the company Ceregene [138]. Twelve patients, each suffering from advanced PD, received bilateral intraputaminial injections of AAV2-neurturin (at one of two doses) and were followed for twelve months. Results showed that neurturin-treated patients displayed reductions in their off-medication UPDRS score, decreases in the time spent in the ‘off’ period and reductions in dyskinesias, without any adverse side-effects [138]. However, a subsequent double-blind phase 2 trial, which involved 58 PD patients, was reported to have failed. In this trial, intraputaminial AAV2-neurturin did not have superior effects on motor function than sham surgery, when assessed after twelve months, and only modest benefits were recorded after eighteen months. Serious adverse side-effects were reported in about a third of the neurturin-treated patients [139]. Ceregene began to recruit PD patients for a new double-blind trial using AAV2-neurturin in September 2010 ([www.ceregene.com/press\\_101910.asp](http://www.ceregene.com/press_101910.asp)). This multi-centre phase 2 trial will involve about 52 patients, half of whom will receive intraputaminial AAV2-neurturin and half of whom will receive sham surgery. It is hoped that a new dosing regimen, designed to maximise the delivery of the neurturin gene throughout the degenerating nigrostriatal system, will be the key to a promising outcome from this trial. This hypothesis is based on a post mortem analysis on the brains of some of the patients who had received AAV2-neurturin (and who had died of unrelated causes), which found that neurturin had been retrogradely transported from the intraputaminial injection site to the substantia nigra [140]. The company subsequently administered AAV2-neurturin to both the substantia nigra and the striatum of

six PD patients and, based on analysis of the safety data from these six patients, are now recruiting patients for the second phase of this trial.

#### **4. Conclusion**

Despite the recent disappointing results in clinical trials with GDNF and neurturin, there remains an optimism that NTF will prove to be useful in PD therapy (see [7]). Optimisation of delivery methods is needed and vital information is being gleaned in this respect from studies in animal models, such as grafting of encapsulated cells expressing NTF and viral-mediated NTF delivery. It is likely that NTF will be most applicable in the early stages of the disease, to provide neuroprotection to the remaining nigrostriatal dopaminergic neurones before extensive neuronal loss has occurred. For future clinical trials, optimisation of surgical and infusion protocols, as well as careful patient selection, will be critical to advance this promising therapeutic approach. Animal studies have provided signs that NTF therapy may be more applicable in young patients than in old, and also that patients with earlier, less severe, disease stages may be more responsive to this type of therapy than those at advanced disease stages.

Researchers are currently investigating the use of stem or progenitor cells as a possible alternative to freshly-dissected embryonic midbrain for transplantation in PD patients. The use of such cells would alleviate some of the ethical and practical concerns associated with the use of fresh embryonic tissue. It is likely that NTF could be used to improve stem cell therapy for PD, both to enhance the survival of transplanted dopaminergic precursor cells, and to induce a dopaminergic cell fate in unspecified progenitors.

In conclusion, much research still remains to be conducted in the area of NTF therapy for PD. In the case of those factors which have been tested in clinical trials (GDNF and neurturin) optimisation of the surgical delivery procedures and patient selection will be critical to the ongoing development of this therapeutic approach. Exploration of novel delivery mechanisms, such as viral vector-mediated delivery and cell encapsulation, which has been tested in animal models of PD to deliver GDNF, will be critical. More information is needed about factors such as GDF5 and CDNF, which have shown promise in preclinical models of PD. Knowledge of the receptors and signal transduction pathways that are involved in the neurotrophic and protective effects of these factors will aid the future development of safe and targeted therapeutics.

## **5. Conflict of interest**

The authors have no conflict of interest in relation to the present work.

## **6. References**

1. Toulouse A., Sullivan A.M., Progress in Parkinson's disease-where do we stand? *Prog Neurobiol* 2008, 85, 376-392.
2. Fearnley J.M., Lees A.J., Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain* 1991, 114 ( Pt 5), 2283-2301.
3. Hoehn M.M., Yahr M.D., Parkinsonism: onset, progression and mortality. *Neurology* 1967, 17, 427-442.
4. Dauer W., Przedborski S., Parkinson's disease: mechanisms and models. *Neuron* 2003, 39, 889-909.
5. Long-Smith C.M., Sullivan A.M., Nolan Y.M., The influence of microglia on the pathogenesis of Parkinson's disease. *Prog Neurobiol* 2009, 89, 277-287.
6. Lin L.F., Doherty D.H., Lile J.D., Bektesh S., Collins F., GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 1993, 260, 1130-1132.
7. Aron L., Klein R., Repairing the parkinsonian brain with neurotrophic factors. *Trends Neurosci* 2011, 34, 88-100.
8. Theofilopoulos S., Goggi J., Riaz S.S., Jauniaux E., Stern G.M., Bradford H.F., Parallel induction of the formation of dopamine and its metabolites with induction of tyrosine hydroxylase expression in foetal rat and human cerebral cortical cells by brain-derived

- neurotrophic factor and glial-cell derived neurotrophic factor. *Brain Res Dev Brain Res* 2001, 127, 111-122.
9. Krieglstein K., Suter-Crazzolara C., Fischer W.H., Unsicker K., TGF-beta superfamily members promote survival of midbrain dopaminergic neurons and protect them against MPP+ toxicity. *Embo J* 1995, 14, 736-742.
  10. Widmer H.R., Schaller B., Meyer M., Seiler R.W., Glial cell line-derived neurotrophic factor stimulates the morphological differentiation of cultured ventral mesencephalic calbindin- and calretinin-expressing neurons. *Exp Neurol* 2000, 164, 71-81.
  11. Eggert K., Schlegel J., Oertel W., Wurz C., Krieg J.C., Vedder H., Glial cell line-derived neurotrophic factor protects dopaminergic neurons from 6-hydroxydopamine toxicity in vitro. *Neurosci Lett* 1999, 269, 178-182.
  12. Hou J.G., Lin L.F., Mytilineou C., Glial cell line-derived neurotrophic factor exerts neurotrophic effects on dopaminergic neurons in vitro and promotes their survival and regrowth after damage by 1-methyl-4-phenylpyridinium. *J Neurochem* 1996, 66, 74-82.
  13. Ma D., Wang X., Han J., NIH 3T3 cells or engineered NIH 3T3 cells stably expressing GDNF can protect primary dopaminergic neurons. *Neurol Res* 2000, 22, 538-544.
  14. Clarkson E.D., Zawada W.M., Freed C.R., GDNF reduces apoptosis in dopaminergic neurons in vitro. *Neuroreport* 1995, 7, 145-149.
  15. Sawada H., Ibi M., Kihara T. *et al.*, Neuroprotective mechanism of glial cell line-derived neurotrophic factor in mesencephalic neurons. *J Neurochem* 2000, 74, 1175-1184.
  16. Clarkson E.D., Zawada W.M., Freed C.R., GDNF improves survival and reduces apoptosis in human embryonic dopaminergic neurons in vitro. *Cell Tissue Res* 1997, 289, 207-210.
  17. Xing B., Xin T., Zhao L., Hunter R.L., Chen Y., Bing G., Glial cell line-derived neurotrophic factor protects midbrain dopaminergic neurons against lipopolysaccharide neurotoxicity. *J Neuroimmunol* 2010, 225, 43-51.
  18. Burke R.E., Antonelli M., Sulzer D., Glial cell line-derived neurotrophic growth factor inhibits apoptotic death of postnatal substantia nigra dopamine neurons in primary culture. *J Neurochem* 1998, 71, 517-525.
  19. Borgal L., Hong M., Sadi D., Mendez I., Differential effects of glial cell line-derived neurotrophic factor on A9 and A10 dopamine neuron survival in vitro. *Neuroscience* 2007, 147, 712-719.
  20. Martin D., Miller G., Cullen T., Fischer N., Dix D., Russell D., Intranigral or intrastriatal injections of GDNF: effects on monoamine levels and behavior in rats. *Eur J Pharmacol* 1996, 317, 247-256.
  21. Ramaswamy S., Soderstrom K.E., Kordower J.H., Trophic factors therapy in Parkinson's disease. *Prog Brain Res* 2009, 175, 201-216.
  22. Beck K.D., Valverde J., Alexi T. *et al.*, Mesencephalic dopaminergic neurons protected by GDNF from axotomy-induced degeneration in the adult brain. *Nature* 1995, 373, 339-341.
  23. Bowenkamp K.E., Hoffman A.F., Gerhardt G.A. *et al.*, Glial cell line-derived neurotrophic factor supports survival of injured midbrain dopaminergic neurons. *J Comp Neurol* 1995, 355, 479-489.
  24. Hoffer B.J., Hoffman A., Bowenkamp K. *et al.*, Glial cell line-derived neurotrophic factor reverses toxin-induced injury to midbrain dopaminergic neurons in vivo. *Neurosci Lett* 1994, 182, 107-111.
  25. Opacka-Juffry J., Ashworth S., Hume S.P., Martin D., Brooks D.J., Blunt S.B., GDNF protects against 6-OHDA nigrostriatal lesion: in vivo study with microdialysis and PET. *Neuroreport* 1995, 7, 348-352.
  26. Bowenkamp K.E., Lapchak P.A., Hoffer B.J., Miller P.J., Bickford P.C., Intracerebroventricular glial cell line-derived neurotrophic factor improves motor function

- and supports nigrostriatal dopamine neurons in bilaterally 6-hydroxydopamine lesioned rats. *Exp Neurol* 1997, 145, 104-117.
27. Fox C.M., Gash D.M., Smoot M.K., Cass W.A., Neuroprotective effects of GDNF against 6-OHDA in young and aged rats. *Brain Res* 2001, 896, 56-63.
  28. Kearns C.M., Gash D.M., GDNF protects nigral dopamine neurons against 6-hydroxydopamine in vivo. *Brain Res* 1995, 672, 104-111.
  29. Sauer H., Rosenblad C., Bjorklund A., Glial cell line-derived neurotrophic factor but not transforming growth factor beta 3 prevents delayed degeneration of nigral dopaminergic neurons following striatal 6-hydroxydopamine lesion. *Proc Natl Acad Sci U S A* 1995, 92, 8935-8939.
  30. Shults C.W., Kimber T., Martin D., Intrastratial injection of GDNF attenuates the effects of 6-hydroxydopamine. *Neuroreport* 1996, 7, 627-631.
  31. Winkler C., Sauer H., Lee C.S., Bjorklund A., Short-term GDNF treatment provides long-term rescue of lesioned nigral dopaminergic neurons in a rat model of Parkinson's disease. *J Neurosci* 1996, 16, 7206-7215.
  32. Sullivan A.M., Opacka-Juffry J., Blunt S.B., Long-term protection of the rat nigrostriatal dopaminergic system by glial cell line-derived neurotrophic factor against 6-hydroxydopamine in vivo. *Eur J Neurosci* 1998, 10, 57-63.
  33. Rosenblad C., Martinez-Serrano A., Bjorklund A., Intrastratial glial cell line-derived neurotrophic factor promotes sprouting of spared nigrostriatal dopaminergic afferents and induces recovery of function in a rat model of Parkinson's disease. *Neuroscience* 1998, 82, 129-137.
  34. Kirik D., Rosenblad C., Bjorklund A., Mandel R.J., Long-term rAAV-mediated gene transfer of GDNF in the rat Parkinson's model: intrastratial but not intranigral transduction promotes functional regeneration in the lesioned nigrostriatal system. *J Neurosci* 2000, 20, 4686-4700.
  35. Kirik D., Winkler C., Bjorklund A., Growth and functional efficacy of intrastratial nigral transplants depend on the extent of nigrostriatal degeneration. *J Neurosci* 2001, 21, 2889-2896.
  36. Aoi M., Date I., Tomita S., Ohmoto T., The effect of intrastratial single injection of GDNF on the nigrostriatal dopaminergic system in hemiparkinsonian rats: behavioral and histological studies using two different dosages. *Neurosci Res* 2000, 36, 319-325.
  37. Rosenblad C., Kirik D., Bjorklund A., Sequential administration of GDNF into the substantia nigra and striatum promotes dopamine neuron survival and axonal sprouting but not striatal reinnervation or functional recovery in the partial 6-OHDA lesion model. *Exp Neurol* 2000, 161, 503-516.
  38. Tomac A., Lindqvist E., Lin L.F. *et al.*, Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. *Nature* 1995, 373, 335-339.
  39. Gash D.M., Zhang Z., Ovadia A. *et al.*, Functional recovery in parkinsonian monkeys treated with GDNF. *Nature* 1996, 380, 252-255.
  40. Miyoshi Y., Zhang Z., Ovadia A. *et al.*, Glial cell line-derived neurotrophic factor-levodopa interactions and reduction of side effects in parkinsonian monkeys. *Ann Neurol* 1997, 42, 208-214.
  41. Iravani M.M., Costa S., Jackson M.J. *et al.*, GDNF reverses priming for dyskinesia in MPTP-treated, L-DOPA-primed common marmosets. *Eur J Neurosci* 2001, 13, 597-608.
  42. Zhang Z., Miyoshi Y., Lapchak P.A. *et al.*, Dose response to intraventricular glial cell line-derived neurotrophic factor administration in parkinsonian monkeys. *J Pharmacol Exp Ther* 1997, 282, 1396-1401.

43. Gerhardt G.A., Cass W.A., Huettl P., Brock S., Zhang Z., Gash D.M., GDNF improves dopamine function in the substantia nigra but not the putamen of unilateral MPTP-lesioned rhesus monkeys. *Brain Res* 1999, 817, 163-171.
44. Grondin R., Zhang Z., Yi A. *et al.*, Chronic, controlled GDNF infusion promotes structural and functional recovery in advanced parkinsonian monkeys. *Brain* 2002, 125, 2191-2201.
45. Maswood N., Grondin R., Zhang Z. *et al.*, Effects of chronic intraputaminal infusion of glial cell line-derived neurotrophic factor (GDNF) in aged Rhesus monkeys. *Neurobiol Aging* 2002, 23, 881-889.
46. Choi-Lundberg D.L., Lin Q., Chang Y.N. *et al.*, Dopaminergic neurons protected from degeneration by GDNF gene therapy. *Science* 1997, 275, 838-841.
47. Mandel R.J., Spratt S.K., Snyder R.O., Leff S.E., Midbrain injection of recombinant adeno-associated virus encoding rat glial cell line-derived neurotrophic factor protects nigral neurons in a progressive 6-hydroxydopamine-induced degeneration model of Parkinson's disease in rats. *Proc Natl Acad Sci U S A* 1997, 94, 14083-14088.
48. Bilang-Bleuel A., Revah F., Colin P. *et al.*, Intrastratial injection of an adenoviral vector expressing glial-cell-line-derived neurotrophic factor prevents dopaminergic neuron degeneration and behavioral impairment in a rat model of Parkinson disease. *Proc Natl Acad Sci U S A* 1997, 94, 8818-8823.
49. Choi-Lundberg D.L., Lin Q., Schallert T. *et al.*, Behavioral and cellular protection of rat dopaminergic neurons by an adenoviral vector encoding glial cell line-derived neurotrophic factor. *Exp Neurol* 1998, 154, 261-275.
50. Kozlowski D.A., Connor B., Tillerson J.L., Schallert T., Bohn M.C., Delivery of a GDNF gene into the substantia nigra after a progressive 6-OHDA lesion maintains functional nigrostriatal connections. *Exp Neurol* 2000, 166, 1-15.
51. Kojima H., Abiru Y., Sakajiri K. *et al.*, Adenovirus-mediated transduction with human glial cell line-derived neurotrophic factor gene prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopamine depletion in striatum of mouse brain. *Biochem Biophys Res Commun* 1997, 238, 569-573.
52. Connor B., Kozlowski D.A., Schallert T., Tillerson J.L., Davidson B.L., Bohn M.C., Differential effects of glial cell line-derived neurotrophic factor (GDNF) in the striatum and substantia nigra of the aged Parkinsonian rat. *Gene Ther* 1999, 6, 1936-1951.
53. Mandel R.J., Snyder R.O., Leff S.E., Recombinant adeno-associated viral vector-mediated glial cell line-derived neurotrophic factor gene transfer protects nigral dopamine neurons after onset of progressive degeneration in a rat model of Parkinson's disease. *Exp Neurol* 1999, 160, 205-214.
54. Kordower J.H., Emborg M.E., Bloch J. *et al.*, Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science* 2000, 290, 767-773.
55. Emborg M.E., Moirano J., Raschke J. *et al.*, Response of aged parkinsonian monkeys to in vivo gene transfer of GDNF. *Neurobiol Dis* 2009, 36, 303-311.
56. Palfi S., Leventhal L., Chu Y. *et al.*, Lentivirally delivered glial cell line-derived neurotrophic factor increases the number of striatal dopaminergic neurons in primate models of nigrostriatal degeneration. *J Neurosci* 2002, 22, 4942-4954.
57. Brundin P., Barker R.A., Parmar M., Neural grafting in Parkinson's disease Problems and possibilities. *Prog Brain Res* 2010, 184, 265-294.
58. Rosenblad C., Martinez-Serrano A., Bjorklund A., Glial cell line-derived neurotrophic factor increases survival, growth and function of intrastratial fetal nigral dopaminergic grafts. *Neuroscience* 1996, 75, 979-985.

59. Apostolides C., Sanford E., Hong M., Mendez I., Glial cell line-derived neurotrophic factor improves intrastriatal graft survival of stored dopaminergic cells. *Neuroscience* 1998, 83, 363-372.
60. Granholm A.C., Mott J.L., Bowenkamp K. *et al.*, Glial cell line-derived neurotrophic factor improves survival of ventral mesencephalic grafts to the 6-hydroxydopamine lesioned striatum. *Exp Brain Res* 1997, 116, 29-38.
61. Sullivan A.M., Pohl J., Blunt S.B., Growth/differentiation factor 5 and glial cell line-derived neurotrophic factor enhance survival and function of dopaminergic grafts in a rat model of Parkinson's disease. *Eur J Neurosci* 1998, 10, 3681-3688.
62. Yurek D.M., Glial cell line-derived neurotrophic factor improves survival of dopaminergic neurons in transplants of fetal ventral mesencephalic tissue. *Exp Neurol* 1998, 153, 195-202.
63. Sinclair S.R., Svendsen C.N., Torres E.M., Martin D., Fawcett J.W., Dunnett S.B., GDNF enhances dopaminergic cell survival and fibre outgrowth in embryonic nigral grafts. *Neuroreport* 1996, 7, 2547-2552.
64. Tang F.I., Tien L.T., Zhou F.C., Hoffer B.J., Wang Y., Intranigral ventral mesencephalic grafts and nigrostriatal injections of glial cell line-derived neurotrophic factor restore dopamine release in the striatum of 6-hydroxydopamine-lesioned rats. *Exp Brain Res* 1998, 119, 287-296.
65. Wang Y., Tien L.T., Lapchak P.A., Hoffer B.J., GDNF triggers fiber outgrowth of fetal ventral mesencephalic grafts from nigra to striatum in 6-OHDA-lesioned rats. *Cell Tissue Res* 1996, 286, 225-233.
66. Mehta V., Hong M., Spears J., Mendez I., Enhancement of graft survival and sensorimotor behavioral recovery in rats undergoing transplantation with dopaminergic cells exposed to glial cell line-derived neurotrophic factor. *J Neurosurg* 1998, 88, 1088-1095.
67. Mendez I., Dagher A., Hong M. *et al.*, Enhancement of survival of stored dopaminergic cells and promotion of graft survival by exposure of human fetal nigral tissue to glial cell line-derived neurotrophic factor in patients with Parkinson's disease. Report of two cases and technical considerations. *J Neurosurg* 2000, 92, 863-869.
68. Bauer M., Meyer M., Grimm L. *et al.*, Nonviral glial cell-derived neurotrophic factor gene transfer enhances survival of cultured dopaminergic neurons and improves their function after transplantation in a rat model of Parkinson's disease. *Hum Gene Ther* 2000, 11, 1529-1541.
69. Ostefeld T., Tai Y.T., Martin P., Deglon N., Aebischer P., Svendsen C.N., Neurospheres modified to produce glial cell line-derived neurotrophic factor increase the survival of transplanted dopamine neurons. *J Neurosci Res* 2002, 69, 955-965.
70. Behrstock S., Ebert A., McHugh J. *et al.*, Human neural progenitors deliver glial cell line-derived neurotrophic factor to parkinsonian rodents and aged primates. *Gene Ther* 2006, 13, 379-388.
71. Date I., Shingo T., Yoshida H. *et al.*, Grafting of encapsulated genetically modified cells secreting GDNF into the striatum of parkinsonian model rats. *Cell Transplant* 2001, 10, 397-401.
72. Shingo T., Date I., Yoshida H., Ohmoto T., Neuroprotective and restorative effects of intrastriatal grafting of encapsulated GDNF-producing cells in a rat model of Parkinson's disease. *J Neurosci Res* 2002, 69, 946-954.
73. Yasuhara T., Shingo T., Muraoka K. *et al.*, Early transplantation of an encapsulated glial cell line-derived neurotrophic factor-producing cell demonstrating strong neuroprotective effects in a rat model of Parkinson disease. *J Neurosurg* 2005, 102, 80-89.
74. Sajadi A., Bensadoun J.C., Schneider B.L., Lo Bianco C., Aebischer P., Transient striatal delivery of GDNF via encapsulated cells leads to sustained behavioral improvement in a bilateral model of Parkinson disease. *Neurobiol Dis* 2006, 22, 119-129.

75. Kishima H., Poyot T., Bloch J. *et al.*, Encapsulated GDNF-producing C2C12 cells for Parkinson's disease: a pre-clinical study in chronic MPTP-treated baboons. *Neurobiol Dis* 2004, 16, 428-439.
76. Sautter J., Tseng J.L., Braguglia D. *et al.*, Implants of polymer-encapsulated genetically modified cells releasing glial cell line-derived neurotrophic factor improve survival, growth, and function of fetal dopaminergic grafts. *Exp Neurol* 1998, 149, 230-236.
77. Lindvall O., Wahlberg L.U., Encapsulated cell biodelivery of GDNF: a novel clinical strategy for neuroprotection and neuroregeneration in Parkinson's disease? *Exp Neurol* 2008, 209, 82-88.
78. Kotzbauer P.T., Lampe P.A., Heuckeroth R.O. *et al.*, Neurturin, a relative of glial-cell-line-derived neurotrophic factor. *Nature* 1996, 384, 467-470.
79. Akerud P., Alberch J., Eketjall S., Wagner J., Arenas E., Differential effects of glial cell line-derived neurotrophic factor and neurturin on developing and adult substantia nigra dopaminergic neurons. *J Neurochem* 1999, 73, 70-78.
80. Horger B.A., Nishimura M.C., Armanini M.P. *et al.*, Neurturin exerts potent actions on survival and function of midbrain dopaminergic neurons. *J Neurosci* 1998, 18, 4929-4937.
81. Oiwa Y., Yoshimura R., Nakai K., Itakura T., Dopaminergic neuroprotection and regeneration by neurturin assessed by using behavioral, biochemical and histochemical measurements in a model of progressive Parkinson's disease. *Brain Res* 2002, 947, 271-283.
82. Rosenblad C., Kirik D., Devaux B., Moffat B., Phillips H.S., Bjorklund A., Protection and regeneration of nigral dopaminergic neurons by neurturin or GDNF in a partial lesion model of Parkinson's disease after administration into the striatum or the lateral ventricle. *Eur J Neurosci* 1999, 11, 1554-1566.
83. Tseng J.L., Bruhn S.L., Zurn A.D., Aebischer P., Neurturin protects dopaminergic neurons following medial forebrain bundle axotomy. *Neuroreport* 1998, 9, 1817-1822.
84. Hoane M.R., Gulwadi A.G., Morrison S., Hovanesian G., Lindner M.D., Tao W., Differential in vivo effects of neurturin and glial cell-line-derived neurotrophic factor. *Exp Neurol* 1999, 160, 235-243.
85. Cass W.A., Peters L.E., Neurturin effects on nigrostriatal dopamine release and content: comparison with GDNF. *Neurochem Res* 2010, 35, 727-734.
86. Grondin R., Zhang Z., Ai Y. *et al.*, Intrapatamenal infusion of exogenous neurturin protein restores motor and dopaminergic function in the globus pallidus of MPTP-lesioned rhesus monkeys. *Cell Transplant* 2008, 17, 373-381.
87. Li H., He Z., Su T. *et al.*, Protective action of recombinant neurturin on dopaminergic neurons in substantia nigra in a rhesus monkey model of Parkinson's disease. *Neurol Res* 2003, 25, 263-267.
88. Rosenblad C., Kirik D., Bjorklund A., Neurturin enhances the survival of intrastriatal fetal dopaminergic transplants. *Neuroreport* 1999, 10, 1783-1787.
89. Fjord-Larsen L., Johansen J.L., Kusk P. *et al.*, Efficient in vivo protection of nigral dopaminergic neurons by lentiviral gene transfer of a modified Neurturin construct. *Exp Neurol* 2005, 195, 49-60.
90. Kordower J.H., Herzog C.D., Dass B. *et al.*, Delivery of neurturin by AAV2 (CERE-120)-mediated gene transfer provides structural and functional neuroprotection and neurorestoration in MPTP-treated monkeys. *Ann Neurol* 2006, 60, 706-715.
91. Gasmi M., Brandon E.P., Herzog C.D. *et al.*, AAV2-mediated delivery of human neurturin to the rat nigrostriatal system: Long-term efficacy and tolerability of CERE-120 for Parkinson's disease. *Neurobiol Dis* 2007, 27, 67-76.
92. Gasmi M., Herzog C.D., Brandon E.P. *et al.*, Striatal Delivery of Neurturin by CERE-120, an AAV2 Vector for the Treatment of Dopaminergic Neuron Degeneration in Parkinson's Disease. *Mol Ther* 2007, 15, 62-68.

93. Herzog C.D., Dass B., Holden J.E. *et al.*, Striatal delivery of CERE-120, an AAV2 vector encoding human neurturin, enhances activity of the dopaminergic nigrostriatal system in aged monkeys. *Mov Disord* 2007, 22, 1124-1132.
94. Herzog C.D., Brown L., Gammon D. *et al.*, Expression, bioactivity, and safety 1 year after adeno-associated viral vector type 2-mediated delivery of neurturin to the monkey nigrostriatal system support cere-120 for Parkinson's disease. *Neurosurgery* 2009, 64, 602-612; discussion 612-603.
95. Bartus R.T., Herzog C.D., Bishop K. *et al.*, Issues regarding gene therapy products for Parkinson's disease: the development of CERE-120 (AAV-NTN) as one reference point. *Parkinsonism Relat Disord* 2007, 13 Suppl 3, S469-477.
96. Milbrandt J., de Sauvage F.J., Fahrner T.J. *et al.*, Persephin, a novel neurotrophic factor related to GDNF and neurturin. *Neuron* 1998, 20, 245-253.
97. Akerud P., Holm P.C., Castelo-Branco G., Sousa K., Rodriguez F.J., Arenas E., Persephin-overexpressing neural stem cells regulate the function of nigral dopaminergic neurons and prevent their degeneration in a model of Parkinson's disease. *Mol Cell Neurosci* 2002, 21, 205-222.
98. Baloh R.H., Tansey M.G., Lampe P.A. *et al.*, Artemin, a novel member of the GDNF ligand family, supports peripheral and central neurons and signals through the GFRalpha3-RET receptor complex. *Neuron* 1998, 21, 1291-1302.
99. Cass W.A., Peters L.E., Harned M.E., Seroogy K.B., Protection by GDNF and other trophic factors against the dopamine-depleting effects of neurotoxic doses of methamphetamine. *Ann N Y Acad Sci* 2006, 1074, 272-281.
100. Gardell L.R., Wang R., Ehrenfels C. *et al.*, Multiple actions of systemic artemin in experimental neuropathy. *Nat Med* 2003, 9, 1383-1389.
101. Hall A.K., Miller R.H., Emerging roles for bone morphogenetic proteins in central nervous system glial biology. *J Neurosci Res* 2004, 76, 1-8.
102. Krieglstein K., Suter-Crazzolara C., Hotten G., Pohl J., Unsicker K., Trophic and protective effects of growth/differentiation factor 5, a member of the transforming growth factor-beta superfamily, on midbrain dopaminergic neurons. *J Neurosci Res* 1995, 42, 724-732.
103. O'Keefe G.W., Hanke M., Pohl J., Sullivan A.M., Expression of growth differentiation factor-5 in the developing and adult rat brain. *Brain Res Dev Brain Res* 2004, 151, 199-202.
104. Storm E.E., Huynh T.V., Copeland N.G., Jenkins N.A., Kingsley D.M., Lee S.J., Limb alterations in brachypodism mice due to mutations in a new member of the TGF beta-superfamily. *Nature* 1994, 368, 639-643.
105. Lingor P., Unsicker K., Krieglstein K., Midbrain dopaminergic neurons are protected from radical induced damage by GDF-5 application. Short communication. *J Neural Transm* 1999, 106, 139-144.
106. O'Keefe G.W., Dockery P., Sullivan A.M., Effects of growth/differentiation factor 5 on the survival and morphology of embryonic rat midbrain dopaminergic neurones in vitro. *J Neurocytol* 2004, 33, 479-488.
107. Clayton K.B., Sullivan A.M., Differential effects of GDF5 on the medial and lateral rat ventral mesencephalon. *Neurosci Lett* 2007, 427, 132-137.
108. Wood T.K., McDermott K.W., Sullivan A.M., Differential effects of growth/differentiation factor 5 and glial cell line-derived neurotrophic factor on dopaminergic neurons and astroglia in cultures of embryonic rat midbrain. *J Neurosci Res* 2005, 80, 759-766.
109. Sullivan A.M., Opacka-Juffry J., Hotten G., Pohl J., Blunt S.B., Growth/differentiation factor 5 protects nigrostriatal dopaminergic neurones in a rat model of Parkinson's disease. *Neurosci Lett* 1997, 233, 73-76.

110. Sullivan A.M., Opacka-Juffry J., Pohl J., Blunt S.B., Neuroprotective effects of growth/differentiation factor 5 depend on the site of administration. *Brain Res* 1999, 818, 176-179.
111. Hurley F.M., Costello D.J., Sullivan A.M., Neuroprotective effects of delayed administration of growth/differentiation factor-5 in the partial lesion model of Parkinson's disease. *Exp Neurol* 2004, 185, 281-289.
112. O'Sullivan D.B., Harrison P.T., Sullivan A.M., Effects of GDF5 overexpression on embryonic rat dopaminergic neurones in vitro and in vivo. *J Neural Transm* 2010, 117, 559-572.
113. Lindholm P., Saarma M., Novel CDNF/MANF family of neurotrophic factors. *Dev Neurobiol* 2010, 70, 360-371.
114. Lindholm P., Voutilainen M.H., Lauren J. *et al.*, Novel neurotrophic factor CDNF protects and rescues midbrain dopamine neurons in vivo. *Nature* 2007, 448, 73-77.
115. Petrova P., Raibekas A., Pevsner J. *et al.*, MANF: a new mesencephalic, astrocyte-derived neurotrophic factor with selectivity for dopaminergic neurons. *J Mol Neurosci* 2003, 20, 173-188.
116. Choi J.M., Hong J.H., Chae M.J. *et al.*, Analysis of mutations and the association between polymorphisms in the cerebral dopamine neurotrophic factor (CDNF) gene and Parkinson disease. *Neurosci Lett* 2011, 493, 97-101.
117. Lindholm P., Peranen J., Andressoo J.O. *et al.*, MANF is widely expressed in mammalian tissues and differently regulated after ischemic and epileptic insults in rodent brain. *Mol Cell Neurosci* 2008, 39, 356-371.
118. Palgi M., Lindstrom R., Peranen J., Piepponen T.P., Saarma M., Heino T.I., Evidence that DmMANF is an invertebrate neurotrophic factor supporting dopaminergic neurons. *Proc Natl Acad Sci U S A* 2009, 106, 2429-2434.
119. Voutilainen M.H., Back S., Porsti E. *et al.*, Mesencephalic astrocyte-derived neurotrophic factor is neurorestorative in rat model of Parkinson's disease. *J Neurosci* 2009, 29, 9651-9659.
120. Hellman M., Arumae U., Yu L.Y. *et al.*, Mesencephalic astrocyte-derived neurotrophic factor (MANF) has a unique mechanism to rescue apoptotic neurons. *J Biol Chem* 2011, 286, 2675-2680.
121. Voutilainen M.H., Back S., Peranen J. *et al.*, Chronic infusion of CDNF prevents 6-OHDA-induced deficits in a rat model of Parkinson's disease. *Exp Neurol* 2011, 228, 99-108.
122. Leitner M.L., Molliver D.C., Osborne P.A. *et al.*, Analysis of the retrograde transport of glial cell line-derived neurotrophic factor (GDNF), neurturin, and persephin suggests that in vivo signaling for the GDNF family is GFRalpha coreceptor-specific. *J Neurosci* 1999, 19, 9322-9331.
123. Tomac A., Widenfalk J., Lin L.F. *et al.*, Retrograde axonal transport of glial cell line-derived neurotrophic factor in the adult nigrostriatal system suggests a trophic role in the adult. *Proc Natl Acad Sci U S A* 1995, 92, 8274-8278.
124. Nutt J.G., Burchiel K.J., Comella C.L. *et al.*, Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology* 2003, 60, 69-73.
125. Kordower J.H., Palfi S., Chen E.Y. *et al.*, Clinicopathological findings following intraventricular glial-derived neurotrophic factor treatment in a patient with Parkinson's disease. *Ann Neurol* 1999, 46, 419-424.
126. Gill S.S., Patel N.K., Hotton G.R. *et al.*, Direct brain infusion of glial cell line-derived neurotrophic factor in Parkinson disease. *Nat Med* 2003, 9, 589-595.
127. Love S., Plaha P., Patel N.K., Hotton G.R., Brooks D.J., Gill S.S., Glial cell line-derived neurotrophic factor induces neuronal sprouting in human brain. *Nat Med* 2005, 11, 703-704.

128. Patel N.K., Bunnage M., Plaha P., Svendsen C.N., Heywood P., Gill S.S., Intraputaminal infusion of glial cell line-derived neurotrophic factor in PD: A two-year outcome study. *Ann Neurol* 2005, 57, 298-302.
129. Slevin J.T., Gerhardt G.A., Smith C.D., Gash D.M., Kryscio R., Young B., Improvement of bilateral motor functions in patients with Parkinson disease through the unilateral intraputaminal infusion of glial cell line-derived neurotrophic factor. *J Neurosurg* 2005, 102, 216-222.
130. Lang A.E., Gill S., Patel N.K. *et al.*, Randomized controlled trial of intraputaminal glial cell line-derived neurotrophic factor infusion in Parkinson disease. *Ann Neurol* 2006, 59, 459-466.
131. Tatarewicz S.M., Wei X., Gupta S., Masterman D., Swanson S.J., Moxness M.S., Development of a maturing T-cell-mediated immune response in patients with idiopathic Parkinson's disease receiving r-metHuGDNF via continuous intraputaminal infusion. *J Clin Immunol* 2007, 27, 620-627.
132. Hovland D.N., Jr., Boyd R.B., Butt M.T. *et al.*, Six-month continuous intraputaminal infusion toxicity study of recombinant methionyl human glial cell line-derived neurotrophic factor (r-metHuGDNF) in rhesus monkeys. *Toxicol Pathol* 2007, 35, 676-692.
133. Barker R.A., Continuing trials of GDNF in Parkinson's disease. *Lancet Neurol* 2006, 5, 285-286.
134. Lang A.E., Langston J.W., Stoessl A.J. *et al.*, GDNF in treatment of Parkinson's disease: response to editorial. *Lancet Neurol* 2006, 5, 200-202.
135. Penn R.D., Dalvi A., Slevin J. *et al.*, GDNF in treatment of Parkinson's disease: response to editorial. *Lancet Neurol* 2006, 5, 202-203.
136. Richardson R.M., Kells A.P., Rosenbluth K.H. *et al.*, Interventional MRI-guided Putaminal Delivery of AAV2-GDNF for a Planned Clinical Trial in Parkinson's Disease. *Mol Ther* 2011.
137. Evans J.R., Barker R.A., Neurotrophic factors as a therapeutic target for Parkinson's disease. *Expert Opin Ther Targets* 2008, 12, 437-447.
138. Marks W.J., Jr., Ostrem J.L., Verhagen L. *et al.*, Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. *Lancet Neurol* 2008, 7, 400-408.
139. Marks W.J., Jr., Bartus R.T., Siffert J. *et al.*, Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol* 2010, 9, 1164-1172.
140. Bartus R.T., Herzog C.D., Chu Y. *et al.*, Bioactivity of AAV2-neurturin gene therapy (CERE-120): differences between Parkinson's disease and nonhuman primate brains. *Mov Disord* 2011, 26, 27-36.

Table 1: Effects of GDNF on dopaminergic neurones *in vitro*

<b>Effect</b>	<b>Reference</b>
Increases Tyrosine Hydroxylase expression	[8]
Promotes survival of mesencephalic cultures	[6, 9]
Promotes morphological differentiation	[10]
Protects from MPP+ neurotoxicity	[12, 13]
Protects from 6-OHDA neurotoxicity	[11]
Decreases apoptosis	[14, 15, 16]
Protects from LPS neurotoxicity	[17]

**Table 2: Neurotrophic factors in clinical trials**

<b>Neurotrophic factor</b>	rh-methionyl-GDNF	rh-methionyl-GDNF	rh-methionyl-GDNF	rh-methionyl-GDNF	AAV2-NRTN	AAV2-NRTN
<b>Trial type</b>	MC,R,DB,PC	Open-label	Open-label	MC,R,DB,PC	Open-label	MC, DB, SSC
<b>Delivery Site</b>	ICV	IPu	IPu	IPu	IPu	IPu
<b>No. Patients</b>	50	5	10	33	12	58
<b>Age / Duration of disease (years)</b>	58±8 / 11±6	54.2±6 / 19±9.8	57.9±9.3 / 8.7±3.6	56±7.2 / 9.7±3.9	57±8 / 11±3	60±7 / 10±3
<b>Benefits</b>	No	Yes	Yes	No	Yes	No
<b>Side-effects</b>	LS, Nausea, Anorexia	LS	LS, Headaches	LS, Anti-GDNF antibodies	Headaches	Headaches
<b>Reference</b>	[124]	[126, 128]	[129]	[130]	[138]	[139]

Abbreviations: rh recombinant human, MC Multi-centre, R Randomized, DB Double-blind, PC Placebo-controlled, SSC Sham surgery-controlled, ICV Intracerebroventricular, IPu IntraputaminaI, LS L'Hermitte's sign