Review

GDNF-induced cerebellar toxicity: A brief review

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ABSTRACT

Recombinant-methionyl human glial cell line-derived neurotrophic factor (GDNF) is known for its neurorestorative and neuroprotective effects in rodent and primate models of Parkinson’s disease (PD). When administered locally into the putamen of Parkinsonian subjects, early clinical studies showed its potential promise as a disease-modifying agent. However, the development of GDNF for the treatment of PD has been significantly clouded by findings of cerebellar toxicity after continuous intraputamenal high-dose administration in a 6-month treatment/3-month recovery toxicology study in rhesus monkeys. Specifically, multifocal cerebellar Purkinje cell loss affecting 1–21% of the cerebellar cortex was observed in 4 of 15 (26.7%; 95% confidence interval [CI]: 10.5–52.4%) animals treated at the highest dose level tested (3000 μg/month). No cerebellar toxicity was observed at lower doses (450 and 900 μg/month) in the same study, or at similar or higher doses (up to 10,000 μg/month) in subchronic or chronic toxicology studies testing intermittent intracerebroventricular administration. While seemingly associated with the use of GDNF, the pathogenesis of the cerebellar lesions has not been fully understood to date. This review integrates available information to evaluate potential pathogenic mechanisms and provide a consolidated assessment of the findings. While other explanations are considered, the existing evidence is most consistent with the hypothesis that leakage of GDNF into cerebrospinal fluid during chronic infusions into the putamen down-regulates GDNF receptors on Purkinje cells, and that subsequent acute withdrawal of GDNF generates the observed lesions. The implications of these findings for clinical studies with GDNF are discussed.

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1. Introduction

1.1. GDNF biology

Glial cell line-derived neurotrophic factor (GDNF), originally isolated from a rat glioma cell line in the early 1990s (Lin et al., 1993), is a distant member of the transforming growth factor-β superfamily and a founding member of the GDNF family of ligands (GFL), which includes neurturin, artemin and persephin (Airaksinen and Saarma, 2002). GDNF is a potent trophic factor for midbrain dopaminergic neurons, central noradrenergic neurons, spinal motor neurons and a variety of peripheral neurons. Outside the nervous system, it acts as a morphogen in kidney development and regulates spermatogonial differentiation (Airaksinen and Saarma, 2002).

Following intracellular processing, GDNF is secreted as a glycosylated mature protein of 134 amino acids (Lin et al., 1993). The active compound is a disulfide-bonded homodimer of approximately 30.4 kDa. As it lacks a specific carrier protein or transporter at endothelial cells, GDNF does not cross the blood-brain barrier (Kastin et al., 2003). In the adult human brain, GDNF is expressed at very low levels, with the highest concentrations in the caudate nucleus, putamen and substantia nigra (43–70 pg/mg protein), significantly lower concentrations in the cerebellum and frontal cortex (10–15 pg/mg protein), and undetectable concentrations (<8 pg/ml) in cerebrospinal fluid (CSF) (Mogi et al., 2001).

Cellular responses to all GDNF family ligands are mediated by a multicomponent receptor complex consisting of the membrane-anchored GDNF family ligand receptor (GFRα) and transmembrane RET receptor tyrosine kinase (Airaksinen and Saarma, 2002). Four different GFRα proteins (GFRα-1–4) with unique binding affinities for each ligand have been identified. GDNF preferentially binds to GFRα-1, but also interacts with GFRα-2 and GFRα-3, although at lower affinities (Airaksinen and Saarma, 2002). Effective GDNF signaling via GFRα-1-RET additionally requires the presence of heparan sulfate glycosaminoglycans which serve as high abundance, low-affinity receptors on the cell surface and in the extracellular matrix (Barnett et al., 2002; Tanaka et al., 2002). In cells lacking RET, especially in the forebrain, cortex and inner ear, the neural cell adhesion molecule (NCAM) has been identified as an alternate signaling receptor for GDNF, again requiring the co-expression of GFRα-1 for high affinity binding (Paratcha et al., 2003; Sariola and Saarma, 2003).

1.2. GDNF as a disease-modifying agent

Since its discovery, GDNF has received considerable attention as a drug candidate for the treatment of a variety of neurological diseases, most prominently Parkinson’s disease (PD), with the understanding that the molecule needs to be delivered directly to the tissue of interest so as to bypass the blood-brain barrier to achieve meaningful tissue levels (Allen et al., 2013). In toxin-induced rodent and nonhuman primate models of PD, GDNF has been reproducibly shown to have both neurorestorative and neuroprotective effects and to improve motor function when delivered into the cerebral ventricles or directly into the dopamine-deficient striatum or substantia nigra (Tomac et al., 1995; Gash et al., 1996; Bjorklund et al., 1997; Zhang et al., 1997; Grondin et al., 2002).

Based on these promising animal data, GDNF was tested in four clinical studies enrolling a total of 99 subjects with PD. In the first study, monthly intracerebroventricular (ICV) bolus injections of GDNF failed to provide clinical benefit relative to placebo and were associated with a number of gastrointestinal side effects including nausea, anorexia and vomiting and induced both weight loss and hyponatremia in over half of the subjects (Nutt et al., 2003). A postmortem analysis in one of the GDNF-treated study subjects showed no GDNF immunoreactivity and no appreciable increase in putaminal tyrosine hydroxylase immunoreactivity relative to age-matched control cases with PD (Kordower et al., 1999). Therefore, ICV delivery was subsequently replaced with intraputaminal (IPu) delivery using implantable pumps. In addition, intermittent bolus administration was replaced with continuous administration by infusion, as the pumps required minimal basal infusion rates to maintain proper function (Gash et al., 2005).

With these changes, GDNF showed strong signs of efficacy in two uncontrolled open-label Phase I studies (Gill et al., 2003; Slevin et al., 2005), but was not significantly different from placebo in a randomized placebo-controlled Phase II study (Lang et al., 2006). In contrast to the ICV study, GDNF was found to be well tolerated and clinically safe in all of the IPu studies (Gill et al., 2003; Slevin et al., 2005; Lang et al., 2006), although more than half of the subjects treated with GDNF developed clinically asymptomatic immune responses with binding antibodies to the protein, including 5 subjects with neutralizing antibodies (Tatarewicz et al., 2007).

1.3. GDNF pharmacokinetics

Both in mammalian cell cultures and in vivo, GDNF undergoes N-terminal proteolytic cleavage of 31–37 amino acid-long fragments including the main heparin-binding site of the molecule (Lau, 1996). Truncated GDNF remains biologically active in soluble form, although not in immobilized matrix-bound form which requires interaction with the heparan sulfate chains of syndecan-3, a transmembrane proteoglycan receptor, for proper signal transduction (Bespalov et al., 2011).

Plasma concentrations in normal rhesus monkeys after single ICV infusions of exogenous GDNF (recombinant-methionyl human GDNF, r-methHuGDNF) at different doses (100 and 500 μg) were detectable only sporadically within the first hour post dosing, the highest individual value being 2.11 ng/ml (Lau, 1996). By contrast, mean peak GDNF concentrations in CSF (C_{CSF}) were 3554 ng/ml (100 μg dose) and 33,975 ng/ml (500 μg dose), respectively (Lau, 1996). The terminal half-life in CSF of GDNF after these doses was found to be assay-dependent. With an assay detecting only full-length GDNF, the terminal half-life was 34 h, while it was almost threefold longer (92 h) with an assay capturing both full-length and truncated GDNF, indicating that in vivo processing of GDNF occurs in CSF (Lau, 1996). The pharmacokinetics of GDNF in CSF of normal rhesus monkeys were further found to hinge on the proximity of the sampling site (lumbar spine or cisterna magna) to the site of administration (intrathecal lumbar or ICV). After administration of the same single doses, higher peak concentrations, larger areas under the curve and longer half-lives were found at the sampling site that was closer to the site of administration than at the more distant sampling site (Wong, 2003a,b). Binding of GDNF to local heparan sulfate glycosaminoglycans is considered...
the most likely explanation for this observation (Barnett et al., 2002; Tanaka et al., 2002).

Single infusions of 3 or 15 µg of GDNF (infusion volume, \( V; 10 \mu L \); concentration in the infusate, \( C; 0.3 \) or 1.5 µg/µL) in normal rat striatum resulted in substantially increased striatal tissue concentrations of GDNF (Hadaczek et al., 2010). Basal concentrations of endogenous GDNF in striatum were reported as 0.04–0.05 ng/mg protein in this study. Three days post infusion, GDNF concentrations were 13.0 ng/mg protein at the low-dose level and 59.8 ng/mg protein at the high-dose level. At both dose levels, GDNF tissue concentrations rapidly decreased after Day 3, but remained elevated as compared to baseline over periods of 2 (low-dose level) to 4 (high-dose level) weeks (Hadaczek et al., 2010).

More recently, a study of the tissue clearance of GDNF following single doses of 0.5, 1, 3 or 5 µg (\( V; 5 \mu L \); \( C; 0.1, 0.2, 0.6 \) or 1.0 µg/µL) into the striatum of normal rats reported results that were consistent with the findings of the above study and confirmed the extended dose-dependent pharmacokinetics of exogenous GDNF in striatum (Taylor et al., 2013). At the 1.0 µg/µL level, GDNF tissue concentrations were significantly increased both 2 and 4 weeks post infusion; at the 0.6 µg/µL level, they were significantly increased only 2 weeks post infusion; and at the 0.1 and 0.2 µg/µL levels, no discernible changes in GDNF tissue concentrations were observed (Taylor et al., 2013).

### 1.4. GDNF CNS-related toxicity program

Consistent with the need to deliver GDNF directly to the tissue of interest so as to bypass the blood–brain barrier, the GDNF toxicology program undertaken to support the clinical development of GDNF had a special focus on studies testing ICV, IT and IPu administration, using the rhesus monkey (Macaca mulatta) as the standard model. Altogether, 11 studies involving 205 animals were performed, including 6 ICV studies (\( N = 86 \)), 1 IT study (\( N = 17 \)) and 4 IPu studies (\( N = 102 \)). None of the ICV/IT studies and only one of the IPu studies have been published previously (Hovland et al., 2007). Of particular importance, the program included 4 studies testing the effects of long-term drug administration over 3–6 months in a total of 140 animals. Three of these studies employed intermittent dosing ICV/IT protocols at biweekly or monthly intervals (Orr, 1996; Orr, 1997b; Crosby Tompkins, 1998), while the fourth one employed a continuous dosing IPu protocol (Boyd, 2005a,b). Dosing details for these subchronic and chronic studies are presented in Table 1. In order to make the studies comparable across the different dosing regimens, dose levels are temporally normalized and detailed on the basis of GDNF cumulative monthly doses.

Animals in the ICV/IT studies were dosed through a subcutaneous access port connected to a catheter which was implanted in a lateral ventricle or the lumbar spine, respectively. Dosing consisted of intermittent bolus infusions, and maximum GDNF dose levels tested with ICV/IT were 10 mg given biweekly for 1 month (ICV) (Orr, 1997a) or monthly for 3 months (IT) (Crosby Tompkins, 1998) and 3 mg given biweekly for 3 months (ICV) (Orr, 1996) or monthly for 6 months (ICV) (Orr, 1997b).

No mortality occurred during these studies. Acute clinical signs observed on the treatment days included retching, vomiting, salivation, lethargy and tremor; incidence and onset were dose-related. These reactions were more severe with biweekly dosing than with monthly dosing. Compared to control animals, administration of GDNF was associated with reductions in mean body weight, body weight gain and/or food and water consumption. The magnitude of these effects was generally modest and appeared to be dose-related, although weight differences relative to control animals did not generally reach the level of statistical significance (Orr, 1996; Orr, 1997a,b; Crosby Tompkins, 1998).

Histopathological effects included two main types of findings, (1) increased inflammatory changes in various regions including meninges, ventricular system, spinal cord, spinal nerve roots, perineurial tissue and catheter tract; and (2) axonal sprouting with Schwann cell hyperplasia in dorsal spinal nerve roots, sympathetic nerves and/or pia mater. Both types of changes were dose-related in all studies and appeared to be at least partially reversible after one (inflammation) or three (Schwann cell changes) months of recovery (Orr, 1996; Orr, 1997a,b; Crosby Tompkins, 1998). In the 3-month IT administration study, axonal degeneration in the lumbosacral spinal region was seen as an additional finding at dose levels of 1750 µg/month or more (Crosby Tompkins, 1998). Although a GDNF-specific drug effect cannot be ruled out, it seems more likely that the increased inflammatory changes (and secondary axonal degeneration after IT administration) represented a nonspecific response to high local concentrations of a heterologous protein resulting from the combination of high doses and GDNF binding to local heparan sulfate glycosaminoglycans (Barnett et al., 2002; Tanaka et al., 2002). In contrast, the Schwann cell effects are probably attributable to the intrinsic pharmacologic activity of GDNF (Hoke et al., 2003).

For the IPu studies, animals were implanted with a single unilateral IPu catheter (1 mm outer diameter) that was connected to a microinfusion pump implanted in the abdominal wall. The pump reservoirs were refilled with infusion solution on a monthly basis, and dosing occurred via continuous infusion at infusion rates of 6–24 µL/h (Boyd, 2005a,b; Boyd, 2006; Hovland et al., 2007).

In a subacute IPu study, 20 animals were treated with vehicle or 30, 100 or 500 µg GDNF per day (\( C; 0.1, 0.33 \) or 1.67 µg/µL), corresponding to 900, 3000 or 15,000 µg/month. All infusions were delivered at a constant rate of 12.5 µL/h for 1 month (Boyd, 2005b).

Treatment with GDNF was generally well tolerated, although sustained body weight loss and reduced body weight gain relative to control animals were observed at dose levels of 3000 µg/month or more. At the 15,000 µg/month dose, clinical laboratory alterations including hyponatraemia, hypochloremia, hyperkalemia, hypocholesterolemia, changes in the plasma protein profile and aciduria were observed after 3 weeks of treatment or later. One of

### Table 1

Toxicology studies testing long-term delivery of GDNF to targets within the blood–brain barrier in rhesus monkeys (Orr, 1996; Orr, 1997b; Crosby Tompkins, 1998; Hovland et al., 2007).

<table>
<thead>
<tr>
<th>Route</th>
<th>Tx/Ry [months]</th>
<th>N</th>
<th>Dosing scheme</th>
<th>Cumulative GDNF dose [µg/month]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent dosing into cerebrospinal fluid compartment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IT</td>
<td>3/0</td>
<td>17</td>
<td>Monthly bolus administration</td>
<td>0 300 1750 10,000</td>
</tr>
<tr>
<td>ICV</td>
<td>3/1</td>
<td>24</td>
<td>Biweekly bolus administration</td>
<td>0 600 2000 6000</td>
</tr>
<tr>
<td>ICV</td>
<td>6/3</td>
<td>27</td>
<td>Monthly bolus administration</td>
<td>0 75 500 3000</td>
</tr>
<tr>
<td>Continuous dosing into putamenal parenchyma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPu</td>
<td>6/3</td>
<td>72</td>
<td>Continuous, unilateral infusion</td>
<td>0 450 900 3000</td>
</tr>
</tbody>
</table>

\( N \): number of animals in study; \( \text{Tx/Ry: duration of treatment/recovery period; IT: intrathecal; ICV: intracerebroventricular; IPu: intraputamenal.} \)
5 rhesus monkeys treated at this dose had to be sacrificed early because of a moribund condition associated with dehydration, hyperthermia, recency and clinical laboratory abnormalities (Boyd, 2005b). Necropsy did not reveal any explanation for the condition, and the findings could not be replicated in a repeat study treating 3 additional animals at the same dose (Boyd, 2005a). No drug-related gross lesions or organ weight changes were observed at necropsy in any of the other animals, and no drug-related microscopic changes were noted outside the CNS (Boyd, 2005b).

Within the CNS, both thickened meninges and Schwann cell hyperplasia were observed in the 3000 μg/month and 15,000 μg/month groups and were considered to be related to the intrinsic activity of GDNF. In addition, nonspecific perivascular leukocyte infiltrates were observed along the catheter tract and/or at the infusion site in all groups and increased in incidence and severity as a function of dose. In the 15,000 μg/month group, 3 animals had local edema, 2 of them together with focal necrosis and degenerated neurons (Boyd, 2005b). These findings were reflected in end-of-treatment magnetic resonance imaging (MRI) scans, where a hyperintense diffuse T-2 weighted signal and T-2 and T-1 weighted ring lesions with localized gadolinium enhancement were seen at the infusion sites in the 3000 μg/month and 15,000 μg/month groups. The above findings were interpreted as a standard nonspecific response to a foreign protein (Boyd, 2005b). Similar findings including significant vacuolations at the infusion site were previously made in rats 5 weeks after single retronigral infusion with GDNF at a very high concentration (G; 25 μg/μL; rate: 1.0 μL/min; Vi; 4 μL) (Bownenkamp et al., 1995). However, the same observations were made in control animals receiving cytochrome C at the same concentration and infusion protocol. This, together with the fact that the findings were markedly reduced when the GDNF/cytochrome C concentrations were halved while the infusion volumes were doubled to keep the total protein dose constant, indicated that the effects were not related to the intrinsic activity of GDNF, but to local accumulation of protein (Bownenkamp et al., 1995).

On the basis of these findings, the no observed adverse effect level (NOAEL) in this study was determined to be 900 μg/month (Boyd, 2005b).

In a chronic IPu study, 72 animals were treated with vehicle or 15, 30 or 100 μg GDNF per day (G; 0.1, 0.2 or 0.67 μg/μL), corresponding to 450, 900 or 3000 μg/month (Hovland et al., 2007). All infusions were given at a constant rate of 6.25 μL/h for 6 months (6/sex/group), followed by a 3-month recovery period (3/sex/group) (Hovland et al., 2007).

Twelve unscheduled sacrifices and 1 death occurred over the course of the study. The single mortality and 11 of the sacrifices were secondary to pump-related complications. The remaining sacrifice (900 μg/month) was due to disseminated intravascular coagulopathy, which was probably secondary to chronic exposure to GDNF as a human antigen. No other possibly immune-mediated events were observed, and clinical observations, including physical, electrocardiographic, neurological and ophthalmological examinations, were unremarkable over the course of the study. No toxicologically significant hematology, serum chemistry, coagulation, or urinalysis parameters were observed. Furthermore, CSF total cell count and chemistry profiles were unremarkable (Hovland et al., 2007).

Statistically significant drug-related body weight loss and marked reductions in body weight gain relative to control animals were observed in the 3000 μg/month group. Changes in food consumption paralleled the body weight effects. A rebound in body weight gain occurred during the recovery period. No relevant test article-related gross observations or organ weight changes were noted at the end of treatment or recovery necropsies (Hovland et al., 2007).

On-treatment MRI scans showed a circumferential increase in the uptake of gadolinium contrast at the catheter tip in T-2 weighted sequences which was often superimposed with a diffuse T-2 fast spin echo (FSE) hyperintense signal in all groups. However, the frequency of the T-1 contrast findings increased over time in the 3000 μg/month group, while it remained relatively constant in all other groups. At 6 months, the frequency was higher in the 3000 μg/month group (66.7%) than in the 450 μg/month (28.6%) and 900 μg/month (26.7%) groups, with the control group having the lowest frequency (13.3%). Similarly, the frequency of the diffuse T-2 FSE hyperintense signal at 6 months was higher in the 3000 μg/month group (93.3%) than in the 450 μg/month (71.4%) and 900 μg/month (73.3%) groups, and the control group again had the lowest frequency (40.0%). Evaluating these findings in their entirety, it was concluded that they were likely the result of localized edema and disruption of the blood–brain barrier due to inflammation following local protein accumulation (Hovland et al., 2007).

GDNF immunohistochemistry demonstrated the presence of GDNF at the infusion site around the catheter tip in a radially diffuse pattern in all actively treated groups. In general, the intensity of staining increased with increasing dose and tended to be most pronounced in white matter tracts. In animals that developed a cavity or pronounced inflammatory reaction at the catheter site, the infused GDNF tended to be trapped within the cavity or inflammatory zone. Staining was consistently present in actively treated animals at the end of recovery, although less pronounced than at the end of treatment (Hovland et al., 2007).

Meaningful microscopic observations in actively treated animals were limited to the CNS and/or closely associated structures. Specific changes including meningeal Schwann cell hyperplasia and meningeal thickening due to axonal proliferation, either underlying the medulla oblongata or overlying various spinal cord segments, were observed in the 900 μg/month (1/9) and 3000 μg/month (7/10) groups at the end of treatment and in the 3000 μg/month (3/5) group at the end of recovery. Notable nonspecific microscopic lesions included slight-to-mild inflammation at or adjacent to the catheter tract or infusion site. The infiltrates differed only slightly between actively treated animals and controls, in particular at 450 μg/month and were not considered clinically relevant up to 900 μg/month (Hovland et al., 2007).

Very importantly, this study yielded unexpected signs of cerebellar toxicity in 4 of 15 animals given 3000 μg/month (Hovland et al., 2007). The potential mechanisms underlying this finding are discussed in detail in the following sections.

On the basis of the aggregate findings from this study, including the cerebellar lesions, the NOAEL was determined to be 900 μg/month (Hovland et al., 2007).

2. Cerebellar lesions

At the end of the 3-month recovery period, multifocal cerebellar Purkinje cell loss affecting approximately 21% of the cerebellar cortex was observed in one animal treated for 6 months at the 3000 μg/month dose in the chronic IPu study (Hovland et al., 2007). Triggered by this finding, a systematic review of all prospectively prepared and additional for-cause cerebellar sections from the other animals in this study and of all available cerebellar sections from the animals in the other subchronic and chronic toxicity studies of the program (as presented in Table 1) was performed. This analysis revealed similar, but less extensive (1–3% of cerebellar cortex) lesions in three other animals given 3000 μg/month in the chronic, continuous dosing IPu study. Two of these animals belonged to the recovery cohort, the third one to the main study cohort. In contrast, no lesions were found in any
animal treated with 450 μg/month or 900 μg/month in this study, or in any animal treated in any of the other subchronic and chronic toxicity studies. In summary, the occurrence of cerebellar Purkinje cell loss was confined to the 3000 μg/month dose group in the continuous dosing IPu study, where lesions were observed in 4 of 15 (26.7%; 95% confidence interval [CI]: 10.5–52.4%) treated animals (Hovland et al., 2007).

The severity of lesions in affected animals was generally minimal to moderate, but varied considerably by focus and by animal. Microscopic findings ranged from patchy Purkinje cell loss with proliferation of Bergman’s glia, astrocytosis and vacuolation in the molecular layer to more extensive lesions with translobar, nearly full thickness loss of Purkinje cells. This was associated with minimal to moderate atrophy of the molecular layer (suggesting degeneration of Purkinje cell dendrites) and granule cell loss at some foci (Fig. 1). The loss of granule cells was probably the result of retrograde degeneration caused by the loss of their postsynaptic targets, i.e. Purkinje cells (Sarna and Hawkes, 2003; Cendelin, 2014).

Increased glial fibrillary acidic protein (GFAP) staining in affected areas was indicative of proliferation or thickening of astrocytic processes (Fig. 2).

None of the lesions were associated with increased Fluoro-Jade B staining which would have been indicative of ongoing and progressive neuronal degeneration. Also, there were no signs of microgliosis, suggesting that the lesions were mature and inactive, with no evidence of ongoing pathogenesis (Hovland et al., 2007).

Cumulatively, the lesions affected approximately 21% of the cerebellar cortex in the animal that demonstrated the toxicity, and 1%, 3% and 3% in the other three affected animals. Mapping of the lesions showed that they were distributed randomly throughout the cerebellar cortex among multiple folia in both hemispheres and the vermis, with no predilection for any specific region (Hovland et al., 2007).

While the study was not specifically designed to detect subtle changes in motor function, there was no evidence of neurological deficit or motor dysfunction in affected animals at cage side observations or neurological/physical examinations. Motor changes that might have been expected include ataxia, in particular gait ataxia, and tremor (Wolf et al., 1996; Tolbert et al., 2001; Sarna and Hawkes, 2003). A strong association appears to exist between the extent and type of spatial loss of Purkinje cells and the severity of clinical signs (Wolf et al., 1996). Therefore, the most likely explanation for the absence of motor changes in the study is the limited extent and random distribution of the lesions across the cerebellar cortex of the affected animals. This would also explain why standard sequential MRI did not reveal any findings, and why the brains of the affected animals were grossly unremarkable at visual evaluation at necropsy (Hovland et al., 2007).

3. Potential pathogenic mechanisms

The observed cerebellar lesions were unexpected and as far as can be determined, there are no other published reports that GDNF produces Purkinje (or other neuronal) cell loss in any species. Potential pathogenic mechanisms are considered below.

3.1. Excitotoxicity-mediated mechanisms

Purkinje cells are the largest neurons in the cerebellar cortex and play a central role in motor coordination and motor learning. They
receive and integrate excitatory input via parallel fibers from the ponto-cerebellar pathway and via climbing fibers from the olivo-cerebellar pathway. A remarkable feature of Purkinje cell circuitry is its territorial organization. Each Purkinje cell receives input at its distal dendrites from 10⁷ to 10⁸ parallel fibers, although only one or two of these originate from the same granule cell. Thus, excitation of a single parallel fiber only weakly depolarizes the Purkinje cell that it innervates. By contrast, the same cell is powerfully and exclusively innervated at its proximal dendrites via hundreds of synapses formed by a single climbing fiber originating in the inferior olive (Watanabe, 2008). Not surprisingly, the robust excitatory innervation of Purkinje cells makes them particularly vulnerable to glutamate-mediated excitotoxicity (Sarna and Hawkes, 2003).

3.1.1. Ponto-cerebellar pathway

Although the putamen and cerebellum have no direct functional connection, the cerebellum receives input from the putamen via the direct and indirect motor pathways and further via the cortico-ponto-cerebellar pathway. It is possible that GDNF-induced sustained increase in putaminal dopamine activity would lead to lasting overstimulation of the direct motor pathway coupled with inhibition of the indirect pathway. This would involve increased inhibitory output (through the direct pathway) and decreased excitatory output (through the indirect pathway) to the internal globus pallidus, both resulting in a disinhibition of thalamic motor neurons. Conceivably, increased glutamatergic stimulation of the neocortex resulting from this thalamic disinhibition could then lead to sustained overstimulation of the cerebellar cortex and excitotoxicity-mediated degenerative foci. This overstimulation would occur via the cortico-ponto-cerebellar pathway involving the cortico-pontine, mossy and parallel fiber systems.

There are, however, two important points that argue against the hypothesis that the observed cerebellar lesions may have been caused by an excitotoxic mechanism. First, the specific architecture of the parallel fiber system inevitably leads to activation of major Purkinje cell clusters because, as described above, depolarization of a single Purkinje cell requires simultaneous excitatory input from a large number of granule cells which in turn project to and activate large numbers of adjacent Purkinje cells. Recruitment of the requisite number of granule cells is enabled by extensive branching of mossy fibers in white matter which ensures that any single mossy fiber axon simultaneously projects to a large number of granule cells (Llinás et al., 2003). Therefore, excitotoxic lesions following activation of the cortico-ponto-cerebellar pathway would be expected to show a replicable contiguous and homogeneous distribution pattern across individual lobules or the entire cerebellar cortex rather than the spotted, heterogeneous distribution pattern observed in the affected animals.

Second, while continuous intrastriatal infusions produced significant increases in putaminal dopamine metabolites in nonhuman primates, the corresponding increase in putaminal dopamine concentrations was modest and insignificant (Maswood et al., 2002; Grondin et al., 2002). More importantly, the increase was regionally limited and not different from the increase induced by continuous IV infusion (Grondin et al., 2002). This, together with the observation that the clinical effects of continuous dosing intrastriatal and intermittent dosing IV administration in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys were highly similar (Gash et al., 1996), leads to the conclusion that cerebellar lesions, if induced as hypothesized above, should also have been observed in at least some monkeys in the ICV toxicity studies; however, this was not the case.

3.1.2. Olivo-cerebellar pathway

The olivo-cerebellar pathway involves distinct three-element loops between an individual olivary subnucleus, zonally organized Purkinje cells and a central cerebellar nucleus. The olivary subnucleus of any given loop projects contralaterally to one or more longitudinal zones of Purkinje cells that in turn project to a given cerebellar target nucleus. The same cerebellar nucleus also receives collaterals from and reciprocally projects to the olivary subnucleus of the loop (De Zeeuw et al., 1998). The anatomical unit consisting of a particular Purkinje cell zone with its specific olivary input and the innervation of the associated cerebellar nucleus has been termed a cerebellar module (De Zeeuw et al., 1998).

The modular organization of the olivo-cerebellar circuitry forms the anatomical basis of the neuropathological findings observed after systemic administration of the indole alkaloids, ibogaine and harmaline. Both agents induce sustained activation of neurons in the inferior olive leading to selective excitatory injury and degeneration of Purkinje cells in narrow parasagittal bands in the cerebellar vermis (O’Hearn and Molliver, 1993; O’Hearn and Molliver, 1997). The absence of any such pattern in the continuous dosing IPu GDNF study makes it highly unlikely that the GDNF-induced cerebellar lesions were due to excitotoxicity mediated via the olivo-cerebellar pathway. On the contrary, exogenous GDNF has been shown to delay hereditary Purkinje cell degeneration via intact olivo-cerebellar projections (Hess et al., 2003).

3.2. Ischemia

Purkinje cells are particularly sensitive to ischemia and other forms of energy depletion, presumably due to reduced resistance to the intense synaptic input from the inferior olive upon restoration of blood flow (Welsh et al., 2002). Consistent with this hypothesis, the patterned loss of Purkinje cells after global brain ischemia resembles the ibogaine-induced striped pattern of neurodegeneration. In addition, elimination of climbing fiber input from the inferior olive has been shown to protect Purkinje cells from early ischemic damage (Welsh et al., 2002). The absence of any discernible pattern in the distribution of cerebellar lesions in the continuous dosing IPu GDNF study essentially precludes an ischemic event as the underlying mechanism. This is further supported by the absence of neuropathology in other highly ischemia-sensitive brain areas such as the striatum or the CA1 area of the hippocampus.

3.3. GDNF immunogenicity

As expected of a recombinant human protein administered to nonhuman primates, GDNF was highly immunogenic. As detailed in Table 2, high incidences of binding (88%) and neutralizing (40%) anti-GDNF antibodies were found in the serum of drug-treated animals at least once during the study (Hovland et al., 2007).

The respective incidences in CSF were 14% for both types of antibodies, and all animals with antibodies in CSF also tested positive for antibodies in serum (Boyd, 2006; Hovland et al., 2007). The incidence of binding antibodies in serum peaked early in the study, on Day 2, when 81% of drug-treated animals tested positive,

<table>
<thead>
<tr>
<th>Dose group</th>
<th>Serum antibodies</th>
<th>CSF antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Binding</td>
<td>Neutralizing</td>
</tr>
<tr>
<td>0 µg/month</td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td>450 µg/month</td>
<td>69%</td>
<td>38%</td>
</tr>
<tr>
<td>900 µg/month</td>
<td>94%</td>
<td>50%</td>
</tr>
<tr>
<td>3000 µg/month</td>
<td>100%</td>
<td>33%</td>
</tr>
<tr>
<td>All active dose groups</td>
<td>88%</td>
<td>40%</td>
</tr>
</tbody>
</table>

Incidence reflects positive responses at one or more time points.
while only 66% tested positive at Week 26 and 47% at the recovery time point. The incidence of neutralizing antibodies in serum gradually increased from Day 2 (21%) to Week 26 (34%), before it went down again to 20% at the recovery time point (Boyd, 2006). CSF samples also showed higher antibody incidences later in the study (Week 26 vs. Week 4) but were negative for both binding and neutralizing antibodies at the recovery time point (Boyd, 2006; Hovland et al., 2007). Only the incidence of binding antibodies in serum appeared to be dose dependent, while no dose dependency was observed for neutralizing antibodies in serum or for antibodies of any type in CSF (Hovland et al., 2007).

Consistent with expectations based on the low number of B cells in CSF (0.004 × 10⁶ cells/L) (de Graaf et al., 2011), these findings suggest that antibody formation occurred as a result of systemic exposure. Consistent systemic exposure to GDNF was encountered via the surgical implantation of the pump/catheter system and the pump refill and flushing procedures that were performed at 2-week intervals, starting at surgery, i.e. approximately 4 weeks prior to the initiation of study treatment (Hovland et al., 2007).

Computer analysis of the human GDNF sequence revealed two immunodominant major histocompatibility complex (MHC) class II T cell epitopes within the C-terminal domain of the molecule between amino acids 31–43 and 69–81 (Tatarewicz et al., 2007). The effect of these intrinsic T cell epitopes may have been exposed or potentially augmented in the recombinant protein that is produced in *Escherichia coli* and lacks glycosylation at amino acids 49 and 85 of the endogenous molecule. This supports the hypothesis that the presence of neutralizing antibodies in serum and the appearance of antibodies in CSF was caused by a T-cell-mediated maturing immune response (Tatarewicz et al., 2007).

Importantly, a correlation of Purkinje cell loss and the presence of anti-GDNF antibodies in serum or CSF could not be established (Hovland et al., 2007). Specifically, no animal with Purkinje cell loss presented with pre-existing antibodies and although all four affected animals, like all unaffected animals in the 3000 µg/month dose group, developed binding antibodies in serum during treatment with GDNF, only two also tested positive for neutralizing antibodies and none had antibodies in CSF.

There were signs of nonspecific inflammation locally along the catheter tracts and at the infusion sites at the end of the treatment period in all treatment groups. The changes were more consistent and more pronounced in drug-treated animals, specifically in the two high-dose groups (Hovland et al., 2007). The inflammatory reaction was typically characterized by lymphocyte/eosinophil infiltrates at the catheter tract or in the perivascular space adjacent to the catheter tract (Hovland et al., 2007). By contrast, no such inflammatory reactions and in particular, no parenchymal T cell margination, was observed in areas of Purkinje cell loss (Hovland et al., 2007).

### 3.4. Exposure to GDNF in cerebrospinal fluid

Several interesting observations can be made with respect to GDNF CSF:

1. GDNF CSF was quantifiable in the vast majority of on-treatment samples in all active groups, although individual values were highly variable within any given dose group. This very likely reflects significant leakage of drug from the infusion site into the CSF due to the use of large (1 mm outer diameter), non-step design catheters, with the individual extent of leakage depending on factors such as the exact location of the catheter tip (Chen et al., 1999; Morrison et al., 1999; Krauze et al., 2005; Fiandaca et al., 2008; Allard et al., 2009; Yin et al., 2009a).

2. As shown in Table 3, mean GDNF C_{CSF} increased with dose at both sampling times during treatment (Weeks 4 and 26). Similarly, the frequency of quantifiable levels also increased with dose, and all animals in the 3000 µg/month group had quantifiable levels after 4 weeks.

3. Mean GDNF C_{CSF} nominally decreased between Weeks 4 and 26 in all groups. An appreciable 93% decrease was seen in the low-dose group, while the mid-dose group remained effectively unchanged (~6%), and the high-dose group showed a 51% decrease. Assuming no change in the rate of GDNF transfer into the CSF compartment, this pattern would be consistent with a modest induction over time of the assumed GDNF processing in CSF.

4. No quantifiable GDNF C_{CSF} values were found at the end of the recovery period which is consistent with pharmacokinetic data indicating that the terminal half-life of GDNF in CSF following single dose IV administration in rhesus monkeys is 34 h (Lau, 1996).

At first glance, these observations could be taken to support the hypothesis that exposure to high GDNF C_{CSF} may have caused the cerebellar lesions. However, a correlation between the presence of Purkinje cell loss and peak GDNF C_{CSF} values could not be established at a group level. Specifically, although the animal with the single highest GDNF C_{CSF} value (no. 34: 161,775 pg/mL) was among the affected animals, 8 of 11 unaffected animals had peak values that were well within the range of the affected animals (1710–161,775 pg/mL). The individual values of all animals in the high-dose group are presented in Table 4.

Purkinje cells are known to express both GFRα-2 and c-RET mRNA (Burzalin and Gundlach, 1999). While GDNF preferentially binds to GFRα-1, it also binds to GFRα-2, although at lower affinities

### Table 3

Mean GDNF levels (pg/mL) in cerebrospinal fluid (Hovland et al., 2007).

<table>
<thead>
<tr>
<th>Dose group</th>
<th>On treatment</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
<td>26 weeks</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
</tr>
<tr>
<td>-----------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>450 µg/month</td>
<td>15</td>
<td>297</td>
</tr>
<tr>
<td>900 µg/month</td>
<td>15</td>
<td>602</td>
</tr>
<tr>
<td>3000 µg/month</td>
<td>15</td>
<td>12,200</td>
</tr>
</tbody>
</table>

N: number of samples at sampling time; BLQ: below the limit of quantification.

### Table 4

Individual GDNF levels (pg/mL) in cerebrospinal fluid of animals in the high dose group (3000 µg/month) (Hovland et al., 2007).

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>On treatment</th>
<th>End of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
<td>26 weeks</td>
</tr>
<tr>
<td>Affected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>2099</td>
<td>BLQ</td>
</tr>
<tr>
<td>34</td>
<td>161,775</td>
<td>BLQ</td>
</tr>
<tr>
<td>71</td>
<td>2940</td>
<td>4409</td>
</tr>
<tr>
<td>72</td>
<td>1710</td>
<td>BLQ</td>
</tr>
<tr>
<td>Unaffected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>2569</td>
<td>171</td>
</tr>
<tr>
<td>32</td>
<td>3578</td>
<td>5185</td>
</tr>
<tr>
<td>33</td>
<td>936</td>
<td>8607</td>
</tr>
<tr>
<td>35</td>
<td>1948</td>
<td>15,968</td>
</tr>
<tr>
<td>64</td>
<td>1355</td>
<td>2345</td>
</tr>
<tr>
<td>65</td>
<td>8887</td>
<td>30,968</td>
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<tr>
<td>66</td>
<td>1093</td>
<td>15,392</td>
</tr>
<tr>
<td>67</td>
<td>1203</td>
<td>2403</td>
</tr>
<tr>
<td>68</td>
<td>1630</td>
<td>NS</td>
</tr>
<tr>
<td>69</td>
<td>562</td>
<td>BLQ</td>
</tr>
<tr>
<td>70</td>
<td>1065</td>
<td>144</td>
</tr>
</tbody>
</table>

BLQ: below the limit of quantification. NS: no sample. N/A: not applicable.
(Airaksinen and Saarma, 2002). Not unexpectedly, therefore, Purkinje cells are responsive to GDNF in vitro, suggesting that GDNF is a potent survival and differentiation factor for these neurons (Mount et al., 1995). Consistent with these findings, GDNF injected into the cisterna magna improves cerebellar Purkinje neuron function in aged F344 rats (Bickford et al., 2001). It is unlikely therefore that chronic exposure to GDNF would cause Purkinje cells to become atrophic. Indeed, there is evidence to suggest that chronic exposure to GDNF in CSF has protective effects on Purkinje cells in vivo, as chronic ICV infusion of GDNF over 4 weeks rescues Purkinje cells from hereditary degeneration in the shaker mutant rat model (Tolbert et al., 2001). This is consistent with findings that GDNF protects responsive neurons from excitotoxicity, possibly through its ability to diminish free radical production (Gratacos et al., 2001; Alberch et al., 2002; Cheng et al., 2004). Collectively, these data support the conclusion that exposure of Purkinje cells to high concentrations of GDNF over an extended time period would not have been toxic but indeed would have been protective against degenerative and excitotoxic mechanisms.

3.5. Withdrawal of GDNF in cerebrospinal fluid

While the findings reviewed in Section 3.4 make it unlikely that exposure to GDNF directly induces Purkinje cell loss, they raise the related question of whether abrupt termination of exposure to high concentrations of GDNF in CSF may have been the culprit event.

If this were the case, it would be predicted that lesions would be observed exclusively in animals whose GDNF CSF values were below the limit of quantification at necropsy, i.e., in animals that were “withdrawn”. This was indeed the case. Apart from the five recovery animals in the high-dose group, there were two main group animals that (like two of the recovery animals as well) had GDNF CSF values below the limit of quantification at the end of treatment (although the reasons for this are unknown and may have been due to catheter migration or peripheral disconnection). Three of the animals with Purkinje cell lesions belonged to the recovery group, while the fourth one was one of the two main group animals above. Hence, 4 of 7 high-dose animals with no measurable levels of GDNF CSF at necropsy had lesions, as compared to 0 of 8 high-dose animals that were still exposed to measurable concentrations. The probability that this split occurred by chance is 0.026 (7 × 6 × 5 × 4/15 × 14 × 13 × 12), suggesting that withdrawal played a causal role in the pathogenesis of the lesions (Hutchinson, 2008).

Further insight can be gained by analyzing the individual patterns of GDNF CSF over time in the high-dose animals (Fig. 3).

The graph reveals that the four affected animals shared a common pattern of GDNF CSF over time that was otherwise seen in only one unaffected animal in the recovery group (no. 35). In particular, all of these animals had GDNF CSF values >1700 pg/mL at Week 4 and/or Week 26, and decreased to values below the limit of quantification at necropsy. Moreover, animals that presented with high values only at Week 4 (no. 28, 34, 71, and 72) had mild to minimal lesions with 1–3% of the cerebellar cortex affected, while animal no. 71 that presented with high values both at Week 4 and Week 26 had moderate lesions with 21% of the cerebellar cortex affected. Animal no. 35 presented with a high value at Week 26 and remained unaffected. All other animals (1) never exceeded the threshold of 1700 pg/mL (no. 68, 69, and 70), (2) had a more gradual decrease between Week 4 and Week 26 (no. 29), or (3) had values above the threshold at necropsy (no. 32, 33, 64, 65, 66, and 67, as well as mid-dose animals no. 19 and 56 (Hovland et al., 2007)).

In aggregate, these data suggest that the observed Purkinje cell loss was caused by abrupt withdrawal following extended exposure to GDNF CSF values >1700 pg/mL. All of the affected animals shared the respective, characteristic pattern of GDNF CSF over time, and 4 of 5 animals that showed this pattern were affected. Also, although the number of sampling time points was limited, it seems that the length of exposure was correlated with the severity of the lesions.

These data are consistent with the hypothesis that chronic exposure to high GDNF CSF in normal animals leads to suppression of endogenous GDNF synthesis and receptor down-regulation on Purkinje cells (Salvatore et al., 2006). While direct evidence for GDNF-induced down regulation of GDNF receptors on cerebellar Purkinje neurons is lacking, receptor down-regulation as an important mechanism to attenuate growth factor-activated receptor tyrosine kinase signaling is well established (Schlessinger, 2000). Ligand binding leads to receptor clustering in coated pits on the cell surface, followed by endocytosis, intracellular trafficking and lysosomal degradation, processes that are in part regulated by ligand-induced receptor ubiquitination (Schlessinger, 2000; Dikic and Giordano, 2003; Thien and Langdon, 2005; Arevalo et al., 2006). These mechanisms can abrogate receptor tyrosine kinase signaling irreversibly (Alsina et al., 2012).

There is no reason to believe that this general principle does not also apply to GDNF and its receptors. In fact, it is known that the expression of GDNF receptors is not static and can undergo dynamic changes. For example, GFRα-1 and GFRα-2 are progressively down-regulated in postnatal spinal motoneurons (Zhang and Huang, 2006), and expression to toxins such as MPTP and rotenone effects a robust down-regulation of c-RET in neurons (Hirata and Kiuchi, 2007). Also, there are a number of studies reporting that chronic long-term over-expression of GDNF has adverse effects on both intact and Parkinsonian nigrostriatal neurons (Georgievskia et al., 2004a; Winkler et al., 2006). A potential explanation for the latter findings is a direct negative feedback mechanism involving GDNF receptors.

If receptor down-regulation is followed by abrupt withdrawal of exogenously delivered GDNF, cells can become atrophic and ultimately die via a caspase-dependent non-mitochondrial pathway (Yu et al., 2003). The finding that Purkinje cells predominantly express GDNF family ligand receptor GFRα-2 (Burazin and Gundlach, 1999) further supports this hypothesis. As GDNF binds to GFRα-2 at lower affinities than to GFRα-1 (Airaksinen and Saarma, 2002), higher GDNF concentrations and extended exposure would be needed to induce receptor down-regulation, and this would explain why lesions were observed exclusively in the high-dose group.

Fig. 3. Individual GDNF Levels (pg/mL) in cerebrospinal fluid of animals in the high-dose group (3000 µg/month) (Hovland et al., 2007). Animals grouped together on the left side (no. 28, 34, 71, 72) had cerebellar lesions, animals grouped together on the right side (no. 29, 32, 33, 35, 64–70) did not have lesions. Animals 34, 35, 70, 71, 72 were in the recovery group, all other animals were in the main group. Values below the level of quantification are represented as 2 pg/mL. The 26-week sample of animal no. 68 is missing.
The fact that cerebellar lesions were never observed in the intermittent dosing ICV studies provides additional support for the receptor down-regulation hypothesis. Animals in these studies were exposed to very high peak GDNF $C_{CSF}$ values immediately after dosing. As reported in Section 1.3, mean peak GDNF $C_{CSF}$ after single dose ICV administration of 500 μg GDNF (1/6 of the highest dose given in the ICV toxicity studies) was approximately 34 μg/ml (Lau, 1996), i.e. 3–6 × 10^3 higher than mean GDNF $C_{CSF}$ at the high-dose level in the continuous dosing IPu study. However, in the intermittent dosing ICV studies, exposure to high-GDNF $C_{CSF}$ values of Purkinje cells was likely of insufficient duration to induce sustained down-regulation of the GFR–receptor. As discussed earlier, the elimination of GDNF from CSF following ICV administration in rhesus monkeys fits a tri-exponential model with half-lives of 0.24, 6.6 and 34 h (Lau, 1996). This suggests that exposure to GDNF in CSF ended within approximately one week after dosing in the intermittent dosing ICV studies.

Therefore, cerebellar Purkinje cells were left unexposed for 3 weeks before the next treatment took place with monthly dosing, and still for one week before the next treatment took place with biweekly dosing. Of note, CSF samples from the biweekly dosing study which were taken 2 days after the last dosing already yielded GDNF $C_{CSF}$ values well below the hypothesized “critical threshold” of 1700 pg/ml in 2 of 4 animals both in the low-dose group (600 μg/month) and the mid-dose group (2000 μg/month). Only in the high-dose group (6000 μg/month) were the values consistently above the threshold of 1700 pg/ml at 2 days after the last dosing (Orr, 1996). No GDNF measurements in CSF are available from the monthly dosing ICV study, since all CSF samples were accidentally discarded before the analysis.

4. Clinical studies

Chronic, continuous delivery of GDNF into the putamen has been investigated in 3 clinical studies to date, including two open-label Phase I studies and a double-blind, placebo-controlled Phase II study (Gill et al., 2003; Patel et al., 2005; Slevin et al., 2005; Lang et al., 2006; Slevin et al., 2007). Overall, 49 subjects with PD participated in these studies, 32 of whom were exposed to GDNF over a period of up to 43 months. Doses ranged from 90 to 2592 μg/month, the most commonly used dose being 900 μg/month. This translates to a safety margin of 14 (range: 4.9–140) relative to the no-observed-adverse-effect-level (NOAEL) of 900 μg/month in the continuous dosing IPu toxicity study (Hovland et al., 2007), when a standard scaling factor of ≥14 is used to adjust for the differences between normal rhesus monkeys and Parkinsonian humans in brain weight (95 g vs. 1350 g) (Nieuwenhuys et al., 1998) or brain volume (97 cm³ vs. 1470 cm³) (Yin et al., 2009b). Notably, the above approach to the calculation of safety margins is valid only for toxicities in brain regions that are remote to the putaminal site of delivery, e.g. cerebellum. By contrast, safety margins for local parenchymal toxicities would have to be calculated on the basis of $C_L$ and without considering spatial differences.

In none of the subjects were there any clinical signs or symptoms of cerebellar atrophy. Upon identifying cerebellar lesions in the continuous dosing IPu toxicity study in rhesus monkeys, MRI scans from 9 of 10 subjects enrolled in one of the Phase I studies were systematically analyzed to determine whether subtle volumetric or intensity changes could be detected in the cerebellum or elsewhere following GDNF treatment for over 1 year (Chebrulé et al., 2006). The analysis revealed no significant cerebellar differences in any of the subjects (difference image analysis), no significant morphometric differences between pre- and post-GDNF scans (voxel-based group morphometric analysis), and no signal abnormalities in the cerebellum on fluid-attenuated inversion-recovery (FLAIR) images (clinical scan review). One subject who participated in the other Phase I study died from an unrelated myocardial infarction 3 months after the cessation of treatment with GDNF (Love et al., 2005). The drug had been infused unilaterally at doses of 432–1296 μg per month over a total of 43 months. Autopsy showed no evidence of cerebellar atrophy, while there was a focal increase in astrocytic gliosis around the catheter tract and limited inflammation at the catheter tip, surrounded by a local increase in tyrosine hydroxylase-immuno-positive nerve fibers indicating significant biological activity of GDNF (Love et al., 2005).

5. Implications for clinical research

The clinical development program of GDNF in PD which was halted in 2004 has recently been resumed employing a novel intermittent dosing, convection-enhanced delivery (CED) paradigm for local drug administration into the putamen (Bienemann et al., 2011; Gill et al., 2012) [https://www.clinicaltrialsregister.eu/ctr-search/trial/2011-003866-34/GB]. CED is a technique that has been introduced to overcome the limitations of diffusion-based delivery (Bobo et al., 1994). It is enabled by a continuous local pressure gradient that is generated by a pressurized infusion system and forces the infusate from the site of deposition through the extracellular space (Bobo et al., 1994; Allard et al., 2009). The resulting bulk flow leads to reproducible, homogeneous drug distribution throughout clinically significant volumes of brain parenchyma (Bobo et al., 1994; Morrison et al., 1994; Lieberman et al., 1995). Unlike diffusion, CED is not significantly influenced by the concentration and molecular weight or particle size of the infused agent (Bobo et al., 1994; Lieberman et al., 1995; Croteau et al., 2005; Allard et al., 2009).

All previous clinical studies testing IPu administration of GDNF employed continuous infusion schemes at flow rates of 0.1 μL/min or less (Gill et al., 2003; Patel et al., 2005; Slevin et al., 2005; Lang et al., 2006; Slevin et al., 2007), supplemented with short 2-min pulses at 10.9 μL/min every 6 h in one of the Phase I studies (Slevin et al., 2005; Slevin et al., 2007). It has since been established that flow rates of 0.5 μL/min or more are needed to induce effective convection (Fiandaca et al., 2008; Allard et al., 2009). Application of the low-flow Phase II delivery protocol in rhesus monkeys produced minimal putaminal coverage and highly variable and irregular distribution of GDNF (Salvatore et al., 2006). It should be borne in mind that utilization of CED-enabling flow rates continuously over extended times would inevitably lead to drug distributions beyond the desired target volume (“flooding”) and leakage into white matter and/or the CSF compartment. As leakage into the CSF is likely the first step in the chain of events that eventually leads to cerebellar lesions (Vareniuk et al., 2008; Fiandaca et al., 2008), this must be avoided. Therefore, this hypothesis would dictate that chronic CED can only be utilized safely (from a cerebellar toxicity perspective) with intermittent dosing regimens.

In addition to the generic technical requirement for the intermittent use of CED, there is a biological case for intermittent delivery of GDNF. Notably, the activity of GDNF, both upstream as assessed by dopamine turnover and downstream as assessed by local synaptophysin concentrations, has been shown to remain elevated over several weeks after single dose administration via CED into the striatum of normal rats (Hadaczek et al., 2010; Taylor et al., 2013). This is consistent with earlier behavioral data in Parkinsonian nonhuman primates showing sustained functional improvements over 4 weeks following single intranigral, intracaudate or ICV administration (Gash et al., 1996). Finally, and perhaps most importantly, intermittent delivery would be projected to reduce the possibility of generating ligand-induced
receptor down-regulation both on target and non-target neurons. At the same time, intermittent delivery is associated with the use of markedly lower total doses (temporally normalized), thus increasing the safety margins relative to the cerebellar toxicity-based NOAEL of 900 μg per month determined in the continuous dosing IPu toxicology study (Howland et al., 2007).

In conclusion, review of the available evidence suggests that further clinical testing of intraputaminaly administered GDNF can be safely pursued in PD subjects as long as the molecule is delivered intermittently and via CED.

Conflict of interest

The authors are employees of and investors in MedGenesis Therapeutics Inc.

Transparency document

The Transparency document associated with this article can be found in the online version.

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