CHAPTER 1

Cell replacement and gene-therapy strategies for Parkinson’s and Alzheimer’s disease

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Abstract

Parkinson’s disease and Alzheimer’s disease are the most common neurodegenerative diseases in the elderly population. Given that age is the most important risk factor in these diseases, the number of patients is expected to rise dramatically in the coming years. Therefore, an effective therapy for these diseases is highly sought. Current treatment brings only temporary symptomatic relief and does not result in halting the progression of these diseases. The increasing knowledge on the molecular mechanisms that underlie these diseases enables the design of novel therapies, targeted at degenerating neurons by creating an optimal regenerative cellular environment. Here, we review the progress made in the field of cell-replacement and gene-therapy strategies. New developments in the application of embryonic stem cells and adult neuronal progenitors are discussed. We also discuss the use of genetically engineered cells in neuronal rescuing strategies that have recently advanced into the clinic. The first trials for the treatment of Alzheimer’s disease and Parkinson’s disease with this approach are ongoing.
Introduction

Parkinson’s disease (PD) and Alzheimer’s disease (AD) are the two most common neurodegenerative diseases associated with age. PD is primarily a movement disorder and at least 1% of people suffer from it by age 70 years [1]. AD is characterized by progressive memory loss and is the most common cause of dementia in the elderly. Approximately 7% of those aged over 65 years and approximately 40% of those aged over 80 years are affected by this disease [2]. Owing to aging of the population worldwide and lack of a cure, the number of PD and AD cases will grow substantially the next two to three decades [3–5]. Therapies used today are only relief based and are unable to halt the progression of PD and AD. Therefore, there is a great need to find new effective regenerative therapeutic strategies that can stop the development and progression of these neurodegenerative diseases and that will improve a patient’s quality of life.

Over the past 20 years, a substantial progression in our understanding of the molecular mechanisms underlying PD and AD has been achieved, enabling the development of novel therapeutic strategies to cure these diseases. The main hallmarks of both disorders is neuronal degeneration and neuronal death; therefore, cell replacement strategies are currently regarded as a potential therapy by either transplanting embryonic stem cells or neural progenitors [6]. In addition, due to the progress in gene-manipulation strategies, gene therapy also becomes an attractive approach, where cells engineered to express neurotrophic factors have been transplanted with the aim to promote neuronal survival and rescue neuronal function [7,8].

In this review, we will critically discuss emerging cell replacement and gene therapies for PD and AD and the current status of applying these experimental therapies in clinical trials.

Parkinson’s disease

Pathogenesis

PD is a progressive disease and clinical symptoms, such as rest tremor, rigidity, balance impairment and slowness of movement, only manifest in the late stages. Braak and colleagues described in detail six stages of the disease based on the pathological hallmarks, that is, α-synuclein build up in the Lewy bodies (LB) and Lewy neurites (LN) and degeneration of the dopaminergic (DAergic) neurons in the substantia nigra (SN) [9]. The loss of DA neurons in the SN and the loss of DAergic innervations in the striatum are the cause of movement disabilities described in PD (Figure 1). Apart from these well-known motor disabilities, additional symptoms present themselves, such as sleep disruption, depression, fatigue, constipation and anxiety.
The cause of PD in the majority of the patients suffering from the nonhereditary form of the disease is as yet unknown. A role of environmental factors in the development of the disorder has been suggested. In the early twentieth century, it has been observed that a viral infection can cause nigral degeneration [10]. Additionally, it has been shown that toxic substances, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, paraquat, maneb and epoxomicin, can induce Parkinsonism [1]. Some of these substances (e.g., MPTP and epoxomicin) are used today in research to induce PD-like symptoms in animals, thus generating a model for the disease.

In the past 10 years, genetic mutations were identified that cause or are involved in PD. Single point mutations in the α-synuclein, parkin, DJ-1, PTEN-induced putative kinase 1 (PINK-1), dardarin (LRRK2) [1] and ubiquitin carboxyl-terminal esterase L1 (UCH-L1) [11] genes have been shown to be involved in the pathogenesis of PD [12]. The discovery of these genetic factors revealed that an impaired energy metabolism, a deficient ubiquitin–proteasome pathway and an inhibition of neurotransmitter release are likely to be involved in the pathogenesis of the disease.
It is expected that, in the non-monogenic forms of PD, genetic factors are important as well, since many PD patients are known to have an affected family member [13]. Genes such as tau, semaphoring 5A, α-synuclein, fibroblast growth factor 20 and nuclear receptor-related 1 (Nurr1) are all implicated in the development of PD [1,14]. Interestingly, different variants of semaphorin 5A were associated with PD in a Taiwanese population. However, this could not be confirmed in a Finish population study, prompting a debate regarding this gene and its role in PD development [15]. Current genome-wide screening studies will contribute to our knowledge of the molecular mechanisms involved in PD pathogenesis.

**Treatment**

All current PD therapies focus on restoring the DA levels by either the oral administration of the DA precursor levodopa (L-DOPA), which supplements the low level of endogenous DA, or inhibition of the breakdown of endogenous DA by treatment with the monoamine oxidase type B (MAO-B) inhibitor, selegiline. DA agonists are also used to directly stimulate the DA receptors [1,16]. Unfortunately, these therapies are all symptomatic treatments and do not prevent the progression of the DAergic neuronal degeneration and, more importantly, are not capable of curing PD. In the later stages of the disease, L-DOPA becomes ineffective or, even worse, causes severe side effects, such as the development of dyskinesias (fragmented or incomplete movements) and the prolongation of the ‘off’ time (time of enhanced symptomatic activation) [17]. Surgical treatment by deep brain stimulation is practiced to further modify dyskinesias and decrease the ‘off’ time [18]. Although advanced, this treatment does not stop the progression of the disease either [1], therefore there is a great need to develop new regenerative therapeutic strategies that directly target the degenerating DAergic neurons or their innervation areas.

**Alzheimer’s disease**

**Pathogenesis**

AD is characterized by a progressive cognitive decline and memory loss [7]. The classical pathological changes in the brain of AD patients include deposition of α-amyloid plaques, the presence of neurofibriillary tangles, gliosis and neuronal atrophy.

The neuronal degeneration and loss of synapses progresses over time and are widespread in AD, beginning with the entorhinal cortex and hippocampus and progressing to the neocortex, amygdala, thalamus and SN [19]. A main feature is the degeneration of the cholinergic neurons in the nucleus basalis of Meynert, which leads to a reduction in the cholinergic innervation of the cortical and subcortical regions [20]. This reduction in acetylcholine correlates with
the clinical and pathological severity of AD and is currently the main target for therapy (Figure 2) [21].

The majority of AD cases are nonfamilial with an unknown etiology. Many risk factors have been implicated to contribute to AD [22], such as old age, head injury, smoking, diabetes and exposure to heavy metals, such as copper or aluminium [23,24].

Genetic screenings have so far revealed the causative role of three genes in early-onset familial AD. Missense mutations in presenilin 1 (PS1), presenilin 2 (PS2) and the amyloid precursor protein (APP) gene all caused autosomal dominant AD [25]. Furthermore, the presence of one or two apolipoprotein E 4 (ApoE4) alleles has been shown to be the main genetic risk factor for AD [26]. Over the years, many other genes have been associated with the disease, such as sortilinrelated receptor (SORL1), LRP and α2macroglobulin [27,28]. The genetic forms of AD have taught us a lot about the molecular mechanism underlying amyloid plaque formation. Oxidative stress and an impaired ubiquitin–proteosome system have also been implicated in AD [29]. However, the cellular pathways that cause cholinergic neuron degeneration and tangle formation in AD are still elusive.

Treatment

Currently, there is no cure for AD and today’s treatments bring relatively small symptomatic relief to the patients, without having an effect on the progression of the disease. The most common therapy is the prescription of acetyl cho-
linesterase inhibitors, such as donepezil, tacrine or rivastigmine, which reduce the rate of acetylcholine breakdown and increase its concentration in the brain, resulting in a modest cognitive improvement [30]. Another drug, memantine, is a novel NMDA receptor antagonist that targets the glutamatergic pathway [31]. Light therapy and electrical stimulation have also been used as treatments for AD. These treatments slightly improve the patient’s cognitive function [32,33]. As with PD, there is also an urgent need for effective regenerative therapeutic approaches for AD that prevent neuronal degeneration or rescue dying neurons.

**Cell-replacement therapy**

Over the last decades, cell-replacement therapy has emerged as a potential treatment for neurodegenerative diseases such as PD and AD. We will discuss different cell-replacement strategies based on human fetal mesencephalic brain tissue, embryonic stem cells, neural progenitor cells (NPCs) and genetically engineered cells. Figure 3 shows the different cell types and defines the terminology used in this review. Most research has been performed in the field of PD, since the clinical symptoms are connected to the degeneration of the DAergic neurons mainly restricted to the SN and therefore can be easily targeted. This contrasts with the possibilities in AD, where the widespread pathological changes are a great challenge for cell-replacement treatment [17,34]. Recent extensive assessment of brain pathology in PD patients reveals that, in PD, there is also a widespread neuropathology [9], indicating the cell replacement aimed at the SN might not be sufficient as a therapy for PD. To create a successful cell-replacement therapy, five main goals must be achieved:

- Establishment of sufficient amounts of viable cells (DAergic or cholinergic) for transplantation
- Successful axon extension
- Formation of functional synapses
- Stable and long-term integration of the cells into the host brain circuitry
- Evidence of functional recovery

**First cell-transplantation trials in PD & AD**

The first clinical trials concentrated on transplantation of DAergic cell types with the intention of creating a new local source of DA. Animal studies have shown that functional synaptic integration of grafted DAergic neurons is possible [35,36]. However, to date, similar methods have failed to reconstitute the lost neuronal circuitry of the DA system in patients. Adrenal medulla tissue was first transplanted [37], resulting in a slight short-term improvement in behavior; however the graft did not survive [6]. Human fetal ventral mesencephalic tissue has also been grafted in the striatal area of PD patients. Over 300 patients
have been transplanted worldwide with this tissue [6], showing high graft survival, reinnervation of the striatum and long-lasting symptomatic relief in small initial trials. However, double-blind studies failed to prove group improvement, but rather indicated individual patient differences and dyskinesia development [38,39]. Long-term effect studies showed DA storage and release by the graft tissue contributed to some symptomatic relief. The disease, however, was still progressing [40]. The differences in these trials and the ethical and practical reasons make this therapeutic approach unsatisfactory [41]. These issues were recently extensively discussed by Winkler and colleagues, and lie beyond the scope of this review [42]. In AD, cholinergic-rich tissue [43] and peripheral cholinergic neurons [44] were transplanted into an AD rat model with nucleus basalis of Meynert lesions. These animal trials showed a memory improvement, which indicated a partial neuronal rescue in the disease model. No clinical trials in AD patients have been initiated with this method.
**Embryonic stem cells as regenerative tools in degenerative diseases**

Embryonic stem (ES) cells are defined as pluripotent, undifferentiated cells that can proliferate and have the capacity of self-renewal and differentiation into different types of specialized cells from all three primary germ layers. They are derived from the inner mass of the embryonic blastula. ES cells were first isolated from mice in 1981 [45], later from primates in 1995 [46] and finally from humans in 1998 [47]. Hope exists that ES cells may prove to provide an inexhaustible source of neurons and glia for therapies that are aimed at cell replacement or neuronal protection in neurodegenerative disorders [34]. A lot of research has been focused on developing the best method of using ES cells in a therapeutic approach. Cell-replacement therapy for PD can be targeted to specific brain areas, the SN or the caudate putamen, as these areas are directly connected to the clinical symptoms of the disease (Figure 1) [17,34]. Unfortunately, this technique is more difficult to apply to AD patients, since the neuropathology is too widespread throughout the brain (Figure 2).

**Animal ES cells**

To date, the research on ES cells has progressed to the level of animal testing. In PD, these models are mostly behavioral models with a chemically induced SN injury. Table 1 summarizes some of the animal studies performed, showing the progression in this field.

A prerequisite to prevent tumor formation after transplantation is ES cell differentiation (Table 1). Many different techniques were used to produce the most efficient differentiation procedure with the highest yield of DAergic neurons. Mimicking developmental signaling was thought to be the best method to differentiate ES cells. Lee and colleagues were the first to obtain a high yield of over 30% tyrosine hydroxylase (TH)-positive cells marking DAergic neurons in vitro by applying mitogens and specific signaling molecules [48]. They have developed a protocol for ES cell differentiation into CNS progenitors and further maturation into DAergic and serotonergic neurons expressing all the cell-specific markers, such as Nurr1 for midbrain neurons. Kim and colleagues improved the protocol by the use of a stable Nurr1-expressing ES cell line derived by transfection of cells with Nurr1 cDNA [49]. Later, Barberi and colleagues demonstrated that, by coculturing ES cells with stromal cells that present developmental signals, the same goal could be reached [50]. Recently, it was shown that the Lmx1 and MSX1 homeodomain proteins are important in the generation of DA neurons from ES cells [51]. Transfection of ES cells with Lmx1a cDNAs driven by a nestin promoter, resulted in a robust generation of TH-positive neurons. Furthermore, the stromal cellderived-inducing activity (SDIA) technique enabled generation of DA neurons by co-culturing mouse ES cells with PA6 cells (stromal cells derived from skull bone marrow). This technique was very efficient and, in 92% of the differentiated ES cells, expression of TH was observed [52]. A similar technique was applied on primate ES cells [53], generating a high yield of TH-positive cells.
<table>
<thead>
<tr>
<th>Study and year</th>
<th>Type of cells</th>
<th>Transplantation site</th>
<th>Assessing technique</th>
<th>Results</th>
<th>Side effects</th>
<th>Ref</th>
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<tbody>
<tr>
<td>Deacon et al., (1998)</td>
<td>Undifferantiated mouse ES cells</td>
<td>Mouse and rat striatum</td>
<td>Cell survival and differentiation into TH+ neurons</td>
<td>Some neuronal differentiation noted, but no integration into the host brain</td>
<td>Not mentioned</td>
<td>[132]</td>
</tr>
<tr>
<td>Bjorklund et al., 2002; Tai et al. (2004)</td>
<td>Undifferantiated mouse ES cells</td>
<td>Rat PD model brain</td>
<td>Cell differentiation, behavioural tests and MRI</td>
<td>Cells positive for differentiation and functional recovery present in the animal. 24% of grafts showed no survival</td>
<td>20% of animals developed teratomas and had to be sacrificed before the end of the experiment</td>
<td>[91,133]</td>
</tr>
<tr>
<td>Kim et al., (2002)</td>
<td>In vitro ES derived TH+ cells by high Nurr1 gene expression</td>
<td>Striatum of 6-OHDA rat model</td>
<td>Cell survival and integration into the brain and behaviour tests.</td>
<td>Cells extended their axons into the striatum, formed functional synaptic connections and the animal showed recovery behaviour</td>
<td>No teratoma formation, but further observation is recommended</td>
<td>[49]</td>
</tr>
<tr>
<td>Takagi et al., (2005)</td>
<td>In vitro ES derived DA neurons with the use of stromal cell derived inducing activity (SDIA) and FGF and FGF20 singaling</td>
<td>Primate MPTP model of PD</td>
<td>Behaviour studies and functional imaging</td>
<td>Behaviour recovery and DA activity.</td>
<td>No tumor formation 3 months after transplantation, but longer observation suggested</td>
<td>[134]</td>
</tr>
<tr>
<td>Rodriguez-Gomez et al. (2007)</td>
<td>In vitro mouse ES-derived DA neurons</td>
<td>Ipsalateral striatum of mouse 6-OHDA PD model</td>
<td>Behaviour studies, DA release, reuptake and synapse stimulation with PET imaging</td>
<td>Behavioral improvement and long-term DA release from the graft. Postsynaptic DA D2 receptor normalized in the striatum</td>
<td>No teratoma formation</td>
<td>[135]</td>
</tr>
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</table>

**Table 1.** Rodent and primate embryonic stem cell transplantation studies in Parkinson’s disease animal models. 6-OHDA: 6-hydroxy dopamine; DA: Dopamine; ES: Embryonic stem; FGF: Fibroblast growth factor; MPTP: 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine; MRI: Magnetic resonance imaging; PET: Positron emission tomography; SDIA: Stromal cell-derived inducing activity; TH: Tyrosine hydroxylase.
(35%). Such a technique provides, in principle, a stable and unlimited source of primate neuronal cells.

Since undifferentiated ES cells may cause teratoma formation after transplantation, a method of complete irreversible differentiation into DA neurons and reliable, reproducible purification of the newly formed DA neurons is the biggest challenge. The field is turning now towards the use of the developmental signaling present in the CNS since these molecular pathways play a critical role in the generation of DA neurons. Smidt and Burbach defined a molecular scheme for ES differentiation leading to DAergic neurons. First, an early ES cell induction of Shh, Fgf8 and Wnt1 is needed and, shortly afterwards, the induction of Lmx1a and Lmx1b as well as Oxt2 and Foxa2 will start off the differentiation procedure. Foxa1, En1 and En2 induction would move the differentiation process into the next stage when the cells express Ptx3, Nr4a2 and Ilf1 and, as a consequence, TH and Vmat2 expression would be upregulated, at which point the cells represent mature DAergic neurons of the SN or ventral tegmental area [54,55]. Cholinergic neurons can also be differentiated from ES and neural stem cells. Embryonic skeletal muscle extract [56] and bone morphogenetic protein 2 [57] were both shown to induce stem cell differentiation into the cholinergic neuron phenotype. More studies identifying the developmental cues responsible for this neuronal subtype, such as the finding that the Islet-1 transcription factor is specific for cholinergic neurons [58], are needed to develop a similar molecular scheme for cholinergic neuron differentiation.

**Human ES cells**

Similarly to animal ES cells, the use of human ES (hES) cells as a tool for cell-replacement therapies is still at an early stage. Table 2 summarizes the experiments performed in the search for the most optimal conditions for hES cell differentiation into DA neurons. With each trial, the DAergic cell numbers increased; however, when applied in transplantation studies, these hES cell-derived neurons did not integrate into the host brain and did not result in functional recovery in the PD animal models. Table 3 summarizes animal studies performed with these cells.

Apart from the ethical problems concerning the use of hES cells, many practical concerns emerge. A significant problem lies in the differentiation methods of hES cells, since nonhuman stromal cells or other tumor cell lines are used to induce lineage specificity. If hES cells are to be used as a therapeutic tool, they will have to be cultured under animal-free conditions (i.e., not grown on, for example, a mouse cell monolayer) to avoid any animal viral infection of human tissue. Recently, this was achieved by Roy and colleagues, who used human fetal midbrain astrocytes for co-culture [59]. The use of different hES cell lines also poses a problem since they may differ in their ability to generate DAergic neurons and may have different functional activity in the host brain. For instance, in the study by Ben-Hur and colleagues, hES cells were able to improve animal
behavior after a lesion [60], which was in contrast to Shultz and colleagues, who performed the same experiment but failed to show a functional recovery [61]. Another major problem is the minimal survival rate of the hES-derived neurons in the host brain after transplantation. These cells either do not survive the graft procedure or they die in the rodent brain after transplantation. This was not observed with other mammalian ES cells, indicating significant differences between species. Finally, and probably the most troubling issue, is the inability to completely differentiate all ES cells. This is a necessity since purifying the differentiated from the undifferentiated cells has not been successful [62]. Some of the ES cells may still proliferate and may cause teratoma formation after they have been transplanted into the host brain. This and other aforementioned problems must be solved before an ES cell-replacement therapy will be safe enough for starting a clinical trial [63].

**Alternative use of ES cells: gene modification and/or environmental ‘chaperones’**

The cell-replacement strategy with ES cells is still in a very early stage of development. Their migration ability and their ‘chaperone’ attribute are also important and may lead to alternative uses of ES stem cells in future therapies. ES cells can be used in *ex vivo* gene therapeutic approaches as delivery vehicles. Cultured ES cells can be genetically modified by using either plasmid-based or lentiviral or retroviral vectormediated cDNA transduction [17]. After transplantation, the genetically modified ES cell and its progeny, in case of integration of the cDNA in the DNA of the ES cells, will express the gene of interest *in vivo* [64]. Additionally, the ‘chaperone’ character of ES stem cells (i.e., to nurse and support host neurons) is an interesting feature, which might be related to either the secretion of growth factors by the stem cells or expression of specific surface proteins.

<table>
<thead>
<tr>
<th>Study</th>
<th>Cell line</th>
<th>Differentiation technique</th>
<th>Results</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>Park et al., (2004)</td>
<td>MB03</td>
<td>Basic FGF cell induction</td>
<td>20% TH+ neurons but no confirmation of midbrain DA neurons.</td>
<td>[136]</td>
</tr>
<tr>
<td>Perrier et al., (2004)</td>
<td>H1, H9 and HES-3</td>
<td>Use of stromal feeder-based differentiation system [50] and midbrain DA neuronal induction by FGF8 and SHH signaling</td>
<td>79% TH+ midbrain DA neurons derived.</td>
<td>[137]</td>
</tr>
<tr>
<td>Yan et al., (2005)</td>
<td>H1 and H9</td>
<td>FGF8, SHH and Sox1 treatment</td>
<td>60% of TH+ cells also expressed midbrain DA neuron markers. These cells were electrophysiologically active and released DA in activity-dependent manner.</td>
<td>[138]</td>
</tr>
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</table>

*Table 2. Human embryonic stem cells differentiation in vitro. DA: Dopamine; FGF: Fibroblast growth factor; SHH: Sonic hedgehog; TGF: Transforming growth factor; TH: Tyrosine hydroxylase.*
<table>
<thead>
<tr>
<th>Study</th>
<th>Cell line</th>
<th>Differentiation technique</th>
<th>Transplantation site</th>
<th>Assessing technique</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schulz et al., (2004)</td>
<td>BG01 and</td>
<td>Suspension growth in HepG2 liver tumor cell-conditioned medium and later in conventional serum-free medium.</td>
<td>6-OHDA rat striatum</td>
<td>Cell survival and behaviour tests</td>
<td>Very few TH+ cells found at the site of the graft.</td>
<td>[61]</td>
</tr>
<tr>
<td>Ben-Hur et al., (2004)</td>
<td>HES-1</td>
<td>Exposure to bone morphogenetic protein antagonist noggin</td>
<td>6-OHDA animal model</td>
<td>Cell survival and behavioral tests</td>
<td>0.5% TH+ cells in vitro, improvement in behaviour. 12 weeks after transplantation 0.2% of transplanted cells TH+</td>
<td>[60, 63]</td>
</tr>
<tr>
<td>Zeng et al., (2004)</td>
<td>BG01</td>
<td>PA6 stromal cell co-culture</td>
<td>6-OHDA lesioned rodent striatum</td>
<td>Cell survival and lineage</td>
<td>Few surviving TH+ neurons 5 weeks post-transplantation, many mesodermal lineage cells</td>
<td>[139]</td>
</tr>
<tr>
<td>Park et al., (2005)</td>
<td>HSF-6</td>
<td>Use of stromal feeder-based differentiation system and midbrain DA neuronal induction by FGF8 and SHH signaling</td>
<td>Striatum of PD rat model</td>
<td>Cell survival and behavior tests</td>
<td>In vitro 40% of cells TH+ DAergic lineage with capability of releasing DA. In vivo no TH+ cells found at the site of the graft and no behavior changes</td>
<td>[140]</td>
</tr>
<tr>
<td>Breder-lau et al. (2006)</td>
<td>SA0002.5</td>
<td>PA6 stromal cell co-culture for 16, 20 or 23 days</td>
<td>6-OHDA lesion rat striatum</td>
<td>Cell survival and lineage, behaviour test</td>
<td>Low DA neuron phenotypes of the graft. No behavioral changes. Severe teratoma development in 16-day differentiation condition</td>
<td>[141]</td>
</tr>
<tr>
<td>Roy et al. (2006)</td>
<td>Hi and H9</td>
<td>SHH and FGF8 exposure and co-culture with telomerase-immortalized fetal midbrain astrocytes</td>
<td>6-OHDA lesion rat striatum</td>
<td>Cell survival and lineage, behaviour test</td>
<td>21% of TH cells at 10 weeks post-graft and declining with time. 6 weeks post-graft, significant behavior changes noted. Graft also showed the presence of nondifferentiated cells</td>
<td>[59]</td>
</tr>
<tr>
<td>Martinat et al., (2006)</td>
<td>H9</td>
<td>Co-culture with bone marrow-derived stromal cells (SIDA protocol) and transduced with lentiviral vectors expressing Nurr1 and Ptx3</td>
<td>6-OHDA lesion mouse striatum</td>
<td>Cell survival and lineage, behaviour test</td>
<td>45% TH+ cells and behavior improvement</td>
<td>[142]</td>
</tr>
<tr>
<td>Sonntag et al. (2007)</td>
<td>H7 and H9</td>
<td>Exposure to bone morphogenetic protein antagonist noggin and stromal cell co-culture</td>
<td>6-OHDA lesion rat striatum</td>
<td>Cell survival and lineage, behaviour test</td>
<td>H7-derived grafts contained TH+ neurons and some animals showed behavioral improvement. Teratoma growth was noted</td>
<td>[143]</td>
</tr>
<tr>
<td>Iacovitti et al. (2007)</td>
<td>H9, HUES7 and 8</td>
<td>Use of chemically defined rhuman-derived media additives and substrata</td>
<td>6-OHDA lesion rat striatum</td>
<td>Cell survival and lineage, behaviour test</td>
<td>Robust cell survival with neuronal lineage. Many cells express TH</td>
<td>[144]</td>
</tr>
</tbody>
</table>

**Table 3.** Human embryonic stem cell transplantation studies in Parkinson's disease animal models. 6-OHDA: 6-hydroxy dopamine; DA: Dopamine; FGF: Fibroblast growth factor; PD: Parkinson's disease; SDIA: Stromal cell-derived inducing activity; SHH: Sonic hedgehog; TH: Tyrosine hydroxylase.
molecules. More research is needed to clarify this phenomenon. In spinal cord injury, this effect, along with the remyelination of the injured axons by transplanted precursor cells, could have contributed to the restoration of locomotion [65]. There are even plans to make use of this ES cell ‘nursing’ characteristic observed in rat spinal cord injury and to inject ES cells into patients with paralyzing spinal cord injuries [66].

**Neural progenitor cells**

Progenitor cells are multipotent cells that divide asymmetrically giving rise to a daughter progenitor cell and a cell that will differentiate into a neuron or a glial cell (Figure 3). NPCs are a promising source of cells for transplantation.

**Embryonic & adult neural progenitors**

With the discovery of neurogenesis in the adult hippocampus [67] and olfactory bulb [68], the dogma of the CNS as an unregenerative, hardwired tissue was discredited. Multipotent neural progenitors and lineage-specific NPCs were shown to be present in the brain (Figure 3) [69]. In the mammalian adult brain, two main neurogenic niches were identified: the subgranular zone of the dentate gyrus in the hippocampus [70,71] and the subventricular zone (SVZ) in the walls of the lateral ventricle of the brain [72,73]. Additionally, neural progenitors were found in other areas of the adult brain, such as the cortex [74], SN [75,76] and ependymal layer of the third ventricle [77]. The presence of neural progenitors in the SN is still in dispute [69,75,78]. Also, in the human brain, neurogenesis was observed in the SVZ [79] and hippocampus [71]. Recently, it has been shown that the rostral migratory stream is also present in the human brain. Newly born neurons travel from the SVZ to the olfactory bulb along the rostral migratory stream [80].

Neural progenitors became attractive candidates for cell-replacement therapy. Flax and colleagues were the first to explore the characteristics of the human NPCs *in vitro* and *in vivo* [81]. Their experiments show that human embryonic NPCs are capable of expansion *in vitro* and are also capable of producing neurons, astrocytes and oligodendrocytes. Additionally, this group also showed that these human cells can be transplanted into an animal model and that they survive and even replace cells lost due to injury [81]. NPCs apparently have the ability to respond to local environment-derived signals at the site of the injury. NPCs can migrate toward a lesion area, differentiate into neurons and establish synaptic contacts with the local neurons [69,78]. This demonstrates that these cells can be useful tools in future therapy for neurodegeneration. On the other hand, Jain and colleagues transplanted NPCs in the PD 6-OHDA animal model and showed that, although capable of migration, these cells move out of the graft site, not influenced by the DA neuronal loss in the SN [82].

As in ES cell research, the use of NPCs in transplantation procedures comes with some apprehensions. One concern relates to the method of culturing and
differentiating NPCs. Until recently, there was a problem with establishing a stable human NPC culture with a constant ability to sustain ex vivo mitosis in culture [83]. Walton and colleagues resolved this issue by developing an optimal protocol to culture human adult progenitor cells that were highly expandable [83]. In addition to the culture conditions, the method of differentiation is also important. New ways of stimulating NPCs into differentiation have been developed, such as the use of different growth factors, retinoic acid or cAMP stimulation [69,84]. However, before the NPCs can be used in the clinic, these procedures have to be consistent and proven to be safe.

A further concern relates to the phenotype of the transplanted NPCs. Should the cells be in an undifferentiated form, or should they be stimulated in vitro to differentiate into specific neuronal cells? Both types of cells show survival in the graft [85,86], although undifferentiated human precursor cells show a better migration behavior towards the lesion, whereas the differentiated precursors have higher survival rate with more neurons present in the graft [87,88]. A study by Burnstein and colleagues compared two cell types as candidates for transplantation in a PD model, but they could not show recovery or additional DAergic neuronal differentiation. In this study, the cells survived in the graft, but they did not integrate into the host system [88]. This brings us to the final concern with this technique, regarding the functional significance of the transplantation method. Functional recovery can only take place when there is integration of donor cells into the host neuronal network and restoration of function. Table 4 summarizes transplantation experiments with the use of NPCs in animal models for PD. The various results of these experiments, plus the possibility of tumor growth induced by these NPCs [89], make this cell-replacement method still risky and it is necessary to understand the mechanism of progenitor cell differentiation in more detail before using them as a therapeutic tool.

**NPCs as environmental ‘chaperones’**

While performing transplantation experiments, Ourednik and colleagues made a very interesting discovery concerning the behavior of NPCs after grafting into the MPTP mice model [90]. They not only noticed a conversion of NPCs into DAergic neurons, but they also found that the majority of the new TH-positive neurons belonged to the host. This may be indicating a ‘rescue’ of the host neurons by the NPC graft. The NPCs may play a role as ‘chaperones’ of the host cells and promote their regrowth. These cells could have an inherent capacity to preserve and reactivate cells through their natural expression of trophic and neuroprotective substances. Therefore, the presence of NPCs at the injury site by itself may alter the microenvironment, resulting in the rescue of the impaired host neurons. This approach provides an alternative to the cell-replacement method, where the NPCs do not necessarily have to substitute the impaired neurons, but they may just simply promote their growth and stimulate their activity [90]. Others also share this view and suggest a new role of NPCs as chaperones
<table>
<thead>
<tr>
<th>Study</th>
<th>Cell type</th>
<th>Transplantation site</th>
<th>Assessing technique</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanchez-Pernaute et al., (2001)</td>
<td>Human fetal midbrain precursor cells</td>
<td>Striatum of 6-OHDA rat PD model</td>
<td>Behavioral tests and graft examination</td>
<td>High surviving cell rate of neurons and trend in behavior improvement</td>
<td>[145]</td>
</tr>
<tr>
<td>Svendsen et al., (1996)</td>
<td>Human fetal mesencephalon neuronal stem cells</td>
<td>Striatum of DA-depleted rat PD model</td>
<td>Graft examination</td>
<td>Most cells in the graft showed glial lineage</td>
<td>[146]</td>
</tr>
<tr>
<td>Snyder et al., (1997)</td>
<td>Multipotent NPCs</td>
<td>Mouse neocortex with targeted apoptotic neuronal degeneration</td>
<td>Graft examination</td>
<td>Little survival and 15% differentiation of NPC into neurons</td>
<td>[147]</td>
</tr>
<tr>
<td>Lepore et al., (2004)</td>
<td>Rat fetal neuronal and glial lineage-restricted NPCs</td>
<td>Rat adult hippocampus, striatum and spinal cord (intact and injured)</td>
<td>Graft examination</td>
<td>Survival of mature lineage-restricted cells in the graft and integrated into the host brain</td>
<td>[87]</td>
</tr>
<tr>
<td>Ahn et al., (2004)</td>
<td>DA cells derived from neuronal stem cells</td>
<td>Rat DA-denervated striatum.</td>
<td>Graft examination and behavioral tests</td>
<td>No migration to the neuronal degenerative areas and no change in behavior</td>
<td>[148]</td>
</tr>
<tr>
<td>Lepoer et al., (2006)</td>
<td>Rat neuronal and glial lineage restricted NPCs</td>
<td>Rat adult and developing striatum</td>
<td>Graft examination</td>
<td>NPC differentiation and integration into the host brain</td>
<td>[86]</td>
</tr>
<tr>
<td>Jain et al., (2006)</td>
<td>Human expanded neuronal precursor cells</td>
<td>Rat striatum of 6-OHDA OD model prior, at the same time, or after the lesion</td>
<td>Presence of donor cells in SN, frontal cortex and globus pallidus</td>
<td>Although capable of migration, NPCs do not show specific tropism for the sites of DA neuron loss</td>
<td>[82]</td>
</tr>
</tbody>
</table>

**Table 4.** Neural progenitor cell-transplantation studies in Parkinson’s disease animal models. 6-OHDA: 6-hydroxy dopamine; DA: Dopamine; NPC: Neural progenitor cell; PD: Parkinson’s disease; SN: Substantia nigra.
that offer neuroprotection and mediate rescue of specific populations of degenerating neurons in the host brain [91].

**NPCs used in molecule delivery to the diseased brain**

As an alternative to cell replacement, NPCs can be used as delivery vehicles due to their favorable migratory capabilities. There are at least three important factors influencing the migratory behavior of progenitor cells: inflammation, reactive gliosis and angiogenesis [17]. During inflammation, neurotransmitters released by microglia [17] and the release of attractive chemicals by reactive astrocytes both promote neuronal survival and progenitor migration [92]. This also accounts for angiogenesis, where the activated endothelial cells secrete neuronal chemoattractants, such as vascular growth factor [17]. An indication that this process takes place was shown in a study by Jin and colleagues. They provided evidence that neurogenesis was induced by stroke in the human brain [93].

The migration capacity of the NPCs made them a good vehicle for the delivery of small molecules into the brain. NPCs may be manipulated in vitro to express certain molecules by a gene-transfer method by either cDNA transfection or lentivirus or retrovirus transduction techniques [17]. A wide variety of therapeutic genes can be expressed by progenitor cells depending on their later function in vivo. In the case of PD, NPCs have been used to deliver glial cell-derived neurotrophic factor (GDNF). GDNF is a potent neurotrophic factor for SN DAergic neurons, preventing the progression of neuronal loss, maintaining neuronal connections and function, and inducing an additional regenerative response in these neurons [94]. An experiment with the use of GDNF-expressing NPCs showed successful integration and differentiation of these cells and, most importantly, showed a stable GDNF expression for up to 4 months post-transplantation [94]. An improvement of animal behavior indicated that the GDNF NPCs prevented DAergic neuron degeneration in the SN.

NPCs can also be used in AD as delivery vehicles of therapeutic molecules. The character of AD imposes a greater problem with specific molecule delivery, since the damage in the brain is spread over many areas. The use of the homing qualities of NPCs can allow delivery of molecules such as enzymes or antibodies to the amyloid deposition areas [17,95]. These experiments are still ongoing, but the possibility of such therapeutic approaches may have a significant impact.

A new interesting approach of in vivo genetic modification of NPCs by a lentiviral-vector encoding the green fluorescent protein (GFP) as a marker protein was recently explored by Consiglio and colleagues [96]. The lentivirus was injected into the SVZ of mice and labeled the NPCs. As time progressed, the daughter NPCs were expressing the marker protein, showing an efficient long-lived transfer of GFP. This experiment demonstrated the feasibility of a new gene-therapy strategy that creates a continuous source of expression of a therapeutic gene in new-born neurons, by transducing the NPCs in vivo (Figure 4).
Other progenitor cells

Bone marrow adult progenitor cells became an interesting candidate for cell-replacement therapy due to their accessibility. Recently, these cells were shown to generate neurons in the brain of mice in vivo [97]. Although the number of neuronal cells derived from the bone marrow progenitor cells was low, this study showed that tissue-specific stem cells were able to transdifferentiate [6]. Interestingly, this approach also seems to be possible in humans. Cogle and colleagues looked at the brains of three sex-mismatched female bone marrow transplantation patients [98]. At 6 years after the transplantation, they found hippocampal neurons containing a Y chromosome in all transplanted patients. In total, 1% of the neurons found in the female brains contained one Y chromosome and 1–2% of glial cells was made up of male astrocytes and microglia. This study supports the theory that hemopoietic cells can indeed transdifferentiated and demonstrates that this process can take place in human patients, indicating its future impact for therapy [98]. Another approach presents a possibility to differentiate human and mouse bone marrow progenitor cells into neuronal cells in
Schwarz and colleagues transplanted such cells expressing human TH into a rodent model of PD and demonstrated significant functional recovery [100]. Some doubt was put on this hypothesis, where two studies argued that no new neurons were made from these stem cells, but rather they occasionally fuse with host neurons [101,102]. Interestingly, further investigations of female patients who were transplanted with male bone marrow patients showed that the Y chromosome present in neuronal cells in these patients’ brains could not be explained by cell fusion [103,104].

**Gene therapy**

Gene therapy for neurodegenerative diseases involves the delivery of genes encoding protective or restorative molecules into an area of interest in the brain with the aim of preventing neuronal loss or atrophy, or to promote axonal regeneration and synapse formation. Successful gene therapy will either prevent further functional decline or promote the restoration of neuronal function. In current research, the predominant molecules used in this approach are neuronal growth factors. When delivered to areas of neuronal degeneration in the brain, they promote neuronal survival and counteract or reverse atrophy. Compelling evidence from animal studies suggests that neurotrophic factors can potentially halt the progression of neurodegeneration in diseases such as PD and AD, resulting in improved motor and cognitive performance [105–107].

Delivery of neurotrophic factors to the CNS is difficult. Injection in the bloodstream is not optimal since most proteins do not cross the blood–brain barrier and systemic injection of certain growth factors results in strong peripheral side effects. There are three main ways of molecular delivery of large molecules to the brain:

- Use of transplanted cells genetically engineered to secrete the molecule of interest (previously discussed)
- Delivering the protein itself
- Direct transfer of genes using viral vectors [108]

Viral vector-mediated gene delivery allows specific targeting of diseased tissue, high local expression of the substance of interest and low spread of the molecule into other regions of the brain. Viral vector-mediated gene transfer thus allows for long-term and local expression of a foreign gene after a single injection of a viral vector [107–109].

In this section, we will discuss gene delivery in PD and AD models, as well as cover the ongoing clinical trials of neurotrophic factor delivery in these diseases (Table 5).
<table>
<thead>
<tr>
<th>Study</th>
<th>Animal</th>
<th>Gene delivery vehicle</th>
<th>Transplantation site</th>
<th>Assessing technique</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parkinson's disease</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Backlund et al., (1985); Sayles et al., (2004)</td>
<td>Adrenal medulla tissue neuronal transplantation</td>
<td>Transplantation into striatum or caudate nucleus</td>
<td>Increased in L-DOPA ‘on’ phase, disappeared after 18 months, no graft survival</td>
<td>High level of mortality</td>
<td>[6,37]</td>
</tr>
<tr>
<td>Sayles et al., (2004); Freed et al., (2002); Olanow et al., (2003)</td>
<td>Fetal VM transplantation</td>
<td>Transplantation into striatal area</td>
<td>Graft survival and reinnervation. No treatment effects on PD</td>
<td>Development of dyskinesias</td>
<td>[6, 39, 62]</td>
</tr>
<tr>
<td>Mendez et al., (2005)</td>
<td>Fetal midbrain transplantation</td>
<td>Cell suspension transplantation into striatum</td>
<td>Graft survival and reinnervation, minimal inflammation reaction</td>
<td>Unknown</td>
<td>[160]</td>
</tr>
<tr>
<td>Titan Pharmaceuticals, Inc.</td>
<td>Spheramine cell therapy</td>
<td>Stereotactic injection of human post-mortem retinal pigmented epithelial cells producing L-DOPA attached to microcarriers into DA-deficient brain regions</td>
<td>Improvement in motor function 12 months post-treatment, sustained for 24 months</td>
<td>Unknown</td>
<td>[205]</td>
</tr>
<tr>
<td>Kordower et al., (1999); Nutt et al., (2003); Dass et al., (2006)</td>
<td>GDNF protein injection</td>
<td>Intraventricular protein injection</td>
<td>No improvements</td>
<td>Nausea, vomiting, confusion, hallucinations and dyskinesias</td>
<td>[107, 111, 112]</td>
</tr>
<tr>
<td>Gill et al., (2003); Slevin et al., (2003); Dass et al., (2006)</td>
<td>GDNF protein injection</td>
<td>Chronic infusion of protein into postcommissural putamen with the use of a pump</td>
<td>Functional improvement, 1-year increase of DA storage in putamen, reduced dyskinesias</td>
<td>No side effects</td>
<td>[107, 113, 114]</td>
</tr>
<tr>
<td>Dass et al., (2006); Amgen biotechnology</td>
<td>GDNF protein injection</td>
<td>Double-blind study with intraputameanal injection</td>
<td>Study prematurely terminated- no difference in placebo group</td>
<td>Immunological reaction noted in 10% of the patients</td>
<td>[107]</td>
</tr>
<tr>
<td>Dass et al., (2006); Ceregene, Neurologix and Avigen, Inc.</td>
<td>AAV-NTN, AAV-GAD and AAV-AADC gene delivery</td>
<td>Striatum injection, subthalamical injection and second striatum injection, respectively</td>
<td>AAV-NTN trial shows good tolerance to the vector and reduction of disease symptoms by 40%</td>
<td>No side effects reported</td>
<td>[107]</td>
</tr>
<tr>
<td><strong>Alzheimer's disease</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Eriksdotter et al., (1998)</td>
<td>NGF protein injection</td>
<td>Intracerebroventricular protein injection</td>
<td>Slight improvement in cognitive function</td>
<td>Dull constant back pain and weight reduction</td>
<td>[120]</td>
</tr>
<tr>
<td>Tuszniski et al., (2005)</td>
<td>Ex vitro NGF expression</td>
<td>Injection of NGF-expressing fibroblasts to the cholinergic basal forebrain</td>
<td>NGF-induced trophic effect, cholinergic connections are reconnected and cognitive impairment slowed down</td>
<td>No side effects reported</td>
<td>[7]</td>
</tr>
</tbody>
</table>
**Parkinson’s disease**

In PD, gene therapy has the potential to deliver neurotrophic factors to the anatomical areas of the brain that are affected: the SN, putamen and striatum. GDNF was demonstrated to prevent degeneration of DAergic neurons and, out of all growth-promoting factors, it is so far the most consistent in its action [110]. Because of these attributes, GDNF is the most researched growth factor in relation to PD and it was the first to be used in clinical trials. Two methods of GDNF administration have been used: either direct administration of the protein or delivery of the GDNF gene.

**GDNF protein delivery**

After demonstrating the potency of GDNF in reversing degeneration of DA neurons in animal models, the first clinical trials were endorsed [111,112]. GDNF protein was intraventricularly injected into PD patients, but failed to produce improvements in disease symptoms. This may be due to a failure to deliver GDNF to the site of pathology, such that no nigral neurons were reached by the factor. This procedure caused serious side effects, including nausea, anorexia, vomiting, confusion, hallucinations, paraesthesias, headaches and dyskinesias, indicating that the protein reached unwanted peripheral targets. The diffusion of GDNF in the brain parenchyma was very poor, with little GDNF passing the ventricular ependyma and, consequently, no GDNF reached the nigral neurons [107]. These studies showed that it is not only the neurotrophic activity of the growth factor that matters, but that the site and method of delivery are essential for the growth factor treatment to be successful.

Two other open-label clinical trials were initiated where GDNF was chronically infused using pumps into the postcommissural putamen (an area of the brain that displays the greatest DA loss and directly connects to the motor cortex [107]) of PD patients [113,114]. Both of these trials showed functional improvement with a long-term (1 year) increase of DA storage in the putamen and a corresponding improvement in the processing of motor output [113]. Additionally, dyskinesia scores were significantly reduced in both studies. No side effects were noted, showing that site-specific direct GDNF treatment may be safe and beneficial.

To further explore the impact of direct GDNF protein delivery, in 2004, Amgen biotechnology [201] performed a double-blind, placebo-controlled study where GDNF was injected into the putamen of 34 PD patients [107]. The study was prematurely terminated after 6 months due to lack of clinical improvements.

**Table 5.** (Previous page). Clinical trials in Parkinson’s disease and Alzheimer’s disease patients. AADC: Aromatic L-amino acid decarboxylase; AV: A deno-associa ted virus; GAD: Glut amic acid decarboxylase; GDNF: Glial cell line-derived neurotrophic factor; L-DOPA: Levodopa; NGF: Nerve growth factor; NTN: Neurturin; PD: Parkinson’s disease; VM: Ventral mesencephalic.
in the group treated with GDNF compared with the placebo-controlled group. Additionally, an immunological reaction against GDNF was noted in 10% of patients. Parallel results from monkey studies showed irreversible brain damage in the animals treated with high-dose GDNF. Fortunately, this was not noted in patients, presumably because the dose of GDNF was much lower [107].

A complicating factor of GDNF protein infusion is the fact that intraparenchymal protein diffusion may be uncontrollably affecting other cell types in surrounding brain areas. Although there are no eminent side effects in PD patients treated for a maximum of 1 year, these issues may lead to problems following long-term treatment regiments [107].

**GDNF gene delivery**

Compared with protein delivery, gene delivery offers several advantages. It is technically more practical, since it does not require permanent intraparenchymal catheter implantation and refills of the minipumps. Additionally, a number of carefully spaced small stereotactic injections of a viral vector carrying a transgene in a defined brain region ensure the continuous and more targeted local production of a therapeutic transgene (Figure 4).

GDNF gene delivery to the brain was investigated in several ways in animal models for PD (Table 6). The main issues addressed in these experiments were the identification of the best viral vector and finding the most optimal site for delivery of the transgene in order to create the best conditions for neuronal protection and/or neuronal restoration by GDNF.

Some experiments were designed in such a way that viral GDNF was injected into the animal before the administration of the neurotoxin, to show neuron-protective characteristics of this growth factor. The site of the injection plays a crucial role in the successful rescue of the DA neurons. The striatum is suggested to be the optimal site of viral GDNF injection and this will most likely be the area that will be genetically modified in future clinical trials [107]. Additionally, adeno-associated virus (AAV) vectors were found to be the most efficient and safe gene-delivery vehicles. Although prelesion viral vector-mediated expression of GDNF results in neuronal protection, these observations cannot be directly translated to a treatment for PD since the vector encoding GDNF would have to be injected after the initiation of neuronal degeneration. Therefore, other studies examined the effectiveness of GDNF to rescue injured DA neurons after induction of the lesion and showed that GDNF could indeed improve motor behavior and decrease cell death (Table 6).

**Table 6.** Rodent and primate GDNF gene-delivery trials in Parkinson’s disease animal models. 6-OHDA: 6-hydroxy dopamine; AAV: Adeno-associated virus; A d: Adenovirus; DA: Dopamine; GDNF: Glial cell line-derived neurotrophic factor; HSV: Herpes simplex virus; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD: Parkinson’s disease; SN: Substantia nigra; TH: Tyrosine hydroxylase.
<table>
<thead>
<tr>
<th>Study</th>
<th>Animal</th>
<th>Gene delivery vehicle</th>
<th>Transplantation site</th>
<th>Assessing technique</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kirik et al., (2000)</td>
<td>6-OHDA animal model</td>
<td>Ad-GDNF</td>
<td>Intrastratial, intranigral and intraventricular</td>
<td>Cell death, TH+ cell count and behaviour tests</td>
<td>Only intrastratal injection could preserve TH+ neurons</td>
<td>[149]</td>
</tr>
<tr>
<td>Choi-Lundberg et al., (2000)</td>
<td>6-OHDA animal model</td>
<td>Ad-GDNF</td>
<td>Intrastratal</td>
<td>Cell survival and behaviour tests</td>
<td>40% of DA neurons rescued, behavior improvement and severe inflammatory response</td>
<td>[150]</td>
</tr>
<tr>
<td>Natsume et al., (2001)</td>
<td>6-OHDA animal model</td>
<td>HSV-GDNF and HSV-Blc-2</td>
<td>Intranigral</td>
<td>Cell death and TH+ cell count</td>
<td>50% cell survival and strong inflammatory response</td>
<td>[151]</td>
</tr>
<tr>
<td>Kirik et al., (2000)</td>
<td>6-OHDA animal model</td>
<td>AAV-GDNF</td>
<td>Intrastratal</td>
<td>Cell death, TH level and behaviour tests</td>
<td>High DA neuronal protection, striatal reinervation and behavior improvement</td>
<td>[152]</td>
</tr>
<tr>
<td>Georgievska et al., 2002</td>
<td>6-OHDA animal model</td>
<td>Lentivirus-GDNF</td>
<td>Intrastratal</td>
<td>Cell survival</td>
<td>65-77% cell survival, neuronal reinnervation and minimal inflammation reaction</td>
<td>[153]</td>
</tr>
<tr>
<td>Kojima et al., 1997</td>
<td>MPTP animal model</td>
<td>AAV-GDNF</td>
<td>Intrastratal</td>
<td>DA levels</td>
<td>Higher DA levels</td>
<td>[154]</td>
</tr>
<tr>
<td>Eslamboli et al., (2003)</td>
<td>6-OHDA primate animal model</td>
<td>AAV-GDNF</td>
<td>Intrastratal and intranigral</td>
<td>Cell death and behavior studies</td>
<td>Model cell protection and protection of motor function</td>
<td>[155]</td>
</tr>
<tr>
<td></td>
<td>After lesion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wang et al., (2002)</td>
<td>6-OHDA rat</td>
<td>AAV-GDNF delivery 4 weeks post lesion</td>
<td>Intrastratal</td>
<td>Cell death, TH+ cell count and behavior tests</td>
<td>SN cell death diminished, motor recovery</td>
<td>[157]</td>
</tr>
<tr>
<td>Kordower et al., (2000)</td>
<td>MPTP young primate PD model</td>
<td>Lenti-GDNF 1 week post-lesion</td>
<td>Intracaudate, intraputamen and intranigral injections</td>
<td>PET imaging, behaviour tests and TH+ cell count</td>
<td>Reduction of motor disability, 300% increase in striatal activity and TH+ neurons present</td>
<td>[158]</td>
</tr>
<tr>
<td>Palfi et al., (2002)</td>
<td>MPTP aged primate PD model</td>
<td>Lenti-GDNF 1 week post lesion</td>
<td>Intrastratal</td>
<td>TH+ cell count</td>
<td>7-fold increase of striatal TH+ neurons</td>
<td>[159]</td>
</tr>
</tbody>
</table>

**Study** and **Gene delivery vehicle** refer to the study and gene delivery vehicle used in the experiments. **Transplantation site** indicates the site where the transplantation was performed. **Assessing technique** describes the methods used to assess the results. **Results** summarize the outcomes of the experiments. **Ref.** provides the reference number for each study.
Other gene-therapy approaches in PD treatment

The trophic factor neurturin (NTN), a member of the GDNF family, was identified in 1996 and is an alternative candidate to GDNF gene therapy. Research on AAV–NTN proved that this molecule can provide structural and functional protection of nigrostriatal neurons [107]. These results formed the basis of a Phase I clinical trial by Ceregene with AAV–NTN delivery into the striatum of 12 advanced PD patients. Recent initial results showed good tolerance for the viral vector and reduction of the disease symptoms by approximately 40%. The company is preparing for a Phase II clinical trial [202]. Two other clinical trials have also begun using either AAV-glutamic acid decarboxylase (GAD; an enzyme that catalyzes the synthesis of the GABA neurotransmitter) gene delivery to the subthalamic nucleus of twelve patients [203] and AAV aromatic L-amino acid decarboxylase (AADC; an enzyme that converts L-DOPA into DA) delivery to the striatum [107,204]. No results are reported yet. AAV–GAD injected into primate PD models was well tolerated by the animals and proved to have potential therapeutic characteristics [115].

Alzheimer’s disease

Gene therapy is also used as a therapeutic experimental approach for AD. The widespread neuronal and synaptic loss throughout the brain creates a challenge to target the degeneration of neurons [34]. Thus, again, as in the cell-replacement strategies, AD is very difficult to tackle with gene therapy due to its extended brain pathology. However, it is possible to target selected areas of neuronal degeneration, such as the nucleus basalis of Meynert, with neurotrophic factors that promote cell type-specific neuronal restoration. The cholinergic neurons in this area are known to degenerate in AD patients (Figure 2) and this finding has been corroborated by animal studies. A good candidate for neurotrophic gene therapy is nerve growth factor (NGF), since it has been shown to be a trophic factor preventing both lesion-induced and agerelated atrophy of basal forebrain cholinergic (BFC) neurons in rodents and nonhuman primates [21,116,117]. Furthermore, it was shown that, in the Ts65Dn mouse, a model for Down’s syndrome, BFC neurons degenerate and that these mice had a deficit in NGF retrograde transport [118]. Down’s syndrome patients suffer from AD pathology, and the T65Dn can therefore also be a model for certain aspects of AD. Also, aged AD11 mice expressing NGF antibodies showed a dramatic neurodegenerative phenotype similar to AD with both β-amyloid depositions and tau intracellular accumulation and degeneration of BFC cholinergic neurons [119]. These observations imply that NGF is an essential factor for the maintenance of the cholinergic system.
**NGF protein delivery**

The first clinical trial using NGF as a therapeutic molecule was performed with three AD patients, where the NGF protein was administered into the ventricles. Due to side effects, such as strong constant back pain and loss of weight, in all three patients, the trial was suspended [120]. The intranasal administration of NGF protein has been studied as an alternative to NGF delivery in the brain ventricles [121]. De Rosa and colleagues used an AD11 mouse model and showed that the intranasal route of administration of NGF did result in an improvement of the cognitive deficit [121]. A potential problem associated with this method of delivery is the nonspecific targeting. We know from both AD and PD research that the site of growth factor application is crucial for the success of the treatment as well as for the avoidance of side effects. NGF is known to cause pain, and its intranasal application may lead to the diffusion of NGF to unwanted sites.

**NGF gene delivery**

A first Phase I clinical trial with NGF gene therapy in AD has been completed [7]. In this trial, eight patients were treated using an ‘ex vivo’ gene therapy strategy. Fibroblasts of the patients were genetically modified to express NGF and were grafted into the nucleus basalis of Meynert. Studies using rodent and primate models have demonstrated the feasibility, effectiveness and safety of this approach [21,116,122,123].

In this clinical trial, three potentially beneficial effects of NGF gene therapy in AD were observed. First, PET studies showed a widespread increase in glucose uptake by cortical neurons after 6–8 months. This suggests that the cholinergic projections to the cortex are re-activated by NGF. Second, a decrease in disease progression was presented based on cognition assessment with the use of the Mini-Mental State Examination scores. As this study progresses, the reduction in the rate of cognitive decline is hoped to become even more significant. Finally, NGF-induced ‘trophic’ effects in the brain, including robust cholinergic axonal sprouting around and into the site of the NGF expression, were noted. The authors realized that, although this is a small group and an open-label study with no placebo control, these results are very encouraging [7]. No negative effects of NGF administration (weight loss or pain) were noted in the AD patients, even up to 2 years post surgery. Direct AAV-mediated expression of NGF is the next step to determine the safety and effectiveness of gene therapy for AD [202].

Concern and criticism towards using NGF as a therapeutic molecule was presented, after it was shown that β-amyloid precursor protein may be induced by NGF [124]. In this scenario, rather than reducing neuronal degeneration, NGF would accelerate the pathology of AD. To test this hypothesis, Tuszynski and colleagues designed an experiment where NGF was ex vivo delivered in the parenchym of an aged primate brain [21]. This study not only found no increase of β-amyloid plaque deposition in NGF-treated monkeys, but also showed a reduc-
tion of age-related atrophy of cholinergic neurons by NGF delivery into the basal forebrain.

Questions remain on the safety of long-term constant NGF expression in the human brain. Since the genetically modified fibroblasts have been implanted into the brains of the patients, there is no feasible way to control the NGF expression if side effects would occur. Also, following injection of AAV–NGF into the brain, NGF will be expressed constitutively, leading to constant release of NGF from the transduced neural cells. This issue could be resolved by the use of a regulatable viral vector [109,125]. The problem is that the tetracycline (Tet) repressor-protein that controls the transgene expression is a foreign protein that elicits an immunological response [126]. In the future, this problem could potentially be solved by using modified Tet repressor-proteins that are less immunogenic.

Conclusion & future perspective

The field of cell replacement and gene therapy as a new therapy for PD and AD has evolved enormously over the last years. Currently, no cure is available for these diseases. The application of embryonic and neural stem cells has opened new avenues to treat PD and AD, as well as the feasibility of delivering and expressing genes encoding neurotrophic factors in the human brain, which can be regarded as a breakthrough. However, none of the cell-replacement and gene-therapy strategies reported so far result in complete rescuing or restoration and functional repair of the damaged brain areas. Targeting molecular mechanisms with current genome-wide genotyping of large populations of patients [127], large-scale gene expression profiling [128] and proteomics studies [129] will pinpoint novel molecular pathways involved in the process of degeneration in PD and AD. Furthermore, new developments, such as the identification of noncoding microRNAs [130,131], may have an impact on our understanding of the maturation of neuronal progenitor cells, recruitment of these cells for neural repair and promotion of neuronal regeneration. Our increasing knowledge on the molecular mechanisms underlying PD and AD will enable the development of novel cell- and gene-based therapies. Each of these strategies, or perhaps a combination of the two, could result in genuine repair and rescue of degenerating DAergic and cholinergic neurons in the future, thereby ideally bringing not only symptomatic relief but also stopping the progression of these neurodegenerative diseases (Figure 4).
Executive summary

- The early neuron-replacement therapies did relieve some of the symptoms of Parkinson's disease (PD) in several of the patients, but did not cure the disease.
- Animal models for PD and Alzheimer's disease (AD) only partially mimic the disease, therefore better animal models covering the full clinical spectrum of PD and AD are required.
- The first clinical trial transplanting genetically engineered cells that secrete nerve growth factor has advanced the field of genuine restorative treatments for neurodegenerative diseases enormously.
- Novel direct gene-therapy approaches in AD patients have the potential to again bring the field a step forward.
- The discovery of adult human neuronal stem cells revealed a new and important population of cells that can be stimulated and targeted to treat PD and AD in the future.
- Advances in molecular analyses, such as population genotyping and genome-wide expression studies, will contribute to our understanding of the pathogenesis of PD and AD.

Acknowledgements

The authors would like to thank Prof. Dr DF Swaab (NIN, Amsterdam) for providing us with a picture of the NBM in Figure 2 and we are grateful to the Netherlands Brain Bank, (NIN, Amsterdam) for providing the human post-mortem brain material and for the neuropathological stainings.
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