

Treating Neurodegenerative Disorders: Towards Detailed Pathophysiology and Precision Medicine

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Abstract – The following paper reviews current literature on disease etiology, pathophysiology, and therapeutic options of neurodegenerative disorder (NDs). We first discuss current directions of disease management and treatment, and highlight technical hindrances of current gene therapy-based treatments as well as significant ethical, regulatory, and efficacy-based concerns present in current stem cell treatment options for Alzheimer's, Parkinson's and Huntington's Disease. We then suggest a narrower focus for future research on identifying molecular, pathological, and biomarker-based disease progression that can in turn inform the development of novel approaches in developing ND therapies.

INTRODUCTION

Neurodegenerative diseases (NDs) encompass a wide range of acute and chronic conditions characterised by the loss of neuron and glial cells in the brain or spinal cord. In acute conditions, due to stroke or spinal cord injury, different types of neurons within a restricted brain region die over a short amount of time. In chronic conditions, there is either a selective neuronal loss of specific sub-populations (such as dopamine neurons in Parkinson's or medium spiny neuron loss in Huntington's disease), or widespread degradation of many neuronal types over several years. For both conditions, no cures currently exist. Current clinical approaches adopted can only target symptomatic treatment and disease management, while clinical applications of novel therapies developed for NDs have been limited.

This paper reviews the current literature on both implemented and developing therapies for Alzheimer's (AD), Parkinson's (PD), and Huntington's (HD) disease, three of the most common and debilitating chronic NDs. The symptoms, affected brain regions, and some molecular changes have been identified for several NDs, some of which are summarized in Table 1¹. Novel therapies have been developed in animal models to target these observed pathological and molecular changes, but significant ethical, economic, regulatory, safety, and etiological weaknesses still hinder their widespread clinical application.

Within the context of the two main developing therapies examined, gene and stem cell therapy, specific focuses for research are suggested. Major milestones in research that must

be reached before clinical application begin with better understandings of disease initiation and progression. Molecular pathological classification through the identification of biomarkers can provide the greatest cross-methodological benefits in furthering effective and personalized treatment of NDs. Fundamentally, a more thorough understanding of underlying ND pathophysiology can be accomplished through biomarker identification, stem cell-based disease modelling, and genetic research. Combined, this knowledge will help develop more personalized and ultimately more effective novel therapies for treating neurodevelopmental disorders.

PART 1: CURRENT APPROACHES: DISEASE MANAGEMENT

Current clinical approaches to addressing AD, PD, and HD all center on disease management and symptom alleviation. The use of pharmacological agents, physical therapy, and caregiving aid is efficient and affordable, but are ultimately only forms of palliative care rather than effective treatments.

Four acetylcholinesterase inhibitors (donepezil, galantamine, rivastigmine, tacrine) and one NMDA receptor antagonist (memantine) constitute the five main medications currently used to provide temporary symptomatic relief to AD patients (Desai & Grossberg, 2005). The first four enhance cholinergic function by reducing rates of acetylcholine breakdown (as reduced ACh levels is related to memory and cognitive decline in AD), while memantine reduces AD-related excitotoxicity (observed in AD & related to neuronal death). Further behavioural, psychosocial, and caregiving provisions are used to improve quality of life for AD patients.

Current therapies for PD are arguably the most well-developed and effective among these three discussed NDs. Drugs such as levodopa or dopamine agonists used in earlier stages of care can restore some motor function, but their efficacy decreases dramatically over time. Deep brain stimulation (DBS) and rehabilitation techniques provide further symptomatic therapies for PD (Mizuno, 2014).

Treatments for HD, like those for AD and PD, are only able to reduce symptom severity. Various pharmacological treatments are available to reduce chorea. As the disease progresses, physical, speech, and language therapies are used to rehabilitate physical and cognitive function (Busse et al., 2012).

¹ Table 1 based on: <http://www.nhs.uk/Conditions/>
<http://www.neural.org.uk/store/assets/files/20/original/NeuroNumbers.pdf>;

<https://www.ncbi.nlm.nih.gov/books/NBK1352/>;
<https://neurodiscovery.harvard.edu/challenge>

Gaps in Current Treatment Techniques

Techniques currently available to treat neurodegenerative diseases are able to provide temporary symptomatic relief, but are critically unable to halt the rapid deterioration and progression of neurodegeneration.

Though the complete causes of AD and PD have not been fully characterised, neurofibrillary tangles, α -amyloid plaques, decreased synaptic function, and cortical atrophy observed in AD and the loss of dopaminergic neurons characteristic of PD can be addressed by gene therapy and stem cell-based therapies. These two therapies can also be applied to treat the root causes of HD, which has been attributed to an expanded trinucleotide repeats, the mHTT protein, and consequent neostriatal neuronal loss. However, their widespread clinical application has been limited by underdeveloped understandings of disease etiology and mechanisms of therapeutic action. Insights into the molecular mechanisms of NDs lie at the crux for the development of novel targets of disease-modifying therapies. Therefore, efforts to further gene and stem cell-based therapies (discussed in Parts 2 & 3) will benefit from an increased focus on biomarker research (discussed in Part 4) to elucidate disease pathophysiology.

PART 2: GENE THERAPY²

The theory behind the use of gene therapy to treat neurodegenerative disease is simple: deliver a transgene that either corrects or replaces a defective gene to cure a complex disease using a transfer vector. However, the numerous technical difficulties associated with gene therapy are daunting in complexity: the transfer vector must be easy to produce, safe for patients and the environment, target specific cells efficiently, and not trigger a deleterious immune reaction; simultaneously, the appropriate transduced transgene must be expressed at the optimal level for an optimal duration (Sarkis & Mallet, 2005). All these factors that must be taken into consideration can only be optimized with increased knowledge of disease etiology, which can be mediated through biomarker research.

Gene Therapy Trials

Gene therapy vectors are either viral or non-viral. Currently, the most common viral vectors are adeno-associated viruses (AAVs) and lentiviruses. Both infect non-dividing and dividing cells, but lentiviral vectors integrate into host genomes while AAVs do not. Thus, lentivirus integration can confer long-term, stable transgene expression, but poses a risk of integrational mutagenesis. Non-viral vectors (mostly used for HD treatment) have more localized effects, require higher

dosage levels, and confer only transient gene expression that is normally insufficient to fully treat chronic ND.

Viral Techniques

A series of viral vector-mediated gene therapy trials has been conducted for AD and PD, summarized in the table below. In these trials, AAV2-mediated transgenes were injected into patients' brains, but treatment results either fail to meet or achieve the same level of therapeutic benefit as current standards of care. It is important to emphasize that the efficacy of gene therapy depends critically on the therapeutic gene delivered; this can only be improved by better understandings of the etiology of neurodegenerative diseases.

Non-Viral Techniques

Huntington's is a genetically acquired disease with a known etiology (CAG repeats in the ITI5 huntington gene), for which non-viral gene therapy techniques carry significant therapeutic potential. Carroll et al. (2011) have developed ASOs that selectively bind to exonic and intronic SNP (single nucleotide polymorphisms) sites on the mutant huntingtin protein (mHTT), thus selectively silencing gene expression of mutated regions on the mHTT. The experiment used primary cells from HD-patients with transgenic mouse lines, and the developed ASOs achieved potent and selective allele-specific knockdown of mHTT both in vitro and in vivo.

This presents the most exciting potential treatment for Huntington's, as ASO delivery to adult CNS can be achieved through simple intrathecal or intracerebroventricular infusion rather than viral delivery. Further, ASO dosing can be precisely controlled or stopped when necessary. Kordasiewicz et al. (2012) have shown that transient infusion of ASOs catalysing RNase H-mediated degradation of mHTT in mouse and non-human primate models delays disease progression and sustains HD disease reversal that outlasts ASO-mediated mHTT knockdown. Thus, clinical trials should be conducted to test the efficacy of gene therapy either through direct ASO infusion, or combined with iPSC-mediated transplantation (correcting disease mutations of patient-derived iPSCs with ASOs, then transplanting corrected NPCs).

Low Efficacy in Gene Therapies

Promising proof-of-principle concepts have been presented in Phase I trials, which have shown that gene therapy techniques targeting AD & PD are safe and well-tolerated. However, therapeutic efficacy in further clinical trials remains weak, in that observed improvements failed to exceed placebo effect or current standards of care for AD and

² This section is based on readings from O'Connor & Boulis (2015), as accredited in *Works Cited*

PD. Non-viral ASO-mediated silencing of mHTT expression has proved efficacious in HD-animal models but have not yet moved to clinical trials. In sum, success by design meets failure of efficacy within these gene therapy studies.

Moving forward, future research must focus first on expanding choices of possible transgenes for delivery, as improvements in vectors and delivery methods depend crucially on efficacy of the transgene delivered. Greater understanding in disease etiology will help identify these gene targets. A significant area of development lies in the identification of biomarkers of NDs, which would not only improve gene therapy techniques, but also allow for earlier diagnoses, continuous monitoring of the progression of therapeutic effects, and the development of precision medicine.

PART 3: STEM CELL THERAPY³

Stem cell and iPSC research has significantly furthered neurodegenerative disease modelling, diagnosis, and transplant- and drug-based therapies. A plethora of therapies developed in animal models have failed to translate into effective clinical trials due to differences in mammalian genomes and embryonic development (Begley & Ellis, 2012). Human-based stem cell research generates not only potential ND therapies, but also presents clearer pathophysiological models for those diseases. In particular, the development of induced pluripotent stem (iPS) cells has presented a unique approach for studying signalling pathways, growth control, and disease mechanisms in previously inaccessible human brain tissue.

The main types of stem cells used for neurodegenerative therapies are embryonic, mesenchymal, progenitor, and iPS cells (Singh et al., 2016). Embryonic stem cells are pluripotent and hold excellent potential to restore damage caused by brain injury or neurodegeneration; however, their ability for unlimited self-renewal poses a high risk for tumour formation post-engraftment, limiting the range of their clinical application. Mesenchymal stem cells are multipotent and immunomodulating (derived from autologous source; do not trigger a host immune response), and thus find a relatively widespread application for neurodegenerative treatment. However, their use in genetic diseases, wherein autologous sources contain the same genetic predisposition to the disease, is limited. Neural progenitor cells (NPC) and neural stem cells (NSC) are derived from either fetal or adult neural tissue, carry limited differentiation and tumour-formation potentials, but are difficult to isolate and thus lower in availability. iPSCs, generated from somatic

tissue and reprogrammed using TFs into embryonic stem cell-like states, offer novel sources of autologous cellular therapy.

Application of the appropriately selected type of stem cell to treat neurodegenerative diseases is mediated through two primary mechanisms. First, stem cells can replace or stabilize neuronal networks that experience both widespread or specific subpopulation-based neuronal loss. Second, stem cells can provide environmental enrichment through neurotrophic support to prevent further neuronal degradation. These mechanisms will be discussed in the context of specific neurodegenerative diseases below. Following this discussion, I'll emphasize that the focus of stem cell research should first lie in exploiting its means of modelling disease pathophysiology and providing an in vitro screening tool for screening efficacy and safety of drugs, then on its direct therapeutic applications. I'll then highlight the potential iPSC research holds in future developments of precision medicine.

Alzheimer's

Complementing current pharmacological AD treatments that regulate neurotransmitter activity (Section 1.1), stem cell (SC)-based therapies for AD can also help restore degeneration and loss of cholinergic neurons, and increase levels of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) that are lowered in AD. In mouse models, combinatorial upregulation of BDNF with NPC transplants promoted neurogenesis and protected neuronal function. Thus, NPC-mediated stem cell-based therapy may offer an enhanced approach to preventing AD-associated degradation of cholinergic synapses and neurons (Tuszynski, 2007).

Wang et al. (2006) compared transplants of ESC-derived NSC to transplants of ESC alone into mouse AD models. As suggested in Section 3.0, ESC-transplanted mice developed teratomas (ESCs: unlimited self-renewal capabilities, high risk of tumour formation), while transplanted NSCs successfully differentiated into stable cholinergic neurons, improving memory and learning. Genetically modified NSCs for BDNF expression has been shown in many rodent studies to reduce AD symptoms (Lindvall & Kokaia, 2010). In these mouse models, increased hippocampal synaptic density, increased cognitive function, and enhanced cell-cell synaptic communication, as associated with increased BDNF expression (Blurton-Jones et al., 2009; Li et al., 2009; Xuan et al., 2008). NGF has also been implicated in preventing neuro-degeneration and reducing amyloid toxicity

³ This section is based on readings from Singh et al. (2016), Kumar et al. (2016), Lunni et al. (2011), Goldman (2005), and Chmielnicki & Goldman (2005), accredited in *Works Cited*

(Tuszynski et al., 2007). In addition to AAV2-mediated gene therapy techniques discussed in Section 2.1.1, autologous fibroblasts genetically modified to express NGF implanted into patient forebrains have been found to slow rates of cognitive decline in an open-label phase 1 clinical trial (Tuszynski et al., 2005). However, fibroblasts are immobile and cannot slow degeneration across large brain regions. Transplanted stem cells can efficiently migrate and release growth factors to many damaged sites, but their use in AD treatment has yet to be fully explored. Another technique to increase NGF release to treat AD is with encapsulated cell biodelivery (ECB) implants, for which the first clinical trial was conducted in 2008 by the Danish company NsGene. Encapsulated retinal pigment epithelial cells releasing NGF were implanted into the basal forebrains of six AD patients (Wahlberg et al., 2012). At 12 months, implants were retrieved and low but persistent NGF secretion was detected in half of the subjects. Thus, both ECB implantation and stem cell-based gene therapy may provide future methods for enhancing NGF expression to counteract cholinergic neuronal death.

Although NSC transplantations into the adult forebrain of patients may be able to increase cholinergic and cognitive aptitude, they are unable to target the main molecular cause of the disease: accumulation of β -amyloid plaques (Blurton-Jones et al., 2009). Nonetheless, the ability of NSC transplants to enhance cholinergic function, synaptic transmission, and neuronal function is suggested by animal studies and replicated by initial clinical trials (Tuszynski, 2007). Combining growth factor overexpression with NPCs' ability to integrate across many neural networks thus presents an exciting potential for limiting AD pathogenesis. Continued research must be conducted before established SC-based treatments can be developed for AD therapy.

Parkinson's

Earlier open-label studies of human fetal ventral mesencephalic tissue (hfVM) transplants in Parkinson's patients yielded successful long-term PD symptomatic relief, but these results failed to replicate in subsequent placebo-controlled, double-blind trials (Brundin et al., 2010). Success has been observed in using genetically modified ESCs and iPSCs to derive neurons with the dopaminergic (DA) phenotype, but these techniques have not yet been translated into clinical studies.

Clinical trials using hfVM tissue transplants in the 1990s (Madrazo et al., 1988; Lindvall et al., 1990) found impressive restoration of dopaminergic neurotransmission and motor-symptom alleviation. In these open-label clinical studies, PET scans showed long-lasting evidence of functionally-integrated dopaminergic neurons forming

from transplanted tissue, restored DA release in vivo, and reactivation of cortical motor areas. However, the design of these open-label trials contains significant bias and thus deserve cautious interpretation. Further, the occurrence of recurring graft-induced dyskinesias (GIDs) in consequent double-blind trials, in addition to heavy ethical concerns raised over the use of fetal tissue as a therapeutic source, has heavily impeded the clinical development of this form of hfVM-based therapy (Brundin et al., 2010). Instead, the focus has been shifted to ESCs and iPSCs: mouse model studies have found that overexpression of Nurr1, FGF-8, and Shh signalling in mouse ESCs and iPSCs has successfully induced midbrain dopaminergic phenotypes and improved functional recovery (Kim et al., 2003; Cooper et al., 2010). A major concern for ESC clinical transplants, however, is the risk for tumour formation observed in animal models (Lindvall & Kokaia, 2010). As PD patients have normal life expectancies, even a slim risk of tumour formation is unacceptable.

Initial suggestions of hfVM transplant-mediated PD therapy have been significantly hindered by ethical and methodological issues, while the induction of ESCs and iPSCs into dopaminergic neurons is cumbersome, potentially nonhomogeneous (may contain traces of undifferentiated cells that can lead to teratomas), and currently unsubstantiated by clinical studies. Thus, progress made in stem cell-based PD treatments have been slower than for AD. However, iPSC-derived dopaminergic neurons have provided critical insights into the pathophysiology of PD, by providing a SC-based human disease model. Abnormalities in mitochondrial and dopamine homeostasis, as well as mutations in Parkin and PTEN-induced putative kinase 1 (PINK1) previously noted in animal models have been substantiated by similar data from studies using PD patient-derived iPSC neurons (Van Laar et al., 2010; Cai et al., 2012).

Though stem cell technology has yet to develop clinically-relevant PD therapies, it has proved effective in modelling Parkinson's. In particular, mutation-bearing iPSCs has helped study PD physiology, elucidate underlying signalling cascades modulating the disease, identify PD biochemical markers, and screen efficiency of therapeutic compounds.

Huntington's

Applications of stem cell techniques in HD studies have paralleled those for AD and PD. More specifically, fetal striatal grafting into HD patients have provided transient symptom improvements, but its mechanisms are poorly understood and its use is limited by ethical constraints (Bachoud-Lévi et al., 2000). Both iPSCs and ESCs have been

used to establish differentiation protocols for medium spiny neurons (MSN)— these transplantations have shown successful neural circuit development and integration, but are currently restricted to animal models (Viegas et al., 2011; Precious & Rosser, 2012).

Initial clinical trials using fetal-derived tissue, iPSCs, or ESCs as sources of MSNs have observed reduced motor and cognitive dysfunction in HD patients. In addition, decreased neuronal loss was observed in HD rodents with NPC transplants engineered to overexpress GDNF, as compared to control mice with unmodified NSC transplants, emphasizing the potential of stem cell-based therapies to provide environmental enrichment and protect endogenous neurons (Pineda, 2007).

For AD, PD, and HD, similar trends in stem cell-based therapies can be observed. Early cell-based therapies using fetal ventral mesencephalic tissue (for PD) and fetal striatal tissue (for HD) transplants provided initial support for successful symptomatic relief. However, widespread clinical applications of human fetal tissue transplants has been limited by significant ethical and scarcity issues associated with the nature of the tissue source. Subsequent clinical trials have failed to consistently replicate initial data and observed deleterious side effects (teratoma formation, GIDs, immunorejection). Thus, the focus of stem cell therapies has shifted to ESCs derived NSCs induced to certain cell fates or gene expressions (increased BDNF/NGF expression for AD, dopaminergic phenotypes for PD, and MSN for HD); however, these induced populations are usually not pure, long-term graft survival is generally low, and any ES cells escaping in vitro differentiation carries the potential or yielding teratomas and tumours following transplantation.

Above all, the development of effective stem cell-based therapies is ultimately hindered by our current lack of knowledge on the mechanisms of action of transplanted stem cells, and how stem cell proliferation, differentiation, survival, and migration are controlled in a pathological environment. The mechanisms of cellular protection provided by SC-mediated trophic support are also not fully understood (McBride et al., 2004). Because current potential risks outweigh validated benefits in their therapeutic applications, research on human stem cells and patient-derived iPSCs should first be focused on disease modelling and used for screening drugs and other therapeutics. Successful development of clinical therapies can begin once more precise disease pathophysiology and mechanisms of SC action post-transplantation are identified.

In sum, the use of stem cells for transplantation has generated enormous ethical, technical, efficacy, and safety-related debates. It is true that SC replacement therapy can

stabilize and regenerate entire networks of neuronal loss and prevent further neurodegeneration by providing environmental and trophic factors against toxic, neurodegenerative factors. However, with current literature, the greatest functionally validated potential of SCs lies in their unique ability to model disease pathophysiology and mechanisms of action. Patient-derived iPSC cells may be a particularly tremendous treatment option for personalized medicine in the future, but for now serve as prominent tools for understanding disease etiology on a highly personalized level, as they carry the patient's genotype, disease mutations, and also account for environmental influences on the individual's disease.

PART 4: PRECISION MEDICINE & BIOMARKERS

In discussing future developments of neurodegenerative therapies, it is critical to emphasize the importance of elucidating the molecular etiologies of NDs. Pathologically altered proteins and protein aggregations are fundamental characteristics of neurodegenerative diseases and should be of paramount focus for further research.

I've alluded to the importance of biomarker research in aiding developments of novel therapies during Part 2 & 3's discussion of gene therapy and stem-cell based treatments. This is because molecular classifications of NDs can detail the nature of pathologically dysfunctional proteins and correlate corresponding clinical symptoms to genetic abnormalities. Ongoing biomarker research has already identified extracellular deposits of A β fibrils and aggregation of abnormally-phosphorylated tau proteins in AD, the presence of Lewy body deposits, altered dopamine transporters in PD, and protein misfolding and aggregation associated with mHTT proteins in HD (Kovacs, 2016). Thus, the ability of biomarker data to identify core disease pathology has already been validated, and therapies targeting these protein abnormalities (through immunotherapy, gene therapy, SC-transplants) have yielded promising results in animal and some clinical models. However, the most exciting potential of biomarker research lies in its ability to characterise patient-specific environment-disease dynamics and gene variations, and consequently provide personalized diagnoses. In the emerging era of precision medicine, the use of biomarkers (detected in body fluids or PET imaging) can provide earlier and more accurate ND diagnosis, and their identified protein dysfunctions can be targeted in translational research using novel therapies.

Looking forward, biomarker research will contribute greatly to developing pathophysiological disease models, improving immunotherapy, gene therapy and SC-based transplant methods, and moving us closer to personalized

cures for neurodegenerative diseases. Similarly, stem cell research can be applied to experimentally model NDs. Patient-derived iPSCs are especially well-suited to uncover specific disease pathophysiologies as they carry the patient's genotype and bear patient-specific disease

mutations and gene-environment interactions. Thus, I believe that research focusing on biomarkers, patient-derived iPSCs, and genetic studies are currently most necessary and potentially most beneficial for current experimental and clinical endeavours for ND cures.

Table 1: Overview of Neurodegenerative Disorders

Disorder	~Incidence (total in US)	Brain Region Affected	Molecular Changes	Symptoms
Alzheimer's Disease (AD)	5,000,000	Hippocampus, amygdala, neocortex, basal forebrain	<ul style="list-style-type: none"> · Neurofibrillary tangles (hyperphosphorylation of tau proteins) · β-amyloid plaques (amyloid peptide aggregates) 	Dementia (cognitive, psychiatric, behavioural)
Amyotrophic Lateral Sclerosis (ALS)	30,000	Motor neurons	<ul style="list-style-type: none"> · Motor neuron degeneration in spinal cord, brain stem, primary motor cortex 	Difficulty walking, weakness in limbs/arms, slurred speech, muscle cramps and twitching
Parkinson's Disease (PD)	1,000,000	Substantia nigra	<ul style="list-style-type: none"> · Dopaminergic neuron loss from nigrostriatum and substantia nigra pars · Build-up of Lewy bodies, α-synuclein aggregates 	Tremors, bradykinesia, impaired balance and posture, loss of automatic movements
Huntington's Disease (HD)	30,000	Basal ganglia, neostriatum	<ul style="list-style-type: none"> · Trinucleotide (CAG) repeat in ITI5 huntington gene · Loss of medium spiny neurons (MSN) · Toxicity of mHTT: protein misfolding & aggregation 	Muscle problems (rigidity, dystonia), involuntary jerking movements, abnormal eye movements
Spinal Muscular Atrophy (SMA)	9,000	Motor neurons	Mutations in SMN1 gene	Muscular atrophy, balance and coordination deficits

Table 2: Current Therapies for AD, PD, & HD

Disease	Pharmacological Treatments	Other Treatments
Alzheimer's disease	<ul style="list-style-type: none"> · Acetylcholinesterase inhibitors (donepezil, galantamine, rivastigamine, tacrine) · NMDA receptor antagonist (memantine) 	<ul style="list-style-type: none"> · Caregiving · Behavioural therapy (reduce problem behaviours) · Psychosocial interventions (emotion and cognition regulation)
Parkinson's disease	Dopamine replacement (levodopa, carbidopa, selegeline)	<ul style="list-style-type: none"> · Deep brain stimulation (of thalamus, globus pallidus, subthalamic nucleus) · Physical therapy
Huntington's disease	Chorea treatment (tetrabenazine, benzodiazepines, neuroleptics)	<ul style="list-style-type: none"> · Caregiving · Physical therapy · Speech and language therapy

Table 3: Viral Vector-Mediated Gene Therapies (AD & PD)

Disease	Study / Trial Codes	Gene therapy	Method of Delivery	Current status
Alzheimer's disease	Rafii et al., 2014 (NCT00876863)	AAV2-NGF -- NGF: nerve growth factor // dysregulated in AD, neuroprotector/cellular therapy efficacy	Injection into basal forebrain	· Phase I successful*, therapy well-tolerated, NGF-induced axonal sprouting observed · Phase II in progress *success defined by meeting safety requirements and showing functional improvement
Parkinson's disease	LeWitt et al., 2011 (NCT00643890)	AAV2-GAD -- GAD: glutamic acid decarboxylase // rate-limiting biosynthetic enzyme for GABA, reduces excitotoxicity	Injection into subthalamic nucleus	Phase I & II successful, but symptom improvements achieved were not better than current standards of care
Parkinson's disease	Bartus et al., 2011 (NCT00400634; NCT00985517)	AAV2-NRTN -- NRTN: neurturin // delivers support to dopaminergic neurons	Injection into striatum & putamen	· Phase I successful, observed increased NRTN expression · Phase II showed no improvement in clinical outcome over controls
Parkinson's disease	Azzouz et al., 2002 (NCT00627588; NCT01856439)	Lentivirus-TH, AADC, GCH1 (*exciting tricistronic vector: cells transduced express all 3 enzymes) -- TH: tyrosine hydroxylase / AADC: aromatic amino acid dopa decarboxylase / GCH1: GTP cyclohydrolase I // three genes encode rate-limiting dopamine biosynthesis enzymes	Injection into striatum & putamen	· Phase I (open label) successful, improved motor function correlating with increasing dose · Currently optimizing delivery method before Phase II (double-blinded randomized trial)
Parkinson's disease	Muramatsu et al., 2015 (NCT02418598)	AAV2-AADC -- (as discussed above)	Injection into putamen	· Phase I safety trial successful, stable AADC expression 4 years post-delivery · Phase II recruiting, in progress
Parkinson's disease	(NCT00229736)	AAV2-AADC	Intrastriatal infusion	· Phase I safety trial successful

Table 4: Overview of Current Literature on SC-based ND therapies

Disease	Stem Cell Applications	Goal of SC-based Therapies	Current Therapeutic Progress
Alzheimer's disease	<ul style="list-style-type: none"> · NSCs · ESCs 	<ul style="list-style-type: none"> · Restore loss of cholinergic neurons · Increase BDNF & NGF trophic factors to promote neurogenesis 	<ul style="list-style-type: none"> · Successful basal forebrain grafts of NGF-secreting fibroblasts (open-label trial, Tuszynski, 2007) · Development of encapsulated cell biodelivery implants to increase NGF release (NsGene) · No current clinical trials testing SC-transplants in AD
Parkinson's disease	<ul style="list-style-type: none"> · ESCs 	<ul style="list-style-type: none"> · Restore nigrostriatal dopaminergic neuronal loss 	<ul style="list-style-type: none"> · Successful clinical open-label hfVM transplants (Lindvall et al., 1990; Madrazo et al., 1988) – provided long-term symptomatic relief, but failed to replicate in subsequent double-blind trials (which observed GIDs) · Successful animal studies using ESCs & iPSCs to derive DA phenotype, not yet translated to clinical trials due to risk of tumour formation and technical difficulties
Huntington's disease	<ul style="list-style-type: none"> · MSNs 	<ul style="list-style-type: none"> · Restore medium spiny neuron loss 	<ul style="list-style-type: none"> · Successful clinical fetal striatal grafting (Bachoud-Lévi et al., 2000)– provided transient symptom improvements, but limited by ethical constraints & poorly-understood mechanism of action · Successful animal studies using ESCs & iPSCs to restore MSN loss, not yet translated to clinical trials

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