



Published in final edited form as:

Ann Neurol. 2011 September ; 70(3): 353–361. doi:10.1002/ana.22487.

Stem Cell Technology for Neurodegenerative Diseases

J. Simon Lunn, PhD, Stacey A. Sakowski, PhD, Junguk Hur, PhD, and Eva L. Feldman, MD PhD

Department of Neurology – University of Michigan, Ann Arbor, MI

Abstract

Over the past 20 years, stem cell technologies have become an increasingly attractive option to investigate and treat neurodegenerative diseases. In the current review, we discuss the process of extending basic stem cell research into translational therapies for patients suffering from neurodegenerative diseases. We begin with a discussion on the burden of these diseases on society, emphasizing the need for increased attention towards advancing stem cell therapies. We then explain the various types of stem cells utilized in neurodegenerative disease research, and outline important issues to consider in the transition of stem cell therapy from bench to bedside. Finally, we detail the current progress regarding the applications of stem cell therapies to specific neurodegenerative diseases, focusing on Parkinson's disease, Huntington's disease, Alzheimer's disease, amyotrophic lateral sclerosis and spinal muscular atrophy. With a greater understanding of the capacity of stem cell technologies, there is growing public hope that stem cell therapies will continue to progress into realistic and efficacious treatments for neurodegenerative diseases.

Neurodegenerative diseases are characterized by the loss of neurons in the brain or spinal cord. Acute neurodegeneration may result from a temporally discrete insult, such as stroke or trauma, leading to a localized loss of neurons at the site of injury. Chronic neurodegeneration may develop over a long period of time and results in the loss of a particular neuronal subtype or generalized loss of neuronal populations. In the brain, Alzheimer's disease (AD) and Huntington's disease (HD) result in widespread loss of neurons, while Parkinson's disease (PD) involves the specific and localized loss of dopaminergic (DA) neurons in the substantia nigra. In the brainstem and spinal cord, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) involve the degeneration and loss of motor neurons (MNs). While these conditions all exhibit unique neuronal pathologies, the exact mechanisms for neuronal loss are complex, making the identification of efficacious treatments elusive.

The lack of effective therapies for these neurological diseases creates an enormous burden on society. In the USA, approximately 7 million people are living with AD, HD, PD, ALS or SMA (Fig. 1A; <http://www.alz.org/>, <http://www.apdaparkinson.org/>, <http://www.hdsa.org/>, <http://www.alsa.org/>, <http://www.smafoundation.org/>). Projected research spending by the National Institutes of Health (NIH) in 2011 for these 5 diseases totals \$768 million, and spending allocation is proportional to the number of individuals affected by each disease (Fig. 1A; <http://report.nih.gov/rcdc/categories/>). In the USA, an estimated 5.3 million individuals suffered from AD in 2010, making it by far the most prevalent neurodegenerative disorder, carrying an estimated health care cost of \$172 billion (<http://www.alz.org/>). PD affects up to an estimated 1.5 million people in the USA, with approximately 50,000 new cases diagnosed each year, and the estimated costs of PD stood at

\$11 billion per 500,000 affected Americans in 2009.¹ Furthermore, HD and ALS each affect 30,000 Americans, and SMA affects 25,000 Americans.

Despite the destructive nature of these diseases, the number of affected individuals, and health care costs surpassing billions of dollars, there is a stunning lack of treatment options. Recently, cellular therapies have earned increased attention as potentially feasible novel therapies. Analysis of published scientific manuscripts demonstrates that <2% of all papers per disease field examine the application of stem cells (Fig. 1B). However, it is likely that these figures will increase as the field of cellular therapy advances. In the past 5 years this percentage rose for each neurodegenerative disease analyzed (Fig. 1B) and NIH spending on stem cell research doubled between 2006 and 2011 (Fig. 1C; <http://report.nih.gov/rcdc/categories/>). While stem cell therapies in the USA are methodically examined via cautiously designed trials and basic laboratory science, the lack of available and effective treatments has prompted people suffering with neurologic diseases to turn elsewhere for “stem cell treatments”. Reports of “stem cell treatments” from clinics in China and India are followed by glowing patient testimonials; however, these treatments are not the result of rigorous trials investigating safety and efficacy. Analysis of 7 spinal cord injury patients receiving treatment at a clinic in China included uncertainties concerning the type of cells utilized, appropriate delivery for the level of injury, and ultimate presence of any clinical benefit.² Since this analysis, improvements in cell quality and identification, delivery accuracy and targeting, and reduced post-operative complications may have been made but have clearly not been documented.

Neurodegenerative diseases create a tremendous burden on society and despite decades of research, effective treatments do not exist. Cellular therapies are attractive options and the application of stem cell research to neurodegenerative diseases is rapidly expanding. In the current review, we detail the current state of stem cell research for neurodegenerative diseases, beginning with a brief introduction to the various stem cell technologies available. We also describe the rationale and remaining hurdles associated with transitioning stem cell therapies from bench to bedside. Finally, we discuss the current data and progress for translational stem cell therapies for AD, PD, HD, ALS and SMA.

STEM CELL TECHNOLOGY

Stem cells have the capacity to proliferate and differentiate into multiple cellular lineages. There are different classifications of stem cells that reflect the range of possible cell types they can produce, and the ways in which the stem cells are derived. These include embryonic stem (ES) cells, progenitor cells, mesenchymal stem cells (MSCs), and induced pluripotent stem (iPS) cells (Fig. 2). To appreciate the potential applications of stem cell technology to neurodegenerative diseases, it is important to understand the characteristics of the various stem cell types available and the potential impact of cellular therapies on disease mechanisms.

Stem cell classifications

Each stem cell type possesses certain qualities and advantages, and the rationale for utilizing each one depends on the desired applications and outcomes. ES cells are derived from the inner cell mass of a developing blastocyst and are pluripotent, possessing the capacity to give rise to all three germ layers. Progenitor cells, which are derived from more developed fetal or adult tissues, are multipotent, meaning they give rise to more restricted lineages than ES cells. These potential lineages are usually determined by the germ layer of origin. For example, neural progenitor cells (NPCs), or neural stem cells (NSCs), are capable of differentiating to cell types within a neural lineage. NPCs may be derived directly from fetal or adult neural tissue, or by directed differentiation of ES cells via cell culture

manipulation.^{3, 4} Along with this limited differentiation potential, NPCs also appear to have a more restricted self-renewal potential; while the self-renewal state may be maintained in culture, cells stop proliferating and start to differentiate when transplanted *in vivo*.⁵ MSCs are an alternative source of multipotent self-renewing cells and are derived from adult bone marrow. Naturally, they differentiate to produce osteoblasts, chondrocytes and adipocytes; however, there is evidence that they can transdifferentiate to a neural lineage.⁶ MSCs provide an accessible alternative to ES cells and potentially circumvent the need for immunosuppression in cellular therapies since they are derived from an autologous source. Using autologous cells, however, may be less desirable when dealing with genetic diseases because the cells may possess the same genetic predisposition to disease. For example, MSCs derived from ALS patients exhibit diminished growth and differentiation capacity, however, these issues may be circumvented by enhancing cell culture techniques and establishing optimal cell passage numbers.^{7, 8}

More recently, the development of iPS cells provide an additional source of autologous stem cells for modeling and treating diseases. iPS cells are generated from somatic tissue such as fibroblasts and are reprogrammed into ES-like cells by the addition of select transcription factors. The original approach utilized Oct 3/4, Klf, Sox2 and c-Myc⁹, and multiple research groups have now accomplished successful reprogramming of fibroblasts using various combinations of factors delivered by vector, virus, protein or RNA-mediated approaches.¹⁰⁻¹³ While many neurological disorders rely on complex genetic rodent models or chemical treatments that may not fully represent human neurodegenerative diseases, these cells afford options for disease modeling and provide novel sources for autologous cellular therapies. It should be noted that residual alterations from the genetic reprogramming required to induce pluripotency are possible^{14, 15}; therefore, careful characterization of patient iPS lines must be performed. With the continued advancement of iPS technology, however, directed differentiation of patient iPS cells may be utilized to model human disease processes for mechanistic and therapeutic discovery.

Cellular therapy strategies and applications

“Cellular therapies” utilize cell or tissue grafts to treat diseases or injury (Fig. 2). Treatment objectives of stem cell therapies typically center on cellular replacement or providing environmental enrichment. Cellular replacement for neurodegenerative diseases involves the derivation of specific neuronal subtypes lost in disease and subsequent grafting into affected areas of the nervous system. The newly transplanted neurons may then integrate, synapse and recapitulate a neural network similar to the one lost in disease. Alternatively, stem cells may provide environmental enrichment to support host neurons by producing neurotrophic factors, scavenging toxic factors or creating auxiliary neural networks around affected areas. Many strategies for environmental enrichment utilize stem cells to provide *de novo* synthesis and delivery of neuroprotective growth factors at the site of disease. Growth factors such as glial-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF) are protective in neurodegenerative disease models and provide *in situ* support at the main foci of disease.¹⁶⁻²¹ The appropriate objective of cellular therapy for each neurodegenerative disease must be based on the specific neuronal pathology of each disorder. While cellular replacement may be effective in diseases like PD where a specific neuronal subpopulation is lost, ALS is most likely to benefit from cellular therapies that enrich the local spinal cord environment to support the remaining MNs. Factors such as how well grafted neurons integrate and migrate within the host tissue, and the distances that axons must extend to reach their targets, must be considered when determining the potential efficacy of cellular therapies for neurodegenerative diseases.

CELLULAR THERAPY FOR NEURODEGENERATIVE DISEASES

Selecting the appropriate stem cell type and understanding the desired mechanism of support is only one step in developing and translating cellular therapies to patients. The course from bench to bedside is long and complex; and while each disease and cellular therapy is unique, certain universal issues must be considered for a safe transition to patient therapies.²²⁻²⁴ Table 1 describes some of these issues that are pertinent to the development of any clinical trial, and describes some of the issues that arise along these lines for stem cell therapies. This transition from bench to bedside may take well over a decade of *in vitro*, *in vivo* and large animal studies (Fig. 3). As we acknowledge and overcome these issues, however, advances in the field of translational stem cell therapy will continue to gain momentum. Next, we will discuss the potential for therapeutic success, supported approaches, and current progress in translating stem cells from bench to bedside for specific neurodegenerative diseases.

Alzheimer's disease

AD, the most frequent form of dementia, is characterized by memory loss and cognitive decline.²⁵ As the disease progresses, there is a widespread loss of neurons and synaptic contacts throughout the cortex, hippocampus, amygdala, and basal forebrain.²⁶ While the exact pathology of AD remains unclear, pathologic hallmarks include A β plaques and neurofibrillary tangles.^{26, 27} There is an increased risk of developing AD with age, and the majority of AD cases are late-onset, developing after 65 years of age.^{28, 29} With an increasingly aging population, the burden from AD is anticipated to rise.

Current treatment options for AD are centered on regulating neurotransmitter activity. Enhancing cholinergic function improves AD behavioral and cognitive defects.³⁰ Targeting the cholinergic system using stem cell therapies may provide environmental enrichment. Neurogenesis in the hippocampus decreases as we age and is exacerbated in AD^{31, 32}; therefore cellular therapies which enhance neurogenesis or replace lost neurons may also delay the progression of AD. Enhancing BDNF levels, which are decreased with age and in AD, promotes neurogenesis and protects neuronal function.³³ Rodent AD models receiving NPC grafts demonstrate increased hippocampal synaptic density and increased cognitive function associated with local production of BDNF.¹⁹ Similarly, BDNF upregulation along with NPC transplants also improves cell incorporation and functional outcomes in an AD rat model.²¹ Nerve growth factor (NGF) production is another mechanism of cellular therapy efficacy. Genetically engineered patient fibroblasts that produce NGF are currently being examined in a phase I trial for AD.^{34, 35} Integration of NGF-fibroblasts into a major cholinergic center of the basal forebrain provided some benefit to AD patients.³⁴ The Danish company NsGene (<http://nsgene.dk/>) is currently developing an NGF-releasing therapy using encapsulated epithelial cells. Combining engineered growth factor overexpression with the benefits of NPC integration into neural networks may provide an enhanced approach to treating AD. Furthermore, given the widespread neuronal loss involved in AD pathogenesis, targeting multiple systems simultaneously may be advantageous.

Parkinson's disease

PD results from the progressive loss of DA neurons in the substantia nigra.³⁶ Patients suffer from severe motor deficits manifesting as tremors, muscle rigidity and unstable gait and posture. Current treatment options include deep brain stimulation or therapies that aim to increase dopamine levels by providing a dopamine precursor, L-dopa, or providing dopamine agonists.³⁷⁻³⁹ These treatments are effective early in disease to alleviate symptoms, but long term efficacy is uncertain, they do not correct the deficit, have long-term side-effects and become increasingly ineffective with PD progression. Cellular

approaches for PD, on the other hand, focus on the replacement of lost DA neurons. Initial cellular therapies for PD utilized fetal ventral midbrain tissue as a source of DA neurons. Clinical trials had varying degrees of success, but they supported cellular therapies for a potential functional benefit in PD.²³ Potential limitations of utilizing fetal tissue, however, include ethical concerns and the ability to obtain adequate amounts of tissue for treatment. Alternatively, ES cells offer sources for large-scale production of neurons which acquire a midbrain DA phenotype.^{40, 41} Grafting both ES- and MSC-derived DA neurons into rat PD models results in functional recovery.^{42, 43} The ability to produce patient-specific DA neurons has recently been demonstrated using iPS cells.⁴⁴ Transplantation of these cells into a rodent PD model improved functional deficits and demonstrated cell integration in the host tissue.⁴⁵ These reports are among the first to demonstrate a therapeutic use for iPS cells in a neurodegenerative disease.

While studies maintain cellular replacement as a viable approach for treating PD, environmental enrichment may also support existing DA neurons and slow or prevent further degeneration. Growth factor therapy through direct delivery or viral-based systems protects against neuronal decay in PD.^{46, 47} MSCs and NPCs engineered to produce growth factors such as BDNF, VEGF, GDNF and IGF-I provide prolonged local growth factor production *in situ*. Transplantation of growth factor-producing MSCs and NPCs protects DA neurons and promotes functional recovery in rodent models of PD.^{18, 48-54} Taken together, the combination of cellular replacement and environmental enrichment may improve the efficacy of cellular therapies for PD.

Huntington's disease

HD is an autosomal dominant polyglutamine disease caused by the accumulation of CAG repeats in the *huntingin* gene.⁵⁵ Onset typically occurs in the fourth to fifth decade of life, with a disease course of approximately 20-30 years.⁵⁶ HD manifests with involuntary motor activity, dementia, personality changes and cognitive impairment associated with the progressive loss of medium spiny neurons (MSNs).⁵⁷ Loss of these GABAergic neurons in the striatum is also accompanied by degeneration in the cortex, brain stem and hippocampus.⁵⁷ Despite the known genetic basis for HD, insight into disease mechanisms and identification of effective therapies remain elusive.

Cellular therapies have provided some of the only positive treatment outcomes for HD. Initial therapies utilized fetal-derived tissue⁵⁸, and grafting using the whole ganglionic eminence offered an optimal source of MSNs for HD.⁵⁹ The transplantation of neural cells and striatal grafts into rodent HD models demonstrated that MSNs integrate and form circuitry in the host.^{60, 61} Translation of fetal tissue grafting into HD patients prompted slight transient improvements and a period of stabilization prior to the inherent decline.⁶² Key issues still remain, based on the ethical implications of utilizing fetal tissues and the dangers associated with cellular therapies such as graft overgrowth and the presence of non-neuronal cells within grafts.⁶² Overall, the relative safety of the technique has been demonstrated in trials for both HD and PD.^{22, 23, 58, 59}

Stem cells also have the potential to restore functional loss of MSNs in HD. Striatal injections of NPCs into HD rodents demonstrate incorporation as well as migration to secondary sites associated with the disease.⁶³ The resulting functional improvements confirmed that isolated cell types provide similar functional benefits to those observed with fetal tissue, although mechanisms of cellular therapy protection were not examined. To address the role of environmental enrichment in cellular therapy for HD, NPCs engineered to overexpress GDNF were transplanted into HD rodents. While unmodified NSCs provided no neuroprotective effects, NPCs expressing GDNF protected neurons and promoted

functional recovery.^{18, 20} This study validates that environmental enrichment and protection of endogenous neurons may lead to functional recovery.

Amyotrophic lateral sclerosis

ALS is an adult-onset disorder involving the degeneration and loss of MNs. Patients present with loss of coordination and muscle strength with transition to complete loss of muscle control. Death typically results from respiratory failure within 2-5 years of diagnosis. Multiple cell types and mechanisms are likely involved in ALS pathogenesis⁶⁴, which makes the development of conventional drug therapies difficult. Cellular therapies for ALS provide both an integrating neural component and environmental enrichment to support and protect MNs from degeneration.^{16, 17} Assessment of several stem cell types, including NPCs and MSCs, in ALS rodent models demonstrates that systemic and direct intraspinal injection ameliorates disease progression^{5, 65-71}, suggesting intervention prior to the irreversible loss of critical MN numbers may improve outcomes. Because it is crucial to protect the remaining MNs in ALS, the ability of stem cells to provide environmental enrichment via GDNF, VEGF and IGF-I expression has also been examined, as these growth factors all confer neuroprotection to MNs.¹⁷ MN axonal degeneration precedes symptom onset and loss of MNs in ALS; therefore, providing distal support to MNs at neuromuscular junctions may also prevent neurodegeneration. Distal production of GDNF in muscle protects neuromuscular junctions and promotes MN protection, likely by retrograde transport.⁷¹ Overall, the literature supports targeting cellular therapies to maintain MNs in the spinal cord and provide environmental enrichment to MNs and neuromuscular junctions.⁷²

These supporting studies have created the foundation for the first Phase I trial for ALS using fetal spinal cord-derived NPCs (<http://neuralstem.com/>). The cells utilized in the trial integrate safely into the spinal cord, synapse and interact with host MNs, and also provide a source of growth factors upon direct spinal cord injection in rodents.^{5, 73} Delivery optimization and safety was further established in minipigs.^{74, 75} NPCs are being delivered to non-ambulatory, and ultimately ambulatory, ALS patients through direct lumbar and cervical intraspinal injections to demonstrate the safety of the procedure and lack of toxicity from the cellular therapy. Because cellular therapies for ALS are designed to provide support and enrichment to existing MNs, it is likely that treatment efficacy in future trials will be best examined in earlier stage patients.

Spinal muscular atrophy

SMA involves the selective loss of MNs and presents with a broad range of onset and severity. SMA type I is the leading genetic cause of infantile mortality⁷⁶ and is characterized by early onset severe muscle weakness and fatality within 2 years. SMA is caused by a mutation or loss of the *SMN1* gene⁷⁷, and the resulting decrease in SMN protein levels contribute to MN loss. In humans, low levels of SMN protein may be produced by alternative splicing variants encoded by the *SMN2* gene. Current pharmaceutical developments and gene therapy treatments focus on regulating *SMN2* to treat SMA. Cellular therapies, however, have been examined in mouse models of SMA, where grafting of ES-derived NPCs protected MNs from degeneration and improved survival.^{78, 79} It is possible that for SMA, transient rescue of the developmental loss of SMN may be sufficient to confer efficacy, which may not be the case for other neurodegenerative diseases where long-term degeneration of the transplanted cells is a valid concern.

FUTURE CHALLENGES

Neurodegenerative diseases create a tremendous societal burden due to their devastating nature, cost, and lack of effective therapies. Cellular therapies offer great promise for the

treatment of these diseases, and research progress to date supports the utilization of stem cells to offer cellular replacement and/or provide environmental enrichment to attenuate neurodegeneration. In diseases where specific subpopulations of cells or widespread neuronal loss are present, cellular replacement may reproduce or stabilize neuronal networks. In addition, environmental enrichment may provide neurotrophic support to remaining cells or prevent the production or accumulation of toxic factors that harm neurons. In many cases, cellular therapies provide beneficial effects through both mechanisms.

Many questions still remain unanswered and certain issues must be addressed as we continue the translation of cellular therapies from the bench to bedside (Table 1). The pathophysiology of each neurodegenerative disease discussed in this review is unique, and thus requires careful attention to these topics: Which type of cells offer the best approach to treat this disease? What do we expect the stem cells to do, and what outcomes are predicted? How do we anticipate patients early and later in the disease course will respond to treatments? As we begin to design clinical studies that take into account these questions and learn lessons from the trials currently underway, we are poised to maximize the potential of cellular therapies to provide much-needed treatments for neurodegenerative diseases.

Acknowledgments

The authors would like to thank Ms. Judith Boldt for excellent secretarial support during the preparation of this manuscript. Funding is provided by the A. Alfred Taubman Medical Research Institute and the Program for Neurology Research & Discovery. SAS is supported by the NIH (T32 NS007222-28).

REFERENCES

1. Dorsey ER, Constantinescu R, Thompson JP, et al. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology*. 2007; 68:384–386. [PubMed: 17082464]
2. Dobkin BH, Curt A, Guest J. Cellular transplants in China: observational study from the largest human experiment in chronic spinal cord injury. *Neurorehabil Neural Repair*. 2006; 20:5–13. [PubMed: 16467274]
3. Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. *Cell*. 2002; 110:385–397. [PubMed: 12176325]
4. Gaspard N, Vanderhaeghen P. Mechanisms of neural specification from embryonic stem cells. *Curr Opin Neurobiol*. 2010; 20:37–43. [PubMed: 20080043]
5. Yan J, Xu L, Welsh AM, et al. Extensive neuronal differentiation of human neural stem cell grafts in adult rat spinal cord. *PLoS Med*. 2007; 4:e39. [PubMed: 17298165]
6. Satija NK, Singh VK, Verma YK, et al. Mesenchymal stem cell-based therapy: a new paradigm in regenerative medicine. *J Cell Mol Med*. 2009; 13:4385–4402. [PubMed: 19602034]
7. Cho GW, Noh MY, Kim HY, et al. Bone marrow-derived stromal cells from amyotrophic lateral sclerosis patients have diminished stem cell capacity. *Stem Cells Dev*. 2010; 19:1035–1042. [PubMed: 20030561]
8. Choi MR, Kim HY, Park JY, et al. Selection of optimal passage of bone marrow-derived mesenchymal stem cells for stem cell therapy in patients with amyotrophic lateral sclerosis. *Neurosci Lett*. 2010; 472:94–98. [PubMed: 20117176]
9. Yamanaka S. Induction of pluripotent stem cells from mouse fibroblasts by four transcription factors. *Cell Prolif*. 2008; 41(Suppl 1):51–56. [PubMed: 18181945]
10. Cho HJ, Lee CS, Kwon YW, et al. Induction of pluripotent stem cells from adult somatic cells by protein-based reprogramming without genetic manipulation. *Blood*. 2010; 116:386–395. [PubMed: 20439621]

11. Yakubov E, Rechavi G, Rozenblatt S, Givol D. Reprogramming of human fibroblasts to pluripotent stem cells using mRNA of four transcription factors. *Biochemical and biophysical research communications*. 2010; 394:189–193. [PubMed: 20188704]
12. Judson RL, Babiarz JE, Venere M, Belloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. *Nat Biotechnol*. 2009; 27:459–461. [PubMed: 19363475]
13. Hanley J, Rastegarlarlari G, Nathwani AC. An introduction to induced pluripotent stem cells. *Br J Haematol*. 2010; 151:16–24. [PubMed: 20666774]
14. Gore A, Li Z, Fung HL, et al. Somatic coding mutations in human induced pluripotent stem cells. *Nature*. 2011; 471:63–67. [PubMed: 21368825]
15. Hussein SM, Batada NN, Vuoristo S, et al. Copy number variation and selection during reprogramming to pluripotency. *Nature*. 2011; 471:58–62. [PubMed: 21368824]
16. Suzuki M, Svendsen CN. Combining growth factor and stem cell therapy for amyotrophic lateral sclerosis. *Trends Neurosci*. 2008; 31:192–198. [PubMed: 18329734]
17. Lunn JS, Hefferan MP, Marsala M, Feldman EL. Stem cells: comprehensive treatments for amyotrophic lateral sclerosis in conjunction with growth factor delivery. *Growth Factors*. 2009; 27:133–140. [PubMed: 19294549]
18. Behrstock S, Ebert AD, Klein S, et al. Lesion-induced increase in survival and migration of human neural progenitor cells releasing GDNF. *Cell Transplant*. 2008; 17:753–762. [PubMed: 19044202]
19. Blurton-Jones M, Kitazawa M, Martinez-Coria H, et al. Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease. *Proc Natl Acad Sci U S A*. 2009; 106:13594–13599. [PubMed: 19633196]
20. Ebert AD, Barber AE, Heins BM, Svendsen CN. Ex vivo delivery of GDNF maintains motor function and prevents neuronal loss in a transgenic mouse model of Huntington's disease. *Experimental neurology*. 2010; 224:155–162. [PubMed: 20227407]
21. Xuan AG, Long DH, Gu HG, et al. BDNF improves the effects of neural stem cells on the rat model of Alzheimer's disease with unilateral lesion of fimbria-fornix. *Neurosci Lett*. 2008; 440:331–335. [PubMed: 18579298]
22. Lo B, Parham L. Resolving ethical issues in stem cell clinical trials: the example of Parkinson disease. *J Law Med Ethics*. 2010; 38:257–266. [PubMed: 20579249]
23. Brundin P, Barker RA, Parmar M. Neural grafting in Parkinson's disease Problems and possibilities. *Prog Brain Res*. 2010; 184:265–294. [PubMed: 20887880]
24. Leigh PN, Swash M, Iwasaki Y, et al. Amyotrophic lateral sclerosis: a consensus viewpoint on designing and implementing a clinical trial. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2004; 5:84–98. [PubMed: 15204010]
25. Reitz C, Brayne C, Mayeux R. Epidemiology of Alzheimer disease. *Nat Rev Neurol*. 2011
26. Castellani RJ, Rolston RK, Smith MA. Alzheimer disease. *Dis Mon*. 2010; 56:484–546. [PubMed: 20831921]
27. Eckman CB, Eckman EA. An update on the amyloid hypothesis. *Neurol Clin*. 2007; 25:669–682. vi. [PubMed: 17659184]
28. Kukull WA, Bowen JD. Dementia epidemiology. *Med Clin North Am*. 2002; 86:573–590. [PubMed: 12168560]
29. Campion D, Dumanchin C, Hannequin D, et al. Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. *American journal of human genetics*. 1999; 65:664–670. [PubMed: 10441572]
30. Bird, TD. *Alzheimer Disease Overview*. 1993.
31. Shruster A, Melamed E, Offen D. Neurogenesis in the aged and neurodegenerative brain. *Apoptosis*. 2010; 15:1415–1421. [PubMed: 20339917]
32. Drapeau E, Arous D Nora. Stem cell review series: role of neurogenesis in age-related memory disorders. *Aging Cell*. 2008; 7:569–589. [PubMed: 18221417]
33. Li G, Peskind ER, Millard SP, et al. Cerebrospinal fluid concentration of brain-derived neurotrophic factor and cognitive function in non-demented subjects. *PLoS One*. 2009; 4:e5424. [PubMed: 19412541]

34. Tuszynski MH. Nerve growth factor gene therapy in Alzheimer disease. *Alzheimer Dis Assoc Disord.* 2007; 21:179–189. [PubMed: 17545746]
35. Tuszynski MH, Thal L, Pay M, et al. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nature medicine.* 2005; 11:551–555.
36. Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain.* 1999; 122(Pt 8):1437–1448. [PubMed: 10430830]
37. Poewe W. Treatments for Parkinson disease--past achievements and current clinical needs. *Neurology.* 2009; 72:S65–73. [PubMed: 19221317]
38. Benabid AL, Chabardes S, Mitrofanis J, Pollak P. Deep brain stimulation of the subthalamic nucleus for the treatment of Parkinson's disease. *Lancet Neurol.* 2009; 8:67–81. [PubMed: 19081516]
39. Krack P, Batir A, Van Blercom N, et al. Five-year follow-up of bilateral stimulation of the subthalamic nucleus in advanced Parkinson's disease. *The New England journal of medicine.* 2003; 349:1925–1934. [PubMed: 14614167]
40. Kim HJ. Stem cell potential in Parkinson's disease and molecular factors for the generation of dopamine neurons. *Biochimica et biophysica acta.* 2011; 1812:1–11. [PubMed: 20713152]
41. Morizane A, Darsalia V, Guloglu MO, et al. A simple method for large-scale generation of dopamine neurons from human embryonic stem cells. *Journal of neuroscience research.* 2010; 88:3467–3478. [PubMed: 20981866]
42. Cui YF, Hargus G, Xu JC, et al. Embryonic stem cell-derived L1 overexpressing neural aggregates enhance recovery in Parkinsonian mice. *Brain.* 2010; 133:189–204. [PubMed: 19995872]
43. Li M, Zhang SZ, Guo YW, et al. Human umbilical vein-derived dopaminergic-like cell transplantation with nerve growth factor ameliorates motor dysfunction in a rat model of Parkinson's disease. *Neurochem Res.* 2010; 35:1522–1529. [PubMed: 20658188]
44. Cooper O, Hargus G, Deleidi M, et al. Differentiation of human ES and Parkinson's disease iPS cells into ventral midbrain dopaminergic neurons requires a high activity form of SHH, FGF8a and specific regionalization by retinoic acid. *Mol Cell Neurosci.* 2010; 45:258–266. [PubMed: 20603216]
45. Hargus G, Cooper O, Deleidi M, et al. Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *Proc Natl Acad Sci U S A.* 107:15921–15926. [PubMed: 20798034]
46. Laganieri J, Kells AP, Lai JT, et al. An engineered zinc finger protein activator of the endogenous glial cell line-derived neurotrophic factor gene provides functional neuroprotection in a rat model of Parkinson's disease. *J Neurosci.* 30:16469–16474. [PubMed: 21147986]
47. Manfredsson FP, Okun MS, Mandel RJ. Gene therapy for neurological disorders: challenges and future prospects for the use of growth factors for the treatment of Parkinson's disease. *Curr Gene Ther.* 2009; 9:375–388. [PubMed: 19860652]
48. Xiong N, Zhang Z, Huang J, et al. VEGF-expressing human umbilical cord mesenchymal stem cells, an improved therapy strategy for Parkinson's disease. *Gene therapy.* 2010
49. Falk T, Zhang S, Sherman SJ. Vascular endothelial growth factor B (VEGF-B) is up-regulated and exogenous VEGF-B is neuroprotective in a culture model of Parkinson's disease. *Mol Neurodegener.* 2009; 4:49. [PubMed: 20003314]
50. Eberling JL, Kells AP, Pivrotto P, et al. Functional effects of AAV2-GDNF on the dopaminergic nigrostriatal pathway in parkinsonian rhesus monkeys. *Human gene therapy.* 2009; 20:511–518. [PubMed: 19254173]
51. Garbayo E, Montero-Menei CN, Ansorena E, et al. Effective GDNF brain delivery using microspheres--a promising strategy for Parkinson's disease. *J Control Release.* 2009; 135:119–126. [PubMed: 19154763]
52. Emborg ME, Ebert AD, Moirano J, et al. GDNF-secreting human neural progenitor cells increase tyrosine hydroxylase and VMAT2 expression in MPTP-treated cynomolgus monkeys. *Cell Transplant.* 2008; 17:383–395. [PubMed: 18522241]

53. Ebert AD, Beres AJ, Barber AE, Svendsen CN. Human neural progenitor cells over-expressing IGF-1 protect dopamine neurons and restore function in a rat model of Parkinson's disease. *Experimental neurology*. 2008; 209:213–223. [PubMed: 18061591]
54. Jourdi H, Hamo L, Oka T, et al. BDNF mediates the neuroprotective effects of positive AMPA receptor modulators against MPP+–induced toxicity in cultured hippocampal and mesencephalic slices. *Neuropharmacology*. 2009; 56:876–885. [PubMed: 19371576]
55. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell*. 1993; 72:971–983. [PubMed: 8458085]
56. Foroud T, Gray J, Ivashina J, Conneally PM. Differences in duration of Huntington's disease based on age at onset. *J Neurol Neurosurg Psychiatry*. 1999; 66:52–56. [PubMed: 9886451]
57. Vonsattel JP, DiFiglia M. Huntington disease. *Journal of neuropathology and experimental neurology*. 1998; 57:369–384. [PubMed: 9596408]
58. Clelland CD, Barker RA, Watts C. Cell therapy in Huntington disease. *Neurosurg Focus*. 2008; 24:E9. [PubMed: 18341412]
59. Kelly CM, Dunnett SB, Rosser AE. Medium spiny neurons for transplantation in Huntington's disease. *Biochemical Society transactions*. 2009; 37:323–328. [PubMed: 19143656]
60. Nakao N, Ogura M, Nakai K, Itakura T. Embryonic striatal grafts restore neuronal activity of the globus pallidus in a rodent model of Huntington's disease. *Neuroscience*. 1999; 88:469–477. [PubMed: 10197767]
61. Isacson O, Deacon TW, Pakzaban P, et al. Transplanted xenogeneic neural cells in neurodegenerative disease models exhibit remarkable axonal target specificity and distinct growth patterns of glial and axonal fibres. *Nature medicine*. 1995; 1:1189–1194.
62. Freeman TB, Cicchetti F, Bachoud-Levi AC, Dunnett SB. Technical factors that influence neural transplant safety in Huntington's disease. *Experimental neurology*. 2011; 227:1–9. [PubMed: 20849848]
63. McBride JL, Behrstock SP, Chen EY, et al. Human neural stem cell transplants improve motor function in a rat model of Huntington's disease. *J Comp Neurol*. 2004; 475:211–219. [PubMed: 15211462]
64. Ilieva H, Polymenidou M, Cleveland DW. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *The Journal of cell biology*. 2009; 187:761–772. [PubMed: 19951898]
65. Yan J, Xu L, Welsh AM, et al. Combined immunosuppressive agents or CD4 antibodies prolong survival of human neural stem cell grafts and improve disease outcomes in amyotrophic lateral sclerosis transgenic mice. *Stem cells (Dayton, Ohio)*. 2006; 24:1976–1985.
66. Xu L, Yan J, Chen D, et al. Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. *Transplantation*. 2006; 82:865–875. [PubMed: 17038899]
67. Corti S, Locatelli F, Donadoni C, et al. Wild-type bone marrow cells ameliorate the phenotype of SOD1-G93A ALS mice and contribute to CNS, heart and skeletal muscle tissues. *Brain*. 2004; 127:2518–2532. [PubMed: 15469951]
68. Corti S, Nizzardo M, Nardini M, et al. Systemic transplantation of c-kit+ cells exerts a therapeutic effect in a model of amyotrophic lateral sclerosis. *Hum Mol Genet*. 2010; 19:3782–3796. [PubMed: 20650960]
69. Garbuzova-Davis S, Willing AE, Zigova T, et al. Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. *J Hematother Stem Cell Res*. 2003; 12:255–270. [PubMed: 12857367]
70. Suzuki M, McHugh J, Tork C, et al. GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. *PLoS ONE*. 2007; 2:e689. [PubMed: 17668067]
71. Suzuki M, McHugh J, Tork C, et al. Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS. *Mol Ther*. 2008; 16:2002–2010. [PubMed: 18797452]
72. Lunn JS, Sakowski SA, Federici T, et al. Stem cell technology for the study and treatment of motor neuron diseases. *Regen Med*. 2011; 6:201–213. [PubMed: 21391854]

73. Xu L, Ryugo DK, Pongstaporn T, et al. Human neural stem cell grafts in the spinal cord of SOD1 transgenic rats: differentiation and structural integration into the segmental motor circuitry. *J Comp Neurol.* 2009; 514:297–309. [PubMed: 19326469]
74. Usvald D, Vodicka P, Hlucilova J, et al. Analysis of dosing regimen and reproducibility of intraspinal grafting of human spinal stem cells in immunosuppressed minipigs. *Cell Transplant.* 2010
75. Raore B, Federici T, Taub J, et al. Cervical multilevel intraspinal stem cell therapy: assessment of surgical risks in Gottingen minipigs. *Spine (Phila Pa 1976).* 2011; 36:E164–171. [PubMed: 21099736]
76. Lefebvre S, Bulet P, Liu Q, et al. Correlation between severity and SMN protein level in spinal muscular atrophy. *Nat Genet.* 1997; 16:265–269. [PubMed: 9207792]
77. Wee CD, Kong L, Sumner CJ. The genetics of spinal muscular atrophies. *Curr Opin Neurol.* 2010; 23:450–458. [PubMed: 20733483]
78. Corti S, Nizzardo M, Nardini M, et al. Embryonic stem cell-derived neural stem cells improve spinal muscular atrophy phenotype in mice. *Brain.* 2010; 133:465–481. [PubMed: 20032086]
79. Corti S, Nizzardo M, Nardini M, et al. Neural stem cell transplantation can ameliorate the phenotype of a mouse model of spinal muscular atrophy. *J Clin Invest.* 2008; 118:3316–3330. [PubMed: 18769634]
80. Johe KK, Hazel TG, Muller T, et al. Single factors direct the differentiation of stem cells from the fetal and adult central nervous system. *Genes & development.* 1996; 10:3129–3140. [PubMed: 8985182]
81. Guo X, Johe K, Molnar P, et al. Characterization of a human fetal spinal cord stem cell line, NSI-566RSC, and its induction to functional motoneurons. *J Tissue Eng Regen Med.* 2010; 4:181–193. [PubMed: 19950213]

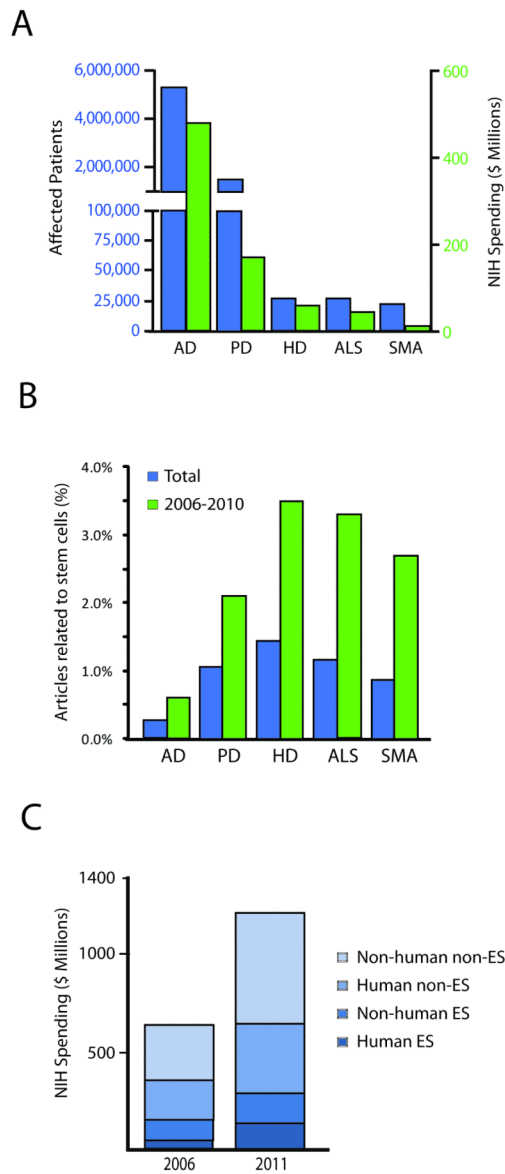


Figure 1. Analysis of the current state of stem cell research for neurodegenerative diseases

A. Comparison of approximate number of affected individuals in the USA (blue) and anticipated breakdown of NIH spending on diseases in 2011 (green). **B.** Portion of published literature focusing on stem cell technologies for individual neurodegenerative diseases overall (blue) and between 2006 and 2010 (green). Literature mining was performed using the MeSH terms “Alzheimer disease”, “Parkinson disease”, “Huntington disease”, “amyotrophic lateral sclerosis”, “Muscular atrophy, spinal” and “stem cell”. **C.** NIH spending on stem cell technologies in 2006 and 2011 by stem cell type and species.

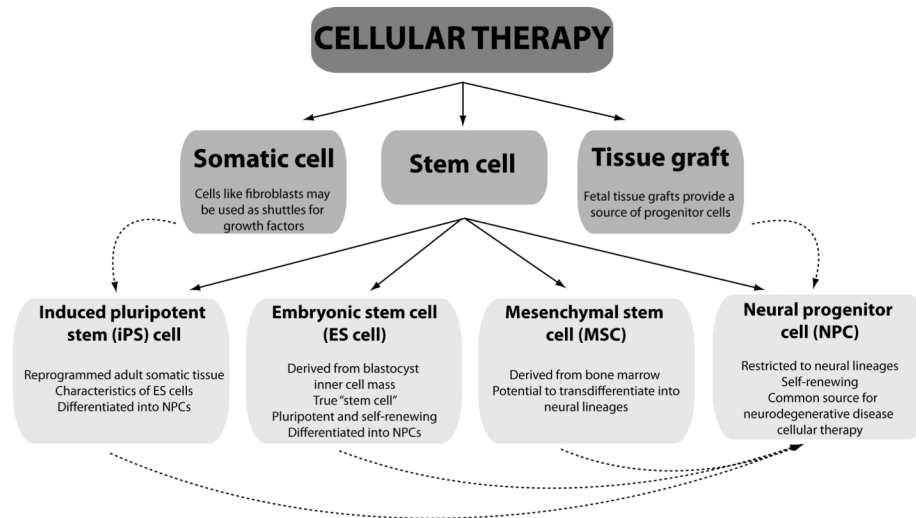


Figure 2. Stem cell technology and cellular therapy classifications

Cellular therapy involves the treatment of diseases using cells or tissue grafts. Various types of stem cells may be utilized, each possessing unique characteristics and advantages depending on the desired outcomes. Selecting the appropriate stem cell and treatment mechanisms for each disease is necessary to support the translation of cellular therapies for neurodegenerative diseases from bench to bedside. Solid arrows represent divisions within each category. Dashed arrows represent sources of iPS cells and NPCs.

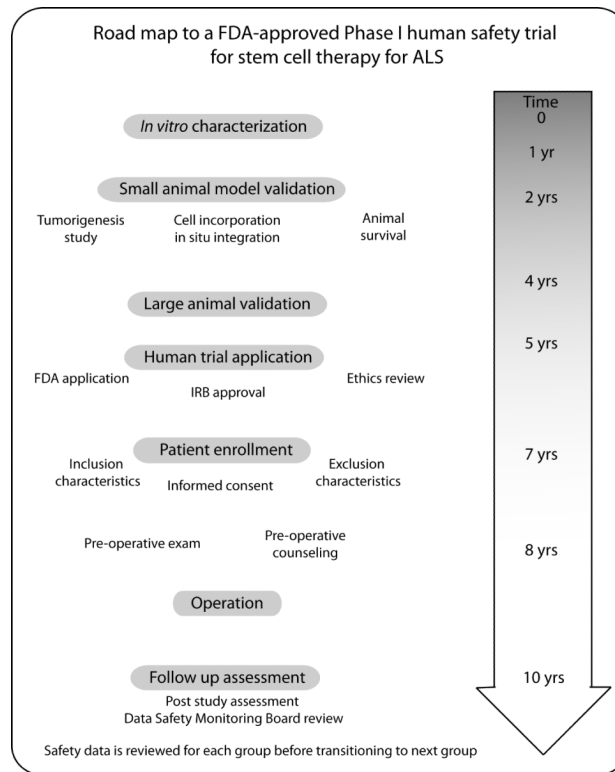


Figure 3. Translating a stem cell-based treatment for ALS from the bench to patients

Overview of the stages involved in translating a stem cell therapy from the bench to patients, using the current ALS stem cell trial as a representative timeline. Supporting studies began in the late 1990's with the development of the HSSC line utilized in the trial and transitioned from HSSC characterization, through validation in animal models and stages required for human applications^{5, 72, 80, 81}, and finally to clinical trial approval in 2009^{5, 78-80}. The timeline in this figure reflects over a decade of preclinical supporting studies for the current ALS trial. This road map provides an outline of the rigorous stages required for the translation of stem cell therapies from the bench to bedside that may be applied to multiple neurodegenerative diseases.

Table 1

Common considerations when translating stem cell therapies to neurodegenerative disease patients.

Inclusion/exclusion criteria	Enrolling late-stage patients may prevent loss of quality of life Late-stage patients may mask any positive effects due to the intervention occurring too late in the disease course
Realistic expectation	Informed consent forms must clearly illuminate the goals of the study Safety trials vs. efficacy trials Expectations of therapeutic effects based on disease state at intervention
Controlled study	Ideal study is a double-blind placebo study Late-stage patients may mask any positive effects not observed due to the intervention occurring too late in disease Original PD studies offered control arms treatment after a 1-year follow-up which confuses interpretation of efficacy
Immunosuppression	While the brain remains an immunologically privileged site due to the blood-brain-barrier, there is evidence that this barrier can be compromised in disease Studies into cell graft survival demonstrate that immunosuppression increases that survival of graft tissue
Potential side effects	Prevent/minimize potential side effects (i.e. meningitis, fever) Avoid exacerbation of disease and tumor formation Risk vs. quality of life
Safety of cellular therapy administration	Consider CNS accessibility and safety of delivery methods Pros/cons of systemic delivery, lumbar puncture or stereotactic injection are important

Abbreviations: PD, Parkinson's disease; CNS, central nervous system.