### Title

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### Journal

Expert Opinion on Biological Therapy, 16(8)

### ISSN

1471-2598



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### Publication Date

2016-08-02

### DOI

10.1080/14712598.2016.1183641

Peer reviewed

Taylor & Francis
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Expert Opinion on Biological Therapy

ISSN: 1471-2598 (Print) 1744-7682 (Online) Journal homepage: https://www.tandfonline.com/loi/iebt20

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**To cite this article:** Peter Deng, Johnathon D. Anderson, Abigail S. Yu, Geralyn Annett, Kyle D. Fink & Jan A. Nolta (2016) Engineered BDNF producing cells as a potential treatment for neurologic disease, Expert Opinion on Biological Therapy, 16:8, 1025-1033, DOI: 10.1080/14712598.2016.1183641

**To link to this article:** https://doi.org/10.1080/14712598.2016.1183641

Accepted author version posted online: 09 May 2016.
Published online: 21 May 2016.

EXPERT OPINION ON BIOLOGICAL THERAPY, 2016
VOL. 16, NO. 8, 1025–1033
http://dx.doi.org/10.1080/14712598.2016.1183641

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REVIEW

# Engineered BDNF producing cells as a potential treatment for neurologic disease

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**ABSTRACT**

**Introduction**: Brain-derived neurotrophic factor (BDNF) has been implicated in wide range of neurological diseases and injury. This neurotrophic factor is vital for neuronal health, survival, and synaptic connectivity. Many therapies focus on the restoration or enhancement of BDNF following injury or disease progression.

**Areas covered**: The present review will focus on the mechanisms in which BDNF exerts its beneficial functioning, current BDNF therapies, issues and potential solutions for delivery of neurotrophic factors to the central nervous system, and other disease indications that may benefit from overexpression or restoration of BDNF.

**Expert opinion**: Due to the role of BDNF in neuronal development, maturation, and health, BDNF is implicated in numerous neurological diseases making it a prime therapeutic agent. Numerous studies have shown the therapeutic potential of BDNF in a number of neurodegenerative disease models and in acute CNS injury, however clinical translation has fallen short due to issues in delivering this molecule. The use of MSC as a delivery platform for BDNF holds great promise for clinical advancement of neurotrophic factor restoration. The ease with which MSC can be engineered opens the door to the possibility of using this cell-based delivery system to advance a BDNF therapy to the clinic.

**ARTICLE HISTORY**
Received 4 March 2016
Accepted 25 April 2016
Published online 20 May 2016

**KEYWORDS**
BDNF; Mesenchymal Stem Cell; Gene Therapy; Huntington's disease; Neurodegeneration

## 1. Brain-derived neurotrophic factor introduction

There is an increasing body of evidence for the importance of neurotrophins (NTF) and their role in developmental and mature neuronal cell health. The first NTF, nerve growth factor (NGF), was discovered in the 1950s by a group of embryologists,[1] and over the past 50 years additional NTFs such as brain-derived neurotrophic factor (BDNF) in pig brain in 1982,[2] neurotrophin 3 (NT3),[3] and neurotrophin 4 (NT4) [4] have been discovered. In the mature brain, NTFs have two primary functions. First, NTFs offer trophic support that enhances neurogenesis and neuroprotection. Second, NTFs are potent modulators of synaptic plasticity, a defining role in learning throughout life. BDNF is of potential interest due to the widespread prevalence of TrkB, BDNF's preferential receptor in the central nervous system (CNS), and its role in potentiating neuronal cell fate through various canonical signaling cascades [5] that influence survival, neuronal outgrowth,[6,7] and plasticity.[8] Loss of BDNF has been implicated in a number of neurodegenerative diseases,[9] while restoration or addition of exogenous BDNF has shown a physiologic therapeutic effect [10–12] (Figure 1).

This review will briefly describe the gene expression and signal transduction pathways of NTFs, the role of BDNF in the developing and mature nervous system, and its indication in a number of neurodegenerative diseases. The review will also summarize the preclinical and clinical research on BDNF, challenges with *in vivo* delivery of BDNF, and application to new disease indications.

### 1.1. BDNF mechanism

NTFs are a class of growth factors with vital roles in the development of mature neuronal systems. Mature neurotrophin action is mediated by high-affinity tropomyosin receptor kinase (Trk) family of receptors which are signaled by dimerization of two receptor molecules. The Trk receptor family consists of a TrkA, TrkB, and TrkC subtype, and activation is characterized by intracellular autophosphorylation and ensuing secondary signaling cascades following receptor dimerization. NTFs demonstrate preferential binding to specific Trk receptors. NGF preferentially binds to TrkA, BDNF and NT4 to TrkB, and NT3 to TrkC.[13] Interestingly, p75NTR has equally low affinity for mature NTFs but high-affinity toward pro-neutrophins. BDNF is initially synthesized as a precursor protein known as preproBDNF that is cleaved to proBDNF that is further cleaved into mature BDNF by tissue plasminogen activator. When bound to TrkB, mature BDNF induces receptor dimerization, and autophosphorylates tyrosine residues that initiates secondary cascades that regulate neurogenesis, synaptic plasticity, and apoptosis.[14] This is achieved by three major signaling pathways: the phosphatidyl inositol 3-kinase (PI3K)–serine–threonine kinase (AKT), mitogen-activated protein kinase (MAPK) extracellular-related kinase (ERK) pathway, and the phospholipase C y (PLCy)-Cam Kinase (CaMK) pathway. Interestingly, studies have shown that both proBDNF and mature BDNF have varying functions by different intracellular pathways. The binding of proBDNF to p75NTR

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**Article highlights**

- Brain-derived neurotrophic factor is a critical neurotrophin regulating cell survival, neuronal outgrowth, and plasticity.
- Brain-derived neurotrophic factor regulates neurogenesis, synaptic plasticity, and apoptosis via PI3K-AKT, MAPK, ERK, and y-CaM kinase pathway.
- Dysregulation of brain-derived neurotrophic factor has been implicated in many disorders including Alzheimer's, Parkinson's, and Huntington's disease.
- Delivery of brain-derived neurotrophic factor has been the major hurdle to its use in a clinical setting.
- Engineered mesenchymal stem cells may circumvent many of the issues surrounding brain-derived neurotrophic factor delivery.

This box summarizes key points contained in the article.

has been shown to promote long-term depression in rodent hippocampal neurons, in contrast with long-term potentiation by mature BDNF binding to TrkB.[15]

BDNF is translated and released in an activity-dependent manner. The *Bdnf* gene consists of 11 exons in humans and a single exon coding for the proBDNF protein. *Bdnf* transcription is controlled by nine different promoters that are active in a developmental, tissue specific, and activity-dependent manner.[16] Transcription of *Bdnf* is initiated at each of the 5′ noncoding exons that are spliced into the common proBDNF protein-coding 3′ exons. Alternatively, there are variable ATG sequences upstream of the exons I, VII, and VIII in humans, resulting in production of proBDNF with variable amino acid lengths at the N-terminal end of the protein.[16] *Bdnf* has two alternative polyadenylation sites, resulting in two distinct species of mRNA with either a long or short 3′UTR. While BDNF mRNA and protein are highly expressed in the hippocampus, cerebral cortex, amygdala, and cerebellum in the mammalian brain,[17] these BDNF transcripts are distributed in a structurally distinct manner as well, with short 3′ UTR transcripts present in the somata of hippocampal neurons, while the long 3′ UTR transcripts are found in the dendrites.[18] Studies in mice with a truncated long 3′ UTR demonstrated deficits in synaptic pruning, enlargement of dendritic spines, and selective impairment of long-term potential of dendrites but not the soma of hippocampal neurons.[19] BDNF is expressed to a lower extent in non-neural tissues such as heart, kidney, lung, and testis.

The multiple promoters present in *Bdnf* results in context-dependent responses to intracellular processes and extracellular disturbances. Promoter IV is of particular interest in that its expression is dependent on multiple transcriptional regulators: both $Ca^{2+}$ response elements and $Ca^{2+}$ sensitive transcription factors such as cyclic AMP-responsive element binding protein (CREB),[20] and methylated CpG binding protein.[21] Membrane depolarization and activation of glutamate receptors such as *N*-methyl-D-asparate (NMDA) receptors enhance BDNF expression.[22] $Ca^{2+}$ dependent neural activity induced by NMDA receptors stimulates transcriptional expression of BDNF due to multiple $Ca^{2+}$-response elements in exons I and IV of *Bdnf*.[23] Interestingly, CREB is tightly controlled by multiple signaling pathways triggered by $Ca^{2+}$ such as the rapidly accelerated

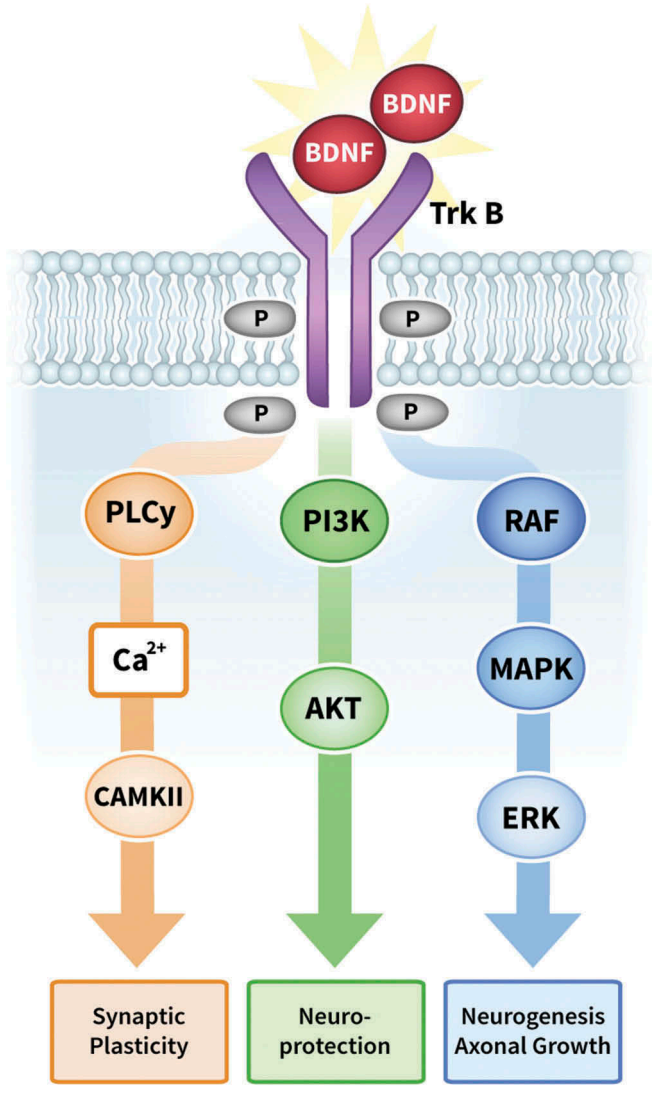


Figure 1. BDNF function acts through various canonical signaling pathways once bound to its preferred receptor TrkB to influence survival, neuronal outgrowth, synaptic plasticity, learning, neurogenesis and neuroprotection. Loss of BDNF has been indicated in a number of neurodegenerative diseases, while restoration or addition of exogenous BDNF has shown a physiologic therapeutic effect.

fibrosarcoma/MAPK/ERK and the CaMK; important pathways for sensory stimuli and learning. Together, this suggests that BDNF expression is strongly regulated by CREB; however, CREB activation can be due to a myriad of $Ca^{2+}$ induced pathways.

The proBDNF precursor is a 32-kDa protein generated in the endoplasmic reticulum and then cleaved to create 13-kDa mature BDNF protein via furin protease in the *trans*-Golgi network (TGN).[24] The pro-region of BDNF binds to the lipid-raft associated sorting receptor carboxypeptidase E (CPE) in the TGN and subsequently BDNF is shuttled into secretory vesicles, relaying the importance of the pro-region as a sorting motif.[25] A mutation (val66met) in the pro-region results in poorer sorting of BDNF into vesicles [26] and results in hippocampal volume loss [27], and altered experience dependent plasticity in motor neurons in human Alzheimer's disease (AD) patients.[28] BDNF-containing vesicles are transported to secretory sites by motor protein

complexes and can be released from the cell soma axon and dendrites in a depolarization-dependent manner *in vitro* [29] in response to $Ca^{2+}$ influx as mentioned earlier. Interestingly, mutant *Huntingtin*, the causative gene in Huntington's disease (HD), appears to attenuate vesicular transport of BDNF along microtubules in neurons [30] and wild-type *Huntingtin* acts as a transcription factor to enhance cortical BDNF.[31] Repression element silencing factor-1 (REST), a master regulator of neuronal expression, has been shown to have a strong association with *Huntingtin* and its normal function disrupted in presence of mutant *Huntingtin*. REST is normally found within the cytoplasm; however, mutant *Huntingtin* causes translocation of REST into the nucleus and represses several targets including BDNF.[32,33] This phenomenon is reversed upon silencing of REST.[34]

The proposed mechanism of BDNF action as a neuronal growth factor and synaptic regulator has been explored in several studies. Axonal outgrowth in hippocampal neurons is mediated by stabilization of cAMP/PKA activity following BDNF-induced local elevation of TrkB, driving axon initiation in undifferentiated neurites.[35] Both *in vitro* and *in vivo* studies have shown that BDNF promotes axon elongation and branching of sensory neurons [36] as well as increased dendritic outgrowth in BDNF-overexpressing neurons in rat cortical slice cultures.[37] Increased expression of BDNF in transgenic mice demonstrated preganglionic and axonal hypertrophy as well as increased synaptic innervation of sympathetic neurons.[11] Adult mutant mice with absent TrkB signaling during development showed less presynaptic terminals, reduced excitatory number, and altered structural morphology of synapses in the hippocampus.[38] Furthermore, aberrant branching and pruning of thalamic axon terminals was observed in conditional TrkB knockout mice in the thalamus.[39]

Decreased synaptic proteins and vesicle release have been shown in animal models with reduced TrkB signaling [40] whereas BDNF exposure results in increased number of docked synaptic vesicles in cultured hippocampal neurons.[41] BDNF potentiates neuronal circuitry by modulating synaptic efficiency. Exogenous BDNF increases glutamate transmission in rodent brain slices of the hippocampus [42] and visual cortex [43] and favors increased insertion of both NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors in the post-synapse of excitatory neurons,[44] important functions for learning. BDNF is a strong modulator for axonal development and synaptic connectivity in the mammalian brain.

The neurogenic potential of BDNF has been well known due to the intricate relationship between its preferential receptor, TrkB, to cell survival and proliferation pathways. However, BDNF has been implicated in a number of disease phenotypes such as ischemic stroke and epileptic seizure.[45] BDNF and TrkB expression in brain structures that are vulnerable to temporal lobe epilepsy (amygdala, etc.) are elevated following epileptic insult and acute infusion of BDNF into the murine brain appears to induce seizure.[46,47] However, opposing studies have shown that chronic infusion of BDNF reduces potential of reaching an epileptic state [48] and BDNF conditional knockout mice did not appear to demonstrate significant altered epileptic state.[49] Together, this suggests that while BDNF may have a role in epilepsy, it is not entirely well understood.

### 1.2. BDNF therapies

Deficits in BDNF have been an observed phenomenon in a number of diseases. Post-mortem studies in AD patients have shown significantly decreased BDNF expression in the hippocampus, temporal, and frontal cortex.[50] Spinocerebellar ataxia type 6 showed reduced BDNF mRNA expression in human cerebellum and sequestration of BDNF to abnormal loci in the neuron.[52] Reduced BDNF mRNA is observed in dopaminergic neurons in Parkinson's disease (PD) in comparison to normal counterparts and reduced neuronal density was correlated to total BDNF mRNA expression,[51] suggesting that loss of BDNF compromised the well-being of neighboring neurons.

BDNF impairment has been related to a number of neurocognitive disorders (reviewed in [56]), particularly in anxiety [57,58] and may contribute to the cognitive and emotional disturbances observed in HD. It is interesting to note that *Huntingtin* appears to be a regulator of BDNF transport and presence of the mutant protein results in aberrant transport and expression in hippocampal and cortical neurons, respectively.[30] Association of REST with mutant *Huntingtin* results in reduction of BDNF expression.[32,33] The BDNF protein is necessary for medium spiny neurons (MSNs) survival;[54] however, striatal neurons only produce very low levels of neurotrophic factors and are dependent on BDNF to be produced in the cerebral cortex and transported via corticostriatal tracts to MSNs.[59] While it has been reported in experimental models of neurodegenerative diseases that deep-brain stimulation, specifically in the subthalamic nucleus, can increase BDNF protein and mRNA levels in the striatum, functional recovery was only observed in the presence of the stimulation,[60] thereby limiting the long-term benefits without chronic exposure to deep-brain stimulation.

Interestingly, it was found that patients with symptomatic HD and lower levels of BDNF serum concentrations had significantly worse motor and cognitive performances than individuals with normal BDNF levels.[54,55] It is known that BDNF is very important for survival and differentiation of striatal neurons and lower BDNF may be a reason why these neurons deteriorate in HD.[55] Objective biochemical measures can allow one to monitor the progression of HD, and peripheral BDNF levels may become a potential marker to measure the state of the disease and/or the effectiveness of a given treatment.[55]

There have been considerable efforts in developing BDNF as a therapeutic, given the large amount of evidence for its role as a neuroprotective and neurogenic element and associated deficiencies in a number of diseases. Treatment with exogenous BDNF demonstrated sparing of forebrain cholinergic neurons [61] and reduction in Aβ peptide [10] in rat brains. Early BDNF treatment in transgenic AD mice ameliorated cell loss and increased synaptophysin in the entorhinal cortex, while also demonstrating enhanced hippocampal fear conditioning.[62] This same effect was observed in nonhuman

primates.[63] Enhanced striatal innervation and reduced cell loss were observed in a Parkinsonian nonhuman primate model following BDNF infusion.[64] Arrest of hippocampal neurogenesis results in impaired contextual fear conditioning following directed irradiation of the hippocampus,[65,66] suggestive that BDNF treatment results in enhanced neurogenesis and synaptic connection.

Marked sparing of motor neuron damage from acute spinal lesions is observed after administration of BDNF.[67–69] Environmental enrichment through increased exercise rescued low BDNF levels in R6 HD mice, reducing weight loss and spared motor symptoms.[70] Interestingly, BDNF has been implicated in some forms for neuropathic pain. Diabetic mice showed synaptic dysfunction and both reduced BDNF mRNA and protein.[71] *Gamma*-Aminobutyric acid (GABA) is down regulated in the dorsal horn of spinal lesioned mice. This effect is ameliorated by BDNF-induced GABA release following intrathecal injections of NTF, resulting in reduced mechanical hypersensitivity and thermal hyperalgesia in treated animals.[72] Allodynia and hyperalgesia induced by chronic constriction injury of the sciatic nerve in rats were reversed 1-week following adeno-associated virus (AAV)-BDNF injections, suggesting a potential therapy for chronic pain.[73] More recently, cerebellar injections of a herpesviral vector encoding BDNF prevented apoptosis of cerebellar granule cells and ataxic phenotype in an induced Fredreich's ataxia mouse model.[74]

Despite encouraging preclinical data using BDNF, the ability to deliver NTFs into the brain remains a challenge. NTFs are large, polarized proteins that do not readily cross the blood–brain barrier (BBB), necessitating delivery of NTFs directly into the CNS. BDNF administration in animal models of amyloid lateral sclerosis (ALS) demonstrated robust cell survival in spinal motor neurons and corticospinal neurons.[75] Ensuing clinical trials demonstrated BDNF presence in the cerebral spinal fluid following intrathecal administration of recombinant BDNF. While generally well-tolerated, high dose BDNF groups experienced adverse elements such as sleep disturbance and abnormal behavioral effects that necessitated dose reduction.[76] The large-scale, Phase III BDNF Study Group Trial enrolled 1135 patients and did not demonstrate statistical significance in a survival effect following subcutaneous recombinant BDNF administration.[77] Later, clinical trials were performed in smaller numbers and demonstrated the same lack of statistical significance following intrathecal BDNF infusion. Retrospective analysis suggests NTF administration either subcutaneously or intrathecally are unable to reach degenerating neurons [78,79] or cross the BBB [80–82] effectively. It is important to note that the associated adverse events observed during the BDNF Study Group were due to dose and not an innate toxicity from BDNF. A similar phenomenon was seen in mice with AAV-BDNF overexpression at high levels.[83]

## 2. Delivery of BDNF in the CNS

With the knowledge that BDNF is neuroprotective, researchers have attempted to increase BDNF production from endogenous cells within the striatum via adenoviral injections [84,85] or through AAV vector injections into the striatum [86] prior to quinolinic acid lesions. Both groups observed an increase in the numbers of cells expressing Dopamine- and cAMP-regulated phosphoprotein of 32 kDa (Darpp32; specific to MSNs) relative to lesioned control animals. Interestingly, in the latter group, an AAV was also utilized to increase glial-derived neurotrophic factor release.

New approaches in identifying an optimal delivery method for BDNF have been explored in recent years. Transplantation of neural stem cells in an alpha-synuclein mouse improved performance in cognitive and motor domains following restoration of BDNF expression,[87] mimicking effects seen in AAV-mediated BDNF.[88] Nanoparticle-mediated therapy elevated BDNF in the hippocampus mitigated levels of Aβ and tau proteins while augmenting neuronal cell survival in AD transgenic rats.[89] Overexpression of BDNF using mesenchymal stem cells (MSCs) in HD mouse models have shown amelioration of behavioral deficits in HD mouse models,[90] increased striatal volume and neurogenesis.[12]

Effective delivery of BDNF for neurological disorders remains a major challenge due to its very short half-life, which severely limits the effectiveness of the recombinant protein. Several studies have examined various exogenous delivery methods that may be utilized to translate BDNF-based therapeutics to the clinic. Benraiss et al. used AAV vectors to express BDNF in striatal neurons and demonstrated that AAV delivery of BDNF-induced neurogenesis and promoted a longer lifespan in a murine model of HD.[91] Interestingly, this benefit was potentiated by a factor secreted by mesenchymal stem/stromal cells, noggin.[92–94] Despite this success, the use of AAV in the clinic has proven difficult due to host immunogenicity to the virus and limited biodistribution.[95] In comparison, MSCs demonstrate paracrine effects such as local immune modulation and release of beneficial factors such as human growth factor, fibroblast growth factor-2, insulin growth factor 1 and vascular endothelial growth factor (VEGF), BDNF, Dickkopf-related protein 1, b-NGF that favor growth.[96] Furthermore, MSCs home to sites of inflammation, apoptosis, and hypoxia, [97] making it an attractive targeting vehicle for sites of injury.

Our group at the Institute for Regenerative Cures at the University of California, Davis have tested the safety and efficacy of genetically engineered human bone marrow MSCs in transgenic HD mouse models and published the results of our investigative new drug-enabling studies in *Molecular Therapy*.[12] These cells were prepared in a clinically compliant manner, mimicking cells proposed for a Phase I clinical trial. The YAC128 and R6/2 transgenic HD mouse models were used for behavioral and histological studies following transplantation to measure efficacy of MSC/BDNF as a putative therapy for HD.

Human MSC genetically engineered to produce BDNF reduced the total striatal atrophy observed in HD mice by approximately 50%, as compared to untreated transgenic mice. It was observed that engineered MSC also induced significant neurogenesis, a potential mechanism of recovery following BDNF administration. Engineered MSC also provided significant behavioral recovery in transgenic mouse model of HD. Following transplantation, treated mice displayed significantly less anxiety-like behaviors than untreated transgenic

mice. These results, along with the abundance of peer-reviewed articles (see [98]) provide compelling evidence for the proposed use of genetically engineered MSC as a candidate therapy for changing the trajectory of disease progression in patients diagnosed with early-stage HD.

MSCs were chosen as the delivery platform for BDNF since they are known to secrete a variety of neurotrophic and other factors that reduce inflammation, reduce programmed cell death, enhance connections between neurons, and reduce cell toxicity.[98] MSCs have been shown to be readily engineered using viral vectors to robustly deliver growth factors.[99,100] Using gene-modified MSCs as a delivery strategy addresses certain safety concerns involved with the direct use of viral vectors, as MSCs do not permanently engraft into host tissues. In addition, MSCs do not require immunosuppression following allogeneic transplantation, and have a strong, demonstrable safety profile in clinical trials.[96,101] Most importantly, MSCs have been shown to be well-tolerated in phase I/II clinical trials without adverse events in a variety of diseases with no tissue matching. Currently, Atherys, SanBio, and Brain-Storm Therapeutics have all concluded Phase II clinical trials with MSCs with no reported adverse effects in either acute or chronic neurodegenerative insults such as ischemic stroke or amyotrophic lateral sclerosis. Importantly, Brain-Storm Cell Therapeutics concluded a phase I/IIa clinical trial in patients with amyotrophic lateral sclerosis using autologous MSCs induced to express neurotrophic factor (NurOwn) with mild and transient adverse effects reported. Strikingly, treated ALS patients demonstrated slowed disease progression following the conclusion of the phase IIa trial with improvements in breathing and reduced motor decline compared to pretreatment.[102]

MSC/BDNF combine the beneficial effects of BDNF administration to the striata along with the benefits of MSC secreted factor supplementation (e.g. noggin). Unlike direct BDNF delivery via viral vector injection or recombinant protein administration into the brain, MSCs migrate into areas of tissue damage and have been shown to have numerous tissue healing effects (reviewed in [98]). Studies have shown that MSCs do not permanently engraft into host tissues; however, the duration and strategic localization of BDNF production should be adequate to produce a beneficial effect that will outlast the survival of MSCs.[12,103]

### 2.1. Alternative disease indications for MSC-delivered BDNF

The adult nervous system has limited repair capabilities after injury due to intrinsic neuronal properties which prevent effective reinnervation. However, remaining uninjured axons can develop a few collaterals that reinnervate denervated neurons and this response is increased by NTFs such as BDNF. In addition, severed neurons may be rescued from degenerative atrophy and apoptosis upon treatment with BDNF.

Moreover, increased interest in BDNF as a co-therapeutic for acute spinal injury has been expressed due to its role in synaptic modulation and axonal regeneration. Enhanced BDNF is observed in subpopulations of motor nuclei in spinalized rats that underwent exercise, suggesting BDNF as marker for increased motor innervation.[104] Reduced BDNF is observed in both spared and resected dorsal root ganglia following partial ganglionectomy, but BDNF increased significantly several days following operation in spared ganglia, suggesting a role for BDNF in neuroplasticity following acute injury.[105] Increased BDNF application in spinal lesions in mice demonstrated increased sprouting of injured neurons.[106]

While multiple studies have demonstrated that BDNF can promote axonal regeneration and rewiring of injured nerve fibers, outcomes vary in their therapeutic effect. BDNF treatment may benefit from complementary signaling proteins such as those produced by MSC. After spinal cord injury, pro-apoptotic factors are typically upregulated; however, BDNF may induce pro-survival signaling cascades and rescue neurons that take part in restructuring neuronal circuitry.

Reinnervation of muscles is also critical to re-establishing the functional activity of the muscle–skeletal apparatus after peripheral nerve injuries. Several studies have determined that BDNF is critical to the repair of damaged peripheral nerves, and is upregulated via epigenetic mechanisms for several days post-injury.[107–109] Studies have demonstrated that BDNF induced axonal regeneration and axonal sprouting from the proximal end of cut nerves into denervated nerve stumps.[110,111] Taken together, numerous studies have demonstrated that BDNF-related therapies may prove beneficial for spinal cord and peripheral nerve injuries (Figure 2).

## 3. Expert opinion

BDNF is a crucial NTF for development and mature neuronal health. It is highly implicated in numerous neurological diseases such as AD, PD, HD and other neurodegenerative disorders, making it a prime therapeutic agent. Countless studies have shown the therapeutic potential of BDNF in promoting axonal regrowth, preserving synaptic strength, sparing neuronal loss in a number of neurodegenerative disease models, and facilitating reinnervation of neurons in acute CNS injury. Despite numerous preclinical studies providing evidence on the therapeutic potential of BDNF, there has been difficulty in translating this work to the clinic. The methods to deliver BDNF to the CNS in a safe and physiologically relevant manner have historically been lacking. The use of recombinant protein is fraught with issues of protein degradation, immune response, and an inability to cross the BBB in appreciable quantities. The use of viral vectors also runs the risk of immune response as well as insertional mutagenesis and the potential of overloading the endogenous neurons with excess BDNF, a concern in cases of epilepsy. As such, researchers must adapt existing technologies and develop new methods to surmount the current challenges in delivering NTFs clinically.

The use of MSC as a delivery platform for NTF, specifically BDNF, holds great promise to advance the clinical use of NTF replacement or supplementation. The ease with which MSC can be engineered opens the door to the possibility of using this cell-based delivery system to advance poly-therapeutics such as trophic support in addition to potent gene editing molecules. Our research team at the Institute for Regenerative Cures at the University of

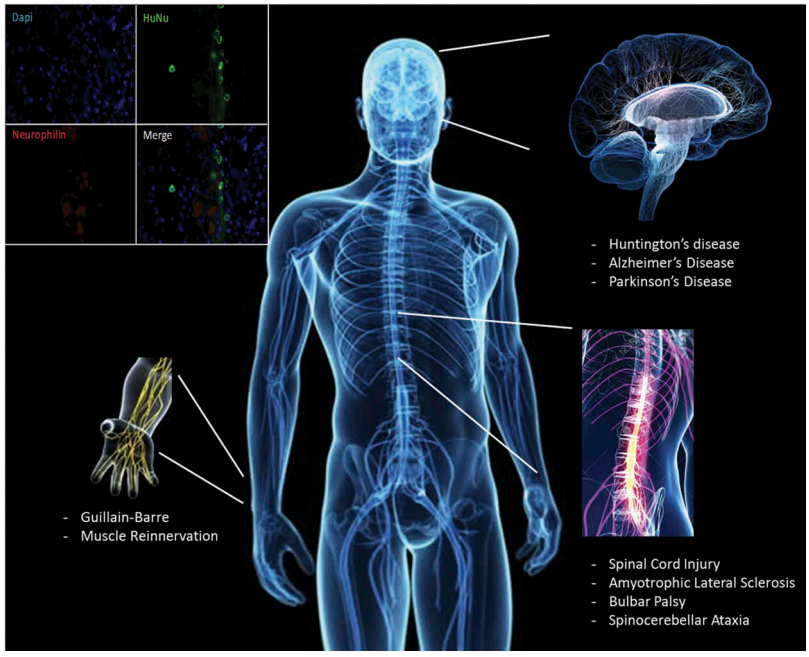


Figure 2. Therapeutic intervention using supplementation or replacement of BDNF has been implicated in a number of neurodegenerative disorders, spinal cord injuries, and nerve reinnervation. The upper left panel displays human MSC genetically engineered to produce BDNF in green (HuNu) following transplantation in the mouse brain with new axonal growth cones shown in red (Neurophilin).

California, Davis has specifically focused on translating promising preclinical data using MSC as a delivery platform for trophic factors including BDNF and VEGF for HD and critical limb ischemia, respectively. We have validated the preclinical efficacy and safety of MSC engineered to produce BDNF in transgenic mouse models of HD. The results from our animal studies indicate that transplantation of genetically engineered MSC are safe and provide significant behavioral and neuropathological benefits in two transgenic mouse models of HD.

We are currently conducting duration, biodistribution, and large animal (porcine) studies as recommended by the FDA for Investigational New Drug approval to potentially start a Phase I safety and tolerability study transplanting genetically engineered MSC in early-stage HD patients (HD-CELL). In anticipation of HD-CELL our group is conducting a Phase I observational trial (PRE-CELL NCT01937923) to longitudinally assess disease progression prior to potentially enrolling patients in a therapeutic trial. The parallel design of the preclinical and clinical observations not only pre-identifies a patient population that would be eligible to receive our MSC/BDNF stem cell product, but allows each subject to essentially serve as their own control when addressing disease progression. We feel that our strong preclinical data, along with the robust evidence in the literature that MSC-based delivery of BDNF or other NTF holds great promise to treat not only HD, but other neurological diseases or damage to the CNS.

## Acknowledgments

Support for this project was provided by a NIH NIGMS Predoctoral Fellowship T32GM099608 (Deng), NSF GRFP 2011116000, NIH T32-GM008799, NSF GROW 201111600, T32-HL086350 NIH (Anderson), NRSA Postdoctoral Fellowship F32NS090722 (Fink), a NIH Director's transformative award 1R01GM099688 (Nolta), The Stewart's and Dake Family Gift (Nolta/Fink), California Institute for Regenerative Medicine (CIRM) DR2-05415 (Wheelock/Nolta), and philanthropic donors from the HD community, including the Roberson family and Team KJ.

## Declaration of interests

This paper was supported by funds from the NIH, California Institute for Regenerative Medicine, and unrestricted philanthropic gifts. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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• of interest
•• of considerable interest

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