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Neurogenesis: Gene-Based Strategies for Treating Ischemic Stroke

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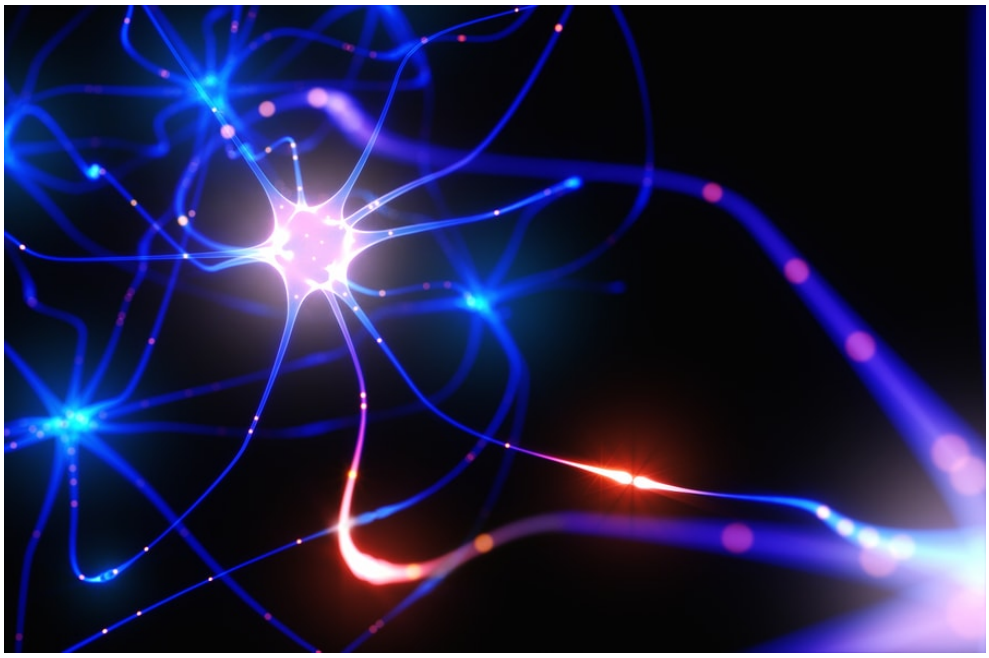

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Abstract

Stroke is one of the predominant causes of long-term disability and death, and is characterized by the cessation of blood flow to part of the brain, preventing it from accessing oxygen and nutrients needed for ATP production and proper function. Ischemic stroke, the most prevalent type of stroke, involves an obstruction such as a blood clot in a blood vessel. Current treatments seek to remove the obstruction and restore blood flow, but are time-limited and do not reverse tissue damage once it has occurred. The endogenous response to ischemic stroke involves an increase in neurogenesis but falls short in producing functional recovery. Gene therapy that enhances neurogenesis has provided encouraging results in animal models. Preclinical studies in this area have utilized neurotrophic factors that can promote migration of cells from the subventricular zone (SVZ). The transcription factor NeuroD1 converts reactive glial cells, which increase during ischemia, into neurons that integrate into the existing circuits of the brain. The clinical value of gene therapy depends upon the development of safe and efficient administration methods. In this review, we draw from scientific literature to evaluate the relevant genes and vectors for treating ischemic stroke through neurogenesis, and discuss strategies to overcome current limitations of gene therapy in human patients.

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Introduction

Stroke is a leading cause of death, accounting for 5.5 million deaths worldwide each year[1]. In the United States, stroke is attributed to 1 of every 19 deaths, and one person will suffer from a stroke every 40 seconds. While 80% of those who suffer from strokes survive, many are impaired with permanent disability. Although several types of strokes exist, including ischemic, hemorrhagic and transient ischemic attacks, the majority of research is focused on ischemic stroke, which makes up 80% of cases[2]. Ischemic stroke results from an obstruction of blood vessels that lead to the brain and causes an impairment of function[1]. This arises from atherosclerosis due to cerebral thrombosis, a blood clot that develops at the fatty deposits, or cerebral embolism, a blood clot that forms in large arteries in the neck, chest or heart and travels into the smaller blood vessels of thebrain[3]. Based on the duration that the brain is deprived of blood and the region that is blocked, strokes can bring about temporary or permanent disabilities, if not death[1].

While stroke continues to be among the most debilitating illnesses, current treatments for stroke are limited by their time of administration and most notably cannot reverse tissue damage once it has occurred. The most prominent and vastly used ischemic stroke treatments created thus far are emergency interventions given to stroke patients within a few hours of stroke onset, and are limited in safety and availability. These include thrombolysis, the chemically induced breakdown of blood clots, and thrombectomy, the surgical removal of the blood clots with a catheter[4]. A stroke patient must receive the standard thrombolytic treatment, tissue plasminogen activator (tPA), within 4 ½ hours of onset, and patients above 80 years of age must receive tPA within 3 hours[5]. Moreover, tPA comes with the risk of hemorrhage, neurologic decline and orolingual angioedema[6], and its positive outcome rate is estimated to be only 43%[5]. Thrombectomy is a more versatile option because of its ability to treat patients with a proximal occlusion, unlike thrombolysis. Still, it is restricted by a six-hour window from the onset of symptoms and is associated with risks such as hemorrhage, reocclusion and cerebral edema[7,8].

Neurogenesis

A strategy for treating ischemic stroke by gene therapy involves increasing neurogenesis, the process by which neurons are formed by neural stem cells (NSCs). Neurogenesis is the basis of cognitive function and allows the brain to respond to new stimuli[9]. Through constant refining and moderation of these neurons, the brain is able to adapt to cognitive, environmental, and pathophysiological demands. During prenatal and embryonic stages of human development, the majority of neurons are formed, but the process is continued through adulthood at a decreased rate in localized zones[10].

During prenatal neurogenesis, the central nervous system (CNS) consists of NSCs, specifically radial glial cells (RGCs), that generate neurons[11]. The RGCs occupy a majority of the prenatal ventricular zone and move from symmetric to asymmetric cell division. As asymmetric division and proliferation occurs, the RGCs separate, allowing for the creation of neural progenitor cells (NPCs) and intermedial neuronal precursors. After further separation, new neurons are formed[12]. As these neurons migrate through the embryo, they generate neural circuitry and increase in maturation. The rate of this prenatal neurogenesis and the type of neurons formed depend on genetic and molecular factors, although prenatal neurogenesis in humans typically occurs at gestational week 5 and is thoroughly developed by week 34[13].

Although the majority of neurogenesis occurs before a human is born, the neurogenic processes that occur throughout the lifespan continue to play a significant role in brain function[9]. In contrast to prenatal neurogenesis, which occurs throughout the entire CNS, neurogenesis during adulthood occurs primarily in the subventricular zone (SVZ) and subgranular zone (SGZ)[15]. Stem cells formed during adulthood do not spontaneously activate, but instead are dormant in the brain and only activate in response to new cognitive or environmental signals[16]. Along with the lack of spontaneous growth and decreased proliferation of neurons during adulthood, the density, plasticity, and ability to repair neurons also diminishes over time[17]. These factors all contribute to a decreased ability of

the brain to recover after injury. Because neurological impairments, including those caused by ischemic stroke, are more prevalent among older populations, the creation of healthy neurons after neurological injury is rarely sufficient to induce functional recovery[18].

Pathophysiology of Ischemic Stroke

In ischemic stroke, the obstruction of blood vessels to the brain limits cerebral oxygen and glucose supply, causing tissue damage. This reduction in oxygen and glucose due to inadequate cerebral blood flow initiates biochemical cascades that lead to cell death[19]. Oxygen and glucose are required to produce ATP, the key source of energy in cells, and as a result of ischemia, ATP levels decline[20]. This prevents the function of ion pumps that rely upon ATP to maintain ionic gradients. Failure of the Na^+/K^+ ATPase pump, which normally transports K^+ into the cell and Na^+ out of the cell, causes excess intracellular Na^+ and extracellular K^+. Intracellular Ca^{2+} levels rise because of Ca^{2+} pump failure[21], and the binding of Ca^{2+} to synaptotagmin stimulates glutamate release to the synapse. Excess glutamate leads to the hyperexcitation of NMDA glutamate receptors, providing positive feedback for intracellular Ca^{2+} levels[22]. In a phenomenon known as excitotoxicity, excess glutamate sets the stage for mechanisms of cell death. Excess intracellular Ca^{2+} levels activate endonucleases, lipases, phosphatases and proteases[23], which contribute to cell membrane disruption, cytoskeletal breakdown, DNA fragmentation, free radical production and mitochondrial damage. These processes lead to cell death by apoptosis or necrosis and irreversible tissue damage[19].

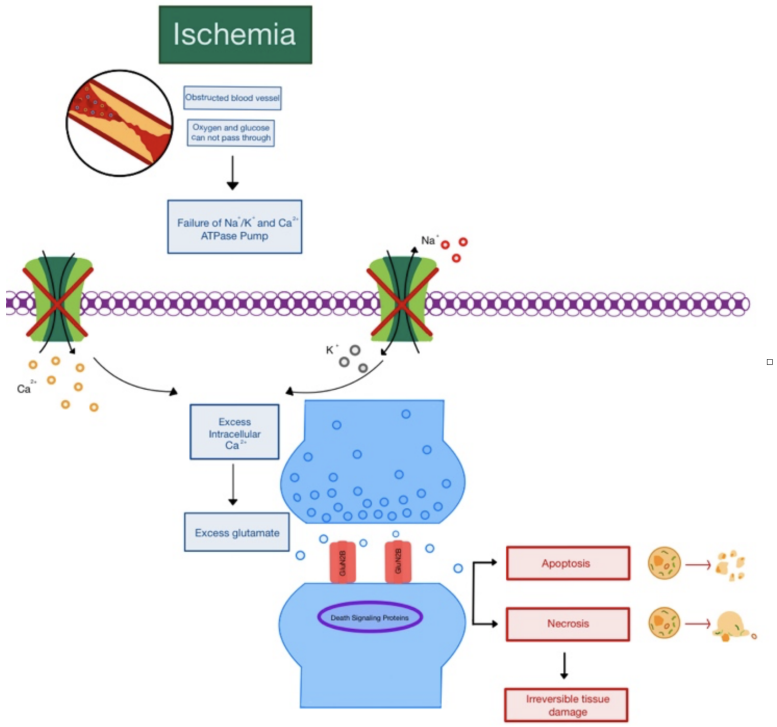

Figure 1: Pathway from ischemic stroke to tissue damage in the brain. *Obstruction of blood vessels leading to the brain reduces oxygen and glucose supply, which are required for ATP production. Ca^{2+} and Na^{+}/K^{+} pumps rely on ATP and their failure in ischemic conditions causes ion imbalances. Excess intracellular Ca^{2+} leads to glutamate excitotoxicity and cell death.*

Ischemic stroke additionally results in neuroinflammation. Inadequate blood supply activates microglia, leading to inflammation through cytokine upregulation, disruption of the blood-brain barrier (BBB) and the production of reactive oxygen species. In astrocytes, inflammatory factors like glial fibrillary acidic protein (GFAP) result in reactive gliosis and glial scar formation, and failure of the Na^{+}/K^{+} ATPase pump leads to the swelling of astrocytes. Ischemia obstructs the connection between astrocytes and endothelial cells at the BBB, allowing peripheral inflammatory cells to breach[24].

The glial scar—primarily made up of astrocytes and microglia—forms to protect the brain after stroke but prevents the regeneration of neurons. In

ischemic stroke, astrocytes undergo morphological and functional changes, proliferating and overlapping with each other during reactive gliosis while GFAP is upregulated. The scar has neuroprotective effects, such as providing a barrier between healthy and damaged tissue and limiting the spread of tissue damage[25,26]. However, it impedes the restoration of neural function in stroke[25] because axons physically cannot cross the scar, and reactive astrocytes produce growth-inhibiting factors that inhibit axon extension. While eliminating reactive astrocytes increases tissue damage, due to their benefits[15], strategies to mitigate the obstructive effects of the scar and restore long-term function would be favorable in treating stroke.

Neurogenesis increases as an endogenous response to ischemic stroke, but this is insufficient to restore function after stroke[27]. This process is driven by NSCs in the SVZ, which proliferate and differentiate due to an upregulation of neurotrophic factors[28]. The mechanism behind NSC proliferation and differentiation in post-stroke neurogenesis is reported to be induced by H19, a long non-coding RNA[29]. Stroke patients experience a level of functional improvement in the first few months following stroke due to self-repair[30], so enhancing neurogenesis would be a promising approach to improve function after stroke.

In vivo animal models are typically used for preclinical research of ischemic stroke and allow researchers to replicate the pathophysiology of the condition in humans with far more complexity than in vitro models[31]. The standard method to model focal ischemic stroke in animals is through the occlusion of the middle cerebral artery (MCA)—which is easily accessible and the most common site of stroke in humans—by mechanical devices, blood clots or pharmacological agents[32,33]. MCA occlusion is often induced by inserting an intraluminal thread into the internal carotid artery via the external carotid artery to block the MCA. This leads to significant damage in local brain tissue within three hours but can be lacking in precision, despite its non-invasiveness. More direct access to the MCA requires craniectomy, the surgical opening of the skull, which exposes the brain to the atmosphere and poses a risk for cerebrospinal fluid leakage, infection and unintended damage[34].

Gene Therapy to Enhance Neurogenesis

To induce neurogenesis by gene therapy, genes are typically administered through a vector. To successfully deliver a gene in vivo, we would have to identify the appropriate transgenes for the local recruitment of endogenous stem cells. This technique could be used to slow the spread of neural decline or generate a neural niche in regions that did not already express the necessary signals or factors[35]. Vectors are preferred over the delivery of naked DNA because of their greater efficiency, and viral vectors take advantage of viruses' evolved ability to alter gene expression in cells they infect[36]. Viruses deliver genetic information by infecting the cell directly, but in gene therapy, they are modified so they are unable to transfer unwanted information and result in pathogenicity. Delivery of vectors is carried out in two ways: in vivo, where the gene is delivered directly into the patient, and ex vivo, where cells are removed from the patient before gene delivery and then inserted back into the patient[37].

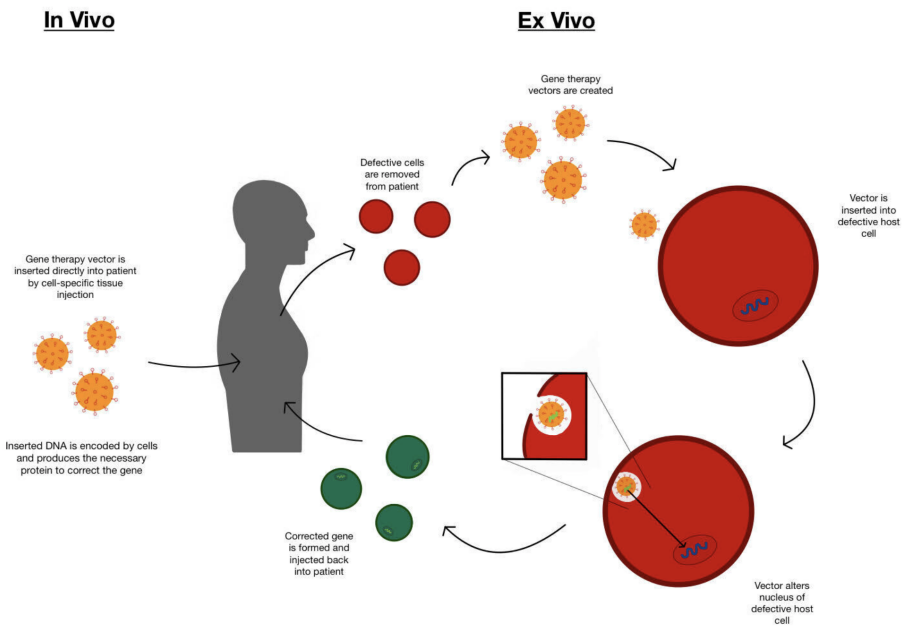

Figure 2: Schematic of gene therapy methods. *In vivo gene therapy often involves direct delivery of a vector into the body to alter gene expression. Ex vivo gene therapy is performed by removing cells from the body, delivering the vector into cells and transplanting altered cells into the body.*

Each viral vector offers unique advantages in gene therapy but has particular drawbacks. A class of vectors that is rising in popularity in gene therapy utilizes the adeno-associated virus (AAV)[38] because of its low immunogenicity and thus its propensity for an undesired immune response. Integration of AAV into the host genome depends on the presence of the rep gene, which exists in wild-type AAV. Recombinant AAV (rAAV) does not contain rep and therefore helps limit side effects[39]. A key disadvantage of AAVs that do not integrate into the host genome is that subsequent doses, if needed, would have a reduced efficacy due to an adaptive immune response. To combat this, methods to remove anti-AAV antibodies from the bloodstream, including plasmapheresis and IgG-cleaving endopeptidases, have been developed and shown effectiveness[37]. Serotypes of AAV have also been engineered to improve its permeability to the BBB, which is necessary for peripheral administration in treating CNS disorders like stroke[40]. For example, AAV-PHP.eB has the ability to transduce most neurons in the cortex and striatum and efficiently promote gene expression in the CNS[41].

Another viral vector being investigated for use in ischemic stroke models is the adenoviral vector, which offers greater capacity and transfection efficiency. A study of adenovirus-mediated gene transfer to an ischemic brain model found that this vector provided effective expression of transgene at the nonischemic and peri-ischemic areas[42]. However, adenoviral vectors are limited by their high immunogenicity, and high doses can lead to inflammation[43]. Lentiviral vectors, derived from the retrovirus family, have lower immunogenicity than other retrovirus-based vectors and most notably integrate into the host genome. This ability makes them useful for stimulating stable long-term expression. At the same time, genomic integration puts target cells at risk for insertional mutagenesis[44]. Unlike lentiviral vectors, retroviral vectors have limited use in gene therapy to promote neurogenesis because they only transduce dividing cells, and neurons are non-dividing cells[45]. All viral vectors have advantages and drawbacks and should be chosen on a case-by-case basis, but rAAV would generally be favorable to enhance neurogenesis in humans. Low immunogenicity should be prioritized from a therapeutic standpoint, and this is a defining characteristic of rAAV.

Although less common than viral vectors, another area of interest in gene therapy for stroke is the delivery of genes by non-viral means. In general, non-viral vectors limit the risk of cytotoxicity, immunogenicity and mutagenesis, making them an attractive alternative to viral vectors for therapeutic use in humans, while being less costly. Their effectiveness is hindered by their relatively low transfection efficiency, specificity and duration of expression[46]. The conventional method of non-viral gene delivery is polyethylenimine (PEI), a polymer that is associated with cytotoxic risks due to its high positive charge density[47]. Conjugation of PEI with deoxycholic acid to carry the anti-inflammatory gene heme-oxygenase 1 can help to overcome this challenge and reduce infarct size from ischemic stroke[48]. Despite the potential of non-viral vectors, their viral counterparts remain the most prevalent type of vector in research and existing drugs because of their efficiency, diversity and adaptability[49].

Neurotrophic Factors in Gene Therapy

Neurotrophic factors promote the growth and survival of neurons and are the primary group of molecules that can be overexpressed through gene therapy to induce neurogenesis. They serve an array of other functions in treating stroke[50]. Neurotrophic factors play roles in the development of axons, dendrites and synapses[51], and are endogenously upregulated in response to stroke[52]. Neurotrophins, a family of neurotrophic factors, target transmembrane receptors Trk and p75-NTR. Neurotrophins bind, causing dimerization and transphosphorylation of Trk. This activates PLCγ, which produces IP3 and DAG to enhance intracellular Ca^{2+} release and promote synaptic plasticity via protein kinase C. The growth of axons and dendrites is mediated by a separate pathway featuring Trk, Ras and ERK. The PI3-K pathway activates serine/threonine kinase to drive neuronal survival[53]. Several neurotrophic factors that have been investigated in gene therapy research pertaining to ischemic stroke, and their benefits and limitations, will be assessed in this section.

The first neurotrophic factor to be discovered and the one that is most well-understood is nerve growth factor (NGF)[50], a neurotrophin that promotes both neurogenesis via upregulation of growth-associated protein 43 (GAP-43) and angiogenesis via upregulation of vascular endothelial growth factor (VEGF)[54]. Serum NGF levels are inversely correlated with post-stroke function, potentially indicating a therapeutic link[55]. Pseudolentiviral gene therapy with β-NGF, a subunit of the factor with very similar effects, in the rat hippocampus increases neurogenesis and reduces apoptosis following ischemic stroke, as reported by Cao et al. (2018). Cognitive functional recovery was demonstrated by improved Morris water maze performance of rats that received β-NGF[56]. Non-invasive routes of NGF administration have been investigated as well. In a study by Zhu et al. (2011), intranasal delivery in rats improved survival in the striatum and SVZ but failed to enhance cell proliferation in those regions[57]. NGF has been explored in relation to non-viral vectors, in part to overcome the impermeability of NGF to the blood-brain barrier. In vitro carbon nanotube delivery showed that NGF treatment promotes neuron survival in a sustained manner, although its in vivo applications remain unclear[58]. Albumin nanocarriers successfully promoted neurite growth in vitro. However, in vivo delivery of NGF in combination with U0126, which inhibits the MEK pathway involved in ischemia, did not significantly reduce infarct size compared to U0126 alone, suggesting that this treatment is lacking in efficacy[59]. NGF delivery with exosomes in the ischemic cortex of mice resulted in long-lasting neuroprotection and indications of neurogenesis, but functional recovery was not tested[60]. Clinical trials of NGF in Alzheimer's disease and Parkinson's disease patients revealed negative side effects, such as weight loss and back pain due to nociceptive activation by NGF. The effect of NGF on nociception must be mitigated and methods for efficient transport across the BBB must be developed to make it an effective therapeutic option[61].

Brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin, is another factor that induces neurogenesis and helps prevent apoptosis. Overexpression of BDNF protects neurons from glutamate excitotoxicity and has been looked at as a potential therapeutic option in stroke patients[62]. At the same time, excess BDNF in the forebrain can have

negative effects on learning and memory[63]. As with NGF, stroke is associated with a reduction in BDNF levels in the bloodstream[64]. AAV-BDNF gene therapy targeting the SVZ, a key location of adult neurogenesis, of rats two weeks before contralateral MCA occlusion increases the migration of NPCs from the SVZ and contributes to behavioral recovery. It failed to reduce infarct size significantly, contradicting the findings of previous studies into the factor[65]. Gene therapy utilizing BDNF has also been used to reduce symptoms of stroke in preclinical research, including post-stroke pain and depression. This suggests that BDNF plays roles in both enhancing neurogenesis and reducing inflammation[66,67]. A clinical trial conducted in 1999 tested the effectiveness of BDNF, administered subcutaneously, in patients with amyotrophic lateral sclerosis, a neurodegenerative disease. Although side effects were minimal, the trial was unable to establish a significant benefit of BDNF treatment. Still, it had success in patients with early respiratory impairment and altered bowel function, a side effect of the treatment, indicating a need for further research[68]. Future trials with BDNF would likely benefit from more refined methods of gene transfer into subjects.

Neurotrophin-3 (NT-3) is part of the same family as NGF and BDNF and plays a distinct role in neurogenesis during development[53]. It is involved in the survival, proliferation and differentiation of neurons, as well as in the plasticity and regeneration of glutamatergic neurons. NT-3 is a contributor to revascularization and has anti-inflammatory properties[69]. Duricki et al. (2016) investigated the effectiveness of NT-3 in gene therapy treatment for stroke. AAV-mediated intramuscular delivery of the factor in adult and elderly rats 24 hours after stroke resulted in neuroplasticity, as observed through the sprouting of corticospinal axons, and behavioral recovery. Neuroprotection was not displayed due to the delay in treatment, and rats experienced minor inflammation from AAV injection[70]. NT-3 binds to TrkC receptors, which are not found on adult nociceptors, making it advantageous over the pain-regulating NGF. Duricki et al. (2019) confirmed this benefit in the forepaws of adult and elderly rats. Furthermore, human clinical trials in patients with neuropathy have not revealed significant side effects[71]. NT-3 appears to be a safer alternative to other members of the

neurotrophin family, and future research into its effect on neuron survival could reinforce a need for clinical studies in stroke patients.

Neurotrophic factors that are not part of the neurotrophin family have also attracted the attention of researchers in gene therapy. Among these is ciliary neurotrophic factor (CNTF), an important contributor to endogenous neurogenesis that occurs after stroke. CNTF promotes proliferation of cells from the SVZ[72]. Its continuous administration into the lateral ventricle for four weeks after MCA occlusion in rats had the capacity to reduce infarct size of the cortex and degeneration of the thalamus, while improving spatial learning[73]. MacLaren et al. (2006) found that CNTF has protective effects on ganglion cells, following intravitreal administration of AAV-CNTF[74]. Although not directly related to ischemic stroke, this study reveals the therapeutic applications of CNTF in preventing tissue damage from CNS injury that occurs in ischemic stroke. A clinical trial by Chew et al. (2019) using CNTF in patients with macular telangiectasia, a neurodegenerative condition of the retina, had success in promoting retinal neuroprotection[75]. While this trial showed minimal side effects, it is important to note in future human applications that CNTF and its neurogenic effects are linked to satiety and weight loss[76].

Glial cell-derived neurotrophic factor (GDNF) promotes neurogenesis in striatal neurons and is involved in synaptic plasticity. It primarily acts on dopaminergic neurons, stimulating the release of the neurotransmitter dopamine in the striatum[77]. Dopamine plays a major role in sensorimotor function, and research shows that ischemic stroke contributes to motor deficits through the loss of dopaminergic neurons[78]. Beker et al. (2022) reported that intracerebral lentiviral delivery of GDNF 10 days before MCA occlusion led to an increase in the expression of the dopaminergic neuron transcription factor Nurr1 and reduced degeneration of neurons in mice. The treatment did not appear to increase the number of striatal dopaminergic neurons following ischemic stroke[77]. Beker et al. (2020) observed that lentiviral GDNF delivery in mice induces both neurogenesis and angiogenesis and reduces the glial scar in the peri-infarct region. In addition, motor recovery was demonstrated[79]. Clinical trials using GDNF to

treat Parkinson's disease have not shown a significant improvement in motor function compared to a placebo group. Trials in which GDNF was delivered directly to the putamen had greater success, indicating that it could be useful through more efficient routes of administration[80].

Insulin-like growth factor 1 (IGF-1) contributes to neurogenesis and angiogenesis. Low levels of IGF-1 are associated with more severe tissue death in stroke patients, and it is produced as part of the endogenous response to stroke. Zhu et al. (2009) intracerebrally delivered AAV-IGF-1 after MCA occlusion, which was shown to promote neurogenesis by enhancing proliferation and migration of NSCs from the SVZ. Its ability to increase vascular density promotes cerebral blood flow[81]. Okoreeh et al. (2017) reported that AAV5-IGF-1 six to eight weeks before MCA occlusion was unable to reduce infarct size by targeting astrocytes. Behavioral recovery and a reduction in neuroinflammation were still observed[82]. The effect of IGF-1 on neurogenesis to treat diseases like stroke is yet to be clinically researched. Future preclinical studies are required to investigate its neuroprotective abilities before application in humans.

Vascular endothelial growth factor (VEGF) primarily induces angiogenesis but is also known to enhance neurogenesis in the SVZ and SGZ. Wang et al. (2007) shed light on the connection between VEGF overexpression and neurogenesis. Liposomes, a non-viral vector, were used to transfer VEGF into the lateral ventricle of the ischemic rat brain, increasing striatal neurogenesis and reducing the size of infarction[83]. Markosyan et al. (2020) tested adenoviral vectors carrying VEGF in combination with GDNF and neural cell adhesion molecule as a preventative option for ischemic stroke in rats. Intrathecal delivery reduced infarct size and brought about functional recovery of neurons, though neurogenesis was not observed[84]. Acute upregulation of VEGF is associated with disruption of the BBB, which must be considered for clinical trials and could be mitigated by combining VEGF with other angiogenic factors[85].

Conversion of Glial Cells to Neurons

A related approach to promote the formation of neurons after stroke involves neuroregeneration through the conversion of reactive glial cells, which proliferate in the post-stroke brain, into neurons. The endogenous response to ischemic stroke features the formation of the glial scar, largely made up of reactive astrocytes and microglia, which is driven by GFAP[25,26]. Considering the hindering effects of the scar and the low auto-regenerative abilities of the CNS, it would be favorable to convert components of the scar into neurons. Inducing neurogenesis through the conversion of reactive glial cells would both reduce the size of the glial scar and potentially restore neuronal function. The neural transcription factor neurogenic differentiation 1 (NeuroD1), which plays a major role in prenatal neurogenesis, has been the primary focus of gene therapy research aiming to convert glial cells into functional neurons as a treatment for stroke[86]. NeuroD1 binds to regulatory elements of neuronal development genes to promote neuronal migration and transcription via conversion of heterochromatin to euchromatin, a process that resembles the maturation of neurons seen in the prenatal stage[86,87]. Although several other transcription factors have been identified, including Neurogenin-2, Olig2 and Ascl1, and have been experimented in different combinations, they do not display as much efficiency or efficacy as NeuroD1 alone[88].

In vivo NeuroD1-based gene therapy studies have shown that the conversion of reactive glial cells to neurons promotes functional recovery from ischemic stroke. A study by Ge et al. (2019) using non-human primates (NHPs), which model the human brain more closely than rodents, showed that the overexpression of NeuroD1 in reactive astrocytes of the cortex successfully led to their conversion into neurons. After stroke induction, it was revealed that NeuroD1-AAV increased neuronal density and synaptic and dendritic markers, while protecting parvalbumin interneurons, in treated areas. A 90% reduction in targeted astrocytes was observed, as well as decreases in reactive microglia and macrophages. These changes were initiated between 10 and 30 days after the onset of stroke and lasted between two months and one year, suggesting that NeuroD1-mediated gene therapy is long-lasting and has a more generous

time window than existing treatments[89]. Jiang et al. (2021) reported that lentiviral delivery of NeuroD1 into the peri-infarct region of mice, one week following stroke onset, resulted in 66% of infected cells being marked as mature neurons, reduction in astrogliosis, and increases in synaptic plasticity and sensorimotor function[88]. A study of NeuroD1-AAV in mice by Chen et al. (2020) showed the ability of this factor to promote functional recovery from ischemic stroke, with 30% to 40% of neurons in the motor cortex being regenerated after NeuroD1 was expressed in reactive astrocytes[90]. Altogether, preclinical research into NeuroD1 has been encouraging, because it accomplishes two critical objectives in reversing tissue damage from stroke: reducing the obstructiveness of the glial scar and increasing neurogenesis. Nonetheless, gene therapy with NeuroD1 must be performed in a regulated manner, because eliminating the glial scar leads to adverse outcomes[15].

Practical Considerations

While preclinical in vivo studies have shown success in promoting functional recovery from ischemic stroke through gene therapy, several limitations still exist that prevent moving forward with treatments in humans. There have been few clinical trials in humans, many of which have produced inconclusive or discouraging results. Although studies involving rodents and NHPs serve as an important basis for our understanding of gene therapy, the differences between their genomes from humans could create unsuccessful results in the clinical stage.

One of the main obstacles in using gene therapy to treat CNS disorders like stroke in humans is the BBB. The vast majority of drugs including the factors discussed cannot pass through this barrier, but to minimize the invasiveness of gene therapy, vectors expressing therapeutic genes must be able to cross in an efficient manner. Therapeutic options that bypass the BBB require direct injection into the brain or high peripheral doses and present a significant risk to the patient[40,91]. High doses of AAV to treat X-linked tubular myopathy led to liver dysfunction, sepsis and the deaths of two patients in a 2020 clinical trial[92]. Intravenous administration is a non-invasive route, though it requires genes to cross the BBB. It additionally

lacks specificity, increasing the likelihood of off-target effects on peripheral organs[93]. The development of vectors that can efficiently cross the BBB would improve the feasibility of intravenous gene therapy. Research in this area has largely focused on serotypes of AAV, such as AAV-PHP.eB, although these variants reduce the already small packaging capacity of the vector[91,93]. In more recent strategies, vectors cross the BBB by paracellular transport through disruption of the barrier or by attaching to a transport receptor[94]. Intranasal delivery has received interest as a minimally invasive method of bypassing the BBB[95]. NGF has been administered intranasally in rat stroke models and had success in promoting neurogenesis[54].

Another aspect that must be considered is the risk for viral-mediated gene therapy treatment to provoke an immune response. Gene therapy with rAAV is promising for in vivo treatment of CNS diseases such as stroke because it is associated with low immunogenicity and no integration into the host genome[39]. However, this lack of integration is also a key drawback of rAAV, because anti-AAV antibodies are produced by the adaptive immune system, attenuating subsequent doses[37]. Existing approaches to remove these antibodies from the bloodstream, as outlined earlier, would make rAAV gene therapy far less practical in humans. Inducing neurogenesis through gene therapy may additionally target unwanted cells, allowing for potentially harmful results. Vectors that can integrate into the host genome, such as lentiviral and wild-type AAV vectors, present the greatest risk for insertional mutagenesis[44]. Therefore, the use of vectors that do not integrate may presently be the safest option.

Safety of gene therapy in humans requires that side effects are minimized. The target cells of genes must be as restricted as possible, which is dependent on how well the promoters used are able to target desired cells. Cell-type specificity of promoters remains a challenge, especially for intravenous administration[93]. Gene therapy that enhances neurogenesis to treat stroke aims to target neurons in the hippocampus and lateral ventricles, the two primary locations of adult neurogenesis. Finneran et al. (2021) reported that the promoters CAMKIIα and human synapsin 1 allow high-level gene expression specific to neurons, including those in the

hippocampus, and prevent peripheral expression when used with AAV. Use of CAMKIIα resulted in lower aberrant expression in the heart, lung and muscle compared to human synapsin 1[96]. For gene therapy utilizing NeuroD1, it would be beneficial to target reactive glial cells to optimize their conversion into neurons. The standard promoter used for expression in astrocytes, the main component of the glial scar, is GFAP, but its large size is unfavorable for AAV, which has a small capacity. gfaABC$_1$D is a smaller promoter that also targets astrocytes but research into its specificity has been inconclusive[97]. Taschenberger et al. (2017) investigated the MicroRNA124 (miR124)target sequence, which is specific to neurons, as a means of reducing off-target effects. The study demonstrated that miR124 greatly reduces off-target gene expression in neurons, although the level of expression was less than desired[98].

Future Directions

Although the factors examined in this review have generally shown promise in gene therapy for stroke in the preclinical stage, minimal clinical research has been conducted in relation to stroke specifically, and success in humans has been limited. Key areas of future research that would help open the door for gene therapy in humans include vectors that can efficiently cross the BBB and minimize aberrant gene expression. Improving the transfection efficiency of non-viral vectors could potentially make them a more practical option than viral vectors for gene therapy in humans, and thus should be an area of future research interest. Non-viral vectors are far lower than viral vectors in immunogenicity, cytotoxicity and mutagenesis. To make them a more practical option in gene therapy to treat stroke, strategies to improve their specificity and transfection efficiency must be further researched[46].

To gain a fuller understanding of ischemic stroke and the effectiveness of gene therapy as a treatment option, animal stroke models must be refined. The standard model for focal cerebral ischemia, intraluminal MCA occlusion, can lead to hyperthermia by blocking hypothalamic blood supply and subarachnoid hemorrhage if the suture is not inserted properly[99]. Laser doppler flowmetry has attracted attention as a way to accurately guide the

suture, but studies into its benefits for modeling MCA occlusion have had mixed results[100,101]. Alternative models must also be investigated so that new treatments encompass less common forms of ischemic stroke. Only 50.8% of ischemic stroke arise from the MCA but the vast majority of in vivo models used to evaluate gene therapy use MCA occlusion. Small-vessel and brainstem strokes, for example, combine to make up 24.2% of cases. Developing techniques to access deeper regions of the brain would help overcome this disproportion[102]. Further research into stroke models would improve the likelihood of translating preclinical successes in gene therapy into viable treatments for humans.

Gene therapy using neurotrophic factors and NeuroD1 must be carefully controlled in its duration to avoid the development of tumors. Accumulating evidence has implicated them in tumor neurogenesis, the formation and proliferation of cancer cells. NGF is overexpressed in breast, gastric, liver, lung, ovarian, pancreatic, skin and thyroid cancers. Cancer cells secrete NGF and contribute to tumor neurogenesis by acting on Trk receptors on nerves. Moreover, NGF is linked to heightened pain experienced by cancer patients[103]. Other neurotrophins, including BDNF, are upregulated in various types of cancer[104]. Studies have indicated that NeuroD1 is expressed in small cell lung and colorectal cancers[105,106]. However, the risk of cancer from these factors in gene therapy for stroke is yet to be thoroughly investigated.

Finally, the enhancement of neurogenesis through gene therapy must be further investigated in relation to symptomatology. Preclinical studies have focused on histological markers like growth of axons and dendrites and proliferation of neurons. Although previous research has indicated that adult neurogenesis from the hippocampus contributes to learning and memory[107], the direct effects of neurogenesis on cognitive recovery from stroke must be studied in greater detail, and the implementation of new measures would be beneficial. The factors discussed in this review had a wide range of effects in reducing the symptoms of ischemic stroke, so the direct role of neurogenesis remains largely unclear.

Conclusion

As one of the most common causes of death and disability around the world, ischemic stroke has become the focus of a growing number of studies related to gene therapy. Where current treatments may fail, gene therapy can reverse the pathophysiology of stroke and greatly extend the period in which patients can be treated after stroke and still experience functional recovery. Treatment can be administered to increase neuron proliferation and growth. Vectors expressing genes for neurogenic molecules have shown the capacity to augment the endogenous response to stroke, which involves low-level neurogenesis, in preclinical studies. Overexpression of neurotrophic factors, including the neurotrophins NGF, BDNF, NT-3, as well as CNTF, GDNF, IGF-1 and VEGF, may have the potential to enhance neurogenesis and aid in recovery. However, clinical trials have uncovered side effects that should be explored in greater detail. From a safety perspective, rAAV is the most favorable viral vector for stroke gene therapy due to its low immunogenicity, and methods for cell-type specificity should be refined. An alternative approach to neurogenesis is the conversion of glial cells to neurons. This tactic would employ NeuroD1 to reduce the size of the glial scar while simultaneously working to restore neuronal function. To progress further from preclinical in vivo studies to treatment in humans, further research is required to investigate less invasive methods for efficient gene therapy. A formidable obstacle is the BBB, which is extremely difficult to cross with minimal invasiveness. More research is needed into routes of administration and side effects to ensure success in human clinical trials.

References

1. Donkor ES. Stroke in the 21st Century: A Snapshot of the Burden, Epidemiology, and Quality of Life. *Stroke Res Treat*. 2018;2018:3238165. Published 2018 Nov 27. doi:10.1155/2018/3238165

2. Boehme AK, Esenwa C, Elkind MS. Stroke Risk Factors, Genetics, and Prevention. *Circ Res*. 2017;120(3):472-495. doi:10.1161/CIRCRESAHA.116.308398

3. Chugh C. Acute Ischemic Stroke: Management Approach. *Indian J Crit Care Med*. 2019;23(Suppl 2):S140-S146. doi:10.5005/jp-journals-10071-23192

4. Gravanis I, Tsirka SE. Tissue-type plasminogen activator as a therapeutic target in stroke. *Expert Opin Ther Targets*. 2008;12(2):159-170. doi:10.1517/14728222.12.2.159

5. Boehme AK, Esenwa C, Elkind MS. Stroke Risk Factors, Genetics, and Prevention. *Circ Res*. 2017;120(3):472-495. doi:10.1161/CIRCRESAHA.116.308398

6. Rabinstein AA. Update on Treatment of Acute Ischemic Stroke. *Continuum (Minneap Minn)*. 2020;26(2):268-286. doi:10.1212/CON.0000000000000840

7. Derex L, Cho TH. Mechanical thrombectomy in acute ischemic stroke. *Rev Neurol (Paris)*. 2017;173(3):106-113. doi:10.1016/j.neurol.2016.06.008

8. Krishnan R, Mays W, Elijovich L. Complications of Mechanical Thrombectomy in Acute Ischemic Stroke. *Neurology*. 2021;97(20 Suppl 2):S115-S125. doi:10.1212/WNL.0000000000012803

9. Ming GL, Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. *Neuron*. 2011;70(4):687-702. doi:10.1016/j.neuron.2011.05.001

10. Mira H, Morante J. Neurogenesis from embryo to adult – lessons from flies and mice. *Frontiers in Cell and Developmental Biology*. 2020;8. doi:10.3389/fcell.2020.00533

11. Iacopetti P, Michelini M, Stuckmann I, Oback B, Aaku-Saraste E, Huttner WB. Expression of the antiproliferative gene TIS21 at the onset of neurogenesis identifies single neuroepithelial cells that switch from proliferative to neuron-generating division. *Proc Natl Acad Sci U S A*. 1999;96(8):4639-4644. doi:10.1073/pnas.96.8.4639

12. Sidman RL, Rakic P. Neuronal migration, with special reference to developing human brain: a review. *Brain Res*. 1973;62(1):1-35. doi:10.1016/0006-8993(73)90617-3

13. Budday S, Steinmann P, Kuhl E. Physical Biology of Human Brain Development. *Frontiers in Cellular Neuroscience*. 2015;9. doi:10.3389/fncel.2015.00257

14. Lois C, García-Verdugo JM, Alvarez-Buylla A. Chain migration of neuronal precursors. *Science*. 1996;271(5251):978-981. doi:10.1126/science.271.5251.978

15. Huang L, Wu ZB, Zhuge Q, et al. Glial scar formation occurs in the human brain after ischemic stroke. *Int J Med Sci*. 2014;11(4):344-348. Published 2014 Feb 11. doi:10.7150/ijms.8140

16. Lim DA, Alvarez-Buylla A. The Adult Ventricular-Subventricular Zone (V-SVZ) and Olfactory Bulb (OB) Neurogenesis. *Cold*

Spring Harb Perspect Biol. 2016;8(5):a018820. Published 2016 May 2. doi:10.1101/cshperspect.a018820

17. Apple DM, Kokovay E. Vascular niche contribution to age-associated neural stem cell dysfunction. *American Journal of Physiology-Heart and Circulatory Physiology*. 2017;313(5). doi:10.1152/ajpheart.00154.2017

18. Cuartero MI, García-Culebras A, Torres-López C, et al. Post-stroke Neurogenesis: Friend or Foe?. *Front Cell Dev Biol*. 2021;9:657846. Published 2021 Mar 23. doi:10.3389/fcell.2021.657846

19. Rama R, García JC. Excitotoxicity and oxidative stress in acute stroke. *Ischemic Stroke - Updates*. October 2016. doi:10.5772/64991

20. Orellana-Urzúa S, Rojas I, Líbano L, Rodrigo R. Pathophysiology of Ischemic Stroke: Role of Oxidative Stress. *Curr Pharm Des*. 2020;26(34):4246-4260. doi:10.2174/1381612826666200708133912

21. Khoshnam SE, Winlow W, Farzaneh M, Farbood Y, Moghaddam HF. Pathogenic mechanisms following ischemic stroke. *Neurol Sci*. 2017;38(7):1167-1186. doi:10.1007/s10072-017-2938-1

22. Sekerdag E, Solaroglu I, Gursoy-Ozdemir Y. Cell Death Mechanisms in Stroke and Novel Molecular and Cellular Treatment Options. *Curr Neuropharmacol*. 2018;16(9):1396-1415. doi:10.2174/1570159X16666180302115544

23. Belov Kirdajova D, Kriska J, Tureckova J, Anderova M. Ischemia-Triggered Glutamate Excitotoxicity From the Perspective of Glial Cells. *Front Cell Neurosci*. 2020;14:51. Published 2020 Mar 19. doi:10.3389/fncel.2020.00051

24. Jayaraj RL, Azimullah S, Beiram R, Jalal FY, Rosenberg GA. Neuroinflammation: friend and foe for ischemic stroke. *J Neuroinflammation*. 2019;16(1):142. Published 2019 Jul 10. doi:10.1186/s12974-019-1516-2

25. Wang H, Song G, Chuang H, et al. Portrait of glial scar in neurological diseases. *Int J Immunopathol Pharmacol*. 2018;31:2058738418801406. doi:10.1177/2058738418801406

26. Linnerbauer M, Rothhammer V. Protective Functions of Reactive Astrocytes Following Central Nervous System Insult. *Front Immunol*. 2020;11:573256. Published 2020 Sep 30. doi:10.3389/fimmu.2020.573256

27. Rahman AA, Amruta N, Pinteaux E, Bix GJ. Neurogenesis After Stroke: A Therapeutic Perspective. *Transl Stroke Res*. 2021;12(1):1-14. doi:10.1007/s12975-020-00841-w

28. Marques BL, Carvalho GA, Freitas EMM, et al. The role of neurogenesis in neurorepair after ischemic stroke. *Semin Cell Dev Biol*. 2019;95:98-110.

29. Fan B, Pan W, Wang X, et al. Long noncoding RNA mediates stroke-induced neurogenesis. *Stem Cells*. 2020;38(8):973-985. doi:10.1002/stem.3189

30. Cuartero MI, García-Culebras A, Torres-López C, et al. Post-stroke Neurogenesis: Friend or Foe?. *Front Cell Dev Biol*. 2021;9:657846. Published 2021 Mar 23. doi:10.3389/fcell.2021.657846

31. Llovera G, Pinkham K, Liesz A. Modeling Stroke in Mice: Focal Cortical Lesions by Photothrombosis. *J Vis Exp*. 2021;(171):10.3791/62536. Published 2021 May 6. doi:10.3791/62536

32. Fluri F, Schuhmann MK, Kleinschnitz C. Animal models of ischemic stroke and their application in clinical research. *Drug Des Devel Ther*. 2015;9:3445-3454. Published 2015 Jul 2. doi:10.2147/DDDT.S56071

33. Hermann DM, Popa-Wagner A, Kleinschnitz C, Doeppner TR. Animal models of ischemic stroke and their impact on drug discovery. *Expert Opin Drug Discov*. 2019;14(3):315-326. doi:10.1080/17460441.2019.1573984

34. Li Y, Zhang J. Animal models of stroke. *Animal Model Exp Med*. 2021;4(3):204-219. Published 2021 Sep 15. doi:10.1002/ame2.12179

35. Marr RA, Thomas RM, Peterson DA. Insights into neurogenesis and aging: Potential therapy for degenerative disease? Future neurology. Published July 1, 2010. Accessed March 27, 2022.

36. Genet N, Hirschi KK. Understanding neural stem cell regulation *in vivo* and applying the insights to cell therapy for strokes. *Regen Med*. 2021;16(9):861-870. doi:10.2217/rme-2021-0022

37. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. *Signal Transduct Target Ther*. 2021;6(1):53. Published 2021 Feb 8. doi:10.1038/s41392-021-00487-6

38. Chen YC, Ma NX, Pei ZF, et al. A NeuroD1 AAV-Based Gene Therapy for Functional Brain Repair after Ischemic Injury through In Vivo Astrocyte-to-Neuron Conversion. *Mol Ther*. 2020;28(1):217-234. doi:10.1016/j.ymthe.2019.09.003

39. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov*. 2019;18(5):358-378. doi:10.1038/s41573-019-0012-9

40. Liu D, Zhu M, Zhang Y, Diao Y. Crossing the blood-brain barrier with AAV vectors. *Metab Brain Dis*. 2021;36(1):45-52. doi:10.1007/s11011-020-00630-2

41. Chan KY, Jang MJ, Yoo BB, et al. Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. *Nat Neurosci*. 2017;20(8):1172-1179. doi:10.1038/nn.4593

42. Ooboshi H, Ibayashi S, Takada J, Yao H, Kitazono T, Fujishima M. Adenovirus-mediated gene transfer to ischemic brain: ischemic flow threshold for transgene expression. *Stroke*. 2001;32(4):1043-1047. doi:10.1161/01.str.32.4.1043

43. Lukashev AN, Zamyatnin AA Jr. Viral Vectors for Gene Therapy: Current State and Clinical Perspectives. *Biochemistry (Mosc)*. 2016;81(7):700-708. doi:10.1134/S0006297916070063

44. Parambi DGT, Alharbi KS, Kumar R, et al. Gene Therapy Approach with an Emphasis on Growth Factors: Theoretical and Clinical Outcomes in Neurodegenerative Diseases. *Mol Neurobiol*. 2022;59(1):191-233. doi:10.1007/s12035-021-02555-y

45. Chen YH, Keiser MS, Davidson BL. Viral Vectors for Gene Transfer. *Curr Protoc Mouse Biol*. 2018;8(4):e58. doi:10.1002/cpmo.58

46. Zu H, Gao D. Non-viral Vectors in Gene Therapy: Recent Development, Challenges, and Prospects. *AAPS J*. 2021;23(4):78. Published 2021 Jun 2. doi:10.1208/s12248-021-00608-7

47. Xu D, Su Y, Xu Q, Huang T, Chen Z, Zhang T. Uniform iron oxide nanoparticles reduce the required amount of polyethylenimine in the gene delivery to mesenchymal stem cells. *Nanotechnology*. 2021;33(12):10.1088/1361-6528/ac4066. Published 2021 Dec 24. doi:10.1088/1361-6528/ac4066

48. Oh J, Lee MS, Jeong JH, Lee M. Deoxycholic Acid-Conjugated Polyethylenimine for Delivery of Heme Oxygenase-1 Gene in Rat Ischemic Stroke Model. *J Pharm Sci*. 2017;106(12):3524-3532. doi:10.1016/j.xphs.2017.07.020

49. Sainz-Ramos M, Gallego I, Villate-Beitia I, et al. How Far Are Non-Viral Vectors to Come of Age and Reach Clinical Translation in Gene Therapy?. *Int J Mol Sci*. 2021;22(14):7545. Published 2021 Jul 14. doi:10.3390/ijms22147545

50. Lorenzini L, Baldassarro VA, Stanzani A, Giardino L. Nerve Growth Factor: The First Molecule of the Neurotrophin Family. *Adv Exp Med Biol*. 2021;1331:3-10. doi:10.1007/978-3-030-74046-7_1

51. Hernández-Echeagaray E. Neurotrophin-3 modulates synaptic transmission. *Vitam Horm*. 2020;114:71-89. doi:10.1016/bs.vh.2020.04.008

52. Larpthaveesarp A, Ferriero DM, Gonzalez FF. Growth factors for the treatment of ischemic brain injury (growth factor treatment). *Brain Sci*. 2015;5(2):165-177. Published 2015 Apr 30. doi:10.3390/brainsci5020165

53. Houlton J, Abumaria N, Hinkley SFR, Clarkson AN. Therapeutic Potential of Neurotrophins for Repair After Brain Injury: A Helping Hand From Biomaterials. *Front Neurosci*. 2019;13:790. Published 2019 Aug 2. doi:10.3389/fnins.2019.00790

54. Li X, Li F, Ling L, Li C, Zhong Y. Intranasal administration of nerve growth factor promotes angiogenesis via activation of PI3K/Akt signaling following cerebral infarction in rats. *Am J Transl Res*. 2018;10(11):3481-3492. Published 2018 Nov 15.

55. Luan X, Qiu H, Hong X, et al. High serum nerve growth factor concentrations are associated with good functional outcome at 3 months following acute ischemic stroke. *Clin Chim Acta*. 2019;488:20-24. doi:10.1016/j.cca.2018.10.030

56. Cao JY, Lin Y, Han YF, et al. Expression of nerve growth factor carried by pseudotyped lentivirus improves neuron survival and cognitive functional recovery of post-ischemia in rats. *CNS Neurosci Ther*. 2018;24(6):508-518. doi:10.1111/cns.12818

57. Zhu W, Cheng S, Xu G, et al. Intranasal nerve growth factor enhances striatal neurogenesis in adult rats with focal cerebral ischemia. *Drug Deliv*. 2011;18(5):338-343. doi:10.3109/10717544.2011.557785

58. Hassanzadeh P, Arbabi E, Atyabi F, Dinarvand R. Nerve growth factor-carbon nanotube complex exerts prolonged protective effects in an in vitro model of ischemic stroke. *Life Sci*. 2017;179:15-22. doi:10.1016/j.lfs.2016.11.029

59. Feczkó T, Piiper A, Ansar S, et al. Stimulating brain recovery after stroke using theranostic albumin nanocarriers loaded with nerve growth factor in combination therapy. *J Control Release*. 2019;293:63-72. doi:10.1016/j.jconrel.2018.11.017

60. Yang J, Wu S, Hou L, et al. Therapeutic Effects of Simultaneous Delivery of Nerve Growth Factor mRNA and Protein via Exosomes on Cerebral Ischemia. *Mol Ther Nucleic Acids*. 2020;21:512-522. doi:10.1016/j.omtn.2020.06.013

61. Rocco ML, Soligo M, Manni L, Aloe L. Nerve Growth Factor: Early Studies and Recent Clinical Trials. *Curr Neuropharmacol*. 2018;16(10):1455-1465. doi:10.2174/1570159X16666180412092859

62. Gaidin SG, Turovskaya MV, Gavrish MS, et al. The selective BDNF overexpression in neurons protects neuroglial networks against OGD and glutamate-induced excitotoxicity. *Int J Neurosci*. 2020;130(4):363-383. doi:10.1080/00207454.2019.1691205

63. Cunha C, Angelucci A, D'Antoni A, et al. Brain-derived neurotrophic factor (BDNF) overexpression in the forebrain results in learning and memory impairments. *Neurobiol Dis*. 2009;33(3):358-368. doi:10.1016/j.nbd.2008.11.004

64. Chaturvedi P, Singh AK, Tiwari V, Thacker AK. Brain-derived neurotrophic factor levels in acute stroke and its clinical implications. *Brain Circ*. 2020;6(3):185-190. Published 2020 Sep 30. doi:10.4103/bc.bc_23_20

65. Yu SJ, Tseng KY, Shen H, Harvey BK, Airavaara M, Wang Y. Local administration of AAV-BDNF to subventricular zone induces functional recovery in stroke rats. *PLoS One*. 2013;8(12):e81750. Published 2013 Dec 2. doi:10.1371/journal.pone.0081750

66. Chen HH, Zhang N, Li WY, et al. Overexpression of brain-derived neurotrophic factor in the hippocampus protects against post-stroke depression. *Neural Regen Res*. 2015;10(9):1427-1432. doi:10.4103/1673-5374.165510

67. Shih HC, Kuan YH, Shyu BC. Targeting brain-derived neurotrophic factor in the medial thalamus for the treatment of central poststroke pain in a rodent model. *Pain*. 2017;158(7):1302-1313. doi:10.1097/j.pain.0000000000000915

68. A controlled trial of recombinant methionyl human BDNF in ALS: The BDNF Study Group (Phase III). *Neurology*. 1999;52(7):1427-1433. doi:10.1212/wnl.52.7.1427

69. de Miranda AS, de Barros JLVM, Teixeira AL. Is neurotrophin-3 (NT-3): a potential therapeutic target for depression and anxiety?. *Expert Opin Ther Targets*. 2020;24(12):1225-1238. doi:10.1080/14728222.2020.1846720

70. Duricki DA, Hutson TH, Kathe C, et al. Delayed intramuscular human neurotrophin-3 improves recovery in adult and elderly rats after stroke. *Brain*. 2016;139(Pt 1):259-275. doi:10.1093/brain/awv341

71. Duricki DA, Drndarski S, Bernanos M, et al. Stroke Recovery in Rats after 24-Hour-Delayed Intramuscular Neurotrophin-3 Infusion. *Ann Neurol*. 2019;85(1):32-46. doi:10.1002/ana.25386

72. Kang SS, Keasey MP, Arnold SA, Reid R, Geralds J, Hagg T. Endogenous CNTF mediates stroke-induced adult CNS neurogenesis in mice. *Neurobiol Dis*. 2013;49:68-78. doi:10.1016/j.nbd.2012.08.020

73. Kumon Y, Sakaki S, Watanabe H, et al. Ciliary neurotrophic factor attenuates spatial cognition impairment, cortical infarction and thalamic degeneration in spontaneously hypertensive rats with focal cerebral ischemia.

Neurosci Lett. 1996;206(2-3):141-144. doi:10.1016/s0304-3940(96)12450-2

74. MacLaren RE, Buch PK, Smith AJ, et al. CNTF gene transfer protects ganglion cells in rat retinae undergoing focal injury and branch vessel occlusion. *Exp Eye Res*. 2006;83(5):1118-1127. doi:10.1016/j.exer.2006.05.019

75. Chew EY, Clemons TE, Jaffe GJ, et al. Effect of Ciliary Neurotrophic Factor on Retinal Neurodegeneration in Patients with Macular Telangiectasia Type 2: A Randomized Clinical Trial. *Ophthalmology*. 2019;126(4):540-549. doi:10.1016/j.ophtha.2018.09.041

76. Xu B, Xie X. Neurotrophic factor control of satiety and body weight. *Nat Rev Neurosci*. 2016;17(5):282-292. doi:10.1038/nrn.2016.24

77. Beker MÇ, Beker M, Çağlayan AB, et al. Striatal dopaminergic neurons as a potential target for GDNF based ischemic stroke therapy [published online ahead of print, 2021 Nov 13]. *Turk J Med Sci*. 2021;10.3906/sag-2108-268. doi:10.3906/sag-2108-268

78. Lohmann S, Grigoletto J, Bernis ME, et al. Ischemic stroke causes Parkinson's disease-like pathology and symptoms in transgenic mice overexpressing alpha-synuclein. *Acta Neuropathol Commun*. 2022;10(1):26. Published 2022 Feb 24. doi:10.1186/s40478-022-01327-6

79. Beker M, Caglayan AB, Beker MC, et al. Lentivirally administered glial cell line-derived neurotrophic factor promotes post-ischemic neurological recovery, brain remodeling and contralesional pyramidal tract plasticity by regulating axonal growth inhibitors and guidance proteins. *Exp Neurol*. 2020;331:113364. doi:10.1016/j.expneurol.2020.113364

80. Gash DM, Gerhardt GA, Bradley LH, Wagner R, Slevin JT. GDNF clinical trials for Parkinson's disease: a critical human dimension. *Cell Tissue Res*. 2020;382(1):65-70. doi:10.1007/s00441-020-03269-8

81. Zhu W, Fan Y, Hao Q, et al. Postischemic IGF-1 gene transfer promotes neurovascular regeneration after experimental stroke. *J Cereb Blood Flow Metab*. 2009;29(9):1528-1537. doi:10.1038/jcbfm.2009.75

82. Okoreeh AK, Bake S, Sohrabji F. Astrocyte-specific insulin-like growth factor-1 gene transfer in aging female rats improves stroke outcomes. *Glia*. 2017;65(7):1043-1058. doi:10.1002/glia.23142

83. Wang YQ, Guo X, Qiu MH, Feng XY, Sun FY. VEGF overexpression enhances striatal neurogenesis in brain of adult rat after a transient middle cerebral artery occlusion. *J Neurosci Res*. 2007;85(1):73-82. doi:10.1002/jnr.21091

84. Markosyan V, Safiullov Z, Izmailov A, et al. Preventive Triple Gene Therapy Reduces the Negative Consequences of Ischemia-Induced Brain Injury after Modelling Stroke in a Rat. *Int J Mol Sci*. 2020;21(18):6858. Published 2020 Sep 18. doi:10.3390/ijms21186858

85. Shen F, Fan Y, Su H, et al. Adeno-associated viral vector-mediated hypoxia-regulated VEGF gene transfer promotes angiogenesis following focal cerebral ischemia in mice. *Gene Ther*. 2008;15(1):30-39. doi:10.1038/sj.gt.3303048

86. Guo Z, Zhang L, Wu Z, Chen Y, Wang F, Chen G. In vivo direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease

model. *Cell Stem Cell*. 2014;14(2):188-202. doi:10.1016/j.stem.2013.12.001

87. Pataskar A, Jung J, Smialowski P, et al. NeuroD1 reprograms chromatin and transcription factor landscapes to induce the neuronal program. *EMBO J*. 2016;35(1):24-45. doi:10.15252/embj.201591206

88. Jiang MQ, Yu SP, Wei ZZ, et al. Conversion of Reactive Astrocytes to Induced Neurons Enhances Neuronal Repair and Functional Recovery After Ischemic Stroke. *Front Aging Neurosci*. 2021;13:612856. Published 2021 Mar 26. doi:10.3389/fnagi.2021.612856

89. Ge L-J, Yang F-H, Li W, et al. In vivo neuroregeneration to treat ischemic stroke through neurod1 AAV-based gene therapy in adult non-human primates. *Frontiers in Cell and Developmental Biology*. 2020;8. doi:10.3389/fcell.2020.590008

90. Chen YC, Ma NX, Pei ZF, et al. A NeuroD1 AAV-Based Gene Therapy for Functional Brain Repair after Ischemic Injury through In Vivo Astrocyte-to-Neuron Conversion. *Mol Ther*. 2020;28(1):217-234. doi:10.1016/j.ymthe.2019.09.003

91. Mathiesen SN, Lock JL, Schoderboeck L, Abraham WC, Hughes SM. CNS Transduction Benefits of AAV-PHP.eB over AAV9 Are Dependent on Administration Route and Mouse Strain. *Mol Ther Methods Clin Dev*. 2020;19:447-458. Published 2020 Oct 20. doi:10.1016/j.omtm.2020.10.011

92. Morales L, Gambhir Y, Bennett J, Stedman HH. Broader Implications of Progressive Liver Dysfunction and Lethal Sepsis in Two Boys following Systemic High-Dose AAV. *Mol Ther*. 2020;28(8):1753-1755. doi:10.1016/j.ymthe.2020.07.009

93. Gessler DJ, Tai PWL, Li J, Gao G. Intravenous Infusion of AAV for Widespread Gene Delivery to the Nervous System. *Methods Mol Biol*. 2019;1950:143-163. doi:10.1007/978-1-4939-9139-6_8

94. Fu H, McCarty DM. Crossing the blood-brain-barrier with viral vectors. *Curr Opin Virol*. 2016;21:87-92. doi:10.1016/j.coviro.2016.08.006

95. Lochhead JJ, Thorne RG. Intranasal delivery of biologics to the central nervous system. *Adv Drug Deliv Rev*. 2012;64(7):614-628. doi:10.1016/j.addr.2011.11.002

96. Finneran DJ, Njoku IP, Flores-Pazarin D, et al. Toward Development of Neuron Specific Transduction After Systemic Delivery of Viral Vectors. *Front Neurol*. 2021;12:685802. Published 2021 Aug 26. doi:10.3389/fneur.2021.685802

97. O'Carroll SJ, Cook WH, Young D. AAV Targeting of Glial Cell Types in the Central and Peripheral Nervous System and Relevance to Human Gene Therapy. *Front Mol Neurosci*. 2021;13:618020. Published 2021 Jan 11. doi:10.3389/fnmol.2020.618020

98. Taschenberger G, Tereshchenko J, Kügler S. A MicroRNA124 Target Sequence Restores Astrocyte Specificity of gfaABC$_1$D-Driven Transgene Expression in AAV-Mediated Gene Transfer. *Mol Ther Nucleic Acids*. 2017;8:13-25. doi:10.1016/j.omtn.2017.03.009

99. Kumar A, Aakriti, Gupta V. A review on animal models of stroke: An update. *Brain Res Bull*. 2016;122:35-44. doi:10.1016/j.brainresbull.2016.02.016

100. Ingberg E, Dock H, Theodorsson E, Theodorsson A, Ström JO. Effect of laser

Doppler flowmetry and occlusion time on outcome variability and mortality in rat middle cerebral artery occlusion: inconclusive results. *BMC Neurosci*. 2018;19(1):24. Published 2018 Apr 19. doi:10.1186/s12868-018-0425-0

101. Cuccione E, Versace A, Cho TH, et al. Multi-site laser Doppler flowmetry for assessing collateral flow in experimental ischemic stroke: Validation of outcome prediction with acute MRI. *J Cereb Blood Flow Metab*. 2017;37(6):2159-2170.

102. Ng YS, Stein J, Ning M, Black-Schaffer RM. Comparison of clinical characteristics and functional outcomes of ischemic stroke in different vascular territories. *Stroke*. 2007;38(8):2309-2314. doi:10.1161/STROKEAHA.106.475483

103. Griffin N, Faulkner S, Jobling P, Hondermarck H. Targeting neurotrophin signaling in cancer: The renaissance. *Pharmacol Res*. 2018;135:12-17. doi:10.1016/j.phrs.2018.07.019

104. Radin DP, Patel P. BDNF: An Oncogene or Tumor Suppressor?. *Anticancer Res*. 2017;37(8):3983-3990. doi:10.21873/anticanres.11783

105. Ikematsu Y, Tanaka K, Toyokawa G, et al. NEUROD1 is highly expressed in extensive-disease small cell lung cancer and promotes tumor cell migration. *Lung Cancer*. 2020;146:97-104. doi:10.1016/j.lungcan.2020.05.012

106. Li Z, He Y, Li Y, et al. NeuroD1 promotes tumor cell proliferation and tumorigenesis by directly activating the pentose phosphate pathway in colorectal carcinoma. *Oncogene*. 2021;40(50):6736-6747. doi:10.1038/s41388-021-02063-2

107. Disouky A, Lazarov O. Adult hippocampal neurogenesis in Alzheimer's disease. *Prog Mol Biol Transl Sci*. 2021;177:137-156. doi:10.1016/bs.pmbts.2020.09.00