

# Revolutionizing Stroke Recovery: Unveiling the Promise of Stem Cell Therapy

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**Abstract:** Stem cells, renowned for their unique regenerative capabilities, present significant hope in treating stroke, a major cause of disability globally. This review offers a detailed analysis of stem cell applications in stroke (ischemic and hemorrhagic) recovery. It examines therapies based on autologous (patient-derived), allogeneic (donor-derived), and Granulocyte-Colony Stimulating Factor (G-CSF) based stem cells, focusing on cell types such as Mesenchymal Stem/Stromal Cells (MSCs), Bone Marrow Mononuclear Stem Cells (BMMSCs), and Neural Stem/Progenitor Cells (NSCs). The paper compiles clinical trial data to evaluate their effectiveness and safety and addresses the ethical concerns of these innovative treatments. By explaining the mechanisms of stem cell-induced neurological repair, this review underscores stem cells' potential in revolutionizing stroke rehabilitation and suggests avenues for future research.

**Keywords:** stem cell therapy, stroke, brain hemorrhage, autologous stem cells transplantation, allogeneic stem cells transplantation, granulocyte-colony stimulating factor

## Introduction

Stroke, often referred to as a cerebrovascular accident (CVA), stands as a formidable adversary in the realm of neurological disorders. This devastating event occurs when blood flow to a part of the brain is interrupted, leading to the impairment of vital functions controlled by that specific region. Classified into two main types—cerebral ischemia and cerebral hemorrhage—stroke imposes a significant burden on global health, being a leading cause of long-term disability and mortality.<sup>1</sup> It is the second leading cause of death and the third leading cause of death and disability combined worldwide. Between 1990 and 2019, there was a notable increase in stroke-related statistics, with a 70% rise in incident strokes, a 43% increase in deaths from stroke, and a 143% increase in disability-adjusted life years (DALYs).<sup>2</sup>

Despite advances in acute stroke management, including thrombolytic therapy and thrombectomy, the quest for effective treatments to mitigate the long-term consequences of stroke remains paramount.

Recent advancements in neurorestorative therapies, such as neurostimulation and cell transplantation, are pivotal in stroke recovery. Neurostimulation techniques, including cortical and cerebellar stimulation, vagal stimulation, and optogenetics, enhance synaptic plasticity and reorganize neural circuitry. Cell transplantation involves both endogenous strategies to stimulate neural stem cells and exogenous therapies to replace damaged neurons. These multidimensional mechanisms aim to leverage the brain's inherent plasticity, promoting repair and reorganization of neural networks disrupted by stroke.<sup>3</sup>

In this review, we delve into the evolving landscape of stem cell therapy in stroke. The first instance of stem cell therapy for stroke patients involved the use of neuroterocarcinoma cells that were transformed into postmitotic neurons. This pioneering approach was conducted by Kondziolka et al in 1998, marking a significant milestone in the field.<sup>4</sup> The

study provided preliminary data on the safety, feasibility, and tolerability of stem cell therapy in humans, laying the groundwork for subsequent research in this area.

Preclinical research on stem cell therapy for stroke has highlighted both the therapeutic potential and the challenges of this approach. Boltze et al emphasize the use of large animal models (LAMs) due to their gyrencephalic brains, which more accurately mimic human brain anatomy and are suitable for advanced imaging techniques.<sup>5</sup> They note the challenges and benefits of working with LAMs, including their potential for more realistic testing of cell delivery techniques and the exploration of stroke sequelae like cognitive impairment. Stem cells have been implicated in promoting brain repair mechanisms such as angiogenesis, neurogenesis, and gliogenesis, when transplanted into stroke models, thereby facilitating functional recovery after stroke.<sup>6</sup>

Significant research is dedicated to elucidating the mechanisms of immune cell trafficking across the blood-brain barrier (BBB) and how these pathways can be leveraged to improve the targeted delivery of stem cells to the brain.<sup>7</sup> Experimental strategies that have been developed to enhance stem cell migration, including genetic engineering to express specific cell surface proteins mimicking immune cells, cell membrane engineering, stem cell selection and preconditioning, and methods to temporarily increase BBB permeability.<sup>7</sup>

In order to address immune rejection, preclinical studies considered the deletion of genes encoding for HLA molecules that trigger immune responses and the insertion of genes that promote immune tolerance, the use of hypoimmunogenic stem cells, the implementation of safety switch systems that allow for the selective ablation of grafted cells if necessary, as well as innovative approaches to creating “universal” induced pluripotent stem cells (iPSC) lines through genetic modifications.<sup>8,9</sup>

Another critical aspect is the time of initiation of stem cell therapy. Wahl et al found that when immunotherapy targeting a neurite growth-inhibitory protein is administered to encourage new fiber sprouting before intensive training, rats can achieve nearly full recovery of skilled forelimb functions.<sup>10</sup> However, initiating high-intensity training too early, during the growth phase, disrupts this recovery process, leading to improper fiber patterns. Rust and Tackenberg argue that the subacute phase post-stroke, a few days to a week after the incident, might be most conducive for graft survival and integration.<sup>9</sup>

A study by Wang et al revealed the therapeutic potential of 3K3A-APC, an Activated Protein C (APC) analog, in enhancing the reparative properties of transplanted human neural stem/progenitor cells (NSCs) after ischemic stroke in mice.<sup>11</sup>

Rust et al investigated the generation of good manufacturing practice (GMP)-compatible, transgene- and xeno-free neural progenitor cells (NPCs). The NPCs demonstrated stable gene expression over multiple passages and showed significant scalability, with the potential for substantial cell production.<sup>12</sup> In vitro differentiation experiments indicated that upon withdrawal of growth factors, these NPCs primarily matured into active neurons.

Recently the group of Llorente et al created glial enriched progenitor (GEP) cells derived from human-induced pluripotent stem cells (hiPSCs) (hiPSC-GEPs) through an experimental manipulation involving a brief treatment with a prolyl hydroxylase inhibitor, deferoxamine, biasing these cells towards an astrocyte fate.<sup>13</sup> When transplanted into mice suffering from white matter stroke during the subacute period post-stroke, hiPSC-GEPs demonstrated remarkable abilities: they migrated widely within the brain, matured into astrocytes with a pro-repair phenotype, induced endogenous oligodendrocyte precursor proliferation and remyelination, and promoted axonal sprouting. This led to enhanced motor and cognitive recovery compared to other hiPSC-differentiated cell types.

As the field of stem cell research advances, clinical trials have become a pivotal arena for evaluating the safety and efficacy of stem cell-based interventions. These trials, conducted in humans under stringent regulatory oversight, aim to address critical medical needs, ranging from hematological diseases to diabetes, liver cirrhosis, respiratory ailments, and beyond. Recent strides in stem cell therapy have showcased promising outcomes, including the reduction of chronic diabetic complications, significant improvements in liver function, and the synthesis of cartilage tissue for orthopedic applications. The quest for improved methods of prevention, treatment, and diagnosis of stroke has led to an exploration of stem cell therapy's potential. Drawing insights from current clinical trials, we aim to unravel the progress made in the use of stem cells for stroke intervention. Through this comprehensive exploration, we endeavor to guide the future direction of clinical research, fostering a deeper understanding of stem cell therapy's clinical translation in stroke.

## Stem Cell Therapy

Stem cells are a fundamental part of medical science due to their unique characteristics and potential in treating various diseases. They are special cells that have the ability to develop into many different cell types in the body, from muscle cells to brain cells. This makes them an invaluable tool in regenerative medicine and therapy. Stem cells function in two primary ways: they can continuously renew themselves to make exact replicas, and they are the only cells that can differentiate into specialized cells to replenish or repair specific cell types in the body.<sup>14</sup>

Autologous and allogeneic stem cell transplantation represent two critical strategies in regenerative medicine, and their application in stroke patients is an area of growing interest. In autologous transplantation, stem cells are harvested from the patient's own body, such as from bone marrow or peripheral blood.<sup>14</sup> These cells, after being processed, are reintroduced into the patient's body. This approach is primarily valued for its lower risk of immune rejection, as the cells originate from the patient. Allogeneic transplantation, on the other hand, involves using stem cells from a donor, who may be a relative or an unrelated matched donor.<sup>14</sup> This method is crucial when the patient's own cells are not viable for transplantation. Granulocyte Colony-Stimulating Factor (G-CSF) is a growth factor often used to stimulate bone marrow to produce more granulocytes and stem cells and release them into the bloodstream.<sup>15</sup> The G-CSF method, being less invasive than direct stem cell transplantation, presents a promising therapeutic avenue.

In the context of stroke patients, these therapies aim to repair or regenerate damaged brain tissue, potentially restoring lost functions. The principle behind their use in stroke recovery hinges on the ability of stem cells to differentiate into various types of brain cells and promote neural repair. However, the application in stroke patients is still largely experimental and under clinical investigation, with the focus on understanding the optimal cell type, timing, and method of cell delivery, as well as long-term efficacy and safety of these treatments.<sup>16</sup>

The administration of stem cell therapy, while promising for regeneration and functional recovery, is accompanied by potential risks that necessitate careful consideration. These risks include immune reactions, the possibility of tumor formation, and the risk of embolism, especially when stem cells are administered intravenously.<sup>17</sup> This risk of embolism has been observed to increase with the dosage of the infused cells and the velocity at which the infusion is administered.<sup>18</sup> Notably, there have been instances where patients, including those with a history of renal transplantation and chronic kidney disease, experienced thromboembolism following the intravenous infusion of umbilical cord mesenchymal stem cells (UCMSC).<sup>19</sup> Thrombolytic therapy, involving the administration of urokinase and warfarin, was successfully employed to alleviate the symptoms. These cases underline the critical importance of monitoring and preventing thromboembolism and other potential complications, emphasizing that safety remains a paramount concern in the application of stem cell therapy for stroke and other conditions.<sup>19</sup>

Furthermore the necessity of combining immunosuppressive therapy with stem cell therapy remains uncertain. The majority of studies did not employ immunosuppressive therapy after therapy and reported no rejection reactions. Autologous stem cells transplantation is generally considered not to require immunosuppressive therapy.<sup>20</sup> However, allogeneic stem cells, sourced from donors, often necessitate immunosuppression to prevent immune rejection.<sup>20</sup> An exception is mesenchymal stem cells (MSCs), known for their immunomodulatory properties that can diminish rejection risks, potentially reducing the need for immunosuppression.<sup>21,22</sup> Ethical considerations in stem cell transplantation are significant and multifaceted, encompassing issues such as consent, donor selection, genetic manipulation and editing.<sup>23</sup>

There are different types of stem cells, each with its unique potential and application. The main types include:

MSCs originating from early mesoderm development, can be sourced from various tissues. In clinical practice, the most commonly utilized MSCs are Bone Marrow (BM) MSCs, Human Umbilical Cord (HUC) MSCs, and Adipose-Derived (AD) MSCs.<sup>24</sup>

BM-MSCs, known for their therapeutic effects, are easily acquired, can pass through the Blood-Brain Barrier (BBB), and demonstrate the ability to migrate to the injured area.<sup>25</sup> Studies report that BM-MSCs administration can ameliorate neurological deficits, restore Blood-Brain Barrier function, and enhance the overall recovery of neurological function in rats with intracranial hemorrhage (ICH).<sup>26</sup>

HUC-MSCs, obtained in large quantities with minimal invasion, have been applied in the treatment of various neurological diseases, including ICH. Combining minimally invasive hematoma aspiration with HUC-MSC transplantation has shown promise in reducing neural damage and improving neural functions.<sup>27</sup>

AD-MSCs, isolated from adipose tissue, offer advantages such as accessibility, abundance, and low apoptotic rates, making them suitable for large-scale cultivation. Research focusing on ADMSC treatment demonstrates their potential to differentiate into neuron-like and astrocyte-like cells around the hematoma, leading to functional improvement and alleviation of long-term brain degeneration.<sup>28</sup>

Embryonic Stem Cells (ESCs) represent a highly undifferentiated cell type isolated from early embryos or original gonads. Known for their limitless proliferation, self-renewal, and multidirectional differentiation capabilities *in vitro*, ESCs hold great promise in treating central nervous system diseases. While their differentiation into various cell types, including neurons and glial cells, makes them attractive for therapeutic applications, challenges such as the ethical concerns surrounding their use and the unknown molecular mechanisms of cell migration and cytokine regulation need careful consideration.<sup>29</sup>

Hematopoietic Stem Cells (HSCs) derived from various hematopoietic tissues, including bone marrow and umbilical cord blood. Studies suggest that higher levels of circulating HSCs post-ICH are associated with better functional outcomes.<sup>30</sup> Mobilization of HSCs through G-CSF has shown promise in promoting functional recovery not only in ischemic stroke but also in ICH.<sup>31</sup> Convenient acquisition of HSCs in large numbers makes them a potential candidate for broad clinical use.

Neural Stem Cells (NSCs) found in the nervous system exhibit self-renewal and differentiation into neurons or glial cells. NSC transplantation, both exogenous and endogenous, has demonstrated the ability to promote functional recovery in mouse models with ICH and brain ischemia.<sup>32,33</sup>

Activation of endogenous NSCs in pathological conditions and their migration to injury sites contribute to neurorestoration. The neuroplasticity of NSCs, along with their low immunogenicity and high histocompatibility with brain tissue, positions them as a viable alternative for treating stroke.

Induced Pluripotent Stem Cells (iPSCs), a groundbreaking discovery in stem cell research, are reprogrammed from somatic cells using specific transcription factors. Studies have reported therapeutic effects in rat models with ICH and brain ischemia.<sup>34,35</sup> iPSCs can differentiate into neuroepithelium-like and neuroepithelial stem cells, secreting neurotrophic factors. The iPSC technology, addressing ethical concerns and immune rejection issues, holds promise for clinical applications, although challenges like low reprogramming efficiency need further resolution.

The biological mechanisms by which stem cells aid in stroke recovery are multifaceted and complex. In the context of ischemic stroke, stem cells primarily contribute to repair and regeneration through several pathways. They have the potential to differentiate into various neural cell types, replacing damaged neurons and supporting cells in the brain.<sup>36</sup> Additionally, stem cells secrete growth factors and cytokines that promote neuroprotection, angiogenesis, and neurogenesis, thereby creating a more favorable environment for brain repair.<sup>37</sup> They also modulate the immune response to the injury, reducing inflammation, which is a critical factor in limiting further damage post-stroke.<sup>38</sup> Furthermore, stem cells can facilitate synaptic plasticity and neural network reorganization, essential for restoring functional deficits caused by the stroke. Understanding these mechanisms is crucial for optimizing stem cell therapies and enhancing their efficacy in clinical applications for stroke recovery.

## Stem Cell Treatment in ICH

In the field of stem cell therapy for ICH, diverse approaches and routes of administration have been explored across various clinical trials as summarized in the [Table 1](#), each presenting unique insights into the safety and potential efficacy of this innovative treatment.

In the autologous stem cell therapy various stem cell types have been explored. Sobrino et al's study explored the influence of bone marrow-derived CD34+ progenitor cells in 32 ICH patients. The study found a positive correlation between serum CD34+ cell levels and good functional outcomes.<sup>30</sup> Bhasin et al focused on intravenous autologous mesenchymal stem cell (MSC) transplantation in 12 chronic stroke and ICH patients, reporting significant improvements in clinical scores and functional imaging without cell-related side effects.<sup>39,45</sup> Zhu et al treated 206 surgical ICH patients

**Table 1** Summary of the Various Stem Cell Therapy Studies in the Context of Hemorrhagic Stroke

Year	Trial Design/Phase	Author(s)	Study Focus	Number of Patients	Cell Type(s)	Route of Administration	Results
2011	Prospective study	Sobrinho et al <sup>30</sup>	Primary ICH	32	CD34+ progenitor cells (Autologous)	n.a.	Positive correlation between serum levels of CD34+ and good functional outcomes
2011	Non randomised controlled trial	Bhasin et al <sup>39</sup>	Chronic stroke and ICH	12 (9 stroke, 3 ICH)	MSCs (Autologous)	Intravenous	Significant improvements in clinical scores, no side effects
2015	N.A	Zhu et al <sup>40</sup>	ICH post-surgery	206	BMSCs (Autologous)	Injection into perihemorrhage area and subarachnoid space	Significant clinical improvements, no adverse effects
2018	Phase I	Ahn et al <sup>41</sup>	Severe IVH in preterm infants	9	MSCs (Allogeneic)	Intraventricular	Well-tolerated, no serious adverse effects
2013	N.A	Chen et al <sup>42</sup>	Chronic stroke, including hemorrhage	10 (4 with hemorrhage)	Olfactory ensheathing cells, neural progenitor cells, umbilical cord mesenchymal cells, Schwann cells (Mixed)	Intrathecal, Intravenous, Intraparenchymal	Neurological function improvement in all patients
2016	Retrospective analysis of a cohort with cell treated patients	Chang et al <sup>43</sup>	Cerebral hemorrhage post-surgery	24	BM mononuclear cells (Autologous), Umbilical cord mononuclear cells (Allogeneic)	Intracavity post-surgery	Better functional outcomes in cell-transplanted groups
2014	N.A	Xue et al <sup>44</sup>	Cerebral hemorrhage	40 (20 in treatment group)	Neural stem cells (Allogeneic)	Intrathecal (lumbar puncture)	Significant reduction in NIHSS scores, minimal adverse reactions

**Abbreviations:** MSCs, Mesenchymal Stem/Stromal Cells; BMSCs, Bone Marrow Stroma Cells; BM, Bone Marrow; NIHSS, National Institutes of Health Stroke Scale; ICH, Intracranial Hemorrhage; IVH, intraventricular hemorrhage.

with autologous bone marrow stroma cells (BMSCs) injected into the perihemorrhage area and subarachnoid space, observing significant clinical improvements and no transplantation-related adverse effects.<sup>40</sup>

In the allogeneic stem cell therapy, a Phase I clinical trial involved intraventricular transplantation of MSCs in nine preterm infants with severe intraventricular hemorrhage.<sup>41</sup> This study used a dose-escalation approach and reported well-tolerated treatment without serious adverse effects or dose-limiting toxicities. Xue et al investigated neural stem cell intrathecal transplantation in 40 cerebral hemorrhage patients, using lumbar puncture for cell delivery, and reported significant improvements in clinical scores with minimal adverse reactions.<sup>44</sup>

Chen et al's study, conducted from 2003 to 2011 on 10 chronic stroke patients, including four with brain hemorrhage, used a mix of cell types like olfactory ensheathing cells, neural progenitor cells, umbilical cord mesenchymal cells, and Schwann cells.<sup>42</sup> These cells were administered through intraparenchymal, intrathecal, or intravenous routes, with all patients showing neurological improvement.

Chang et al retrospectively analyzed 24 ICH patients treated with conventional surgery and either autologous bone marrow mononuclear cells or allogeneic umbilical cord mononuclear cells.<sup>43</sup> Both cell-transplanted groups showed better functional outcomes than the control group after 5 years.

Each of these studies, across autologous, allogeneic, and mixed stem cell therapy categories, contributes vital insights into the potential of these treatments in improving outcomes for ICH patients. They collectively underscore the diversity in stem cell types, administration routes, and patient demographics within this promising therapeutic field.

## Stem Cell Treatment in Ischemic Stroke

Recent years have witnessed a proliferation of clinical trials focusing on cerebral ischemia. Various intervention methods have been explored, with BMMSCs, and NSCs being the predominant cell types, followed by others such as umbilical cord mesenchymal cells, CD34+ cells, olfactory ensheathing cells, Schwann cells, and dental pulp stem cells.<sup>46,47</sup> Additionally, G-CSF has been employed to indirectly regulate stem cell mobilization.<sup>48,49</sup> The overall safety of stem cells in treating cerebral ischemia is deemed satisfactory, with intervention-related adverse events generally mild or comparable to those in control groups, and no unexpected brain structural abnormalities reported.<sup>50,51</sup> Beyond bone marrow-derived stem cells and CD34+ cells, which are typically transplanted following autologous cell extraction, other cells are challenging to self-extract. Consequently, allogeneic stem cell transplantation is an alternative treatment approach. Encouragingly, none of the clinical trials reported oncological or serious adverse events during follow-up. The number of cell injections varied across trials, ranging from  $5 \times 10^5$  to  $3 \times 10^8$ , predominantly administered as a single injection, with a minority of trials utilizing multiple repeated cell treatments.<sup>52–55</sup> Multiple injections did not exhibit significant safety changes compared to single injections, and there was some improvement in patient symptoms. The injection routes were diverse, with MSCs primarily administered intravenously, while other cell types were delivered through various routes such as the middle cerebral artery (MCA) pathway, intrathecal, via the cerebellomedullary cistern, or directly in the intracerebral infarct area.<sup>42,56,57</sup> Evaluation methods included the use of the modified Rankin scale, NIHSS scale, mRS score, Fugl-Meyer assessment scale, ARAT test, European Stroke Scale (ESS), ESS Motor Subscale (EMS), and Barthel index (BI) to assess cerebral ischemic function before and after intervention. Imaging, blood biochemical measurements of infarct size, blood biomarkers, and other comprehensive assessments were also conducted.<sup>57–59</sup>

In [Table 2](#) we summarize the most important information about clinical trials in ischemic stroke patients.

## Studies Showing Efficacy and Safety/Feasibility

Acute cerebral ischemia, defined within 24 hours to 1 week after onset, has seen varying efficacy in stem cell therapy. Trials involving intravenous infusion of autologous MSCs showed a trend towards motor function improvements and reduced lesion volumes.<sup>60</sup> There was an open-label, observer-blinded trial involving 85 patients with severe middle cerebral artery infarcts. The study showed that intravenous MSC transplantation was safe over a five year period and suggested potential improvements in recovery, as indicated by modified Rankin Scale scores with the degree of improvement correlating with serum stromal cell-derived factor-1 levels.<sup>52</sup>

In a phase I study 25 patients received intravenous administration of autologous bone marrow mononuclear cells (BM MNCs) after acute ischemic stroke. The study reported improvements in functional outcomes in some patients, as measured by the NIH Stroke Scale, Barthel Index, and modified Rankin Scale. It found no severe adverse events related to the treatment over 24 months, indicating safety and feasibility.<sup>61</sup>

In a Phase II randomized, double-blind, placebo-controlled trial study with allogeneic transplantation of Adipose Tissue-Derived Mesenchymal Stem Cells (AMASCIS) patients in the AD-MSC group showed a nonsignificantly lower median NIHSS score. At 24 months of follow-up, however, there were no statistically significant differences in neurological outcomes between the two groups.<sup>51</sup>

Moreover the intra-arterial administration of immunoselected CD34+ Stem Cells for Acute Ischemic Stroke, showed improvements in clinical functional scores (NIHSS, Barthel-Index, mRS) and reductions in lesion volume over a 6-month follow-up period.<sup>67</sup> There were no treatment associated adverse events.

G-CSF has shown potential in mobilizing bone marrow CD34+ stem cells in recent ischemic stroke patients, who showed an improvement in the clinical scores (NIHSS, mRS).<sup>59,83</sup> One of the biggest studies is the AX200 for Ischemic Stroke Trial, a multinational, multicenter, randomized, and placebo-controlled trial with 328 patients. G-CSF was administered intravenously over 72 hours. G-CSF failed to meet primary and secondary endpoints including modified

**Table 2** Summary of Cerebral Ischemia Stem Cell Treatment Studies Classified by Intervention Type

Year Published	Trial Design/Phase	First Author	Stroke Phase	Number of Patients	Intervention Type	Route of Administration	Results	Evidence Type
<b>Autologous Stem Cell Transplantation</b>								
2021	Phase II	Law et al. <sup>57</sup>	Subacute stroke	17	BMMSCs	Intravenous	Improvement in absolute change in median infarct volume. No adverse effects.	Safety & trend for efficacy
2005	Phase I/II	Bang et al. <sup>60</sup>	Acute stroke	30	MSCs	Intravenous	Safe, Improvements in motor function and reduced lesion volumes	Safety & Efficacy
2010	Open-label, observer blinded clinical trial	Lee et al. <sup>52</sup>	Acute stroke	85	MSCs (Autologous)	Intravenous	Safe, potential improvements in recovery	Safety & Efficacy
2019	Phase I	Vahidy et al. <sup>61</sup>	Acute stroke	25	BM MNCs (Autologous)	Intravenous	Improvements in functional outcomes, no severe adverse events	Safety & Efficacy
2020	Phase II	Jaillard et al. <sup>58</sup>	Subacute stroke	31	MSCs (Autologous)	Intravenous	Improvement in motor performance and clinical outcomes	Efficacy
2014	Phase II	Prasad et al. <sup>62</sup>	Subacute stroke	120	BMSCs (autologous)	Intravenous	Safe but no beneficial effect on stroke outcome	Safety, no efficacy
2017	Phase I	Shichinohe et al. <sup>63</sup> (RAINBOW)	Acute ischemic stroke	10	BMSCs (autologous)	Stereotactically administrated	Safe	Safety
2021	Phase III	Lee et al. <sup>64</sup>	Subacute/Chronic stroke	54	MSCs (Autologous)	Intravenous	Higher improvement in Fugl-Meyer score, positive neuroimaging changes	Efficacy
2013	Phase I	Cotten et al. <sup>54</sup>	Chronic stroke	23	Umbilical cord blood cells (Autologous)	Intravenous	Safety, better developmental status for infants with HIE	Safety & trend for Efficacy
2017	Non-randomised controlled trial	Bhasin et al. <sup>45</sup>	Chronic stroke (+ ICH)	6	Bone marrow stem cells (Autologous)	Intravenous	Modest clinical improvements and neural plasticity	Efficacy
2023	Phase II	Moniche et al. <sup>65</sup> (IBIS trial)	Acute stroke	77	BMMNCs (autologous)	Intra-arterial	Safe but no significant improvement at 180 days on the mRS.	Safety
2020	Phase I	Kawabori et al. <sup>46</sup>	Acute stroke	6	BMSCs (Autologous)	Intracerebral	Safe, cell engraftment observed	Safety
2018	Phase II	Savitz et al. <sup>66</sup>	Subacute Stroke	100	ALD-401 Cells (Autologous)	Internal Carotid Artery Intra-arterial	Safe, no adverse events related to treatment, no efficacy demonstrated	Safety, No efficacy

(Continued)

Table 2 (Continued).

Year Published	Trial Design/Phase	First Author	Stroke Phase	Number of Patients	Intervention Type	Route of Administration	Results	Evidence Type
2014	Phase I	Banerjee et al <sup>67</sup>	Acute Stroke	5	CD34+	Intra-arterial	No adverse events, all patients showed improvements	Safety & trend for efficacy
2019	Phase I/IIa	Fang et al <sup>68</sup>	Acute Stroke	18	EPCs (Autologous)	Intravenous	Safe, no toxicity events or infusional or allergic reactions in any treated group	Safety
2018	Prospective, randomized, open-label, blinded–end point	Bhatia et al <sup>69</sup>	Subacute stroke	20	Bone marrow–derived mononuclear cells (BMMNC)	Intraarterial into the ipsilateral middle cerebral artery	Safety, improved clinical outcomes	Safety & Efficacy
2012	Randomized, open-label, parallel-group study	Prasad et al <sup>70</sup>	Acute stroke	10	G-SCF	Subcutaneous	One patient in G-CSF therapy arm died due to raised intracranial pressure. No severe adverse effects were seen in rest of patients	Safety & feasibility
<b>Allogeneic Stem Cell Transplantation</b>								
2024	Phase II/III multicenter, double-blind, parallel-group, placebo-controlled trial	Houkin et al <sup>71</sup> (TREASURE trial)	Acute	206	Multipotent adult progenitor cell product (Multistem)	Intravenous	Safe, well tolerated, no improvement of short-term outcomes	Safety
2022	Phase I	Baak et al <sup>50</sup>	Perinatal arterial ischaemic stroke	10	MSCs (Allogeneic)	Intranasal	Safe, well-tolerated, no serious adverse events	Safety
2022	Phase II	De Celis-Ruiz et al <sup>51</sup> (AMASCIS)	Acute Stroke	13	AD-MSCs (Allogeneic)	Intravenous	Lower median NIHSS score, no other significant differences in outcomes	Efficacy
2016	Phase I	Kalladka et al <sup>72</sup> (PISCES)	Chronic Stroke	13	CTX0E03 (Allogeneic)	Intraputamenal	No adverse events, Improvement in patients with residual upper limb movement	Safety & Efficacy
2020	Open-label, single-arm, multicentre study	Muir et al <sup>73</sup> (PISCES-2)	Chronic Stroke	23	Human neural stem cells (Allogeneic)	Intracerebral	Feasible and safe, improved upper limb function, variable results	Safety & Efficacy
2013	N.a	Jiang et al <sup>74</sup>	Chronic Stroke	4	UCMSCs (Allogeneic)	Intra-arterial	Improved muscle strength and scores, feasible and safe	Safety & Efficacy
2013	Phase II	Chen et al <sup>42</sup>	Chronic Stroke	10	Mixed cell types (Allogeneic)	Various	Enhanced various functions, improved scores	Safety & Efficacy
2019	Phase I/II	Levy et al <sup>56</sup>	Chronic Stroke	36	MSCs (Allogeneic)	Intravenous	Confirmed safety, mild adverse events, improvements in Barthel Index Score	Safety & Efficacy

2013	N.a	Qiao et al <sup>53</sup>	Chronic Stroke	8	Neural stem/progenitor cells and MSCs (Autologous)	Intravenous/through cerebromedullary cistern	Clinical improvements, minimal adverse effects	Safety & Efficacy
2019	Phase I	Pan et al <sup>75</sup>	Subacute and chronic stroke	37	BM-MSCs (Allogenic)	Intrathecal	Safe, feasible, no severe adverse events, minor side effects like headache and fever, increase in WBC and protein concentration in CSF	Safety & Feasibility
<b>Granulocyte Colony-Stimulating Factor (G-CSF) Intervention</b>								
2006	Phase I	Shyu et al <sup>59</sup>	Acute stroke	10	G-CSF	Subcutaneous	Safe, feasible, Improvement in clinical scores	Safety & Efficacy
2006	Phase IIa	Sprigg et al <sup>76</sup>	Subacute ischemic stroke	36	G-CSF	Subcutaneous	G-CSF increased CD34+ count, no difference in serious adverse events between groups	Safety
2011	Phase I	Floel et al <sup>77</sup>	Chronic Stroke	41	G-CSF	Subcutaneous	More frequent adverse events in G-CSF group but generally mild or moderate	Safety
2013	Phase II	Ringelstein et al <sup>78</sup>	Acute Stroke	328	G-CSF	Intravenous	Failed primary endpoints, trend for reduced infarct growth, no adverse events.	Safety & trend for Efficacy
2010	Phase II	Schabitz et al <sup>79</sup> (AXIS)	Acute stroke	44	G-CSF	Intravenous	No significant differences, potential effects in specific patients	Safety & trend for Efficacy
2016	Phase II	Mizuma et al <sup>80</sup>	Acute stroke	49	G-SCF	Intravenous	Well-tolerated, no significant difference in clinical outcomes or infarct volume	Safety & no efficacy
2014	Phase II	Gorthi et al <sup>81</sup>	Acute Stroke	47	G-CSF	Subcutaneous	Better improvement, not statistically significant	Safety & trend for Efficacy
2012	Phase IIb	England et al <sup>31</sup>	Subacute stroke	60	G-CSF	Subcutaneous	Safe, trend towards reduction in lesion volume	Safety & trend for Efficacy
2011	Phase II	Alasheev et al <sup>82</sup> (STEMTHER)	Acute Stroke	20	G-SCF	Subcutaneous	No difference in degree of disability/dependence at 180 days post-stroke	Safety, but no efficacy

**Abbreviations:** BMMSCs, Bone Marrow Mononuclear Stem Cells; MSCs, Mesenchymal Stem/Stromal Cells; BM MNCs, Bone Marrow Mononuclear Cells; BMSCs, Bone Marrow Stroma Cells; UCMSCs, umbilical cord mesenchymal stem cells; ICH, Intracranial Hemorrhage; G-CSF, Granulocyte Colony-Stimulating Factor; EPCs, Endothelial Progenitor Cells; AD, Adipose-Derived.

Rankin scale score and NIH Stroke Scale score at day 90. However, a trend for reduced infarct growth in the G-CSF group was noted.<sup>78</sup>

The study “AXIS: A Trial of Intravenous Granulocyte Colony-Stimulating Factor in Acute Ischemic Stroke” was a phase IIa trial that tested various intravenous G-CSF doses in acute ischemic stroke patients. It involved 44 patients receiving G-CSF doses ranging from 30 to 180 µg/kg over 3 days. The study reported no significant differences in clinical outcomes between treatment and placebo groups. However, exploratory analyses suggested potential treatment effects in patients with larger baseline DWI lesions. The study concluded that G-CSF was well-tolerated even at high dosages.<sup>79</sup>

In an open-label randomized controlled trial, 47 patients the group that received G-CSF showed better improvement on all three stroke scales (NIHSS, mRS, Barthel-Index) compared to the control group. However, the percentage improvement between the two groups was not statistically significant. The study provided preliminary evidence that G-CSF therapy is potentially safe, feasible, and tolerable in patients with acute ischemic stroke and may lead to a better functional outcome.<sup>81</sup>

Subacute cerebral ischemia, occurring 1–3 weeks after onset, demonstrated reliable safety in stem cell therapy. The efficacy correlated with individual patient differences. Trials involving IV injection of autologous bone marrow-derived MSCs within 7–30 days showed improvement in motor performance and task-related primary motor cortex (MI) in functional MRI, reduction in median absolute infarct volume and favorable clinical outcomes based on the Modified Rankin Scale and Barthel Index scores.<sup>57,58,70</sup> Patients with Intra-Arterial Infusion of Autologous BMMSCs Stem Cells after subacute ischemic stroke showed a tendency towards good clinical outcomes (mRS less than 2) compared to the control group ( $p < 0.068$ ).<sup>69</sup> However, there is a need for larger studies for validation.

A phase IIb trial involved 60 patients treated with G-CSF at a dose of 10 µg/kg subcutaneously for 5 days, 3 to 30 days post-ischemic stroke. The trial concluded that G-CSF is safe and observed a trend towards a reduction in MRI ischemic lesion volume in treated patients.<sup>31</sup>

Chronic phase treatment, initiated three weeks after ischemic stroke, currently lacks effective solutions. However, significant improvement in behavioral endpoints and recovery of neurological and motor function has been observed in a considerable number of patients after stem cell treatment.

The study by Lee et al investigated the efficacy of intravenous mesenchymal stem cells (MSCs) for motor recovery after ischemic stroke.<sup>64</sup> This clinical trial involved 54 patients within 90 days after a middle cerebral artery territory infarct and found that the improvement ratio of the Fugl-Meyer assessment score was significantly higher in the MSC group. Neuroimaging measures indicated that stem cell-based therapy can protect the corticospinal tract against degeneration and enhance positive changes in network reorganization to facilitate motor recovery after stroke.<sup>64</sup>

Additionally, several studies have focused on autologous stem cell transplantation for patients with chronic stroke, involving smaller patient cohorts. This reflects the exploratory and preliminary nature of this therapeutic approach. Michael L. Levy’s team conducted a phase I/II study assessing intravenous allogeneic mesenchymal stem cells in 36 chronic ischemic stroke patients.<sup>56</sup> This study involved dose-escalation and expanded safety cohorts, confirming the safety of the treatment with mild adverse events reported. Preliminary efficacy estimates suggested significant improvements in the Barthel Index Score over 12 months, indicating potential benefits for chronic stroke patients. Qiao et al assessed the cotransplantation of neural stem/progenitor cells and mesenchymal stromal cells in 8 ischemic stroke patients, showing clinical improvements in more than half of the patients with minimal adverse effects.<sup>53</sup> Jiang et al explored the feasibility of delivering mesenchymal stem cells via catheter in 4 patients, noting improvements in muscle strength and modified Rankin scale scores.<sup>74</sup> Bhasin et al involved 6 chronic stroke patients, where bone marrow stem cells were used, demonstrating modest clinical improvements and neural plasticity.<sup>39</sup> Cotten et al investigated autologous umbilical cord blood cell therapy in 23 infants with hypoxic-ischemic encephalopathy, highlighting the therapy’s safety and suggesting better developmental status (Bayley score) in the first year follow up.<sup>54</sup>

Similarly, the implantation of the allogeneic human neural stem cell line CTX0E03 into the ipsilateral putamen of cerebral infarction showed improvement in patients with residual upper limb movement.<sup>72</sup> Muir’s study on the intracerebral implantation of allogeneic human neural stem cells focused on improving upper limb function after stroke, finding the treatment feasible and safe, with no cell-related adverse events, although the results varied among patients.<sup>73</sup> Infusion of allogeneic umbilical cord mesenchymal stem cells (UCMSCs) into the M1 segment of the middle cerebral artery also improved muscle strength and modified Rankin scale scores in chronic ischemic stroke patients.<sup>74</sup> Finally, the

study by Chen and colleagues involved ten patients receiving a combination of different cell types, including olfactory ensheathing cells, neural progenitor cells, umbilical cord mesenchymal cells, and Schwann cells.<sup>42</sup> These cells were administered through various methods such as intracranial parenchymal and intrathecal implantation, as well as intravenous administration. The treatment led to enhanced speech, muscle strength, balance, and improved scores on the Barthel Index and Clinic Neurologic Impairment Scale. Notably, reported adverse events were mostly related to surgical procedures or pre-existing conditions rather than the cell transplantation itself.

Together, these studies illustrate the potential of autologous and allogeneic stem cell therapies in the treatment of chronic stroke, demonstrating safety and varying degrees of efficacy. They mark important steps in understanding the application and impact of different cell types in stroke recovery.

## Studies Showing Safety/Feasibility

### Autologous Stem Cell Transplantation

Numerous studies collectively affirm the safety and feasibility of autologous stem cell therapy in ischemic stroke patients. However, some of these studies did not show any efficacy of the treatment. Prasad et al delivered a phase II multicenter randomized trial evaluating the efficacy and safety of intravenous infusion of autologous bone marrow mononuclear stem cells (BMSCs) in 120 patients with subacute ischemic stroke.<sup>62</sup> The study found no significant difference between the BMSCs arm and the control arm in these outcomes. Additionally, safety outcomes, including adverse events and new area of 18 fluorodeoxyglucose uptake, were similar in both arms, indicating that intravenous infusion of BMSCs is safe.

A smaller, Phase 1, open-label, uncontrolled trial involved seven patients with severe neurological deficits, where BMSCs were extracted from each patient's iliac bone and cultured using human platelet lysate (PL). The cells were labeled with superparamagnetic iron oxide for tracking with magnetic resonance imaging (MRI) and then stereotactically administered around the infarcted area. There were no severe adverse events reported during the surgical and follow-up periods for a year, indicating that an intraparenchymal administration of stem cell therapy could be safe.<sup>63</sup> A Spanish randomized, multicentre, Phase 2 controlled clinical trial involving 77 acute ischemic stroke patients found that intra-arterial bone marrow mononuclear cells (BMMNCs) therapy was safe, but it did not result in a significant improvement in modified Rankin Scale (mRS) scores at 180 days when compared to the control group. There were no notable differences in adverse events among the groups, except for two patients in the low-dose BMMNC group who experienced groin hematomas after cell injection.<sup>65</sup> The study by Savitz et al was a Phase 2 randomized sham-controlled trial investigating the intracarotid artery infusion of autologous bone marrow-derived ALD-401 cells in patients with recent stable ischemic stroke.<sup>66</sup> ALD-401 is an enriched population of aldehyde dehydrogenase bright stem cells, capable of reducing neurological deficits in animal models. The study involved patients aged 30–83 with confirmed first-time middle cerebral artery ischemic stroke. Results showed no significant difference in primary efficacy endpoints between the treatment and placebo groups. The study did not report any serious clinical adverse events related to the treatment, though there was a higher incidence of asymptomatic restricted diffusion lesions on MRI in the treatment group.

A smaller scale study investigated the safety and preliminary efficacy of autologous ex vivo expanded endothelial progenitor cells (EPCs) injected intravenously in patients with acute cerebral infarct.<sup>68</sup> The trial found no significant differences in mortality or neurological/ functional improvement between the EPC group and the placebo group. It highlighted the treatment's safety, with only a small incidence of atrial fibrillation in the EPC group and no reported toxicity or allergic reactions.

### Allogeneic Stem Cell Transplantation

In recent studies exploring allogeneic stem cell therapy for stroke, three notable approaches have been examined.

The Phase 2/3 TREASURE randomized multicenter clinical trial in Japan, evaluated the safety and efficacy of the allogeneic stem cell therapy of a bone marrow-derived, allogeneic, multipotent adult progenitor cell product (MultiStem) for acute ischemic stroke.<sup>71</sup> This multicenter, double-blind, parallel-group, placebo-controlled trial recruited 206 participants with acute ischemic stroke between November 15, 2017, and March 29, 2022. Participants, were randomly

assigned to receive either an intravenous single dose of 1.2 billion MultiStem cells or a placebo within 18 to 36 hours of stroke onset.

The study found that while MultiStem therapy was safe, but it did not significantly improve short-term outcomes at 90 days compared to the placebo. The primary endpoint, a composite measure of excellent outcomes involving the modified Rankin Scale (mRS), the National Institutes of Health Stroke Scale (NIHSS), and the Barthel index as well the secondary endpoints, including the distribution of mRS scores at days 90 and 365, and the rate of excellent outcomes at day 365, did not differ significantly between the two groups. The frequency of adverse events was similar between the treatment and placebo groups, with no grade 3 or 4 allergic reactions reported.

The PASSIoN study investigated the intranasal administration of bone marrow-derived mesenchymal stromal cells (MSCs) in neonates with perinatal arterial ischemic stroke.<sup>50</sup> This study involved ten neonates and demonstrated the method's safety and feasibility, with no serious adverse events reported.

An open-label clinical study from China involving 37 patients with various neurological diseases, including brain ischemic stroke examined the safety and feasibility of intrathecal allogeneic bone marrow-derived mesenchymal stromal cell (BM-MSC). Patients received four consecutive intrathecal injections of bone marrow-derived -MSCs at one-week intervals and were followed up for at least six months. The study found that the highest adverse event was a slight ache at the injection site (4.11%), followed by fever (3.42%) and mild headache (2.05%), with no severe adverse events reported. Although white blood cell (WBC) counts in cerebrospinal fluid (CSF) increased in 30 patients, and protein concentrations in CSF exceeded the normal range in 26 patients, other CSF indicators remained normal, and there were no signs of CNS infection. Hematological and imaging examinations showed no abnormalities. Compared to previous studies, the incidence of adverse events in this study was consistent or even lower for headache, fever, nausea, and neck pain.<sup>55</sup> The same results appeared from a smaller group of patients (six patients ranging from 3 to 85 years old) with ischemic brain stroke, suggesting that intrathecal approach could be safe independently from age.<sup>75</sup>

These studies collectively highlight the evolving landscape of stem cell therapy in stroke treatment ranging from neonates to the elderly, focusing on innovative routes of administration. While demonstrating a favorable safety profile, these studies pave the way for further research to explore the effectiveness of these methods in improving clinical outcomes for stroke patients.

## Granulocyte Colony-Stimulating Factor (G-CSF)

In exploring the administration routes and efficacy of Granulocyte Colony-Stimulating Factor (G-CSF) in stroke treatment, four studies stand out, each varying in administration method and patient stages.

The first study employed intravenous administration in 49 acute ischemic stroke patients.<sup>80</sup> This study, while confirming the safety of G-CSF, did not find significant improvements in neurological function or reductions in infarct volume compared to placebo.

The remaining three studies utilized subcutaneous G-CSF administration but differed in patient demographics and stroke stages.

The second study included 41 chronic stroke patients.<sup>77</sup> Despite its reasonable tolerability, the study did not demonstrate significant efficacy improvements in primary endpoints, such as hand motor function.

The STEMATHER trial involved 20 patients with carotid region ischemia treated within 48 hours of stroke onset.<sup>82</sup> This study, focusing on acute ischemic stroke, similarly found no significant differences in neurological impairment or disability at 180 days post-stroke, indicating that while G-CSF is safe, its effectiveness remains uncertain in acute stroke treatment.

Finally, the STEMS trial included 36 subacute ischemic stroke patients.<sup>76</sup> It demonstrated G-CSF's ability to effectively mobilize stem cells and its overall tolerability, but like the others, it did not report significant efficacy improvements.

In summary, while these studies collectively underscore the safety and tolerability of both intravenous and subcutaneous G-CSF administration in acute, subacute, and chronic stroke patients, they consistently highlight a lack of substantial evidence for G-CSF's efficacy in improving stroke outcomes across these different stages.

## Ethics

The ethical implications of stem cell therapy in stroke are multifaceted and significant. They stem primarily from the source of stem cells, particularly embryonic stem cells, which raise concerns regarding the moral status of embryos.<sup>84,85</sup> The use of induced pluripotent stem cells, derived from adult cells, offers a potential ethical alternative but also poses concerns about long-term safety and genetic manipulation. Informed consent, especially in vulnerable stroke populations, is another critical issue, requiring clear communication about potential risks and benefits.<sup>84</sup> Additionally, there's a need to ensure equitable access to these therapies, avoiding disparities in healthcare. The ethical landscape necessitates ongoing dialogue among scientists, ethicists, patients, and policymakers to balance innovation with ethical responsibility.

## Future Directions

Future preclinical research in stem cell therapy should focus on animal models mirroring the clinical conditions of human stroke in a reliable and relevant manner. This includes replicating the pathophysiology of human stroke, associated risk factors, and the specific details of drug treatment such as doses, timing, and modes of administration, covering various reproducible models of permanent and transient focal cerebral ischemia in animal species like rats and mice.<sup>86,87</sup>

Moreover there are pending results of important clinical studies. The TOOTH study investigates intracranial autologous stem cell administration for chronic stroke,<sup>88</sup> while the J-REPAIR trial explores dental pulp stem cells in acute ischemic stroke, potentially redefining treatment protocols.<sup>89</sup> These studies are expected to provide crucial insights for future therapy development.

Future research in stem cell therapy for stroke should prioritize comparative studies to identify the most effective stem cell types. Emphasis should be placed on optimizing timing and delivery methods for post-stroke treatment, and monitoring long-term effects to ensure safety and sustained benefits. Deepening the understanding of the mechanisms by which stem cells facilitate neurological recovery is crucial. Developing personalized therapies tailored to individual patient profiles and stroke types, and exploring combination therapies with neurorestorative techniques such as neurostimulation are also key. Addressing ethical and regulatory issues, especially concerning embryonic stem cells, and educating healthcare providers and the public, are essential for advancing stem cell therapy as a comprehensive treatment for both ischemic and hemorrhagic stroke.

## Conclusions

Stem cell therapy for ischemic and hemorrhagic stroke has demonstrated promising results. Approaches like autologous and allogeneic stem cell transplantation have shown neurological improvements, with a diversity of cell types like MSCs, HSCs, and NSCs highlighting the field's potential. However, it remains largely experimental, necessitating further research on cell type, timing, and delivery methods. The integration of Granulocyte Colony-Stimulating Factor (G-CSF) in some therapies has shown potential for enhanced recovery. While safety is consistently reported and feasibility is established in most studies, efficacy varies. This variability underscores the need for comprehensive research to optimize treatment protocols and fully understand the therapeutic potential of stem cells, including G-CSF's role in stroke recovery.

## Disclosure

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