BRAIN
REVIEW ARTICLE

Brain repair mechanisms after cell therapy for stroke

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Cell-based therapies hold great promise for brain repair after stroke. While accumulating evidence confirms the preclinical and clinical benefits of cell therapies, the underlying mechanisms by which they promote brain repair remain unclear. Here, we briefly review endogenous mechanisms of brain repair after ischaemic stroke and then focus on how different stem and progenitor cell sources can promote brain repair.
Specifically, we examine how transplanted cell grafts contribute to improved functional recovery either through direct cell replacement or by stimulating endogenous repair pathways. Additionally, we discuss recently implemented preclinical refinement methods, such as preconditioning, microcarriers, genetic safety switches and universal (immune evasive) cell transplants, as well as the therapeutic potential of these pharmacologic and genetic manipulations to further enhance the efficacy and safety of cell therapies.
By gaining a deeper understanding of post-ischaemic repair mechanisms, prospective clinical trials may be further refined to advance post-stroke cell therapy to the clinic.

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Introduction

Stroke remains a leading cause of disability and death, affecting one in four people after the age of 25.[1-3] Approximately 87% of strokes are ischaemic, in which a blood vessel supplying the brain is obstructed.[1] More than half of stroke survivors experience various degrees of impairments that affect their somatosensory function, movement, speech and/or cognitive function, establishing an unmet medical need. Therefore, the development of novel regenerative therapies outside the confines of conventional treatments (Box 1) is critical.[4]

Stem cell therapy holds great promise for the treatment of many disorders of the CNS, including stroke.[5-7] Although preclinical findings in animal models of stroke provide a large body of evidence for functional recovery (reviewed by Sandu *et al.*,[8] Laso-García *et al.*[9] and Zhang *et al.*[10]), clinical translation of cell-based treatments has proven to be challenging due to several reasons such as high cell mortality rate after transplantation, poor integration with the host tissue and lack of control over cell differentiation in the injured brain.[11,12]

Several cell types have been proposed to enhance post-ischaemic brain repair, including primary mesenchymal stem cells (MSCs), neural stem and progenitor cells (NSC/NPCs), glia and pericytes.[13] Apart from MSCs (abundant and easily obtained from peripheral blood), cell grafts are commonly generated from patient or donor-induced pluripotent stem cells (iPSCs). Since iPSCs can be derived from blood cells, expanded to large quantities and differentiated into many cell types, they represent an ideal resource for therapeutic purposes with fewer ethical concerns than embryonic stem cells (ESCs). A detailed overview of the therapeutic potential of different cell sources for stroke has recently been reviewed elsewhere.[5,14-17]

Unlike single-target pharmaceuticals, cell-based therapies have pleiotropic effects as they can activate multiple cellular and molecular mechanisms in both the transplanted graft and the host tissue, which makes efforts to decipher stem cells' mode of action exceptionally challenging. Cell therapies have been proposed to either contribute to brain repair through direct replacement of host brain cells and integration into the damaged brain circuits and/or by the secretion of pro-regenerative signalling molecules that stimulate endogenous regeneration.[18-21] For instance, indirect effects of cell-based therapies include not only modulation of the inflammatory response to stroke injury[22] and glial scar formation,[18] but also stimulation of post-stroke angiogenesis,[23-25] neurogenesis[26-28] and blood–brain barrier (BBB) integrity.[29-32]

Here, we present a summary of recent progress in the development of stem cell therapies for long-term treatment of post-ischaemic stroke treatment, along with our current understanding of brain repair mechanisms by which cell grafts can enhance brain regeneration and functional recovery after stroke. The overall focus of this review is to critically appraise the therapeutic potentials of cell-based therapies for stroke recovery and to provide suggestions for future research direction.

Pathophysiology and brain repair after stroke

Ischaemic strokes are defined by the interruption of blood flow in a brain artery. Patients experiencing large vessel occlusions lose, on average, 120 million neurons, 830 billion synapses and 714 km of myelinated fibres, ageing the brain by 3.6 years per hour.[33,34] Apart from neural injury, stroke causes a massive local and systemic inflammatory response and severe damage to components of the BBB. These detrimental events are followed by a neuroplasticity phase which activates endogenous mechanisms of repair and tissue remodelling in an attempt to regenerate the damaged tissue and restore the lost functions.

Molecular and cellular tissue damage after stroke

The abrupt reduction of blood flow during a stroke episode (below 20% of baseline blood flow levels[35]) causes immediate and irreversible neuronal damage characterized by hyperacute excitotoxic and necrotic cell death in the stroke core, known as infarct. Owing to its perfusion by collateral blood arteries, the region directly adjacent to the infarct, also called the penumbra, is an area of intermediate blood flow (below 30%–40% of baseline blood flow levels[35]) sufficient to maintain cellular integrity but not electric activity of neurons or their metabolic functions. Since collateral circulation is inadequate to maintain the neuronal demand for oxygen and glucose long-term, neurons in the penumbra die if reperfusion is not

Box 1 Current status of clinically approved stroke treatments

The only US Food and Drug Administration (FDA)-approved treatments in stroke focus on re-establishing reperfusion of the occluded vessel to restore normal cerebral blood flow levels. To achieve recanalization, two distinct strategies are available: (i) mechanical clot retrieval via endovascular thrombectomy; and (ii) pharmacological thrombolysis using tissue plasminogen activator (t-PA). Although these approaches have shown substantial clinical trial success, only a small percentage of stroke patients benefit from them due to the narrow therapeutic window of 4.5-h after stroke onset. This leaves behind a substantial portion of untreated stroke patients with no medical therapy to promote repair and recovery. Long-term management of ischaemic stroke is often limited to monitoring vital parameters and standard emergency care, providing preventative measures such as antithrombotic treatment with aspirin or low-molecular-weight heparins.

During the chronic phase, stroke treatments involve long-term neurorehabilitation to prevent the development of further complications, such as progressive stiffening of the limbs, and stimulate neural plasticity. Despite advances in stroke patient care, rehabilitation remains insufficient to induce complete functional recovery. To date, no clinical trials have succeeded in alleviating stroke survivors' neurological impairment.

Today, it is critical to develop new therapies that promote brain repair and functional recovery after stroke. Among all the therapeutic strategies currently in development, cell therapy has shown promise in enhancing brain repair in preclinical models. The crucial advantage of transplanted cells is their ability to activate multiple endogenous repair processes after stroke.

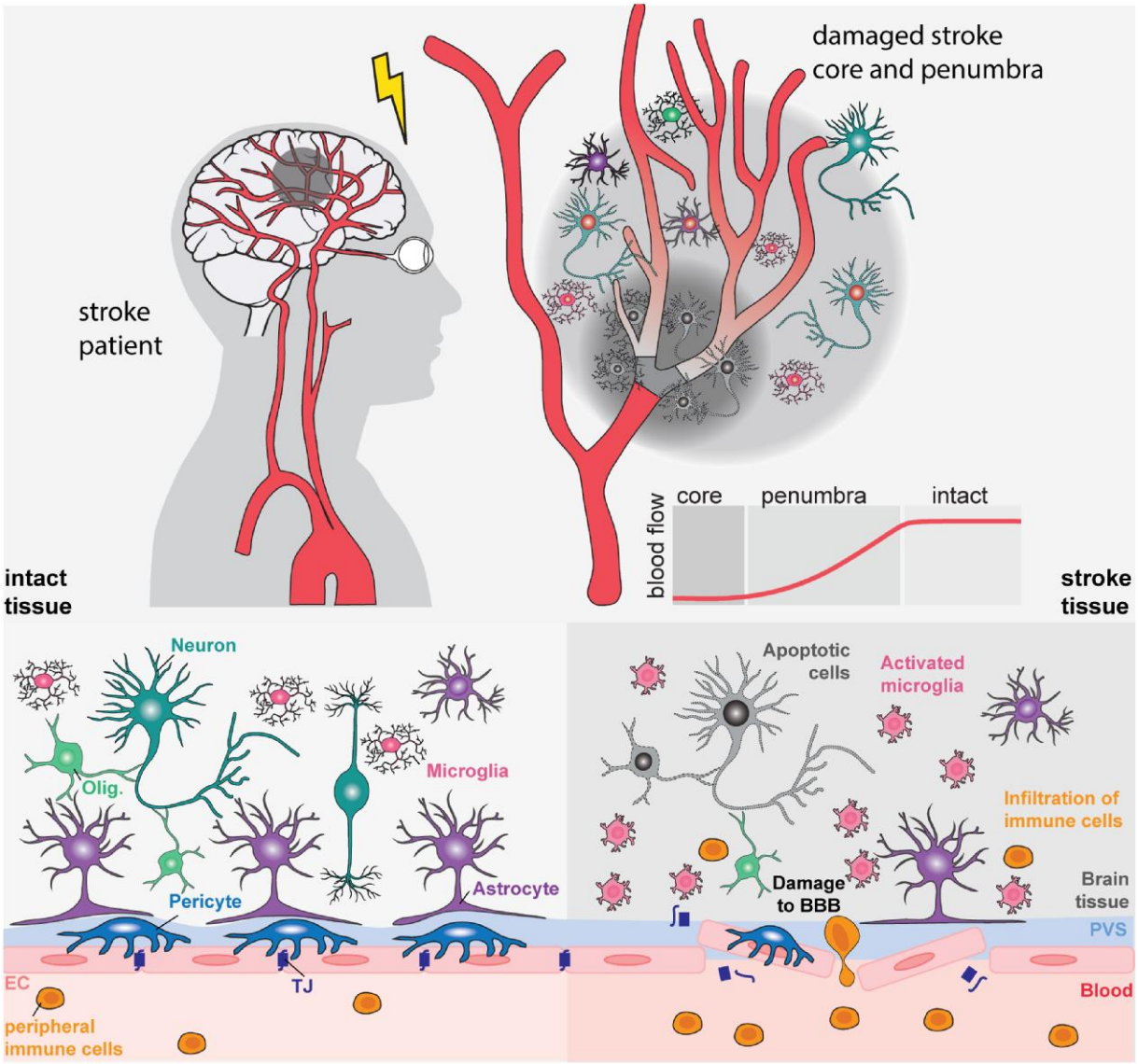

Figure 1 Pathophysiology of stroke. An ischaemic stroke usually occurs when a brain artery becomes blocked, leading to a reduction in oxygen and nutrients in the affected brain areas. These regions can be divided into: the stroke core, an area with severe cerebral blood flow reduction and irreversible necrotic cell death (<10 ml/100 g/min); the penumbra, an area with reversible damage (17–10 ml/100 g per min); and normal, intact tissue (>17 ml/100 g per min). Damage to stroke tissue includes necrotic and apoptotic cell death, damage to the blood–brain barrier, activation of resident microglia and infiltration of peripheral immune cells. EC = endothelial cell; olig = oligodendrocyte; TJ = tight junction.

established during the early hours, spreading the necrotic core towards the healthy tissue.

Interestingly, while neurons are very sensitive to hypoxia, non-neural cells like endothelial cells and pericytes are more resistant and can survive within the stroke-injured areas, even after a permanent ischaemic stroke.[36] At the vascular level, stroke induces significant changes to endothelial cell receptor expression, matrix degradation, detachment of astrocyte end-feet,[37] and pericytes,[38,39] all of which lead to BBB damage within the first 30 min and up to weeks after stroke onset in preclinical animal models.[37,40] The injury-induced vascular dysfunction is further aggravated during the acute phase by a series of cellular and molecular events. The acute secretion of the vascular endothelial growth factor (VEGF) in neurons, astrocytes and endothelial cells (ECs) of the penumbra can increase BBB permeability and elevate the risk of hemorrhagic transformation.[41-43] High levels of VEGF lead to the formation of new, immature vessels that lack tight junction proteins and pericyte coverage.[40,44,45] Activation of microglia, infiltration of peripheral immune cells through the opened BBB and release of pro-inflammatory cytokines (e.g. TNFα, IL1β, IL6, IFN-γ) and metalloproteases (MMPs) such as MMP-2 and MMP-9 further exacerbates vascular permeability, neuroinflammation and cell death.[46-48] (Fig. 1).

Within days to weeks, the lesion site turns into an empty cavity devoid of structural features necessary for cell infiltration and tissue growth.[25,49,50] The morphological remodelling of perilesional astrocytes into an astrocytic scar creates a physical barrier to tissue growth and compartmentalizes the cavity. Importantly, the length of the remodelling and involved processes can vary considerably depending on the sex, species and stroke size.[4,51,52] This compartmentalized cavity can accept a large volume of transplant without further damaging the surrounding healthy parenchyma,[53,54] which

makes extracellular matrix (ECM)-mimetic engineered biomaterials[55] and stem cell transplantation within the lesion site promising approaches for stroke treatment.[56,57] The subacute phase after injury is also characterized by progressive injury affecting neurons that survived the initial injury and leads to axon and glial degeneration due to the altered energy metabolism in preclinical models.[58,59] Moreover, a persistent secondary neurodegeneration process occurs, leading to neuronal loss, neuroinflammation and accumulation of neurotoxic proteins in distant brain regions that are anatomically connected to the infarct site but were initially unaffected by the cerebral blood flow (CBF) reduction from the stroke.[60,61]

This phase of acute cell death and tissue remodelling is followed by a phase of neuroplasticity and repair, during which neuronal and synaptic formation and remapping, gliogenesis, neurogenesis, vascular repair and restoration of the BBB happen. In patients, the neuroplasticity window is estimated to peak during the first 3 months[62] and to weaken after 6–12 months after stroke.[63,64] Although partial functional recovery in chronic stroke is possible,[65] it often requires intensive rehabilitation therapy and predominantly occurs through compensatory processes rather than 'true' regeneration.[66,67]

Endogenous stroke recovery

Post-stroke neural plasticity, also referred to neuroplasticity, can be defined as the ability of the brain to rewire and reorganize its neural circuits in response to injury in an attempt to adapt to the structural, metabolic and cellular changes associated with the tissue and functional loss. Plasticity involves both the de novo creation and modifications of existing neurons, axons, synapses and neuronal connections, persist for several months after stroke and primarily occurs in the peri-lesional area that surrounds the infarct.[68] During this phase, the transcriptional signature indicates an upregulation of genes and signalling pathways involved in axonal growth, vascular development, dendritic sprouting, synapse formation, adhesion molecules and cytoskeletal rearrangements in experimental rodent models of stroke.[50,69-72]

Neuroplasticity after stroke

Studies using rodent models of stroke indicate that genes associated with neuroplasticity, such as *Srr1*, *Gap43*, *Cap23* and *Marcks*, *c-Jun*, and genes coding for neurotrophic factors, such as *Ngf*, *Bdnf*, *Nt-3*, *Fgf-2*, *Igf-1* and *Gdnf*, are upregulated in sequential waves from Day 3 after injury.[69,71,73] Subsequently, cell adhesion molecules and cytoskeletal reorganization-associated genes such as *L1cam*, *Cdkn1a*, *Scg10* and *Sclip* are expressed. A solid body of evidence indicates that neuroplasticity is also associated with the reduction of growth inhibitory factors. The main axonal growth inhibitors in the adult and damaged CNS are myelin-associated proteins (e.g. Nogo-A/RNT4A, MAG) and axonal guidance molecules (e.g. netrins, ephrins, semaphorins). The regulation of these factors shows unique expression at different stages after stroke,[50,71,74] and appears to be age-dependent.[69,75] More recently some of the axonal growth inhibitors have been shown to be release and exhibit long-range signalling.[76,77] Loss- and gain-of-function studies indicate that the inhibition of pro-repair genes and activation of inhibitory genes impair functional recovery in animal models of stroke.[50,68,78-82]

Recent studies have shown a high heterogeneity of neuroplasticity in different brain areas. In rodents, non-human primates and humans, stroke lesions in the sensorimotor cortex were found to induce substantial remapping of sensorimotor functions over a period of 8 weeks.[4,83] As an example, the forelimb M1 area responds to stroke by inducing axonal sprouting from corticospinal fibres in the intact hindlimb M1 areas, converting a hindlimb neuron into a forelimb-projecting neuron. In addition, neurological functions from large infarcted areas can be recovered through unaffected regions of the contralateral hemisphere where sufficient capacity for structural remodelling is still present. Studies in both rodents and humans show that other brain circuits such as the ones in the somatosensory areas, may have weaker neuroplasticity potential with less remapping observed after stroke.[84-86] Even within the motor cortex, experimental studies have shown that spontaneously recovered mice after stroke have a distinct ipsi- and contralesional motor cortex transcriptome to littermates that show no recovery involving Adora2a, Drd2 and Pde10a-mediated cyclic AMP (cAMP) signalling.[73]

The formation of new neurons, or neurogenesis, may also contribute to neuroplasticity and repair after stroke. Early experimental data suggests that NPCs from the subventricular zone can proliferate, migrate towards the lesion site and differentiate into mature neurons. Additionally, experimental stroke can activate NPCs within and around the ischaemic areas, extending beyond the conventional neurogenic zones like the subventricular zone and the dentate gyrus.[87-89] A solid body of evidence shows that experimental enhancement of neurogenesis is associated with improved recovery,[90-93] while its inhibition is linked to impaired recovery.[93] Although NPCs have been well characterized throughout the years, their migration pattern from the neurogenic niche towards the lesion site is still poorly understood, as animal studies show that the route, distance and efficiency of migration are highly variable and depend on the severity, location and cellular activity of the lesion.[90]

Various other cell types play a significant role in stimulating neurogenesis and plasticity after injury, including immune and vascular cells.[94,95] Experimental studies have shown that endothelial cells are capable of releasing growth factors that stimulate vascular growth and remodelling in the peri-infarct area,[96,97] which in turn enhances NSC proliferation and migration towards the lesion site, while guiding their infiltration in the peri-infarct area[49,98] to stimulate neuronal plasticity.[98,99] In turn, NPCs increase angiogenesis via the secretion of VEGF,[100] demonstrating a bidirectional relationship between vascular and neuronal remodelling.[101,102]

Other important cell types for plasticity are pericytes and glial cells. For instance, pericytes as part of the neurovascular unit have been shown to regulate angiogenesis, CBF and repairment of the BBB after stroke,[103,104] and their absence leads to neurovascular uncoupling.[105,106] On the other hand, a subset of pericytes, termed type A pericytes, has recently been described to form a fibrotic scar tissue inhibiting neural plasticity in several neurological injuries, including stroke.[107,108]

Astrocytes not only have the capability to eliminate neurotransmitters and other molecules associated with excitotoxicity but are also important in neurovascular coupling and enhancing synaptogenesis by promoting the formation of new functional synapses.[109-111] Oligodendrocyte progenitors differentiate into mature oligodendrocytes, facilitating axon remyelination and improving white matter integrity,[112,113] while microglia have been shown to reduce inflammation and release trophic factors such as BDNF, thereby promoting synaptogenesis.[114]

Despite the many advances made in recent years, the exact mechanism by which all these neuroplasticity processes interact

with each other to promote the repair and recovery of lost functions is not fully elucidated. As an example, although neurogenesis was evidenced to contribute to healing in animal models, whether it can also contribute to stroke patients' functional recovery remains uncertain, as the vast majority of newly generated NPCs die within their migratory path before reaching the lesion site or integrating into pre-existing functional neuronal networks. Furthermore, adult neurogenesis is more robust in rodents, which have shorter turnover and maturation cycles, compared to primates, including humans.[115] The age-dependent decline of adult neurogenesis in the mouse[116] and human brain[117,118] may further limit the therapeutic impact for stroke patients with an average age of 70 years.[119] Overall, in humans, evidence of any functional contribution of adult neurogenesis to recovery remains limited and under-investigated.[120-122]

Such experimental uncertainties continue to hinder the development of clinically relevant solutions for stroke treatment. Over 50% of stroke survivors suffer from persistent motor and/or cognitive deficiencies that significantly affect their quality of life and increase their vulnerability to other comorbidities such as hypertension, cancer, diabetes and congestive heart failure.[123] With the current therapeutic approach, most improvement occurs in the first few weeks post-stroke, with a plateau after 3–6 months with not much motor recovery subsequently. Cognitive dysfunction, including those of the language domains, can still achieve some improvements with training even at the chronic phase, after 6 months. The process of functional recovery involves not only the enlargement of dendritic trees and the creation of new connections by ipsilateral neurons but also the sprouting of contralateral cells to support the damaged brain area. However, such extreme responses can even be detrimental and hinder functional recovery.[124] With the biology of recovery involving multiple interrelated pathways not yet fully known, the road toward a full recovery remains challenging; and newer approaches, including cell therapy, are essential to explore and advance the understanding and treatment of stroke recovery.

Vascular formation and repair of the blood–brain barrier

During the subacute phase after stroke, the vascular system and the associated components of the BBB and the peri-vascular ECM undergo extensive remodelling in the peri-lesional area. During this phenomenon, known as post-stroke angiogenesis, new blood vessels are formed, pericytes are recruited to the vessel wall and tight junctions are re-established to partially restore BBB integrity. Increasing evidence shows that the efficiency of post-stroke angiogenesis is age-dependent as EC proliferation and remodelling of brain vessels weakens with age in patients[125] and rodents.[75]

Animal studies indicate that all major angiogenesis-associated signalling factors are transcriptionally upregulated after stroke, e.g. *Vegfa*, *Vegfr2*, *Tie2*, *Angpt2*, *Pdgf*, *Cxcl1* and *Pecam1*.[50,75] VEGF secretion and activation of its receptor VEGFR2 are required for post-stroke angiogenesis.[49,50] Increased peri-lesional vascular growth has been shown to be associated with functional recovery in experimental and human stroke.[49,50,126,127] As an example, increased CBF is correlated with the upregulation of the VEGF-eNOS-NO pathway and glucose intake in the peri-lesional area,[128] VEGF expression is increased in the serum and the brain of stroke patients[129,130] and increased microvascular density around the lesion is correlated with longer survival in patients.[126,131]

Cerebral arteries and capillaries are supported by smooth muscle cells and pericytes, which play a major role in the BBB permeability and integrity[105,132,133] through their cellular crosstalk with ECs. During post-stroke angiogenesis, EC-secreted PDGFB binds to its receptor PDGFRB on the pericyte's surface, while pericyte-secreted Angpt1 binds to its receptor Tie2 on the EC's surface.[134,135] Enhanced pericyte coverage of peri-lesional vascular walls has been shown to improve BBB integrity and contribute to tissue repair and functional recovery.[49,50,136]

Despite clear evidence of the role VEGF plays in brain repair and neuroplasticity, developing VEGF-based therapies remains challenging due to the effect of VEGF on BBB permeability. Indeed, multiple studies have shown that the brain injection of VEGF can worsen the inflammatory response to stroke by increasing the risk of haemorrhagic transformation in newly formed, immature vessels,[43] known to have impaired tight junction protein and incomplete pericyte coverage.[40,44,45] Indeed, sustained BBB permeability after stroke is correlated with worse neurological outcome in rodents and stroke patients.[137] Adverse effects include enhanced peripheral immune cell infiltration, neurotoxicity and vasogenic oedema, which all lead to enhanced neuronal loss and infarct volume.[138,139]

Learning from clinical trials: efficacy and challenges of clinical cell therapy in stroke

Cell therapies have emerged as a promising therapeutic strategy for stroke and reached clinical testing two decades ago.[11,12] Initially, immortalized neural cell lines, fetal cell sources and adult MSCs were used as a cell source (Fig. 2). Cell therapies for stroke are generally considered safe and a large body of evidence from preclinical and larger phase 2 and phase 3 clinical trials reported only sporadic adverse events and generally a good safety profile (Table 1 and Supplementary Table 1).[18,29,142,146,151,152] Comprehensive reviews and meta-analyses on the clinical efficacy and safety of cell therapy for stroke have been discussed elsewhere.[11,153-155]

Although many cell sources proved effective in preclinical models, their subsequent translation to clinical applications has been met with inconsistent outcomes. Within the clinical landscape, challenges included limited efficacy, inefficient delivery methods, poor graft survival and differentiation rate and/or ethical concerns.[13] Recent advances in genetic cell manipulation and the iPSC technology have enhanced the potential and applications of cell therapy.[156] Cell sources for iPSCs can be isolated from donors, expanded indefinitely and differentiated into well-characterized cell types such as NSCs and glia (Fig. 2).

As an example, iPSC-derived dopaminergic precursor cells and iPSC-derived retinal epithelial cells were found to be safe and effective in treating Parkinson's disease[157,158] and age-related macular degeneration.[159,160] In stroke research, there is a preferential use of iPSC-derived cell sources in preclinical studies[18,29,152,161] that has not yet been translated into clinical applications. The majority of clinical trials instead used primary MSCs as a cell source, especially when choosing a systemic route of administration in acute stroke patients. Immortalized NSC cell lines were primarily administered directly into the brain, mostly in chronic stroke patients.[144,162,163]

Over the past 5 years, a limited number of smaller studies have demonstrated that the application of MSCs via intravenous or intraarterial administration leads to notable improvements in the National Institutes of Health Stroke Scale (NHSS), modified Rankin Scale (mRS) and/or Barthel Index (BI) when compared to control groups of stroke patients in the acute/subacute[141,143,145,147,149,164] and chronic phase[147,148] post stroke. The

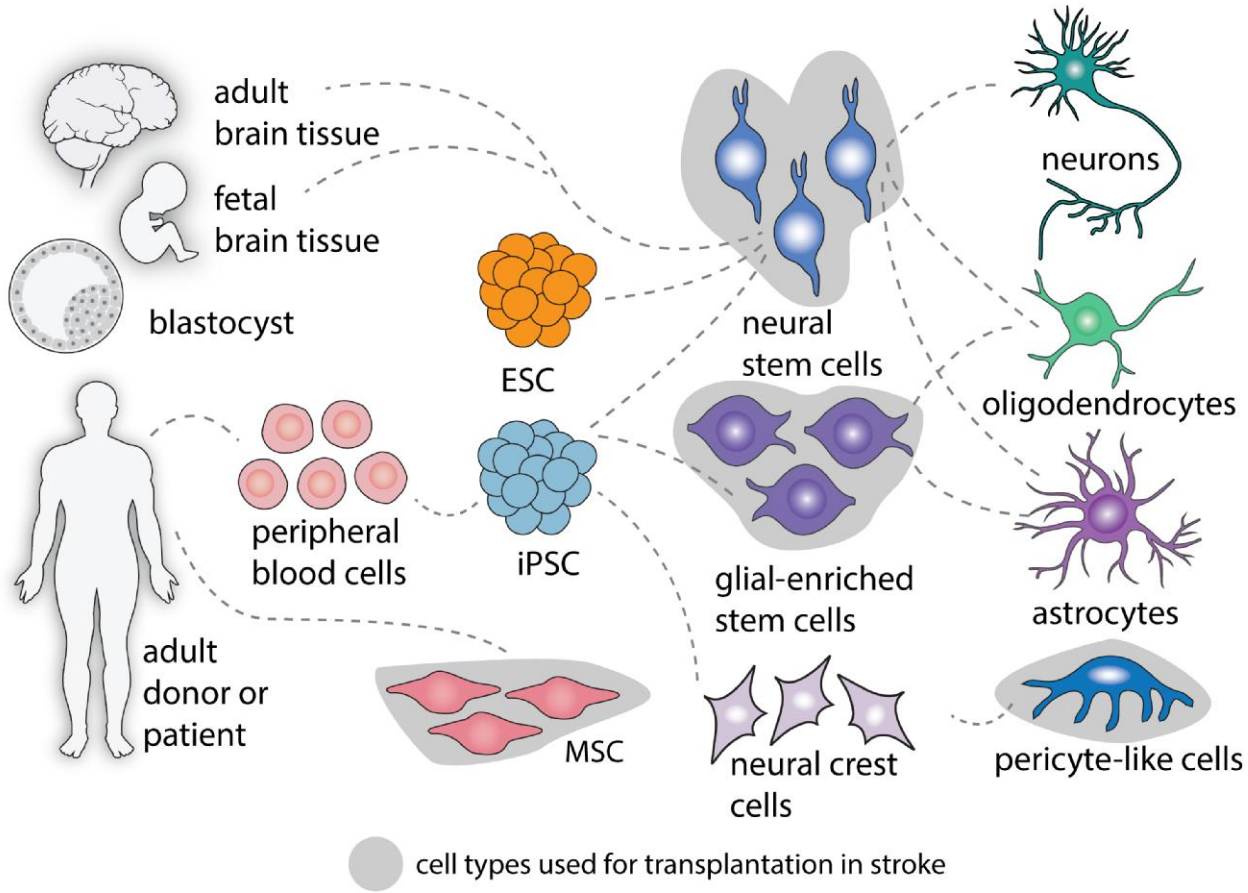

Figure 2 Cell types for cell therapy after stroke. Cell transplants can be derived either from the embryonic/fetal stages or the adult human. Adult stem cells comprise mesenchymal stem cells (MSCs) or differentiated cells from the peripheral blood reprogrammed to generate induced pluripotent stem cells (iPSCs). Both embryonic stem cells (ESC) and iPSCs can be differentiated into neural or glial-enriched stem cells to generate neurons, oligodendrocytes or astrocytes. Pericyte-like cells can be generated from iPSCs through neural crest cell intermediates.

efficacy of MSCs has only been partially confirmed by a more extensive phase 3 randomized controlled trial, STARTING-2, enrolling 60 stroke patients. Intravenous application of MSCs led to diminished corticospinal tract degeneration and enhanced neural network connectivity, as assessed through neuroimaging, along with improved lower extremity motor function according to the Fugl-Meyer Assessment.[141] However, the primary outcome in STARTING-2, as measured by mRS after 90 days, reported no significant differences between the groups.[142] No significant functional improvement was observed in a more recent randomized, controlled, multicentre trial (IBIS trial) that recruited 77 patients. The intraarterial transplantation of autologous bone marrow mononuclear cells in stroke patients at 180 days showed no improvement in mRS scores.[151] Parallel results were seen using allogenic bone marrow-derived adult progenitor cells, specifically MultiStem. In the phase 2, multicentre, double-blind, randomized and controlled MASTERS trial, intravenous MultiStem administration 24–48 h post-stroke failed to enhance the neurological outcome at 90 days.[150] However, the follow-up trials MASTERS-2 (a phase-3 study recruiting 300 patients) and TREASURE (a phase 2/3 study that exclusively recruited 206 patients from Japan[165]) are currently exploring the impact of the same MultiStem cell source but administered within a tighter window of 18–36 h after stroke. The TREASURE trial recently reported enhanced functional outcomes in an exploratory subgroup analysis with no corrections for multiple comparisons in the mRS and BI.[166] However, the trial did not meet its primary end point of achieving an 'excellent outcome'—characterized by combined improvements in mRS, NIHSS and BI scores—at the 90-day mark.[140] The MASTERS-2 trial is still in the recruitment phase and has not yet been finalized. As of now, no detailed data from the MASTERS-2 trials have been released for peer-reviewed publication.

Besides MSCs, a genetically modified neural stem cell line, CTX0E03, has been tested in chronic stroke patients through the PISCES series of trials. Both PISCES-I and PISCES-II involved the administration of CTX0E03 cells to chronic stroke patients via stereotactic injection.[144,163] The larger PISCES-II trial, a phase 2a, multicentre trial, administered 20 million cells to 23 patients, resulting in improved upper limb function at 3, 6 and 12 month intervals.[144] However, the subsequent randomized, controlled phase 2b trial, PISCES-III, began but was prematurely terminated, yielding no results.[167]

An overview of major clinical trials with results using stem cells for stroke is summarized in Table 1 and Supplementary Table 1. Comprehensive analysis and excellent reviews on the efficacy of cell therapies for stroke in clinical trials have been performed in recent studies.[154,155,168,169]

Despite the large body of preclinical and clinical data, only a small fraction of studies looked into the mechanistic insights and molecular crosstalk between grafted stem cells and stroke-injured

Table 1 Selected recently completed clinical trials with results from using stem cells to treat stroke

Route	Cell type	Cell origin	Cell dose	Transplanted patients, *n*	Control patients, *n*	Stroke phase	Adverse events	Major findings	Year
IV	BM multipotent adult progenitor cell (TREASURE)[140]	Allogenic	1.2×10^9	104	102	Acute/subacute	No	No improvement in 'excellent' outcomes at 90 days compared with placebo measured in mRS, NHSS, Barthe index	2024
IV	BM-MSC[141]	Autologous	–	31	13	Subacute	Not measured	Significant better FM and improved connectivity	2022
IV	BM-MSC[142]	Autologous	–	39	15	Acute/subacute	No	No significant improvement in mRS, but better leg motor function	2021
IV	BM-MSC[143]	Autologous	$100, 300 \times 10^6$	16	15	Subacute	No	Improvement in motor-NIHSS, motor FM score and task-related fMRI. But no significant improvement in NIHSS, mRS. BI	2020
IC	NSC (cell line CTX0E03)[144] (PISCES-II)	Allogenic	2×10^7	21	0	Subacute/chronic	Transient procedural events	Action research arm test improvement (only in patients with upper limb function)	2020
IV	BM-MSC[145]	Allogenic	$0.5, 1, 1.5 \times 10^6$	15 + 21	0	Chronic	Only mild adverse events possibly related to procedure	Improvement in BI, NIHSS compared to baseline	2019
IA	BM-aldehyde dehydrogenase bright stem cells (RECOVER)[146]	Autologous	3.08×10^6	60	40	Subacute	Only mild/moderate adverse events	Demonstrated safety, no significant improvement in mRS, NIHSS, BI	2019
IC	Modified BM-MSC (SB623)[147,148]	Allogenic	$2.5, 5, 10 \times 10^6$	18	0	Chronic	Adverse events e.g. headache, caused by surgical procedure	ESS, NIHSS, FM, FMMS improvement. No difference in mRS	2018
IA	BM-MNC[149]	Autologous	5×10^8	10	10	Subacute	No	Improving trends in NIHSS, mRS, BI compared to baseline	2018
IV	BM multipotent adult progenitor cell (MASTERS)[150]	Allogenic	$400, 1200 \times 10^6$	67	62	Acute	No	No significant improvement in NIHSS, mRS, BI	2017

Stroke phase: acute, <7 days; subacute, 7 days–6 months; chronic, >6 months. AD-MSC = adipose-derived mesenchymal stem cells; BI = Barthel Index; BM-MNC = bone marrow mononuclear cells; BM-MSC = bone marrow mesenchymal stem cells; CD34+ HSC = CD34+ haematopoietic stem cells; ESS = European Stroke Scale; FM = Fugl-Meyer Assessment; FMMS = Fugl-Meyer Motor Score; IA = intra-arterial; IC = intracranial; ICV = intracerebroventricular; IT = intrathecal; IV = intravenous; mRS = modified Rankin Scale; NIHSS = National Institute of Health Stroke Scale; NSC = neural stem cells; UCB = umbilical cord blood.

brains.[170,171] Clinical trials have produced mixed outcomes so far. This suggests that a more comprehensive understanding of the underlying mechanisms could refine various aspects of cell therapies. Two general beneficial mechanisms have been suggested for cell therapies in stroke. The first mechanism is the direct cell replacement that allows grafted cells to differentiate into mature neurons or glia and functionally integrate into damaged host's brain circuits. Depending on the cell source, the graft homing can directly contribute to e.g. restoration of brain function, BBB integrity or CBF in the damaged regions. Second, grafted cells may contribute indirectly through e.g. supportive release of pro-regenerative factors that enhance or prolong endogenous regeneration programs and ultimately lead to improved recovery.[10,172]

Mechanistic insights from preclinical studies on cell therapy for stroke

Cell replacement after stroke

Multiple animal studies have investigated the integration of NPC grafts into the host circuits through viral-assisted tracing of their synaptic connections,[173] the monosynaptic inputs from grafted neurons,[173,174] or through the inhibition of their neuronal activity (using chemogenetic or light-induced inhibition), which results in partial impairment of gained motor recovery.[18,25,173] When transplanted into the damaged visual cortex, neural grafts exhibited functional calcium (Ca^{2+}) activity in response to visual stimuli[175] and, similarly, showed responses to physiological sensory stimuli when grafted in the somatosensory cortex.[174] Both studies demonstrate the successful functional integration of grafted neurons.

Enhanced functional integration of iPSC-NPCs grafts has recently been shown by optochemogenetic activation. After transplanting genetically modified iPSC-NPCs with the optochemogenetics fusion protein (LMO3) into the post-stroke brains of young and old mice, repeated stimulation improved graft integration in the peri-infarct regions and fostered recovery.[176] Earlier mechanistic studies have shown that an important pathway guiding migration and integration is CXCR4/SDF1-a. CXCR4-expressing NSC migrate/move towards astrocytic and endothelial SDF-1a expression that is upregulated within the injured brain regions.[177] Newer studies have also found that CXCR4 genetic up-regulation, preconditioning of cell grafts and loss of LRP1, a regulator of CXCR4 can further enhance the homing process towards the stroke area.[178-180]

Direct functional replacements in cell therapy have also been investigated for non-neural cells. In contrast to NPC grafts, genetic ablation (induction of cell death after transplantation using diphtheria toxin[18,25]) of glial-enriched progenitor cells did not contribute to loss of functional benefits after transplantation in a rodent model of white matter stroke, suggesting that these cells promote recovery through an indirect mechanism.[18] Transplanted iPSC-derived pericyte-like cells were localized within the vasculature in the ischaemic border zone and were associated with reduced BBB opening and improved functional recovery.[29] In addition, multiple studies suggest that, although MSCs can survive in the brain over 2 weeks post-transplantation, they likely do not have the ability to transdifferentiate to neural lineages *in vivo*,[181] despite conflicting *in vivo* studies in which unspecific uptake of fluorescent and DNA tracers were found in astrocytes and neurons.[182]

The extent of direct cell replacement depends not only on the selected cell source but may also be considerably influenced by the timing of cell therapy. Generally, it is believed that the capacity to integrate new cells into a damaged circuitry is lower in chronic stroke due to the substantial remodelling in the transition from the acute to the chronic phase of stroke.[183]

One of the most important limitations of direct cell replacement lies in the short survival of newly generated neurons that are thought to protect the infarcted brain by releasing factors rather than by replacing lost tissue. Survival of transplanted cells is usually not an outcome of clinical trials limiting our knowledge. Also, heterogeneity in differentiation capacity is reported depending on tissue sources and donor characteristics (i.e. age, gender, comorbidities), which may hamper the functional properties of MSCs. Preclinical evidence suggests a role for pro-survival treatment in this field, including preconditioning, pretreatment or overexpression of antiapoptotic, neurotrophic and homing mediators.[13,156] Also, to improve survival and facilitate replacement, microcarriers have been successfully used in preclinical studies. As compared to cell culture media or saline, hydrogels or scaffolds protect cells from the hostile stroke environment and carries neurotrophic and protective factors, further improving graft survival.[184-187] Lastly, the use of structured tissue (i.e. brain organoids) rather than dissociated cells may offer cell-cell and cell-matrix protection and improve graft survival and vascularization after transplantation.[161,188-190]

Indirect beneficial effects of cell therapies

Although cellular integration into the host tissue is possible, the majority of cell transplantation studies in stroke have found that the beneficial effects on repair and recovery are stimulated through indirect mechanisms that include immunomodulation, release of pro-regenerative factors that stimulate vascular and neural growth and ECM remodelling in the peri-infarct tissue, all of which activate the endogenous repair machinery of the brain and promote repair and recovery after stroke (Fig. 3). The main findings from preclinical studies that have used cell therapy for stroke treatments are summarized in Table 2 and Supplementary Table 2.

Immunomodulation

Although acute inflammatory responses post-stroke can also be beneficial,[137] as they facilitate tissue repair by secreting neurotrophic factors and clearing debris, persistent inflammation can contribute to secondary injury to viable tissue by releasing pro-inflammatory cytokines and other cytotoxic products.[48,192,193] Besides the classical pro-inflammatory M1 and anti-inflammatory M2 classification, transcriptomic profiling of microglia has revealed a dynamic range of activation states, including mixed phenotypes, following stroke.[70,193,194]

Experimental studies have found that transplantation of MSC, iPSC and ESC-derived NSC results in gene and protein upregulation of anti-inflammatory cytokines such as IL-10 and reduction of pro-inflammatory cytokines including TNFα and IFN-γ,[19,32,195,196] both of which have been linked to improved stroke outcome.[19-21,171,197,198] Furthermore, various cell grafts have been shown to release neurotrophic factors such as NGF, GDNF and BDNF, known to inhibit T-cell activation and leucocyte infiltration, which overall modulate the immune response to injury.[27,199] Transplanted MSCs were also associated with enhanced TGF-β expression, which inhibits MCP-1 secretion and limits $CD68^+$ macrophage infiltration into the lesion.[27,200] Transplanted NSCs have also been shown to reduce chronic CNS inflammation by reducing succinate levels in the CSF and decreasing immune cell infiltration and secondary damage.[201] Reduced post-stroke inflammation is

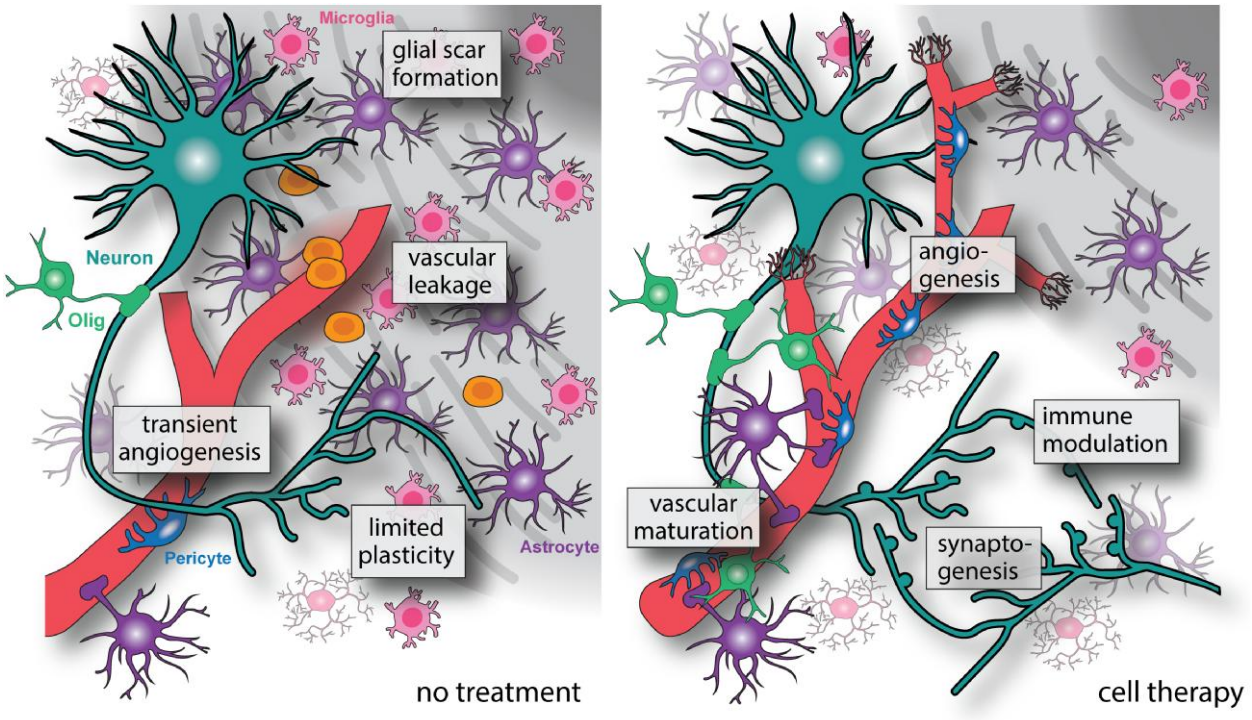

Figure 3 Indirect mechanisms of cell therapy for brain repair. Indirect effects of cell therapy include improved vascular repair and maturation through the release of pro-angiogenic factors in the peri-infarct region. Immunomodulation and an increased anti-inflammatory signature reduce secondary damage and decrease glial scar formation. The release of neurotrophic factors by cell grafts also increases axonogenesis and synaptogenesis in damaged areas.

associated with reduced infarct volume and improved recovery.[19,21,24,26] Similarly, comparable anti-inflammatory effects were observed when delivering stem cell-derived extracellular vesicles containing anti-inflammatory cytokines.[202-205]

A very recent alternative theory, termed the 'bioreactor' hypothesis, attempts to determine how systemically delivered cells induce immunomodulatory effects without integrating the brain.[181] This theory lies in the cells' ability to migrate and integrate peripheral organs such as lungs, spleen and other lymphoid tissue, interact with the host's immune cells and generate an anti-inflammatory environment that is amenable for tissue remodelling and repair in the injured brain.[181] In experimental stroke research, pioneering studies demonstrated functional transplantation of primary human umbilical cord blood without graft entry into the CNS, indicating an alternative peripheral recovery mechanism, such as migration towards the spleen.[206] More recent experimental stroke studies confirmed that multipotent adult progenitor cells improve post-stroke recovery by elevating anti-inflammatory cytokines such as Il-10 and increase the presence of regulatory T cells.[207,208] Interestingly, the removal of spleens mitigated the effect of cell therapy in stroked rats, indicating the spleen's essential role in these therapeutic responses.[207] Also, inflammation in other peripheral tissues, such as the gut, can regulate T-cell mediated inflammation and recovery after stroke.[209]

Vascular repair and remodelling

In recent years, a solid body of evidence from both preclinical and human studies has shown that post-stroke angiogenesis plays a major role in activating endogenous repair mechanisms and functional recovery after injury,[43,102] and its inhibition is systematically associated with delayed or worsened repair and recovery.[49,50] Once delivered into the injured brain, transplanted stem cells, including MSCs and NSCs, are known to produce pro-angiogenic factors such as VEGF, ANG1, FGF2, PDGFA and HIF-1a, resulting in increased vascular density and expression of angiogenic receptors in the peri-infarct tissue.[23-25,28,195] Cell-mediated angiogenic responses are largely induced by the expression of VEGF by the transplants or by the tissue directly adjacent to the graft.[210] Ang1 signalling in MSCs cell grafts was found to activate angiogenesis and CBF after stroke,[211] and increased FGF2 levels were found to induce endothelial and pericyte proliferation and migration in the peri-infarct area after experimental stroke.[212] Mechanistically, transplanted bone-marrow mononuclear cell grafts have been shown to activate endogenous angiogenesis by transferring small molecules including glucose via gap junctions, thereby providing metabolic support to the stroke-injured tissue.[213,214]

Functional vascular repair also requires the re-establishment of the neurovascular unit to restore the BBB integrity. This is clearly illustrated in studies in which preservation and restoration of the BBB via NSC transplantation is associated with increased expression of tight junction proteins (claudin-5, occludin, ZO-1) and dystroglycan, decreased BBB leakage[171,210] and reduced stroke volume and neurological deficits after stroke.[171,199,215] In addition, studies investigating the effect of MSC brain injection found a direct correlation with the inhibition of the vascular key adhesion molecule ICAM-1, known to play a major role in the transmigration of peripheral immune cells, through the AMP-activated protein kinase (AMPK) pathway.[199] Transplantation of MSCs also reduced MMP-9 activity (but not the MMP-2 activity) and BBB leakage in stroked mice.[199,216] Studies on MSC-derived extracellular vesicles (EVs) showed comparable angiogenic and neuroprotective effects to transplanted cell grafts.[202,217]

Table 2 Selected recent preclinical studies using cell therapy for stroke

Route	Cell source	Ischaemia	Species	Animals, *n*	Sex	Stroke phase	Dose	Major findings	Year
IP	iPSC-NSC[171]	Permanent	Mouse	40	Male and female	Chronic	2.5×10^5	Improved angiogenesis, reduced inflammation, axonogenesis, reduced BBB leakage, improved functional outcome	2024
IP	iPSC-organoids[161]	Permanent injury on visual cortex	Rat	36	Male	Chronic	Organoids	Survival, differentiation, neuronal projections to visual system and spontaneous and visually-evoked neural activity of engrafted organoid cells (GFP, IHC, rabies virus retrograde tracing, herpes simplex virus anterograde tracing, electrophysiological recordings)	2023
IP	Human brain-derived iSCs[191]	Permanent	Mouse	118	N/A	Chronic	1.0×10^4 1.0×10^5	Improved behavioural recovery (wire hang, basket, open-field, hot plate, Y-maze, passive avoidance-learning, sucrose preference and tail suspension tasks); homing and differentiation of engrafted cells (GFP, IHC); activation of endogenous NSCs (IHC)	2023
IP	NSC[19]	Temporary	Mouse	75	Male	Subacute	1.0×10^5	Reduced infarct size (TTC); reduced inflammation TNFα, IL6, IL1β, ICAM1 and extracellular matrix disassembly MMP3, MMP9 (PCR); brain transcriptome (bulk RNAseq)	2022
IP	Glia-enriched progenitors[18]	Temporary	Mouse	114	Male	Acute–chronic	0.9×10^5	Reduced infarct size (BDA); improved behavioural recovery (grid walking, cylinder test, novel object recognition, fear conditioning); homing, survival, proliferation and differentiation of engrafted cells (GFP, IHC); improved axon growth and connection (BDA, IHC); improved remyelination and oligodendrocyte proliferation (IHC)	2021
IP	Control and p5-primed ADMSCs[23]	Temporary	Rat	40	Male	–	1.0×10^6	No change in infarct volume (IHC NeuN); improved behavioural recovery (beam walking, sticky-tape, cylinder test, water maze); reduced microglia apoptosis (IHC, p5-ADMSCs only); increased angiogenesis (IHC); survival of engrafted cells (IHC)	2021
IA	MSCs[20]	Temporary	Rat	30	Female	Acute	1.0×10^5	Reduced infarct volume (TTC); improved behavioural recovery (NDS, Rotarod); reduced brain inflammasome activity caspase 1, ASC, IL1β, ASIC1a, NLRP1, NLRP3 (WB)	2021
IV	hPSC-pericytes[29]	Temporary	Mouse	110	Male	Subacute	1.0×10^6	Reduced infarct size (TTC); improved behavioural recovery (NDS, corner test, sticky-tape, Rotarod); reduced neuron apoptosis (TUNEL, IHC); improved BBB (water content, permeability assay, TEM, WB, IHC); homing, vessel investment and pericytic differentiation of engrafted cells (DsRedE2, IHC); greater benefits by hPSC-PC than fibroblast, similar to brain pericyte; the role of midline in neuroprotection by hPSC-PC	2020

Stroke phase at time point of cell transplantation: acute, <1 day; subacute, 1 day–7 days; chronic, >7 days. Animal numbers in some studies were estimated based on the methods section and figure legends. ADMSCs = adipose-derived mesenchymal stem cell; BBB = blood–brain barrier; BDA = biotinylated dextran amine; fMRI = functional MRI; IA = intra-arterial; IP = intraparenchymal; IHC = immunohistochemistry; iPSCs = Induced pluripotent stem cells; iSC = induced multipotent stem cells; IV = intravenous; MSCs = marrow stromal cells; NDS = neurologic deficit score; NPCs = neural progenitor cells; NSCs = neural stem cells; TTC = 5-triphenyltetrazolium chloride staining; WB = western blot.

One suggested mechanism is that EVs can promote angiogenesis after experimental stroke by inhibiting autophagy through STAT3 activation.[217] Similar results were also found in animal studies of spinal cord injury, in which MSC-derived EVs were associated with a reduction in pericyte detachment from capillaries through inactivation of the NF-kB pathway.[218]

Glia and glial scar formation

The glial scar forms a major physical barrier to axonal infiltration at the lesion site and limits the regenerative process. Reduced glial scar formation has been associated with improved stroke recovery[49,50] and beneficial effects following post-stroke cell therapy.[219] While the pharmacological degradation of glial scar components (such as chondroitin sulfate proteoglycans) prior to NSC transplantation enhances recovery following CNS injury,[220] the presence of the scar remains necessary as its absence reduces MSC graft integration and beneficial effects on functional outcomes.[221] Molecular studies point out astrocyte heterogeneity, indicating inflammatory states that can differentially regulate axonal sprouting and support the neuroinflammatory response to injury.[222,223]

Earlier preclinical studies in spinal cord injury and stroke animal models suggest that endogenous NSCs from neurogenic niches play a major role in producing astrocytes that contribute to scar formation through a Notch /Thrombospondin-4 (Thbs4) signalling mechanism.[224,225] The inhibition of NSC proliferation, blockage of Notch signalling and deficiency in Nuclear factor I (a regulator of Thbs4) were associated with impaired glial scar formation, increased neuronal apoptosis, BBB defects and increased haemorrhagic transformation.[224,225] Transplantation of CDK5-knockdown astrocytes in an experimental global cerebral ischaemia model showed recovery of the neurovascular unit integrity by stimulating the production of endogenous BDNF and improving functional recovery.[226] Furthermore, the direct lesion environment may influence the therapeutic effect of astrocytes as NSCs transplanted in close proximity to the lesion preferentially differentiate towards a naturally occurring repair astroglial phenotype.[227]

Glial cells can further contribute to the proliferation and differentiation of oligodendrocyte progenitor cells (OPCs) to myelinating oligodendrocytes. One underlying mechanism is through astrocytic release of TIMP-1 and modulation of the surrounding ECM.[228] In a subcortical white matter stroke model, transplanted glial progenitor cells promoted endogenous OPC proliferation and axonal sprouting that led to improved motor and cognitive recovery.[18] Furthermore, local stereotaxic transplantation of OPCs into a stroke model reduced infarct volume and protected the BBB via Wnt/β-catenin signalling acutely after stroke.[229]

Neural repair

Indirect repair of neural circuits can occur through several mechanisms after cell therapy. Transplanted MSCs and NSCs have shown to induce endogenous neurogenesis and gliogenesis.[191,230] For instance, the migration and differentiation of endogenous cells are enhanced following MSC cell therapy via the PI3K-Akt signalling pathway.[231] The release of general chemokines such as SDF-1 and NRG1 was shown to increase the proliferation of endogenous NPCs.[21] Furthermore, there is accumulating evidence that many signalling pathways overlap between angiogenic and neurogenic processes.[50,102] For instance, the pro-angiogenic factor VEGF also regulates neurogenesis and survival of neural progenitor cells after stroke,[49] and axon guidance molecules such as Nogo-A and Netrin 1 have been shown to be important for post-stroke angiogenesis,[50,232-234] as guidance cues to direct newly formed NPCs and newly formed neurons towards the injury.

To improve engrafted cell survival and homing, NSC can be preconditioned or engineered to overexpress factors that are neurotrophic, antiapoptotic, improve differentiation, or facilitate revascularization. For instance, BDNF pretreatment of transplanted NSCs reduced stroke size and improved sensorimotor recovery after stroke,[235] overexpression of neurotrophin-3 in NSC improves survival and reduces glial activation and results in smaller brain lesion volume.[236] GDNF overexpressing NSCs promote neuronal survival and migration of transplanted cells by improving the injury-microenvironment.[237,238] Over-expression of angiogenic factors such as VEGF, HIF-1a and angiopoietin in NSCs improve functional recovery by balancing the proapoptotic environment and facilitating angiogenesis.[239] Other approaches to reducing graft apoptosis by overexpressing Akt, Bcl-2 or survivin have also proved effective at the experimental level.[156,240]

Molecular crosstalk between grafted stem cells and stroked brain tissue

Advances in transcriptomic technologies now allow the identification of molecular interactions between grafted cells and the stroke-injured host tissue. A recent study showed that transplantation of iPSC-derived NSC promotes long-term functional recovery after cerebral ischaemia.[171] Single-cell profiling of grafted cells revealed that the graft primarily differentiated towards GABAergic-like cells and communicated with host tissue via regeneration-associated pathways such as Neurexin (NRXN), Neuregulin (NRG), Neural Cell Adhesion Molecule (NCAM) and SLIT signalling pathways.[171]

To further understand the molecular crosstalk and identify paracrine factors between graft and host after post-stroke cell therapy, a recent molecular study used translating ribosome affinity purification (TRAP) sequencing to separate and identify both transcriptomes 7 days post stroke.[170] As expected, most enriched pathways from host tissue were associated with stroke, immune response, cell death and brain plasticity. Analysis of the graft transcriptome revealed more than 400 differentially expressed genes from NSC grafted in stroke-injured versus naïve brains. These genes were enriched in pathways linked to cell differentiation, neurogenesis, gliogenesis, synaptic plasticity and lipid metabolism,[170] confirming findings from experimental cell therapy studies for stroke.[18,19,26,219,241] The study also confirms the importance of different microenvironments after stroke depending on the proximity to the core.[227]

The interaction of secreted factors and identification of canonical pathways between graft and host revealed molecular candidates such as BMP6 from the stroked brain induce canonical BMP signalling in the graft and hNSCs secrete noggin, a factor that has been shown in earlier studies to be important for stroke brain recovery.[242]

Current challenges and prospective strategies for the clinical implementation of cell therapy in stroke

Cell therapies have been recognized by the Stroke Treatment Academic Industry Roundtable (STAIR) as one of the most promising approaches to improving stroke outcome.[243] However, several limitations have also been recognized to hinder the translation of cell therapy for stroke into clinical practice, including age,[8]

comorbidities[244,245] and medications,[246] which are mostly not accounted for in preclinical trials. This disparity in the design of preclinical and clinical studies may contribute to the limited efficacy of cell therapies in stroke patients.[247] To address these limitations and propose future research directions, guidelines were proposed by academic and industry experts in the field during Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS) meetings.[248-250] These guidelines recommend, for example, the use of preclinical models that reflect the targeted patient population (sex, stroke model, age, disease comorbidities) and the combination of cell therapies with medications predominantly used by stroke patients (antiplatelets, antihypertensives, statins).[250] For instance, the therapeutic effects of stem cells on stroke in diabetic rodents can vary considerably compared to non-diabetic rodents, potentially leading to adverse outcomes, including haemorrhage and BBB leakage.[244,251]

Furthermore, the majority of preclinical studies use transient models of stroke, which mimic the recanalization therapy in patients.[252] However, many patients do not receive recanalization therapy due to the short therapeutic window or contraindications. Additionally, the success rate of recanalization therapy in removing occlusions is only 6–30%,[253] suggesting that permanent occlusion models might better reflect the clinical scenario.

Inconsistent outcomes from preclinical and clinical trials can also be attributed to the functional outcome measures, as recognized by the STEPS consortium.[250] Functional tests in rodents should be highly sensitive to detect long-term impairments and include tasks such as reaching, foot fault tests or deep-learning assisted gait analysis.[62,254,255] Simpler tasks are often useful to identify acute stroke or exclude animals but may not be appropriate to quantify long-term therapeutic efficacy. Similar challenges are also present in clinical settings. The commonly used mRS score is a relatively gross measurement[256] that may not be able to detect meaningful improvements, especially in small trials with a heterogeneous cohort of stroke patients. New concepts for parallelizing preclinical and clinical trial designs have been proposed by the STEPS consortium to accelerate clinical translation. In these studies, preclinical efficacy results can be tested in early-stage safety clinical trials. Next, information about the target patient population can then be incorporated into more advanced confirmative preclinical efficacy studies, which are followed by larger clinical efficacy trials.[250] Additionally, beyond outcome measures, future trials may support their findings with recent advancements in fluid biomarkers.[130,257-259]

As the pathology of ischaemic stroke is very heterogeneous, the potential of different cell sources may strongly depend on the stroke subtype. Glial-enriched cells are likely more suitable for white matter strokes, whereas NPCs have been preferentially tested in cortical strokes mainly affecting the grey matter.[18] The delivery route of cell grafts can occur either by local brain transplantations directly to the injury site or through a systemic blood injection. While during acute stroke there is evidence that the disrupted BBB may allow more effective migration of cell grafts to the brain,[260] chronic strokes likely require a local transplantation to ensure delivery of cell grafts in sufficient quantities.

In parallel, recent advancements in the genetic engineering of cell grafts may facilitate more efficient migration across the restored BBB by overexpressing surface peptides known to facilitate the migration of peripheral immune cells to the brain.[156] Cell graft delivery could also be further improved using recently developed self-assembling microcarriers based on microbubble propulsion.[261,262] If cell grafts are not derived from the patient, the immune incompatibility with the recipient represents another limitation 0.[263] To avoid graft rejection, long-term treatment with immunosuppressive drugs is usually required for successful allogenic cell therapies.[158] However, new strategies are developing to enhance the immunocompatibility of cell grafts by genetic modification of human leukocyte antigen (HLA) genes to create 'universal' or HLA-matched cell lines and the generation of large iPSC biobanks.[264-266]

One of the biggest clinical concerns is likely the undesired transformation of transplanted stem cells into malignant tumours. Therefore, establishing a cell safety system to selectively eliminate the cell grafts following undesired transformation must be considered.[267] For instance, a commonly used approach is the expression of herpes simplex virus thymidine kinase and treatment with the prodrug ganciclovir, which has been shown to selectively remove grafted cells in preclinical and clinical settings.[156,268] Alternatively, the knock-in of inducible caspase 9 (iCasp9) has been successfully implemented in preclinical and clinical studies. Upon administration of chemical inducer AP1903, iCasp9 becomes activated, leading to apoptotic signalling and subsequent apoptosis of the transduced cell graft.[156,268]

In future clinical trials involving cell grafts for stroke, iPSC-based cells are likely to become the preferred source. This is due to their easy accessibility, expandability and ability to give rise to customized cell types, as has been demonstrated in recent studies on other neurological and ophthalmological diseases.[159,269] To address the logistical, regulatory and cost challenges associated with using autologous iPSCs, allogenic cell sources that have undergone in-depth HLA characterization and are stored in a biobank are likely to offer a more practical solution. This approach could make off-the-shelf cell products more readily available to a broader population.[270] The scope of HLA coverage may further be increased by using genetically engineered, universal iPSCs that facilitate immune evasion.[264,266] These modifications will likely require safety measures for iPSC-therapies, which could be implemented using genetic safety switches, a method already applied, e.g. in clinical T-cell therapies for lymphoma.[271] If the anticipated cell therapeutic mechanism relies on the bystander effect, current clinical trials, such as MASTERS, suggest that systemic transplantation of cell grafts should ideally occur within the first 36 h after stroke onset.[150] However, clinical data from the PISCES trial series indicate that neural cell sources could also be beneficial for chronic stroke patients when locally transplanted into the brain.[144] Looking forward, local transplantation might not even be necessary if newly developed cell graft delivery systems like genetic brain shuttles[156] or navigating microrobots[261,262] prove effective in guiding cell sources to injury sites. These advancements may also facilitate the translation of stem cell therapies for a broader range of neurological disorders involving neuronal loss.[183,272,273]

Conclusion

Cell therapy remains a promising regenerative strategy for stroke. The multimodal effects of cell grafts have great potential to improve long-term recovery in stroke patients. Despite this potential, the varied outcomes of existing clinical trials highlight the importance of a more comprehensive mechanistic understanding. Recent advancements in preclinical research offer insights into the molecular interactions between cell grafts and the host at a single-cell level. Other preclinical work indicates how genetic engineering of cell grafts can improve current clinical challenges such as immunocompatibility, graft delivery and safety of cell therapies. An

increasing understanding of the underlying therapeutic mechanisms will also help to identify the ideal cell sources and tailor customized therapies to stroke patients. Future clinical trials may implement these recent preclinical advancements and investigate whether they produce more consistent beneficial effects, paving the way for the integration of cell-based therapies into standard stroke treatments in the future.

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Competing interests

L.L. is coinventor on the International Patent WO/2020/226993 filed in April 2020; the patent relates to the use of antibodies which specifically bind interleukin-1a to reduce various sequelae of ischemia-reperfusion injury to the central nervous system.

Supplementary material

Supplementary material is available at *Brain* online.

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