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Promoting Brain Repair and Regeneration After Stroke: A Plea for Cell-based Therapies

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Abstract

Purpose of Review: After decades of hype, cell-based therapies are emerging into the clinical arena for the purposes of promoting recovery after stroke. In this review we discuss the most recent science behind the role of cell-based therapies in ischemic stroke and the efforts to translate these therapies into human clinical trials.

Recent Findings: Preclinical data support numerous beneficial effects of cell-based therapies in both small and large animal models of ischemic stroke. These benefits are driven by multifaceted mechanisms promoting brain repair through immunomodulation, trophic support, circuit reorganization, and cell replacement.

Summary: Cell-based therapies offer tremendous potential for improving outcomes after stroke through multimodal support of brain repair. Based on recent clinical trials, cell-based therapies appear both feasible and safe in all phases of stroke. Ongoing translational research and clinical trials will further refine these therapies and have the potential to transform the approach to stroke recovery and rehabilitation.

Keywords

neurogenesis; stem cells; transplantation; stroke recovery; neuroplasticity; brain regeneration

Introduction

Stem cell therapies are emerging in the clinical arena, and bringing with them renewed hope for novel therapeutic approaches to promoting brain repair after stroke. The concept of regenerative medicine in central nervous system injury dates back more than a century, when Santiago Ramon y Cajal observed, “In adult centers the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated. It is key for the science of the future to change, if possible, this decree.”[1] Over several decades we have learned much about the potential for regeneration in the CNS, with the recognition of neural stem and progenitor cells (NSPs) persisting in the brain throughout life. Reynolds and Weiss first demonstrated the ability to isolate multipotential progenitors from the brains of adult rodents.[2] Animal models then demonstrated increased neurogenesis from these progenitors after stroke in both the immature and aged brain. Attention has more recently turned toward

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transplantation of exogenous cells to support and augment endogenous repair mechanisms. Originally stymied by ethical considerations surrounding the use of embryonic stem cells (ESCs), the brakes have been released by a plethora of mechanisms for generating neural progenitors from adult tissues. These include most notably induced pluripotent stem cells (iPSCs) which can be generated from an individual's own somatic cells. Today we have tremendous capabilities to generate many different specific cell types. In many ways this has outpaced our ability to study the effects of different cell types as means of therapy. In this review article we will discuss the variety of cell-based therapies under investigation, possible mechanisms of action, and the current evidence available from human clinical trials. Finally we propose a roadmap for future research to accelerate the development and optimization of cell-based therapies as critical treatments for stroke recovery.

Pathways for Cell-Based Therapies

The term "stem cell" has existed in the literature for more than a century, and by strict definition necessitates the characteristic capacities for self-renewal and differentiation into other cell types.[3] Stem cells range from pluripotent ESCs from which entire organisms arise, to more restricted organ-specific stem cells. Experimental observations also suggest that stem cells and their progeny exist on a continuum, with at least some potential of bidirectional phenotypic lability.[4] As applied to regenerative medicine, the key characteristics of stem cells present a double-edged sword. The expansion and multipotential differentiation capacities are therapeutically promising, but also present the feared possibility of tumorigenicity.[5-7] Many of the cell types that have been investigated in stroke have been are either more restricted progenitors or stem cells that have been modified to limit this risk, but nonetheless are commonly referred to collectively as stem cell therapy.

Exogenous Cell Administration

ESCs are derived from blastocysts and represent the most pluripotent cell state available for potential therapeutic purposes.[8-10] This pluripotency also raises concerns regarding tumorigenicity following transplantation.[5, 11] These cells can be directed *in vitro* toward neural lineages, as reviewed elsewhere.[12-14] Most experimental approaches have used such directed differentiation prior to transplantation to reduce the risk of uncontrolled expansion. After transplantation in preclinical stroke models, ESCs have can engraft and survive for up to 12 weeks.[15-18] Some studies have demonstrated migration of transplanted cells whether transplanted ipsilesionally or contralesionally,[16] but others have not observed significant migration.[15] These cells can differentiate into multiple neuronal subtypes as well as glia,[16, 17] develop electrophysiological properties of mature neurons, [16] and form structural connections within the host brain.[18]

NSPs are more restricted stem cells. They are able self-replicate, but differentiation is restricted neuronal and glial subtypes.[19, 20] In addition to ESCs and iPSCs, NSPs can be derived from fetal and adult tissue.[21, 22] Adult NSPs reside in the subventricular zone (SVZ) in the wall of the lateral ventrical and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus.[23] While in general considered multipotential, NSPs may actually have region-specific lineage restrction.[24] NSPs have been administered directly

into the brain either through stereotactic neurosurgery or intra-arterially in preclinical animal models of stroke, and a recent meta-analysis found many pleiotropic benefits on behavioral and structural outcomes.[25, 26]

Mesenchymal stem cells (MSCs) reside in tissue of mesodermal lineage such as adipose tissue, bone marrow, umbilical cord blood, and others.[27] The first identified and most commonly used MSCs are bone-marrow derived MSCs, a subset of bone marrow mononuclear cells (BMMNCs).[28] Along with the ability to differentiate into a range of mesenchymal tissue, MSCs can also differentiate into ectodermal and endodermal lineages, including neural cells.[29, 30] This possibly due to an even more specific subset of MSCs, the recently described multilineage-differentiating stress enduring (Muse) cells that comprise a small portion of bone marrow-derived MSCs.[31] These cells may also play a role in the unique ability of MSCs to migrate towards areas of injury and spontaneously differentiate and integrate with damaged tissue. [32, 31, 33] MSCs can be isolated and expanded from patients as an autologous source of cells, thus reducing the risk of immune system activation.[34-36] MSCs have both anti-inflammatory and neurotrophic effect with the ability to secrete multiple factors including BDNF, NGF, FGF, and VEFG.[37]

Induced pluripotent stem cells (iPSCs) are dedifferentiated somatic cells, most commonly fibroblasts, transformed via induction of defined transcription factors.[38-40] Similar to ESCs, iPSCs are returned to their pluripotent state and have the ability to differentiate into different neuronal cell types, including NSCs.[41, 42] Unlike ESCs, however, autologous iPSCs have less immunogenicity due to their derivation from the patient's own tissue, avoid the moral and legal issues surrounding the cultivation and use of ESCs, and afford nearly limitless customization.[43-45]. Transplantation of iPSC-derived NSPs leads to regeneration of mature and functional neurons and axonal projections through trophic support and enhances neurogenesis and angiogenesis following ischemic stroke, promoting improved neurologic outcomes.[46-49]

Endogenous Neurogenesis

Once considered to be a static organ, we now know that the brain has the capacity to generate new cells during postnatal neurodevelopment and long after. Joseph Altman first demonstrated new cells being born in the adult rodent brain using 3H-thymidine incorporation assays.[50] Kaplan and Hinds later confirmed similar results demonstrating newly born neurons in the rat dentate gyrus and olfactory bulb using electron microscopy. [51] Adult neurogenesis is now a well-established feature of the rodent brain, occurring in discrete neurogenic niches: the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus.[52, 53] Despite these early findings, the issue of adult neurogenesis remained contentious due to the unknown source of these cells and primate research that suggested adult neurogenesis may be limited to rodents.[54] Postnatal neurogenesis has since been confirmed in the human hippocampus, taking advantage of patients who had received the thymidine analog bromodeoxyuridine (BrdU) as chemotherapy and thus labeled newborn cells at the time of treatment.[55] More recent studies suggest that basal levels of endogenous neurogenesis in humans are very low, with the possible exception of the perinatal period.[56, 57] Animal models have repeatedly

demonstrated increased neurogenesis after stroke, both in immature and adult rodents.[58-63] Key questions remain as to the functional importance of this apparent regenerative response, but numerous studies have demonstrated correlations between behavioral recovery after stroke.[64] In humans, evidence is much more sparse given technical limitations, but some studies hint that a similar phenomenon may occur.[65, 66]

Other avenues for promoting cell-based therapy

In addition to stem cells themselves, a number of adjunctive technologies are emerging with the potential to further advance these therapies. The use of bioscaffolds such as biologically derived and synthetic hydrogels greatly aid in the transplantation and subsequent survivability of exogenous stem cells in the stroke cavity.[67, 68] These substances allow for in situ tissue regeneration and provide a non-reactive matrix that can act both as structural support system for stem cells as well as a vehicle for drug delivery.[69, 70] Imaging techniques including optical imaging, magnetic resonance imaging (MRI), and positron emission tomography (PET) offer the ability to track and monitor cells from the point of administration[71-73] Cells to be transplanted are labelled with magnetic markers, typically superparamagnetic iron oxide (SPIO) nanoparticles. In addition to the primary function as MRI markers, SPIO-labeled cells can be physically manipulated via an external magnet through fluid compartments, potentially indicating a method of manual direction through the ventricular system.[74]. These technologies may open even further avenues for the application of cell-based therapies in stroke.

Mechanisms of Action

The holy grail of stem cell therapy is to replace cells that are lost or damaged as a consequence of disease or injury. In the context of stroke, this is an enormous ask given that a stroke indiscriminately destroys all brain tissue, often leaving behind a region devoid of the infrastructure that was laid down during development. In order to achieve cell replacement, therapies will have to accomplish (i) delivery of cells to the infarct territory; (ii) allowing or promoting the differentiation of those cells into a diverse population including various types of neurons, glia, and blood vessels; and (iii) re-establishment of complex connections and networks both locally and remotely. Fortunately cell-based therapies provide numerous mechanisms for enhancing repair of the brain following injury, independently of actual cell replacement.[75]

Modulation of neuroinflammation

Stroke represents an evolution of injury over time, from acute necrosis due during ischemia to secondary cell death due to inflammation.[76] An overly simplistic view of inflammation would suggest that proinflammatory cytokines and the cellular immune response aggravate injury, impair neurogenesis, and impede neural repair after stroke.[77, 78] The true interaction between inflammation and the regenerative response to the brain is likely much more complex, and some inflammatory mediators may actually help to promote repair.[79, 80] Microglia play a biphasic role in ischemic stroke with shifting polarization between pro-inflammatory and anti-inflammatory phenotypes, a phenomenon that can be targeted therapeutically with cell-based therapy in preclinical models.[81-83] Accumulating evidence

in both humans and animals support a significant role for immunomodulation as one pillar of stem cell therapies in enhancing recovery after stroke.[84-88] This mechanism of action is particularly applicable to peripherally administered stem cells because they can exert their effects through the systemic immune system rather than requiring direct localization near the stroke.

Remodeling of Neural Networks and Cell Replacement

Data from animal models human patients suggests that after ischemic stroke neural circuitry in areas surrounding damaged tissue reorganizes to regain previously lost function.[89] These changes include axon sprouting, dendritic remodeling, and new synapse formation, and can be facilitated by functionally-directed rehabilitation.[90-94] Expanding evidence suggests that stem cells promote neural circuit regeneration through multiple intertwined mechanisms, promoting repair reorganization of existing cells as well as limited incorporation of new cells into the regenerating circuit.

One important mechanism through which stem cells promote neural circuit remodeling is secretion of neurotrophic factors. Infusion of mesenchymal stem cells engineered to express brain-derived neurotrophic factor (BDNF), placental growth factor (PGF), glial cell-line derived neurotrophic factor (GDNF), or vascular endothelial growth factor (VEGF) and angiopoietin into rodent models of ischemic stroke improved functional outcomes.[95-98] The functional improvement in these experiments correlated with decreased infarct volume and improved vascular regrowth into the injured parenchyma. Although cells can be engineered to overexpress neurotrophic factors, MSCs exposed to the ischemic post-stroke environment also appear to inherently upregulate production of neurotrophic factors.[99] Neurotrophic factors are known to be crucial to neural circuit development at sequential stages of development, from promoting neurogenesis, through dendrite and axon growth, to synaptogenesis and synaptic refinement.[100] Cell-based therapies may act in part by re-inducing developmental programs of neural circuit formation.[101] Emerging evidence also suggests that exosomes may provide a critical mechanism by which stem cells exert their effects in promoting remodeling after injury.[102]

Indeed, all of the anticipated effects of neurotrophic signaling in the stroke-damaged brain have been observed after stem cell transplant. When transplanted into the ischemic brain, exogenous NSPs can augment neurogenesis and angiogenesis from resident precursors thus increasing the population of cells that may potentially be integrated into the recovering circuit.[103-105] Transplanting human NSPs into stroke-injured brain also promotes remodeling of both neuronal axons and dendrites, with increased connectivity within damaged circuits and improved axon function as evidenced by increased cargo transport along the length of axons.[106] Accompanying *in vitro* studies suggest that these effects were at least in part mediated by VEGF and thrombospondin.

Bystander or paracrine effects are clearly important factors underlying the efficacy of stem cells in promoting repair and regeneration, but cell replacement likely has a role as well. Arvidsson and colleagues observed that less than 20% of newly generated cells survived and matured into NeuN-expressing neurons.[59] Despite this sobering fact, a minority of cells do survive, migrate into sites of injury, and even functionally integrates into local circuitry,

developing similar electrophyiological signatures compared to pre-existing neighbors.[107] There is evidence that stem cells can generate mature neurons that form functional afferent and efferent connections. Neural precursor cells derived from explants of immature medial ganglionic eminence (which developmentally is the source of inhibitory interneurons) were directly implanted into stroke-damaged brain, and found to differentiate into neurons.[108, 109] These explant-derived neurons received functional synaptic connections, as measured electrophysiologically by postsynaptic potentials, and were able to generate action potentials, although the target of their connectivity was not defined. Following transplantation, iPSCs that had been primed toward cortical neuronal phenotypes also functionally integrate into damaged circuitry following transplantation.[49] These cells differentiated into both excitatory and inhibitory mature neurons (as assessed both immunohistochemically and electrophysiologically) and received functional synaptic inputs from native cortex. While most effort has emphasized neuronal production, some investigators have also observed oligodendrogenesis.[110] Understanding the role of glia in both promoting and limiting regeneration in the brain will be critical for further promotion of cell-based therapy.[111, 112]

Clinical Trials of Cell-based Therapy in Stroke

Based upon encouraging results from preclinical studies of cell-based therapies in animal models of stroke, investigators have embarked on pioneering human studies over the past two decades. Most of these have been small, open-label, single arm studies. Table 1 summarizes many of the published trials to date. The majority of these clinical trials have been initial phase I/II trials of feasibility and safety, with small numbers of patients and often not randomized or controlled.

In one of the earliest efforts, Kondziolka and colleagues investigated the effects of stereotactic transplantation of human embryonic carcinoma-derived precursor cells (termed LBS-neurons) in chronic basal ganglia stroke. In their first study they found slight improvements in the European Stroke Scale at 6 months compared to the patients' baseline, but in their follow up phase II study there were no significant differences between transplanted patients and control patients.[113, 114] In both studies there were no adverse cell-related events, although procedure-related complications did occur. One of the major criticisms of these studies was the use of a cancer-derived cell line and the risk for tumorigenicity given limited follow up of only one year. This led to a pilot study of porcine embryonic precursor cells derived from the lateral ganglionic eminence, but this study was terminated by the FDA after 2/5 patients developed adverse events.[115]

An alternative approach has utilized an immortalized human neural stem cell line derived from fetal cortical brain tissue (CTX0E03 cell line). These cells have been engineered with a retrovirally-delivered c-mycERTAM transgene to allow large scale expansion and banking.[116] *In vivo* models have demonstrated rapid epigenetic silencing of this transgene within the first week after transplantation, supporting a low risk of uncontrolled expansion and tumorigenicity.[117] In a phase I safety trial (PISCES), Kalladka and colleagues transplanted increasing doses of these cells into the ipsilesional putamen of 11 men with ischemic stroke 14-51 months prior to enrollment.[118] Importantly their trial did not

include immunosuppression as preclinical models suggested that low immunogenic responses to the CTX0E03 cells. The primary outcome of this phase I trial was safety, and they saw no significant adverse events that they attributed to the cell therapy, but several related to the neurosurgical procedure. While not powered or designed for efficacy, several patients did experience improvements in multiple measurement scales including the modified Rankin scale. While typically patients are not expected to make significant improvements at the timepoints in this study, it is not possible to attribute causality to the cell therapy in the absence of a control group. An important caveat to this study is that only men were included to reduce the risk of incidental exposure to tamoxifen, a commonly used treatment for breast cancer, because the transgene is under control of a tamoxifen-inducible promoter. Whether the safety of this treatment will be generalizable to women remains to be seen. A phase II has recently been completed, but not yet published. The company's website indicates that although the primary endpoint was not met, but enough benefits were observed in some subjects to prompt planning of a definitive trial.

The previously described studies used neuronal precursors, others MSC transplantation has also been explored in clinical trials. Steinberg and colleagues used bone marrow-derived MSCs that had been transiently transfected with Notch1 to promote differentiation to a neuronal lineage.[119] These cells were stereotactically implanted to multiple locations in the peri-infarct tissue under MRI guidance, with a goal of bracketing the stroke with stem cells. Transplantations were performed in the chronic phase at a mean of 22 months after stroke (range 7-36). Similar to prior studies, adverse events were rare and largely attributable to the neurosurgical procedure rather than the cells. In 18 patients, there were only 4 treatment-emergent adverse events that were possibly related to cell therapy and none that were probably or definitely deemed attributable to cell therapy, but there were 22 adverse events with a possible/probable/definite relationship to the neurosurgical procedure (most commonly headache). Similar to the PISCES trial, it is difficult to draw strong conclusions on efficacy in the absence of a control group, but the investigators observed statistically significant improvements in the European Stroke Scale, the NIH Stroke Scale, and the Fugl-Meyer at timepoints when substantial improvement would typically be unexpected.

Honmou and colleagues investigated IV infusion of autologous MSCs in the subacute to chronic phase of ischemic stroke, and they observed no significant adverse effects.[120] Interestingly they did see an increased rate of improvement in NIHSS in the first 1-2 weeks post-infusion, but there was no control group and evaluators were not blinded. Additionally, many of these patients received infusion within 3 months after stroke, a time window in which some spontaneous recovery of impairment is expected. They also saw progressive reduction of lesion volumes, reaching a mean of 20% reduction at 1 week post-infusion compared to 1 day after infusion, at a time when such lesion evolutions may not be expected.[121]

The application of cell-based therapies during the acute phase of stroke has mostly been limited to systemic administration of bone marrow derived precursors (MSCs, MAPCs, BMMCs). The MASTERS trial is one of the largest studies to date and was performed in a multicenter, placebo-controlled, double-blinded fashion.[85] Bone marrow derived stem/progenitor cells were administered intravenously between 24 and 48 hours after stroke onset.

There was no difference in the primary or secondary safety endpoints of dose-limiting toxicity, neurological worsening due to the investigational product, secondary infections, or laboratory/cardiac abnormalities. While overall the frequency of treatment emergent adverse events were more common in the treatment group, these were mostly deemed mild to moderate. The primary efficacy endpoint was the multivariate global stroke recovery at 90 days (mRS ≤ 2, 75% improvement in NIHSS, and Barthel Index ≥ 95). Exploratory analyses suggested benefit in terms of excellent outcome (defined as mRS ≤ 1, BI ≥ 95, and NIHSS ≤ 1) at one year. Additionally when considering only those patients treated within 24-36 hours, mRS shift analysis and excellent outcome at 90 days both favored MAPC treatment, and the one year outcomes were even more strongly in favor of MAPC treatment. The authors interpretation of these results posited that MAPC treatment may ameliorate secondary inflammation after stroke, and that these benefits may take even more time to become evident than our typical 90 day outcomes. They also note the suggestion that time window of treatment may be important.

Conclusions

The momentum behind cell-based therapies for stroke recovery remains substantial, but while early studies have shown hints of promise true efficacy has not yet been achieved. In 2007, investigators from academia, government, and industry convened a consortium to lay a path forward, and from this emerged the Stem Cells as an Emerging Paradigm in Stroke (STEPS) series of guidelines.[122-124] Preclinical studies have shown that stem cells through both immunomodulatory mechanisms and through post-stroke neural circuit remodeling in a twofold manner: by enhancing mechanisms of intrinsic circuit remodeling (secreting neurotrophic factors, increasing neurogenesis, and promoting plasticity), and by maturating into neurons that directly incorporate into the neural circuit. Whether these mechanisms are independent or synergistically bound requires further exploration. While the quality of clinical evidence remains limited, safety and feasibility have been demonstrated for multiple cell types, routes of administration, and times of administration. Future studies should establish biomarkers so that as clinical trials progress we will be able to re-evaluate biological targets to optimize efficacy. An iterative process between the clinic and the laboratory is essential to refine the approach for cell-based therapy and ultimately reach the desired endpoints. Bioengineering advances promise to allow customization of both cells and scaffolds to enhance therapeutic benefits.[125-127] No therapies in current standard clinical practice improve outcomes beyond the proportional recovery expected from spontaneous biological repair mechanisms.[128] Cell-based therapies offer the potential to dramatically shift the paradigm of stroke rehabilitation and recovery. It is imperative that we continue to refine and drive these therapies toward the goal of improving functional restoration in our patients.

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standards(including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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Table 1.

Published clinical trials of cell-based therapies in ischemic stroke. Quality of evidence provided is based on GRADE assessment of the trial design.[142] Phases of stroke: Acute = within the first week; Subacute = 1 week to 3 months; Chronic = more than 3 months. Abbreviations: MSC – mesenchymal stem cells; BMMNC – bone marrow mononuclear cells; hNSP – human neural stem/progenitor cells; SAE – significant adverse event; mRS – modified Rankin Scale; BI – Barthel Index; FM – Fugl Meyer scale; ESS – European Stroke Scale; NIHSS – NIH Stroke Scale.

		Stroke phase	Cell type	Mode of delivery	Treatment	Control	Safety concerns	Outcomes	Quality of evidence
Kondziolka[114]	2000	Chronic 6m-5y	hNSP	Surgical	12	N/A	No SAE	-Improved ESS mean 2.9 pts at 6 months -No change in NIHSS or BI at 6 months -No change in infarct volume -Increased [F18]FDG uptake on PET in 6/11	Low -open label -single arm
Kondziolka[113]	2005	Chronic 1-5y	hNSP	Surgical	18	4 No sham	No SAE	-Improvement in ESS mean 2.7 pts at 6 months compared to 0.75pts in control group (p=0.148)	Moderate -randomized -observer blinded
Rabinovich[129]	2005	Chronic 4m-2y	Fetal tissue	Intrathecal	10	11 No sham	Fever, meningismus within 48h transplant	-Improved Karnovskii functional activity scale, quality of life	Very low -nonrandomized -unblinded -nonstandard outcome measure
Savitz[115]	2005	Chronic 1.5-10y	porcine lateral ganglionic eminence cells	Surgical	5	N/A	Enhancing MRI lesions in 2/5 patients	-Improvement of NIHSS by 1 point over 4 years in 3/5 patients and by 4 points in 1 patient -Subjective functional improvement in 3/5 patients	Very low -open label -single arm -subjective outcome
Bang[130]	2005	Subacute 4-5w	autologous MSC	Intravenous	5	25	No SAE	-Improved BI at 3 and 6m (not significant at 12m)	Moderate -randomized -controlled -observer blinded
Suarez-Monteguido[131]	2009	Chronic 3y-8y	BMMNC	Surgical	5	N/A	No SAE	-Imporved motor function (NIHSS and SSS) -Improved spasticity 1.Ashworth) -Improved BI (mean 4 pts at 6m, 10 pts at 12m)	Very low -small number -single arm -multiple outcomes
Lee[132]	2010	Subacute 4-5w	autologous MSC	Intravenous	16	36 No sham	No difference v. ctl	-Improved mRS shift -Improved survival (HR 0.344)	Moderate -randomized -controlled -observer blinded
Savitz[133]	2011	Acute 24-72h	autologous BMMNC	Intravenous	10	historical	No SAE	-710 patients with better 90d mRS than expected based on historical controls	Very Low -single arm -unblinded

		Stroke phase	Cell type	Mode of delivery	Treatment	Control	Safety concerns	Outcomes	Quality of evidence
								−5/9 mRS 0-2 at 6m −7/9 BI ≥90 at 6m	
Honmou[120]	2011	subacute to chronic 43-133d	autologous MSC	Intravenous	12	N/A	No SAE	-Increased daily rate of change in NIHSS during first week after infusion −20% reduction in lesion size 1 week after infusion	Very Low -single arm -unblinded
Friedrich[134]	2012	Acute 3-7d	autologous BMMNC	Intravenous	20	N/A	No SAE	-7/20 patients with “satisfactory” improvement in NIHSS at 24 hours or mRS at 90 days.	Very Low -single arm -unblinded
Moniche[135]	2012	Acute 5-9d	Autologous BMMNC	Intraarterial	10	10 No sham	Seizure in 2 treated patients at 3m	-no significant difference in mRS or BI at 90d -increased BNGF at 8 days compared to controls	Low -Nonrandomized -Controlled -Observer blinded
Bhasin[136]	2011	Chronic 3m-1y	Autologous MSC	Intravenous	6	6 Matched for age, chronicity, lesion size, severity	No SAE	-no significant difference in FM, BI, Ashworth at 6m	Low -Nonrandomized -Controlled -Unblinded
Bhasin[137]	2012	Chronic 3m-2y	autologous BMMNC	Intravenous	12	12	No SAE	-improved BI at 24w	Low -Nonrandomized -Controlled -Unblinded
Bhasin[138]	2013	Chronic 3m-2y	Autologous MSC v BM-MNC	Intravenous	20	20	No SAE	-Improvement in 24w BI compared to controls	Low -Nonrandomized -Controlled -Unblinded -multiple comparisons
Bhasin[139]*	2017	Chronic 3m-1y	Autologous MSC	Intravenous	6	6	No SAE	-No significant difference in FM or BI at 4y	Low -Nonrandomized -Controlled -Unblinded
Wang[140]	2013	Chronic 1-7y	autologous BMMNC	Intravenous	8	N/A	No SAE	-Mean decrease NIHSS 3.1pts at 12m -Mean increase BI 20.6pts at 12m	Very Low -Single arm -Unblinded
Prasad[141]	2014	Subacute 1-4w	autologous MSC	Intravenous	58	60 No sham	No SAE	-No significant difference in mRS shift or BI at 6m	Moderate -Randomized -Observer blinded
Kalladka[118]	2016	Chronic 6-60m	hNSC	Surgical	11 Dose-escalation	N/A	2 SAE related to procedure (not to the cells)	-improved mRS by 1 grade in 4/11 patients	Low -Single arm -Unblinded
Steinberg[119]	2016	Chronic 6-60m	Allogeneic modified MSC	Surgical	18 Dose-escalation	N/A	2 AEs definitely or probably related to surgery,	-Increase mean ESS by 6.88 points at 12m	Low -Single arm -Unblinded

		Stroke phase	Cell type	Mode of delivery	Treatment	Control	Safety concerns	Outcomes	Quality of evidence
							none related to cells	-Decrease mean NIHSS by 2 points at 12m -Increase mean total FM by 19.2 points at 12m -Increase mean motor FM 11.4 points at 12m -no change in mRS at 12m -no clear dose response	
Hess[85]	2017	Acute 24-48h	MAPC (MultiStem)	Intravenous	65	61 Placebo	23% treatment-related adverse event (halitosis, fever/chills, nausea	-Primary outcome (mRS≤2, NIHSS improvement ≥ 75%, BI≥95) not significantly different at 90d -BI≥95 favored MAPC at 1y -mRS≤1 favored MAPC at 1y -Excellent outcome (mRS≤1, NIHSS≤1, & BI≥95) favored MAPC at 1y -Secondary outcomes more strongly significant for patients treated within 36h	High -Randomized -double-blinded -placebo-controlled