



# Neuroprotective and neurorestorative actions of mesenchymal stromal cell-derived small extracellular vesicles in the ischemic brain

Chen Wang<sup>1,2</sup> , Bernd Giebel<sup>3</sup> , Dirk M. Hermann<sup>1,2\*</sup> 

<sup>1</sup>Department of Neurology, University Hospital Essen, University of Duisburg-Essen, D-45122 Essen, Germany

<sup>2</sup>Center for Translational and Behavioral Neurosciences, University Hospital Essen, University of Duisburg-Essen, D-45122 Essen, Germany

<sup>3</sup>Institute for Transfusion Medicine, University Hospital Essen, University of Duisburg-Essen, D-45122 Essen, Germany

**\*Correspondence:** Dirk M. Hermann, Department of Neurology, University Hospital Essen, University of Duisburg-Essen, Hufelandstr. 55, D-45122 Essen, Germany. [dirk.hermann@uk-essen.de](mailto:dirk.hermann@uk-essen.de)

**Academic Editor:** Aurel Popa-Wagner, University of Medicine and Pharmacy Craiova, Romania

**Received:** May 25, 2022 **Accepted:** August 9, 2022 **Published:** October 09, 2022

**Cite this article:** Wang C, Giebel B, Hermann DM. Neuroprotective and neurorestorative actions of mesenchymal stromal cell-derived small extracellular vesicles in the ischemic brain. *Explor Neurosci.* 2022;1:61–74. <https://doi.org/10.37349/en.2022.00005>

## Abstract

Ischemic stroke is a highly prevalent condition that frequently results in life-long disability and death. Considerable efforts have been made to establish treatments that prevent secondary ischemic damage and promote stroke recovery. Until now, the recanalization of occluded blood vessels via thrombolysis and thrombectomy, although highly potent, remains the only treatment in humans that enhances stroke outcome. Small extracellular vesicles are non-replicating, nano-sized (70–150 nm) lipid bilayer-enclosed vesicles, which have shown remarkable biological activities in various physiological and pathophysiological contexts. When administered post-stroke, mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) induce neuroprotection, promote brain remodeling and plasticity, and enhance neurological recovery in rodents and non-human primates via mechanisms that involve immunomodulation and anti-inflammation. In this review, experimental studies on the therapeutic actions of MSC-EVs in animal stroke models are summarized and perspectives for clinical translation are outlined.

## Keywords

Ischemic stroke, exosome, microparticle, anti-inflammation, immunomodulation, leukocyte, microglia, polymorphonuclear neutrophil

## Introduction

Stroke is one of the most common causes of death and long-term disability. Each year, stroke causes approximately 101.5 million cases, 143 million disability-adjusted life years (DALYs), and 6.6 million deaths worldwide. These cases and disability numbers are still increasing annually, imposing great economic burdens on societies [1, 2]. The most common type of stroke is ischemic stroke, which results from the occlusion of a cerebral vessel by a clot. Despite the extensive attempts in developing ischemic stroke treatments, only intravenous thrombolysis and endovascular thrombectomy have hitherto been shown to be effective in

© The Author(s) 2022. This is an Open Access article licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



clinical settings. Both aim at removing the clot and restoring blood flow to the brain. Due to their narrow applicable time windows, only a small proportion of patients with ischemic benefit from these treatments.

Ischemic stroke activates components of innate and/or adaptive immunity and induces brain inflammatory responses, which besides removing damaged tissue exacerbate ischemic brain injury [3, 4]. Following a stroke, immune responses and neuroinflammation persist throughout all phases of injury, from early tissue damage to late brain tissue remodeling [3, 4]. Specifically, polymorphonuclear neutrophils (PMNs) have been shown to play a critical role in the exacerbation of ischemic brain injury in the acute stroke phase and in the postischemic brain remodeling and angiogenesis in the post-acute phase [5, 6]. Although animal studies have yielded promising results on immunomodulatory therapies targeting poststroke leukocyte brain entry, these approaches have without exception failed in clinical trials [7]. Among others, this study's failure may be attributed to the wide utilization of animal models that lack prevalent vascular risk factors (e.g., age, hyperlipidemia, diabetes) and/or the use of treatments that act too unspecifically to achieve clinically sustainable improvements in stroke patients.

Recently, mesenchymal stromal cell (MSC)-derived small extracellular vesicles (EVs) have emerged as a potential new therapeutic agent for stroke treatment. EVs are nano-sized lipid bilayer-enclosed vesicles (70–150 nm) that are released by virtually all cell types. Apparently, EVs are essential components of an ancient cargo and communication system, we are just getting aware of. A proportion of EVs transmit complex signals from sending cells to selected target cells within the close and distant environment. Applying EVs from the right cell types, we gain new vehicles to interfere with biological processes and use them as novel therapeutic agents [8, 9]. In contrast to their parental MSCs, MSC-derived EVs are easier to handle and can be sterilized by filtration. Since EVs are non-replicating, they lack the risk of endogenous malignant transformation. Therefore, MSC-EVs became a promising tool for the development of novel stroke therapies.

This review summarizes existing evidence on the neuroprotective and neurorestorative effects of MSC-EVs in ischemic stroke, defining the role of immunomodulatory and anti-inflammatory actions in their recovery-promoting responses. Clinical perspectives of MSC-EVs are discussed and challenges for the translation from bench to bedside are outlined.

## Stroke statistics

Stroke is the second leading cause of death and the third leading cause of death and disability worldwide [2]. Generally, stroke can be classified into two major subtypes, namely ischemic stroke and hemorrhagic stroke, caused by brain vessel blockage or rupture, respectively. Of all strokes, 87% are ischemic [1]. Globally, in 2019 approximately 77.2 million people suffered from ischemic stroke and a total of 3.3 million people died [1, 10]. Stroke statistics exhibit sex-related differences. Age-specific stroke incidence is similar between males and females at ages up to 55 years, but greater in males than females at ages 55–75 years [11, 12]. Age-standardized rates of deaths and DALYs from ischemic stroke are substantially greater in males than females, but prevalence rates are greater in females than males [13]. Due to population growth and aging, stroke incidence and prevalence rates are annually raising, imposing further economic burdens on societies [1, 11, 13].

## Limited therapeutic options for ischemic stroke

So far, only two treatments have succeeded in clinical trials and become standard treatments for acute ischemic stroke. Both of them aim at the restoration of cerebral blood flow for the salvageable tissues in the penumbra. The first one is intravenous thrombolysis with recombinant tissue-plasminogen activator (tPA) and the second is endovascular thrombectomy. In 1995 tPA was proven to significantly improve stroke patients' neurological outcome within 3 h of symptom onset, regardless of age, severity, and stroke subtype [14]. Thereafter, in 1996 tPA was approved by the United States Food and Drug Administration as a thrombolytic drug. A later clinical trial expanded the time window for administration of intravenous tPA from 3 h to 4.5 h of symptom onset [15], and using mismatch concepts inclusion criteria have even been expanded recently under well-defined clinical conditions [16]. Although tPA represents great progress in the development of stroke treatments, it has many drawbacks, such as narrow time windows,

strict contraindications, and potential life-threatening complications. In fact, only up to 15% of patients with acute ischemic stroke are eligible for tPA in the United States [17, 18]. On the contrary, endovascular thrombectomy with a stent retriever device was proven to improve stroke outcomes up to 24 h after symptom onset [19, 20] and also as an add-on treatment to thrombolysis [21–23]. Although the number of patients eligible for thrombolysis and thrombectomy is on the rise [23–25] and the patients treated with one of the two treatment options is currently between 20–30% in well-established stroke centers, additional neurorestorative treatments are urgently needed, which allow improving stroke recovery once an ischemic injury has occurred.

## Mechanisms of secondary brain injury poststroke

Ischemic stroke activates brain-resident microglia and recruits blood-derived leukocytes, causing secondary brain injury (reviewed in [3, 4]). Microglia are the resident macrophages in the central nervous system that act as first-line innate immune defense. Microglia are activated when they sense danger signals, namely danger-associated molecular patterns released from the ischemic tissue, such as high-mobility group box 1, histones, and heat shock proteins [26–28]. Activated microglia display distinct phenotypical characteristics and polarize between a pro-inflammatory (M1-like) phenotype and an anti-inflammatory (M2-like) phenotype [29–31]. Activated M1-like microglia further recruit peripheral leukocytes into the brain by endothelial adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) [32–34]. Consequently, brain-invading leukocytes aggravate brain damage through production of reactive oxygen species (ROS), matrix metalloproteinases (MMPs), and cytokines/chemokines, further leading to blood-brain barrier (BBB) disruption, brain edema, hemorrhagic transformation, and neuronal death [35–40]. To limit excessive inflammation, the peripheral immune system may transiently adopt an immunosuppressive lymphopenic state. This poststroke immunosuppression facilitates infections, such as pneumonia, which worsen stroke outcome and increase mortality [41]. Interestingly, microglia depletion increases neuroinflammation and brain injury in acute ischemic stroke [42]. This observation emphasizes that microglia have important brain protective roles in ischemic stroke pathology, perhaps via their M2-like phenotype.

PMNs are the most abundant type of leukocytes in humans and act as first-line immune response to ischemic stroke [6]. It has been suggested that PMNs play a decisive role in poststroke brain injury and neurological recovery (reviewed in [5]). PMNs already appear in brain parenchyma at 12 h and peak within 24–72 h after ischemic stroke [43, 44]. The number of brain-infiltrating PMNs is positively correlated with the severity of ischemic injury and neurological outcome [32, 45]. Specifically, the brain accumulation of PMNs due to impaired microglial phagocytosis worsens stroke outcomes [46], and high neutrophil-lymphocyte ratios in poststroke peripheral blood indicate poor neurological recovery [47]. Therefore, targeting PMNs might be a strategy for stroke therapy. Indeed, preclinical studies have shown that depletion of PMNs or prevention of PMN brain entry by anti-lymphocyte antigen 6 complex locus G (Ly6G) antibody or by C-X-C motif chemokine receptor 2 or very-late-antigen-4 (VLA-4) blockade, respectively, reduces neurological deficits and infarct volume [5, 32, 45, 48]. Yet, PMN depletion has recently been shown to compromise brain microvascular remodeling and angiogenesis in the post-acute stroke phase [49], presumably by preventing the release of proteases and ROS that facilitate extracellular matrix remodeling [6]. Hence, the role of PMNs in ischemic stroke pathology differs between the acute and post-acute stroke phase. Treatments targeting PMNs appropriately need to consider treatment timing in order to ensure that beneficial actions are not put at risk by interventions administered in the wrong stroke phase.

Lymphocytes, especially T lymphocytes, are likewise associated with deleterious effects in acute ischemic stroke [48]. However, their early detrimental effects in experimental ischemic stroke are not related to adaptive immunity [50]. T cells accumulate in the brain lesion and mediate postischemic damage as early as 24 h after reperfusion [51, 52]. It has been reported that recombination activating gene 1 (RAG1)-deficient mice (which are T cell and B cell-deficient) are protected from focal cerebral ischemia and this protection is attributed to T cells because RAG1<sup>-/-</sup> mice reconstituted with T cells are no longer protected from the injury [50, 53]. More specifically, among T cell subpopulations, CD4<sup>+</sup>, CD8<sup>+</sup>, and  $\gamma\delta$  T cells have been shown to have detrimental roles in acute ischemic brain injury through mechanisms involving release of cytotoxic cytokines [51, 54], while

regulatory T cells (Tregs) were reported to have neuroprotective effects by secreting the protective cytokine interleukin (IL)-10 [55]. Thus, the role of T cells in ischemic stroke pathology is cell subset-dependent. A comprehensive understanding of T cell biology is key for the development of T cell-targeting stroke treatments.

## Lessons from the failed stroke trials targeting leukocyte infiltration

Although preclinical studies have demonstrated the detrimental role of leukocyte brain infiltration in ischemic stroke, immunomodulatory strategies targeting leukocyte brain entry have repeatedly failed in clinical trials (reviewed in [7]). For example, natalizumab, a humanized monoclonal antibody against VLA-4, did not influence infarct growth [56] and enlimomab, a murine monoclonal antibody against ICAM-1, failed to show benefits and even increased mortality in patients with acute ischemic stroke [57]. The failures can be attributed, at least partially, to the scarce utilization of animals with different sex, age, and comorbidity in stroke research. In line with previous reports, our group observed that aged mice exhibited worse cerebral blood flow recovery following postischemic reperfusion, greater neurological deficits, larger infarct volume and brain edema, and higher BBB permeability than young mice after intraluminal middle cerebral artery occlusion (MCAO) [58], similar to aged rats exhibiting more severe motor-coordination deficits and larger brain infarcts than young rats after permanent distal MCAO [59]. Moreover, the aggravated stroke outcome is associated with the exacerbated age-related immune alteration, that is, more severe peripheral immune responses and brain infiltration of leukocytes in aged ischemic brains. Specifically, PMN frequency and activation and activated CD8<sup>+</sup> T cell counts are significantly increased in the peripheral blood, and the number of PMNs, T cells, and ED1<sup>+</sup> macrophages is elevated in the ischemic brain of aged rodents [45, 58, 59]. Comorbidities, such as hyperlipidemia, substantially influence ischemic injury and stroke outcome (reviewed in [60]). We have shown that hyperlipidemic mice, fed with a cholesterol-rich Western diet, have increased BBB permeability and brain edema, impaired cerebral blood flow, attenuated cerebral angiogenesis and vascular remodeling, and disturbed stroke recovery [61–63]. This hyperlipidemia-exacerbated ischemic brain injury is associated with increased blood neutrophilia and brain PMN infiltration [32, 63]. Since comorbid conditions alter poststroke immune responses and have a huge impact on stroke outcome, immunotherapies targeting leukocytes should be thoroughly evaluated in preclinical studies under conditions of risk factors. Besides, leukocytes have both detrimental and beneficial roles in ischemic stroke pathology, and unfortunately, the immunomodulatory therapies that failed invariably also blocked the beneficial actions. For instance, PMNs besides their injury-promoting actions have recently been reported to have a pro-angiogenic role in the post-acute stroke phase [6, 49], and natalizumab, which inhibits T cell brain invasion, similarly prevents the brain entry of cytotoxic CD8<sup>+</sup> effector cells and of neuroprotective Tregs. There is a need for more targeted therapies in the stroke field that selectively influence the detrimental immune cell actions without influencing the beneficial ones.

## MSCs for stroke treatment

MSCs and their secretome have meanwhile emerged as such therapies. MSCs are multipotent non-hematopoietic progenitor cells with the potential to develop along the osteogenic, chondrogenic, and adipogenic lineage [64]. Since their discovery in the bone marrow by Friedenstein et al. [65] in the 1960s, the therapeutic potential of MSCs has been explored in various disease models and more than 1,000 registered clinical trials (<https://clinicaltrials.gov/>) [66]. MSCs have attracted great attention because of their easy sampling access from a variety of tissues including bone marrow, adipose tissue, and umbilical cord, their low immunogenicity due to the low expression of major histocompatibility complex (MHC)-I and no expression of MHC-II, and their potent immunomodulatory capabilities that regulate wide-ranging immune cells such as monocytes/macrophages, PMNs, natural killer cells, T cells, and B cells [8, 64, 67, 68]. Over the past two decades, preclinical studies have demonstrated the therapeutic efficacy of MSCs in ischemic stroke, where MSC administration reduces ischemic brain damage as well as neurological deficits, and promotes neurological recovery, associated with peripheral blood immunomodulation, anti-inflammation, increased angiogenesis, and increased neurogenesis [69–73]. Based on these promising preclinical data, multiple clinical trials of MSCs

as a therapy for ischemic stroke have been registered (Table 1). The mechanism of action (MoA) of MSC in ischemic stroke was—as in many other diseases—at first assumed to be cell replacement [74]. Due to the observation that infusion of MSC-derived conditioned medium likewise improved disease outcomes in various settings, a paracrine MoA was discussed quickly [75]. As a possible mediator, MSC-EVs have been discussed. Indeed, in a head-to-head study in mice, Doeppner et al. [69] have demonstrated that MSC-EV preparations are equally effective as their parental MSCs in reducing poststroke motor-coordination deficits and promoting long-term angiogenesis and neurogenesis.

**Table 1.** Registered clinical trials of MSCs and MSC-EVs as therapeutics in ischemic stroke

Identifier	Country	Intervention	Tissue source	Origin	Route	Phase	Status
NTC00875654	France	MSCs	Bone marrow	Autologous	Intravenous	II	Completed
NTC00908856	United States	MSCs	Bone marrow	Autologous	Intravenous	I	Withdrawn
NTC01091701	Malaysia	MSCs	Not defined	Allogeneic	Intravenous	I/II	Withdrawn
NCT01297413	United States	MSCs	Bone marrow	Allogeneic	Intravenous	I/II	Completed
NCT01461720	Malaysia	MSCs	Bone marrow	Autologous	Intravenous	II	Unknown
NCT01468064	China	MSCs	Bone marrow	Autologous	Intravenous	I/II	Completed
NCT01678534	Spain	MSCs	Adipose tissue	Allogeneic	intravenous	II	Completed
NCT01714167	China	MSCs	Bone marrow	Autologous	Intracerebral	I	Unknown
NTC01716481	South Korea	MSCs	Bone marrow	Autologous	Intravenous	III	Unknown
NCT01849887	United States	MSCs	Bone marrow	Allogeneic	Intravenous	I/II	Withdrawn
NCT01922908	United States	MSCs	Bone marrow	Allogeneic	Intravenous	I/II	Withdrawn
NCT02378974	South Korea	MSCs	Umbilical cord	Not defined	Intravenous	I/II	Completed
NCT02564328	China	MSCs	Bone marrow	Autologous	Intravenous	I	Unknown
NTC02580019	China	MSCs	Umbilical cord	Not defined	Intravenous	II	Unknown
NTC03176498	China	MSCs	Umbilical cord	Allogeneic	Intravenous	I/II	Suspended
NTC03186456	China	MSCs	Umbilical cord	Allogeneic	Intravenous	I	Suspended
NCT03356821	Netherlands	MSCs	Bone marrow	Allogeneic	Intranasal	I/II	Completed
NCT03384433	Iran	MSC-EVs	Not defined	Allogeneic	Intraparenchymal	I/II	Recruiting
NCT04093336	China	MSCs	Umbilical cord	Allogeneic	Intravenous	I/II	Recruiting
NCT04097652	China	MSCs	Umbilical cord	Not defined	Intravenous	I	Recruiting
NCT04280003	Spain	MSCs	Adipose tissue	Allogeneic	Intravenous	II	Recruiting
NCT04434768	China	MSCs	Umbilical cord	Allogeneic	Intravenous and intraarterial	I	Recruiting
NCT04590118	China	MSCs	Not defined	Allogeneic	Intravenous	I/II	Recruiting
NCT04811651	China	MSCs	Umbilical cord	Not defined	Intravenous	II	Recruiting
NCT04953663	China	MSCs	Bone marrow	Allogeneic	Intravenous	I/II	Recruiting
NTC05008588	Indonesia	MSCs	Umbilical cord	Not defined	Intraparenchymal	I/II	Not yet recruiting
NCT05158101	Antigua and Barbuda	MSCs	Umbilical cord	Allogeneic	Intravenous	I	Recruiting
NCT05292625	Vietnam	MSCs	Umbilical cord	Allogeneic	Intravenous and intrathecal	I/II	Recruiting

## MSC-EVs for stroke treatment

EVs can be classified based on their size and cellular origin as exosomes, microvesicles, and apoptotic bodies. Exosomes (70–150 nm in diameter) are formed as intraluminal vesicles by the late endosomal compartment and are released into the extracellular environment when multivesicular bodies (MVBs) fuse with the plasma membrane. Microvesicles (100–1,000 nm) are formed and released by budding from the cell plasma membrane, and apoptotic bodies (> 500 nm) are formed as fragments of apoptotic cells [8, 9]. The term “EVs” is generally used to refer to all experimentally-obtained extracellular vesicles due to the lack of technology to separate given subtypes [76]. It is widely assumed that therapeutic actions of EVs mainly

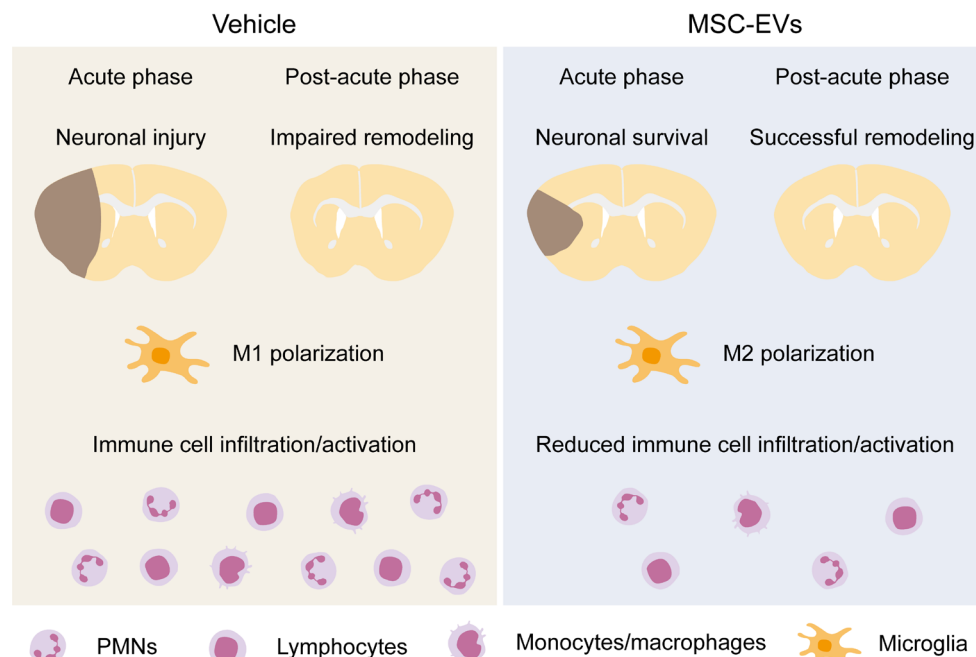


derive from exosome-sized EVs which are also referred to as small EVs [8, 66, 76]. MSC-EVs have been reported in preclinical models to protect against more than 30 different diseases [8, 66, 77]. In ischemic stroke, MSC-EVs have been shown by others and us to induce neuroprotection and promote neurological recovery and brain remodeling in rodents following systemic delivery [45, 49, 58, 59, 69, 78–83]. While initial studies have been performed in young mice and rats [45, 49, 69, 78–83], additional studies recently examined the MSC-EV effects in aged mice, rats, and non-human primates [58, 59, 84–87]. In aged (15–24-month-old) male and female mice exposed to intraluminal MCAO, our group recently showed that MSC-EVs, when systemically administered immediately after reperfusion or with 6 h delay, induced neuroprotection, reduced functional neurological deficits, and reduced brain leukocyte infiltrates [58]. In aged (19–20-month-old) male Sprague-Dawley rats exposed to permanent distal MCAO, we showed that systemically administered MSC-EVs promoted motor-coordination recovery, reduced brain macrophage infiltrates in periinfarct tissue, and increased periinfarct angiogenesis and neurogenesis [59]. In aged (16–26-year-old) rhesus monkeys (*Macaca mulatta*) with cortical cold injury, systemic MSC-EV administration was found to improve fine motor and coordination recovery, reduce microglial-mediated neuroinflammation, and enhance oligodendrocyte-mediated myelin maintenance [84–86]. In a single study using middle-aged (12-month-old) mice subjected to thromboembolic MCAO, Webb et al. [87] observed no protective effects of systemically administered MSC-EVs on neurobehavioral recovery, infarct volume, and peripheral immune response. The lack of neuroprotection by MSC-EVs in this latter study might be attributed to the heterogeneity of MSCs, as we previously demonstrated that MSC-EV preparations exhibited considerable donor-to-donor variability in terms of anti-inflammatory and neuroprotective properties [45]. The joint evidence of these studies provides important information that supports the clinical translation of MSC-EVs. Cell-free MSC-EV therapies have numerous advantages over MSC treatments. First, MSC-EVs are much easier to handle since they can be sterilized by filtration. Secondly, the biological activity of EVs can be predicted more precisely than that of MSCs, since they hardly respond to environmental changes. Finally, in contrast to cells EVs lack endogenous tumor transformation potential. These traits make MSC-EVs particularly attractive as stroke treatments. Mechanistically, the neuroprotective and neurorestorative actions of MSC-EVs in ischemic stroke are closely related to their potent immunomodulatory and anti-inflammatory effects.

## Immunomodulatory and anti-inflammatory effects of MSC-EVs

Immunomodulatory and anti-inflammatory actions of MSC-EVs were clinically documented in our worldwide first case report of MSC-EV application in a human patient with steroid-refractory acute graft-versus-host disease (GvHD), in which MSC-EV treatment effectively reduced the GvHD symptoms and modulated the patient's immune response by reducing the levels of proinflammatory cytokines and increasing the levels of anti-inflammatory cytokines [88]. Our preclinical animal studies evaluating the therapeutic efficacy of MSC-EVs in ischemic stroke have also correlated the MSC-EV-induced neuroprotection and neurological recovery to their immunomodulatory activities [45, 58, 59, 69]. As discussed above, immune responses and neuroinflammation are hallmarks in ischemic stroke pathophysiology. Notably, MSC-EVs effectively reduce poststroke microglial-mediated neuroinflammation and promote microglial polarization from the M1 to the M2 phenotype [59, 85, 89–92], and reduce brain infiltration of leukocytes, namely of PMNs, monocytes/macrophages, and lymphocytes [45, 58, 59, 93, 94], as summarized in Figure 1. As a result, the decreased expression of pro-inflammatory factors and increased anti-inflammatory mediators are noted in the ischemic brain [89–91, 93–95]. Besides, MSC-EVs successfully reverse poststroke lymphopenia in peripheral blood [45, 69, 94]. The mechanisms underlying MSC-EV-induced immunomodulation are not fully elucidated. We have previously shown that PMNs play a pivotal role in the MoA of MSC-EVs in ischemic stroke [45]. PMN depletion using anti-Ly6G delivery mimicked the effects of MSC-EVs on reducing neurological deficits and brain ischemic injury as well as on reducing brain-infiltrating leukocyte counts after intraluminal MCAO; however, MSC-EVs did not have any synergistic effects regarding further reduced stroke injury and brain immune cell infiltrates in PMN-depleted mice [45]. Interestingly, MSC-EVs did not influence peripheral blood immune response in sham-operated mice [45], indicating that the immunomodulation

of MSC-EVs is pathophysiology-dependent. In addition, MSC-EVs did not influence the PMN frequencies and activation states in the peripheral blood of both young and aged stroke mice, but they did so in the ischemic brain [45, 58]. Given that intravenously infused MSC-EVs firstly encounter peripheral blood immune cells and PMN depletion abolishes the neuroprotective effects of MSC-EVs, PMNs might be the site of immunomodulatory action of MSC-EVs in ischemic stroke [45, 49, 58]. Alternatively, MSC-EVs may target macrophages/microglia, as supported by the findings that MSC-EVs promote macrophage/microglia polarization from the M1-like to the M2-like phenotype and reduce the macrophage/microglia-associated brain inflammatory response [59, 85, 89–92].



**Figure 1.** Immunomodulatory effects of MSC-EVs in the ischemic brain. MSC-EVs induce ischemic neuroprotection in the acute stroke phase and promote brain tissue remodeling in the post-acute stroke phase. As underlying mechanisms, immunomodulatory effects involving M2 microglia polarization and reduced brain immune cell infiltration and activation play a decisive role. (This figure is built upon graphic elements of previous publications [45, 58] with self-owned copyrights.)

## Clinical translation of MSC-EVs

There is meanwhile growing evidence that systemically administered MSC-EVs reduce ischemic injury and promote neurological recovery in animal stroke models. Studies in young mice and rats [45, 49, 69, 78–83] have meanwhile been complemented by studies in aged mice [58], rats [59], and non-human primates [84–86]. Considering the low immunogenicity potential of MSCs and MSC-EVs and an own worldwide first case report of MSC-EV application in a steroid-refractory human GvHD patient, in which MSC-EV treatment was well tolerated and effectively reduced the patient's GvHD symptoms [88], MSC-EV treatment in humans is likely safe. Indeed, clinical phase I/II studies demonstrated the safety of intravenous transfusion of allogeneic MSCs in human stroke patients [96, 97]. Currently, there is only one registered clinical trial on MSC-EVs for ischemic stroke (Table 1), but others will undoubtedly follow. A major risk with respect to the clinical translation of MSC-EVs is the heterogeneity of MSCs [98] and MSC-EV products [99]. The extent of MSC-EV heterogeneity is partially dependent on donors, tissue origins, cell culture conditions, EV isolation, and storage conditions [76]. We have previously demonstrated that even within the same manufacturing workflow, MSC-EV preparations obtained from three different healthy donors exhibited considerable heterogeneity in terms of neuroprotection and immunomodulatory properties in mice exposed to intraluminal MCAO [45]. Hence, the development of potency assays for MSC-EV products is an essential task for translational projects [66, 99]. Potency testing of biologics including MSC-EV products is highly recommended for early clinical trials and becomes mandatory for phase III clinical trials in human patients. By fulfilling these needs, we should become able to successfully translate MSC-EVs to clinical practice.

## Abbreviations

BBB: blood-brain barrier

EVs: extracellular vesicles

GvHD: graft-versus-host disease

MCAO: middle cerebral artery occlusion

MoA: mechanism of action

MSC: mesenchymal stromal cell

MSC-EVs: mesenchymal stromal cell-derived extracellular vesicles

PMNs: polymorphonuclear neutrophils

tPA: tissue-plasminogen activator

## Declarations

### Author contributions

CW drafted the manuscript. All authors revised it and approved the submitted version.

### Conflicts of interest

DMH and BG hold U.S. and E.U. patents on extracellular vesicles for the treatment of inflammatory conditions (US9877989B2; EP2874634B1).

### Ethical approval

Not applicable.

### Consent to participate

Not applicable.

### Consent to publication

Not applicable.

### Availability of data and materials

Not applicable.

### Funding

Supported by German Research Foundation (389030878, 405358801) and Federal Ministry of Education and Science (3DOS; 161L0278B). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Copyright

© The Author(s) 2022.

## References

1. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al.; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2021 update: a report from the American Heart Association. *Circulation*. 2021;143:e254–743.
2. GBD 2019 Stroke Collaborators. Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol*. 2021;20:795–820.



3. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med*. 2011;17:796–808.
4. Tobin MK, Bonds JA, Minshall RD, Pelligrino DA, Testai FD, Lazarov O. Neurogenesis and inflammation after ischemic stroke: what is known and where we go from here. *J Cereb Blood Flow Metab*. 2014;34:1573–84.
5. Hermann DM, Gunzer M. Polymorphonuclear neutrophils play a decisive role for brain injury and neurological recovery poststroke. *Stroke*. 2019;50:e40–1.
6. Mohamud Yusuf A, Hagemann N, Ludewig P, Gunzer M, Hermann DM. Roles of polymorphonuclear neutrophils in ischemic brain injury and post-ischemic brain remodeling. *Front Immunol*. 2022;12:825572.
7. Veltkamp R, Gill D. Clinical trials of immunomodulation in ischemic stroke. *Neurotherapeutics*. 2016;13:791–800.
8. Börger V, Bremer M, Ferrer-Tur R, Gockeln L, Stambouli O, Becic A, et al. Mesenchymal stem/stromal cell-derived extracellular vesicles and their potential as novel immunomodulatory therapeutic agents. *Int J Mol Sci*. 2017;18:1450.
9. Cocozza F, Grisard E, Martin-Jaular L, Mathieu M, Théry C. Snapshot: extracellular vesicles. *Cell*. 2020;182:262–262.e1.
10. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396:1204–22. Erratum in: *Lancet*. 2020;396:1562.
11. Gorelick PB. The global burden of stroke: persistent and disabling. *Lancet Neurol*. 2019;18:417–8.
12. GBD 2016 Stroke Collaborators. Global, regional, and national burden of stroke, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. 2019;18:439–58.
13. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al.; GBD-NHLBI-JACC Global Burden of Cardiovascular Diseases Writing Group. Global burden of cardiovascular diseases and risk factors, 1990–2019: update from the GBD 2019 Study. *J Am Coll Cardiol*. 2020;76:2982–3021. Erratum in: *J Am Coll Cardiol*. 2021;77:1958–9.
14. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. 1995;333:1581–8.
15. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, et al.; ECASS Investigators. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med*. 2008;359:1317–29.
16. Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, et al.; WAKE-UP Investigators. MRI-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med*. 2018;379:611–22.
17. Schwamm LH, Ali SF, Reeves MJ, Smith EE, Saver JL, Messe S, et al. Temporal trends in patient characteristics and treatment with intravenous thrombolysis among acute ischemic stroke patients at Get With The Guidelines-Stroke hospitals. *Circ Cardiovasc Qual Outcomes*. 2013;6:543–9.
18. Decker-Palmer M, Wu N, Biscossi M, Savitz SI. Abstract TP6: current trends in thrombolytic use for acute ischemic stroke. *Stroke*. 2020;51:ATP6.
19. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al.; DEFUSE 3 Investigators. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med*. 2018;378:708–18.
20. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al.; DAWN Trial Investigators. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med*. 2018;378:11–21.
21. Evans MRB, White P, Cowley P, Werring DJ. Revolution in acute ischaemic stroke care: a practical guide to mechanical thrombectomy. *Pract Neurol*. 2017;17:252–65.

22. Josephson SA, Kamel H. The acute stroke care revolution: enhancing access to therapeutic advances. *JAMA*. 2018;320:1239–40.
23. Candio P, Violato M, Leal J, Luengo-Fernandez R. Cost-effectiveness of mechanical thrombectomy for treatment of nonminor ischemic stroke across Europe. *Stroke*. 2021;52:664–73.
24. Weber R, Krogias C, Eyding J, Bartig D, Meves SH, Katsanos AH, et al. Age and sex differences in ischemic stroke treatment in a nationwide analysis of 1.11 million hospitalized cases. *Stroke*. 2019;50:3494–502.
25. MacKenzie IER, Moeini-Naghani I, Sigounas D. Trends in endovascular mechanical thrombectomy in treatment of acute ischemic stroke in the United States. *World Neurosurg*. 2020;138:e839–46.
26. Wang C, Liu XX, Huang KB, Yin SB, Wei JJ, Hu YF, et al. Preconditioning with recombinant high-mobility group box 1 induces ischemic tolerance in a rat model of focal cerebral ischemia-reperfusion. *J Neurochem*. 2016;137:576–88.
27. Schuhmann MK, Kollikowski AM, März AG, Bieber M, Pham M, Stoll G. Danger-associated molecular patterns are locally released during occlusion in hyper-acute stroke. *Brain Behav Immun Health*. 2021;15:100270.
28. Yamaguchi A, Jitsuishi T, Hozumi T, Iwanami J, Kitajo K, Yamaguchi H, et al. Temporal expression profiling of DAMPs-related genes revealed the biphasic post-ischemic inflammation in the experimental stroke model. *Mol Brain*. 2020;13:57.
29. Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S, et al. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke*. 2012;43:3063–70.
30. Sardari M, Dzyubenko E, Schmermund B, Yin D, Qi Y, Kleinschnitz C, et al. Dose-dependent microglial and astrocytic responses associated with post-ischemic neuroprotection after lipopolysaccharide-induced sepsis-like state in mice. *Front Cell Neurosci*. 2020;14:26.
31. Manrique-Castano D, Dzyubenko E, Borbor M, Vasileiadou P, Kleinschnitz C, Roll L, et al. Tenascin-C preserves microglia surveillance and restricts leukocyte and, more specifically, T cell infiltration of the ischemic brain. *Brain Behav Immun*. 2021;91:639–48.
32. Herz J, Sabellek P, Lane TE, Gunzer M, Hermann DM, Doeppner TR. Role of neutrophils in exacerbation of brain injury after focal cerebral ischemia in hyperlipidemic mice. *Stroke*. 2015;46:2916–25.
33. Huber JD, Campos CR, Mark KS, Davis TP. Alterations in blood-brain barrier ICAM-1 expression and brain microglial activation after  $\lambda$ -carrageenan-induced inflammatory pain. *Am J Physiol Heart Circ Physiol*. 2006;290:H732–40.
34. Lindsberg PJ, Carpe'n O, Paetau A, Karjalainen-Lindsberg ML, Kaste M. Endothelial ICAM-1 expression associated with inflammatory cell response in human ischemic stroke. *Circulation*. 1996;94:939–45.
35. Shi K, Zou M, Jia DM, Shi S, Yang X, Liu Q, et al. tPA mobilizes immune cells that exacerbate hemorrhagic transformation in stroke. *Circ Res*. 2021;128:62–75.
36. Salas-Perdomo A, Miró-Mur F, Urra X, Justicia C, Gallizioli M, Zhao Y, et al. T cells prevent hemorrhagic transformation in ischemic stroke by P-selectin binding. *Arterioscler Thromb Vasc Biol*. 2018;38:1761–71.
37. Casas AI, Geuss E, Kleikers PWM, Mencl S, Herrmann AM, Buendia I, et al. NOX4-dependent neuronal autotoxicity and BBB breakdown explain the superior sensitivity of the brain to ischemic damage. *Proc Natl Acad Sci U S A*. 2017;114:12315–20.
38. Romanic AM, White RF, Arleth AJ, Ohlstein EH, Barone FC. Matrix metalloproteinase expression increases after cerebral focal ischemia in rats. *Stroke*. 1998;29:1020–30.
39. Gidday JM, Gasche YG, Copin JC, Shah AR, Perez RS, Shapiro SD, et al. Leukocyte-derived matrix metalloproteinase-9 mediates blood-brain barrier breakdown and is proinflammatory after transient focal cerebral ischemia. *Am J Physiol Heart Circ Physiol*. 2005;289:H558–68.
40. Yu L, Su X, Li S, Zhao F, Mu D, Qu Y. Microglia and their promising role in ischemic brain injuries: an update. *Front Cell Neurosci*. 2020;14:211.

41. Shi K, Wood K, Shi FD, Wang X, Liu Q. Stroke-induced immunosuppression and poststroke infection. *Stroke Vasc Neurol.* 2018;3:34–41.
42. Marino Lee S, Hudobenko J, McCullough LD, Chauhan A. Microglia depletion increase brain injury after acute ischemic stroke in aged mice. *Exp Neurol.* 2021;336:113530.
43. Garcia JH, Liu KF, Yoshida Y, Lian J, Chen S, del Zoppo GJ. Influx of leukocytes and platelets in an evolving brain infarct (Wistar rat). *Am J Pathol.* 1994;144:188–99.
44. Gelderblom M, Leyppoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, et al. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. *Stroke.* 2009;40:1849–57.
45. Wang C, Börger V, Sardari M, Murke F, Skuljec J, Pul R, et al. Mesenchymal stromal cell-derived small extracellular vesicles induce ischemic neuroprotection by modulating leukocytes and specifically neutrophils. *Stroke.* 2020;51:1825–34.
46. Otxoa-de-Amezaga A, Miró-Mur F, Pedragosa J, Gallizioli M, Justicia C, Gaja-Capdevila N, et al. Microglial cell loss after ischemic stroke favors brain neutrophil accumulation. *Acta Neuropathol.* 2019;137:321–41.
47. Xue J, Huang W, Chen X, Li Q, Cai Z, Yu T, et al. Neutrophil-to-lymphocyte ratio is a prognostic marker in acute ischemic stroke. *J Stroke Cerebrovasc Dis.* 2017;26:650–7.
48. Neumann J, Riek-Burchardt M, Herz J, Doeppner TR, König R, Hütten H, et al. Very-late-antigen-4 (VLA-4)-mediated brain invasion by neutrophils leads to interactions with microglia, increased ischemic injury and impaired behavior in experimental stroke. *Acta Neuropathol.* 2015;129:259–77.
49. Gregorius J, Wang C, Stambouli O, Hussner T, Qi Y, Tertel T, et al. Small extracellular vesicles obtained from hypoxic mesenchymal stromal cells have unique characteristics that promote cerebral angiogenesis, brain remodeling and neurological recovery after focal cerebral ischemia in mice. *Basic Res Cardiol.* 2021;116:40.
50. Kleinschnitz C, Schwab N, Kraft P, Hagedorn I, Dreykluft A, Schwarz T, et al. Early detrimental T-cell effects in experimental cerebral ischemia are neither related to adaptive immunity nor thrombus formation. *Blood.* 2010;115:3835–42.
51. Yilmaz G, Arumugam TV, Stokes KY, Granger DN. Role of T lymphocytes and interferon- $\gamma$  in ischemic stroke. *Circulation.* 2006;113:2105–12.
52. Jander S, Kraemer M, Schroeter M, Witte OW, Stoll G. Lymphocytic infiltration and expression of intercellular adhesion molecule-1 in photochemically induced ischemia of the rat cortex. *J Cereb Blood Flow Metab.* 1995;15:42–51.
53. Hum PD, Subramanian S, Parker SM, Afentoulis ME, Kaler LJ, Vandenbark AA, et al. T- and B-cell-deficient mice with experimental stroke have reduced lesion size and inflammation. *J Cereb Blood Flow Metab.* 2007;27:1798–805.
54. Shichita T, Sugiyama Y, Ooboshi H, Sugimori H, Nakagawa R, Takada I, et al. Pivotal role of cerebral interleukin-17-producing  $\gamma\delta$ T cells in the delayed phase of ischemic brain injury. *Nat Med.* 2009;15:946–50.
55. Liesz A, Suri-Payer E, Veltkamp C, Doerr H, Sommer C, Rivest S, et al. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat Med.* 2009;15:192–9.
56. Elkins J, Veltkamp R, Montaner J, Johnston SC, Singhal AB, Becker K, et al. Safety and efficacy of natalizumab in patients with acute ischaemic stroke (ACTION): a randomised, placebo-controlled, double-blind phase 2 trial. *Lancet Neurol.* 2017;16:217–26.
57. Enlimomab Acute Stroke Trial Investigators. Use of anti-ICAM-1 therapy in ischemic stroke: results of the enlimomab acute stroke trial. *Neurology.* 2001;57:1428–34.
58. Wang C, Börger V, Mohamud Yusuf A, Tertel T, Stambouli O, Murke F, et al. Postischemic neuroprotection associated with anti-inflammatory effects by mesenchymal stromal cell-derived small extracellular vesicles in aged mice. *Stroke.* 2022;53:e14–8.

59. Dumbrava DA, Surugiu R, Börger V, Ruscu M, Tertel T, Giebel B, et al. Mesenchymal stromal cell-derived small extracellular vesicles promote neurological recovery and brain remodeling after distal middle cerebral artery occlusion in aged rats. *GeroScience*. 2022;44:293–310.
60. Menet R, Bernard M, ElAli A. Hyperlipidemia in stroke pathobiology and therapy: insights and perspectives. *Front Physiol*. 2018;9:488.
61. ElAli A, Doeppner TR, Zechariah A, Hermann DM. Increased blood-brain barrier permeability and brain edema after focal cerebral ischemia induced by hyperlipidemia: role of lipid peroxidation and calpain-1/2, matrix metalloproteinase-2/9, and RhoA overactivation. *Stroke*. 2011;42:3238–44.
62. Zechariah A, ElAli A, Hagemann N, Jin F, Doeppner TR, Helfrich I, et al. Hyperlipidemia attenuates vascular endothelial growth factor-induced angiogenesis, impairs cerebral blood flow, and disturbs stroke recovery via decreased pericyte coverage of brain endothelial cells. *Arterioscler Thromb Vasc Biol*. 2013;33:1561–7.
63. Herz J, Hagen SI, Bergmüller E, Sabellek P, Göthert JR, Buer J, et al. Exacerbation of ischemic brain injury in hypercholesterolemic mice is associated with pronounced changes in peripheral and cerebral immune responses. *Neurobiol Dis*. 2014;62:456–68.
64. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. *Nat Biotechnol*. 2014;32:252–60.
65. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation*. 1968;6:230–47.
66. Gimona M, Brizzi MF, Choo ABH, Dominici M, Davidson SM, Grillari J, et al. Critical considerations for the development of potency tests for therapeutic applications of mesenchymal stromal cell-derived small extracellular vesicles. *Cytotherapy*. 2021;23:373–80.
67. Zhou T, Yuan Z, Weng J, Pei D, Du X, He C, et al. Challenges and advances in clinical applications of mesenchymal stromal cells. *J Hematol Oncol*. 2021;14:24.
68. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. *Nat Rev Nephrol*. 2018;14:493–507.
69. Doeppner TR, Herz J, Görgens A, Schlechter J, Ludwig AK, Radtke S, et al. Extracellular vesicles improve post-stroke neuroregeneration and prevent postischemic immunosuppression. *Stem Cells Transl Med*. 2015;4:1131–43.
70. Brenneman M, Sharma S, Harting M, Strong R, Cox CS Jr, Aronowski J, et al. Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. *J Cereb Blood Flow Metab*. 2010;30:140–9.
71. Li Y, Chen J, Chen XG, Wang L, Gautam SC, Xu YX, et al. Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. *Neurology*. 2002;59:514–23.
72. Wei L, Fraser JL, Lu ZY, Hu X, Yu SP. Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. *Neurobiol Dis*. 2012;46:635–45.
73. Sarmah D, Agrawal V, Rane P, Bhute S, Watanabe M, Kalia K, et al. Mesenchymal stem cell therapy in ischemic stroke: a meta-analysis of preclinical studies. *Clin Pharmacol Ther*. 2018;103:990–8.
74. Anderson JD, Pham MT, Contreras Z, Hoon M, Fink KD, Johansson HJ, et al. Mesenchymal stem cell-based therapy for ischemic stroke. *Chin Neurosurg J*. 2016;2:36.
75. Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. *J Cell Biochem*. 2006;98:1076–84.
76. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the international society for extracellular vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. 2018;7:1535750.
77. Shah R, Patel T, Freedman JE. Circulating extracellular vesicles in human disease. *N Engl J Med*. 2018;379:958–66.

78. Kuang Y, Zheng X, Zhang L, Ai X, Venkataramani V, Kilic E, et al. Adipose-derived mesenchymal stem cells reduce autophagy in stroke mice by extracellular vesicle transfer of miR-25. *J Extracell Vesicles*. 2020;10:e12024.
79. Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic administration of exosomes released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. *J Cereb Blood Flow Metab*. 2013;33:1711–5.
80. Xin H, Katakowski M, Wang F, Qian JY, Liu XS, Ali MM, et al. MicroRNA-17–92 cluster in exosomes enhance neuroplasticity and functional recovery after stroke in rats. *Stroke*. 2017;48:747–53. Erratum in: *Stroke*. 2017;48:e137.
81. Xin H, Wang F, Li Y, Lu QE, Cheung WL, Zhang Y, et al. Secondary release of exosomes from astrocytes contributes to the increase in neural plasticity and improvement of functional recovery after stroke in rats treated with exosomes harvested from MicroRNA 133b-overexpressing multipotent mesenchymal stromal cells. *Cell Transplant*. 2017;26:243–57.
82. Xin H, Li Y, Liu Z, Wang X, Shang X, Cui Y, et al. MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. *Stem Cells*. 2013;31:2737–46.
83. Zhang ZG, Buller B, Chopp M. Exosomes — beyond stem cells for restorative therapy in stroke and neurological injury. *Nat Rev Neurol*. 2019;15:193–203.
84. Moore TL, Bowley BGE, Pessina MA, Calderazzo SM, Medalla M, Go V, et al. Mesenchymal derived exosomes enhance recovery of motor function in a monkey model of cortical injury. *Restor Neurol Neurosci*. 2019;37:347–62.
85. Go V, Bowley BGE, Pessina MA, Zhang ZG, Chopp M, Finklestein SP, et al. Extracellular vesicles from mesenchymal stem cells reduce microglial-mediated neuroinflammation after cortical injury in aged Rhesus monkeys. *GeroScience*. 2020;42:1–17.
86. Go V, Sarikaya D, Zhou Y, Bowley BGE, Pessina MA, Rosene DL, et al. Extracellular vesicles derived from bone marrow mesenchymal stem cells enhance myelin maintenance after cortical injury in aged rhesus monkeys. *Exp Neurol*. 2021;337:113540.
87. Webb RL, Kaiser EE, Scoville SL, Thompson TA, Fatima S, Pandya C, et al. Human neural stem cell extracellular vesicles improve tissue and functional recovery in the murine thromboembolic stroke model. *Transl Stroke Res*. 2018;9:530–9.
88. Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, Doeppner TR, et al. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. *Leukemia*. 2014;28:970–3.
89. Venkat P, Zacharek A, Landschoot-Ward J, Wang F, Culmone L, Chen Z, et al. Exosomes derived from bone marrow mesenchymal stem cells harvested from type two diabetes rats promotes neurorestorative effects after stroke in type two diabetes rats. *Exp Neurol*. 2020;334:113456.
90. Liu X, Zhang M, Liu H, Zhu R, He H, Zhou Y, et al. Bone marrow mesenchymal stem cell-derived exosomes attenuate cerebral ischemia-reperfusion injury-induced neuroinflammation and pyroptosis by modulating microglia M1/M2 phenotypes. *Exp Neurol*. 2021;341:113700.
91. Zhao Y, Gan Y, Xu G, Hua K, Liu D. Exosomes from MSCs overexpressing microRNA-223-3p attenuate cerebral ischemia through inhibiting microglial M1 polarization mediated inflammation. *Life Sci*. 2020;260:118403.
92. Yang HC, Zhang M, Wu R, Zheng HQ, Zhang LY, Luo J, et al. C-C chemokine receptor type 2-overexpressing exosomes alleviated experimental post-stroke cognitive impairment by enhancing microglia/macrophage M2 polarization. *World J Stem Cells*. 2020;12:152–67.
93. Dabrowska S, Andrzejewska A, Strzemecki D, Muraca M, Janowski M, Lukomska B. Human bone marrow mesenchymal stem cell-derived extracellular vesicles attenuate neuroinflammation evoked by focal brain injury in rats. *J Neuroinflamm*. 2019;16:216.



94. Haupt M, Zheng X, Kuang Y, Lieschke S, Janssen L, Bosche B, et al. Lithium modulates miR-1906 levels of mesenchymal stem cell-derived extracellular vesicles contributing to poststroke neuroprotection by toll-like receptor 4 regulation. *Stem Cells Transl Med*. 2021;10:357–73.
95. Ai Z, Cheng C, Zhou L, Yin S, Wang L, Liu Y. Bone marrow mesenchymal stem cells-derived extracellular vesicles carrying microRNA-221-3p protect against ischemic stroke via ATF3. *Brain Res Bull*. 2021;172:220–8.
96. Levy ML, Crawford JR, Dib N, Verkh L, Tankovich N, Cramer SC. Phase I/II study of safety and preliminary efficacy of intravenous allogeneic mesenchymal stem cells in chronic stroke. *Stroke*. 2019;50:2835–41.
97. Díez-Tejedor E, Gutiérrez-Fernández M, Martínez-Sánchez P, Rodríguez-Frutos B, Ruiz-Ares G, Lara ML, et al. Reparative therapy for acute ischemic stroke with allogeneic mesenchymal stem cells from adipose tissue: a safety assessment: a phase II randomized, double-blind, placebo-controlled, single-center, pilot clinical trial. *J Stroke Cerebrovasc Dis*. 2014;23:2694–700.
98. Phinney DG. Functional heterogeneity of mesenchymal stem cells: implications for cell therapy. *J Cell Biochem*. 2012;113:2806–12.
99. Giebel B. On the function and heterogeneity of extracellular vesicles. *Ann Transl Med*. 2017;5:150.