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The application of mesenchymal stem cells in the treatment of traumatic brain injury: mechanisms, results, and problems

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Summary

Mesenchymal stem cells (MSCs) are multipotent stromal cells that can be derived from a wide variety of human tissues and organs. They can differentiate into a variety of cell types, including osteoblasts, adipocytes, and chondrocytes, and thus show great potential in regenerative medicine. Traumatic brain injury (TBI) is an organic injury to brain tissue with a high rate of disability and death caused by an external impact or concussive force acting directly or indirectly on the head. The current treatment of TBI mainly includes symptomatic, pharmacological, and rehabilitation treatment. Although some efficacy has been achieved, the definitive recovery effect on neural tissue is still limited. Recent studies have shown that MSC therapies are more effective than traditional treatment strategies due to their strong multi-directional differentiation potential, self-renewal capacity, and low immunogenicity and homing properties, thus MSCs are considered to play an important role and are an ideal cell for the treatment of injurious diseases, including TBI. In this paper, we systematically reviewed the role and mechanisms of MSCs and MSC-derived exosomes in the treatment of TBI, thereby providing new insights into the clinical applications of MSCs and MSC-derived exosomes in the treatment of central nervous system disorders.

Keywords: Mesenchymal stem cell, Microglia, Regenerative medicine, Traumatic brain injury, Exosome

Introduction

Traumatic brain injury (TBI) is one of the leading causes of death and disability on a global scale. Despite the exploration of some therapeutic approaches, such as surgical and pharmacological interventions and rehabilitation, outcomes have been less than optimal (Carbonara *et al*., 2018; Williamson *et al*., 2020; Dams-O'Connor *et al*., 2023). The majority of patients with brain injury continue to experience long- or short-term complications, such as neurodegenerative diseases, motor dysfunction, psychiatric disorders, cardiovascular diseases, and metabolic disorders (Howlett *et al*., 2022; Kornblith *et al*., 2022; Lai *et al*., 2022; Pelo *et al*., 2023; Ruchika *et al*., 2023), indicating a poor prognosis (Li *et al*., 2020; Lu *et al*., 2023). To date, it is well-accepted that the mechanisms of brain injury treatment are complex and multifaceted, and the precise treatment methods and mechanisms remain elusive (Ma *et al*., 2019; Kattan *et al*., 2023). Mesenchymal stem cells (MSCs) are pluripotent stem cells with the ability to self-renew and differentiate into a diverse range of cell types, including chondrocytes, osteoblasts, adipocytes, etc. Presently, stem cell therapy is a burgeoning therapeutic approach for central nervous system (CNS) disorders (Zhang *et al.*, 2021a; Borlongan and Rosi, 2022). A plethora of experiments have demonstrated that MSCs possess neuroprotective, neurogenic, and immunomodulatory properties, and can stimulate cell proliferation and angiogenesis by mitigating inflammatory responses, while also secreting a multitude of bioactive molecules that participate in tissue regeneration; these findings offer a promising outlook for the development of treatments for brain injuries (Andrzejewska *et al.*, 2021; Li and Sundström, 2022; Monsour *et al.*, 2022).

TBI

As one of the most common diseases of the CNS, TBI is known as acquired organic damage to head or neck tissues caused by the direct or indirect action of external mechanical stresses (Menon *et al*., 2010); the most common causes include traffic accidents, falls, etc., ultimately causing degeneration and death of CNS cells (Rosenfeld *et al*., 2012). It can affect people of all ages, especially young individuals under 40 years of age, and is the leading cause of death and disability in this population (Maas *et al*., 2017). Globally, more than 50 million people are reported to suffer from TBI each year, resulting in an additional economic expenditure of $400 billion per year (Maas *et al*., 2017). In addition, patients who survive TBI have a poor prognosis and most have many sequelae, including cognitive and memory deficits, motor behavior disorders, visual impairment, and various psychological problems, etc. (Fox *et al.*, 2019; Semple *et al.*, 2019; Lambez and Vakil, 2021; Subramanian *et al.*, 2022). In addition, TBI is a known risk factor for chronic neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, etc.) (Li *et al.*, 2017b; Brett *et al.*, 2022), and TBI can cause a variety of temporary or permanent neurological changes, severely affecting the quality of life of patients. It is one of the main causes of long-term disability and is becoming a major global public health event (Mollayeva *et al*., 2018) and a worldwide health problem (Li and Sundström, 2022).

TBI is a complex and highly heterogeneous injury that is usually classified into primary and secondary injuries according to the pathological process (Hill *et al.*, 2016;

Stocchetti *et al.*, 2017). Primary injury refers to localized intracranial brain tissue damage caused by direct violence, or when a percussive injury is caused at the site of the blow, due to the transient action of external forces thus causing early tissue destruction and deformation, resulting in acute hemorrhage, necrotic cell death, nerve fiber breakage, neuronal loss, and efferent dysfunction, which occur immediately at the time of injury (Tian *et al.*, 2008). In contrast, secondary injury is further damage caused by the pathophysiological changes induced by the primary injury (Hukkelhoven *et al.*, 2006), where a series of cascade reactions, caused by changes in the local microenvironment of the injury, lead to renewed damage to surviving neural tissue. It usually occurs minutes to days or even months after the primary injury and can lead to diffuse and persistent damages (Pearn *et al.*, 2017).

Current treatment for TBI includes symptomatic treatment (surgery, hyperbaric oxygen therapy), as well as pharmacological treatment and rehabilitation (Aertker *et al.*, 2016; Maas *et al.*, 2017; Robinson, 2021). Although some effects have been achieved to control the progression of the disease and alleviate patients' symptoms to some extent, the definitive recovery effect on neurological tissues remains limited. In severe patients, current interventions such as craniotomy, debridement, and decompression only focus on relieving physical symptoms to keep the patient alive but do not directly address the underlying damage at the biochemical and cellular levels, and post-trauma recovery still relies mainly on endogenous healing mechanisms to restore brain function (Walcott *et al.*, 2012; Zhou *et al.*, 2016; Galgano *et al.*, 2017; Chu and Gao, 2022). Therefore, the urgent need to identify potential therapeutic targets

and introduce new treatments to improve the clinical prognosis of patients with TBI appears to be crucial (Yang *et al*., 2017; Khellaf *et al*., 2019; Jarrahi *et al*., 2020).

Microglia in TBI

The immune system of the CNS is a highly efficient network of mononuclear phagocytes composed mainly of innate immune cells (Xu *et al*., 2022), in which tissue-resident macrophages, also known as microglial cells, are the most abundant (Van Deren *et al*., 2022), accounting for 5-15% of the brain cells of the adult brain (Aguzzi *et al.*, 2013; Frost and Schafer, 2016; Liu *et al.*, 2021). The dynamic movement of microglia is considered to be the vanguard sentinel for monitoring neuronal activity and detecting local cerebral changes and switching on a specific response pattern (DiBona *et al.*, 2019; Umpierre and Wu, 2021), and thereby play a key role in the immune defense of the CNS (Kin *et al*., 2021).

In the CNS, the neuroinflammatory process after brain injury is mainly mediated by microglia, which are rapidly activated after the onset of TBI, as they are unable to efficiently transmit antigens when exposed to external stimuli, however, their plasticity allows them to rapidly adapt to changes in the microenvironment, respond differently to different stimuli, and maintain the ability to make functional transitions in response to changes in the internal environment (Donat *et al*., 2017; Liu *et al*., 2023), generating multiple response phenotypes with neuroinflammatory and neuroprotective properties (Borst *et al*., 2021). Similar to peripheral macrophages, microglia are usually classified into two categories based on their role upon activation: classically activated M1-type

microglia and alternatively activated M2-type microglia (Xiong *et al*., 2016), which can respectively lead to neurodegeneration or tissue repair (Minhas *et al*., 2021; Wang *et al*., 2022). Therefore, microglia-targeted therapies have a promising application in the treatment of CNS diseases (Prinz *et al*., 2021). Ideal therapies should target microglia, modulate them from a pro-inflammatory phenotype to an anti-inflammatory phenotype, reduce neuroinflammation and neuronal apoptosis, improve neurological function, and promote neurogenesis and functional recovery after TBI. Although some drugs have been shown to be effective in *in vitro* experiments, most conventional drugs cannot readily cross the blood-brain barrier (BBB) (Pardridge, 2016), and no drugs have yet been shown to act as modulators of microglia directional polarization in clinical settings. Therefore, it would be valuable to explore new ways to modulate microglia polarization to reduce the neuroinflammatory response after trauma.

MSCs

Stem cells, as a specific type of undifferentiated or incompletely differentiated cell capable of self-renewal, have the capacity for clone formation and directed differentiation into different types of tissue cells (Ma *et al.*, 2018; Somredngan *et al.*, 2023). Stem cells can regulate cellular inflammation (Zhao *et al*., 2021), attenuate the destruction of neurons (Cheng *et al*., 2018), promote angiogenesis and nerve regeneration (Yin *et al*., 2020), and have important applications in regenerative medicine (Hoang *et al*., 2022).

MSCs are non-hematopoietic stem cells derived from the mesoderm that have both

a fibroblast-like morphology and the ability to differentiate into different cell types such as osteoblasts, adipocytes, and chondrocytes, and have been widely studied due to their unique biological properties (Lim and Khoo, 2021) (Fig. 1). They are not only widely available and easy to obtain from almost all tissue sources *in vivo*, such as bone marrow, adipose tissue, umbilical cord, and dental pulp (Andrzejewska *et al*., 2019), but are also easy to isolate, culture, expand, and purify, with good survival characteristics (Cai *et al*., 2020), retaining stem cell characteristics after multiple passages. Furthermore, MSCs also have homing properties (Lin *et al*., 2017). Under normal conditions, MSCs are in a quiescent state, but when stimulated by biological signals such as tissue injury, MSCs will be activated and will home to the site of injury according to the injury environment, replacing damaged cells, differentiating into the corresponding functional cells, and integrating into the damaged tissues of the host to improve the function of the damaged site (Xia *et al.*, 2014; Zhang *et al.*, 2016; Li *et al.*, 2017a). Due to the strong multi-directional differentiation potential, self-renewal ability, low immunogenicity, and homing, MSCs are currently considered ideal seed cells for the treatment of injurious diseases, providing new possibilities and great promise in the field of tissue repair and regenerative medicine (Mishra *et al*., 2020).

In recent years, stem cell-derived exosomes, in addition to stem cell transplantation, have opened new avenues for treating a wider range of diseases. Recent studies found that exosomes are nanosized vesicles, with a diameter of about 40-160 nm, a lipid bilayer membrane structure, and a complex internal composition, secreted by different types of cells (Camussi *et al*., 2011; Azmi *et al*., 2013). These microvesicles can carry

therapeutic loads such as proteins, lipids, RNA, enzymes, metabolites, and other important cellular molecules, and have good biological properties that reflect the state of the mother cell (Stremersch *et al*., 2016). Exosomes are released from the source cells and then taken up by the target cells, interacting with the receptors on the target cells and transporting the proteins, lipids, and nucleic acids they carry as signaling molecules to the receptor cells, thereby altering the different biological behaviors and functional states of these cells (Xu *et al*., 2013), facilitating the transfer of information between cells (Pegtel and Gould, 2019) to regulate physiological homeostasis and control disease progression (Xunian and Kalluri, 2020).

Studies have shown that stem cell therapy is more effective than traditional therapeutic strategies and improves the quality of life of patients (Wu *et al.*, 2018; Tien *et al.*, 2019; Chen *et al.*, 2021). There is growing evidence that functional recovery after brain injury may also benefit from stem cell therapy (Weston and Sun, 2018; Das *et al.*, 2019), and this is now considered one of the most promising therapies for the treatment of TBI (Chen *et al.*, 2020; Bjorklund *et al.*, 2021) (Fig. 1).

Application of MSCs for TBI and Possible Mechanisms

MSC-mediated therapy for TBI is shown below and in Table 1. Due to experimental limitations, most *in vitro* studies have had difficulty reproducing secondary brain injury after TBI and, therefore, the therapeutic experiences with MSC presented in this review focus on primary injury associated with TBI. However, previous experiments have also demonstrated greater loss of brain tissue at the site of injury 14 days after TBI, which

is consistent with the course of secondary injury (Ni *et al.*, 2019). After an episode of TBI, without intervention, more and more brain tissue is lost in and around the site of injury, and the exacerbated secondary injury leads to excessive neuronal cell death. In addition, exosome administration reduced post-traumatic brain tissue loss and reduced lesion size compared with the 14-day PBS group, which simultaneously validates previous findings that MSCs are equally beneficial for post-traumatic secondary injury (Li *et al.*, 2011).

Modulation of the immune response and suppression of inflammation

TBI is followed by a neuroinflammatory response that produces microglia at the site of injury (Yan *et al*., 2022), which act as first responders to the CNS and rapidly initiate an immune response after injury. Cellular rupture following primary brain injury activates microglia as antigens, transforming them from phenotype M0 to the pro-inflammatory and neurotoxic M1 phenotype. Although microglia normally exhibit a mixed M1 and M2 phenotype with a dynamic balance of anti-inflammatory and pro-inflammatory properties after the onset of injury (Wolf *et al*., 2018), the secondary inflammatory response inhibits the activation of their anti-inflammatory M2 phenotype and converts them to a predominantly pro-inflammatory M1 phenotype (Gardner *et al*., 2018). In contrast, in the later stages of inflammation, microglia are activated to an anti-inflammatory M2 phenotype that suppresses the inflammatory state and functions as a tissue repair agent (Xin *et al*., 2021). Related studies (Lv *et al*., 2018; Wu *et al*., 2021) have shown that by polarizing microglia from phenotype M1 to M2, the inflammatory

response after TBI can be attenuated, thereby promoting recovery from injury.

Evidence suggests that the presence of MSCs and their exosomes in a pro-inflammatory environment plays an important role in both inhibiting microglia activation and modulating the cellular phenotype to attenuate inflammation (Heo *et al*., 2019; Maiti *et al*., 2019). Ni *et al*. (2019) injected rat bone marrow MSCs (BMSCs)-derived exosomes into a TBI rat model via the medial orbit and found a decrease in M1-type microglial cell markers around the lesion area and a significant increase in the expression of M2-type microglial cell markers in the treatment group. Compared with the control group, the exosome-treated group significantly suppressed the expression levels of pro-inflammatory cytokines, demonstrating that BMSC-exo have the effects of regulating the immune response and suppressing inflammation. Moreover, Li *et al*. (2017) showed, for the first time, that exosomes produced from stem cells from human exfoliated deciduous teeth (SHED-exo) have therapeutic effects on TBI in rats. SHED-exo were injected locally into the TBI rat model, and the exosome-treated group was able to significantly reduce the cellular markers of the pro-inflammatory microglia M1 phenotype and induce the differentiation of microglia to the M2 phenotype in a dose-dependent manner, suggesting that SHED-exo could alter the polarization of microglia to attenuate neuroinflammation after injury (Li *et al.*, 2017c). In a recent study, Ruppert *et al*. injected human adipose-derived mesenchymal stromal cells (ADMSCs) via the tail vein into a rat model of controlled cortical impact at an early (three days) and delayed (14 days) time after injury (Ruppert *et al*., 2020). The expression of M1 ($CD32^+$, $CD86^+$) and M2 ($CD163^+$) microglia markers was analyzed by flow cytometry, and the

results demonstrated that ADMSCs effectively reduced M1 microglia at three days post-injury; the percentage of $CD163^+$ microglia and the M2/M1 ratio of the treatment group increased significantly at 14 d post-injury, suggesting that treatment with ADMSCs had an inhibitory effect in TBI and play a major role in immunomodulation and promoting homeostasis *in vivo*. Kodali *et al*. investigated the effect of a single intranasal (IN) administration of human MSC-derived extracellular vesicles (MSC-EV) in TBI and demonstrated that MSC-EV naturally enriched with activated microglial regulatory miRNAs inhibited the chronic activation of NLRP3-p38/MAPK signaling after TBI at an optimal IN dose, which reduced the release of pro-inflammatory cytokines and exerted a preventive effect against persistent brain dysfunction (Kodali *et al.*, 2023a).

Neuroprotection and promotion of nerve regeneration

The occurrence of vascular hemorrhage, impaired synaptic plasticity, disruption of BBB integrity, neuronal apoptosis, and necrosis resulting from TBI ultimately leads to loss of neurological function. Consequently, the primary approaches for achieving neurological recovery following TBI involve minimizing neuronal loss and promoting neurogenesis (Vandenbark *et al*., 2019).

MSCs could not only promote the proliferation and differentiation of primitive neural stem cells but also differentiate into neuronal cells and glial cells with strong regenerative behaviors to compensate for damaged brain cells (Lian *et al*., 2021). Additionally, the paracrine effects of MSCs (Kamei *et al.*, 2007; Marsh and Blurton-

Jones, 2017) can promote neurogenesis and support neuronal survival and development by secreting a variety of neurotrophic and chemotactic factors (Bothwell, 2014; Popova *et al*., 2017) to alleviate secondary neurological injuries and be effective in neuroprotection after TBI (Yan *et al*., 2019). Studies have shown that MSCs can significantly improve neurological function after TBI (Mastro-Martínez *et al.*, 2015; Yuan *et al.*, 2020b), partly because they can actively interact with microglia to proliferate, migrate, and release a variety of growth factors after brain injury, which promotes neural repair after TBI (Burda *et al*., 2016; Jassam *et al*., 2017). In a previous study, Xu *et al*. collected a secretion set of human ADMSCs under hypoxic conditions and injected TBI rats with ADMSCs via the tail vein for seven days. Neurological function in TBI rats was assessed by the neurological severity score and Morris water maze tests; the data demonstrated that ADMSCs reduced the neurological deficits and cognitive deficits in TBI rats (Xu *et al.*, 2020a). Chen *et al*. injected human ADMSCs intravenously into rats three hours after TBI and examined the recovery of neurological function by using the neurological function assessment angle test, and found that the ADMSC-treated group had no immunological side effects, effectively protected the structural integrity of the brain, and significantly enhanced neurological function after TBI (Chen *et al.*, 2020b). MSC exosomes (MSC-exo) were shown to play an important role in the recovery of neurological function after TBI (Yang *et al*., 2017; Das *et al*., 2019), and one possible mechanism is that exosomes act as carriers to facilitate intercellular communication by transferring microRNAs, thereby promoting synaptic growth and nerve regeneration (Xin *et al*., 2012). Furthermore, the integration of MSCs

with low-intensity transcranial ultrasound therapy and the gelatin hydrogel system can synergize with host cells to establish a defense mechanism aimed at attenuating cerebral edema, decreasing the extent of damage within the region of injury, increasing neuronal survival, and inducing neuroprotective mechanisms (Zhang *et al*., 2018; He *et al*., 2019; Tan *et al*., 2020). The TBI model used in an experiment conducted by Darkazalli *et al*. induced a significant increase in the number of neoplastic cells in the subventricular zone, confirming that MSCs are activated only in response to injury and, in the absence of injury, intravenous injection of MSCs does not alter the baseline level of endogenous cell proliferation in the subventricular zone. This suggested a relationship between endogenous neural progenitor cells and the ability of MSCs to prevent TBI-induced depression and other behavioral deficits (Darkazalli *et al.*, 2016). Another recent study conducted by Kodali *et al*. investigated the effect of MSC-EV treatment after TBI on preventing the decline in hippocampal neurogenesis and synaptic loss in the chronic phase of TBI. It was experimentally demonstrated that a single IN dose of MSC-EV 90 minutes after TBI attenuates TBI-induced declines in BDNF-ERK-CREB signaling, hippocampal neurogenesis, and synapses (Kodali *et al.*, 2023b).

Promotes blood vessel formation

Angiogenesis can improve the plight of brain tissue ischemia by providing oxygen and nutrients to the brain, thereby promoting structural remodeling of damaged brain tissue to repair dysfunction after TBI (Xiong *et al*., 2010, 2015). In a previous study, Guo *et al*. transplanted mice BMSCs into TBI mice. Next, brain tissues were isolated

from the mice 14 days post-transplantation, and the distribution of blood vessels in the brain tissues was visualized by immunohistochemistry. The data showed that the group treated with BMSCs stained more microvessels than TBI mice, suggesting a potential mechanism by which BMSC transplantation promotes microvessel formation in brain tissue after TBI, which may improve function by promoting angiogenesis (Guo *et al*., 2017). Shi *et al*. intravenously transplanted mouse MSCs over-expressing hypoxia-inducible factor (HIF)-1 alpha within six hours of injury in TBI mice and demonstrated that mice in the treatment group exhibited significantly more angiogenesis, as well as an increase in vascular endothelial growth factor and erythropoietin expression measured by quantitative RT-PCR and western blotting. Accordingly, the over-expression of HIF-1 alpha augmented the ability of BMSCs to induce functional recovery after TBI by stimulating angiogenesis (Shi *et al*., 2018). A series of recent studies focused on the use of MSC-exo as a potential therapeutic agent for TBI, demonstrating an improved vascular plasticity capacity in the exosome intervention group in animal models (Yang *et al*., 2017; Willing *et al*., 2020; Mot *et al*., 2023). Zhang *et al*. subjected TBI rats to a single intravenous injection of miR-17-92 cluster-enriched exosomes one day after injury. Five weeks later, brain tissues were taken for immunohistochemical analysis and stained with 5-bromo-2'-deoxyuridine/endothelial barrier antigen to detect newly generated endothelial cells. In comparison with the exosome-empty treatment, the exosome treatment group formed an increased number of endothelial cells and enhanced angiogenesis, showing that increased cerebral vascular density and neovascularization played a role in functional recovery after TBI

(Zhang, *et al.*, 2021b).

Inhibit apoptosis

Adverse outcomes of TBI are usually associated with cell apoptosis (Butterfield and Reed, 2016). In a previous study, Aaron *et al*. established a swine TBI model and received human MSC-exo treatment one hour after injury. Seven days after treatment, Bcl 2-associated X proteins (Bax), which are well-known pro-apoptotic proteins that play a key role in mediating apoptosis, were compared between brain tissues of the various groups. The results showed that the expression of Bax in the exosome treatment group was significantly lower than in the control group, which proved that exosome treatment was related to the attenuation of apoptotic markers, and promoted cell survival and proliferation to achieve the improvement of TBI through the inhibition of apoptosis (Williams *et al.*, 2020b). In another study, Chen *et al*. demonstrated that human ADMSC-derived exosome (ADMSC-exo) treatment promoted functional recovery in TBI rats by ADMSC-exo intra-cerebroventricular micro-injections into a weight-loss-induced rat model of TBI within 24 hours of injury, as evidenced by NeuN immunofluorescent staining of mature neurons in the brain tissue and TUNEL staining of apoptotic cells in the border zone of the lesion, partly through the inhibition of apoptosis (Chen *et al.*, 2020a). Because more and more studies have demonstrated that transplantation of MSC-exo improves functional recovery after TBI in rats, Xu *et al*. tested a new hypothesis using the injection of brain-derived neurotrophic factor (BDNF)-induced MSC-Exo through the tail vein into a rat model of TBI. They

confirmed that the BDNF-induced MSC-exo could inhibit apoptosis better than rats MSC-exo following TBI, a mechanism that may be related to the high expression of miR-216a-5p, by TUNEL staining (Xu *et al.*, 2020b).

Results of pre-clinical studies

Transplanted MSCs can migrate across the BBB into damaged brain tissue and exert therapeutic effects through multi-directional differentiation, paracrine secretion, and the release of exosomes.

Previous studies demonstrated the ability of transplanted MSCs to differentiate into astrocytes and neuron-like cells in rat models of brain injury, as well as to stimulate neural regeneration and improve motor-sensory function, thereby facilitating functional recovery and slowing disease progression after TBI (Anbari *et al*., 2014; Hasan *et al*., 2017). Zhang *et al*. intravenously administered BMSCs into a rat TBI model assessed behavioral outcomes, measured cytokines in brain tissue homogenates, and analyzed their effects on neuroinflammation. They discovered that MSC treatment decreased the area of brain injury following TBI, decreased the presence of microglia in the injured brain parenchyma, and could improve neurological recovery after TBI by upregulating TNF-α-stimulated gene 6 protein, reducing peripheral blood leukocyte density at the injury site, increasing anti-inflammatory cytokines, and decreasing pro-inflammatory cytokines (Zhang *et al*., 2013). Hu *et al*. performed immunofluorescence and histopathological examinations to assess BMSC survival and TBI lesion volume by pre-injecting the calpain inhibitor MDL28170 into the lesion site 30 minutes after TBI,

followed by a local injection of green fluorescent protein-labeled BMSCs from rat sources into the site of brain injury in TBI rats 24 hours after TBI. The results showed that MDL28170 improves the BMSC transplantation microenvironment by increasing the survival of BMSCs and enhances neurological recovery after TBI, suggesting that new combinational therapeutic strategies can be employed to advance the role of transplanted BMSCs in TBI (Hu *et al*., 2019).

Current studies found that exosome therapy, as an anti-inflammatory agent, can achieve similar therapeutic effects as MSCs in animal models of TBI (Tsiapalis and O'Driscoll, 2020) and promote regeneration of neuronal cells and astrocytes by facilitating neural vascular remodeling (angiogenesis and neural regeneration), inhibiting inflammatory responses in the area of injury, and decreasing neuronal apoptosis, which in turn ameliorates the adverse effects of TBI, avoids secondary injuries (Mot *et al*., 2023), and facilitates functional recovery from TBI (Zhang *et al*., 2017). Zhang *et al*. injected BMSC-exo into the tail vein of rats 24 hours after TBI injury and assessed the recovery of cognitive and sensorimotor functions by the modified Morris water maze, neurological severity score, and foot-fault tests. This study demonstrated that exosome treatment significantly increased the number of neonatal neuroblasts and mature neurons in the dentate gyrus (DG) as well as the number of neonatal endothelial cells in the lesion border zone and the DG, in addition to improving the recovery of cognitive and sensorimotor functions and decreasing brain inflammation. They demonstrated, for the first time, that exosomes produced by MSCs were effective in improving functional recovery by reducing inflammation in rats after

TBI, at least in part, by promoting endogenous angiogenesis and neurogenesis (Zhang *et al*., 2015). In a recent study, Williams *et al*. observed changes in various indices seven days post-injury by establishing a swine TBI model and treating it early with a single dose of human BMSC-exo one hour after injury. The results showed that the Neurologic Severity Scoring of animals in the exosome-treated group was significantly lower in the first four days after injury, which proved that their neurological recovery was also significantly faster. Moreover, the average brain injury size of exosome-treated animals was significantly reduced, along with the expression levels of several inflammatory factors detected by ELISA. Furthermore, the ability to promote neuroprotection, improve the integrity of the BBB, and promote neuroplasticity confirmed the effectiveness of exosome treatment (Williams *et al.*, 2020a,b). In conclusion, data on MSC-derived exosomes in porcine and rodent models of TBI yielded consistent results for increasing the levels of anti-inflammatory factor expression in the brain after TBI, reducing inflammation, and improving cognitive function, thus providing a new cell-free therapy for TBI and other neurological disorders (Willing *et al.*, 2020).

Studies show that cell number and stem cell route of administration play a key role in the success of improving organ dysfunction after cell therapy (Chen *et al.*, 2020b). Pal *et al*. previously conducted an experiment in which MSC transplantation was performed at two different doses (2 and 5 million cells/kg body weight) and two different routes of transplantation (injury site and lumbar puncture) in an animal model of CNS injury (Pal *et al.*, 2010). The results showed that visualization of the transplanted MSCs took place at the site of injury rather than of injection, the

transplantation of MSCs significantly improved motor and sensory function in the experimental group, and the results were dose-related, confirming that the determining factor in the outcome of MSC transplantation is not the number of grafts but the number of MSCs that can successfully migrate to the injured area. Thus, facilitating the migration of MSCs to the site of injury is an important factor in their use for the treatment of CNS injuries. In addition, several studies have shown that, in CNS disorders, repeated multiple injections increase the number of effective MSCs and enhance the recovery of neurological function at damaged tissue sites, improving the effectiveness of treatment (Li *et al.*, 2010; Kim *et al.*, 2015).

Common routes of MSC administration reported to date include intravenous, intracranial, intrathecal, local cerebral, medial orbit, IN, etc. Among the advantages of administering MSCs intravenously, the most important property is that it is simple and non-invasive, the disadvantages are that cell migration to the lesion is low, not all of the infused cells reach the site of the injury, cells may be trapped far from the injury, such as in the liver, spleen, gastrointestinal tract, and lungs, and only a small percentage of cells may enter the injured brain region, raising concerns about the number of cells reaching the target organ (Sanchez-Diaz *et al.*, 2021; Petrou *et al.*, 2022). Intrathecal injection may be associated with local tissue damage and might be a more favorable and safer route for repeated delivery of MSCs to the brain (Kim *et al.*, 2015). The IN route has also recently been validated as a potentially safe and simple alternative for MSC treatment of CNS disease (Kodali *et al.*, 2023a,b).

Clinical trials

Despite the time and effort spent by researchers and clinicians on TBI research and treatment, most early treatments, unfortunately, did not achieve very satisfactory results, and there has been a lack of successful neuroprotective treatments for TBI. The dilemma has not improved until recent years, when more and more neuroprosthetic strategies such as stem cell therapy have been applied to TBI research and treatment (Schepici *et al*., 2020). The bone marrow precursor cell (BMPC) or bone marrow mononuclear cell (BMMNC) fraction contains mesenchymal and hematopoietic stem cells. Numerous preclinical studies have shown that these cells, as components of the bone marrow, can preferentially migrate to the site of brain injury and differentiate into neurons and cell-supporting tissues, improving functional outcomes in animals (de Leeuw *et al.*, 2020; Huang *et al.*, 2020; Sherif *et al.*, 2021). BMMNCs and their derived cells (BMSCs) can provide neuroprotection in TBI, and significant therapeutic advances have been made in repairing neural structures and re-establishing neurological function (Liem *et al*., 2019; Huang *et al*., 2020; Takamura *et al*., 2020). Thus, the cells involved in the clinical trials outlined here include MSCs, BMPCs, and BMMNCs.

We conducted a comprehensive search of the clincaltrials.gov database using the terms "head injury" and "stem cells". A total of 36 clinical studies were identified, and we screened only clinical trials involving the use of MSCs in the treatment of patients with TBI, obtaining a total of 14 clinical trials, as shown in Table 2. We then conducted further PubMed searches using the terms "traumatic brain injury" and "mesenchymal

stem cells" or "mesenchymal stromal cells" or "bone marrow mononuclear cells" or "bone marrow precursor cells". Each article type was then limited to "clinical trials" to find any other published studies that were not already registered in Clinictrials.gov. Autologous BMMNCs were used in most of these experiments, followed by stem cells of autologous adipose origin, in addition to other sources such as BMSC and umbilical cord-derived mesenchymal stem cells (UCMSC). The dose of cells used in these clinical trials also varied, ranging from 1×10^6 to 2×10^8 cells, and the route of transplantation for the experiments was mostly intravenous, but also IN, among others.

1. Clinical Trials Recorded in Clinicaltrial.Gov

BMMNCs

NCT01575470: The main objective of this research was to assess the safety and impact on functional recovery by using autologous BMMNCs for treating acute severe TBI in adults. It recruited 25 volunteers between the ages of 18 and 55 with Glasgow Coma Scores (GCS) between 5 and 8 all of whom also had an initial injury that occurred within 24 hours. The research commenced by collecting bone marrow (5 ml/kg body weight) from these individuals within 36 hours of the trauma. This was followed by administering a solitary intravenous dose of self-derived BMMNCs at 6, 9, and 12×10^6 cells per kilogram of body weight, representing low, medium, and high quantities, respectively, within 48 hours of the individual's injury. The subjects were then assessed for plasma inflammation before treatment, after treatment, and during one and six months of follow up and the healing effect was also assessed by GCS changes and

cerebrovascular accident (CVA). The study findings indicated that after intravenous administration of BMMNCs, the subjects experienced no serious adverse effects, a trend toward downregulation of plasma levels of the major inflammatory cytokines, and a clear tendency toward significant preservation of white matter volume in the low- and medium-dose treatment groups compared with the untreated patient population. This trial is the initial experiment to examine BMMNCs as therapy for TBI and affirms that intravenous autologous BMMNCs are both secure and viable for treating adults with severe TBI (Cox *et al*., 2017).

NCT02525432: This study is a phase 2b study of NCT01575470, designed to investigate the impact of intravenous infusion of autologous BMMNCs on brain structure and neurocognitive/functional outcomes in adults who had experienced severe TBI. Thirty-seven adults aged 18 to 55 years with a GCS between 3 and 8, suffering from non-penetrating closed head trauma were recruited for the study and randomized at a 3:2 ratio into two groups, an autologous BMMNC infusion group and a placebo control group. Using a Bayesian adaptive dose escalation design, the treatment begins with the minimum dosage of 6×10^6 cells/kg body weight and gradually increases to a higher dosage of 9×10^6 cells/kg body weight. The experimental group will undergo a simulated bone marrow extraction and receive saline solution as a substitute. To measure the macroscopic and microstructural properties of gray matter (GM) and white matter (WM) regions, the study will utilize high-resolution anatomical magnetic resonance imaging (MRI) and diffusion tensor imaging. Additionally, neuroinflammatory biomarkers will be analyzed in cerebrospinal fluid and plasma

samples, and group comparisons will be conducted. During the 14 days following the infusion, subjects will be carefully observed for any infusion-related toxicities and complications, while receiving the typical standard of care for TBI. Evaluations of safety and results will occur at 1, 6, and 12 months after the injury. The research is presently in progress and is anticipated to conclude by April 2024.

The safety of using autologous BMMNCs for treating severe TBI in children was assessed in study NCT00254722. Ten children, aged 5 to 14 years, with a GCS between 5 and 8, who had sustained injury within 24 hours, received intravenous administration of 6×10^6 autologous BMMNCs/kg body weight over a period of 48 hours. The safety of the procedure was evaluated based on the children's logistic organ dysfunction score, MRI data, and neurologic function indices. The findings indicated that every patient survived, the majority had a positive outlook, and there were no instances of infusion-related toxicity, thus verifying the safety and suitability of bone marrow collection and intravenous mononuclear cells as a therapeutic approach for severe TBI in children (Cox *et al*., 2011).

In 2013, a subsequent study numbered NCT01851083 was carried out to investigate the impact of intravenous administration of autologous BMMNCs on the structural and neurocognitive/functional results of the brain following serious injury in young individuals. Researchers randomized 47 children between the ages of 5 and 17 with GCS scores between 3 and 8 into experimental and control groups. They administered a single dose of 6×10^6 cells/kg or 10×10^6 cells/kg body weight of BMMNCs to the experimental group, while the control group was injected with a 0.9% sodium chloride

placebo within 48 hours of injury. Quantitative diffusion tensor magnetic resonance imaging (DTMRI) metrics, assessed and compared with untreated controls following injury, were used to validate the safety of intravenous administration of autologous BMMNCs as well as its role in influencing structural and neurocognitive/functional outcomes in the brain. The results of this study have not been published.

The objective of research NCT05293873 is to assess the safety and effectiveness of transplanting mononuclear cells derived from the patient's own bone marrow for the treatment of neurological complications following TBI. Adult volunteers, aged 20 to 50, of any gender, who have experienced closed head injuries, have been living with a TBI for 6-12 months, and have a Functional Independence Measure (FIM) score below 69, will be included in the study. The main result will be evaluated based on the occurrence of severe adverse events after the transplant, the assessment and grading of patients' functional status using FIM and the Glasgow Extended Outcome Scale (GOS-E). The study is still in the recruitment phase.

ADMSCs

The purpose of the NCT04063215 trial was to assess the safety of Hope Biosciences Adipose-derived Mesenchymal Stem Cell (HB-adMSC) infusion and its therapeutic impact on brain structure, neurocognitive/functional outcomes, and neuroinflammation in adults with subacute and chronic neurologic injury. Twenty-four adults aged 18 to 55 years with GOS-E scores > 2 and ≤ 6 who have had the disease for more than six months were recruited for a single-arm, non-randomized study in which adult patients with

subacute or chronic neurological injuries were infused with HB-adMSCs (2×10^8 total cells per dose) on three occasions. The safety and therapeutic efficacy of the treatment will be assessed by comparing glucose, calcium, albumin, total protein, and total sodium in the blood at six months and one year after the infusions. The study is currently ongoing and is expected to be completed by December 2024.

Study NCT04744051 is a Phase I clinical safety study designed to provide an initial assessment of the safety, tolerability, and clinical remission of symptoms associated with Post-Concussion Syndrome (PCS, also known as Chronic Concussion Syndrome, CCS). Inclusion criteria for volunteers were 20 adults of either sex between the ages of 18 and 65 years old, undergoing randomized assignment into four groups to receive a dose of 50 million, 150 million, or 300 million ADMSCs or placebo, via infusion therapy within one hour. Primary outcomes will be assessed by a 36-item short-form health survey (SF-36), verbal fluency, visual attention and task-switching ability, spatial learning, and memory ability; the study is currently ongoing with an expected completion date of January 2024.

Clinical trial NCT05951777 aims to assess the safety and potential therapeutic effects of intravenous infusion of HB-adMSCs on brain structure, neurocognitive/functional outcomes, and neuroinflammation in adults with TBI and/or hypoxic-ischemic encephalopathy. This study is prospective, randomized, double-blind, and placebo-controlled. The study plans to enroll 51 participants, with inclusion criteria of adults of either sex between the ages of 18 and 55 years with an injury or disease process episode or diagnosis of more than six months to meet both GOS-E scores > 2

and ≤ 6. Subjects will receive three infusions (2×10^8 cells per dose) of autologous HB-adMSCs spaced 14 days apart and will be assessed by telephone monitoring at 4 hours post-infusion and 24 hours post each infusion and by testing blood for glucose, calcium, albumin, total protein, and total sodium to assess whether subjects have infusion-related toxicity. After the last HB-adMSC infusion at 6 and 12 months and 2 years (by phone), if infusion-related adverse events are suspected, safety assessments will be performed more frequently to determine the safety of the treatment and the therapeutic efficacy. The study is currently ongoing and is expected to be completed by December 2026.

BMSCs

Clinical trial NCT02795052 aims to assess the potential improvement in neurological function for patients with specific neurological disorders by isolating and transferring autologous BMSCs into the vascular system and lower third of the nasal cavity. Through single group assignment, the study recruited 500 individuals who are adults aged 18 years and above for BMSC injections administered intravenously and intranasally (in the lower third of the nasal cavity). The main result will be determined by neurologic function before treatment (0 months) and changes in neurologic function at 1, 3, 6, and 12 months after treatment will be compared to the pretreatment using the Neurologic Quality of Life (Neuro-QOL) questionnaire. The research is currently in progress and is anticipated to conclude by July 2024.

The objective of trial NCT03724136 is to assess the effectiveness of autologous BMSCs in enhancing cognitive impairments in individuals with Alzheimer's Disease

and other dementias and improving behavioral and social challenges in adults with autism spectrum disorders. Additionally, this study will examine the efficacy of Near Infrared (NIR) Light and BMSC usage. The research enrolled 100 individuals who were adults aged 18 years or older. These participants were required to have either documented cognitive impairment or a diagnosis of a condition linked to cognitive impairment, like Alzheimer's disease or autism spectrum disorder. The research is categorized into three categories: an intravenous BMSC portion, an intravenous BMSC portion along with near-infrared light exposure, and an intravenous BMSC portion along with a localized BMSC portion administered through the nasal route. Participants will be divided into three groups, and their main results will be observed and reevaluated after 1, 3, 6, and 12 months following the treatment using the Mini-Mental State Examination (MMSE) and the Autism Spectrum Quotient Examination. The research is currently in progress and is anticipated to conclude by October 2024.

MSCs from other sources

The safety and effectiveness of administering UCMSCs through intravenous infusion will be examined in trial NCT05018832 for the purpose of treating TBI. The research aims to recruit 20 individuals, including children, adults, or older adults of any gender who have been diagnosed with TBI. Participants will be administered a single intravenous infusion of UCMSCs, totaling 100 million cells. The safety and effectiveness of the treatment will be assessed by monitoring subjects for any potential adverse events or complications one month before treatment and at 1, 6, 12, 24, 36, and

48 months after treatment. Currently, the study is underway, and it is expected to be completed by November 2025.

Study NCT02742857 aims to demonstrate the potential for reversing brain death by using a combination of intrathecal bioactive peptides, stem cells, laser, transcranial intracranial intravenous laser, and median neurostimulation as an adjuvant in cases of brain death caused by TBI and diffuse axonal damage. Twenty individuals aged 15 to 65 were enrolled in the research, meeting the criteria of being declared deceased by MRI due to TBI and undergoing various interventions (BQ-A peptide extracts, MSCs, transcranial laser therapy, and median neurostimulators). The main objective was to evaluate the reversal of brain death, which was assessed through clinical examination or electroencephalogram. The findings of the research unavailable are as yet.

2. Published Clinical Trials

Tian *et al*. conducted a clinical study to validate the effectiveness and safety of autologous BMSCs for treating TBI through lumbar puncture. Autologous BMSCs were transplanted into the subarachnoid space via lumbar puncture, treating a total of 97 patients. The assessment of long-lasting vegetative condition and physical ability indicated that there were no significant issues following 14 days of therapy. Conversely, a few patients experienced improvement in their brain function, awareness, or physical ability to some degree after undergoing BMSC transplantation. This outcome validates the safety and efficacy of BMSC transforaminal lumbar puncture treatment for TBI. Furthermore, the curative impact is negatively correlated with the age of the patient,

with younger individuals having a higher likelihood of experiencing improvement compared with older individuals. The impact of initiating cell therapy during the subacute phase of TBI is directly correlated with the duration, and the sooner the treatment commences, the more favorable the treatment outcome becomes (Tian *et al*., 2013).

An investigation designed to examine the effects of UCMSC transplantation in patients with complications of TBI was designed by Wang and colleagues. The study involved randomly assigning 40 patients to either a group receiving UCMSC treatment or a control group. Via lumbar puncture, the individuals in the UCMSC treatment category were administered a 2 ml cell suspension consisting of 1×10^7 stem cells. Before and 6 months after UCMSC transplantation, all subjects underwent a Fogel-Meyer assessment and an FIM. After transplantation, the UCMSC group exhibited progress in motor, self-care, and social cognitive skills, as indicated by the assessment results after 6 months. The findings validated that the transplantation of UCMSCs could greatly enhance neurological function in individuals suffering from TBI consequences, thus affirming the efficacy and safety of UCMSCs for treating TBI sequelae (Wang *et al*., 2013).

A retrospective cohort study was carried out by Liao *et al*. utilizing information from the phase 1 clinical trial NCT00254722. The trial involved children between the ages of 5 and 14 who were administered 6 million autologous BMMNCs/kg body weight intravenously within 48 hours of the injury. Assessment metrics like the Pediatric Intensity Level of Therapy scale and the Pediatric Logistic Organ Dysfunction

score were used to compare with pediatric controls matched in terms of age and severity. The research showed that using autologous BMMNC treatment lessened the impact of inflammation during the initial period after TBI and decreased the level of therapy required for children with severe TBI, once again validating the dependability of the preclinical information (Liao *et al*., 2015).

Current problems and possible solutions

Although the brain has limited regenerative functions, techniques to promote and expand the regenerative functions of brain tissue have not yet matured while the complexity and multifaceted nature of post-TBI may be a key reason for clinical treatment failure (Weston and Sun, 2018). To intervene in the natural evolution of post-TBI and improve patient prognosis, multiple therapeutic goals need to be achieved simultaneously.

The efficacy and safety of MSCs as a potential treatment for brain injury remain controversial (Dehghanian *et al*., 2020). For example, MSCs may be contaminated or mutated during *in vitro* culture and processing prior to transplantation (Hu *et al*., 2018), and inappropriate homing and implantation of transplanted cells may lead to the spread of foreign pathogens and may convert the good repairing ability of MSCs into an oncogenic ability that provides energy to cancer cells and promotes tumor growth and metastasis (Chen *et al*., 2019). The potential of MSCs to survive and differentiate after transplantation is limited by a number of factors, such as transplantation rate, survival rate, low proliferation rate, and poor graft exertion. The intravenous route of MSCs is

the least traumatic and causes the least damage to MSCs in the brain tissue (Chrostek *et al*., 2019) but the effect is unsatisfactory. Therefore, various measures to improve the safety of MSC therapy, such as finding the appropriate timing of administration, safe and accurate routes of administration, stable and reliable sources of cells, and perfect methods of cell culture, storage, and transportation, are worth further exploration.

In addition, the possibility of the host's own immune cells generating an immune response to MSCs, such as the risk of thromboembolism and tumor proliferation, should not be ignored (Boltze *et al*., 2015). The use of MSC-exo may avoid the problems associated with cell transplantation; however, further pre-clinical and clinical studies are needed to discover the therapeutic potential of MSCs and MSC-exo, there are insufficient clinical trials to demonstrate the direct efficacy of MSC therapies on the pathological manifestations of TBI (Wang *et al*., 2020). Additionally, long-term, systematic *in vivo* studies are required to clarify the safety of the application of MSCs and MSC-exo before eventual clinical translation.

MSCs are widely available and easy to obtain, and most previous studies have used BMSCs, however, due to some impairment in obtaining BMSCs, alternative sources of MSCs have now been sought (Tomic *et al*., 2011). For example, human UCMSCs avoid the invasive procedures required to harvest BMSCs (Thein-Han and Xu, 2011), ADMSCs can be easily obtained by liposuction, and are not only less immunogenic but also have stronger immunomodulatory properties than MSCs from other sources (Mattar and Bieback, 2015). One of the advantages of tooth-derived stem cells is that they are easy to harvest from pulp, gingiva, and periodontal of wisdom teeth, deciduous

teeth, and pre-molars that need to be extracted for orthodontic reasons, which makes dental stem cells an increasingly important source for regenerative medicine research (Raza *et al*., 2018; Abuarqoub *et al*., 2023).

TBI is an intricate condition, and despite certain advancements in clinical experiments, numerous unresolved issues persist. For instance, clinical studies are scarce, and while a few studies have noted a slight enhancement in patients' sensorimotor function following treatment (Tian *et al*., 2013; Wang *et al*., 2013), there is a dearth of longitudinal studies demonstrating significant positive effects. Consequently, it is imperative to conduct large-scale randomized controlled trials to acquire more dependable clinical data that substantiate our claim. Moreover, the duration of subsequent clinical trials is typically brief (Wang *et al*., 2013; Cox *et al*., 2017), necessitating the creation of novel approaches to assess the enduring safety and effectiveness of cellular therapy. Furthermore, it is necessary to establish uniform evaluation standards in clinical studies, since the evaluation methods differ from one trial to another. Likewise, the magnitude and fluctuation of TBI seem to influence the effectiveness of stem cell treatment (Smith *et al.*, 2021; Alouani and Elfouly, 2022), and an equally crucial aspect is gaining a more profound comprehension of the pathophysiological mechanisms involved in TBI.

Conclusions and future perspectives

TBI poses a very serious risk to society, families, and individuals. Over the past several decades, there has been no effective improvement in the treatment of patients

with TBI, nor has there been a well-established treatment program to stop the progression of the injury. Currently, surgical, physical, pharmacologic, or rehabilitative treatments have not been particularly effective, and few single treatments have been successfully applied in clinical practice.

Both MSCs and MSC-exo have strong therapeutic potential and show great therapeutic promise and protective effects in experimental brain injury. Although they still have some problems in clinical application, they are still regarded to be a very promising treatment for TBI. This also hints at the potential of cell-free therapies, which would overcome important issues related to intrinsic cellular heterogeneity and safety. In addition, the use of MSCs in combination with other drugs may improve therapeutic outcomes compared with monotherapy.

With advances in technology, the use of biomaterials and modified exosomes to deliver MSCs to targeted lesions has been successfully applied in clinical settings, which greatly improves the therapeutic effects, effectively improving the healing of brain injury, and proposes a new therapeutic model (Yuan, Botchway*, et al.*, 2020).

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Declarations

Conflict of interest No potential conflict of interest was reported by the authors.

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Figure Legends

Figure 1. Isolated from bone marrow, adipose tissues, umbilical cord, tooth tissues, etc., mesenchymal stem cells (MSCs) or MSC-derived exosomes are capable of modulating immune responses by increasing microglia M2 polarization, suppressing inflammation, promoting neuroprotection and nerve regeneration, accelerating blood vessel formation, inhibiting apoptosis of neurons, thereby reducing lesion areas, stimulating neural regeneration, improving motor-sensory function, slowing disease progression, and facilitating functional recovery in traumatic brain injury models.

Table 1. The *in vitro* and *in vivo* results, underlying mechanisms of mesenchymal stem cell-mediated therapy for traumatic brain injury.

MSCs sources	In vitro	Culture patterns	*In vitro* effects	In vivo	Application methods for MSCs	Animal models	TBI model	Usage	Dosage	Time to start treatment	Mechanism	Therapeutic effect	Reference
Human adipose tissue	/	/	/	√	HADSC/Exosomes derived from hADSC	Adult male Sprague–Dawley rats	Weight-drop method	Intracerebroventricular microinjection	5.0 x 10^5 cells/ 2.0 x10^{10} particles /mL	24 hours after injury	Suppressed neuroinflammation, reduced neuronal apoptosis, and increased neurogenesis	Facilitated sensorimotor functional recovery	Chen *et al.*, 2020a
Rat bone marrow	/	/	/	√	MSC-IL-10/MSCs- GFP	Male Sprague–Dawley rats	CCI	Stem cell transplantation	/	36 hours post-TBI	Neuroprotection, inhibited apoptosis, suppression of inflammation	Increased autophagy, markers for mitophagy, cell survival, and pre- and post-synaptic function	Maiti *et al.*, 2019
Rat bone marrow	/	/	/	√	Exosomes derived from rats BMSC	Male C57BL/6 mice	CCI	Retro-orbital injection	30 μg	15 minutes after TBI	Decreased the activation of microglia M1 phenotype but increased M2 Phenotype, attenuated cell apoptosis, and inflammation	Reduced lesion area, improved functional recovery	Ni *et al.*, 2019
Human exfoliated deciduous teeth	√	SHED + BV-2 microglia cells, Transwell co-culture	Immunomodulatory effect, prevented polarization of the microglia M1 phenotype, promoted the M2 phenotype	√	SHED /Exosomes derived from SHED	Adult male Wistar rats	Free-falling method	Locally injected	/	/	Immunomodulatory effect, suppression of inflammation	Promoted functional motor recovery	Li *et al.*, 2017b
Human adipose tissue	√	HB-adMSC + LPS/concanavalin A, co-culture	Changed immune activity	√	MSCs	Male Sprague–Dawley rats	CCI	Injected through the tail vein	3×10^6 cells/kg	3 and 14 days post-injury	Modulated the inflammatory response	Improved spatial memory performance	Ruppert *et al.*, 2020
Rat bone marrow	√	BMSCs + collagen-chitosan porous scaffold, co-culture for 48	BMSCs grew well in three-dimensional culture conditions	√	BMSC/BMSC-impregnated collagen-chitosan scaffolds	Male Wistar rats	Free-fall combat injury	Collagen-chitosan scaffolds/stereotactic injection	/	72 hours after TBI	Neuroprotection and promotion of nerve regeneration,	Promoted the recovery of neuropathological injury	Yan *et al.*, 2019

		hours									modulation of immune response		
Mouse bone marrow	√	Hypoxic preconditioning	Inhibited apoptosis	√	BMSC/ HP- treated BMSCs	C57BL/6 J mice	CCI	Injected via intravenous injection	2×10^6 cells	24 hours after TBI	Attenuated neurofilament impairment and demyelination, enhanced BMSC differentiation, modulated the pro-survival mTOR/HIF-1α/VEGF signaling pathway	Ameliorated tissue loss and sensorimotor deficits, improved cognitive function	Yuan *et al.*, 2020a
Rat fat tissue	/	/	/	√	MSC	Adult male Sprague–Dawley rats	TBI surgery	Perilesional MSC-a infusion	2×10^5 cells	24 hours after TBI	Increased the cell density in the hippocampus, increased the number of neuronal progenitors in the hippocampus	Improved motor functional outcome	Mastro-Martínez *et al.*, 2015
Human adipose tissue	/	/	/	√	The secretome of adipose-derived MSCs	Male Sprague–Dawley rats	CCI	Injected through the tail vein	0.1ml/ 250 g	7 days after TBI	Alleviated TBI-induced neuroinflammatory edema, mitigated nerve fiber damage, improved neuroinflammatory environment, limited apoptosis	Mitigated neurological impairment and cognitive deficiency	Xu *et al.*, 2020a
Human Umbilical Cord	/	/	/	√	HUCDMSC	Adult male Sprague–Dawley rats	Weight-drop method	Intravenous injection	1.2×10^6 cells	3 hours after TBI	Inhibited the inflammatory-immune and oxidative stress reaction, augmented angiogenesis/ restoring the blood flow and preserved axonal/neural functional integrity	Improved neurological functions	Chen *et al.*, 2020b
Human umbilical cord	/	/	/	√	HUC-MSCs loaded HA/SA scaffold/ hUC-MSCs	Sprague–Dawley rats	Weight-drop method	In situ injection into the center of the lesion	—	7 days after TBI	Caused milder immune response, promoted differentiation,	Promoted motor, sensory, and balance recovery	Zhang *et al.*, 2018

											proliferation, regeneration, and nutrient supply of nerve cells		
Mouse bone marrow	/	/	/	√	BMSCs	C57BL/6 male mice	CCI	Injected through the tail vein	2×10^6/ μL	/	Attenuated neuronal apoptosis, diminished caspase-3 activation, promoted angiogenesis	Promoted recovery of neurological function, ameliorated impairment of learning and memory	Guo *et al.*, 2017
Mouse bone marrow	/	/	/	√	BMSCs overexpressing hypoxia-inducible factor (HIF)-1 alpha	Male Balb/c mice	CCI	Injected through the tail vein	2×10^6 cells	6 hours after TBI	Stimulated angiogenesis and neurogenesis	Improvement of neurological recovery, reduced brain damage	Shi *et al.*, 2018
Human bone marrow	/	/	/	√	MiR-17–92 Cluster-Enriched Exosomes derived from HBMSCs	Young male Wistar rats	CCI	Injected through the tail vein	100 μg/ rat	24 hours after TBI	Reduced neuroinflammation, enhanced endogenous angiogenesis, and neurogenesis	Improved functional recovery	Zhang *et al.*, 2021
Human bone marrow	/	/	/	√	Exosomes derived from human MSC	Female Yorkshire swine	Computer-controlled cortical impact	Injected through the left external jugular vein catheter	1×10^{12} particles	After 1 hour of shock	Promoted neurological recovery, suppression of inflammation, inhibited apoptosis, promoted neuroplasticity	Reduced the lesion size, promoted neurological recovery	Williams *et al.*, 2020a
Rat bone marrow	√	Cell damage model, PC12 cells	Enhanced migration and inhibited oxidative stress injury	√	Exosomes derived from BM-MSCs(MSC-Exo)/BDNF-induced MSC-Exo	Adult male Sprague–Dawley rats	ECCI	Injected through the tail vein	100 μg	24 hours after surgery	Inhibited inflammation, promoted neuronal regeneration, improved cell migration, inhibited apoptosis	Promoted the recovery of sensorimotor function and spatial learning ability	Xu *et al.*, 2020b
Rat bone marrow	/	/	/	√	MSCs	Adult male Sprague–Dawley rats		Injected through the jugular vein	4×10^6 cells	2 hours after TBI	Reduced brain inflammatory cell infiltration, microglia, and apoptotic cell numbers	Improved neurological recovery, reduced brain water content	Zhang *et al.*, 2013

Rat bone marrow	/	/	/	√	GFP-BMSC/ MDL28170+GFP-BMSC	Male Sprague–Dawley rats	Weight-drop method	Grafted with a microinjection needle in the center of the injured area	1×10^5 cells	24 hours post-injury/ 30 minutes post-injury, 24 hours post-injury	Reduced cell apoptosis, decreased inflammatory effects	Reduced lesion volume, improved survival of transplanted cells, improved neurological function	Hu *et al.*, 2019
Human bone marrow	√	Exosomes from bMSCs in 2D/3D culture	HMSCs seeded in the 3D collagen scaffolds generated significantly more exosomes	√	Exosomes from bMSCs in 2D/3D culture	Adult male Wistar rats	CCI	Injected through the tail vein	3×10^9 particles	24 hours after TBI	Promoted angiogenesis, increased neurogenesis, reduced brain inflammation	Promoted sensorimotor functional recovery, enhanced spatial learning	Zhang *et al.*, 2017
Rat bone marrow	/	/	/	√	Exosomes derived from Mesenchymal Stromal Cells	Adult male Wistar rats	CCI	Injected through the tail vein	100 μg	24 hours after TBI	Promoted angiogenesis, increased neurogenesis, reduced brain inflammation	Enhanced spatial learning, promoted sensorimotor functional recovery	Zhang *et al.*, 2015
Human bone marrow	/	/	/	√	Exosomes derived from human MSC	Female Yorkshire swine	Computer-controlled cortical impact	Injected through the left external jugular vein catheter	1×10^{12} particles	1 hour into shock	Neuroprotection, improved BBB integrity	Reduced brain swelling and lesion size, reduced serum glial fibrillary acidic protein levels	Williams *et al.*, 2020b
Human bone marrow	/	/	/	√	HMSC-EVs	Male C57BL/6J mice	CCI	IN	5 μL/ nostril each time	1 hour after TBI	Neuroprotection, eased synapse loss	Preserved hippocampal neurogenesis	Kodali *et al.*, 2023a
Human bone marrow	/	/	/	√	HMSC-EVs	Adult male C57BL/6J mice	ECCI	IN	40×10^9 EVs in 100 μl of PBS	90 minutes after TBI	Inhibited NLRP3 inflammasome activation, enhanced anti-inflammatory cytokines	Prevented long-term cognitive and mood impairment	Kodali *et al.*, 2023b
Human bone marrow	/	/	/	√	HMSC	Sprague–Dawley rats	CCI	Injected through the tail vein	1×10^6 cells	6 hours following TBI	Inhibited endogenous stem cell proliferation	Reduced lesion volume, improved novel object recognition, and	Darkazalli *et al.*, 2016

prevented the injury-induced depression-like behavior, anhedonia

CCI, controlled cortical impact; eCCI, electric cortical contusion impactor; IN, intranasal; hMSC-EVs, human mesenchymal stem cell-derived extracellular vesicles.

Table 2. Clinical trials of stem cell therapy in TBI

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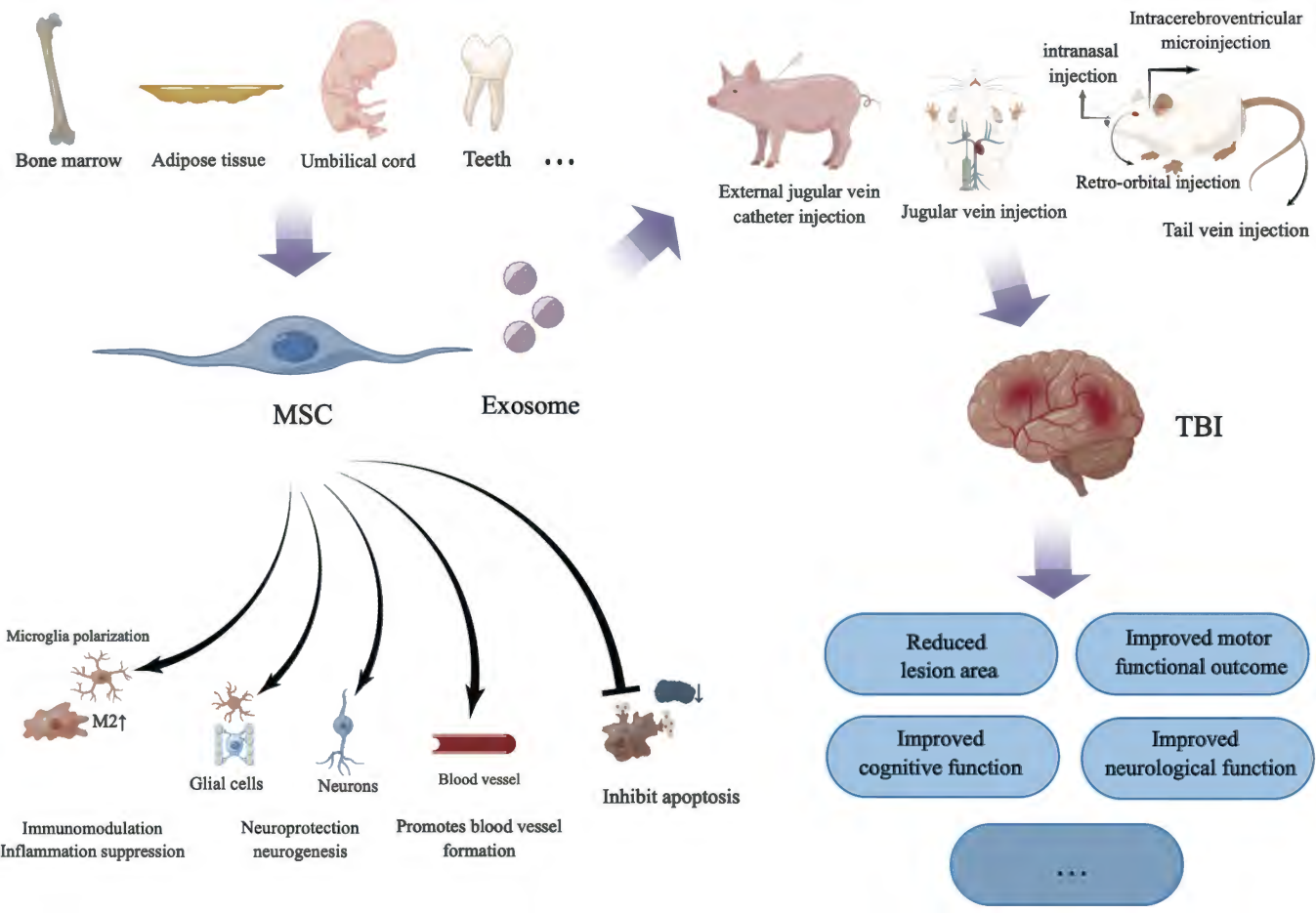
Study Title	Country	Phase	Target Enrollment or Treated Patient Number	Ages	Condition	Cell Type	Cell Source	Route	Dose	Transplant Timing	Assessment Modality	Main Purpose or Major Outcome	Identifier (NCT Number)	References
Safety of Cultured Allogeneic Adult Umbilical Cord Derived Mesenchymal Stem Cell Intravenous Infusion for TBI	Antigua and Barbuda	1	20	Child, Adult, Older Adult	/	Allogeneic	UCMSCs	IV	Single IV injection of 100 million cells	/	/	To investigate the safety and efficacy of intravenous infusion of cultured allogeneic adult UC-MSCs for the treatment of TBI	NCT05018832	/
Safety of Autologous Stem Cell Treatment for Traumatic Brain Injury in Children	USA	1	10	5 to 14 Years (Child)	GCS between 5 and 8, initial injury occurring less than 24 hours	Autologous	BMPC	IV	6 x 10^6 mononuclear cells/kg body weight	Within 36 hours of injury	Neurologic events, infectious morbidity, secondary organ injury	BMPC autografts have been shown to be safe for children after TBI and to improve functional outcomes	NCT00254722	Cox *et al.*, 2011
Autologous Adipose-Derived Mesenchymal Stem Cells for Chronic Traumatic Brain Injury	USA	2	51	18 to 55 Years (Adult)	A GOS-E score > 2 and ≤ 6, disease onset of more than 6 months	Autologous	HB-adMSCs	IV	2 x 10^8 total cells per dose	14-day intervals over a six-week period	Monitoring and evaluation of infusion-related toxicity	To determine the safety of intravenous infusion of HB-adMSCs and their potential therapeutic effects	NCT05951777	/
Stem Cell Therapy in Traumatic Brain Injury	India	1	/	6 Months to 65 Years (Child, Adult, Older Adult)	/	Autologous	BMMNCs	Cell transplantation	/	/	Change in clinical symptoms of TBI, Disability Rating scale, SF-8 scale	To study the effects of stem cell therapy on common symptoms in patients with TBI	NCT02028104	/
A Clinical Trial to Determine the Safety and Efficacy of Hope Biosciences	USA	1/2	24	18 to 55 Years (Adult)	A GOS-E score > 2 and ≤ 6, disease onset of	Autologous	HB-adMSC	IV	2 x 10^8 total cells per dose	14-day intervals over a six-week period	/	To determine the safety of HB-adMSC infusion and the therapeutic efficacy of HB-	NCT04063215	/

Autologous Mesenchymal Stem Cell Therapy for the Treatment of Traumatic Brain Injury and Hypoxic-Ischemic Encephalopathy					more than 6 months							adMSC infusion on brain structure, neurocognitive/ functional outcomes, and neuroinflammation after subacute and chronic neurologic injury in adults		
Autologous Stem Cell Study for Adult TBI (Phase 2b)	USA	2	37	18 to 55 Years (Adult)	GCS between 3 and 8, Within 48 hours of the initial injury	Autologous	BMMNCs	IV	6×10^6 cells/kg body weight to 9×10^6 cells/kg body weight	Within 48 hours of the initial injury	High-resolution anatomical MRI and diffusion tensorm imaging, neuro-inflammatory biomarkers	To determine the effects of intravenous infusion of autologous BMMNC on brain structure and neurocognitive/ functional outcomes after severe TBI in adults	NCT02525432	/
Treatment of Severe Adult Traumatic Brain Injury Using Bone Marrow Mononuclear Cells	USA	1/2	25	18 to 55 Years (Adult)	GCS between 5 and 8, initial injury occurring less than 24 hours	Autologous	BMMNCs	IV	6×10^6 mononuclear cells/kg body weight or 9×10^6 mononuclear cells/kg body weight or 12×10^6 mononuclear cells/kg body weight	Within 36 hours of injury	Neurological events, infectious morbidity, global functional status per the GOS-E	No serious adverse effects were observed in the subjects, and plasma levels of major inflammatory cytokines tended to be downregulated, there was a significant trend toward preservation of white matter volume in the low and intermediate-dose treatment groups compared with untreated patients	NCT01575470	Cox *et al.*, 2017
Pediatric	USA	2	47	5 to 17 Years	GCS	Autologou	BMMNCs	IV	6×10^6	Within 48	DTMRI, Infusional	To determine the	NCT01851083	/

Autologous Bone Marrow Mononuclear Cells for Severe Traumatic Brain Injury				(Child)	between 3 and 8, within 48 hours of the initial injury	s			cells/kg or 10×10^6 cells/kg weight	hours from time of injury	toxicity safety evaluations	effects of intravenous infusion of autologous BMMNC on brain structure and neurocognitive/ functional outcomes following severe TBI in children	
Outcomes of Autologous Bone Marrow-derived Mononuclear Cell Transplantation in the Management of Neurological Sequelae	Vietnam	1/2	50	20 to 50 Years (Adult)	Duration after Brain trauma: 6 - 12 months, FIM < 69, Closed head injury	Autologous	BMMNCs	Cell transplantation	/	BMMNCs will be transplanted at baseline, and the second transplantation will be performed 6 months after the first transplantation	Incidence of adverse events or serious adverse events after transplantation, FIM, GOS-E	To evaluate the safety and efficacy of autologous BMMNC transplantation for the treatment of neurologic sequelae after TBI	NCT05293873 /
Alzheimer's Autism and Cognitive Impairment Stem Cell Treatment Study (ACIST)	USA	/	100	18 Years and older (Adult, Older Adult)	Have documented cognitive impairment or diagnosis of disease associated with cognitive impairment	Autologous	BMSC	IV or intranasal topical or near-infrared light	14 cc of BMSC fraction or 1 cc of BMSC fraction	/	MMSE, Autism Spectrum Quotient Exam	To evaluate the use of autologous BMSCs for the improvement of cognitive deficits in patients with Alzheimer's disease and other dementias, as well as behavioral and social problems in adults with autism spectrum disorders	NCT03724136 /
Neurologic Stem Cell Treatment Study (NEST)	USA	/	500	18 Years and older (Adult, Older Adult)	At least 6 months after onset	Autologous	BMSC	IV and Intranasal (lower 1/3 of the nose)	/	/	Change in Neurologic Function	To determine whether a combination of intravenous BMSCs and topical application of BMSCs to the	NCT02795052 /

												lower 1/3 of the nasal cavity improves neurologic function in patients with certain neurologic disorders		
Use of Adipose-Derived Stem/Stromal Cells in Concussion and Traumatic Brain Injuries (C-TBI)	USA	1	/	16 to 70 Years (Child, Adult, Older Adult)	At least 1-month post mTBI and TBI	Autologous	AD-cSVF	IV	Sterile Normal Saline Suspension AD-cSVF in 500 cc	/	Number of participants with adverse events, MCAS	To seek improvement of long-term residua following adolescent and adult post-traumatic injuries	NCT02959294	/
ATCell™ Expanded Autologous, Adipose-Derived Mesenchymal Stem Cells Deployed Via Intravenous Infusion	USA	1	20	18 to 65 Years (Adult, Older Adult)	/	Autologous	ADMSC	IV	50 million or 150 million or 300 million cells	/	SF-36, Assessment of Visual Attention, Assessment of Contextual Verbal Learning	To provide an initial assessment of the safety, tolerability, and clinical remission of symptoms associated with PCS (CCS)	NCT04744051	/
Non-randomized, Open-labeled, Interventional, Single Group, Proof of Concept Study with Multi-modality Approach in Cases of Brain Death Due to Traumatic Brain Injury Having Diffuse Axonal Injury	India	1	20	15 to 65 Years (Child, Adult, Older Adult)	Individuals declared brain death from a TBI having diffuse axonal injury on MRI	Allogeneic	MSCs	/	/	/	Reversal of brain death as noted in clinical examination or EEG	To investigate the possibility of documenting reversal of brain death using a multimodal approach	NCT02742857	/

IV, intravenous; AD-cSVF, adipose-derived cellular stromal vascular fraction; MCAS, Montreal Cognitive Assessment scale


Bone marrow
Adipose tissue
Umbilical cord
Teeth
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MSC
Exosome
External jugular vein catheter injection
Jugular vein injection
intranasal injection
Intracerebroventricular microinjection
Retro-orbital injection
Tail vein injection
TBI
Microglia polarization
M2↑
Glial cells
Neurons
Blood vessel
Inhibit apoptosis
Immunomodulation
Inflammation suppression
Neuroprotection
neurogenesis
Promotes blood vessel formation
Reduced lesion area
Improved motor functional outcome
Improved cognitive function
Improved neurological function
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