

Review

Cell-Based Therapies for the Treatment of Traumatic Brain Injury: Promises and Trajectories

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Abstract: Traumatic Brain Injury (TBI) is a debilitating condition that poses a significant public health concern. Historically linked to motor vehicle accidents, the epidemiology of TBI has evolved. Falls now emerge as the predominant cause, particularly among older adults. Sport-related TBIs have also garnered increased attention due to concerns regarding long-term neurological sequelae. To date, therapeutic interventions remain limited and have yet to yield substantial clinical benefits. Cell-based therapies offer promising avenues for neural repair and regeneration: endogenous stem cell therapies capitalize on endogenous pools that can be triggered by the injury and further enhanced by therapeutic approaches. In contrast, exogenous cell therapies provide an exogenous source of cells. However, challenges such as age-related decline in neurogenesis, age-related inflammation, and the heterogeneity of TBI present significant hurdles to overcome. Moreover, translating stem cell research from the laboratory to clinical applications necessitates the adherence to good manufacturing practice standards, which presents distinct obstacles. Addressing these challenges requires a multifaceted approach, including careful patient selection in clinical trials, appropriate experimental models, and the optimization of therapeutic techniques. Ultimately, a combination of strategies is likely to yield the most promising outcomes in the pursuit of effective TBI therapies.

Keywords: traumatic brain injury; stem cells; regeneration; therapies; endogenous; exogenous



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1. Epidemiology of Traumatic Brain Injury

Traumatic Brain Injury (TBI) is a debilitating condition that, as of 2010, accounts for more than 2.5 million emergency department (ED) visits, hospitalizations, and deaths in the United States. In the United States alone, it is estimated that around 5.3 million individuals live with a TBI-related disability manifesting as physical, cognitive, and psychological impairment [1]. Historically, TBI most frequently affected young adults as a result of motor vehicle accidents (MVA) [2]. While those remain a substantial source of TBI in low- and middle-income countries and contribute to the growing incidence of TBI worldwide, the implementation of safety regulations in high-income countries has effectively reduced MVA-related injuries [2]. Consequently, there has been a significant shift towards fall-related TBIs amongst older adults, which now constitute the primary cause of TBI globally [2,3]. In the United States, falls are the most common cause of TBI-related ED visits, followed by strikes with an object and MVAs [1].

Although it is difficult to accurately evaluate the proportion of TBI requiring hospitalization, the National Center for Health Statistics National Health Interview Survey (NHIS) estimates that up to 75% of patients with head injuries resulting in loss of consciousness receive medical care, and around 25% require hospital admission [4]. Neurological-related injuries most commonly involve intracerebral hemorrhage (subdural, subarachnoid,

epidural, intraparenchymal, or intraventricular) and skull fracture [5], often requiring a decompressive craniectomy or craniotomy and/or the placement of an external ventricular drain [5]. Patients receiving surgery for TBI have an overall survival of around 7 years, and an overwhelming one-third of patients pass away during the first year [6]. TBI is also associated with significant morbidity. In 2016 alone, an estimated 55 million individuals lived with TBI-related disabilities such as post-traumatic seizure, post-traumatic stress disorder, psychiatric symptoms, sleep and attention disturbance, as well as gait and movement dysfunction [7]. In that same year, TBI led to 8.1 million years of life lived with disability (YLD) [7]. Notably, the annual economic burden associated with TBI in the United States was estimated at approximately USD 56 billion, and accounts for nearly 10% of the total annual health expenses [8,9].

During the last decades, sport-related TBI has gained considerable attention after the long-term consequences of repetitive head impacts garnered media attention [10]. Reports estimate the annual incidence of sport-related TBIs in the United States at up to 3.8 million, although this number might underestimate the correct incidence as most occurrences are unrecognized. In addition, studies in the United States and Europe have estimated that up to 9% of TBIs are related to sports and other recreational activities [10], with numbers reaching as high as 21% in New Zealand [11]. Sports with the highest risk of head injuries include contact and collision sports such as boxing, rugby, and American football, as well as high-velocity sports such as equestrian sports [12]. Notably, repetitive TBI in sports, even when mild, is associated with an increased risk of developing neurodegenerative disorders such as Alzheimer's disease or Parkinson's disease. The prototypic neurological sequela of repetitive TBI is chronic traumatic encephalopathy (CTE), which typically presents in late adulthood with behavioral, cognitive, and/or motor disturbances [12,13]. The incidence of neurological symptoms following sport-related injuries remains to be established. Rates as high as 17% have been reported in a random cohort of 224 professional boxers [14]. Ongoing prospective investigations will help better characterize the incidence in high-risk contact sports [15].

2. Pathophysiology of Traumatic Brain Injury

TBI is characterized by an initial traumatic insult, followed by an intricate cascade of pathophysiological events that constitute the secondary injury [16]. The primary injury is the direct damage incurred by the impact. It includes a spectrum of consequences caused by the mechanical disruption of brain tissue, including diffuse axonal injury (DAI), direct cell membrane rupture, contusion, and hemorrhage [17]. Two types of primary insults can be distinguished: focal and diffuse. Focal injuries typically result in a localized contusion at the impact site, with or without skull fracture [18]. A second contusion (contrecoup) can often be found on the side opposite to the impact. Essentially, these injuries result in a localized disruption of blood supply, with potential hemorrhage and necrosis of neural and glial cells [18]. In contrast, diffuse brain injuries result from acceleration–deceleration forces leading to the stretching and shearing of brain tissue. The prototypic diffuse injury consists of axonal injury in the subcortical and deep white matter of the corpus callosum or brain stem [18].

The secondary injury consists of persistent cellular, molecular, and metabolic disturbances often spanning several years following the initial impact [17]. This complex interplay culminates in deleterious cellular consequences ultimately leading to cell death through both necrosis and apoptosis [17]. Several mechanisms of secondary injury have been described, such as the release of free radicals and oxidative stress, excitotoxicity, mitochondrial dysfunction, and neuroinflammation [18]. Extensive evidence illustrates the release of reactive oxygen species (ROS) following TBI, whether through the activation of inflammatory and excitotoxic pathways, or through mitochondrial dysfunction. These ROS ultimately damage cell membranes, proteins, and DNA, impair synaptic plasticity, and further potentiate mitochondrial dysfunction and excitotoxicity [18]. Following TBI, neuronal cell death and the downregulation of astrocytic glutamate transporters (EAAT1

and EAAT2) lead to the extracellular accumulation of glutamate and other excitatory amino acids [18]. These excitatory amino acids subsequently activate receptors on postsynaptic neurons, inducing neuronal depolarization and intracellular Ca^{2+} accumulation. Excessive intracellular Ca^{2+} impairs mitochondrial function, contributes to ROS formation, and activates pro-apoptotic proteins such as caspases and calpain [18]. Finally, blood–brain barrier disruption leads to the infiltration of circulating immune cells into the injured brain. These immune cells, including neutrophils, monocytes, and lymphocytes, secrete pro-inflammatory cytokines such as $\text{TNF-}\alpha$, which in turn, contribute to cell death [18].

3. Therapeutics for TBI

Given that the primary injury arising from the immediate impact is rapid and typically irreversible, the focus of therapeutic endeavors has revolved around mitigating the biochemical cascades that underlie the secondary injury, with the overarching goal of enhancing brain repair and recovery [17]. For instance, some of the therapies explored included those aimed at reducing excitotoxicity with glutamate receptor antagonists or calcium channel blockers, reducing oxidative stress and mitochondrial dysfunction with corticosteroids, cyclosporine, and progesterone, as well as limiting apoptosis with minocycline, erythropoietin, and progesterone [18]. However, despite optimistic results in preclinical trials, most compounds investigated in phase III clinical trials have been largely unsuccessful, revealing a stark disparity between preclinical promise and clinical reality. Current TBI management guidelines published by the American Association of Neurological Surgeons (AANS) and the Congress of Neurological Surgeons (CNS) focus on the control of physiological parameters such as blood pressure, intracranial pressure, oxygenation, and nutrition among others [19].

In recent years, the identification of neural stem cell (NSC) niches in the mammalian brain, coupled with advancements in the techniques aimed at generating and differentiating NSCs in culture, has led to a surge of interest in developing cell-based therapies to replenish injured neurons and glia [20,21]. Essentially, these therapies include those focused on modulating and enhancing endogenous NSC pools, and those that harness exogenous stem cells [21]. In the mammalian brain, NSCs are primarily located in the subventricular zone (SVZ) around the lateral ventricles, and in the hippocampal dentate gyrus (DG) [22,23]. However, more recent research also demonstrates the presence of NSCs in the adult striatum [24] and subcortical white matter of humans [25,26]. Notably, Behnan and colleagues identified adult neural progenitor cells (aNPCs) in ultrasonic aspirates from neurosurgical procedures, and were able to expand these cells in culture [27].

4. Endogenous Cell Therapy

Substantial evidence derived from rodent models of TBI illustrates that injury triggers the proliferation of neural stem/progenitor cells within the dentate gyrus of the hippocampus and the SVZ [28,29]. Cells derived from each region have differing characteristics. For example, proliferating cells from the SVZ can migrate towards injured cortical and subcortical regions [29] and primarily differentiate into glial cells. In contrast, there is evidence that hippocampal neurogenesis generates neurons capable of integrating into neural networks [30], improving cognitive outcomes and recovery post TBI [20].

To capitalize on this injury-triggered neural response, researchers have explored strategies aimed at potentiating progenitor cell proliferation and differentiation into neurons capable of integration into functional networks [20]. For example, preclinical evidence illustrates the potential role of compounds such as granulocyte colony-stimulating factor (G-CSF) [31], cyclosporin A [32], and metformin [33] in activating endogenous neurogenesis. Similarly, to counteract the differentiation of endogenous stem/progenitor cells into glial cells, researchers successfully expressed the neuronal marker, Sox2, in NG2^+ oligodendrocytes to alter their differentiation profile [34]. More recently, comprehensive strategies capable of enhancing neurogenesis, migration, neural differentiation, myelination, and synapse formation have been undergoing development. An example of such strategies

includes hyaluronan–collagen hydrogels which first act as a structural bridge to facilitate progenitor cell migration, and second, as a platform for the steady release of factors that promote neurogenesis, migration, and synapse formation [35].

An important advantage of endogenous cell therapies over exogenous cell therapies is that they bypass the need for any *in vitro* cell culture which requires good manufacturing practice (GMP) stringency and labor-intensive cell culture, expansion, storage, and transport [36]. In addition, they have a decreased risk of immune rejection compared to exogenous allogeneic cell therapies. However, while therapeutic strategies aimed at promoting endogenous NSCs are indeed attractive and supported by compelling enhancements in behavioral and cognitive outcomes in select preclinical models [20], it is imperative to highlight two key limitations. First, the number of available endogenous cells for manipulation is limited. The total number of cells capable of acting as stem/progenitor cells constitutes a small fraction of the cell population of the brain. Second, the age of the host and therefore endogenous cells is important. Even in mouse models, which have a more robust SVZ neurogenic activity than humans, most evidence derives from studies conducted on young rodents [20,37,38]. This might not necessarily extrapolate to the context of older rodents that better reflect the demographic of patients now presenting with the largest number of TBIs. In fact, it is well established that while younger rodents are endowed with neural stem/progenitor cells, this population becomes depleted with aging [39,40]. For instance, Doublecortin (DCX) positive progenitor cells in the hippocampus decrease by approximately 40% per month in C57BL/6 mice [41]. Similar findings have been reported for the SVZ where electron microscopy imaging over 2–22 months revealed that only residual pockets of neurogenesis remain at 22 months [42]. In humans, SVZ neurogenesis dramatically decreases after a peak in 18-month-old neonates [43–45]. All in all, these age-related deficits in neurogenesis could in part contribute to the exacerbated outcomes observed following brain injury in older adults, both in murine models and in the human population [46].

5. Exogenous Cell Therapies

Alternatively, exogenous cell-based therapies that do not necessarily rely on the presence of endogenous neurogenic cells have gained momentum recently. These therapies rely on the *in vitro* expansion of embryonic, mesenchymal, neural, or induced pluripotent stem cells, that can be implanted into the human brain [47].

5.1. Sources of Exogenous Cells

Various stem cell sources have been investigated for the treatment of neurological diseases, including those isolated from the central nervous system, such as NSCs, and oligodendrocyte progenitor cells (OPCs), as well as peripheral stem cells such as mesenchymal stem cells (MSCs) and olfactory ensheathing cells (OECs) [36]. Importantly, cells can be characterized according to their host of origin: autologous cells are derived from the patient, while allogeneic cells are derived from someone other than the patient. The selection of stem cell sources utilized in clinical trials for TBI is outlined in Table 1. While MSCs and OECs can be obtained from either the patient (autologous) or an allogeneic source, the process is invasive and costly. It requires surgical procedures for harvesting, followed by the *in vitro* expansion and comprehensive molecular and functional characterization of cells [36]. In contrast, NSCs and OPCs are typically derived from embryonic stem cell (ESC) sources, given the challenges associated with autologous harvesting from the central nervous system. ESCs possess totipotent characteristics, allowing them to differentiate into any cell type of the three germ layers. Nevertheless, the associated ethical issues and their tumorigenic potential have raised concerns regarding their clinical use [36]. More recently, advancements in cell fate reprogramming techniques have enabled the derivation of NSCs and OPCs from autologous sources induced into a pluripotent stage. Essentially, these induced pluripotent stem cells (iPSCs) can be generated *in vitro* from adult human cells (typically skin fibroblasts or bone marrow) by expanding them in the presence of certain

transcription factors or via nuclear transfer [36,48]. Generating autologous iPSC-derived NSCs hence reduces the risk of immunologic rejection and offers a strategic advantage in scalability and applicability due to their virtually limitless supply [47,49,50]. Yet, it is important to note that iPSCs often retain epigenetic and residual transcriptomic signatures characteristic of their original source, which might limit their full differentiation into the desired cell fate [51,52].

5.2. Therapeutic Potential of Exogenous Cells

Importantly, these therapies have been harnessed for their direct effect related to replacing damaged neuronal cells, as well as their indirect effects in modulating the neural microenvironment, decreasing inflammation, depositing an extracellular matrix, and providing trophic support (BDNF, GDNF, IGF-1; Figure 1) [36,47]. In fact, numerous preclinical studies have demonstrated the potential of stem cells to localize to the site of injury, differentiate into neuronal-like cells, and improve the histological and behavioral outcomes [58–60]. Similarly, a meta-analysis of 74 preclinical studies on stem cell transplant for spinal cord injury further confirmed the role of stem cells in improving motor recovery [61]. This study uncovered the additive benefit of using a scaffold, and the variability in outcomes resulting from the various injury models. We anticipate that the various mechanisms and severities of TBI could lead to a similar variability in response.

MSCs, a type of multipotent stem cells that can be harvested from autologous bone marrow, adipose tissue, or umbilical cord, possess the capacity to migrate to the injury site and differentiate into neural-like cells expressing neuronal markers such as the neuronal nuclear antigen (NeuN) and the microtubule-associated protein 2 (MAP2) [58,62]. In the context of TBI, these cells have demonstrated efficacy in promoting neuronal regeneration and modulating the immune environment [62], thereby improving both structural and behavioral outcomes [58]. They have been also demonstrated to mitigate the injury-induced excitotoxicity by downregulating the membrane expression of glutamate receptors [63] and modulating their downstream signaling [64]. Alternatively, NSCs and OPCs are tripotent cells capable of differentiating into neurons, astrocytes, and predominantly oligodendrocytes in the case of OPCs. Extensive preclinical evidence illustrates the efficacy of these cell types in homing in on the injured site [65] and facilitating neurological recovery through various mechanisms including trophic factor secretion (BDNF, GDNF), injured axon remyelination, neural circuit remodeling, and extracellular matrix scaffold deposition [36]. In fact, a meta-analysis of 31 published studies demonstrated that the stem cell secretome, including secreted molecules and extracellular vesicles, significantly improves the neurological function and inflammation in rodent models of TBI [66]. Identifying the crucial factors secreted by stem cells and involved in neuroprotection could hence be leveraged for a cell-free therapeutic approach. Additionally, while OECs are not stem cells per se, we discuss them here as they are specialized olfactory glial cells crucial for olfactory nerve regeneration. In fact, the olfactory nerve is unique in its ability to regenerate, and primary sensory olfactory neurons are constantly replaced throughout life, unlike other peripheral nerves [67]. OECs exhibit marked regenerative properties illustrated by a rapid migration to injury sites, the phagocytosis of debris, and the promotion of axon recovery through the secretion of growth factors, as axon guidance cues. The distinctive properties of OECs arise from their phagocytic abilities combined with their low production of pro-inflammatory cytokines [67]. Notably, while OEC transplantation has shown to be safe in several clinical trials [68] and has garnered attention in the context of spinal cord injury, its application for TBI treatment remains limited to a few studies [69,70].

Table 1. NIH registered clinical trials using stem cell therapies for the treatment of traumatic brain injury.

#	NCT#	Phase	Status	Endogenous vs. Exogenous	Age	Tx Intervention	Primary Outcome Measure	Related References/Notes:
1	NCT02525432	2b	Active, not recruiting	Exogenous	18–55 years	Intravenous autologous bone marrow mononuclear cells	Structural properties of gray/white matter on MRI	
2	NCT02028104	1	Withdrawn	Exogenous	6 Months to 65 Years	Intrathecal autologous bone marrow mononuclear cells	Change in clinical symptoms of traumatic brain injury	
3	NCT02416492	2	Completed	Exogenous	18–75 Years	Intracranial allogeneic modified bone marrow–derived mesenchymal stromal/stem cells (SB623)	Change in Fugl-Meyer Motor Scale (FMMS)	Interim analysis demonstrated safety and tolerability with significant improvement from baseline motor status at 6 months compared to controls [53]
4	NCT00254722	1	Completed	Exogenous	5 Years to 14 Years	Intravenous autologous bone marrow precursor cell	Safety	The treatment of severe TBI in children with autologous bone marrow-derived cells is safe and feasible [54]
5	NCT05951777	2	Enrolling by invitation	Exogenous	18–55 Years	Intravenous autologous adipose-derived mesenchymal stem cells	Safety	
6	NCT01575470	1–2	Completed	Exogenous	18–55 Years	Intravenous autologous bone marrow mononuclear cells	Safety	The treatment of severe, adult traumatic brain injury using an intravenously delivered autologous bone marrow mononuclear cell infusion is safe and logistically feasible [55].
7	NCT05018832	1	Not yet recruiting	Exogenous	Age not specified, Child, Adult, Older Adult	Intravenous allogeneic adult umbilical cord derived mesenchymal stem cells	Safety	

Table 1. Cont.

#	NCT#	Phase	Status	Endogenous vs. Exogenous	Age	Tx Intervention	Primary Outcome Measure	Related References/Notes:
8	NCT01851083	2	Completed	Exogenous	5–17 Years	Intravenous autologous bone marrow mononuclear cells	Brain white matter and gray matter structural preservation on DTMRI	An autologous bone marrow transplant was safe and showed the potential for a decreased stay in the ICU and white matter structural preservation [56].
9	NCT02959294	1	Withdrawn	Exogenous	16–70 Years	Parenteral autologous adipose-derived stem/stromal cells	Safety, functional outcome	
10	NCT04063215	1–2	Active, not recruiting	Exogenous	18–55 Years	Autologous adipose-derived mesenchymal stem cells	Safety	
11	NCT04744051	1	Active, not recruiting	Exogenous	18–65 Years	Intravenous adipose-derived stem cells	Health Status using a 36-item Short Form Health Survey (SF-36)	
12	NCT02795052	1	Recruiting	Exogenous	18 Years and older	Intravenous or intranasal autologous bone marrow-derived stem cells	Change in neurologic function, 1, 3, 6, and 12 months post treatment	
13	NCT05293873	1	Unknown status	Exogenous	20–50 Years	Transplant of autologous bone marrow-derived mononuclear cells	Adverse events—Functional independence—Extended Glasgow Outcome Scale up to 12 months post treatment	Study conducted in India, not reviewed by the Indian Council for Medical Research. Listed on the National Institute of Health’s website but not subject to American regulator governance.
14	NCT02742857	1	Completed	Exogenous	15–65 Years	Intrathecal mesenchymal stem cells	Reversal of brain death via clinical exam or electroencephalography	

Table 1. Cont.

#	NCT#	Phase	Status	Endogenous vs. Exogenous	Age	Tx Intervention	Primary Outcome Measure	Related References/Notes:
15	NCT02148367	2	Withdrawn	Endogenous	18–70 Years	Erythropoietin	Number of circulating endothelial progenitor cells	Not directly aimed at enhancing neurogenesis but cited as a possible effect of Erythropoietin. Withdrawn as the primary outcome was not met [57].
16	NCT02226848	2	Withdrawn	Endogenous	18–70 Years	Erythropoietin	Number of circulating endothelial progenitor cells	The same as above.
17	NCT02083445	NA	Completed	Endogenous	20–60 Years	Exercise, muscle electrostimulation, intermittent hypobaric hypoxia	Change in physical and psychological tests	The secondary outcome aims at measuring circulating progenitor cells.
18	NCT00810615	1-2	Completed	Endogenous	19–60 Years	Hyperbaric oxygen therapy	Neuropsychological tests	The secondary outcome included the measurement of CD34 ⁺ circulating stem cells.
19	NCT01239706	2	Unknown status	Endogenous	18–65 Years	Ntx-265 (Human Chorionic Gonadotropin (hCG) and erythropoietin	Safety	hCG and erythropoietin were shown to potentiate neurogenesis in preclinical models.
20	NCT03900182	NA	Terminated	Endogenous	18–65 Years	Hyperbaric oxygen therapy	Neuropsychological tests	The investigators mention the potential for increasing circulating progenitor cells
21	NCT01762475	2	Completed	Endogenous	18–55 Years	Sildenafil	Cerebrovascular reactivity: Blood oxygen level dependent response to hypercapnia	Measures circulating endothelial progenitor cells as the secondary outcome.

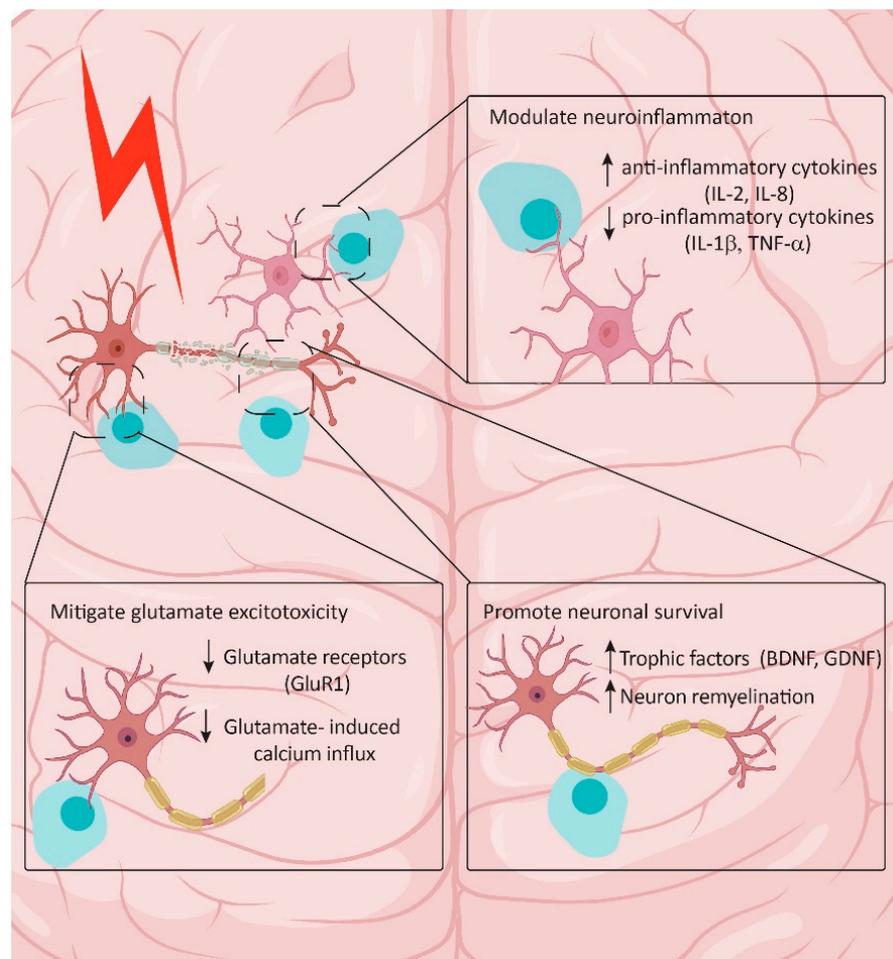


Figure 1. Schematic representation of the indirect protective role of exogenous cell therapies in traumatic brain injury. The upper left panel illustrates the injury induced by TBI. An injured neuron is illustrated in dark red with degraded myelin, an activated microglia is illustrated in pale red, and exogenous stem cells are illustrated in turquoise. We highlight some of the indirect roles of exogenous cell-based therapies in TBI, such as decreasing inflammation in the microenvironment, promoting neuronal survival, and decreasing excitotoxicity. Up arrows designate an increase and down arrows designate a decrease. Part of the figure is prepared with BioRender.com.

To date, despite the substantial preclinical evidence supporting the role of cell-based therapies, clinical trials remain limited to a few early-phase studies. There are no phase 3 clinical trials investigating the efficacy of such therapies in humans, and only a few studies have published their early-phase results (Table 1). While those studies provided evidence of the short-term safety and feasibility of cell-based therapies for TBI, concerns persist about the tumorigenic potential of stem cells, necessitating longer follow-ups [55,71,72]. In addition, whether the approaches undertaken are scalable to larger clinical trials remains questionable. Importantly, although not designed and powered to evaluate efficacy, some of these trials have highlighted the potential of such therapies to improve neurological function and preserve structural integrity, which will require further validation in larger randomized clinical trials [55,71]. Additionally, the influence of age on the success of these therapies was also evident in one of the studies where better recovery was observed among younger patients [71]. It is therefore essential to take this into account when designing phase 3 clinical trials evaluating the efficacy of such therapies. Stratification based on age might therefore be essential to fully explore the potential of these therapies and increase the chances of positive outcomes.

6. Challenges and Strategies to Improve Clinical Translation

A substantial body of preclinical evidence supports the therapeutic potential of cell-based therapies to repair and replace damaged neurons and glia after brain injuries. Clinically, these therapies remain in the early investigative stages of phase I and II clinical trials, despite being under clinical investigation for central nervous system diseases for over two decades [73]. In our search of the National Institutes of Health's (NIH) clinical trial registry, we identified a total of 30 trials citing "Traumatic Brain Injury" as a condition and "Stem cell" as an intervention or other term. Of these, 14 studies employed stem cell transplants, while 7 studies referenced therapies that have the potential to modulate endogenous stem or progenitor cell pools (Table 1). The remaining nine studies were not related to cell therapies. Notably, only 8 studies have been completed, while 4 trials were withdrawn, 2 have an unknown status, and 1 was terminated. Of the completed studies, only a few have published their results illustrating the short-term safety and feasibility. Two-thirds of studies relied on exogenous cell therapies, of which more than half used bone marrow stem cells (both autologous and allogeneic). The most commonly investigated method of cell administration was intravenous.

To date, several clinical trials (NIH-registered and non-NIH registered) have attested to the safety of stem cell administration for TBI. Importantly, those trials have investigated different routes of administration such as intravenous [55], intracerebral [53], intraventricular [74], and intrathecal injections [75]. Similarly, they reported on various cell types such as allogeneic and autologous bone marrow-derived mesenchymal and mononuclear cells, as well as umbilical cord-derived mesenchymal stem cells [53,55,72,75]. However, in most instances, the study period was limited to one year and longer follow-ups are required. In addition, while large-scale phase III studies are lacking, some studies have reported on the preliminary efficacy results. For example, the double-blinded phase II STEMTRA trial (NCT02416492) illustrated that the intracranial implantation of allogeneic bone marrow-derived mesenchymal stromal/stem cells (SB623) in patients with TBI-related motor deficits led to functional motor improvements at 6 months post therapy [53]. The authors note that an important feature of their trial was the use of outcome measurement tools capable of discerning subtle, yet clinically important improvements in neurological function [76]. This is especially important considering that outcome measurement often takes place within 6 to 12 months, where there is limited time for changes to become noticeable on the conventionally used Glasgow Outcome Scale or Glasgow Outcome Scale-Extended. However, no studies have reported on the implantation efficiency or survival of administered cells. While these measurements might not always be possible to perform using conventional methods, new MRI- or radiolabeling-based techniques have allowed the tracking of stem cells in vivo and could be employed in future trials [77,78]. An understanding of implantation efficiency and the survival of administered cells will be crucial in designing future phase III clinical trials, as this will guide in selecting the optimal route of administration and treatment schedule. We outline below other several key limitations that need to be addressed to enhance cell-based therapies for TBI and improve the likelihood of positive outcomes in future clinical trials (Figure 2).

	Challenges	Suggested solutions
Preclinical trials	Preclinical models do not perfectly recapitulate human pathology	Investigate preclinical models more representative of human pathology
	Research rarely performed in aged models	Investigate cell-based therapies in age-relevant animals
	Preclinical work rarely performed under good manufacturing practices	Plan for cell-based therapy research under good-manufacturing practices
Early phase trials	Implantation efficiency and survival of administered cells largely unknown	Use techniques to track administered cells and determine transplantation efficiency and survival
	Efficiency of different routes of administration unknown	Compare the efficiency of different routes of administration
Phase III trials	Different injury types can lead to variable treatment response	Perform a highly specific patient selection according to injury type and severity
	Response to treatment is likely to be age-dependent	Perform a stratified analysis based on age
	Implanted cells likely to require trophic support	Combine strategies that improve implanted cell survival
	Short-term improvements might involve subtle changes in functional outcomes	Use outcome measurement tools capable of discerning subtle yet clinically important changes in functional outcomes

Figure 2. Challenges associated with translating therapies for TBI from preclinical models into clinical trials and suggested solutions.

First, age is likely to play a limiting effect both for endogenous and exogenous cell-based therapies. Aging is characterized by a chronic, low-grade inflammatory state often referred to as “inflammaging” [79]. The aged brain presents a unique microenvironment that has a lower reserve of endogenous stem cells and is less favorable for stem cell proliferation and maturation than young brains [80]. It is therefore likely that older populations will respond differently to cell-based therapies compared to younger patients. Unfortunately, few preclinical studies have been specifically tailored to aged populations, which represents an understudied and clinically relevant area for further research. Basic science and translational studies of TBI in older subjects would potentially elucidate additional mechanisms underpinning age-related disparities in TBI outcomes and reveal avenues for therapeutic interventions targeting unique pathophysiologic changes within older individuals. This can be extended to the clinical realm, where clinical trial design would benefit from a stratification of the patient population based on age. This practice might uncover benefits in a subset of patients, and is supported by preliminary evidence in early-phase trials [71].

Second, the survival of transplanted cells has consistently been low in preclinical models. Only a small percentage of transplanted exogenous cells survive the delivery process and adapt to the new environment. The result is significant cell death and breakdown products which likely contribute to an additional inflammatory cascade at the transplant site [36]. Several strategies have been proposed to address this challenge. To promote implant survival, the concomitant delivery of growth factors and anti-inflammatory agents have been investigated. Immunosuppression to reduce rejection-induced cell death and cytokine release by peripheral inflammatory cells is another strategy. However, a meta-analysis of stem cell transplants for spinal cord injury revealed that immunosuppressive therapy negatively affected motor function recovery and may therefore have unintended

consequences within the healing CNS [61]. Alternatively, biomaterials such as hydrogels have been used to provide a steady release of growth factors important for cell survival and proliferation. Some of these have been designed to mimic the brain's extracellular matrix and provide additional structural support to reduce the rate of anoikis [35,36]. Other strategies have also explored the possibility of engineering the cells themselves to secrete neurotrophic [81] and growth factors directly into their local microenvironment to enhance survival [82]. These strategies have not yet been investigated clinically for TBI, and we suggest that they deserve further exploration.

Third, different delivery techniques might lead to different cell implantation efficiency and survival rates. In our search of NIH-registered clinical trials, we observed that most trials relied on the use of the intravenous injection of cells. However, as mentioned above, there is limited knowledge about the efficiency of these cells trafficking into the damaged brain, and using novel techniques to trace this would be beneficial [78]. Similarly, the efficiency of intracranial delivery also needs to be explored. While local delivery bypasses the limitations imposed by the blood–brain barrier and has been demonstrated to be safe [53], it is important to keep in mind that the injection rate and catheter size need to be optimized. In fact, high injection rates lead to compressive forces, while low injection rates promote cell–cell adhesion and potential clogging of the injection device [36]. Similarly, while large needles or catheters reduce the shearing observed in smaller setups, they create greater parenchymal damage and a potential tract for cellular efflux [36]. In addition, stereotactic-guided injections can be performed with the help of a stabilizing frame.

Fourth, the clinical application of cell therapies requires stringent controls, data logging, and conformity to current good manufacturing practices (cGMP) when producing/manipulating cell therapeutics *in vitro*. This carries several implications including frequent testing for pathogens, quality control checkpoints, chain of custody logging, and several prerequisites for the use of reagents, particularly those of an animal origin. Most laboratory discoveries and translational research do not conform to these strict standards for reasons related to cost, knowledge, and/or difficulty. Unfortunately, this means that translating from the bench to the bedside requires an intervening step to develop and test cell research protocols at a GMP grade using a different set of reagents, equipment, personnel, and sometimes techniques. This can be cost-prohibitive, highly time-consuming, and prone to outright failure. This has been a key challenge in the translation of promising cell therapies from animals to patients.

Finally, TBI is a heterogeneous diagnosis arising from a diverse array of mechanisms, including low- and high-energy direct head impacts, rapid acceleration–deceleration forces, blast waves, and penetrating injuries [17]. It also encompasses a wide spectrum of injury severities that pose challenges to clinical classification and are often complicated by concomitant injuries and comorbidities. To address these complexities, clinical trials need to be designed to select highly specific target patient populations that are most likely to benefit from this intervention before expanding to more generalized populations. In addition, while the initiation of treatment is straightforward for severe injuries characterized by evident neurological dysfunction, challenges persist in predicting neurological dysfunction in patients who present with mild injuries. Consequently, uncertainty lingers regarding the selection of patients with mild or moderate injuries who require treatment and the optimal timing to initiate treatment.

In summary, TBI is a multifaceted challenge characterized by heterogeneity and complex pathobiology. While preclinical trials have demonstrated the potential for stem cell-based therapies to repair and replace damaged CNS cells and enhance functional recovery, significant obstacles hinder their clinical implementation. Addressing age-related disparities, optimizing cell manufacturing and delivery techniques, as well as refining patient selection criteria are essential steps toward realizing the full therapeutic potential of these interventions. A multipronged approach addressing these challenges holds the greatest promise for developing a successful cell-based therapeutic option for individuals living with the consequences of TBI.

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