E-ISSN: 3068-5052
Journal of Regenerative Medicine & Biology Research

ATHENAEUM SCIENTIFIC PUBLISHERS

Review Article

Stem Cell-Based Innovations for Neonatal Hypoxic-Ischemic Encephalopathy

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Citation: McCredie HM, et al. Stem Cell-Based Innovations for Neonatal Hypoxic-Ischemic Encephalopathy. J Reg Med Biol Res. 2026;7(1):1-17.

https://doi.org/10.46889/JRMBR.2026.7109

Received Date: 25-03-2026

Accepted Date: 13-04-2026

Published Date: 20-04-2026

Abstract

Neonatal Hypoxic-Ischemic Encephalopathy (HIE) is a common brain injury initiated, during late gestation or labor, from reduced oxygen and blood supply to the brain. Currently, HIE poses high risk of mortality and development of long-term neurological disabilities among neonates. Despite advances in care, HIE continues to have a persistent incidence rate worldwide. Neurologically, injury promotes a cascade of events including energy failure, excitotoxicity, etc. that diminishes brain tissues. Diagnosis and staging of HIE includes clinical assessments, MRI imaging, as well as biomarkers that allows for treatment initiation to occur. Standard treatment with Therapeutic Hypothermia (TH) slows injury by cooling infant's internal temperature to 33.5°C within a few hours. Despite success, increased rates of treatment-related complications resulting from TH highlight the need for alternative methods of treatment. Experimental studies using a variety of stem cells including MSCs have shown success in preclinical and early-phase trials with neuroprotection and regenerative potential present. These studies have small sample sizes and lack comparative data with consistent limitations to be addressed. Future research must identify an optimized protocol in which one cell type is used. Current findings have suggested combination treatments with stem cells and standard care show promising results, yet continued research is needed.

Keywords: Neonatal; Hypoxic-Ischemic Encephalopathy (HIE); Therapeutic Hypothermia; Mesenchymal Stem Cells

Introduction

Neonatal Hypoxic-Ischemic Encephalopathy (HIE) is a significant brain injury that occurs due to insufficient oxygen and blood supply at the time of birth. Currently, the condition affects approximately 1 in 3 neonates per 1,000 live births in high-income countries, with a greater rate of incidence within low-income countries [1-5]. Additionally, current mortality rates of HIE are around 15-25% with nearly 40-50% of survivors developing long-term neurologic disabilities [1]. These long-term neurological disabilities include cerebral palsy, cognitive impairments and Attention-Deficit Hyperactivity Disorder (ADHD). The severity of HIE injury is classified into three categories - mild, moderate and severe. Classification of injury is based on the National Institute of Child Health and Human Development (NICHD) system. Like Sarnat staging, NICHD grades injury on six neurological domains, including level of consciousness, spontaneous activity, muscle tone, posture, primitive reflexes and autonomic activity [1]. Through this system, infants who have at least one domain are considered to have mild HIE, whilst those with three or more domains are considered moderate or severe HIE.

Standard treatment for HIE remains limited to Therapeutic Hypothermia (TH), which is clinically initiated within six hours of birth. With its common practice, TH has reduced both mortality rates and injury progression amongst most neonates. Despite its success, however, studies have now identified hypothermia as less effective in some preterm infants, infants with delayed HIE recognition and in low-income settings where equipment may be limited. Specifically, findings suggest that TH causes nearly

45% of the treated infants to develop neurological impairments or even death [6]. This significant rate of developing neurological deficit questions the efficacy of therapeutic hypothermia's use in clinical practice, highlighting the need for treatment options that, if initiated early on, can effectively reduce injury progression and initiate tissue regeneration.

With these limitations in mind, researchers have initiated studies regarding stem cell-based interventions as potential contenders for standard treatment in neonates with HIE. Unlike single-target drugs, stem cells have the potential to create a favorable environment for tissue regeneration [7]. Used to treat numerous diseases and conditions, stem cells provide regenerative growth factors that aid in both tissue repair and progression of various complications. Current studies using Mesenchymal Stem Cells (MSCs) have shown great success in reducing hypoxic-ischemic injuries through a reduction of neuronal cell death and nervous tissue inflammation, as well as initiating tissue repair. Varying MSCs have been studied, including umbilical cord tissue-derived, amniotic fluid-derived and placental-derived, bone-marrow-derived and Muse cells, providing insight into the therapeutic promise of cell use. Further findings have shown that reduced mortality, improved cognitive function and a reduction of adverse effects are achieved following stem cell transplantation. Experimental delivery methods including intravenous and intranasal administration, have also been studied and have demonstrated both feasibility and safety in animal models, with early-phase clinical trials providing encouraging preliminary data. Furthermore, one study has projected that combined treatment of therapeutic hypothermia and stem cell transplantation has the highest success rate amongst neonates.

Although studies have shown the potential for stem-cell-based treatments in treating HIE injury, these studies have limitations that must be challenged to transition into medical practice. Current trials statistically lack depth due to small sample size and/or completion of comprehensive trials. Beyond mere sample size, researchers have stated that if studies can identify the optimal cell source, determine appropriate treatment timing and dosage, ensure long-term safety and integrate current therapies like therapeutic hypothermia transition of treatment into practice may be achieved. Differences in global clinical protocol, funding and availability of technological advancements have also complicated these challenges and must be factored into future studies. Thus, an expansion of clinical trials with larger sample sizes and a standardized method of delivery, sourcing and dosage is needed for public clinical usage.

This review redefines current findings on stem cell therapies in treating neonatal hypoxic-ischemic encephalopathy. The review will outline the underlying epidemiology and pathophysiology of HIE, summarize current treatment standards and their challenges, as well as discuss diagnostic tools that support therapy selection. Finally, this review will summarize the limitations identified in preclinical and early-phase studies that must be further studied prior to public use of stem cell transplantation.

Results and Discussion

Epidemiology of HIE

Hypoxic-Ischemic Encephalopathy (HIE) remains one of the leading causes of neonatal mortality and morbidity worldwide and affects roughly one to three infants per 1000 births. With its high incidence throughout the globe, HIE accounts for roughly 22% of neonatal deaths worldwide [8]. Prior to the adoption of standard treatment options, mortality rates for severe HIE ranged between 15% and 82%, whilst moderate HIE rates were around 5% [5].

With standard treatment modalities, mortality rates have decreased, ranging between 15-25% [1]. Throughout the years, incidence rates of HIE have increased. Fig. 1 is a graphical representation of incidence rates between 2012-2019, perinatal HIE incidence rates varied from 1.3 to 2.1 per 1000 live births, with roughly 2.19 infants with HIE in 2018 alone [8].

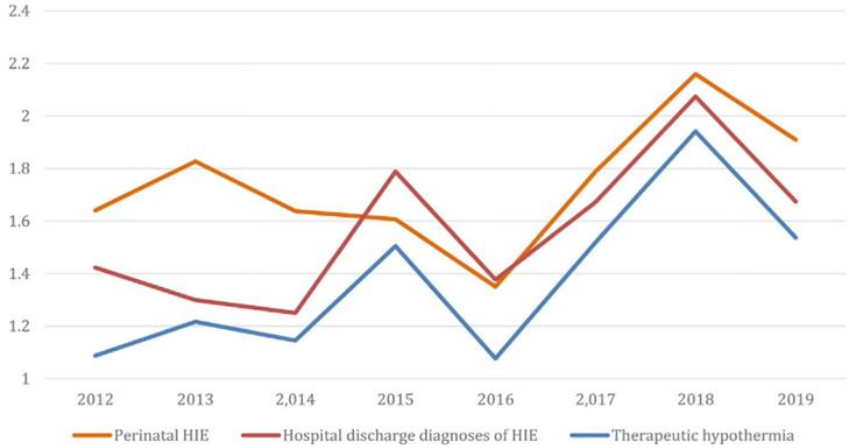

Figure 1: Population incidence rates of perinatal HIE, hospital discharge diagnoses of HIE and therapeutic hypothermia between 2012 and 2019 [8].

The high risk of developing HIE injury depends on a variety of factors. Prenatal and perinatal factors specifically have an increased risk prevalence in the development of hypoxic-ischemic injury. While the exact cause of injury is not known, a higher risk of injury is based upon prenatal factors such as maternal chronic hypertension, preeclampsia and diabetes mellitus, with birth complications like prolonged or obstructed labor, instrumental deliveries and emergency caesarean section additionally putting infants at higher risk for suffering a hypoxia-ischemia injury [3]. In areas with limited resources, delayed diagnosis, prognosis and intervention exacerbate the risk of injury, further contributing to disparities in outcomes between high and low-resource environments. Among those suffering from HIE, survival outcomes remain severe regardless of treatment intervention. Specifically, mortality rates for infants vary with the severity of injury. Studies have found that mortality rates amongst individuals with moderate and severe HIE are ~10% in mild to as high as 85% severe cases [9]. Among survivors of HIE, nearly 30-75% of infants may experience neurological disabilities [9], including attention-deficit disorder, cerebral palsy, epilepsy and cognitive deficits, which impose significant emotional, social and financial burdens. Increased risk for lifelong neurological disabilities further contributes to the need for additional treatments that not only aid in injury prevention but also limit its risk for development of life-altering disorders. While improved neonatal care has reduced mortality in developed regions, higher prevalence and poor prognosis persist where resources may be limited. This persistent issue in global differences contributes to the urgent need for additional treatment therapies to improve both short and long-term consequences of HIE in infants [9]. By understanding the underlying epidemiology of HIE injury, effective interventions such as stem-cell-based therapies can begin to transition from trial to standard practice.

Pathophysiology of HIE

Understanding the progression of hypoxic-ischemic encephalopathy plays a vital role in the administration of treatment options. Over the course of 72 hours, the pathophysiology of hypoxic-ischemic encephalopathy injury undergoes a cascade of both cellular and molecular events initiated during childbirth or gestation. Resulting from reduced cerebral blood flow perfusion and brain hypoxia [10], HIE brain injuries deprive patients of both oxygen and glucose within the brain [11], two of the main power supplies of the brain. This initial insult of injury is characterized as primary energy failure in which oxidative metabolic failure, excitotoxin accumulation, apoptosis, edema and intracellular calcium accumulation occur [11]. In other words, the event disrupts oxidative phosphorylation, thus reducing high-energy phosphorylated compounds-such as adenosine triphosphate (ATP) and phosphocreatine. As reduced ATP levels occur, ionic imbalances and neuronal depolarization further trigger an excessive release of excitatory neurotransmitters like glutamate. Due to such release, excitotoxicity and an uncontrolled influx of calcium occur, increasing the rate of injury (Fig. 2). The increased excitotoxicity results in prolonged glutamate receptor activation, which drives neuronal dysfunction and cell death [11]. Increase in glutamate levels further contributes to excessive calcium intake as high levels of glutamate in the synaptic gap bind to N-methyl-D-aspartate receptors (NMDAR) and their excessive excitation leads to further influx of calcium into cells, further causing cell death through mitochondrial dysfunction, cell membrane damage and DNA breakage [11]. Ultimately, neonatal hypoxic-ischemic injury causes a cascade of events in which increased calcium levels within the brain result in numerous events that exacerbate the severity of neurological injury (Fig. 2).

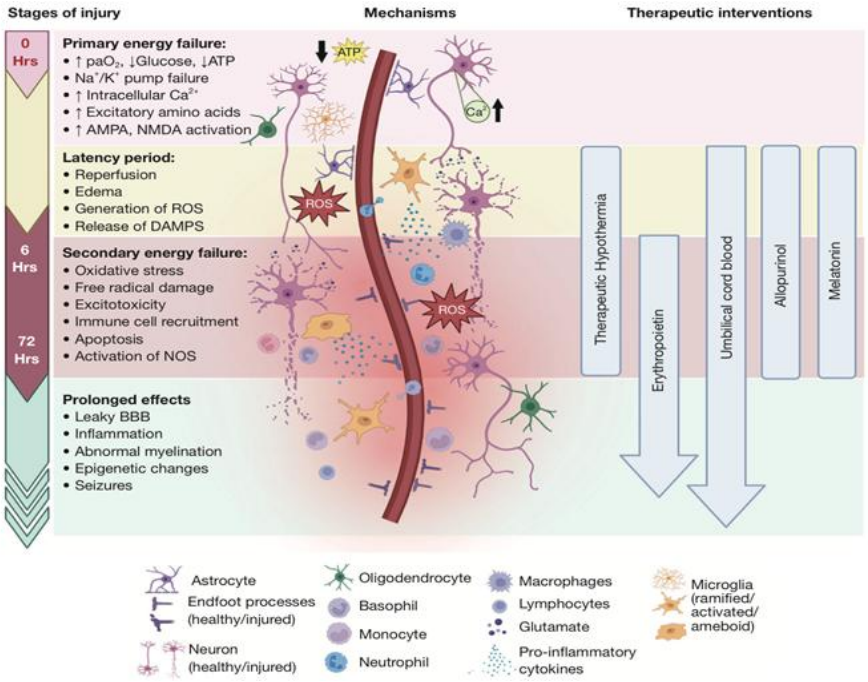

Figure 2: Visual illustration of the cascade of events as neurological injury progresses and corresponding treatment interventions. Abbreviations are as follows: Hypoxic-Ischemic Encephalopathy (HIE), Adenosine Triphosphate (ATP), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA), Reactive Oxygen Species (ROS), damage-associated molecular patterns (DAMPS), NO synthase (NOS), blood-brain barrier (BBB) [5].

Following its initial state of injury, a latent period of injury of around six hours occurs within 30 to 60 minutes after primary energy failure occurs. During this latent period, some recovery of oxidative metabolism, inflammation, blood flow and oxygen supply occurs [7,11]. This recovery period, however, does not stop apoptotic events leading to a secondary energy failure that can emerge within hours to days after initial injury (Fig. 2). This second energy failure continues to increase oxidative stress, excitotoxicity and inflammation of the brain. As injury progresses, an overproduction of free radicals, tissue necrosis and continued cell death occur, leading to the continued initiation of oxidative stress, which is detrimental to neonatal brains. Increased progression of injury initiates a low concentration of antioxidants and high oxygen consumption, in which iron is released. Iron release further initiates a cascade of events leading to the availability of reaction with peroxides, forming free radicals and damage to neuronal tissues [12]. Additionally, these events can be lethal to the survival and function of neurons, leading to a tertiary phase of injury lasting days to years [7]. The tertiary phase of injury is characterized by decreased neural cell plasticity and reduced numbers of neurons [7]. During this phase of injury, the brain continues to go through a detrimental process, including loss of tissue, scarring and loss of brain volume [2]. Yet, evidence of tissue repair has been shown to occur with stem cell treatment.

Clinically, these pathophysiological mechanisms manifest as various infantile abnormalities such as seizures, altered muscle tone, feeding difficulties, respiration difficulties and disturbances of consciousness [5]. Physical symptoms initiated by injury are distinguished as a reflection of injury staging. Typically, HIE-induced seizures are delayed amongst neonates, posing the need for early diagnosis of injury. Amongst those that survive injury, victims of injury tend to have hypotonia, impairment of the bodily organs like the heart, lungs and liver, leading to issues such as pulmonary hypertension, hyperammonemia and other abnormalities later in life [7]. In other words, the delayed progression of injury, such as seizures, can establish a therapeutic window in which treatment intervention as therapeutic hypothermia and emerging regenerative approaches like stem cell transplantation, can excel. A detailed understanding of the cascade injury stages is critical in developing novel treatments to promote repair, improve long-term survival and modulate inflammation.

Diagnostics and Conventional Treatment Approaches

Early diagnosis of HIE is critical for improving survival and minimizing long-term disabilities in neonates. HIE diagnosis typically is based on a combination of perinatal history, clinical examination and adjunctive neurophysiological and imaging

tools (Table 1). The bedside evaluation occurring at birth remains the first step in diagnosing HIE. Clinically, neonates are evaluated using Apgar scoring, assessing muscle tone, skin discoloration, heart rate, reflexes and respiratory effort at one and five minutes after birth. Although not specific for HIE, persistently lower scores of less than 5 at 10 minutes are associated with a higher risk of HIE injury [4, 7, 13-14]. Additionally, Apgar scores below 7/10 suggest perinatal asphyxia, with severe asphyxia diagnosed if the umbilical artery blood pH is lower than 7.0 [4] (see Table 1). To describe the severity of injury, staging systems such as Sarnat scoring and NICHD are often used as a standard practice to describe the severity of injury. Clinically, Sarnat staging scores injury based on multiple factors: muscle tone, reflexes, level of consciousness and seizure activity (see Table 2). These clinical signs, however, may be subtle or delayed and reliance on bedside assessment alone risks under- or overestimating the severity of injury. Overall, clinician evaluation of Apgar scores, cord pH or postnatal blood gas pH, infant's history [5] alongside Sarnat scoring assists in staging the severity of injury prior to treatment intervention.

Modality	Key findings	Clinical use	Limitations/controversies
Apgar Score	Low score (<5 at 5 min)	Early identification of asphyxia	Subjective scoring; poor specificity for HIE; influenced by prematurity/resuscitation
Cord Blood Gas Analysis	pH <7.0; base deficit >12 mmol/L	Confirms metabolic acidosis	Threshold debates: Some guidelines use pH <7.1 or lactate >10 mmol/L
Neuroimaging (MRI/aEEG)	Basal ganglia injury; suppressed aEEG background	Gold standard for injury extent; seizure detection	MRI accessibility limited in resource-poor settings; aEEG interpretation variability
Biomarkers (S100B, NSE)	Elevated serum/cerebrospinal fluid (CSF) levels	Early prognosis; correlates with neuronal injury	Timing-dependent (peak 24–72 h); lack of standardized cutoffs

Table 1: Diagnostic Tools for Hypoxic-Ischemic Encephalopathy. Table showing types of diagnostic tools, findings associated with HIE, its clinical use and the limitations of each diagnostic tool [3].

Stage	Clinical Features	Prognosis	Key Interventions
Mild (stage I)	• Hyperalertness • Normal/increased muscle tone • No seizures	Generally favorable; however, recent studies report subtle cognitive deficits in 10–15% of cases	• Supportive care • Continuous monitoring
Moderate (stage II)	• Lethargy • Hypotonia • Weak/absent reflexes (e.g., Moro reflex) • Seizures	High risk of neurodevelopmental delays (e.g., motor deficits, learning disabilities)	• Therapeutic hypothermia • Anticonvulsants
Severe (stage III)	• Coma • Flaccid tone • Absent reflexes • Refractory seizures	Mortality >50%; survivors often face severe impairments (e.g., cerebral palsy)	• Intensive neuroprotection • Multidisciplinary support

Table 2: Sarnat staging of Hypoxic-Ischemic Encephalopathy. Table depicting HIE injury stages alongside clinical features/symptoms, expected prognosis outcomes and disease progression and key therapeutic interventions [3].

To improve accuracy in diagnosis, neurophysiological monitoring has become central in evaluating HIE injuries. Before birth, cranial ultrasonography is a noninvasive tool used to detect the pre-birth onset of injury and identify abnormalities that may mimic those of HIE [15]. HIE-related abnormalities can also be identified through an ultrasound, but it will take up to 72 hours to see changes in white matter or gray nuclei within the brain. In addition to ultrasonography, systems like Electroencephalography (EEG) and amplitude-integrated EEG (aEEG) are often used to confirm a diagnosis of abnormal brain activity [13]. Both EEG and aEEG have been studied throughout the years to identify a proper protocol for the diagnosis of HIE. Studies have found that the most significant prognostic value of these monitoring systems is best around 48-72 hours after birth [13]. Abnormal EEG background patterns can further confirm a diagnosis of HIE through the discovery of brain lesions or seizure activity, making continuous monitoring a critical diagnostic tool (Table 1).

Diagnostic neuroimaging techniques, particularly Magnetic Resonance Imaging (MRI) with diffusion-weighted sequences, enable visualization of HIE injury patterns. MRI imaging, however, has many limitations worldwide, with significant limitations

in accessibility in low-resource settings and timing protocol of imaging for a proper estimation of injury (Table 1). Regardless of global differences, MRI imagery is the preferred imaging modality during the first week after birth in determining the extent of injury and predicting outcomes of infants with HIE symptoms [15]. MRI imaging findings, including watershed, basal ganglia injury and near-total injuries, can be identified and further help predict HIE events' severity and long-term prognosis (Fig. 3). Various MRI imaging techniques are also utilized to classify injuries, including conventional, diffusion weighted, susceptibility weighted and others. T1-weighted imaging allows for assessing myelination, ischemia depiction and subacute hemorrhaging in regions of the brainstem and basal ganglia, while T2-weighted imaging is useful in white matter and gray-white matter differentiation [15]. Abnormalities identified using diffusion weighted imaging peak at three to five days after injury insult and tend to normalize approximately 11-12 days for infants immediately treated and 6-8 days in non-treated infants, yet DWI images obtained within the first day after birth may lead to an underestimate of injury extent [15]. Studies, however, have found that MRIs performed at 5-7 days of life provide the highest accuracy in diagnosis. Access and delay in imaging remain challenging for many underdeveloped countries, as funding, protocols and other standard precautions may differ.

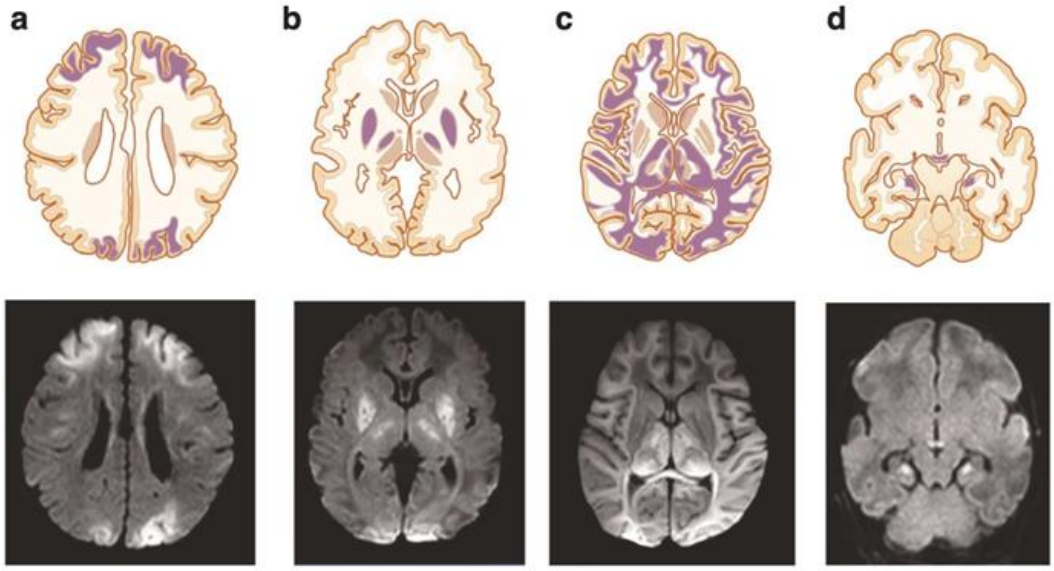

Figure 3: Magnetic Resonance Imaging (MRI) and axial MRI-diffusion weighted images of infants with hypoxic-ischemic encephalopathy taken during the first days of life, portraying common injury patterns. The images below, from left to right, portray a) bilateral watershed injury; b) basal ganglia/thalamic injury; c) near total injury; d) injury of mammillary bodies and hippocampi [13].

To further increase accuracy in diagnostics, biomarkers and proton magnetic resonance spectroscopy have been increasingly investigated to complement clinical and imaging findings of HIE. Magnetic Resonance Spectroscopy (MRS) measures chemical metabolites like choline, creatine, N-acetyl aspartate and lactate levels during injury. In a hypoxic-ischemic injury, N-acetyl aspartate levels decline and remain low, whereas lactate levels peak during the first days of acute brain injury and decline towards normal at the end of the first week of life [13].

Over the years, treatment options for hypoxic-ischemic encephalopathy have significantly increased. Conventional treatment options include erythropoietin, melatonin and single-target drugs. Although treatment options are available, the main course of treatment is Therapeutic Hypothermia (TH). TH is the first-line strategy for limiting the extent of brain damage resulting from ischemic-hypoxic injury induced by perinatal asphyxia. Hypothermic treatment includes head or whole-body cooling within the first six to 24 hours of birth. Studies have shown that rates of TH treatments have increased over the years. Specifically, roughly 1.7 per 1000 infants with HIE were treated with TH compared to 1.2 per 1000 infants in 2012 (Fig. 1) [8]. Further studies have shown that there is a 70% probability of death rate reduction in infants with HIE if hypothermia is utilized within the proper time frame of six to 24 hours post birth [4]. The hypothermia cooling process lasts for 72 hours, during which infants are cooled to 33-34 degrees Celsius with a re-warming period of 0.5 degrees Celsius per hour until core temperature is maintained at 36.5-36.77 degrees [9]. By using this treatment, a decrease in long-term neurological disabilities, cerebral metabolic energy demands and a reduction in inflammation, oxidative and excitotoxic injury and apoptotic cell death is achieved [9]. Despite this, however,

it has been concluded through numerous studies that more than 40-45% of infants with moderate to severe HIE develop adverse outcomes, including epilepsy and cerebral palsy, regardless of TH intervention [6,9]. Furthermore, therapeutic hypothermia has continued to be a poor treatment option for some infants with HIE injury, causing numerous complications for some. Complications such as bradycardia, hypotension, thrombocytopenia and pulmonary hypertension have occurred [6]. For instance, as seen in Fig. 4, one infant, despite treatment with TH, continued to have progression of injury with extensive diffusion restriction in the basal ganglia and thalamus present on a diffusion weighted MRI. Following redirection of care, researchers noted that the infant died. Assessments of HIE patients at 18-22 months old treated previously with TH have revealed that a reduction of adverse effects had occurred. Specifically, one study found that death or IQ scores less than 70 were observed in 47% of TH patients compared to the control groups 62%; death occurred in 28% of TH patients; severe disability or death occurred in 41% of patients compared to the controls 60% [6]. With its high success rate, TH holds a vast number of limitations regardless of its standard practice. Underdeveloped countries that utilize TH treatments have higher rates of poor outcomes, with nearly 40-50% of TH-treated infants either dying or facing neurological disabilities [6]. To improve TH outcomes, preclinical testing of drugs in rat models has been carried out as potential therapies, yet the effectiveness of these drugs has not been established. While outcomes for HIE remain poor in some areas, TH requires sophisticated equipment within an intensive care infrastructure, limiting its availability in many areas where the HIE burden is highest. Additionally, federal protocol differences amongst countries further limit its effectiveness overall.

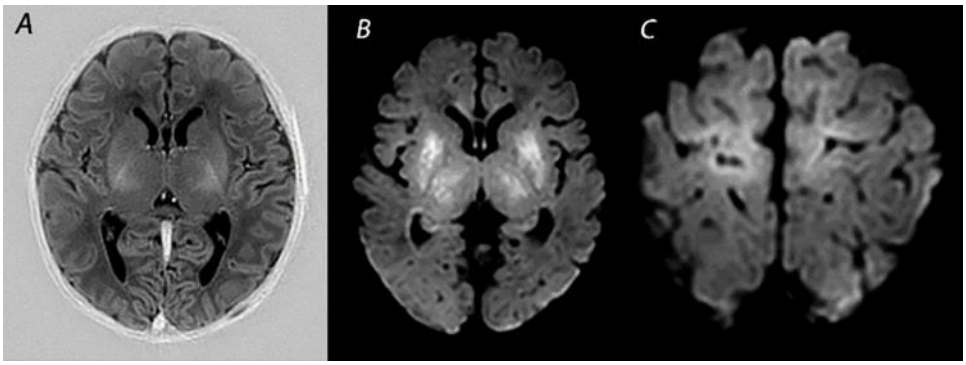

Figure 4: MRI performed on a 5-day-old infant with Apgar scores 0/2/5. Infant received therapeutic hypothermia for HIE injury. Upon MRI imaging, the signal intensity of the posterior limb of the internal capsule appeared normal on T1-weighted imaging (A). Diffusion-Weighted Imaging (DWI), however, indicates extensive diffusion restriction within the basal ganglia, thalami (B) and the perirolandic cortex (C). Despite redirection of care, the infant died [15].

In addition to therapeutic hypothermia, supportive pharmacological therapies are often used to address complications. Experimental adjunctive therapies, including erythropoietin, xenon gas, melatonin and others, have been investigated for potential neuroprotective effects, but none are currently a standard form of care/treatment. Nonetheless, the diagnostic evaluation of HIE relies on integrating clinical staging with adjunctive tools like MRI, EEG/aEEG, etc. Conventional management primarily depends on supportive care and therapeutic hypothermia, which has become the standard of care in high-resource settings. Significant challenges, however, continue to remain, such as the availability of diagnostic tools and the presence of partial neuroprotection in severe HIE patients treated with TH. These limitations in practice underscore the need for novel therapies like regenerative stem cell treatments, which not only limit injury but also promote regenerative repair of brain injury.

Stem Cell-Based Treatments for HIE

With the limitations in standard treatment, stem cell-based treatment innovations have recently emerged with promising effects in slowing injury progression and allowing for quick regeneration of tissues in neonatal hypoxic-ischemic encephalopathy injuries. Unlike conventional treatments that aim to limit further injury, stem cells have shown the potential to actively repair damaged tissue, restore neural networks, as well as modulate pathological cascades [11,16]. Widely available within the human body, stem cells can self-replicate and differentiate into various lineages, thus treating numerous diseases/conditions. These cells further secrete a broad spectrum of growth factors and cytokines that reduce apoptosis, inflammation and promote endogenous neural repair [11]. In treating HIE, stem cells can be directed towards neural progenitors, in which cells that did not survive the hypoxic-ischemic event can be regenerated [4]. Multiple stem cell types are under investigation, with the most common stem cell source being adult and fetal Mesenchymal Stem Cells (MSCs). MSCs have commonly been studied due to their strong possession

of immunomodulatory properties and growth factors that aid in the regeneration of tissues [17]. Specifically, MSCS can differentiate into a variety of mesenchymal tissues such as body, muscle, fat and cartilage cells. In treating HIE, various subtypes of MSCs have been sourced, including umbilical cord-derived stem cells, placental-derived, bone marrow-derived, amniotic fluid stem cells and Muse cells, each offering unique biological advantages towards HIE treatment. Human neural stem cells (hNSCs) have also been studied as a potential transplantation source of stem cells in treating HIE.

A key advantage of stem cell therapy is the alignment with the pathophysiology of HIE injury. As HIE injury evolves through primary, secondary and tertiary phases, there is a therapeutic window in the first 24 hours in which earlier intervention may alter injury trajectory [18]. Therapeutic hypothermia, however, has a limited window of six hours after birth in which the best results have been seen [2-3,9,19-21]. Stem cells can be delivered in various ways, including intravenously, intracranially or intranasally, each of which has been researched in neonates. These regenerative cells can then access the brain, reach the region of injury and provide support during the vulnerable phases of injury. Due to its regenerative properties, the rapid translation from preclinical models to pilot human studies is the start of new treatment options for neonates.

Clinical Trials and Advances

Preclinical Studies

The therapeutic potential of stem cells in neonatal hypoxic-ischemic encephalopathy has been established through preclinical studies. Preclinical studies have consistently demonstrated that stem cells aid in regenerating damaged tissues caused by HIE. Through rodent/animal models, research studies have portrayed the utter safety, initial dose models and therapeutic effects of stem cells. These studies have tested multiple types of stem cells, including varying tissue-derived mesenchymal stem cells and neural stem cells, alongside different routes of administration. Results in the studies have collectively identified reduced symptoms and improved functional recovery of neural tissues following transplantation. Importantly, these studies provide researchers with quantitative guidance on cell dose, route of administration and timing of treatment that can be used for subsequent human trials.

Initial studies using rodent models have demonstrated the safety and efficacy of stem cell transplantation following hypoxic-ischemic injury. For instance, a systematic review by Serrenho, et al., synthesized results from 58 preclinical studies focusing on stem cells isolated from umbilical cord blood, umbilical cord tissues, the placenta and bone marrow [16]. Among these, roughly 83 protocols were identified, with the Rice-Vannucci model of HIE injury induction used in 88% of studies. About 45% of experiments involved 7-day-old rodents and 19 studies used cells isolated from neonatal tissues, encompassing 25 transplantation protocols. Fig. 5 summarizes the distribution of neonatal tissue-derived stem cell types used, showing the number of transplantation protocols developed for each cell type. Regardless of protocol differences, ~80% of preclinical studies reported improvement of cognitive and/or sensorimotor function and a reduction in brain damage. Overall, Serrenho and his researchers concluded that stem cells consistently reduce apoptosis, preserve white matter integrity and improve long-term behavioral outcomes in preclinical models, though they emphasize the need to refine an optimal protocol that promotes the most success in HIE injury.

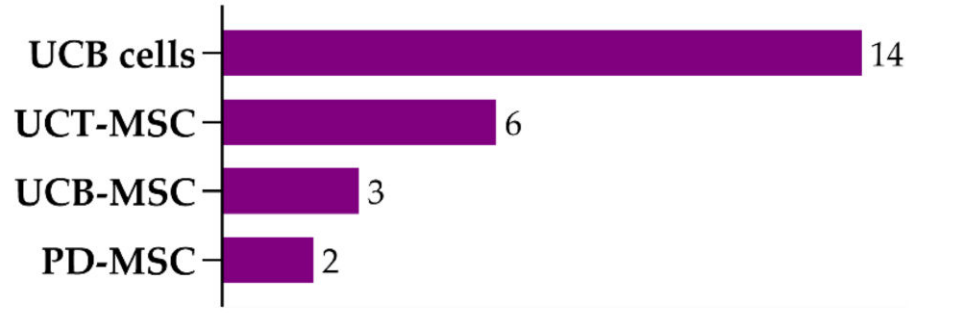

Figure 5: Distribution of the 25 preclinical transplantation protocols identified in the 19 studies using various neonatal tissue-derived stem cell types in 7-day-old rodent models of HIE. Bars represent the number of protocols identified for each cell type. Abbreviations represent Umbilical Cord Blood cells (UBC cells), Umbilical Cord Tissue Mesenchymal Stem Cells (UCT-MSC), Umbilical Cord Blood Mesenchymal Stem Cells (UCB-MSC) and Placenta-Derived Mesenchymal Stem cells (PD-MSC) [16].

Expanding on perinatal derived stem cells, amniotic fluid-derived MSCs offer both multipotency and secretion of trophic factors, offering unique advantages in neuroprotection of neonates. Multiple studies have researched human amniotic fluid MSCs through multiple delivery routes, with intranasal delivery allowing cells to migrate and control neurological inflammation, unlike intraperitoneal delivery. In his review, however, Abe and his researchers highlight that human Amniotic Fluid MSCs (hAFSCs) offer the most cost-effective source of stem cells as they can be easily obtained at birth or by amniocentesis at 15-17 weeks of gestation. In comparison, however, umbilical cord cells may provide some positive results, but as one study found, complications may arise due to inadequate cell sourcing amounts. Used in autologous treatments, hAFSCs can be sourced in greater amounts and offer immunomodulatory properties that can control neurological inflammation, which, if untreated, can exacerbate perinatal brain injury. In 2019, Abe found that intracranially administered hAFCs during the acute phase of HIE injury, finding that decreased apoptosis of neuronal cells, prevented demyelination and improved the size of the hippocampus in the ipsilateral hemisphere of the injured brain occurred following transplantation [22].

Stem cell delivery methods have also continued to be studied, with no optimal delivery method established. In fact, a 2022 preclinical study investigated the optimization of intranasal administration of human Neural Stem Cells (hNSCCs) amongst a sample of 24 neonatal rodents. Human cortical stem cells, obtained from 10-13-week gestation embryos, were cultured under hypoxic conditions (5% O_2, 8.5% CO_2, balanced with N_2 in a three-gas incubator) and labeled with CM-Dil for *in -ivo* cell tracking [23]. HIE injury was induced by cutting the left common carotid artery with an electrocoagulation knife with a one-hour recovery period and cerebral hypoxic-ischemia was induced in the 7-day-old rodents for 90 minutes. Following exposure, rodents were divided into two groups: the ordinary and the optimized transplanted group, each with 12 rats. CM-Dil-labeled hNSCs were intranasally administered at both one and three days post-HI exposure for all rodents. The ordinary transplanted groups received $1x10^6$ normoxic cells suspended in 12 μL of sterile PBS after pretreatment with isoflurane and 100 U hyaluronidase. For the optimized group, however, researchers administered either 8 μL of hyaluronidase or $5x10^5$ cells in 20 μL of sterile PBS, administered in alternating 5 μL portions per nostril via a polyethylene catheter inserted 5mm into the cavity. Following transplantation, researchers gently shook the rats and let them sleep in a supine position for 30 minutes. At 24 and 72 hours after cell administration, rats were euthanized by carbon dioxide inhalation and brain, nasal mucosa and trigeminal nerve tissues were analyzed for cell migration and localization under a fluorescence microscope. Results showed that hypoxic preconditioning of hNSC cells increased cell migratory capacity and CxCR4 expression-2.5 times higher in the pre-treated cells than in untreated cells, suggesting improved chemotactic responsiveness. Additionally, TUNEL analysis of cells concluded that the optimized transplanted group had a larger number of cells within the olfactory epithelium due to catheter use; ordinary transplanted groups with natural inhalation showed almost no hNSCs within the olfactory region at both 24 and 72 hours post administration. Under observation it was noted that stem cells could survive in the nasal cavity for 24 hours but no longer than 48 hours (Fig. 6). Overall, the optimized transplantation protocol improved the success rate of intranasal delivery of cells to ~41.6%, indicating that preconditioning and catheter-assisted administration improved the efficacy of intranasal neural stem cell delivery [23].

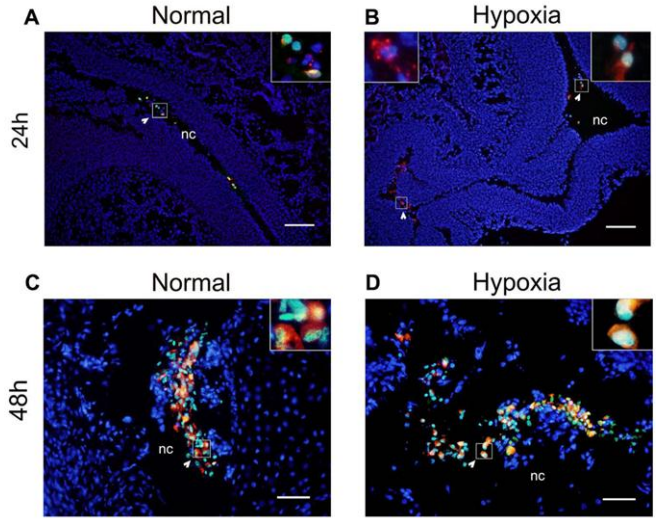

Figure 6: TUNEL analysis of cells after hNSC transplantation under either normxia (ordinary transplant group) or hypoxia (optimized transplant group). Representative pictures represent a merged image of TUNEL staining depict red (CM-Dil), green (TUNEL) and blue (DAPI) fluorescents. Arrows indicate a series of hNSCs presence. Images are as follows: A and B) 24 hours

after intranasal administration, some hNSC nuclei detected as green, indicating cells that underwent apoptosis (white arrow); C and D) 48 hours after transplantation, nearly all cells were positive for apoptosis, regardless of hypoxia (white arrow) [23].

Despite evidence that adult human stem cells can offer unique outcomes, perinatal-derived cells continue to show additional effective promise due to paracrine growth factors. In 2024, one study demonstrated that human placental chorionic plate-derived MSCs (hpcMSCs) improve sensorimotor recovery and promote neovascularization in neonatal HIE rats. With the placenta often discarded after delivery, researchers find that placental MSCs are a less invasive sourcing process that is more proliferation than bone marrow MSCS. Further, these cells hold low immunogenicity, low viral contamination and lack social and ethical controversy which holds great promise in clinical practice. Researchers were given 10 pregnant female Sprague-Dawley rats from the Department of Animal Zoology of Kunming Medical University. After the females gave birth, the 7-day old rat pups were randomly assigned into five groups for *in-vivo* experiments with hpcMSCs. With sample size of ~60, rats were sorted into the following five groups: Sham group (wild type rats subject to right carotid artery exposure, n=20), HI groups (wild type rats subjected to HI injury, n=20), normal saline group (wild type rats with HI injury subject to right ventricle injection of NS, n=5), stem cell group (wild type rats with HI injury subject to right ventricle injection of hpcMSCs, n=5) and IL-3 knockout group (IL-3 KO rats subject to HI injury, n=5). An acute cerebral infarction and neurological impairment model of hypoxic-ischemic injury was established by blocking the right common carotid artery with an electrocoagulate and after body temperature was stabilized at 37 degrees Celsius rats were placed in a hypoxic chamber for two hours with an airflow rate of 1 L/min. After HI models were established, Triphenyl Tetrazolium Chloride (TTC) staining was performed to verify cerebral damage at 24 hours after induction of injury in addition open field test, Morris water maze test, Y-maze test, rotarod test and neurological severity scores were performed assessing cognition, motor ability and anxiety-like behavior of rats at one and two months after HI. Cells provided by the Yunnan Shunxi Stem Cell and Regenerative Medicine Research Center were cultured and suspended within both a trypsin and an 80 μL high-glucose culture medium. Prior to injection, cells underwent immunofluorescence staining indicating that the cells expressed CD90+CD44+CD45-, which was consistent with MSC characteristics. A 5 μL dose of hpcMSCs ($2x10^5$/5 μL) was given to HI rats in three doses of hpcMSCs at one, three and 10 days after hypoxic-ischemic injury in the same lateral ventricle position (4 mm depth). Following injection, immunosuppressant cyclosporin A was intraperitoneally injected daily at 5 mg/kg. Specifically, five rats with hypoxic-ischemic injuries received these three doses of 2x105/5 μL human placental chorionic plate-derived MSCs (hpcMSCs) cells intravenously [24] with the same behavioral testing performed at one and two months after hpcMSCs transplantation (Fig. 7). Compared to the HI group, results of stem cell group identified improved cognitive and locomotion function and anxiety-like behaviors, as seen in Fig. 5.

Long term behavioral experiments indicated that HI rats exhibited significant cognitive, locomotor and anxiety-like behavioral impairments compared to the sham rats at one and two months after injury indicating that HI injury can lead to both acute and long-term neurological impairment (Fig. 7). Fig. 7 summarizes neurological evaluations of rats at two months post transplantation of cells comparing each of the five rat groups. In addition, researchers investigated the underlying mechanisms of hpcMSCs in HIE through protein chip analysis within the right cortex and hippocampus from both the sham and HI group. Protein chip analysis indicated relative expression of the IL-3 (a multi-colony stimulating factor that can improve brain injury) in the HI group compared to the sham group indicating that IL-3 plays a role in HI injury. IL-3 expression, however, was decreased after hpcMSC treatment and showed an exacerbation of injury with removed in the IL-3 knockout group. Researchers note that this inconsistency might be since IL-3 increased after injury and hpcMSCs treatment-maintained expression at normal levels, thus able to protect neurological function in HI rates, whereas HI rats with IL-3 knockout had low levels of IL-3 with severe neurobehavioral impairment [24]. Whilst this study indicates improved neurological function following stem cell transplantation, researchers state that future research into the long-term effects of transplantation is needed alongside further research into IL-3 expression and its relationship with HI injury.

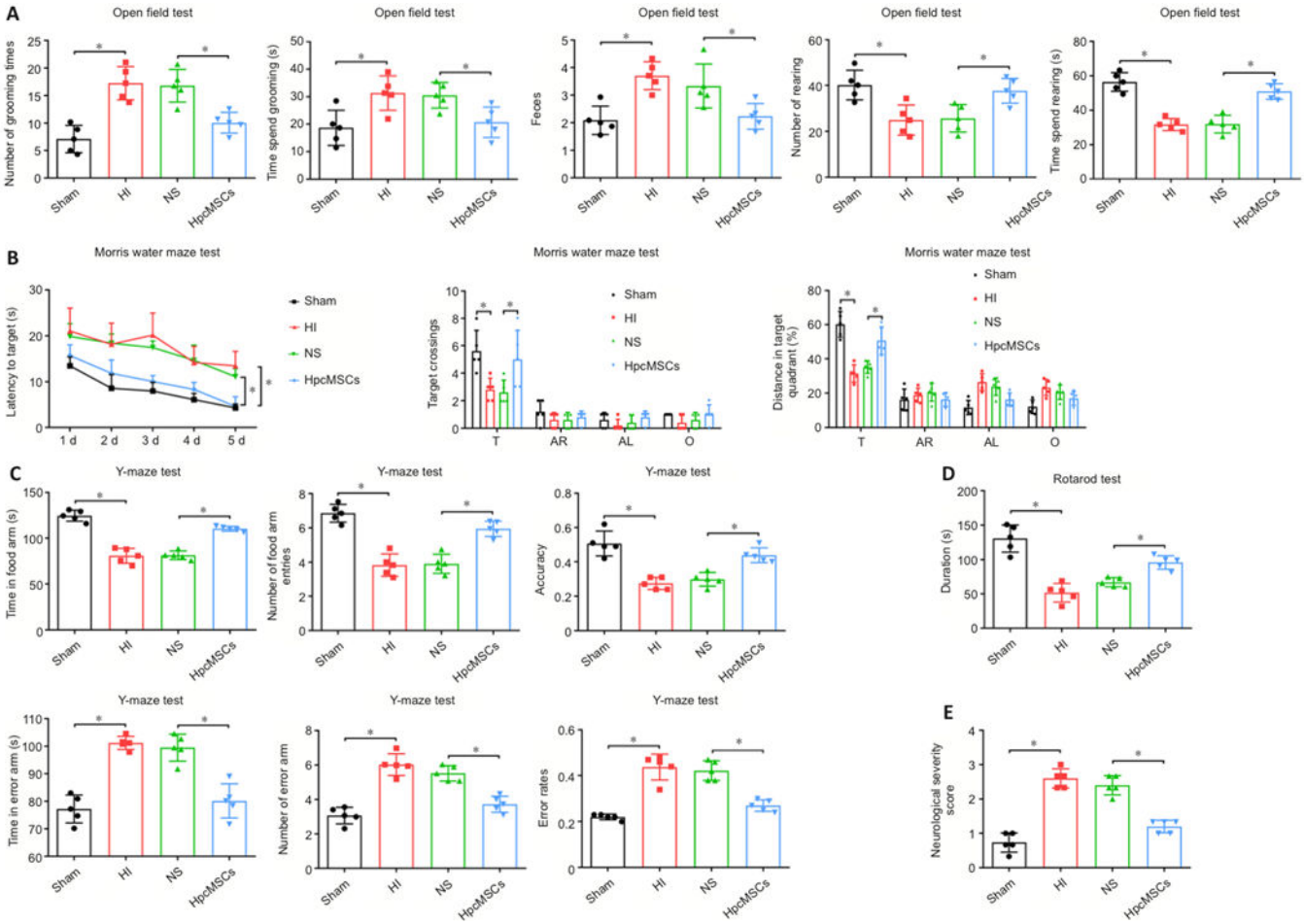

Figure 7: Neurological evaluations of rats at two months post injection. A) Open field test quantifications showing grooming frequency and duration, number of feces, rearing frequency and time spent rearing. B) Morris water maze test performance including latency to target, number of target crossings and distance traveled in target quadrant. C) Y-maze test results showing time and entries in the food and error arms, accuracy, an error rates. D) Graphical representation of the rotarod test duration. E) Graphical representation neurological severity scores. Abbreviations are as followed: adjacent left quadrant (AL), adjacent right quadrant (AR), hypoxia-ischemia (HI), human placental chorionic derived mesenchymal stem cells (hpcMSCs), normal saline (NS), opposite quadrant (O) and target quadrant (T) [24].

Finally, complementary work from Xue, et al., demonstrated, however, that bone marrow-derived mesenchymal stem cells (BMSCs), delivered intraperitoneally at $2x10^5$ cells BMSCs/kg per pup, attenuated neurological deficits by dampening autophagy-related neuronal injury. In this study, researchers tested the efficacy of bone marrow-derived MSCs alongside the effects of Synaptopodin-2 ($SYNPO_2$) in HIE injury repair. $SYNPO_2$ is a functional protein expressed in the heart, smooth and skeletal muscle and neurons that plays a vital role in regulating autophagy and neural morphology and function. In the study, researchers aimed to not only test the effects of BMSC transplantation in HIE models but also identify the role of $SYNPO_2$ in HIE-indued neuronal autophagy and neurological impairment. Sprague Dawley rats were obtained from the Experimental Animal Center of Zunyi Medical University and were used for a variety of reasons: 1-day-old rats were used for cortical primary neuron culture, 3-day-old rats for lentiviral injections, 7-day-old rats for HIE modeling and 30-day-old rats for BMSC extraction [25]. Following injury insult, brain tissues were collected at 24 hours post insult for immunostaining experiments and at 39 days after injury for Nissl and HE stains. Bone marrow mesenchymal stem cells were isolated from 30-day-old SD rats after cervical dislocation and disinfection with 75% ethanol, samples were taking from the femur and tibia and cultured at 37 degrees Celsius in 5% carbon dioxide with a medium change ~48 hours after initial plating with subsequent medium alterations every two days for optimal growth. Hypoxic-ischemic injury was initiated four days after lentivirus injection (~7 days postnatal) through right common carotid artery exposure and blood vessel ligation through electrocoagulation. Afterwards, rats were placed in an anoxic box for 1.5 hours at 8% O_2 + 92% N_2, at 36.6 degrees Celsius with a humidity rate of 60-70%. Within thirty minutes after HI modeling, rats were injected $2x10^5$ μL BMSCs in the lateral ventricle at 1 μL/min for a total of three minutes and were

intraperitoneally injected with cyclosporine A at 10 mg/kg daily until sampling was performed. Prior to injection, behavioral tests were conducted assessing early muscle function and motor including forelimb grip, righting, geotaxis and climbing tests alongside a rotarod, open field, Y-maze and Morris water maze tests for locomotive and cognitive assessment. Autophagy of BMSCs was also performed through immunofluorescence staining and TEM showing that autophagy and markers LAMP1, LC3B and SQSMT1 were increased in HIE rats alongside neuronal brain damage. For those transplanted with BMSCs, however, autophagy markers and neuronal repair decreased indicating that $SYNOPO_2$ depressed neuroprotective effects of BMSCs. Further, protective effects of BMSCS were researched: HIE rats showed increased tissue defects, disordered cell arrangement and widespread neuronal death while transplanted rats hosted increased numbers of normal neurons and Nissl bodies with improvements in right hemisphere defects. Results of behavioral tests indicated that HIE rats had significant impairments in grip strength, rotarod performance and delated recovery time in righting, geotaxis and climbing tests [25]. In comparison, researchers found that treated rats showed increased strength and coordination deficits with enhanced exploratory activity and depressed anxiety-like behavior. In terms of $SYNPO_2$'s role, researchers injected lentivirus (sh$SYNPO_2$ and shNC) into the lateral ventricle of 3-day-old rats finding that overexpression of $SYNPO_2$ depressed the neuroprotective effects of BMSCs and retainment of such when $SYNPO_2$-dPDZ deletion mutations were observed, demonstrating that SYNPO2 contributes to autophagic injury induced by HIE injury. When downregulation of $SYNPO_2$ expression was applied, however, BMSC transplantation reduced autophagy and improved neuronal autophagic injury and dysfunction. Overall, researchers concluded that BMSC treatment down-regulated $SYNPO_2$ expression, reduced neuron apoptosis, suppressed expression of autophagy markers and improved neurological dysfunction in HIE rats, yet interference of $SYNPO_2$ produced neuroprotective effects like BMSC treatment. This study highlights that BMSCs are not only effective in reducing HIE injury and promoting repair but can also modulate autophagy of stem cells through $SYNPO_2$ which could be a potential therapeutic target for stem cell-based treatments.

Collectively, initial preclinical studies have demonstrated that a wide range of stem cell types-including neural, bone marrow-derived and perinatal MSCs-can mitigate brain injury and improve functional outcomes in neonatal HIE animal models. These benefits are largely due to stem cells' paracrine signaling, modulation of inflammation and promotion of tissue repair rather than direct neuronal replacement. While variability in cell type, dosing, timing and delivery route presents challenges, these preclinical studies provide a strong quantitative framework for translation into early-phase human trials. The encouraging efficacy and safety in transplantation have directly informed clinical study design, allowing for the evaluation of such benefits within human neonates with HIE.

Pilot and Clinical Trials

Due to the strong findings identified in preclinical studies, several early-phase clinical trials have been performed evaluating the safety and efficacy of stem cells in human neonates with HIE. Although small in scale, these early-phase studies translate promising animal model findings into human settings, providing further insight into cell dosing, transplantation delivery and raising the question of the potential for combination treatments with the standard-of-care, like therapeutic hypothermia. In most clinical trials, transplantation has been performed via intravenous infusion, though alternative delivery routes have been noted. In these trials, stem cell doses range from $1\text{-}2x10^6$ cells/kg administered within the first 24-48 hours of injury, with higher doses appearing safe in preliminary assessments.

In one of the first dose-escalation studies, Cotton, et al., administered allogenic umbilical cord tissue-derived MSCs to infants undergoing hypothermia. In his study, Cotten and his colleagues performed a Phase 1 pilot trial in neonates treated with therapeutic hypothermia, using umbilical cord MSCs manufactured from placentas of male babies at Duke University Medical Center. Focusing on intravenous dosing and close monitoring for infusion reactions, immune complications and short-term adverse events, the study enrolled a total of six infants; four infants suffered moderate encephalopathy and two infants were classified as having severe encephalopathy. All infants in the study were intravenously administered $2x10^6$ hCT-MSCs/kg over a time frame of 30-60 minutes. Specifically, three infants were given a dose in the first 48 postnatal hours, while the other three received a second dose at two months of age in addition to the first dose during hypothermia and all cells were administered intravenously at a viability rate of >70% [26]. Following transplantation, infants underwent neurological testing at one year through the Bayley Scales of Infant and Toddler Development, 3rd edition, to assess cognitive, language and motor development. Panel reactive antibodies were also monitored at two months and one-year post-infusion, finding that the majority of infants developed anti-class 1 HLA antibodies. Researchers reported that MSC infusion in the acute neonatal period was well tolerated, with developmental functioning within the average to low range.

The 2024 SHIELD trial expanded this approach with a CL2020 muse cell-based product in nine infants. Muse cells, a pluripotent subpopulation of MSCs, were delivered intravenously using a 3+3 dose-escalation design. Cells in the study were obtained from the bone marrow of donors and underwent hypoxic conditions to enhance the population of stage-specific embryonic antigen-3 (SSEA-3)-positive cells [14]. Infants were split into two groups: low-dose and high-dose, based on the degree of injury. Low-dose infants received 1.5 mL of product ($1.5x10^6$ CL2020 cells dispersed in 1.5 mL of acetate Ringer's solution) for two minutes and high-dose infants received 15 mL of product ($1.5x10^7$ CL2020 cells dispersed in 15 mL of acetate Ringer's solution) for 20 minutes. Injection of cells was administered once between days 5 and 14 after birth. Evaluation of infants occurred up until 78 weeks post-administration, finding no serious adverse events. Brain MRIs at 10 days post-injection showed improved preservation of white matter integrity in treated infants and early neurological follow-up suggested reduced rates of severe disability.

A Phase I/II randomized controlled trial was performed in 2025, enrolling a total of 14 neonates. Infants were randomized to therapeutic hypothermia alone versus a combined treatment of TH and intravenous MSCs. Researchers in this study administered 2x106 of Temcell cells (a human MSC product derived from nucleated bone marrow fluid cells) per 1 kg intravenously within 12-36 hours after birth, following core body temperature stabilization at 33-34 degrees Celsius under hypothermia therapy [6]. Temcell was administered twice a week for a total of four weeks with an interval of three days in between each dosing. Following dosing, neurocognitive evaluation was performed, finding that 66.7% of infants in the Temcell combination and 57.1% in the hypothermia therapy-alone group were responsive to treatment. Brain MRI findings at 18 months of age were normal in four patients, grade 2A in 1 and missing in 1 infant of the Temcell combination group; in the hypothermia group, MRI findings were normal in six patients and grade 3 in one patient. Further, the combination group experienced significantly fewer electrographic seizures, faster EEG normalization and improved perfusion metrics on cerebral near-infrared spectroscopy, with no immunologic or systemic complications observed. This study supports the feasibility of integrating stem cell therapy into standard treatment protocols.

Meta-analytic evidence further supports the safety and early efficacy. Panda, et al., synthesized data from multiple pilots and early-phase trials, concluding that stem cells exhibit anti-inflammatory, neuroprotective and regenerative properties that could mitigate HIE [17]. Across all trials analyzed, treated infants demonstrated consistent improvement of neurological function without severe neurodevelopmental impairments at 12-18 months. The review, however, emphasizes that the heterogeneity of stem cell types and delivery methods poses a challenge for the identification of precise mechanisms that are responsible for the observed results, which indicates the need for further study.

Collectively, these clinical trials demonstrate that stem cell therapy for HIE is safe, biologically active and synergistic with therapeutic hypothermia. Although posing data towards the success of stem cell therapy intervention, these studies lack larger sample sizes in which treatment success rates can be easily correlated amongst populations of HIE neonates. While efficacy signals are encouraging, larger Phase III multicenter studies are required to validate neuroprotective effects, refine dosing and determine long-term outcomes.

Challenges and Future Direction

Despite encouraging preclinical and early clinical evidence, stem cell-based therapies for hypoxic-ischemic encephalopathy face several challenges that must be addressed prior to widespread clinical adoption. With the complexity of neonatal HIE brain injury, coupled with the heterogeneity of stem cell source, dosing and delivery routes, significant barriers to the standardization of treatment are established. For proper transition of treatment into clinical use, future research must focus on eradicating limitations whilst supporting the efficacy of stem cell treatment within human neonates.

One challenge present throughout studies is the optimal timing of intervention and optimized stem cell type. Preclinical data consistently demonstrates that early intervention within 24-48 hours of initial injury produces the most effective results, yet this is not easily translated clinically. In clinical settings timing of intervention may not be as precise due to logistical factors. These factors may be delayed diagnosis, parental consent and limited cell processing resources that may push beyond the therapeutic window. To overcome this, however, a clearly defined/optimized protocol during which stem cells can be administered is critically needed. Additionally, determining the ideal cell type and dose for the most effective result needs further study. Trials to date have utilized a range of stem cell products, with the most common being mesenchymal stem cells. As previously

mentioned, MSCs have commonly been studied due to their strong possession of immunomodulatory properties and growth factors that aid in the regeneration of tissues [17]. Although commonly used, however, researchers have studied a variety of mesenchymal stem cells, including bone marrow-derived (BMSCs), human placental chorionic derived (hpcMCS), umbilical cord Tissue-Derived Cells (hCT-MSCs) and Amniotic Fluid-Derived Stem Cells (hAFSCs). In comparison, other studies have utilized neural stem cells and MSC products like Muse and Temcells. Although researchers have no identified abnormalities from cell dosing, the dose-response relationship is poorly characterized, as it remains unclear whether higher doses host greater effects than lower doses. Current studies shows that researchers do not have a definitive dosage for transplantation. For instance, researchers have delivered a range between ~$1x10^6$ to $5x10^6$ cells/kg, indicating that no two studies seem alike. For adoption of stem-cell treatment, future studies that compare dosages and types of stem cell sources are urgently needed to establish which specific stem cell product offers the most consistent long-term benefits.

The route of administration also poses challenges as no set route of transplantation has been established. For instance, throughout various studies, routes of administration have been studied, including intravenous, intracranial, intraperitoneal and intraventricular routes. Intravenous infusion has become the most practical route in neonatal care due to its ability to bypass the need for solute absorption [22]. Researchers have stated that intraventricular routes show positive outcomes in animal studies but carry procedural risks in neonates. In addition, intranasal administration has been established as a promising approach that can bypass the blood-brain barrier, enabling delivery to the central nervous system and is characterized as a noninvasive and easily repeatable form of administration [20]. Overall, a development in the optimized delivery methods is a critical area of research that needs to be studied in the future.

Furthermore, there is growing interest in combination therapies, where stem cells are used alongside standard procedures like therapeutic hypothermia, pharmacologic neuroprotectants, etc. Given the multifactorial cascade of neonatal brain injury events-involving excitotoxicity, oxidative stress, inflammation and cell death-a multimodal approach is likely to provide the greatest benefit/outcomes to neonates, but further research is required.

Summary

Overall, neonatal Hypoxic-Ischemic Encephalopathy (HIE) is a detrimental brain injury resulting from oxygen and blood supply deprivation during late gestation or infant delivery. Although incidence rates differ in various regions, HIE has consistently influenced neonatal mortality and develop of long-term neurological disabilities despite treatment intervention. Increased rates of HIE amongst neonates has continuously been higher in areas with low and middle-resource settings, where limited access to items like advanced diagnostic tools and obstetric care occurs. These global differences in healthcare highlight the need for preventative and effective interventions that can be feasible in resource-limited settings.

HIE injury progression is marked by a cascade of events that overtime diminishes neurological tissues and cognitive function. Beginning with primary energy failure, the injury initiates oxidative metabolic failure, excitotoxin accumulation, apoptosis, edema and intracellular calcium accumulation [11]. Clinically, HIE presents as seizures, hypotonia, respiratory instability and multi-organ dysfunction that reflects both the severity of injury and guide intervention of treatment. Currently, a diagnosis of HIE integrates bedside neurological assessment like APGAR scoring with advanced imaging and electrophysiological techniques. Magnetic Resonance Imaging (MRI) remains the gold standard for evaluating injury patterns and predicting neurodevelopmental outcomes [15]. In addition, electro encephalopathy and emerging serum biomarkers are incorporated into diagnostic pathways to improve sensitivity and allow earlier intervention [13]. Staging of injury includes Sarnat scoring and National Institute of Child Health and Human Development (NICHD) system that assess infants on a variety of areas. These system study infants muscle tone, reflexes, level of consciousness and seizure activity to which allows researchers to establish a prognosis and identify treatment options.

Standard treatment remains as Therapeutic Hypothermia (TH), which cools infants core body temperature, whilst slowing injury progression. Throughout the years, TH has been shown to reduce mortality and disability yet establishes partial neuroprotection of brain tissue. Although a common practice, nearly 50% of infants treated with cooling experiencing adverse neurodevelopmental outcomes [2,9]. In low-resourced countries, it has been identified that roughly 50% of infants treated with TH ultimately face death despite its success in higher-resourced areas. Adjective neuroprotective agents and alternative therapies have been studied, but none have yet demonstrated the efficacy necessary to alter current clinical protocols and practices [19].

To establish a more successful intervention strategy, stem cell-based therapies have emerged as a promising adjunctive therapy to cooling. With their high regenerative properties and easily obtainable sourcing, stem cells offer the potential for both neuroprotection and regeneration of tissues following HIE injury, whilst also slowing the progression of injury if transplanted within 24-48 hours after initial injury. Preclinical studies have demonstrated that mesenchymal stem cells, neural progenitor cells and amniotic fluid stem cells can reduce apoptosis, modulate inflammation and promote synaptic repair in rodent models [16,22,24,25]. These studies, however, have not identified an optimal time frame, cell source and dosage, as well as delivery method providing the most success in treating neonates with HIE injury. Current studies have delivered roughly $1\text{-}5x10^{5\text{-}6}$ cells per animal administered either intranasally, intravenously or intracranially within the first 24-72 hours after injury, with no set protocol in place.

Translation from rodent models to human models have just recently been studied through both clinical and pilot studies. A Phase I trial of umbilical cord tissue-derived mesenchymal stromal cells in 2023 demonstrated safety and feasibility in neonates with HIE, with no serious adverse events reported [26]. Similarly, the SHIELD trial in 2024 investigated Muse cell therapy in combination with TH, confirming tolerability whilst providing preliminary evidence of improved outcomes in neonates [14]. In 2025, however, a randomized Phase 1/II study combining MSCs with TH reported encouraging neuroprotective trends, though larger multicenter trials are needed [6]. Additionally, systematic reviews and meta-analyses of other clinical trials have reinforced the translational potential of stem cell therapy but emphasize the need for standardized protocols regarding cell type, dosage and route of administration [4,17].

Despite technological advances, significant challenges remain that must be addressed for adoption of stem cell-based treatment in standard care. Barriers include variability in cell sourcing, manufacturing under proper conditions, long-term safety data and complexities of delivering therapy in the immediate neonatal period [18,20]. Future research must focus on optimizing sourcing, dosing and delivery methods, establishing long-term follow-up and conducting large-scale randomized trials to validate efficacy across populations [10]. Furthermore, recent studies on integrating stem cell therapy with multimodal approaches-including hypothermia- have started to be studied, which may provide the most effective strategy to mitigate injury and promote repair in HIE neonates [5,7].

Conclusion

In conclusion, neonatal HIE is the most common cause of infant mortality and neurological deficits worldwide. While injury develops a cascade of detrimental events, standard treatment options remain limited. Standard treatment of therapeutic hypothermia has improved outcomes in HIE, yet many infants continue to face neurological deficits making TH insufficient as a standalone treatment. Stem cell therapies represent a new frontier in advanced medicinal technology that has the potential to not only provide neuroprotection from HIE injury progression but also could restore damage to neural networks. Although current data indicates the safety and efficacy in stem cell treatment, further research critically needed to bridge the knowledge gap for adoption into clinical practice. By bridging this knowledge gap, the common goal of providing treatment that exemplifies neurodevelopmental recovery, allowing neonates affected by HIE to achieve their fullest potential, can be achieved.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding Statement

This research did not receive any specific grant from funding agencies in the public, commercial or non-profit sectors.

Acknowledgement

None.

Data Availability Statement

Not applicable.

Ethical Statement

The project did not meet the definition of human subject research under the purview of the IRB according to federal regulations and therefore was exempt.

Informed Consent Statement

Informed consent was taken for this study.

Authors' Contributions

All authors contributed equally to this paper.

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