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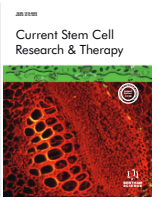
REVIEW ARTICLE

Cell-based Treatment of Cerebral Palsy: Still a Long Way Ahead



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Abstract: ***Background*:** Cerebral palsy (CP) is a permanent neurodevelopmental disorder with considerable global disability. Various rehabilitation strategies are currently available. However, none represents a convincing curative result. Cellular therapy recently holds much promise as an alternative strategy to repair neurologic defects.

***Method*:** In this narrative review, a comprehensive search of the MEDLINE and ClinicalTrials.gov was made, using the terms: "cell therapy" and "cerebral palsy", including published and registered clinical studies, respectively.

***Results*:** The early effects of these studies demonstrated that using cell therapy in CP patients is safe and improves the deficits for a variable duration. Despite such hopeful early bird results, the long-term outcomes are not conclusive.

***Conclusions*:** Due to the heterogeneous nature of CP, personal factors seem essential to consider. Cell dosage, routes of administration, and repeated dosing are pivotal to establish optimal personalized treatments. Future clinical trials should consider employing other cell types, specific cell modifications before administration, and cell-free platforms.

Keywords: Cerebral palsy, cell therapy, clinical trial, cell-based therapy, stem cells, regenerative medicine.

1. INTRODUCTION

Cerebral palsy (CP) is among the leading causes of disability in early childhood, and the overall prevalence of CP is approximately 2:1000 (2.5) live births [1]. CP is a group of constant disorders disturbing the healthy development of movements and posture, interfering with the child's normal activities [2]. The motor deficits in CP patients are usually accompanied by disturbances in normal cognition and behavior [2]. The development of CP has various risk factors. Among them, premature birth is one of the leading causes, and the other possible risk factors include congenital malformations, multiple gestations, perinatal stroke, and fetal growth restriction [1]. Recent findings have implicated that genetic factors, including copy number variants and genetic mutations, play a role development of CP. Although preventing the modifiable risk factors is the foremost approach in avoiding diseases, some risk factors of CP are unmodifiable, and planning for appropriate treatments is inevitable. As demonstrated in CP's definition, the neurologic deficits are permanent. Hence most available management strategies focus on rehabilitation and improving the quality of life. Numerous treatment strategies are available for CP patients, including physical therapies, functional exercises, medicinal therapies, and recently cell-based therapy [3]. Cellular therapy represents an emerging hope in managing neurologic disorders, including CP, and is still evolving rapidly. The present study reviewed the clinical trials evaluating the effectiveness and outcomes of cell therapies in CP patients.

2. CELL THERAPY IN THE MANAGEMENT OF CP

Cellular therapy represents a promising potential for the treatment of various human disorders [4-6]. The initial paradigm for considering stem cells as a potential therapeutic option was differentiation and self-renewal capacity [6]. More recent paradigms have attributed adult stem cells' therapeutic impact to their "stromalness", indicating that mesenchymal stem/stromal cells (MSCs) mainly exert their curative benefit by secreting paracrine factors [7]. MSCs secrete various components, known as the secretome, which promotes regenerative processes. These components include

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growth factors, cytokines, chemokines and, extracellular vesicles [8, 9].

MSCs can be isolated from various tissues throughout the human body. Bone Marrow Mesenchymal Stem/Stromal Cells (BM-MSCs), Adipose Tissue Mesenchymal Stem/Stromal Cells (AT-MSCs), and Umbilical Cord Mesenchymal Stem/Stromal Cells (UC-MSCs) are among familiar sources of stem cells used in clinical trials. Human bone marrow (BM) contains a heterogeneous population of cells, and both hematopoietic and non-hematopoietic stem cells are a small group of these cells [10]. Although the extraction of stem cells from BM requires an invasive procedure, it is still one of the main primary sources of stem cells with low immunogenicity in humans [10]. The therapeutic capacity of BM-MSCs has been reported in various neurologic diseases, including amyotrophic lateral sclerosis, spinal cord injury, and CP [11]. Bansal *et al*. and Purandare *et al*.'s studies are the two clinical trials with long-term follow-up results indicating the beneficial effect of BM-MSCs in CP patients [12, 13]. In the same vein, Koh *et al*. showed that mobilized peripheral blood mononuclear cells (mPBMCs) have the potential as a source of MSCs for the treatment of neurological disorders [14]. These cells can produce neurotrophic factors, including vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and erythropoietin [15]. Rah *et al.* demonstrated that using mPBMCs for CP patients could be beneficial. However, this beneficial effect has a similar impact when considering granulocyte-colony stimulating factor (G-CSF) administration alone. G-CSF can induce stem cell mobilization in cell transplantation. G-CSF has been successfully used in CP patients to mobilize bone marrow stem cells to circulation [16]. Administration of G-CSF and stem cell factors has advantageous effects in reducing infarct brain tissue and angiogenesis [17]. Using G-CSF alongside stem cells can increase the therapeutic effect of cell therapy in CP patients [18]. Similar to G-CSF, which can be utilized as an adjuvant for cellular therapy, enhancement of UCB treatment with erythropoietin has been addressed in the literature [19]. UCMSCs are another source of stem cells that are used for treating CP patients. Cord blood (CB) contains many cells, including mononuclear and stem cells [20]. Hematopoietic and multipotent stem cells in CB made it a potent candidate for cell therapies and regenerative medicine. These cells can differentiate into other cell types and produce various neurotrophic factors [20]. Even the mononuclear cells from CB is thought to produce more neurotrophic factors including BDNF in contrast to peripheral blood mononuclear cells [21]. Regardless of UCMSCs, neural stem cell-like (NSC-like) cells are other stem cells types that is used in treatment of CP patients. Chen *et al*. reported that autologous NSC-like cells derived from MSCs could differentiate into glial and neuronal cells [22]. The superiority of any specific method of cell therapy for CP is not significantly addressed in the literature. Liu *et al*. studied the difference between using BM-MSCs and BM-MNCs in treating CP patients and demonstrated that both of these cells provide significant improvement [23]. In long-term follow-ups, the efficacy of BM-MNCs were similar to rehabilitation group while the BM-MSCs group continued showing significant improvement. This study clearly demonstrated that using BM-MSCs are superior to BM-MNCs in terms of providing long-lasting effects [24].

3. PHYSIOLOGIC EFFECTS OF STEM CELLS IN CP

The main actions of different types of stem cells can be summarized in three main categories, namely cell differentiation, paracrine effect, and regulation of the immune response [24]. Cerebral white matter injury is a common central nervous system pathology in CP patients, which indicates the loss of oligodendrocytes, leading to disruption of nerve conduction [25]. The administration of BM-MNCs can perform repair and remyelination processes in CP patients as these cells can differentiate into oligodendrocytes and astroglial cells [26]. The main challenging question in evaluating stem cells' effect on neurologic diseases, especially those involving the central nervous system, is whether stem cells pass through the blood-brain barrier or perform their activities outside the central nervous system. The blood--brain barrier (BBB) is a disputable issue when considering the intravenous route for cell delivery, targeting the central nervous system. The permeability and integrity of BBB can be affected in different ways, including the use of chemo-radiotherapy or some drugs [20]. It is now demonstrated that stem cells can overcome this issue in their way. Inflammation-induced damage to the blood-brain barrier, which is present in CP patients, increases the permeability of BBB, enabling the transmission of some cells through the barrier [15].

Moreover, pro-inflammatory cytokines released by stem cells may provide a similar inflammation-induced way for easing access through the BBB. IL-8, IL-1α, and monocyte chemotactic protein expressed by CB mononuclear cells are pro-inflammatory and can affect the BBB as well as the migration of leukocytes [27]. Migrated cells entering the brain from the disrupted BBB can regenerate brain tissue after differentiation to microglia cells [14]. Regardless of the localization of stem cells in the human body, it has been demonstrated that mesenchymal stem cells (MSCs) express different factors with specific paracrine effects (Fig. **1**) [28]. BM-MSCs produce some angiogenic and anti-apoptotic factors [28]. These cells secrete VEGF, monokine induced by IFN-γ (MIG), and Monocyte Chemoattractant Protein-1 (MCP-1) to their surrounding environment [28]. MSCs can generally produce various components enhancing angiogenesis, neuroprotection, remyelination, and neuroprotection [29]. Moreover, stem cells produce specific exosomes carrying various molecules, including DNA, RNA, lipids, or proteins. Stem cell-derived exosomes have been shown to significantly improve the heart, kidney, and brain [30-32]. It has been demonstrated that secreted exosomes from MSCs may rescue cognitive function after brain injury [33]. Animal studies revealed that MSCs derived exosomes can improve motor function and cognition following postnatal inflammation injury [34]. Therefore, some studies suggest administering exosomes and other extracellular vesicles instead of MSCs [30].

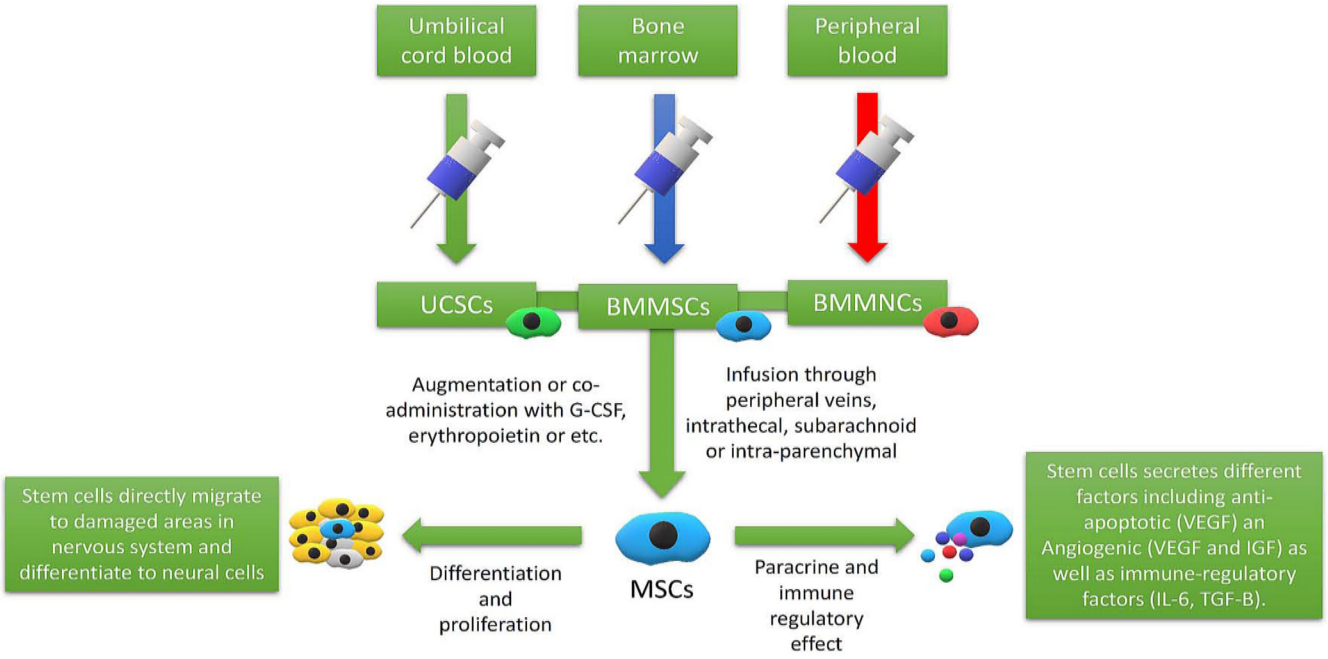

Fig. (1). Demonstractes the possible effects of stem cell therapy in cerebral palsy (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Additionally, stem cells secrete immunomodulatory factors; hence, they may benefit CP patients [35]. It has been shown that immune regulation in CP patients provides a therapeutic outcome. Chernykh *et al*. used cell-based therapy using M2 macrophage in severe CP patients and reported a 5-year lasting improvement in cognition and motor function [36]. M2 macrophage has anti-inflammatory effects and induces active tissue repair [36]. These cells are also able to induce Th2 response, which is helpful in CNS repair and produces insulin growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) [36]. The role of immune system modulation by stem cells in CP patients is briefly evaluated in Kang *et al*. study [19]. They found that an increase in IL-8 is correlated with improved motor function. IL-8 is an angiogenic cytokine that promotes angiogenesis in the nervous system [19]. According to their positron emission tomography (PET) scan, amelioration of brain inflammation was evident, especially in the periventricular area after two weeks of allogeneic UBC therapy [19]. They also demonstrated that frontal motor cortices show increased glucose metabolism, absent in their control group [19].

Furthermore, long-term functional outcomes in CP patients receiving allogeneic UCB correlate with toll-like receptor-4 (TLR-4) and PTX levels. Early elevation of TLR-4 and PTX in the first 12 days is associated with long-term functional outcomes in the first six months. Both TLR-4 and PTX3 are neuroprotective and anti-inflammatory mediators [19].

4. CELLULAR THERAPY OF CP IN CLINICAL TRIALS

Table **1** demonstrates the published clinical studies on cell therapy of CP patients, and Table **2** shows a summary of clinical trials submitted to "ClinicalTrials.gov" until July 2020. Table **1** summarizes the results retrieved from clinical trial studies in MEDLINE searched using the terms "cell therapy" and "cerebral palsy," which were published until July 2020 in English language.

Although overall results provided in this table are favorable regarding cell therapy in CP, some issues still need to be explored. Among the 20 studies summarized in Table **1**, only seven used control groups [19, 22, 24, 37-40]. Moreover, the most extended follow-up period was 24 months reported by three studies [12, 37, 38] and only one study followed a patient for 28 months [18]. Eight studies used UCB as the source of cell therapy [18-20, 37, 38, 40-42], while the rest of the studies used BM-derived stem cells, and only one study used neural stem cell-like cells [22]. Most of the studies evaluated motor improvement as their primary goal of cell therapy in CP patients and demonstrated that administration of different kinds of cells regardless of their administration route or dosage improve motor function [12, 13, 19, 22-24, 37, 38, 40-45].

Moreover, improvement in other domains, including behavioral and sensory functions, has been reported in two studies and reported as the primary goals [13, 40]. Studies

Table 1. Summary of studies using stem cells as a treatment for CP.

Author, year	No. of patients	Population main characteristics	Stem cell type	Cell dosage	Control group	Maximum follow-up	Outcome
Thanh *et al*. [45]	25	2-15 years old GMFCS: 2-5 Previous history of icterus in the neonatal period	BM-MNCs	1st session: 17.4 ± 11.9 × 10^6 MN cells and 1.5 ± 1.4 × 10^6 CD34+ - 2nd session (6 months later): 15.0 ± 12.8 × 10^6 MN cells and 1.1 ± 1.1 × 10^6 CD34+	-	12 months	Improve muscle tone and gross motor function occurred.
Nguyen *et al*. [44]	30	2-15 years old GMFCS level 2-5	BM-MNC (Autologous)	8 mL/kg or (80 mL+ (body weight in kg-10) × 7 mL). Maximum dose: 200 mL	-	6 months	Quality of life, as well as gross motor movement and muscle tone, improved after treatment.
Huang *et al*. [37]	54	3-12 years old CP, according to "The definition, diagnosis criteria, and typing of children cerebral palsy."	hUCB-MSC	4 infusions of hUCB-MSCs (infusions at a fixed dose of 5×10^7)	0.9% normal saline	24 months	Gross motor function measurement and comprehensive functional assessment improved in contrast to the control group
Dong *et al*. [41]	1	4-year-old boy White matter degeneration and softening on MRI, abnormal EEG, convulsion, somnolence, fatigue.	hUCB-MSC	1st session: 7.0×10^6/IT and 5.6×10^6/IV - 2nd session: 1.625×10^7/IT and 3.6×10^6/IV - 3rd session: 2.05×10^7/IT	-	-	Improvement in motor function, language and EMG findings
Sun *et al*. [38]	63	1-6 years old CP with GMFCS level 2-4 or GMFCS level 1 with hemiplegia if they used affected hand as an assist only	hUCB (autologous)	A single dose of $1\text{-}5\times10^7$ nucleated cells/kg autologous cord blood repeating one year later	placebo (TC-99 + 1% dimethyl sulfoxide)	24 months	Stem cell infusion improved brain connectivity and motor function.
Nguyen *et al*. [23]	40	2-15 years old CP of any type related to oxygen deprivation GMFCS level 1-2 and those who had epilepsy were excluded	BM-MNC and BM-MSC (autologous)	1st session: 7.2 × 10^6 MNC/kg and 2.6 × 10^6 CD34+/kg - 2nd session: 17.1 × 10^6 MNC/kg and 1.7 × 10^6 CD34+/kg	-	6 months	Improvement in motor function and muscle rigidity decreased.
Rah *et al*. [39]	57	2-10 years old All types of CP according to their clinical presentation	mPBMCs	mPBMCs TNC 5.97 ± 1.99 × 108/kg, and TNC CD34+ of 3.07 ± 2.1 × 106/kg	Placebo	12 months	Neurodevelopmental improvement was seen either only with GCSF infusion alone.
Liu *et al*. [24]	105	6 to 150 months old diagnosed with Spastic CP GMFCS level 2-5	BM-MNC or BM-MSC	Four transplantations of 1 × 10^6 cells /kg	neurodevelopmental treatment	12 months	Both BM-MNC and BM-MSC groups showed gross and fine motor improvement after 3 months.
Bansal *et al*. [12]	10	moderate-to-severe cerebral palsy	BM-MNC (Autologous)	4.5 × 10^8 mononuclear cells	-	24 months	Motor deficit and communication improved from 6 months to the end of the study.
Wang *et al*. [42]	16	Identical twins aging between 3-12 years Spastic CP	UCB-MSC (Allogeneic)	1.0-1.5 × 10^7 cells repeated 4 times with 5-7 days interval	-	6 months	Motor function significantly improved.
Sharma *et al*. [52]	1	12 years old GMFCS level 3	BM-MNCs (Autologous)	33×10^6 MNCs, diluted in cerebrospinal fluid	-	12 months	Improvement in daily activity and quality of life

(Table 1) Contd….

Author, year	No. of patients	Population main characteristics	Stem cell type	Cell dosage	Control group	Maximum follow-up	Outcome
Kang *et al*. [19]	36	6 months - 20 years old CP patients without genetic syndromes.	UCB (Allogeneic)	>2×10^7 total nucleated cells/kg and < 6×10^7 cell/kg for those older than 4	Placebo material	6 months	Motor function and systemic immune function improved.
Zali *et al*. [43]	12	4-12 years old	BM-derived CD133+ (Autologous)	CD133+ varied from 45 x 10^5 to 176 x 10^5	-	6 months	Stem cell therapy was safe and showed improved motor improvement in some patients.
Mancias-Guerra *et al*. [53]	18	1 month to 8 years old	BM-derived total nucleated cell (Autologous)	13.12 × 10^8 TNCs including 10.02 × 10^6 CD34+ cells (IT) and 6.01 × 10^8 TNCs, with 3.39 × 10^6 cells being CD34+ (IV)	-	6 months	Developmental age increased without severe side effects.
Wang *et al*. [54]	46	6-15 years old CP with abnormal birth history and MRI and mental or motor development retardation GMFCS: 1-5	BM-MSCs	2 x 10^7 cells and repeat in five days interval	-	18 months	The development of patients significantly improved.
Min *et al*. [40]	105	10 months - 10 years old CP according to clinical history and physical exam	UCB (allogeneic)	3 × 10^7 /kg total nucleated cells	rehabilitation	6 months	Cognitive and motor improvement were observed.
Purandare *et al*. [13]	1	6 years old	BM-MNCs (Autologous)	5 infusions with different cell counts	-	24 months	Motor, sensory, and cognitive improvement
Chen *et al*. [22]	30	1-32 years of age Non-progressive neurological disease from infancy or early childhood and developmental retardation	NSC-like	1-2 × 10^7 repeated in 3 weeks	rehabilitation treatment	6 months	Motor function improved after 3 months, but the language quotient did not.
Lee *et al*. [20]	20	2-10 years of age CP caused by hypoxic-ischemic encephalopathy, meningitis, cerebral artery infarction, polymicrogyria, and 9 with unknown causes	UCB-MNC (Autologous)	5.5 ± 3.8 (0.6~15.65) × 10^7/kg TNC	-	6 months	A quarter of patients showed partial neurodevelopmental improvement.
Papadopoulos *et al*. [18]	2	GMFCS level 3	UCB (Autologous)	TNC of 662.4 x 10^6 and 508 × 10^6 in 1st and 2nd patients	-	28 months	Gross Motor Function improvement without side effects

Intravenous (IV); Intra-thecal (IT); Electromyography (EMG); Electroencephalography (EEG); Magnetic resonance imaging (MRI); Human umbilical cord blood mesenchymal stem cell (hUCB-MSC); Gross Motor Classification System (GMFCS); Bone Marrow Mononuclear Cells (BM-MNCs); Bone Marrow Mononuclear stem Cells (BM-MNC); Subarachnoid cavity (SAC); neural stem cell-like (NSC-like); Umbilical cord blood (UCB); Trans femoral cerebral angiography (TCA).

discussed in Tables **1** and **2** used different cell counts, variable doses, and the number of cells administration attempts. Kang *et al*. reported that dosing is a critical issue considering cell therapy in CP patients. They showed that higher doses of allogeneic UCB result in better motor outcomes [19]. Similarly, Sun *et al*. demonstrated that adequate dosing is necessary to achieve encouraging results when considering umbilical cord blood as a treatment opportunity for young children [38]. They reported that the administration of more than 2x10^7 cell/kg from umbilical cord blood improves whole-brain connectivity, as well as motor function, in a one-year follow-up [38]. This study revealed the importance of other developmental therapies on improving patients' symptoms as they showed that even patients in the control group had improved motor function [38]. Overall, as demonstrated in Table **1**, the various concentration of different cells have been used in clinical trials. Determining a standard gold dosage for cell therapy in CP patients is impossible based on the available results. The administration route, which was different in the studied trials, can directly affect the effective dosage. The superiority of separate routes is evaluated in some studies. For example, it was shown that

Table 2. Summary of the clinical trials on cerebral palsy patients (completed or recruiting status).

Trial Identifier	Year of submission / last update	Status	No. of patients	Age of patients (years)	Intervention	Primary outcome
NCT03005249	2016/2018	Recruiting	20	1-12	Neural stem cells therapy	Changes in motor performance
NCT01072370	2010/2018	Recruiting	40	1-12	2 arms: 1st, red-cell depleted, mononuclear cell-enriched cord blood; 2nd, placebo	Safety and adverse events
NCT04098029	2019/2019	Recruiting	90	1-12	3 arms: 1st, High HLA group Cord Blood Mononuclear Cells; 2nd, Low HLA matched Cord Blood Mononuclear Cells; 3rd, drugs, special psychology training, *etc*.	Adverse events, Gross Motor Function, cognitive outcomes and quality of life
NCT01929434	2013/2017	Completed	300	1-14	3 arms: 1st, Rehabilitation; 2nd, no intervention; 3rd, umbilical cord-derived stem cells	Gross motor function measurement
NCT03979898	2019/2019	Completed	1	-	Autologous Adipose Tissue-Derived Mesenchymal Stem Cell	Cognitive improvement
NCT01763255	2013/2014	Completed	8	4-12	2 arms: 1st, Bone marrow-derived CD133 cells; 2nd, no intervention	Motor and sensory function as well as negative events

Human leukocyte antigen (HLA); Granulocyte-colony stimulating factor (G-CSF).

intra-atrial transplantation might not be superior to the intravenous route [19]. It seems that choosing the best route affects the success rate of cell therapy and post-procedure complications. Severe adverse effects, including pneumonia and influenza following allogeneic use of stem cells derived from umbilical cord blood, are also reported in the literature, and still, allogeneic cell therapy should not be considered a completely safe procedure [40].

While most of the studies demonstrated that cell therapy from any route might not have severe complications and has beneficial effects, choosing the best route may depend on the expertness of the cell therapy team and their previous experience with CP patients. The last issue that is addressed in clinical trials is using autologous or allogeneic cells. In some patients, the administration of autologous cells may not be possible. While allogeneic cell transplantation is considered an option, choosing the best-matched HLA subjects will increase success. Fully matched or 1-HLA mismatched patients demonstrated more promising outcomes than those with 2-mismatched HLAs [19].

CONCLUSION AND FUTURE DIRECTIONS

The present study summarized the available clinical trials addressing the outcomes of cell therapy in CP patients. According to the completed trials summarized in Table **1** and the primary outcomes of the ongoing trials, different types of cell therapy in CP patients is safe in the short-term follow-up, and most of the patients experience improvement in motor symptoms and development. However, it is unclear how long the therapy effect would last, and the need for continuous treatment is still unclear. The success rate of stem cell therapy depends on many factors related to the individual's body or transplantation conditions. Wang *et al*. demonstrated that response to cell therapy is related to the genetic background of each patient, and twin patients are most likely to have a similar reaction to the same treatment [42]. CP patients with higher brain structural connectedness and white matter health are more likely to benefit from cell therapy [46]. However, the enrolment criteria of most of the clinical trials mentioned in the present study only rely on the clinical definition of CP in their inclusion criteria rather than considering brain functional imaging. It seems that categorizing the CP patients into different groups according to their brain findings according to any available imaging or functional techniques may provide more transparent results. This issue should also be addressed when performing follow-up studies on patients. As demonstrated in some clinical trials, improvement in brain function can be adequately evaluated by PET scan. The administration of stem cells promoted the frontal lobe's function, resulting in cognitive and fine motor improvement [13]. Although administering various types of stem cells is safe in most patients, the time and cost can be saved by performing such categorizations. When performing such techniques are not available, classification according to other clinical findings also seems helpful. According to the present review, most of the studies employed the Gross Motor Function Classification System (GMFCS) to evaluate their intervention efficacy in CP patients. This approach provided uniformity across most studies; however, GMFCS is not likely to have enough specificity.

Some adverse outcomes could not be considered a complete failure for cell therapy, as neurological deficits may become harder to improve as the patient ages. The patient's age could directly correlate with the improvement of CP symptoms. One possible example has been discussed by Chen *et al*. study as they demonstrated that language improvement might not be achieved in all of their population. While most language development occurs in the first year of life, all patients missed this golden time [22]. Despite the beneficial effects of cellular therapies in the mentioned investigations, the overall efficacy of cell-based therapy in CP patients is not conclusive. As cell therapy's safety and beneficial effect in CP patients is becoming more evident, focusing on those who did not respond well should be outlined in further studies. Performing this approach could narrow clinical indications for using particular cells in specific CP pa-

tients or, more importantly, drawing possible contraindications.

Extracellular vesicles secreted by MSCs (MSCs-EVs) have shown neuroprotective effects in some neurological disorders, including Alzheimer's disease [47, 48], cerebral ischemia [49], and spinal cord injury [50] at the preclinical setting. Due to EVs' circulatory stability and the proven paracrine impact of MSCs, it could be suggested that MSCs-EVs should be further investigated as a cell-free therapeutic approach for treating CP. As stated before, stem cells exert their beneficial impact, at least in part, through releasing neurotrophic factors, *e.g*., BDNF [15]. Genetically modified MSCs, releasing neurotrophic factors, are an encouraging platform for neurotrophic factor delivery. However, there are numerous challenges in utilizing genetically manipulated MSCs [51]. It has commonly been assumed that cell-free platforms for gene manipulation could have fewer safety concerns. Thus, the need for further investigations regarding EVs secreted from genetically modified MSCs is emphasized.

In conclusion, despite preclinical and clinical progress, stem cell therapy for CP remains incomplete and needs optimization. Appropriate cell type, source, derivation methods, correct timing, delivery route, patient demographic, and safety profile must be optimized before clinical trials. Moreover, the present study focused on clinical trials using stem cells. Future studies may focus on studying other cells, including the progenitor cells or even the stem cell-derived extracellular vesicles, as a novel therapeutic approach for treating CP patients.

AUTHORS' CONTRIBUTIONS

Conception and design of the study: RJE, and HRB. Acquisition of data: MS, SM, and ES. Analysis and interpretation of data: MS, ASN, and SM. Drafting or revising the manuscript: ASN, RJS, ES, and HRB. All authors have approved the final article.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

REFERENCES

[1] Stavsky M, Mor O, Mastrolia SA, Greenbaum S, Than NG, Erez O. Cerebral palsy-trends in epidemiology and recent development in prenatal mechanisms of disease, treatment, and prevention. Front Pediatr 2017; 5: 21. http://dx.doi.org/10.3389/fped.2017.00021 PMID: 28243583

[2] Rosenbaum P, Paneth N, Leviton A, *et al.* A report: The definition and classification of cerebral palsy April 2006. Dev Med Child Neurol Suppl 2007; 109: 8-14. PMID: 17370477

[3] Hai-Jing W, Qian Y. A review of the treatment of cerebral palsy in children. 2018; 1(4): 151-8.

[4] Parizadeh S M, Jafarzadeh-Esfehani R, Ghandehari M, *et al.* Stem cell therapy: A novel approach for myocardial infarction. J Cell Physiol 2019; 234(10): 16904-12. http://dx.doi.org/10.1002/jcp.28381 PMID: 30811054

[5] Parizadeh SM, Jafarzadeh-Esfehani R, Hassanian SM, *et al.* Targeting cancer stem cells as therapeutic approach in the treatment of colorectal cancer. Int J Biochem Cell Biol 2019; 110: 75-83. http://dx.doi.org/10.1016/j.biocel.2019.02.010 PMID: 30818083

[6] Mazini L, Rochette L, Amine M, Malka G. Regenerative capacity of Adipose Derived Stem Cells (ADSCs), comparison with Mesenchymal Stem Cells (MSCs). Int J Mol Sci 2019; 20(10): E2523. http://dx.doi.org/10.3390/ijms20102523 PMID: 31121953

[7] Mastrolia I, Foppiani EM, Murgia A, *et al.* Challenges in clinical development of mesenchymal stromal/stem cells: Concise review. Stem Cells Transl Med 2019; 8(11): 1135-48. http://dx.doi.org/10.1002/sctm.19-0044 PMID: 31313507

[8] Kupcova Skalnikova H. Proteomic techniques for characterisation of mesenchymal stem cell secretome. Biochimie 2013; 95(12): 2196-211. http://dx.doi.org/10.1016/j.biochi.2013.07.015 PMID: 23880644

[9] Andrzejewska A, Lukomska B, Janowski M. Concise review: Mesenchymal stem cells: From roots to boost. Stem Cells 2019; 37(7): 855-64. http://dx.doi.org/10.1002/stem.3016 PMID: 30977255

[10] Kucia M, Reca R, Jala VR, Dawn B, Ratajczak J, Ratajczak MZ. Bone marrow as a home of heterogenous populations of nonhematopoietic stem cells. Leukemia 2005; 19(7): 1118-27. http://dx.doi.org/10.1038/sj.leu.2403796 PMID: 15902288

[11] Mukai T, Tojo A, Nagamura-Inoue T. Mesenchymal stromal cells as a potential therapeutic for neurological disorders. Regen Ther 2018; 9: 32-7. http://dx.doi.org/10.1016/j.reth.2018.08.001 PMID: 30525073

[12] Bansal H, Singh L, Verma P, *et al.* Administration of autologous bone marrow-derived stem cells for treatment of cerebral palsy patients: A proof of concept. J Stem Cells 2016; 11(1): 37-49. PMID: 28296863

[13] Purandare C, Shitole DG, Belle V, Kedari A, Bora N, Joshi M. Therapeutic potential of autologous stem cell transplantation for cerebral palsy. Case Rep Transplant 2012; 2012: 825289. http://dx.doi.org/10.1155/2012/825289 PMID: 23259143

[14] Koh H, Hwang K, Lim HY, Kim YJ, Lee YH. Mononuclear cells from the cord blood and granulocytecolony stimulating factor-mobilized peripheral blood: Is there a potential for treatment of cerebral palsy? Neural Regen Res 2015; 10(12): 2018-24. http://dx.doi.org/10.4103/1673-5374.172321 PMID: 26889193

[15] Liu L, Eckert MA, Riazifar H, Kang DK, Agalliu D, Zhao W. From blood to the brain: Can systemically transplanted mesenchymal stem cells cross the blood-brain barrier? Stem Cells Int 2013; 2013: 435093. http://dx.doi.org/10.1155/2013/435093 PMID: 23997771

[16] Moon J H, Kim M J, Song S Y, *et al.* Safety and efficacy of G-CSF mobilization and collection of autologous peripheral blood stem cells in children with cerebral palsy. Transfus Apher Sci 2013; 39(3): 516-21.

[17] Toth ZE, Leker RR, Shahar T, *et al.* The combination of granulocyte colony-stimulating factor and stem cell factor significantly increases the number of bone marrow-derived endothelial cells in brains of mice following cerebral ischemia. Blood 2008; 111(12): 5544-52. http://dx.doi.org/10.1182/blood-2007-10-119073 PMID: 18268092

[18] Papadopoulos KI, Low SS, Aw TC, Chantarojanasiri T. Safety and feasibility of autologous umbilical cord blood transfusion in 2 toddlers with cerebral palsy and the role of low dose granulocyte-

colony stimulating factor injections. Restor Neurol Neurosci 2011; 29(1): 17-22.
http://dx.doi.org/10.3233/RNN-2011-0572 PMID: 21335665

[19] Kang M, Min K, Jang J, *et al.* Involvement of immune responses in the efficacy of cord blood cell therapy for cerebral palsy. Stem Cells Dev 2015; 24(19): 2259-68.
http://dx.doi.org/10.1089/scd.2015.0074 PMID: 25977995

[20] Lee YH, Choi KV, Moon JH, *et al.* Safety and feasibility of countering neurological impairment by intravenous administration of autologous cord blood in cerebral palsy. J Transl Med 2012; 10: 58.
http://dx.doi.org/10.1186/1479-5876-10-58 PMID: 22443810

[21] Fan CG, Zhang QJ, Tang FW, Han ZB, Wang GS, Han ZC. Human umbilical cord blood cells express neurotrophic factors. Neurosci Lett 2005; 380(3): 322-5.
http://dx.doi.org/10.1016/j.neulet.2005.01.070 PMID: 15862910

[22] Chen G, Wang Y, Xu Z, *et al.* Neural stem cell-like cells derived from autologous bone mesenchymal stem cells for the treatment of patients with cerebral palsy. J Transl Med 2013; 11: 21.
http://dx.doi.org/10.1186/1479-5876-11-21 PMID: 23351389

[23] Nguyen LT, Nguyen AT, Vu CD, Ngo DV, Bui AV. Outcomes of autologous bone marrow mononuclear cells for cerebral palsy: An open label uncontrolled clinical trial. BMC Pediatr 2017; 17(1): 104.
http://dx.doi.org/10.1186/s12887-017-0859-z PMID: 28403842

[24] Liu X, Fu X, Dai G, *et al.* Comparative analysis of curative effect of bone marrow mesenchymal stem cell and bone marrow mononuclear cell transplantation for spastic cerebral palsy. J Transl Med 2017; 15(1): 48.
http://dx.doi.org/10.1186/s12967-017-1149-0 PMID: 28235424

[25] Silbereis JC, Huang EJ, Back SA, Rowitch DH. Towards improved animal models of neonatal white matter injury associated with cerebral palsy. Dis Model Mech 2010; 3(11-12): 678-88.
http://dx.doi.org/10.1242/dmm.002915 PMID: 21030421

[26] Akiyama Y, Radtke C, Honmou O, Kocsis JD. Remyelination of the spinal cord following intravenous delivery of bone marrow cells. Glia 2002; 39(3): 229-36.
http://dx.doi.org/10.1002/glia.10102 PMID: 12203389

[27] Aloisi F. Immune function of microglia. Glia 2001; 36(2): 165-79.
http://dx.doi.org/10.1002/glia.1106 PMID: 11596125

[28] Boomsma RA, Geenen DL. Mesenchymal stem cells secrete multiple cytokines that promote angiogenesis and have contrasting effects on chemotaxis and apoptosis. PLoS One 2012; 7(4): e35685.
http://dx.doi.org/10.1371/journal.pone.0035685 PMID: 22558198

[29] McLauchlan D, Robertson NP. Stem cells in the treatment of central nervous system disease. J Neurol 2018; 265(4): 984-6.
http://dx.doi.org/10.1007/s00415-018-8818-7 PMID: 29523970

[30] Jiao Y, Li XY, Liu J. A new approach to cerebral palsy treatment: Discussion of the effective components of umbilical cord blood and its mechanisms of action. Cell Transplant 2019; 28(5): 497-509.
http://dx.doi.org/10.1177/0963689718809658 PMID: 30384766

[31] Jafarzadeh-Esfehani R, Soudyab M, Parizadeh SM, *et al.* Circulating exosomes and their role in stroke. Curr Drug Targets 2020; 21(1): 89-95.
http://dx.doi.org/10.2174/1389450120666190821153557 PMID: 31433753

[32] Parizadeh SM, Jafarzadeh-Esfehani R, Ghandehari M, *et al.* Circulating exosomes as potential biomarkers in cardiovascular disease. Curr Pharm Des 2018; 24(37): 4436-44.
http://dx.doi.org/10.2174/1381612825666181219162655 PMID: 30569849

[33] Kim DK, Nishida H, An SY, Shetty AK, Bartosh TJ, Prockop DJ. Chromatographically isolated CD63+CD81+ extracellular vesicles from mesenchymal stromal cells rescue cognitive impairments after TBI. Proc Natl Acad Sci USA 2016; 113(1): 170-5.
http://dx.doi.org/10.1073/pnas.1522297113 PMID: 26699510

[34] Drommelschmidt K, Serdar M, Bendix I, *et al.* Mesenchymal stem cell-derived extracellular vesicles ameliorate inflammation-induced preterm brain injury. Brain Behav Immun 2017; 60: 220-32.
http://dx.doi.org/10.1016/j.bbi.2016.11.011 PMID: 27847282

[35] Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. Circ Res 2008; 103(11): 1204-19.
http://dx.doi.org/10.1161/CIRCRESAHA.108.176826 PMID: 19028920

[36] Chernykh ER, Kafanova MY, Shevela EY, *et al.* Clinical experience with autologous M2 macrophages in children with severe cerebral palsy. Cell Transplant 2014; 23 (Suppl. 1): S97-S104.
http://dx.doi.org/10.3727/096368914X684925 PMID: 25302537

[37] Huang L, Zhang C, Gu J, *et al.* A randomized, placebo-controlled trial of human umbilical cord blood mesenchymal stem cell infusion for children with cerebral palsy. Cell Transplant 2018; 27(2): 325-34.
http://dx.doi.org/10.1177/0963689717729379 PMID: 29637820

[38] Sun JM, Song AW, Case LE, *et al.* Effect of autologous cord blood infusion on motor function and brain connectivity in young children with cerebral palsy: A randomized, placebo-controlled trial. Stem Cells Transl Med 2017; 6(12): 2071-8.
http://dx.doi.org/10.1002/sctm.17-0102 PMID: 29080265

[39] Rah WJ, Lee YH, Moon JH, *et al.* Neuroregenerative potential of intravenous G-CSF and autologous peripheral blood stem cells in children with cerebral palsy: A randomized, double-blind, crossover study. J Transl Med 2017; 15(1): 16.
http://dx.doi.org/10.1186/s12967-017-1120-0 PMID: 28109298

[40] Min K, Song J, Kang JY, *et al.* Umbilical cord blood therapy potentiated with erythropoietin for children with cerebral palsy: A double-blind, randomized, placebo-controlled trial. Stem Cells 2013; 31(3): 581-91.
http://dx.doi.org/10.1002/stem.1304 PMID: 23281216

[41] Dong H, Li G, Shang C, *et al.* Umbilical Cord Mesenchymal Stem Cell (UC-MSC) transplantations for cerebral palsy. Am J Transl Res 2018; 10(3): 901-6.
PMID: 29636880

[42] Wang X, Hu H, Hua R, *et al.* Effect of umbilical cord mesenchymal stromal cells on motor functions of identical twins with cerebral palsy: Pilot study on the correlation of efficacy and hereditary factors. Cytotherapy 2015; 17(2): 224-31.
http://dx.doi.org/10.1016/j.jcyt.2014.09.010 PMID: 25593078

[43] Zali A, Arab L, Ashrafi F, *et al.* Intrathecal injection of CD133-positive enriched bone marrow progenitor cells in children with cerebral palsy: Feasibility and safety. Cytotherapy 2015; 17(2): 232-41.
http://dx.doi.org/10.1016/j.jcyt.2014.10.011 PMID: 25593079

[44] Nguyen TL, Nguyen HP, Nguyen TK. The effects of bone marrow mononuclear cell transplantation on the quality of life of children with cerebral palsy. Health Qual Life Outcomes 2018; 16(1): 164.
http://dx.doi.org/10.1186/s12955-018-0992-x PMID: 30107811

[45] Thanh LN, Trung KN, Duy CV, *et al.* Improvement in gross motor function and muscle tone in children with cerebral palsy related to neonatal icterus: An open-label, uncontrolled clinical trial. BMC Pediatr 2019; 19(1): 290.
http://dx.doi.org/10.1186/s12887-019-1669-2 PMID: 31438885

[46] Englander ZA, Sun J, Laura Case , Mikati MA, Kurtzberg J, Song AW. Brain structural connectivity increases concurrent with functional improvement: Evidence from diffusion tensor MRI in children with cerebral palsy during therapy. Neuroimage Clin 2015; 7: 315-24.
http://dx.doi.org/10.1016/j.nicl.2015.01.002 PMID: 25610796

[47] Wang SS, Jia J, Wang Z. Mesenchymal stem cell-derived extracellular vesicles suppresses iNOS expression and ameliorates neural impairment in Alzheimer's disease mice. J Alzheimers Dis 2018; 61(3): 1005-13.
http://dx.doi.org/10.3233/JAD-170848 PMID: 29254100

[48] Elia CA, Tamborini M, Rasile M, *et al.* Intracerebral injection of extracellular vesicles from mesenchymal stem cells exerts reduced Aβ plaque burden in early stages of a preclinical model of Alzheimer's disease. Cells 2019; 8(9): E1059.
http://dx.doi.org/10.3390/cells8091059 PMID: 31510042

[49] Han M, Cao Y, Xue H, *et al.* Neuroprotective effect of mesenchymal stromal cell-derived extracellular vesicles against cerebral ischemia-reperfusion-induced neural functional injury: A pivotal role for AMPK and JAK2/STAT3/NF-κB signaling pathway modulation. Drug Des Devel Ther 2020; 14: 2865-76.
http://dx.doi.org/10.2147/DDDT.S248892 PMID: 32764885

[50] Zhou X, Chu X, Yuan H, *et al.* Mesenchymal stem cell derived EVs mediate neuroprotection after spinal cord injury in rats *via* the microRNA-21-5p/FasL gene axis. Biomed Pharmacother 2019; 115: 108818.

[51] Wyse RD, Dunbar GL, Rossignol J. Use of genetically modified mesenchymal stem cells to treat neurodegenerative diseases. Int J Mol Sci 2014; 15(2): 1719-45. http://dx.doi.org/10.3390/ijms15021719 PMID: 24463293

[52] Sharma A, Sane H, Kulkarni P, D'sa M, Gokulchandran N, Badhe P. Improved quality of life in a case of cerebral palsy after bone marrow mononuclear cell transplantation. Cell J 2015; 17(2): 389-94. PMID: 26199918

[53] Mancías-Guerra C, Marroquín-Escamilla AR, González-Llano O, *et al.* Safety and tolerability of intrathecal delivery of autologous bone marrow nucleated cells in children with cerebral palsy: An open-label phase I trial. Cytotherapy 2014; 16(6): 810-20. http://dx.doi.org/10.1016/j.jcyt.2014.01.008 PMID: 24642016

[54] Wang X, Cheng H, Hua R, *et al.* Effects of bone marrow mesenchymal stromal cells on gross motor function measure scores of children with cerebral palsy: A preliminary clinical study. Cytotherapy 2013; 15(12): 1549-62. http://dx.doi.org/10.1016/j.jcyt.2013.06.001 PMID: 24100132