

Progress in clinical trials of stem cell therapy for cerebral palsy

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Abstract

Cerebral palsy is the most common disease in children associated with lifelong disability in many countries. Clinical research has demonstrated that traditional physiotherapy and rehabilitation therapies cannot alone cure cerebral palsy. Stem cell transplantation is an emerging therapy that has been applied in clinical trials for a variety of neurological diseases because of the regenerative and unlimited proliferative capacity of stem cells. In this review, we summarize the design schemes and results of these clinical trials. Our findings reveal great differences in population characteristics, stem cell types and doses, administration methods, and evaluation methods among the included clinical trials. Furthermore, we also assess the safety and efficacy of these clinical trials. We anticipate that our findings will advance the rational development of clinical trials of stem cell therapy for cerebral palsy and contribute to the clinical application of stem cells.

Key Words: adverse events; brain; cell transplantation; central nervous system; cerebral palsy; clinical trials; plasticity; regeneration; stem cell

Introduction

Cerebral palsy (CP) is a group of permanent disorders of the development of movement and posture, often accompanied by disturbances of sensation, perception, cognition, communication and behavior, by epilepsy, and by secondary musculoskeletal problems (Rosenbaum et al., 2007). The prevalence rate is approximately 3 per 1000 births globally, and is much higher in some developing countries (Graham et al., 2016; Van Naarden Braun et al., 2016; Korzeniewski et al., 2018). The causes of CP are complex, and include perinatal stroke, low birth weight, birth complications, gestational age, multiple births, and infection (Korzeniewski et al., 2018). Recent studies show that 14% of patients may have causative single-gene mutations (MacLennan et al., 2015), and the apolipoprotein E (*APOE*) genotype is correlated with the risk and severity of CP (Korzeniewski et al., 2018). Because of the complex etiology of CP, the pathological changes in the brain are highly variable (Brandenburg et al., 2019). Furthermore, a small proportion of patients may have completely normal magnetic resonance imaging (MRI) findings. A European study (Bax et al., 2006) found that 42.5% of children with CP have white matter damage. In addition, basal ganglia lesions, cortical/subcortical lesions, malformations, focal infarcts, and miscellaneous lesions are also shown in MRI. The treatment of CP has continuously evolved. Traditional rehabilitation treatments (Reid et al., 2015) such as physiotherapy, occupational and speech therapy, assistive devices, pharmacological intervention, and surgery such as neurectomy and rhizotomy (Koman et al., 2004; Graham et al., 2016; Jindal et al., 2019) have little efficacy for CP.

Recent studies show that stem cell therapy might effectively cure CP owing to the multi-directional differentiation potential

and migration capabilities of stem cells (Fan et al., 2015; Jantzie et al., 2018; Jiao et al., 2019). Several studies have investigated the mechanisms underlying the effectiveness of stem cells in CP animal models (Zheng et al., 2012). These studies suggest that trophic factor secretion (Bennet et al., 2012), neurogenesis (Carroll and Mays, 2011; Jantzie et al., 2018), immunomodulation (Bennet et al., 2012; Jantzie et al., 2018), angiogenesis (Kiasatdolatabadi et al., 2017) and neuroplasticity (Daadi et al., 2010) are key to their therapeutic effectiveness. An increasing number of studies of stem cell therapy have been carried out for various diseases, including stroke (Bernstock et al., 2017; Liao et al., 2019), amyotrophic lateral sclerosis (Mazzini et al., 2016), Alzheimer's disease (Duncan and Valenzuela, 2017; Reza-Zaldivar et al., 2019), spinal cord injury (Assinck et al., 2017; Mukhamedshina et al., 2019) and Parkinson's disease (Han et al., 2015). Accordingly, transplantation of stem cells is considered a promising and effective treatment for CP as well (Novak et al., 2016).

Children with CP show improvements in gross motor function (Wang et al., 2013; Thanh et al., 2019), fine motor function (Luan et al., 2012), cognition and other symptoms such as salivation, emotional changes and language competence (Dong et al., 2018; Huang et al., 2018) after receiving stem cell infusion. Some of these improvements could be evaluated with rating scales, while others could not. Furthermore, the design of these clinical trials differed substantially, such as in cell subtype, dose and delivery method of the stem cells, and patient background. These differences make it difficult to evaluate and compare the outcomes among the clinical trials. Thus, much more research is needed before stem cells can be used as cell preparations in clinical practice. Here, we summarize recent clinical trials of stem cell therapies for CP, with the aim of allowing researchers to recognize the progress

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Review

and shortcomings of current clinical trials, and so that future clinical trials of stem cell therapies for CP can be more effectively carried out.

Clinical Trial Data Retrieval of Stem Cell Therapy for Cerebral Palsy

An electronic search of the PubMed and ClinicalTrials databases for studies and clinical trials of cerebral palsy from 1971 to 2020 was performed using the following criteria: (“stem cells”[MeSH Terms]) OR (“umbilical cord blood”[MeSH Terms]) AND (“cerebral palsy”[MeSH Terms]). The results were further screened by title, abstract or project information. As of April 28, 2020, there were only 39 clinical trials of stem cell therapy for CP registered on clinicaltrials.gov (**Additional Tables 1 and 2**), of which 18 have been completed. At the same time, some clinical trials not registered on clinicaltrials.gov were also completed and provided some referential results. We performed a literature search with the keywords “cerebral palsy”, “umbilical cord blood” and “stem cell” in PubMed, and the article type was limited to “clinical trials”. A total of 20 articles were retrieved (**Additional Table 3**). The retrieved trials differed in study design, the age and type of the included population, subtype and dose of stem cells, administration method and course of treatment. The trials also varied in assessment tools. Most of these clinical trials are single-arm studies with a small sample size, and are difficult to comparatively evaluate because of the lack of parallel controls. Large-sample randomized controlled trials are needed to obtain more clinically robust data.

Types of Stem Cells Transplanted for the Treatment of Cerebral Palsy

Despite the abundance of stem cell sources, the stem cells used in these clinical trials were mainly derived from the following five tissues: bone marrow (Sharma et al., 2015; Nguyen et al., 2017, 2018), human umbilical cord blood (hUCB)/umbilical cord (UC) (Huang et al., 2018; Okur et al., 2018), fetal brain (Chen et al., 2010; Luan et al., 2012), adipose and peripheral blood (Ruff et al., 2013; Rah et al., 2017) (**Figure 1**). Stem cells produced by these tissues include mesenchymal stromal cells (MSCs) (Wang et al., 2013), total nucleated cells (Mancías-Guerra et al., 2014), neural stem cell-like cells (Chen et al., 2013), CD133-positive enriched bone marrow progenitor cells (Zali et al., 2015) and mononuclear cells (Sharma et al., 2015; Nguyen et al., 2017; Nguyen et al., 2018). In some clinical trials, patients did not exclusively receive stem cells, but cord blood containing stem cell components, and these treatments have also shown therapeutic effectiveness (Min et al., 2013; Sun et al., 2017). Among these, hUCB/UC is the most common source of stem cells, while bone marrow is the second most common. Both of these sources are comparatively abundant, the collection methods are simple, and ethical concerns are fewer. In some studies, the sources are of autologous origin, while in others, they are of allogeneic origin. Because harvesting the tissues can cause great physical and psychological trauma to children, autologous bone marrow stem cells are not a good choice for children with CP. In fact, stem cells of allogeneic origin all exhibit low immunogenicity, which is effective at avoiding immune rejection (Forraz and McGuckin, 2011). Stem cells from different sources have different efficacies, but even stem cells from the same source vary in efficacy during treatment. Liu et al. (2017) compared the efficacy of bone marrow mononuclear stem cells (BMMNCs) and bone marrow MSCs in the treatment of pediatric CP, and found that bone marrow MSCs were better than BMMNCs in the treatment of CP. While neural stem cells are considered ideal cells for the treatment of nerve injury, few studies have applied these cells in CP treatment (Trounson and McDonald, 2015). The fetal

brain is considered the optimal source of neural stem cells, but their use is controversial because of ethical issues, and have only been allowed to be used in some clinical trials in China (Chen et al., 2010; Luan et al., 2012). Peripheral blood and adipose are the least common stem cell sources in these studies, although they have been increasingly used as a source of cells in other fields (Xue and Milano, 2020). Treatment with peripheral blood cells has also shown effectiveness (Rah et al., 2017), but whether adipose-derived stem cells can be effective for CP patients remains to be investigated. Together, the current clinical trials suggest that, regardless of the source and type of stem cells, they all show therapeutic effectiveness for CP. However, further clinical trials are needed to identify the most therapeutically effective stem cell type for CP.

Routes and Methods of Cell Delivery for Cerebral Palsy Patients

Patient characteristics

The age range of enrolled patients in these studies has been large—the youngest was 1 month old (Mancías-Guerra et al., 2014) and the oldest was 32 years old (Chen et al., 2013). The proportion of older patients was very small, and 99% of cases were younger than 15 years, and mainly between 1 and 10 years of age (**Additional Figure 1**). In a double-blind, randomized, placebo-controlled trial, researchers found that among children aged 10 months to 10 years old, the younger patients showed greater improvement than older children (Min et al., 2013). Additional clinical trials are needed to clarify whether this trend is applicable to all patients with CP.

In addition to age, another important aspect of CP is disease classification. Based on clinical manifestations caused by the complex etiologies, CP can be classified into spastic, dyskinetic, ataxic and mixed types (Fan et al., 2015; MacLennan et al., 2015; van Lieshout et al., 2017; Korzeniewski et al., 2018). Although all types of patients have been included in different clinical trials, including quadriplegia, diplegia, athetoid, hemiplegia and hypotonic paralysis, among the many types of CP, researchers have seemingly had a preference for studying patients with spastic CP (**Additional Figure 2**). It has been shown that patients with different types of CP will show different treatment outcomes after receiving stem cell therapy (Sharma et al., 2015), but this still needs to be confirmed by more clinical trials. The severity of gross motor impairment and gender of the patient do not appear to be factors affecting inclusion in studies.

Administration routes

CP patients participating in stem cell clinical trials can receive stem cell transplantation in a variety of ways (**Figure 2**). Lumbar puncture and intravenous injection are the most common routes of administration, brain stereotactic surgery is relatively less used, while nasal administration has never been reported. A clinical trial is currently being conducted to assess the comparative effectiveness of the various administration routes (NCT03414697). Lumbar puncture administration allows drugs to reach the brain through the cerebrospinal fluid circulation, and similarly, researchers have found that stem cells can also reach the brain directly through this route (Kim et al., 2020). Although intrathecal administration is accompanied by some side effects, they can be improved by medical treatment or are spontaneously relieved (Mancías-Guerra et al., 2014; Sharma et al., 2015; Trounson and McDonald, 2015; Nguyen et al., 2017). Stereotactic brain surgery, which also allows stem cells to enter the brain directly, is much more invasive than lumbar puncture. The side effects of stereotactic surgery are relatively serious, such as lateral ventricular blood vessel injury (He et al., 2012), which may cause brain damage, thereby counteracting the therapeutic effect of stem cells. Intravenous injection is also a common route of stem cell administration, but because of the blood-brain barrier, only a small fraction

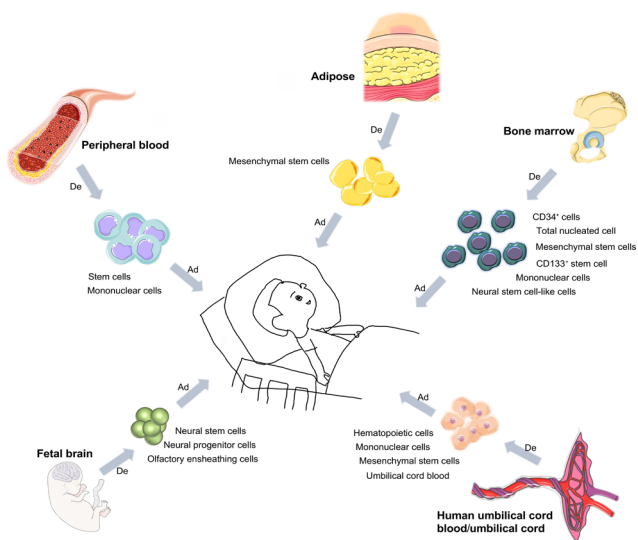


Figure 1 | The stem cell types used in clinical trials for cerebral palsy. The various types of stem cells injected into the human body are not only functionally different, but are also very diverse in origin. Of these, peripheral blood, umbilical cord and adipose sources are relatively abundant, while bone marrow and fetal brain sources are less. Ad: Administration; De: derived.

of the stem cells can enter the brain parenchyma (Sherman et al., 2019). Thus, the regeneration and differentiation of exogenous stem cells in the brain is less efficient. Nonetheless, this administration method has been shown to have some therapeutic effectiveness (Mancías-Guerra et al., 2014; Zhang et al., 2015; McIntosh et al., 2016; Huang et al., 2018), which might be mediated by trophic factors secreted by the stem cells. Nasal administration has been used in clinical practice for many years, but it is a relatively novel method for stem cell therapy. Some investigators have employed this administration route in animal models (Donega et al., 2013). Preclinical studies indicate that stem cells enter the brain through the perineural space between the cribriform plate and the olfactory nerve (Danielyan et al., 2009; Galeano et al., 2018), thereby bypassing the blood-brain barrier (Lochhead and Thorne, 2012). Nasal administration can be said to be the least invasive of these administration methods, and if it also demonstrates better efficacy, it can be widely employed in the future.

Dosage and course

Currently, there is no unified standard for the dosage and transplantation of stem cells. In six trials, stem cells were injected according to kilogram body weight, while in the remaining studies, patients were given a fixed dose of stem cells (Additional Table 3). The trials differed in dose, and furthermore, cells derived from the same source were not administered at the same dose. In children with CP, the maximum dose of stem cells was $5.7 \pm 1.52 \times 10^8$ /kg of peripheral blood mononuclear cells (Rah et al., 2017), while the minimum dose was 2×10^6 of olfactory ensheathing cells (Chen et al., 2010). It has been shown that the dose of stem cells impacts therapeutic effectiveness, with higher doses having a better therapeutic effect (Sun et al., 2017). However, too many stem cells might cause side effects in patients (Gu et al., 2020). Investigators hope to achieve better treatment outcomes by optimizing administration timing as well. The time course of stem cell transplantation differs among the studies, and the number of stem cell infusions may be related to the administration route. Researchers often perform only one administration when using stereotactic brain surgery. In contrast, when transplanting by lumbar puncture or intravenous injection, the researchers are more likely to administer stem cells two to four times, with the interval

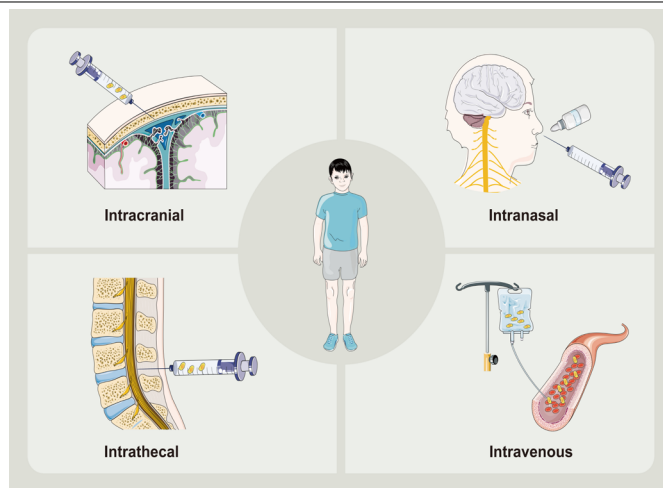


Figure 2 | The common administration routes for cerebral palsy patients in stem cell therapy.

Patients receive stem cell therapy usually through four routes—intrathecal injection, intravenous injection, intranasal injection and intracranial injection. Intranasal injection is noninvasive, while intrathecal and intravenous injection are moderately invasive, but might induce some adverse events. Intracranial is the most invasive, and has the most side effects.

varying from 3–4 days (Wang et al., 2013) to 6 months (Rah et al., 2017). Notably, a 6-year-old Chinese girl who received the most stem cell injections almost recovered completely after 16 intravenous infusions of hUCB-MSCs over the 5-year follow-up period (Zhang et al., 2015). A study showed that the efficacy of stem cell therapy decreased over time by comparing two groups with different transplantation timings (Rah et al., 2017). Perhaps the strategy of multiple injections, which allows patients to receive more stem cells, is an effective solution for ultimately curing patients with CP.

Rehabilitation program

Most patients had received rehabilitation for at least 3 months before stem cell therapy, but without obvious improvement. Rehabilitation for CP is not considered effective, and progress is slow and protracted. Although many patients seem to experience some improvements with rehabilitation, such as in muscle tone and range of motion, these changes may be hard to sustain after rehabilitation stops. Nonetheless, half of the clinical trials used a combination of stem cell therapy and rehabilitation. Most patients received a 6-month rehabilitation regimen (Chen et al., 2010, 2013; Mancías-Guerra et al., 2014; Zhang et al., 2015; McIntosh et al., 2016), and a small proportion were given a 1-year (Luan et al., 2012) or 2-year (Zhang et al., 2015; McIntosh et al., 2016; Huang et al., 2018) program, while the shortest rehabilitation program was 10 weeks (Nguyen et al., 2018). Some clinical studies implemented rehabilitation control groups to compare the therapeutic effectiveness of rehabilitation only with that of rehabilitation combined with stem cell therapy. The results show that patients who received stem cell therapy exhibited a significant improvement compared with those who only received rehabilitation. However, further study is needed to clarify whether the combination of stem cells and rehabilitation has a better therapeutic effect than stem cell therapy alone.

Typical drug

Typical drugs used for CP have limited therapeutic effect and often need repeated administration to maintain effectiveness. The benefits of stem cell therapy may be long-lasting. Because stem cell therapy is still in clinical trials for children with CP, to explore the safety and efficacy of stem cell therapy, patients will avoid using other drugs while participating in clinical trials

Review

unless it is very necessary, so as not to affect the evaluation of the results. Furthermore, some sedatives used in surgery have muscle relaxant and analgesic effects themselves, and patients who still have persistent hypotonia and decreased muscle strength on the second postoperative day have indeed been observed in our clinical trials, and we will avoid objective evaluation during this period to avoid biasing the results.

Safety and Efficacy of Stem Cell Therapy for Cerebral Palsy

Assessment tools

Evaluation of clinical trials includes safety and efficacy evaluations. The safety evaluation generally includes laboratory examination, observation of clinical symptoms and monitoring of adverse events. The assessment of efficacy is mainly divided into scale assessment and objective examination. In the published literature, the types of assessment scales vary widely, commensurate with the variability in improvement in clinical symptoms. Internationally used scales, such as the Gross Motor Function Measure-88/66 and the Gross Motor Function Classification System for gross motor (Alotaibi et al., 2014), the Manual Ability Classification Scale for fine motor and the Modified Ashworth Score for muscle tone, are used for assessment in almost every clinical trial. Clinicians will also generally implement other scales based on nation-specific conditions, such as language competence and communication, which are difficult to evaluate with a unified scale because of differences in culture and language. Furthermore, some improvements, such as in oromotor skills, salivation and psychomotor functions, are difficult to assess because of a lack of assessment scales. Researchers have accordingly tried to evaluate improvement in other ways, including brain MRI and positron emission tomography-CT. However, only a few patients showed structural changes after stem cell therapy on MRI and positron emission tomography-CT (Min et al., 2013; Rah et al., 2017), and most researchers found no imaging changes (Mancías-Guerra et al., 2014; Zali et al., 2015). Brain structural changes take time to manifest, and functional tests may be more sensitive, and the choice of MRI sequence during the evaluation may account for the observed lack of change. Some investigators used computer technology to reconstruct MRI data, and found some positive changes in brain connectivity (Sun et al., 2017). This approach may thus improve assessment. Electroencephalography (EEG) is mainly used as a safety assessment tool, but some investigators found EEG improvements that were associated with improvements in brain function (Huang et al., 2018). There are some limitations in the assessment scales, and errors are inevitable in manual assessment. Furthermore, different scales are required for different symptoms. These factors hinder the circulation and use of standardized scales among countries. MRI and EEG are internationally accepted objective detection methods that have been widely applied in clinical practice. Further innovation of evaluation methods is necessary so that different clinical trial results can be better compared.

Adverse events

In clinical trials, adverse events are of great concern and have been documented in all studies (**Additional Table 3**). Although some serious adverse events did occur, most were mild and could be treated with drugs or spontaneously resolved. Some adverse events appear to be related to the method of administration. The most common adverse events observed in clinical trials were fever, nausea and vomiting, and pain at the site of injection, particularly as lumbar puncture is the preferred method of cell transplantation (He et al., 2012; Wang et al., 2013; Mancías-Guerra et al., 2014; Sharma et al., 2015; Zali et al., 2015; Nguyen et al., 2017; Rah et al., 2017). The most serious adverse events included anesthesia-

related adverse events, laryngeal stridor and lingual edema (Mancías-Guerra et al., 2014), and tiny hemorrhages during the stereotactic surgery (He et al., 2012; Luan et al., 2012). Seizures were found in a few patients, and among these, most had a prior history of seizures. After administration, the seizure frequency increased (Sharma et al., 2015; Zali et al., 2015). Researchers conjectured that the seizures were related to stem cell therapy. However, interestingly, in two clinical trials, patients who had refractory epilepsy or drug-resistant epilepsy exhibited a reduction in seizure frequency after stem cell intervention (DaCosta et al., 2018; Milczarek et al., 2018). Whether stem cells have a therapeutic effect on epilepsy or aggravate the condition is still debatable, and in the future, we need to carry out more relevant clinical studies. Some mood changes like crying (Chen et al., 2013), difficulty in falling asleep (Luan et al., 2012) and irritability (Chen et al., 2013; Rah et al., 2017) were also thought to be associated with stem cell therapy. Overall, stem cell therapy is relatively safe, and nasal administration may be a good choice to limit adverse events related to the route of administration.

Efficacy

Gross motor function

All stem cell therapies have produced improvements in gross motor function, but the improvements have varied. The assessment of gross motor functional improvement mainly includes lying and rolling, sitting, crawling and kneeling, standing, walking, and running and jumping. Stem cells from all sources can be therapeutic for CP patients. The study by Nguyen et al. (2017) showed that 6 months after autologous BMMNCs transplantation, patients showed significant improvements in lying, rolling and sitting, with a proportion of 100%, as well as improvements in walking, running and jumping, with a proportion of 38%. Similar outcomes were observed in other clinical trials using stem cells derived from bone marrow (Chen et al., 2013; Mancías-Guerra et al., 2014; Zali et al., 2015). hUCB/UC, a common source of stem cells, also has an ameliorative effect on gross motor function in CP patients. In a study of allogeneic hUCB-MSC administration, CP children showed great changes in gross motor ability over the 2-year follow-up (Huang et al., 2018). Fetal brain-derived stem cells, including neural progenitor cells (Luan et al., 2012) and olfactory ensheathing cells (Chen et al., 2010), have also been shown to improve gross motor function.

Peripheral blood, an abundant source of stem cells, has been increasingly used in recent years, but rarely in patients with CP. A study by Rah et al. (2017) showed that after peripheral blood mononuclear cell transplantation, 42.6% of the 57 CP patients showed overall improvements in gross motor function, although no changes were observed in gross motor function staging. Although a meta-analysis suggests that MSCs may be the best stem cells for improving gross motor functions (Eggenberger et al., 2019), randomized controlled trials are needed for confirmation.

Fine motor function

In contrast to the significant gross motor function improvements, fine motor changes are not as widespread. Although stem cells from all sources have improved fine motor function, this effect is not observed in all clinical trials. Only a few clinical trials reported fine motor improvements. For example, neural progenitor cells derived from fetal brain can improve hand movement, pinching tiny objects and eye-hand coordination (Luan et al., 2012), and about 10 diplegic CP patients showed better distal hand movements after autologous BMMNC transplantation (Sharma et al., 2015), and a boy's hand function rating improved from level 3 to 2 in the Manual Ability Classification Scale after receiving human UC-MSc transplantation (Okur et al., 2018). Furthermore, after peripheral blood mononuclear cell transplantation, 42.6% of patients showed improvements in fine motor function (Rah et

al., 2017). Fine motor movements are very complex functions, and improvement requires the coordination of various muscles and nerves, including the adjustment of muscle tension. This may make the improvement of fine motor skills more difficult and prolonged.

Muscle tone

Muscle tension is a common problem for many CP patients. The disorders of muscle tension cause the patient to be unable to complete many movements. A decrease in muscle tone has been reported in some clinical trials. Nguyen et al. (2017) observed a decline in the Modified Ashworth score from 3.4 to 2.0, and another study reported a reduction in muscle spasticity from 3.8 to 2.1 after transplantation (Nguyen et al., 2018). Almost all CP patients display muscle tone changes in clinical trials of stem cell therapy (Sharma et al., 2015). However, changes in muscle tone have not been reported in therapy of stem cells derived from hUCB/UC or peripheral blood; however, there is only one report of the latter. Nonetheless, bone marrow and fetal brain-derived stem cells may have some advantages over hUCB/UC-derived stem cells in improving muscle tension.

Cognition

Improvements in cognition with stem cell therapy have been reported in multiple clinical trials. Some studies reported that bone marrow stem cell transplantation results in cognitive improvement (Mancías-Guerra et al., 2014; Sharma et al., 2015; Zali et al., 2015), whereas others did not (Chen et al., 2013; Wang et al., 2013; Nguyen et al., 2017). A similar phenomenon is found with hUCB/UC-derived stem cell therapy (McIntosh et al., 2016; Huang et al., 2018; Okur et al., 2018). Although there are few studies of stem cells derived from fetal brain, significant cognitive increases have been reported (Luan et al., 2012). Perhaps because of the less frequent use of peripheral blood stem cells, no reports of improvement in cognitive function have been reported.

Others

In addition to the above symptoms, many other improvements have been reported in clinical trials. Language competence enhancement is a common finding after stem cell transplantation (He et al., 2012; Mancías-Guerra et al., 2014; Sharma et al., 2015; Huang et al., 2018). Other improvements, such as in self-dependence and social adaptability (He et al., 2012; Mancías-Guerra et al., 2014; Zhang et al., 2015; McIntosh et al., 2016; Huang et al., 2018), visual acuity (He et al., 2012; Sharma et al., 2015), salivation (Wang et al., 2013), and emotional and physical health (Chen et al., 2010; Nguyen et al., 2018) have also been observed in some clinical trials. Some of these changes were assessed with a scale, while others could not. This makes it impossible to carry out significance analysis of these changes. The improvements produced by stem cells are manifold, and there remain challenges in assessment.

Mechanisms Underlying the Effectiveness of Stem Cell Therapy for Cerebral Palsy

It is well-known that stem cells have the ability to differentiate, and under appropriate conditions, become any type of cell in the human body. Stem cells also have the ability to proliferate indefinitely (Jin, 2017). Stem cells have been applied in the clinical study of a variety of neurological diseases (Duncan and Valenzuela, 2017; Burman et al., 2018), and have shown good therapeutic effectiveness and safety for many diseases that cannot be resolved by drugs. Preclinical studies indicate that stem cells also have immunomodulatory actions (Naji et al., 2019) and can secrete cytokines (Huang and Zhang, 2019). Thus, the mechanisms of the therapeutic effectiveness of stem cell therapy for CP likely involves the following: After

entering, stem cells can differentiate into neurons and glial cells, such as astrocytes, and replace damaged cells, and then re-establish connections with other neural cells. Stem cells secrete a variety of cytokines that modulate the inflammatory response, and ensure the survival of neurons and promote angiogenesis. In addition, exogenous stem cells can also induce and accelerate endogenous repair (Huang and Zhang, 2019).

Challenges in the Future Application of Stem Cell Therapy for Cerebral Palsy

With the increase in the number of clinical studies, stem cells are expected to be marketed successively in the near future, bringing new options for the treatment of CP. However, many problems remain to be tackled. The follow-up observation period of current clinical trials is generally short, and the long-term safety and efficacy of stem cell therapy remain to be adequately assessed. It is clear that all types of stem cells have a therapeutic effect in CP. However, because the doses, sources and administration routes differ among the clinical trials, it is currently difficult to declare the best candidate for CP patients. Clinical trials of CP have to date been mainly carried out on younger children, but with the advancement of medical care, many children with CP survive, and it remains unknown whether stem cell therapy can also improve symptoms in older patients. Additionally, it is necessary to identify the optimal administration method to maximize efficacy and minimize side effects, although nasal administration shows promise. Further improvements are needed in efficacy assessment methods. Although scale assessment has been used for many years, the limitations in symptom assessment cannot be ignored. Currently, most scale assessments can only assess the improvement of a certain aspect of clinical symptoms. Furthermore, the scales are prone to human error. New assessment methods, such as positron emission tomography/CT and MRI, have been employed and have shown to be effective in some clinical trials (Eggenberger et al., 2019). However, the radiation exposure from positron emission tomography/CT cannot be ignored. The application of computer analysis of EEG data in other fields (Beatty et al., 2016; Avena-Koenigsberger et al., 2017) may also be applicable to the evaluation of CP. MRI and EEG, as objective examination methods, are sensitive to the changes in brain structure and function. The combination of MRI and EEG using computer data processing techniques may enhance the evaluation of CP.

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Additional files:

Additional Table 1: Completed and recruiting clinical trials of stem cells therapy for CP.

Additional Table 2: Other clinical trials of stem cells therapy for CP.

Additional Table 3: Published clinical trials of stem cell therapy for CP.

Additional Figure 1: The type of CP patients enrolled in clinical trials.

Additional Figure 2: The administration routes of stem cell therapy for CP.

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Additional Table 1 Completed and recruiting clinical trials of stem cells therapy for CP

Project identifier	Sample size	Age (yr)	Intervention model	Status	Phase	Type of stem cell	Infusion route	Start/complete date	Study location
NCT01404663	12	4-12	Single group assignment	Completed	Phase 1	Bone marrow derived CD133+ stem cell	Intrathecal	October, 2011 to May, 2012	Iran
NCT01763255	8	4-12	Parallel assignment	Completed	Phase 1 and 2	Bone marrow derived CD133 cell	Intrathecal	April, 2012 to April, 2014	Iran
NCT03123562	25	1-15	Single group assignment	Completed	Phase 2	Autologous bone marrow mononuclear cell	Intrathecal	April, 2014 to October, 2016	Vietnam
NCT02574923	30	2-15	Single group assignment	Completed	Phase 2	Autologous bone marrow mononuclear cell	Intrathecal	November, 2015 to April, 2018	Vietnam
NCT02569775	40	1-15	Single group assignment	Completed	Phase 2	Autologous bone marrow mononuclear cell	Intrathecal	April, 2014 to August, 2015	Vietnam
NCT01019733	18	1-8	Single group assignment	Completed	Not provided	Autologous bone marrow stem cell	Intrathecal	July, 2009 to January, 2011	Mexico
NCT02983708	57	2-10	Crossover assignment	Completed	Phase 1 and 2	Autologous peripheral blood stem cell	Not provided	August, 2011 to September, 2014	Korea
NCT01193660	105	10mon-10	Parallel assignment	Completed	Not provided	Total nucleated cells from allogenic umbilical cord blood	Intravenous	May, 2010 to April, 2011	Korea
NCT01639404	17	6 mon-20	Single group assignment	Completed	Not provided	Total nucleated cells from allogenic umbilical cord blood	Intravenous/intraarterial	July, 2012 to March, 2013	Korea
NCT02025972	10	Less than 15	Single group assignment	Completed	Not provided	Total nucleated cells from allogenic umbilical cord blood	Not provided	December, 2013 to November, 2015	Korea
NCT01991145	92	10mon-6	Parallel assignment	Completed	Not provided	Total nucleated cells from allogenic umbilical cord blood	Not provided	November, 2013 to June, 2017	Korea
NCT02236065	10	19-75	Single group assignment	Completed	Not provided	Total nucleated cells from allogenic umbilical cord blood	Not provided	August, 2014 to July, 2016	Korea
NCT01528436	37	6 mon-20	Parallel assignment	Completed	Phase 2	Total nucleated cells from allogenic umbilical cord blood	Intravenous/intraarterial	February, 2012 to July, 2012	Korea
NCT03979898	1	3-12	Single group assignment	Completed	Early phase 1	Autologous adipose tissue derived mesenchymal stem cell	Not provided	June, 2017 to May, 2019	Korea
NCT01988584	20	2-10	Crossover assignment	Completed	Phase 2	Umbilical cord blood stem cells/bone marrow stem cell	Not provided	November, 2013 to February, 2018	USA
NCT02599207	15	1-6	Parallel assignment	Completed	Phase 1	Total nucleated cells from allogenic umbilical cord blood	Not provided	November, 2015 to July, 2018	USA
NCT01147653	63	1-6	Crossover assignment	Completed	Phase 2	Total nucleated cells from allogenic umbilical cord blood	Not provided	June, 2010 to March, 2016	USA
NCT01929434	300	1-14	Parallel assignment	Completed	Phase 3	Umbilical cord derived mesenchymal stem cell	Intrathecal	October, 2013 to December, 2016	China
NCT01072370	40	1-12	Crossover assignment	Recruiting	Phase 1 and 2	Autologous cord blood stem cell	Intravenous	January, 2010 to July, 2019	USA
NCT03005249	20	1-12	Parallel assignment	Recruiting	Not provided	Neural stem cell	Not provided	December, 2016 to	China



December, 2019

NCT03791372	25	6 mon-10	Parallel assignment	Recruiting	Phase 1	Total nucleated cells from allogenic umbilical cord blood	Intravenous	October, 2017 to April, 2021	China
NCT03826498	40	1-10	Parallel assignment	Recruiting	Phase 2	Allogenic umbilical cord blood mononuclear cell	Not provided	January, 2018 to January, 2021	Russian
NCT04098029	90	1-12	Parallel assignment	Recruiting	Phase 2	Umbilical cord blood hematopoietic cell	Not provided	Sepember, 2019 to May, 2021	Russian

CP: Cerebral palsy.

Additional Table 2 Other clinical trials of stem cells therapy for CP

Project identifier	Sample size	Age (yr)	Intervention modal	Status	Phase	Type of stem cell	Infusion route	Start/complete date	Study location
NCT03078621	50	2-12	Single group assignment	Active, not recruiting	Phase 1 and 2	Autologous bone marrow-derived stem cells mesenchymal stem cell	Intravenous/intrathecal	September, 2016 to January, 2021	Arabia
NCT03795974	108	4-14	Parallel assignment	Active, not recruiting	Phase 2	Hematopoietic stem cells/mesenchymal stem cell	Intrathecal	July, 2017 to December, 2019	Iran
NCT03414697	44	2-18	Parallel assignment	Active, not recruiting	Not provided	Umbilical cord-derived mesenchymal stem cell	Intravenous/intrathecal/intranasal	April, 2018 to December, 2020	China
NCT03087110	12	1-16	Single Group assignment	Active, not recruiting	Phase 1	Umbilical cord blood cell	Intravenous	March, 2016 to February, 2020	Australia
NCT03130816	90	10 mon -20	Single Group assignment	Active, not recruiting	Phase 1 and 2	Allogeneic umbilical cord blood	Intravenous	July, 2015 to July, 2019	Korea
NCT03473301	90	2-5	Parallel assignment	Active, not recruiting	Phase 1 and 2	Allogeneic umbilical cord blood/umbilical cord tissue-derived mesenchymal stromal cell	Intravenous	April, 2018 to May, 2021	USA
NCT03327467	Not provided	Less than 26	Not provided	Active, not recruiting	Not provided	Total nucleated cells from umbilical cord blood	Intravenous	October, 2017 to December, 2019	USA
NCT04243408	72	2 mon-18	Parallel assignment	Active, not recruiting	Phase 2	Total nucleated cells from allogenic umbilical cord blood	Not provided	January, 2020 to December, 2024	Israel
NCT01978821	Not provided	17-22	Single group assignment	Withdrawn	Phase 1	Autologous bone marrow mononuclear cell	Not provided	August, 2010 to August, 2013	India
NCT01486732	Not provided	6 mon-20	Parallel assignment	Withdrawn	Phase 2	Total nucleated cells from allogenic umbilical cord blood	Intravenous/intraarterial	March, 2013 to July, 2014	Korea
NCT02241395	Not provided	6 mon-35	Single group assignment	Withdrawn	Phase 1	Autologous bone marrow mononuclear cell	Intrathecal	August, 2013 to December, 2018	India
NCT03203941	Not provided	All ages	Not provided	No longer available	Not provided	Autologous cord blood stem cell	Not provided	June, 2017 to May, 2020	USA
NCT04029896	Not provided	≥ 3	Not provided	No longer available	Not provided	Autologous adipose-derived mesenchymal stem cell	Intravenous	July, 2019 to January, 2020	USA
NCT01834664	100	15-70	Single group assignment	Unknown	Phase 1 and 2	Autologous stem cell	Intrathecal	March, 2013 to June, 2016	India
NCT02231242	60	7-9	Crossover assignment	Unknown	Phase 2	Bone marrow total nucleated cell	Intrathecal	September, 2013 to June, 2016	Mexico
NCT01832454	100	3-15	Single group assignment	Unknown	Phase 2 and 3	Bone marrow derived mononuclear cells	Intrathecal	March, 2011 to December, 2015	India

Other clinical trials indicate that the clinical trials of stem cells therapy for cerebral palsy in ClinicalTrials.gov were not included in Additional Table 1. CP: Cerebral palsy.

Additional Table 3 Published clinical trials of stem cell therapy for CP

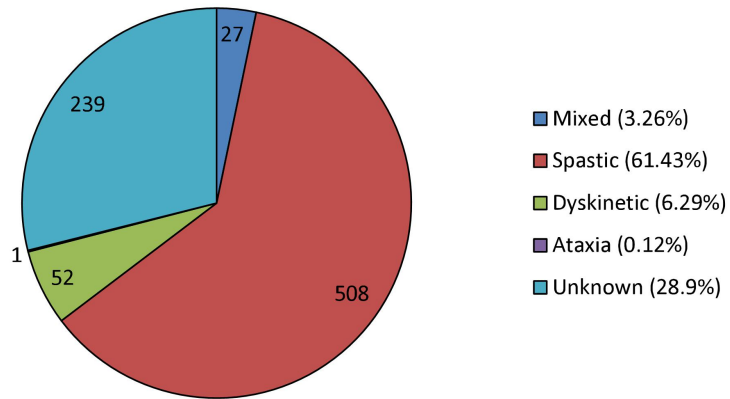
Study	Sample size/number of group	Age (yr)	Type of CP	Type of stem cells	Infusion route and dose	Course	Adverse events	Assessment tool	Improvement
Nguyen et al. (2017)	40/1	2-15	Spastic	Autologous BM MNCs and CD34+ cells	1st time, IT: 27.2×10^6 and 2.6×10^6 /kg, respectively; 2nd time, IT: 17.1×10^6 and 1.7×10^6 /kg, respectively	2 times with an interval of 3 mon	Mild fever (30%), intermittent vomiting (22.5%)	GMFM and MAS	Muscle spasticity, gross motor function
Sharma et al. (2015)	40/1	17mon-22	All types	Autologous BM MNCs	IT: 10.23×10^6	Once	Spinal headache (15%), nausea (7.5%), vomiting (30%), pain at the site of injection (30%) and diarrhea (2.5%), seizure (5%)	PET-CT, GMFCS	Sitting/standing/walking balance, distal hand movements, neck holding, oromotor skills, cognition, leg movements, speech, ambulation, muscle tone of the upper limb/lower limb/trunk, overhead activities, dystonia
Nguyen et al. (2018)	30/1	2-15	Bilateral paresis, hemiplegia	Autologous BM MNCs	IT: a volume of 8 mL/kg to 200 mL maximum	2 times with an interval of 3 mon	No	GMFM, MAS, CP QOL-Child	Gross motor function, muscle spasticity, social well-being and acceptance; feelings about functioning; participation and physical health; emotional well-being; pain and impact of disability; family health
Zali et al. (2015)	12/1	4-12	Ataxic, athetoid, spastic	CD133+ enriched bone marrow progenitor cells	IT: $4.5-17.6 \times 10^6$	Once	Headache (41.7%), nausea and vomiting (41.7%), back pain (91.7%), seizure (8.3%)	GMFM, GMFCS, United Kingdom FIM+ FAM, BBS, MAS, MRI, EEG	Motor function, spasticity and cognitive
Mancías-Guerra et al. (2014)	18/1	1 mon -8	Quadriplegic, triplegic, hypotonic paralysis, paraplegic	Autologous TN Cs and CD34+ cells	IT: 13.12×10^8 range ($4.83-53.87 \times 10^8$) TNCs and 10.02×10^6 (range, $1.02-29.9 \times 10^6$) CD34+ cells ; IV: 6.01×10^8 (range, $1.36-17.85 \times$	Once	Fever (5.6%), headache (11.1%), vomiting (11.1%), neck stiffness (5.6%), lingual edema (5.6%), laryngeal stridor	BDI, MRI	Cognitive, adaptive, personal-social, motor and communication

Author	Year	Sample Size	Condition	Cell Type	Cell Dose	Frequency	Side Effects	Assessments	Outcomes
Zhang et al. (2015)	1/1	6/12	Not available	Allogenic hUC B-MSCs	10^8 TNCs and 3.39×10^6 CD34+ cells IV: 5×10^7	4 times with an interval of a week, and repeated 4 times with an interval of a half year	No	Ashworth spasm assessment, GMFM, MRI, EEG, CDCC, comprehensive function	Gross motor function, language, self-dependence, cognitive ability, and social, adaptation
Okur et al. (2018)	1/1	6	Dystonic, spastic	Allogenic hUC B-MSCs	IT + IV: 15×10^6	4 times with an interval of 15 d	Mild back pain	FIM, GMFCS, MACS, CFSS, Tardieu Scale, TCMS	Gross motor function, self-dependence, trunk control and sitting balance, communication, hand skill
He et al. (2012)	24/1	1 mon-7	Quadriplegic, diplegia, dyskinetic, mixed	Neural stem cells	SS: 3.8×10^6 - 7.3×10^7	Once	Experienced fever (29.2%), vomited (4.2%), sphenotresia (29.2%)	EEG, MRI	Muscular tension in the limbs, the head-controlling action, visual acuity, the communicative competence
Chen et al. (2013)	60/2	1-35	Not available	Neural stem cell-like cells	IT: $1-2 \times 10^7$	2 times with an interval of 3 weeks	Crying	GMFM, Gesell questionnaire	Gross motor function
Rah et al. (2017)	57/2	2-10	Diplegia, hemiplegia,, triplegic, ataxic, athetoid	PBMCs	M1‡ : $4.63 \pm 2.88 \times 10^8$ TNCs and $1.92 \pm 1.99 \times 10^6$ CD34+ cells; M7‡: $6.20 \pm 1.94 \times 10^8$ TNCs and $1.75 \pm 1.07 \times 10^6$ CD34+ cells	Once	Fever (3.5%), Irritability(1.8%), transient hemoglobinuria (5.3%), abdominal pain (1.8%)	GMFCS, GMFM, PEDI, QUEST, DDST-II, MRI, PET-CT	Gross motor function, fine motor function, detailed developmental
Wang et al. (2013)	52/2	6 mon-15	Athetoid, spastic, athetoid/spastic	Autologous BM-MSCs	IT + SS: 2×10^7	Protocol 1‡: intrathecal infusion twice and stereotactic surgery once; Protocol 2‡: intrathecal infusion 4 times. All the interval was 5 d	Low fever (< 37.5°C) and wound aches	GMFM, GMFCS	Gross motor function, the muscular tension, lumbar muscular power and salivation decrease
Huang et al. (2018)	54/2	3-12	Not available	hUCB-MSCs	IV: 5×10^7	4 times with an interval of 1 wk, and repeated once 3 m on later	Upper respiratory tract infection diarrhea, anorexia, constipation	CFA	Gross motor ability and comprehensive function including cognizance, language competence, self-care, motor function, and social adaptability
Luan et	94/2	2-17 mon	Quadriplegic,	NPCs	SS: $8-10 \times 10^6$	Once	Low-grade fever,	GMFM, PDMS-FM	Gross motor function, fine motor

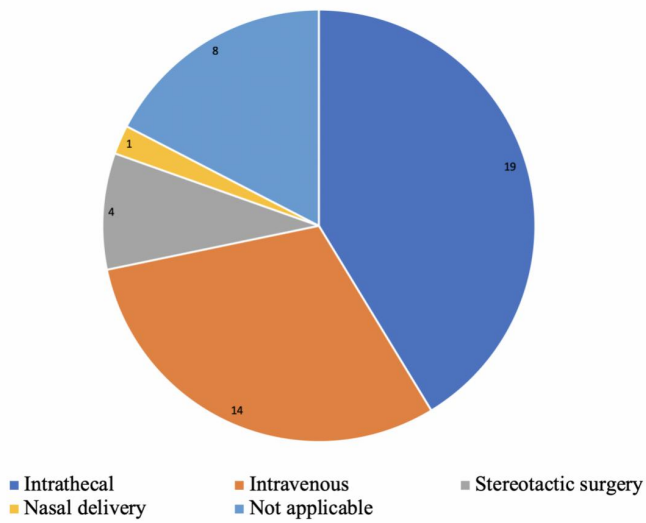
al. (2012)			diplegia, dyskinetic, mixed						function, Psychomotor functions
Chen et al. (2010)	14/2	1-12	Not available	OECs	SS: 2×10^6	Once	No	GMFM, CQS	Neurological function and overall health status
Liu et al. (2017)	105/3	6 mon-12.5	Spastic	BMMNCs and BM-MSCs	IT: $1 \times 10^6/\text{kg}$	4 times with an interval of 3-4 d	Low intracranial pressure reactions: fever	GMFM, FMFM	Gross motor function, fine motor function
Thanh et al. (2019)	25/1	2-15	Spastic	BMMNCs + CD34+ cells	1st time, IT: $17.4 \pm 11.9 \times 10^6$ and $1.5 \pm 1.4 \times 10^6$; 2nd time, IT: $15.0 \pm 12.8 \times 10^6$ and $1.1 \pm 1.1 \times 10^6$	Twice with an interval of 6 mon	Vomiting (32%), local pain (16%), mild fever without any identified infection (4%)	GMFM, GMFCS, MRI, EEG	Gross motor function and a significant decrease in muscle tone values
Dong et al. (2018)	1/1	4	Not available	UC-MSCs	1st time: $7.0 \times 10^6/\text{IT} + 5.6 \times 10^6/\text{IV}$; 2nd time: $1.625 \times 10^7/\text{IT} + 3.6 \times 10^6/\text{IV}$; 3rd time: $2.05 \times 10^7/\text{IT}$	3 times	Not provided	EEG, MRI	EEG and limb strength, motor function, and language expression. The improvement in intelligence quotient was less obvious.
Min et al. (2013)	105/3	10mon-10	Spastic	UCB-TNC	IV: at least $3 \times 10^7/\text{kg}$	Once	Serious: Pneumonia(2.9%), Influenza(2.9%), Death (2.9%); other: upper respiratory tract infection (51.4%), fever (34.3%), loose stool and diarrhea, pneumonia, nausea and vomiting (17.1%), dyspepsia, anorexia, constipation (14.3%), irritability, bronchitis (11.4%), apnea (8.6%), febrile convulsion, urticaria, hirsutism	GMFM, GMFCS, BSID-II, WeeFIM, PEDI, MRI, 18F-FDG-PET/CT	Ameliorated motor, cognitive impairment, self-care

Sun et al. (2017)	63/2	1-6	Hypotonic paralysis, spastic	UCB-TNC	IV: $1.5 \times 10^7/\text{kg}$	Once	(5.7%), seizure, alopecia, acute otitis media, anemia (2.9%) (only potentiated UCB group)	Hives+/- low-grade fever (1.6%)	GMFM, PDMS-2 GMQS, GMFCS, MRI	Gross motor skills connectivity
Gu et al. (2020)	40/2	2-12	Not available	UC-MSC	IV: $4.5\text{-}5.5 \times 10^7$	4 times with an interval of 7 d	Upper respiratory infection (61.54%), diarrhea (38.46%), fever (28.21%), vomiting (20.51%), constipation (10.26%)	Upper respiratory infection (61.54%), diarrhea (38.46%), fever (28.21%), vomiting (20.51%), constipation (10.26%)	GMFM, activities of daily living CFA, 18F-FDG-PET/CT,, MRI	Gross motor and cognitive function

† Protocol 1: child's age were ≥ 5 years old or head circumference ≥ 50 cm; Protocol 2: child's age were < 5 years old or head circumference < 50 cm. ‡ M1: PBMCs first and placebo 6 months later, M7: placebo first and PBMCs 6 months later. BMMNCs: bone marrow mononuclear stem cell; BM-MSC: bone marrow-derived mesenchymal stromal cell; CP: cerebral palsy; TNC: total nucleated cell; PBMC: peripheral blood mononuclear cell; hUCB-MSC: human umbilical cord blood mesenchymal stem cell; NPC: neural progenitor cell; OEC: olfactory ensheathing cell; UC-MSC: umbilical cord mesenchymal stem cell; UCB: umbilical cord blood; IT: intrathecal; IV: intravenous; SS: stereotactic surgery; GMFM: Gross Motor Function Measure; MAS: Modified Ashworth Score; GMFCS: Gross Motor Function Classification System; CP QOL-Child: Cerebral Palsy Quality of Life Questionnaire for children; FIM: Functional Independence Measure; FAM: Functional Assessment Measure; BBS: Berg Balance Scale; BDI: Battelle Developmental Inventory; GMPM: gross motor performance measure; CDCC: CDCC Infant Mental Development Scale; MACS: manual ability classification system; CFSS: Communication Function Classification System; TCMS: Trunk Control Measurement Scale test; PEDI: pediatric evaluation of disability inventory; QUEST: quality of upper extremity skill test; DDST-II: Denver development screening test II; CFA: comprehensive function assessment; PDMS-FM: Peabody Developmental Motor Scale-Fine Motor test; CQS: Caregiver Questionnaire Scale; FMFM: Fine Motor Function Measure; BSID-II: Bayley scales of infant development-II Mental and Motor scales; WeeFIM: functional independence measure for children; PDMS-2 GMQS: Peabody Developmental Motor Scales-2 Gross Motor Quotient scores; PET-CT: positron emission tomography-computed tomography; MRI: magnetic resonance imaging; 18F-FDG-PET/CT: 18F-fluorodeoxyglucose positron emission tomography; EEG: electroencephalogram.



Additional Figure 1 The type of CP patients enrolled in clinical trials.



Additional Figure 2 The administration routes of stem cell therapy for CP.