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Review

Stem cells and cell-based therapies for cerebral palsy: a call for rigor

Lauren L. Jantzie[1], Joseph Scafidi[2] and Shenandoah Robinson[3]

Cell-based therapies hold significant promise for infants at risk for cerebral palsy (CP) from perinatal brain injury (PBI). PBI leading to CP results from multifaceted damage to neural cells. Complex developing neural networks are injured by neural cell damage plus unique perturbations in cell signaling. Given that cell-based therapies can simultaneously repair multiple injured neural components during critical neurodevelopmental windows, these interventions potentially offer efficacy for patients with CP. Currently, the use of cell-based interventions in infants at risk for CP is limited by critical gaps in knowledge. In this review, we will highlight key questions facing the field, including: Who are optimal candidates for treatment? What are the goals of therapeutic interventions? What are the best strategies for agent delivery, including timing, dosage, location, and type? And, how are short- and long-term efficacy reliably tracked? Challenges unique to treating PBI with cell-based therapies, and lessons learned from cell-based therapies in closely related neurological disorders in the mature central nervous system, will be reviewed. Our goal is to update pediatric specialists who may be counseling families about the current state of the field. Finally, we will evaluate how rigor can be increased in the field to ensure the safety and best interests of this vulnerable patient population.

Cerebral palsy (CP), the most common physical disability in childhood, describes a group of permanent disorders that affect movement, muscle tone, and/or posture, and results from non-progressive disturbances of the developing central nervous system (CNS) (1). The prevalence of CP in countries with advanced medical care is 2.22–2.9 per 1,000 (2–4), and is likely higher in economically disadvantaged locations. Motor deficits vary among individuals. To facilitate classification, patients are grouped into five levels of severity using the Gross Motor Function Classification System (5–7). Although the overall prevalence of CP has been stable over time, patterns of motor deficits have shifted. Currently, fewer children born at term are diagnosed with CP, while more preterm survivors have CP (8). A pattern shift was observed in the United States from 2006 to 2010, with an increase in the mildest and most severe CP (Gross Motor Function Classification System I (38 → 48%) and V (17 → 20%), with a reduction in II (16 → 8%) and III (13 → 9%) (IV was stable)) (3). These shifts over relatively short time intervals emphasize the need for clinical trial designs with concurrent controls. Most individuals with CP experience additional neurological comorbidities, related to speech, cognition, behavior, epilepsy, and pain that affect them throughout their lifespan. For example, among people with CP, 75% suffer pain, 50% have intellectual disability, 33% cannot walk, and 25% cannot talk, 25% have epilepsy, 25% have incontinence, and 10% are blind (9). Both motor impairment and non-motor comorbidities vary across individuals, emphasizing that outcome metrics need to assess multiple domains (10). Given the confounding factors of individual deficit patterns from comorbidities, and regional and ethnic/genetic differences in motor deficits, emerging interventions will likely need to be tested in specific populations of patients at risk for, or diagnosed with CP from specific etiologies.

Etiological factors associated with CP include chorioamnionitis and preterm birth, maternal and/or neonatal infections, intrauterine growth restriction, neonatal encephalopathy, infantile traumatic brain injury, and genetic mutations (11–13). Congenital abnormalities in children with CP are more common than appreciated previously (13). Importantly, many children with CP have either multiple overlapping etiologies (14,15) or fail to have a specific cause identified; both factors impact clinical trial design and outcomes (16). Numerous rehabilitative, medical, and surgical interventions help people with CP maximize functional skills (17). Unlike many other neurological conditions where scores have been standardized to compare outcomes for clinical trials, however, rigorous standardized assessments of *multidisciplinary* outcomes for CP interventions are rare (16). No treatment currently exists that cures CP. In sum, the lack of curative interventions has encouraged caregivers to seek promising therapies that may lack scientific and clinical evidence of effectiveness.

[1]Departments of Pediatrics and Neurosciences, University of New Mexico School of Medicine, Albuquerque, New Mexico; [2]Department of Neurology, Children's National Health System, Washington, DC; [3]Division of Pediatric Neurosurgery, Johns Hopkins School of Medicine, Johns Hopkins University, Baltimore, Maryland. Correspondence: Shenandoah Robinson (srobin81@jhmi.edu)

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Box 1 Key issues

Inclusion criteria/patient characteristics
- Precipitating event(s)
- Gestational age
- Chorioamnionitis
- Paripartum stressors and sentinel events
- Neonatal illnesses
  - Sepsis
  - Seizures
  - Bronchopulmonary dysplasia
- Neonatal procedures
- Additional insults in infancy
- Genetic/individual predisposition

Goals of treatment
- Replenish cells
- Restore circuitry
- Normalize cerabral microenvironment
- Attenuate inflammation
- Provide trophic support

Treatment considerations
- Types of stem cells
- Timing of delivery
- Method of delivery
- Location of delivery
- Dosage (How many cells? And how often?)
- Definition of short-term efficacy
- Definition of long-term efficacy
- Individualized treatment
- What is working for similar age groups?
- What is working for similar disease processes?

Figure 1. Key issues regarding stem cell use for cerebral palsy.

Recently, cell-based therapies have been identified as a high-priority research topic for CP. Cell-based therapies might be particularly effective for CP because the CNS damage is non-progressive, and typically involves multiple neural cell types plus CNS inflammation. Both processes are common to other neurological disorders that are already pioneering stem cell research (18–20). The literature pertaining to cell-based therapies has grown considerably over the past 5 years, but the key questions remain (**Figure 1**). Specialists who treat patients with CP have been consistently and appropriately forthright with their concerns (21–26). While cell-based therapies represent new strategies for neural repair, much remains to be learned (27–29). Guidelines for clinical translation of stem cells were released to promote rigorous scientific inquiry and careful ethical deliberations regarding stem cell science (30). Moving forward, reviewers and editors should require compliance with these guidelines. Given the expansive literature on these topics and guidelines for clinical translation (30), several themes and ethical issues related to cell-based therapies have recently been addressed well by others (31). Similarly, powerfully framed discussions of stem cells in the media, and marginalization of vulnerable patients and families in need of hope and access to treatment are reviewed eloquently elsewhere (32). Here, we focus on key scientific aspects of cell-based therapies pertinent to CP, and their translation to early clinical trials.

To move cell-based interventions for the treatment of CP forward, rigorous evaluation of preclinical science is paramount. Developmental programs in the perinatal CNS are intricate, with integrated spatiotemporal cascades that are only partially understood. Preclinical emphasis on the complex dynamic between environmental perturbations, such as ischemia, inflammation, and infection, and their cumulative impact on CNS and musculoskeletal system development are required. Because of the varied etiologies of CP (14,15), and our emerging knowledge of complex neuronal–glial interactions after injury (18), preclinical models that accurately replicate etiologies of CP, the chronic deficits observed in CP, and administration in the chronic phase of CP are paramount. As an example of the rigorous step-wise approach needed, human CNS-derived stem cell lines that successfully repaired thoracic spinal cord injury in rodent models failed to produce improvement with cervical spinal cord injury, the most pressing human form of spinal cord injury that was targeted for early clinical trials (33). This work emphasizes the importance of testing specific agents destined for human trials in clinically relevant and accurate preclinical models (33). Preclinical models that accurately recapitulate CP in each of its etiologies and manifestations are essential, particularly those reflecting chronic motor deficits (34–37). Moreover, preclinical models that encompass placental contributions and inflammation may be of high utility for CNS injury from chorioamnionitis and preterm birth (11,37,38), the leading

cause of CP in the United States. Multifactorial etiologies will be more challenging to model well in animals.

Similarly, clinical outcomes in trials should include specific, reliable *a priori* measures of efficacy that are also used to evaluate current medical and surgical interventions. Outcomes should include the full spectrum of motor impairment in CP, including measures of spasticity and dystonia (10). Specifically, a Common Data Elements approach, such as that used in pediatric traumatic brain injury (39), is needed to standardize outcomes and analyses across trials. To guide human trials, robust tracking of acute and chronic outcomes using age-appropriate, validated measures, as well as risks and benefits are needed. High-resolution imaging of both structural and functional CNS connectivity is essential (40). In addition to childhood imaging biomarkers, neonatal neuroimaging, electrocorticography, and systemic physiologic serum biomarkers are warranted to provide subject stratification and timely feedback for clinical trial design. More sensitive functional outcomes are needed to delineate an individual's natural history from real therapeutic benefits of cell-based therapy. Specific, molecular-targeted biomarkers are needed to capture changes in neural cell development and injury to allow individually tailored application of interventions, similar to the application of precision medicine in cancer treatment.

WHO ARE THE OPTIMAL CANDIDATES FOR TREATMENT?

The etiology of CP is heterogeneous, and some children have CP from mixed or unknown etiologies. To identify who would be best served by a specific cell-based therapy, the etiology and extent of CP for those screened for clinical trials should be clarified, ideally by a combination of clinical history, functional measures, serum biomarkers, and structural and functional imaging. As the etiology of CP largely predicts clinical outcome and therapeutic response, what cell-based therapies can accomplish will be partially defined by the local CNS microenvironment after injury. Notably, etiology may also have bearing on immune response and persistence of an inflammatory reaction that may dictate candidates for therapy (13). Preterm birth and *in utero* exposure to infection and inflammation are known precipitators of CP and sustained systemic prenatal and postnatal inflammation (11,14,41,42). Chorioamnionitis and fetal inflammatory response syndrome (FIRS) are highly associated with the subsequent diagnosis of CP (43–45). Neonatal illnesses including sepsis, epilepsy, and bronchopulmonary dysplasia likely contribute to the functional manifestation of deficits in CP and the therapeutic response. To identify predictors of severe motor impairment at 5 years of age, 1,469 former preemies ($<1{,}250$ g at birth) from the United States, Europe, Israel, and Australia were evaluated (46). Almost 20% had severe motor impairment that could be predicted well by seven neonatal, six term equivalent, and seven infancy variables (46). If a specific cell-based therapy is to be tested in an early clinical trial, a validated scoring system to predict eligibility and stratification is essential. Ideally, stratification would include biomarkers with body fluids and/or imaging/electrocorticography to account for an individual's genetic predisposition and his or her likelihood to respond to treatment. This process would be similar to how cancer treatment has evolved from basic descriptors of histopathology to using molecular and genetic signatures to individualize and tailor therapeutic regimens. Stratification of older infants, toddlers, and children will likely be even more challenging as the perinatal data will be remote and harder to quantify with biomarkers. Imaging biomarkers may prove particularly useful for toddlers and children to improve stratification for early clinical trials.

WHAT ARE THE GOALS OF THERAPEUTIC INTERVENTIONS?

While the primary goals of cell-based therapies is to replenish cells, restore circuitry, normalize the microenvironment, and thus CNS activity, accomplishing this depends on a spectrum of diverse neural cells interacting in concert in the correct spatiotemporal pattern (47). Unlike neurological diseases with specific neuronal sub-populations presumed to be the primary pathogenic targets (i.e., nigrostriatal dopaminergic neurons in Parkinson's disease), most etiologies of CP encompass widespread glial activation and injury, plus loss of multiple neuronal subtypes, including migrating GABAergic interneurons, excitatory neurons, and subplate neurons (48–50). Moreover, multiple types of neural cells are involved in the pathophysiology of CP (51). In this context, some neural cell populations are likely more resistant to injury than others, and by contrast, some cell types are likely more easily replenished than others. For example, pyramidal neurons dictate the position of cortical interneurons through modulation of local neuronal activity and soluble factors in the local microenvironment (52). Replicating such interactions during repair to guide new interneurons will be challenging. Neurons in particular are heavily influenced by adjacent cells including astrocytes, oligodendrocytes, and microglia, and the local extracellular microenvironment. Like neuronal subtypes, multiple subtypes of astrocytes, oligodendrocytes, microglia exist based on morphological, regional, and functional phenotypes (53,54). While morphological classifications and changes are perhaps the most obvious, glial populations vary by brain region and age, each with their own unique developmental trajectories (54,55). Further, astrocytes and microglia are driving forces of the CNS neuroinflammatory response that shapes neuronal and oligodendroglial survival and maturation (56). Thus, the glial inflammatory responses strongly reflect individual patient characteristics and genetic predisposition to both injury and recovery.

The glial neuroinflammatory response is closely intertwined with the local microenvironment with the extracellular matrix, immune cells, cytokines, and chemokines. Injury during development also affects neurotransmitters, receptor subtypes, glutamatergic and GABAergic neurotransmission, and levels of cotransporters (48,57–59). In a study by Lin *et al.* (60), white blood cells from 5-year-old children who were born extremely preterm were collected and challenged with lipopolysaccharide, a bacterial endotoxin used to stimulate

inflammatory responses. Leukocytes from preterm survivors with CP had grossly elevated inflammatory responses characterized by high tumor necrosis factor levels, while preterm survivors without CP did not (60). Even at an unstimulated baseline, the former preemies with CP had much higher levels of inflammatory molecules (60). This study highlights the ongoing role of the immune system in CP pathophysiology, and also emphasizes that immune function may be different in patients with CP. These data also emphasize that clinical biomarkers are needed to assess the state of the immature and mature immune system, systemic, and neuroinflammatory responses in CP patients. This is required to answer the question as to whether those who are predisposed to, or have CP, will respond differently to cell-based therapies in the context of the diverse trajectory of all neural cell populations.

TYPES OF STEM CELLS AND CELL-BASED THERAPIES

The distinguishable phenotypic features of stems cells are an integral component of the understanding the biology and potential for cell-based therapies in CP (61–64). Indeed, to determine their safety, efficacy, and putative mechanisms of beneficial action, stem cell identity is paramount. It is necessary to determine the source of stem cells and thus mechanisms of action associated with cell therapy. Notably, diverse types of stem cells have been identified for potential use in CP (**Figure 2**). Here we focus on those cells most commonly proposed for CP. While autologous cells may superficially seem appealing, allogenic cells are likely better for preterm neonates because of limited quantities, stem cells arising in the context of the etiology of CP (e.g., fetal inflammatory response syndrome), and immune immaturity (43). Both autologous and allogenic stem cells can be challenging to reliably produce for clinical trials (65–67).

Umbilical cord blood (UCB) is a rich source of mononuclear cells that contains high levels of primitive multipotent stem/progenitors, and UCB contains mesenchymal stem cells (MSCs) and a large number of endothelial cell precursors (68,69). Cells from UCB are immune naïve and are also capable of differentiating into other cell phenotypes, including neural lineages (70,71). Despite being relatively easy to obtain, feasible, and potentially efficacious for use in neonatal brain injury, the quantity of umbilical cord blood mononuclear cells obtained from each infant and the amount of stem cells within each batch are variable and heterogeneous. Similarly, preterm babies may not be the best candidates for autologous stem cell transplants as collection volumes are proportional to gestational age, with younger infants yielding the smallest samples; it is unknown how maternal and fetal complications including placental insufficiency, intrauterine growth restriction, and preeclampsia alter the relative proportion of stem and immune cells (72–74). Naturally, this brings concerns about standardization of optimal cell preparation, function, and dosing, which preclinical studies indicate are predictive of efficacy (22,75).

Individual MSCs are readily isolated from placenta, Wharton's jelly, and umbilical cord, as well as adult bone marrow and adipose tissue (76). Regardless of the cell source, each MSC population exhibits a distinguishing and specific set of cell-surface antigens that define multipotency. Typically, MSCs are well tolerated immunologically given their low expression of major histocompatibility complex I and lack of major histocompatibility complex II molecules (77). Wharton's jelly-derived MSCs are less differentiated than bone marrow-derived MSCs, and thus may be more likely to produce neural cells. Similarly, cells derived from Wharton's jelly or UCB show lower immunogenicity, higher proliferative capacity, and increased paracrine potency compared with adult tissue-derived MSCs (78,79).

All types of MSCs can be easily expanded in culture and maintain their undifferentiated state. This is an important consideration when sources of MSCs are an issue and may be

Box 2 Stem cells for potential use in cerebral palsy

Embryonic stem cells (ESCs)

Human amnion epithelial cells (hAECs)

Mesenchymal stem cells (MSCs)
- Umbilical cord blood
- Wharton's jelly (umbilical cord connective tissue)
- Placenta
- Bone marrow
- Adipose tissue

Hematopoietic stem cells (HSCs)
- Bone marrow

Human induced pluripotent stem cells (hiPSCs)
- Somatic cells

Neural stems cells (NSCs)
- Olfactory ensheathing cells
- Oligodendrocyte progenitor cells (OPCs)/glial progenitor cells (GPCs)
- Restricted glial precursors

Figure 2. Type of stem cells for potential use in cerebral palsy.

Box 3 Mechanisms of beneficial action

Acute trophic mechanisms
- Promote cell survival via release of neurotrophic factors and secretion of soluble mediators
  - Support cell migration, proliferation, differentiation, and/or promote angiogenesis and new blood vessel formation
  - Paracrine

Subacute anti-inflammatory mechanisms
- Immune modulation, attenuation of endogenous neuroinflammation, decreased production of excitotoxins, chemokines, and free radicals
  - Secretion of soluble factors
  - Inhibition of lymphocyte proliferation
  - Inhibition of monocyte differentiation into macrophages of dendritic cells
  - Modulation of microglial reactivity and inflammatory profile
  - Modulation of antigen-presenting cell functions
  - Modulate immune cell phenotype

Chronic regenerative mechanisms
- Replacement and/or repair of damaged brain cells brought about by engraftment and proliferation
  - May/may not include differentiation of transplanted cells into new microglia or astrocytes
  - Facilitate cell–cell contact
  - Modulate/repair extracellular matrix and improve microenvironment homeostasis

Figure 3. Putative beneficial mechanisms of action of stem cells for use in cerebral palsy.

necessary to provide a therapeutic dose. While autologous stem cells from UCB can be an advantage because of their ability to bypass host immune cell response and graft rejection (80), there are limitations. Most children with CP do not have bio-banked UCB. In this context, several preclinical and clinical trials have shown that allogeneic UCB stem cells have therapeutic potential for patients with CP (81,82). Full understanding, however, of their safety and efficacy is still limited. One clinical trial for CP has assessed the putative difference between autologous and allogeneic stem cells (83). Treatment with allogeneic UCB stem cells significantly decreased numerous proinflammatory cytokines (interleukin-6, interleukin-1α, tumor necrosis factor-β, and RANTES (regulated on activation, normal T cell expressed and secreted)), and showed improvement in motor and social behavior compared with autologous UCB treatment groups (83). More rigorously designed clinical trials are needed to test both safety and efficacy.

Human-induced pluripotent stem cells (hiPSCs) are being explored for numerous neurological disorders that affect the mature CNS. hiPSCs hold the most promise for disorders where a specific defect, such as an enzyme abnormality, has been identified, and importantly, the several weeks needed to generate the cells will not impact outcome (20,84,85). Given the diverse etiologies and perinatal events associated with CP, hiPSCs perhaps hold less promise for some patients with CP because only a specific cell type is generated after several weeks.

MECHANISMS OF BENEFICIAL EFFECT

Stem cells are multipotent and respond to microenvironmental cues to guide differentiation and cellular activity (86). Although the mechanisms are incompletely understood, transplanted stem cells have proven to be capable of replacing neurons, glia, and vascular cells, and releasing trophic factors. The primary mode of action involved in stem cell-mediated functional recovery with in CP is derived from paracrine effects, and less so from replacement or engraftment (**Figure 3**) (21,26,87,88). Secreted factors facilitate neurogenesis, angiogenesis, synaptogenesis, and immunomodulation (89). Notably, effects of stem cells may differ based on cell origin and the time from administration. Early after administration, restoration of the microenvironment is the mechanism likely driving repair, with notable effects on immune modulation, cell adhesion molecules, including cadherins, selectins, and integrins, and intracellular metabolism (90). In the subacute phase, or weeks after administration, paracrine mechanisms are likely most prominent. Stem cell release of growth factors and other neuromodulators may direct microglial phenotypes, including fine-tuning the local microenvironment to favor anti-inflammatory glial phenotypes (89). Lastly, chronic effects of stem cell therapy may impact neural cell survival, and thus engraftment and formation of direct intracellular interactions to provide maintenance of glial support, neuronal circuits, and myelination of axons (89). Investigators have tested identical cell dosages in a specific injury model, and noted that cells delivered 3 days after the injury were most effective, while cells delivered days earlier or later were not (91). Given that many sick neonates suffer a series of potentially injurious perinatal events, the timing of cell-based treatment, and standardization of dosing regimens during clinical trials becomes even more challenging.

Stem cells also secrete exosomes and other microvesicles designated for precise receptor-mediated intracellular communication. Exosomes carry cargo, including microRNA, proteins, biologically reactive lipids, and receptors that may be beneficial to the injured developing brain. Recently, extracellular vesicles from mesenchymal stromal cells have been shown to rescue cognitive impairments after traumatic brain

injury (92). This investigation is one of several publications showing the benefits of exosomes in repair. Similarly, Drommelschmidt *et al.* (93) demonstrated administration of human MSC-derived exosomes after early postnatal inflammatory injury in rats improved in short- and long-term outcomes, including cognition, motor skills, and microstructural diffusion tensor imaging abnormalities (93). Indeed, exosomes hold significant promise as they may be as efficacious and relatively easier to consistently produce and administer for clinical trials.

Most restoration of function in CP after cell-based interventions is likely due to paracrine effects on the CNS microenvironment, rather than direct cell replacement (88,94). Each stem cell type within the context of its environment expresses a specific set of factors, or "secretome" (95–97). Indeed, the secretome potentially offers a more standardized way to quantify and characterize a cell preparation from a specific source, and even to manipulate it *in vitro* to optimize secreted factors (98). In this way, the cell types and even "dosage strength" could be quantified to allow comparison of interventions across patients and trials, especially from autologous sources.

OPTIMAL LOCATION, DOSAGE, AND TIMING FOR CELL-BASED DELIVERY

Determining the optimal route of stem cell administration is a key issue to be resolved to advance successful clinical translation for the treatment of CP. Currently, numerous routes of administration have been tested including intraventricular, intrathecal, intranasal, intramuscular, intra-arterial, or intravenous (88,91,99,100). Systemic routes may be adequate for modulating inflammation, but are likely suboptimal for treating local CNS lesions because injected cells may be retained in other organs, and by the inability of cells to cross the blood–brain barrier (99). Similarly, intrathecally injected cells may not diffuse to the brain. Direct cerebral injection may be disadvantageous in young, unstable, and medically fragile infants. In the clinical setting where intraventricular devices are required for cerebrospinal fluid diversion or removal, local administration may be more feasible. Injection location within the brain even may be important. Piao *et al.* (101) administered human embryonic stem cell-derived oligodendrocyte progenitors after cranial radiation. Cells injected into the cerebellum improved motor function, while the same cells injected in frontal lobes improved cognition (101). Similarly, embryonic motor neurons form connections with mature motor but not visual areas, while embryonic visual neurons connect with mature visual but not motor areas (102). Taken together, these preclinical studies emphasize the importance of timing, location, and dosing, which are rarely standardized in clinical trials. The current literature suggests that it is somewhat premature to focus on these factors, until preclinical studies demonstrate effective delivery of standardized cell-based preparations to desired targets using clinically relevant paradigms and outcome measures.

Other investigators have studied the impact of dosing regimens, including number of cells, dosing frequency, and cell localization (75,102–104). Drobyshevsky *et al.* (75) elegantly showed early dose–response relationships in efficacy related to human UCB cell number in a rabbit model of CP, using short-term (11 day) outcomes. Infusion of a higher dose of UCB cells mitigated abnormalities in posture, tone, and dystonia, and while half the dose showed limited improvement (75). Moreover, UCB cell tracing with magnetic resonance imaging biomarkers, including Feridex (superparamagnetic iron oxide-labeling) and PCR for human DNA found little penetration of infused cells into the brain in the newborn period, suggesting that short-term benefits were related to paracrine signaling mechanisms as opposed to direct cellular integration or proliferative effects (75). Finally, the timing of cell delivery seems to matter. Cells administered 3 days after injury, but not earlier or later time points, effected repair assessed at 30 days after injury (91). Taken together, these studies emphasize the profound importance of cell type, source, preparation, delivery route, dosing, and timing to efficacy, in addition to confounding factors such as etiology and severity of injury of CP.

TRACKING SHORT- AND LONG-TERM EFFICACY

Owing to the high burden of disease in people with CP, therapeutic efficacy and reliability of putative treatments is paramount. As indicated above, and by many studies, the number, phenotype, and viability of cells administered to each patient or research subject needs to be characterized and defined. Specifically, the optimal number of cells needed in humans with varying CNS injuries and how the phenotype of the cells to be administered relates to their efficacy in a specific CNS injury is unknown. With autologous stem cells, it is unclear how the health of the patient before the cell harvest contributes to the yield, viability, and phenotype, or the downstream paracrine signaling or beneficial effects. For example, do MSCs or amniotic epithelial cells harvested from a baby with chorioamnionitis and fetal inflammatory response syndrome offer a protective capacity? Similarly, would ideal or "healthy" cells be beneficial in an "unhealthy" neural microenvironment such as exists in preterm infants exposed to chorioamnionitis, term infants with hypoxic–ischemic encephalopathy, or infants with brain trauma? These key preclinical and clinical considerations are addressed in **Figures 4 and 5**.

Numerous investigators have attempted to address questions related to efficacy and longevity of stem cells in the brain using sophisticated methods of tracking. In addition to labeling cells with preparations of iron nanoparticles like Feridex and Resovist and evaluating with magnetic resonance imaging, magnetic particle spectroscopy may be a quantitative, easy to interpret, noninvasive form of imaging stem cells that could be used as an adjunct to anatomical imaging modalities (105). Specifically, magnetic particle imaging, a novel tomographic technique, may overcome several limitations of traditional magnetic resonance imaging detection

Box 4 Gaps in preclinical knowledge

Technology/techniques
- How to efficiently and consistently produce cell-based therapies for different disease processes
- What are fast, reliable screens of functional efficacy

Biomarkers of disease process
- How to identify when the local microenvironment is:
  - In need of inflammation modulation?
  - The most receptive to neural repair?

Types of cells to be used
- Are certain cell-based therapies better for specific etiologies or pathophysiology?
- Do different cell-based therapies protect or support different host cells?
- Do certain cell-based therapies have longer efficacy?

Lessons learned from closely related neurological disorders
- Are disease defined by enzyme loss more amiable to repair than those defined by cell loss?
- Does type of cell-based therapy differ based on etiology or patient age?

Long-term efficacy
- How do cell-based therapies mediate sustained repair?
- What biomarkers of repair correlate with functional improvement?

Preclinical modeling
- Does preclinical injury and efficacy reflect the human condition?
- Are outcome measures sensitive enough for translation to humans?

Figure 4. Preclinical knowledge gaps related to stem cell use in cerebral palsy.

Box 5 Key clinical questions

1. What is the most efficient production of cell-based therapies for different disease processes?
2. Do different cell-based therapies protect or support different neural cells?
3. Does maturation of the immune system impact cell-based therapy efficacy?
4. Do different cell-based therapies effect longer recovery than others?
5. Do protocols have to be tailored to patient age?
6. Should cell-based therapies for preterm infants differ from term infants?
7. How do we standardize phenotype, viability, and yield for reproducible dosage and delivery of cell-based therapies across patients and centers?
8. How do we optimize cocktail design (agents, timing, dosage, adjunctive therapies) for each neonate?
9. What adverse events should be monitored in all trials regardless of route of administration?
10. Should allogenic cell-based therapies be matched to individual recipients based on age and sex and etiology of brain injury?
11. Are there short-term outcome measures (30 days−6 months) for clinical trials that predict long-term (2 years+) benefit?
12. How do we repair the neural network in CP, as opposed to just replacing cells?
13. How do we identify when local CNS microenvironment is need of modulation and most receptive to neural repair?

Figure 5. Key clinical questions related to the use of stem cells for cerebral palsy.

superparamagnetic iron oxide approaches by eliminating hot spots and offering a direct detection and quantification of cells. These techniques may be especially useful to tracking short-term efficacy and confirmation of cell placement in clinical investigations.

With respect to long-term tracking of efficacy and effects on brain circuitry, many approaches have been used (106). Important insights into the functional properties of neural grafts generated from stem cells have been gained from studies at the cellular level and also by assessing their functional impact in animal models of disease (106). Patch-clamping experiments have demonstrated that neurons derived from embryonic stem cells or hiPSCs develop electrophysiological properties, including evoked and spontaneous firing of action potentials and evidence for functional afferent input from host neurons after several weeks (106). Taken together, these data support the view that the development and maturation of implanted neurons generated from human cells are protracted. Sophisticated studies using optogenetic approaches have provided more rigorous analyses and evidence that neurons generated from pluripotent stem cells are not only capable of developing intrinsic electrophysiological properties *in vivo* but can also establish functional patterns of afferent and efferent connectivity in the host brain (106,107). Indeed, these studies, and those of others, confirm that neuron growth and myelination can take several weeks to months (106,108). The maturation and electrophysiological properties of hiPSC-derived oligodendrocytes have also been described (85). Importantly, those

investigations revealed that human oligodendrocytes derived from pluripotent stem cells mature with conserved expression of maturation-specific physiological, functional, and channel properties that overlap with rodents, including characteristic developmental switches in glutamate receptor composition (85).

EARLY CLINICAL TRIALS

Most cell-based early trials for CP have shown that stem cell administration is well tolerated and safe (22,109–115). A recent meta-analysis suggests a moderate short-term positive treatment effect on gross motor outcomes (22). Caution is warranted, however, as standardized long-term outcomes used for other CP intervention trial outcomes have not been used in trials reported to date (82,110–115). No trial thus far has reported primary outcomes that are typical for surgical interventions for CP (116), or for cell-based interventions for pediatric neurodegenerative diseases, such as a 10% change in GMFM-66 or GMFM-88 at 24 months (117). It is difficult to determine whether effect is of any clinical significance because of the wide age ranges of the participants studied within individual trials (22), and the need to account for developmental curves in assessing motor outcomes (7,118).

CONCLUSIONS

CP is a collection of multietiologic disorders unified by early CNS disturbances and motor impairments that presents unique challenges to patients, families and caregivers, and physicians. Given the changing needs of people with CP throughout their lifespan and the degree of plasticity in the injured CNS, effective, safe evidence-based therapeutic strategies are paramount. Regarding cell-based therapies, rigorous standardization of cell type, source, preparation, delivery route, dosing, and timing have profound implications for efficacy. Confounding factors such as etiology and changing severity of CP across the lifespan challenge clinical trial design. Just as we expect medications such as antibiotics or chemotherapeutic agents to adhere to stringent guidelines regarding dosing and treatment regimen, cell-based therapies for CP must also undergo similar rigorous standards. Continued identification of mechanisms of genetic, structural, and functional variables that drive impairment and recovery, and the development of biomarkers of CP, will advance scientific support for use of cell-based therapies. Effective interventions during the acute, subacute, and repair phases following injury could possibly mitigate or lessen the long-term disability in person with CP. Increased rigor of both preclinical and clinical efforts is essential to move the field forward and improve precision diagnosis and treatment for each individual with CP.

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