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The Potential for Stem Cells in Cerebral Palsy – Piecing Together the Puzzle

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1) Summary

The substantial socio-economic burden of a diagnosis of Cerebral Palsy (CP), coupled with a positive anecdotal and media spin on stem cell treatments, drives many affected families to seek information and treatment outside of the current clinical and scientific realm. Preclinical studies using several types of stem and adult cells – including mesenchymal stem cells (MSCs), neural precursor cells (NPCs), olfactory ensheathing glia (OEG) and Schwann cells (SCs) - have demonstrated some regenerative and functional efficacy in neurological paradigms. This paper describes the most common cell types investigated for transplant *in vivo* and summarizes the current state of early phase clinical trials. It investigates the most relevant and promising co-administered therapies – including rehabilitation, drug targeting, magnetic stimulation and bioengineering approaches. We highlight the need for adjunctive combinatorial strategies to successfully transfer stem cell treatments from bench to bedside.

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2) Introduction

Cerebral Palsy (CP) comprises a heterogeneous group of non-progressive developmental disorders and is marked by loss of neuromotor function. This is often accompanied by demyelination of cerebral white matter tracts and loss of axons and grey matter. Children with CP often demonstrate concomitant deficits of motor control, cognition, learning and other complex neurological functions. Stem cell therapy has become a topic of interest in the popular media and stem cell transplantation is often perceived as a "cure" for CP and other neurological conditions. This misperception (along with anecdotal claims of efficacy and denial of negative side effects by unregulated foreign clinics purporting to offer "stem cell therapy") presents an unrealistic bias, influencing stakeholders' perceptions of availability, efficacy and safety of "stem cell treatments" for CP (see review[1]). Despite promise in preclinical and clinical trials using stem cells for CP, there remains a knowledge gap surrounding the optimal source and type of cells, timing of treatment and possible mechanisms of action. We present here an updated summary of the current state of stem cell science for CP and describe current relevant clinical trials. This article highlights the need for additional optimization before stem cell treatments can be fully realised as a therapeutic option for CP.

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I. The Etiology of CP

CP is a heterogeneic disability and its etiology is equally multivariate. It remains poorly understood despite a large body of research and literature on the topic. The current "multi-hit" hypothesis surrounding its etiology maintains that alignment of multiple probabilities, rather than a single risk factor, is responsible for CP onset. Indeed, several neonates survive major insults without any evidence of impairment, while others develop severe infarcts almost spontaneously. While an exhaustive description of all causative mechanisms for CP is beyond the scope of this manuscript, the most common risk factors are:

- Preterm birth (and associated complications)
- Intrauterine Growth Restriction
- Maternal infection - viral, bacterial and protozoan (most commonly TORCHS)

T –Toxoplasmosis/ Toxoplasma Gondii

O– "Other" infections (Coxsackievirus, Varicela-Zoster Virus, HIV and Parvovirus B19)

R – Rubella

C – Cytomegalovirus

H – Herpes Simplex Virus

S - Syphilis

- Perinatal and Intrapartum difficulties (ex. hypoxic/ischemic injury)
- Other congenital infections (ex. sepsis, meningitis, encephalitis, tetanus and chorioamnionitis [2–5])

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II. CP Pathophysiology

Inflammation, which may occur through transplacental cytokine passage or by fetal production[6], plays a key role in the pathophysiology of CP. Increased serum concentrations of B-lymphocyte chemo-attractant, ciliary neurotrophic factor (CNTF), epidermal growth factors, IL-12, IL-15, monocyte chemo-attractant protein-3, and others were found to be present in children with CP[7], and there is evidence for maturation-dependent changes in both fetal brain structure and response to immune challenge [8]. Pro inflammatory cytokines - which may act 1) directly on pre-oligodendrocytes, 2) via secondary, effector-mediated processes, or most likely, 3) through a combination of the two - have been linked to periventricular leukomalacia (PVL), the most common physiological cause of CP[9].

Due to GluR subunit composition, the immature oligodendrocyte is intrinsically vulnerable to ischemic and inflammatory insult, particularly to Glutamate-mediated excitotoxicity [10, 11]. Oligodendrocytes express GluR3 and GluR4 receptors during development, but – unlike pre-progenitors or mature oligodendrocytes - fail to express GluR2. This absence of GluR2 leads to enhanced Ca2+ membrane permeability, increased Ca2+ influx and resultant excitotoxic cell death. Therefore, as a result, after global ischemic hypoperfusion, periventricular pre-oligodendrocytes, which are most distal to major cerebral vasculature, are selectively abolished, causing focal necrotic lesions and later widespread apoptosis associated with PVL [12]. These areas spatially correspond with descending corticospinal tracts, leading to the neuromotor symptoms in

the most common CP phenotype. Likewise, unilateral hemorrhagic or hypoxic/ischemic injury leads to neuronal cell death in motor control areas, resulting in the second most common subtype of CP, spastic hemiplegia (Figure 1).

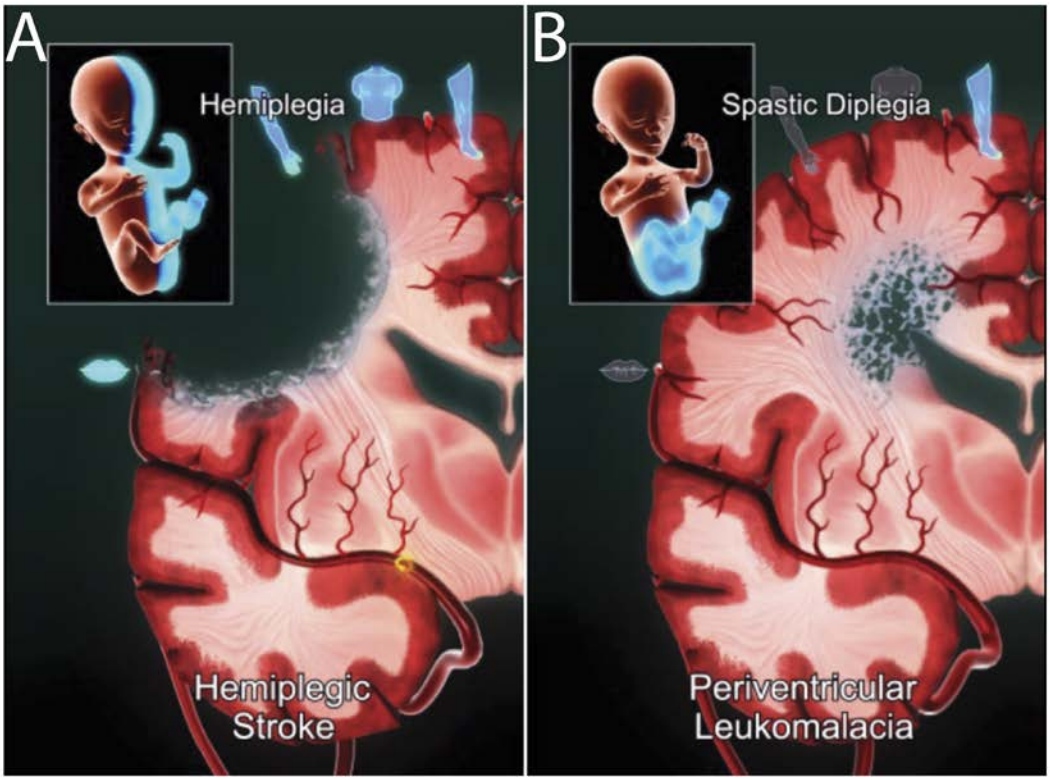

Figure 1: Schematic Diagram Representing the Pathophysiology of Hemiplegic and Diplegic Cerebral Palsy

A) Hypoxic Stroke causes hemispheric loss of cortical tissue perfused by the occluded artery. Affected regions overlap the corticospinal tracts, which control lower limbs, trunk and forelimbs. Resulting gross neuromuscular deficits on the contralateral side of the body translate to hemiplegic cerebral palsy. B) Chronic hypoperfusion causes ischemia in developing periventricular watershed regions of the brain. Since the pre-oligodendrocyte is particularly susceptible to ischemic injury, supporting glial cells are preferentially ablated in this region, causing periventricular leukomalacia. These watershed regions overlap with descending corticospinal axons, and without sufficient signal propagation, many motor neurons are damaged. Altogether, these changes lead to spastic diplegia.

3) Current Clinical Treatments for CP

At this time, non-surgical clinical treatments for CP are restricted primarily to rehabilitation and supportive strategies. Many of the current clinical treatments for CP attempt to promote recovery through stimulation of plasticity via activity-dependent changes in excitability and synaptic strength; this enhanced plasticity could be complimentary to regenerative therapeutics. Future regenerative stem cell strategies are likely to be used in combination with these rehabilitation and physiological measures to facilitate clinical translation.

A. Rehabilitation

Rehabilitation therapy is employed under the premise that regularly utilized neuromotor pathways will strengthen circuit plasticity and also that repeatedly used muscle systems will lead to enhanced co-ordination and function. It is currently the most effective treatment for CP symptoms.

B. Physiotherapy and Occupational Therapy

Physiotherapy (PT) and Occupational Therapy (OT) are the primary health care professions associated with rehabilitation. PT encompasses strengthening of ability, co-ordination, function and movement while OT focuses on application of skills toward

activity-defined goals. However, optimal frequency and duration of PT and OT remain to be fully elucidated (reviewed in [13]).

C. Constraint-Induced Movement Therapy (CIMT) and Bimanual Therapy

CIMT is employed in hemiplegic CP and consists of unilateral constraint on the unaffected side – usually via casting – coupled with shaping techniques and repetitive practice. Alternatively, bimanual training attempts to balance interhemispheric neuromotor competition and involves practiced use of integrated dexterous tasks using both affected and unaffected limbs. Both show promising clinical results, despite little mechanistic preclinical animal data to support or explain their efficacy [14–16].

D. Gaming

Virtual reality simulators and active video games (AVGs) require advanced and co-ordinated leg, arm and body movement. AVGs can increase a child's participation in active rehabilitation, daily living and engagement in social activities. Furthermore, it can improve self image and mental health. A recent systematic review showed an average increase in energy expenditure of 222% (±100%) during AVG play in typically developing children[17].

E. Intrathecal Baclofen Pumps

Baclofen is a gamma-aminobutyric acid (GABA) agonist, which impedes the release of excitatory neurotransmitters in the spinal cord, and is utilized to treat generalized

spasticity or dystonia. Baclofen can be infused via an intrathecal infusion pump,[18] reducing some side effects such as sedation, confusion, dizziness, ataxia, weakness, nausea, hypotension and parasthesia[19].

F. Botulinum Toxin (Botox)

Botulinum toxin (Botox) is a neurotoxin that causes reversible neuromotor blockage (3-6 months duration). It can provide temporary relief of tone, manage spasticity-related pain, promote longitudinal muscle growth and improve general motor function in targeted areas. Strong safety and efficacy evidence exists for its clinical use and low dose injections at multiple sites can be used safely in children [20, 21].

G. Transcranial Magnetic Stimulation (TMS)

TMS utilizes an electromagnetic coil, placed on the scalp, to create electromagnetic pulses that can focally and non-invasively depolarize specific neuronal cortical and sub-cortical targets. Repeated TMS can induce long-lasting activational changes (Long term potentiation (LTP) and long term depression (LTD)) in selected brain areas, particularly those affecting neuromotor control. In animals, intermittent, high-frequency stimulation is generally associated with LTP and longer periods of lower frequency stimulation produce LTD. Although this is promising in model systems, the frequencies and intensities of signals used for LTP can lead to seizures in humans[22]; thus, this treatment needs further work to establish an optimal protocol for delivery.

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4) Stem Cells

There are many types of mature and stem cells which have been used for experimental (and early-phase clinical) treatments in CP and other CNS paradigms. These studies utilize intraparenchymal, intrathecal and intravenous delivery.

I. Pluripotent Stem Cells

A. Embryonic Stem Cells (ESCs)

Embryonic stem cells (ESCs) are derived from the inner cell mass of the developing blastocyst and are pluripotent in nature. The capacity of ESCs to propagate in culture over many passages provides an almost unlimited supply of cells. However,their allogenicity necessitates immunosuppression in transplant scenarios. Furthermore, their ethical derivation (generally requiring destruction of the embryo) remains highly controversial, making alternative sources more attractive.

B. Induced Pluripotent Stem Cells (iPSCs)

Induced pluripotent stem cells (iPSCs) can be derived from human adult somatic tissue and arguably resemble embryonic stem cells morphologically, antigenically and phenotypically, offering an alternative source of pluripotent cells. By upregulating "Yamanaka" transcription factors OCT4, c-Myc, Sox2 and KLF4, [23, 24] iPSCs have been generated from several species, including mouse and human. As they can be prepared from an individual's own cells, iPSCs can potentially reduce the possibility of immune

rejection while circumventing ethical issues that arise from cells directly derived from embryonic origin. The most efficient current derivation methods use viral vectors, and are thus unsuitable for human translation. These methods are further hampered by low iPSC yield and the recent discovery that human iPSCs often display somatic coding mutations as well as epigenetic variability[25]. The recent use of non-integrating vectors, such as the piggybac transposon[26], excisable recombinase systems such as Cre/picornavirus systems[27], ribonucleic acid[28], small molecules[29] and non-integrating viruses [30], have addressed some of the aforementioned concerns.

C. Epigenetic Variability

Although iPSCs share the same genetic signature as their somatic counterparts, differences with pluripotency are at least partially due to epigenetic variability. Indeed, methylation patterns and high-order chromatin organization in large heterochromatin domains are differentially expressed in pluripotent and differentiated cells[31]. Furthermore, this epigenetic variability is cell-type specific. iPSCs derived from distinct somatic populations reveal similarly distinguishable methylation patterns, characteristics and differentiation potential, despite identical iPSC derivation[32, 33]. Functionally, synovial tissue MSCs were more effective in cartilage repair than MSCs derived from bone marrow, muscle or fat, indicating that this epigenetic variability in genetically identical specimens can influence performance *in vivo*[34]. Differential methylation patterns are also observed between ES and iPS-derived pluripotent stem cells[35], indicating that

epigenetic, as well as genetic, reprogramming may be necessary for successful human translation.

D. Fate Restriction

Despite their relative ease of expansion, pluripotent cells display a high degree of plasticity and can form teratomas upon transplant. For ESC or iPSC-derived cells to be used in successful cell therapy, they must be differentiated into multipotent or fate-restricted progenitors. Differentiation can be guided by using a number of factors; however, NPCs created via the default pathway seem to be most effective at turning into myelinating oligodendrocytes *in vivo*[36]. The default pathway differentiates pluripotent cells along a neural lineage by first fate-restricting to a primitive, LIF-dependent, state before reaching a definitive, more differentiated LIF-independent phenotype[37].

Alternatively, methods such as direct lineage reprogramming, or “trans-differentiation” have been used to laterally reprogram cells of one mature phenotype into another. Built on analysis of differential transcriptional regulation made popular by iPS technology, trans-differentiation has been used in neural paradigms to derive neurons or tri-potent neural precursors from fibroblasts[38–41]. Functional neurons have also been generated from hepatocytes, which originate from a different germ layer than neural tissue[38]. *In vivo,* neuroectodermal trans-differentiation has been explored between post-mitotic callosal and corticofugal neurons[39] as well as retinal photoreceptors[40].

II. Adult Stem Cells

A. Neural Precursor Cells (NPCs)

Neural precursor cells (NPCs) are naturally found in the sub-granular region of the hippocampus, sub-ependymal zone of the spinal cord and the sub ventricular area of adult brain. They can also be derived from fetal and embryonic brain tissue. They can generate all three neural cell types - neurons, astrocytes and oligodendrocytes. In addition, NPCs can be differentiated from pluripotent cell sources via the default pathway[37]. When transplanted *in vivo* without pre-conditioning, default pathway derived-NPCs almost exclusively differentiate into glial subtype cells, to the exclusion of neurons[36]. Other groups have used different methods to derive alternative cell types from pluripotent sources (Reviewed in [41]) and the first investigation using iPS-derived NPCs has shown functional efficacy in non-human primate models, albeit with tri-potential *in vivo* differentiation[42].

Preclinically, transplantation of NPCs or more differentiated glial progenitor cells (GPCs) – both adult and pluripotent-derived - in animal models of injury and dysmyelination is associated with migration of cells to the site of injury, remyelination, functional improvement and low rates of tumorigenesis[43–47] (Figure 2).

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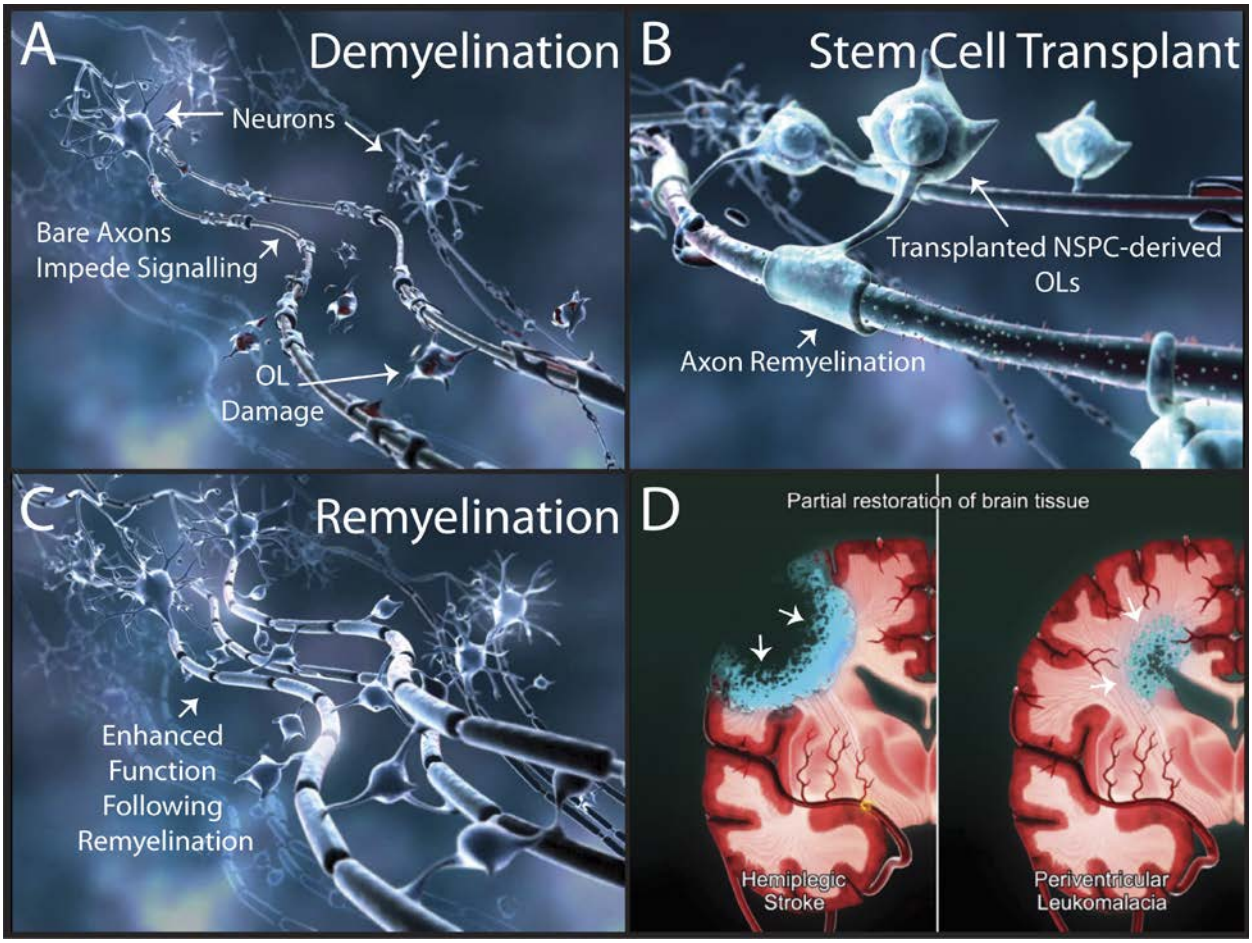

Figure 2: The Function of Neural Precursor Cells for Injury Repair
Hypoxic and hemodynamic insults lead to robust demyelination. B) Transplanted neural precursors can migrate to sites of demyelination and replace lost oligodendrocytes. C) This remyelination often leads to functional recovery and can re-establish axonal signalling. D) Cell transplant also frequently leads to partial restoration of brain tissue.

Furthermore, there is a natural but functionally insufficient propensity for expansion and mobilization of endogenous NPC populations following neurological damage[48]. Although some work has been done to activate or preserve these endogenous populations following injury via drug therapy[49, 50], these studies have been unable to successfully

mobilize numbers of cells comparable to that which can be injected from an expanded exogenous population.

The propensity of many NPCs and GPCs to differentiate into glial supporting cells (to the exclusion of neurons) *in vivo* is advantageous in the context of CP, where demyelination predominates and additional neuronal formation or motor neuron connectivity can be deleterious. Glial progenitor cells (GPCs) arising from neural stem cells are arguably an ideal source of safe and pure oligodendrocyte producing cells for remyelination in demyelination disorders such as hereditary leukodystrophies and CP (see [51] for review). Hence, NPCs (or their derivatives) show the most potential for positive functional results with clinical translation, despite a relatively small number of studies using them in neural injury models.

B. Mesenchymal Stem Cells (MSCs)

Within the bone marrow and umbilical cord exist two major subsets of cells: 1) hematopoietic and endothelial stem cells (CD34+), which form blood cells within the body, and 2) non-hematopoetic mesenchymal stem cells (MSCs, CD34-), which generate bone, cartilage, fat, blood vessels, and connective tissue [52, 53]. MSCs are spindle-shaped, fibroblast-like multipotent adult stem cells with limited capacity for self-renewal, which comprise a small population of the adherent stromal cell fraction. MSCs are traditionally derived from bone marrow, umbilical cord or placenta, although they have also been found in synovium, fat, blood vessels and articular cartilage [54–58].

Immunogenicity of MSCs is comparatively low; they exhibit minimal expression of Human Leukocyte Antigen (HLA)-B[59], and can demonstrate immunosuppressive and anti-inflammatory activity. In the transplant microenvironment, MSCs can influence several other cell types- either through cell-to-cell contact, trophic modulation or a combination of both - including T cells, natural killer cells, dendritic cells, monocytes, and neutrophils. MSCs are known to secrete prostaglandins, VEG-F, FGF, indoleamine 2,3-dioxygenase, soluble HLA-G5, IL-6, IL-10, TGB-β1, HGF, BiNOS, and heme oxygenase- 1 ([60, 61] reviewed in [62]). Although there is some evidence to suggest they can form neural derivatives *in vitro*, this does not occur *in vivo* to a meaningful extent[63]. Hence, the potential therapeutic benefit of MSCs appears to be mainly via immune mediation, angiogenesis, chemotropism and cellular sparing rather than via trans-differentiation into neural myelinating cells.

In preclinical studies of CP, intraperitoneal, intracardiac and intrahemispheric injection of MSCs has resulted in highly variable outcomes (reviewed in [64]). Nevertheless, because hematopoietic niche-derived cells have been used for decades in cell transplant paradigms unrelated to CP, such as leukemia and autoimmune disorders[65], they have promptly moved laterally into safety trials for CP. Considering the inconsistency associated with cell-sourcing and selection conditions currently employed, MSCs, at present, have limited application in chronic cases of human CP.

III. Mature Cells

A. Peripheral Glial Cells

Schwann Cells (SCs) and Olfactory Ensheathing Glia (OEG) are generally agreed to be myelinating cells, found in the PNS and olfactory system respectively. However, they are not "true" stem cells. These cells have myelination capacity and can provide trophic and structural support following injury[66].Historically, SCs were the cell type first studied in transplant paradigms[67]. Because they create potent trophic and physical substrates for axonal growth, proximal sensory and propriospinal axons readily enter and rarely leave SC grafts [68, 69]. The olfactory bulb and the lamina propria of the olfactory mucosa are the main sources of OEG. OEG have shown some promise in SCI models, but mixed results in brain injury models. Their limited application in *in vivo* brain injury models might be due to their origin or culture conditions (reviewed in [70]). Consequently, strategies that employ SC transplant generally involve co-transplanted growth factors, biomaterials[71] or cells such as OEGs.

5) Current Clinical Stem Cell Trials for CP

Currently, eight (one suspended, one completed with results) early phase clinical trials are using stem cells for treatment of CP or its antecedents (according to www.clinicaltrials.gov[72]), using umbilical cord or bone marrow-derived MSCs. Most

consist of treatment in the chronic phase of injury and primarily recruit non gender specific adolescents ~1 to 12 years of age, with diagnosed CP (often mixed etiology), that lack seizures. The only study (from Korea) with results was a double-blinded randomized trial. One hundred and five participants were between 10 months and 10 years of age, with a male: female of ~2:1. They were separated into groups which received either: 1) Allogeneic umbilical cord blood infusion + Erythropoietin (EPO) injection + active rehabilitation, 2) EPO + active rehabilitation, or 3) active rehabilitation, in the chronic phase of injury. Assessment was made at baseline and 1, 3 and 6 months post intervention. Primary outcome measures of motor function and standardized gross motor function were improved in the MSC group compared to other groups. Secondary outcome measures of cognition and neurodevelopmental outcome were also improved in the MSC group compared to other groups (clinical trials identifier: NCT01193660). Despite these results, neurodevelopmental time points beyond 12 months post treatment, coupled with secondary analysis, is required to fully elucidate the potential of this study. Seven clinical trials using NPCs exist in various CNS disorders. The first phase I clinical trial using human ESC derived GPCs for spinal cord injury (clinical trials identifier; NCT01217008) showed promise, but was halted in 2011 due to reported financial difficulties. Since then, fetal-derived NPCs and GPCs have been used as the primary source of NPCs in clinical trials. In other demyelination disorders such as advanced Batten's disease (neuronal ceroid lipofuscinosis) and Pelizaeus-Merzbacher disease, a high intraparenchymal dose of NPCs in multiple injection sites was well tolerated in phase I studies[73, 74].

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Furthermore, there is a growing subversive culture of "stem cell clinics", which operate outside of geographic regulation. These offer "stem cell therapies" for a variety of conditions, based on non-existent or sparse preclinical data. Anecdotal claims of efficacy from such clinics often feed a worrying trend of "Stem Cell Tourism", with individuals and parents seeking treatment or "cures" for their children with developmental disabilities. While a detailed discussion is elsewhere ([75]), financial costs (often exceeding $30,000) and individual risks are high.

6) Combinatorial Strategies

The extent of brain injury in CP is often not constrained to just axonal loss and demyelination. Cognitive and behavioural deficits commonly involve grey matter damage; therefore strategies that preserve all three are important to consider in the context of current rehabilitative strategies and future stem cell approaches. Current strategies for CNS repair have largely focused on two separate approaches to promote recovery: exogenous cell transplantation and endogenous cell stimulation. However, it is increasingly likely that additional approaches, which increase plasticity and structural support (bioscaffolds) as well as immunomodulation, will be required in combination with stem cell transplantation.

I. Growth Factors and Engineered Stem Cells

Growth factors, such as Neurotrophin-3 (NT-3) (which induces neuronal differentiation), granulocyte colony-stimulating factor (G-CSF), stem cell factor and brain derived neurotrophic factor (BDNF), are associated with improved transplant survival and migration. Furthermore, multifaceted strategies involving NPCs, combined with growth factors (NT-3 or multiple trophic factors), chondroitinase or Schwann cells, have increased functional recovery in spinal cord injury (see review [76]).

II. Bioscaffolds

Cross linked hyaluronan (HA)-based synthetic extracellular matrix (sECM)[77–79] and alginate scaffolds[76] can secrete growth factors over days and weeks post-implantation. Their capacity to secrete substrate over a sustained period of time ameliorates the need for repeated invasive injections. In the context of brain injury, where extensive loss of cerebral tissue exists, providing a physical substrate to bridge the tissue gap during cellular regeneration strategies is vital. In experimental SCI, self-assembling peptide nanofibers (SAPs) from peptide amphiphile molecules have been used to bridge the spinal cord cavity. Additionally, use of these SAPs resulted in axonal elongation and glial scar inhibition.[80]. Evidence is emerging that SAPs, from 16 peptide (RADA16-1) molecules, can be effective in brain injury models. They have demonstrated integration into the cavity and a reduction in the number of immune reactive cells local to the lesion (see review[81]). Multifaceted strategies of polymer scaffolds, growth factors and NPC transplantation in experimental SCI[76] indicate an important technological advancement for improved structural support and cell survival in clinical applications of brain injury.

III. Clinical Combinatorial Treatments

Although stem cell science shows great promise for reducing CP symptom load, clinical rehabilitative strategies are currently the *only* standardized treatments that provide benefit. Clinical combinatorial treatments are growing in popularity in attempts to see additive or synergistic effects. Studies using functional neuromuscular electrical stimulation in combination with BTXA injection or wrist splints (see [82] for review), or direct current stimulation (tDSC) combined with CIMT [83] have shown promise, but require greater numbers for complete evaluation.

While one current clinical trial using combinatorial strategies of EPO and cord blood MSCs in CP claims positive results, it is yet unclear what additional benefits combined treatments may have.

7) Conclusions

Despite preclinical progress, media focus and clinical promise, stem cell therapy for CP remains incomplete and in need of optimization. Easily-expandable, patient-specific cellular therapies are on the horizon, but are currently several years (if not decades) away from clinical reality. Despite this, parents of children with developmental disabilities are often unsure and often misguided as to what treatment options exist and where to seek them. The future of stem cell therapy for CP is evolving. A much better

understanding of basic stem cell biology must be established in the field, with appropriate cell type, source, derivation methods and safety profile optimized prior to clinical trials. Also, the correct timing, route of delivery and patient demographic must be taken into consideration when designing early clinical studies. Finally, successful regenerative medicine will involve a multifaceted, combinatorial approach combining current clinical rehabilitation, bioengineering and regenerative stem cell based strategies. Stem cell therapy shows substantial promise in the context of regenerative medicine. Once these important challenges and milestones are overcome, stem cell transplantation for CP could benefit millions of affected children worldwide.

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