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REVIEW

Clinical translation of human neural stem cells

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Abstract

Human neural stem cell transplants have potential as therapeutic candidates to treat a vast number of disorders of the central nervous system (CNS). StemCells, Inc. has purified human neural stem cells and developed culture conditions for expansion and banking that preserve their unique biological properties. The biological activity of these human central nervous system stem cells (HuCNS-SC®) has been analyzed extensively *in vitro* and *in vivo*. When formulated for transplantation, the expanded and cryopreserved banked cells maintain their stem cell phenotype, self-renew and generate mature oligodendrocytes, neurons and astrocytes, cells normally found in the CNS. In this overview, the rationale and supporting data for pursuing neuroprotective strategies and clinical translation in the three components of the CNS (brain, spinal cord and eye) are described. A phase I trial for a rare myelin disorder and phase I/II trial for spinal cord injury are providing intriguing data relevant to the biological properties of neural stem cells, and the early clinical outcomes compel further development.

Background

StemCells, Inc. was formed with the charter of discovering tissue-derived stem cells using the monoclonal antibody-based high speed cell sorting technology platform, previously used for purification of hematopoietic stem cells and peripheral nervous system stem cells [1-4]. More recently, this technology has been used to identify and purify other tissue stem cells, including hair follicle and skin [5], intestinal [6], muscle [7] and cancer stem cells [8,9]. This technology can also be applied to the purification of multi-potent stem cell populations derived from embryonic or induced pluripotent stem cells to eliminate teratogenic precursors. The company employed this strategy to prospectively purify its human central nervous system stem cell (HuCNS-SC®) population for expansion as neurospheres and banking. In this overview, the preclinical data are summarized and rationale provided for advancing these cells into clinical trials involving the brain, spinal cord, and eye.

A seminal finding in advancing regenerative medicine for human neurological disorders was the demonstration that neurogenesis occurs in the human adult brain [10,11]. This discovery, coupled with the identification and expansion of human neural stem cells by our laboratory and others [12-18], has led to a plethora of studies investigating neuroplasticity and regeneration. Though still early, a growing body of data suggests that human neural stem cells or their progenitors might one day repair or replace cells within the diseased or damaged central nervous system (CNS).

The translation of HuCNS-SC to clinical testing has been facilitated by prospective identification, reproducible expansion into cell banks, and stability upon cryopreservation. The availability of small animal models relevant to a range of human conditions has further facilitated efficacy testing and investigation of potential mechanisms of action. Moreover, past experience with cell and tissue transplants into the brains of Parkinson's or Huntington's patients (reviewed in [19-21]) has provided insights into allogeneic long-term survival in the relative immune-privileged niche of the CNS and has paved the way for studies with neural stem and/or progenitor cell products.

About the human central nervous system stem cells

The existence of both mouse and human neural stem cells has been demonstrated by multiple laboratories through growth in tissue culture systems and multi-lineage differentiation in fate mapping studies of cultured cells [22-27]. In 2000, scientists at StemCells, Inc. purified HuCNS-SC [16,28], an adult, tissue-specific stem cell. Each HuCNS-SC bank is created from purified human neural stem cells from a single fetal brain tissue (16 to 20 weeks gestation) using an isolation protocol involving monoclonal antibodies to cell surface markers and high-speed cell sorting.

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The cell expresses high levels of CD133 and low levels of CD24 ($CD133^{+}/CD24^{-/lo}$) and lacks expression of the hematopoietic lineage markers CD45 or CD34. Single $CD133^{+}/CD34^{-}$ $CD45^{-}$ sorted cells can self-renew to form neurospheres with multi-potentiality, hence the qualification as a 'stem cell'. When the $CD133^{+}/CD24^{-/lo}$ cells are grown under defined conditions [15], long-term expandable neurosphere cultures are established. Karyotype and morphological stability have been demonstrated with more than ten passages and in long-term culture. This method of cell isolation and culture has allowed for reproducible generation of human neural stem cell banks. For human clinical application, brain tissues are procured through an approved non-profit tissue procurement agency according to the Good Tissue Practice requirements of the US Food and Drug Administration (FDA).

Differentiation of these cells *in vitro* delineates their multipotency to become astrocytes, oligodendrocytes and different neuronal subtypes [29]. When induced *in vitro* by stimulating media additives, HuCNS-SC show a significant increase in migratory and phagocytic activity as assessed by a quantitative assay of *in vitro* cell function (Figure 1). Moreover, *in vivo* analysis of HuCNS-SC transplants into the brain of immunodeficient mouse models show that the cells seed the neurogenic niche of the subventricular zone, slowly divide, and migrate through different portals, including the rostral migratory stream to the olfactory

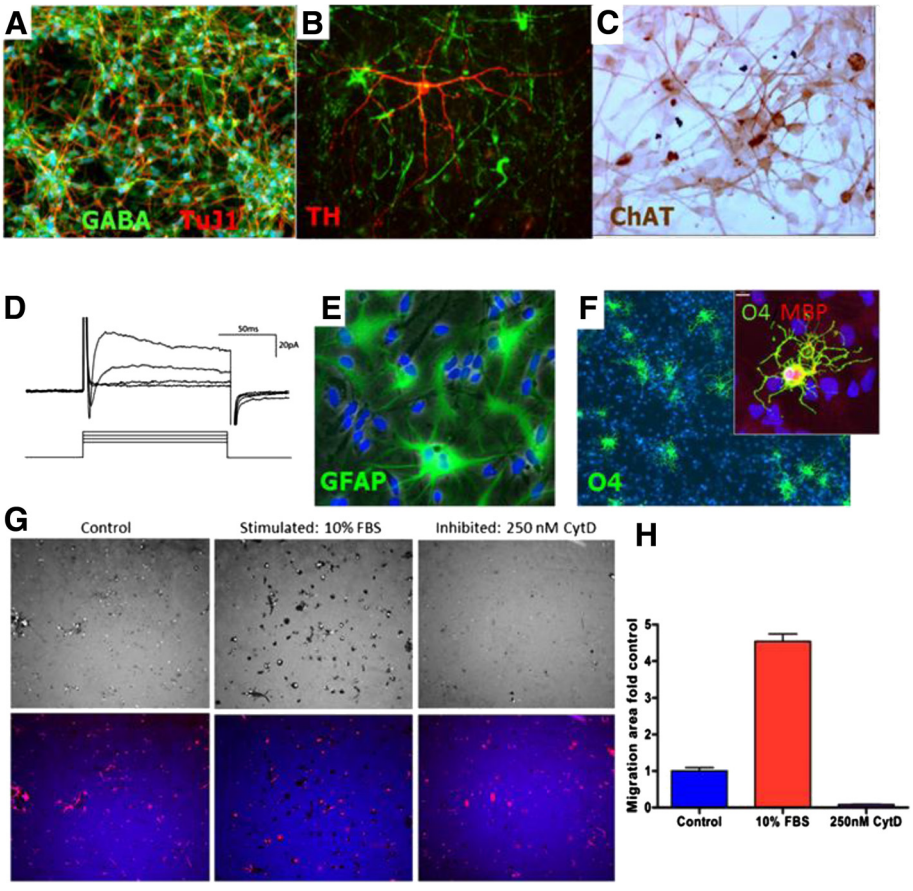

Figure 1 Human central nervous system stem cell (HuCNS-SC) differentiation *in vitro*. Human cells plated in culture become specific neuronal subtypes under defined growth factor conditions. **(A)** Gabaergic (gamma-aminobutyric acid, GABA); **(B)** dopaminergic (tyrosine hydroxylase; TH); **(C)** cholinergic (choline acetyltransferase; ChAT). **(D)** Differentiated neurons mature *in vitro* as shown by voltage-activated sodium and potassium currents from a clamp recording. Adapted from [29]. **(E,F)** HuCNS-SC also differentiate into astrocytes as defined by glial fibrillary acidic protein (GFAP; E) or oligodendrocytes identified by the marker O4 (F). In culture, rare oligodendrocytes can mature to myelin basic protein (MBP)-positive cells (inset of F). **(G)** Images of fluorescent bead lawn in which tracks cleared of beads by migrating/phagocytosing HuCNS-SC appear as black and beads ingested by the cells appear as bright spots (upper panel), and composite images of beads in blue and phalloidin stained cells in red (lower panel). Cells located within cleared track areas (red in lower panel) co-localize with bright spots of phagocytosed beads in upper panel. **(H)** Quantification of migration area (fluorescent beadfree) with high-content assay analysis software. Factors in fetal bovine serum (FBS) significantly enhanced migratory/phagocytosing activity, while it was blocked by the actin polymerisation inhibiting reagent cytochalasin D (CytD). Data represents the results of three independent experiments in triplicate wells.

system (Figure 2). Long-term engraftment (>24 weeks) shows global CNS migration and multi-lineage differentiation (astrocytes, oligodendrocytes, and neurons) in a site-specific manner (Figure 3). These *in vivo* characteristics have formed the basis for initiation of translational studies in select human CNS disorders, discussed below. The company intends to develop the HuCNS-SC as an allogeneic cell therapy for specific CNS disorders based on both neuroprotective and neuronal replacement strategies.

Disease targets

Treating disorders of the CNS has been one of the most challenging areas of modern medicine. Conventional drugs alleviate some symptoms but rarely modify the disease course or halt progression, particularly in neurodegenerative conditions. Regenerative medicine using defined stem or progenitor cells offers the potential to prevent further cell loss (that is, neuroprotection) and/or replace damaged or lost neurons (that is, neuronal replacement). Furthermore, both neuroprotective and neuronal replacement strategies can be envisioned in chronic neurodegenerative (for example, age-related macular degeneration and Alzheimer's disease) and genetic neurodegenerative diseases (for example, neuronal ceroid lipofuscinosis (Batten), leukodystrophies (Pelizaeus-Merzbacher)), as well as injuries to the CNS (for example, spinal cord injury (SCI), stroke and traumatic brain injury). Neuronal cell replacement, as attempted in treating Parkinson's disease, is particularly challenging because of the requirement to restore a precise neuron type in a specific location with proper integration and connectivity into a functional network. Thus, a neuroprotection strategy was envisioned as a more attainable goal for first in-human clinical studies using human neural stem cells. In this regard, StemCells, Inc. is actively engaged in testing HuCNS-SC in several target indications (Table 1).

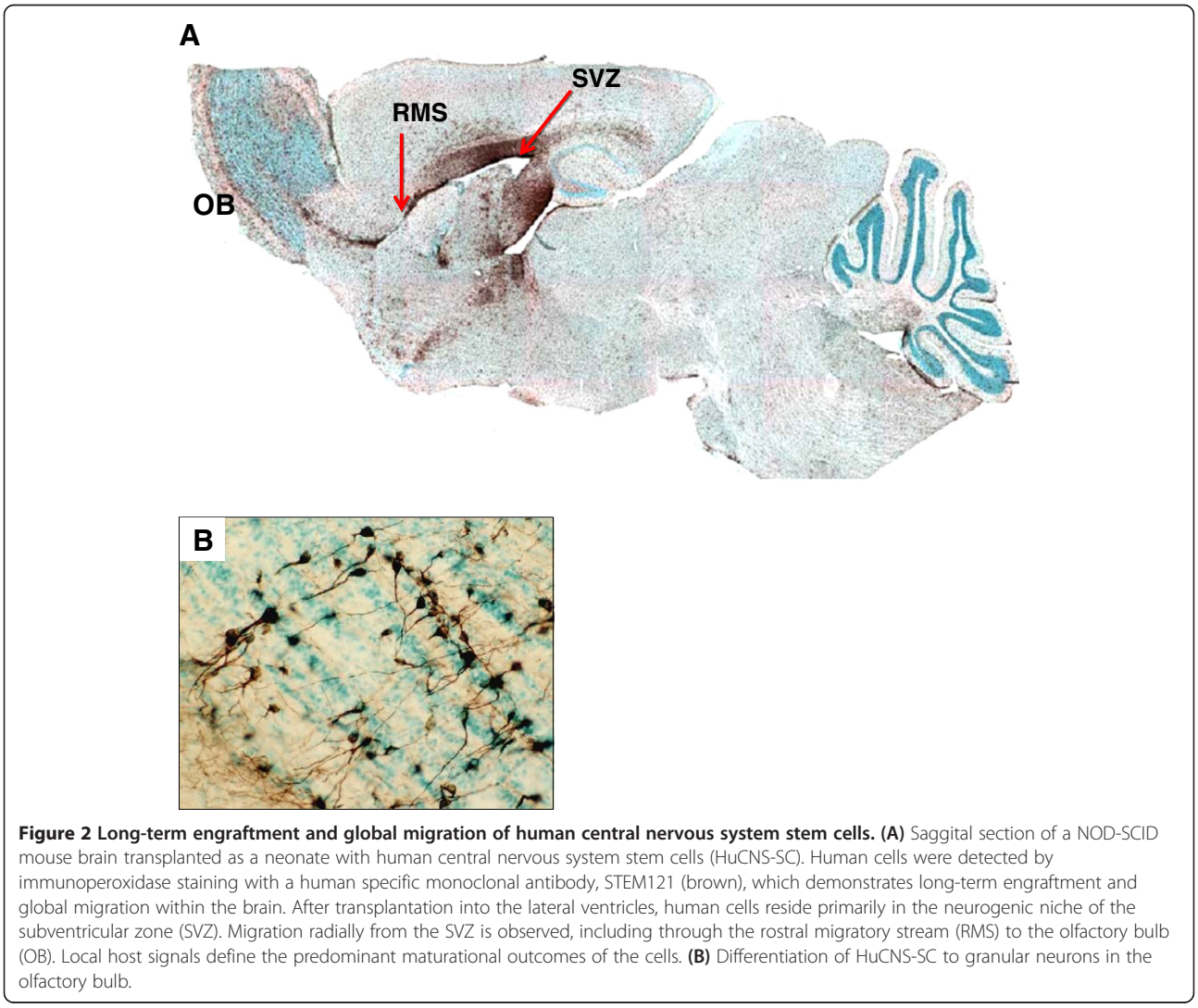

Figure 2 Long-term engraftment and global migration of human central nervous system stem cells. (A) Saggital section of a NOD-SCID mouse brain transplanted as a neonate with human central nervous system stem cells (HuCNS-SC). Human cells were detected by immunoperoxidase staining with a human specific monoclonal antibody, STEM121 (brown), which demonstrates long-term engraftment and global migration within the brain. After transplantation into the lateral ventricles, human cells reside primarily in the neurogenic niche of the subventricular zone (SVZ). Migration radially from the SVZ is observed, including through the rostral migratory stream (RMS) to the olfactory bulb (OB). Local host signals define the predominant maturational outcomes of the cells. **(B)** Differentiation of HuCNS-SC to granular neurons in the olfactory bulb.

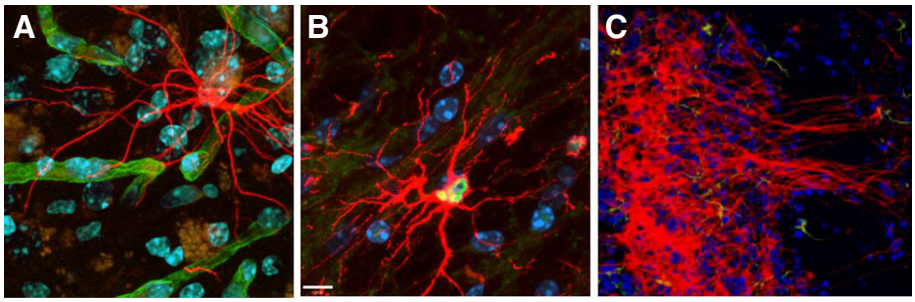

Figure 3 Human central nervous system stem cells (HuCNS-SC) preferentially differentiate to specific lineages depending upon their site of migration. Transplanted HuCNS-SC or their progeny were detected by staining using human-specific antibodies. Cell lineage was determined by morphology or co-staining for lineage markers. **(A)** Human astrocytes (STEM123, hGFAP, red) are observed juxtaposed to mouse blood vessels (beta-dystroglycan, green). **(B)** Human oligodendrocytes (Olig 2; green; STEM121 red) were confined to white matter areas such as the corpus collosum. **(C)** Within the olfactory system, human cells differentiate to granular neurons with long axons (STEM 121, red).

Disease targets for neuroprotective and neuronal replacement strategies

Neuroprotection of host cells can result from several mechanisms, including provision of neurotrophic, angiogenic, immune modulating factors and/or other proteins required for maintenance of healthy neurons. Protection of host neurons can also result from remyelination from new oligodendrocytes. Neuronal replacement strategies aim to replace specific lost or deficient cells, such as in Parkinson's disease. The key attributes of neural stem cells - such as self-renewal to provide a continuous reservoir of factor-producing cells, global CNS migratory properties, and their innate ability to form new normal neurons, astrocytes or oligodendrocytes - position them as attractive novel therapeutics for treating the plethora of neurodegenerative conditions. The translational approach was to first test the neuroprotective properties of the stem cell in the initial introduction to human testing while continuing to accumulate more complex preclinical data supporting neural replacement strategies. The first application of HuCNS-SC as a therapeutic candidate evaluated its safety and preliminary efficacy as a cell-based enzyme delivery system in a neurodegenerative lysosomal storage disease (LSD).

Lysosomal storage diseases affecting the central nervous system

LSDs result from recessive mutations in genes encoding soluble enzymes or structural proteins causing lysosomal dysfunction, accumulation of insoluble storage material, and eventual cell death. Development of effective therapies for the neuropathic LSDs, such as enzyme replacement, is challenged by the presence of the blood-brain barrier, which limits accessibility of intravenously delivered soluble enzyme to the brain. Direct intrathecal and intracisternal delivery of enzyme, protein modifications (such as lipidization and receptor targeting), nanotechnologies, as well as cell-based delivery schemes are all being tested for more effective transport of proteins and drugs to the CNS but currently no strategy has hit a

Table 1 Overview of HuCNS-SC clinical translation programs for central nervous system disorders

CNS area	Disease/injury	Stage	Outcome
Brain	Neuronal ceroid lipofuscinosis (Batten disease): infantile and late-infantile	Phase I completed	Safety, feasibility and tolerability of HuCNS-SC transplants. Post-mortem evidence of long-term donor cell survival in post-mortem 3/6 subjects alive 5 years post-transplant
		Phase Ib suspended	No accrual of eligible subjects
	Pelizaeus-Merzbacher myelin disorder	Phase I completed	MRI evidence of donor-derived myelin and modest gains in neurological function
	Alzheimer's disease	Preclinical	Enhanced synaptic function and restored memory in two AD relevant models
Spinal cord	Thoracic spinal cord injury	Phase I/II in progress	Sensory gains observed in first cohort.
	Cervical spinal cord injury	Preclinical	Improve motor function in SCI mice
Eye	Age related macular degeneration	Phase I/II in progress	Subject accrual ongoing

AD, Alzheimer's disease; CNS, central nervous system; SCI, spinal cord injury.

home-run [30]. The concept of using neural stem cells for the delivery of normal proteins to replace those that are defective or absent was proposed to take advantage of the inherent properties of these cells (reviewed in [31]). Their long-term integration and global distribution throughout the brain parenchyma comprise a mechanism to deliver therapeutic proteins in a direct and sustained manner. Several studies have examined the effect of normal or genetically engineered neural stem cells in specific animal models of LSDs [32-39] and shown these to be viable therapeutic strategies worthy of further investigation.

Neuronal ceroid lipofuscinoses

Of the numerous LSDs, neuronal ceroid lipofuscinoses (NCLs; commonly referred to as Batten disease) exhibit disease pathogenesis predominantly within the CNS. NCLs comprise the most prevalent group of neurodegenerative LSD and consist of at least ten genetically distinct forms. The infantile (CLN1, palmitoyl-protein thioesterase, PPT-1 enzyme deficiency) and late-infantile (CLN2, tripeptidyl-peptidase I, TPP-I enzyme deficiency) genetic subtypes result from gene mutations in soluble lysosomal enzymes [40,41] causing accumulation of lipofuscin material in neurons and eventual cell death. Knockout mouse models for the infantile (*PPT1-/-*) [42] and late-infantile (TPP-I) [43] forms develop progressive and severe neurodegeneration and recapitulate the pathology of the human diseases. As predicted, *in vitro* preclinical studies show HuCNS-SC-based cross-correction of enzyme deficiency through PPT-1 uptake via the mannose 6-phosphate receptor in cultured PPT-1-deficient mouse and human fibroblasts [38]. In order to create a suitable xenotransplantation model for testing the long-term effects of HuCNS-SC, the *PPT-1* knockout mouse was backcrossed to the immune deficient NOD-SCID mouse. Transplantation of HuCNS-SC in the PPT-1 knockout/NOD-SCID brain results in engraftment, migration and a region-specific differentiation pattern similar to that observed in non-neurodegenerative NOD-SCID animals. The HuCNS-SC transplanted mice showed production of functional PPT1 enzyme in whole brain extracts and statistically significant reduction in lipofuscin levels, ranging from 31% in the cortex to >50% in the hippocampus and cerebellum. The reduction in storage material correlated with observed protection of hippocampal neurons (up to 57% of CA1 and 97% of CA2/3) and up to 77% of cortical neurons. The neuroprotective effects of HuCNS-SC transplants through cell-based enzyme cross-correction also delayed the loss of motor function. These data provided the rationale for the first in-human trials using these purified and expanded, allogeneic human neural stem cells.

A phase I open label clinical study was conducted at Oregon Health and Science University by Drs Robert D Steiner and Nathan Selden to evaluate the safety of allogeneic HuCNS-SC administration [44]. The study enrolled six subjects; two with infantile (INCL) and four with late-infantile (LINCL) disease confirmed by detection of mutations in the *PPT-1* or *TPP-I* genes, respectively. Additionally, to consider the equipoise of this first in-human trial, only subjects with severe cognitive (developmental age less than two-thirds of chronological age) and neurological symptoms (significant cerebral atrophy, enlarged ventricles, and marked neurological and neuropsychological impairment) consistent with a very advanced stage of disease were enrolled (Figure 4). The study examined the tolerability of direct neurosurgical implantation into bilateral subcortical and ventricular sites at two dose levels; 500 million or 1 billion cells. The subjects received immunosuppression until the end of the safety study at 12 months. The study revealed the safety of the intervention and transplantation of the cells. At study termination, all remaining subjects consented to participate in a 4-year long-term follow-up protocol, which completed in January 2013. During the trial, one subject died approximately 1 year post-transplantation from causes related to underlying

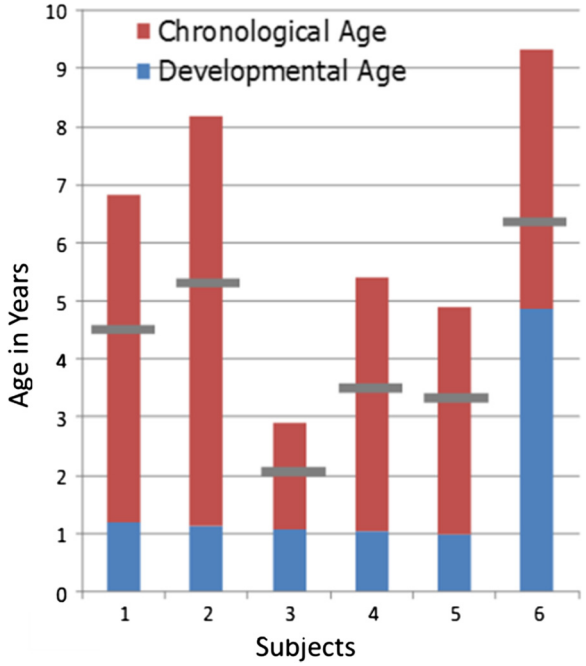

Figure 4 Baseline neuropsychological assessment of neuronal ceroid lipofuscinosis subjects based on the Callier Asuza G-scale. Significant neurological impairment was noted in all subjects prior to transplant. The blue bar denotes developmental age compared to chronological age (red). Eligible patients were required to be less than two-thirds of the patient's chronological age indicated by the gray line.

disease. Two subjects succumbed to their disease during the long-term follow-up study. All families consented to a post-mortem brain examination that revealed severe atrophy consistent with NCL. No adverse histopathological effects on the transplanted HuCNS-SC, such as neoplasia, cystic structures, or immune cell infiltration, were apparent. A molecular analysis was performed on several samples from the post-mortem brains using quantitative PCR analysis to identify the presence of donor cells by histocompatibility antigen differences. Samples were selected from different brain regions to include sites adjacent to and remote from transplant sites. Donor cells were detected in the brains of two subjects, demonstrating long-term survival up to 2.5 years post-transplant and 1.5 years after immunosuppression was stopped. In addition, the distribution of donor-positive samples indicates that these human neural stem cells had migrated away from the transplant sites [44,45]. Demonstration of HuCNS-SC migration within the brain is important when considering future treatment strategies for global and diffuse neurodegenerative diseases such as Alzheimer's disease. The potential of HuCNS-SC to migrate and react to motility-enhancing and chemoattractive stimuli was demonstrated *in vitro* by an array of migration assay systems, one of which is shown as an example in Figure 1.

This clinical trial represents the first demonstration that purified, expanded and cryobanked, allogeneic human neural stem cells can be safely transplanted directly into the brain and are well tolerated in severely afflicted pediatric subjects. Neuropsychological outcomes did not show improvement in the subjects with refractory disease, and alterations in disease course could not be determined in this uncontrolled study. It was noted, however, that patients with the most cerebral atrophy and neurological disability continued to decline whereas those less impacted showed stability [44]. Moreover, the 4-year follow-up of the remaining subjects continues to show a satisfactory safety profile with no emerging safety concerns.

For a neuroprotective strategy to show meaningful clinical outcomes, sufficient numbers of functional host cells must exist at the time of intervention, hence the need to transplant subjects earlier in their disease course. A phase Ib trial in NCL was initiated to examine safety in subjects with early disease and also to determine the impact of HuCNS-SC transplantation on disease progression. The study was suspended before enrolling any subjects due to a lack of available study candidates with less pronounced neurodegeneration at presentation. Of the 22 potential subjects for possible screening, none met the inclusion criteria for the trial. The inability to accrue subjects in clinical trials for rare diseases is a challenge at best, as identifying those earlier in the disease course is compounded by significant delays in proper diagnosis. Establishment of more rapid methods to diagnose genetic diseases in newborns [46] is needed to shorten times to diagnosis and clinical decision making for relevant treatment options.

Myelin disorders

Normal function of the nervous system requires formation and maintenance of the myelin sheath, the insulating layer surrounding nerve axons required for rapid conduction of electrical impulses and axonal integrity. Dysfunction or loss of myelin can lead to severe deficits in neurological function as seen in the leukodystrophies, multiple sclerosis, stroke, and traumatic brain and SCIs. One strategy to preserve neuronal function is through provision of new myelinating oligodendrocytes and supportive astrocytes derived from neural stem cells [47] or glial progenitor cells (reviewed in [48,49]).

Several animal models exist for testing treatment options for myelin disorders, each possessing unique attributes or aspects reflective of the human afflictions (reviewed in [50]). The myelin basic protein (MBP)-deficient shiverer (Shi) mouse is a dysmyelination model widely used to assess myelin production by donor cells [51-56]. The Shi mouse has been crossed to immunodeficient strains to facilitate analysis of transplanted human xenografts [47,53,54,57,58]. *De novo* myelin production from human oligodendrocytes has been observed in the brains of immunodeficient Shi mice (Shi-id) or contused SCI NOD-SCID mice transplanted with HuCNS-SC [47,54]. In these studies, immunohistochemical staining demonstrated that host mouse axons were ensheathed by human myelin derived from transplanted HuCNS-SC. Generation of compact myelin in the injured spinal cord correlated with improved motor function and in the Shi-id brain restored CNS conduction velocity in animals transplanted as asymptomatic neonates or symptomatic hypomyelinated juveniles. Moreover, *ex vivo* magnetic resonance imaging (MRI) of transplanted Shi-id brains detected changes in water diffusivity consistent with increased myelination. In the rodent brain, robust human MBP expression is observed at approximately 6 weeks after HuCNS-SC transplantation [47]. Thus, while other myelin mutant models of human diseases exist, such as the proteolipid protein (PLP) mutants reflective of Pelizaeus-Merzbacher disease (PMD), their shortened life span precludes assessment of the robustness and longevity of neural stem cell-based therapies. The preclinical demonstration of *de novo* myelination from transplanted HuCNS-SC in the Shi-id mouse and the contused SCI NOD-scid mouse provided the rationale to obtain FDA authorization for a phase I/II study in PMD.

PMD is a rare fatal leukodystrophy resulting from mutations of the X-linked gene encoding PLP1, the major

protein of the CNS myelin sheath. PLP1 mutations produce a spectrum of neurological symptoms ranging from severe, or connatal form, to classical, or the milder spastic paraplegia, all resulting from failure to produce functional myelin either due to apoptosis of the oligodendrocytes or abnormal myelin formation [59]. In the most severe connatal group, clinical signs of PMD can present at birth or within the first few weeks as nystagmus (uncontrolled rapid eye movements), difficulty breathing and low muscle tone (hypotonia). The subjects often require a tracheostomy to assist in airway management and a gastrostomy tube shortly after birth. Neurological and developmental milestones are either delayed or never achieved. Patients have severe motor and language impairment, which generally progresses. The onset of severe spasticity can be seen in later childhood. MRI reveals diffuse hypomyelination of both cerebral hemispheres, brainstem and cerebellum. There are no therapeutic options for patients with PMD; only supportive and palliative treatments are available. Death usually occurs within the first decade of life.

A phase I open label study was conducted in four subjects with severe connatal PMD to evaluate the safety and clinical effects of HuCNS-SC transplants into the neurodegenerative, hypomyelinated brain. The trial was conducted by Drs David Rowitch and Nalin Gupta at the University of California, San Francisco. Subjects were all male with confirmed PLP1 mutations, MRI absence of myelin and clinical symptoms consistent with early, severe PMD [60]. Each subject received a total brain dose of 300 million cells through injections into the frontal white matter area of each hemisphere. Immunosuppression was administered for the first 9 months after transplantation. Analysis of safety parameters, including physical and neurological examinations, did not reveal any adverse or serious adverse events considered related to HuCNS-SC transplants. MRI assessments did not show signs of inflammation, gliosis, ischemia, or cystic or neoplastic changes. Diffusion tensor imaging, a non-invasive MRI imaging technique that can measure water diffusivity in the brain, was used as a surrogate to evaluate myelin development in these subjects over time. Decreases in the mean and radial diffusivity (perpendicular to the axon) coupled with increases in fractional anisotropy (FA) are indices of white matter integrity. In each of the four subjects, regions of interest within the corona radiata were examined by these techniques and compared to control regions remote to the transplant sites. The two older subjects (2 and 4) showed the most pronounced increase in FA and decrease in radial diffusivity consistent with new myelin formation. The younger subjects, 1 and 3, also showed increases in FA but were more variable possibly reflective of less mature brain. Table 2 summarizes some of the key clinical and radiological observations by individual subject in the phase I PMD study. Twelve month neurological examinations showed either stable or modest gains in motor or cognitive function in all subjects compared to pre-transplant assessments. Subject 4 had the most pronounced changes, including the ability to follow two-step commands and speak audible words, improved truncal support and development of the ability to take steps with assistance. Neuropsychological assessments also showed small but measurable gains in select subtests. These gains, though modest, are not expected for a progressive, severe neurodegenerative disease. Further testing in a

Table 2 Major neurological and MRI diffusion changes, by subject, for the phase I trial in Pelizaeus-Merzbacher disease

Subject	Neurological and radiological changes
Subject 1 (16 m of age at transplant)	Tracheostomy and gastrostomy at baseline
	Remained neurologically stable, but was noted to have reduced nightly CPAP at 12 months
	Increased FA by MRI
Subject 2 (42 m of age at transplant)	Developed improved truncal support and the ability to take steps with assistance. He also began speaking audible single words and the ability to follow two-step commands
	Increased FA by MRI
Subject 3 (14 m of age at transplant)	Tracheostomy and gastrostomy at baseline
	Developed upper extremity antigravity strength and to take some solid foods by mouth
	Nightly CPAP dependency reduced
	Greatest increase in FA by MRI; but comparable to 'control' regions
Subject 4 (66 m of age at transplant)	Developed improved truncal support and progressed from the use of a walker with significant support at baseline to walking with minimal assistance
	Developed the ability for self-feeding and to follow two-step commands
	Increased FA by MRI

CPAP, continuous positive airway pressure; FA, fractional anisotropy; m, months.

controlled study will be required to demonstrate clinical efficacy of HuCNS-SC transplantation for leukodystrophies such as PMD and other myelin diseases.

Spinal cord injury

Traumatic SCI results in localized destruction of neural tissue from the primary injury followed by secondary injury from inflammation, immune responses and cell apoptosis. These events result in oligodendrocyte death and axonal loss in white matter and neuronal loss in gray matter. Neural stem cell transplantation for SCI represents a unique opportunity to assess an inherent multipronged therapeutic strategy that demonstrated improvement in locomotion in preclinical animal models. Human neural stem cells can provide neuroprotection through provision of secreted neurotropic and angiogenic factors and/or reformation of myelin sheaths from stem cell-derived oligodendrocytes for maintenance of axonal integrity. The transplanted neural stem cells may also contribute to neuroreplacement by differentiating neurons capable of creating synaptic contacts to re-establish bridging circuitry between new neurons and host cells [54].

Our collaborators at the University of California, Irvine, Drs Anderson and Cummings, developed thoracic SCI models in immunodeficient mice to examine the efficacy, mechanism of action, and long-term survival of HuCNS-SC transplants into subacute or chronic injured cords [54,61-63]. The cumulative data spanning approximately 10 years shows that HuCNS-SC transplanted directly into the cord above and below the epicenter of injury restored locomotor function in subacute and chronic SCI mice. Analysis of transplanted spinal cords by dual histochemical staining for human cells and lineage markers showed robust engraftment, migration and differentiation to neurons (26 to 38%), astrocytes (3 to 8%) and oligodendrocytes (48 to 64%) [54,63]. Immunoelectron ultrastructural analysis reveals the formation of compact myelin sheaths by human oligodendrocytes as well as human neurons with synaptic vesicles juxtaposed to host neurons. These results suggest that multiple mechanisms of action may be contributing to functional recovery in these animals. Although the ability to dissect this question remains challenging, one clue to potential mechanisms of action comes from selective ablation of the human cells using diphtheria toxin, which abrogates the regained motor function. This study shows the requirement for continued integration and survival of human cells to maintain restored motor function. Thus, the therapeutic effects of HuCNS-SC seen in SCIs and a hypomyelination disease results from stable integration of newly formed neural cells, in particular myelin-producing oligodendrocytes. In fact, these cells likely impart their full therapeutic potential as a result of both integration and function, as well as provision of neurotrophic support. Another important aspect of these studies was the lack of induced allodynia (abnormal sensitivity to pain) following HuCNS-SC transplantation. These results contrast with those previously reported [64] in which neural stem cell transplants led to functional recovery of hind limbs but development of hypersensitivity (allodynia) in the forepaws due to axonal sprouting. Differences in cell source, animal models and culture methods preclude identification of specific parameters that contribute to the undesired outcome in their study. The positive impact on locomotion coupled with the lack of safety concerns of the purified, expanded and banked HuCNS-SC in the immunodeficient SCI model provided the rationale for initiation of a clinical study in thoracic SCI subjects.

A progressive clinical study design was implemented by the company to test the safety and clinical effects of HuCNS-SC transplants in subjects with chronic thoracic (T2-T11) complete injury (American Spinal Injury Association (ASIA) classification A) progressing to subjects with incomplete (ASIA B or C) injury. The phase I/II trial was authorized by the SwissMedic regulatory authority and is being conducted by Dr Armin Curt (Balgrist Hospital, University of Zurich). The study will enroll 12 subjects who sustained a SCI within 3 to 12 months prior to cell transplantation. Each subject will receive a total fixed dose of approximately 20 million cells injected directly into the thoracic cord near the injury. Dosing of the first cohort, three AISA A subjects, has been completed and a 6-month interim evaluation performed (A Curt, Annual Scientific Meeting of the International Spinal Cord Society, September, 2012). To date, no safety concerns have arisen concerning the surgery or cellular transplant. Considerable gain in sensory function below the injury level was observed in two of the three subjects. This increased sensitivity to touch has evolved over time and was not anticipated in these very severely injured subjects since they were neurologically stable before transplant. Parallel changes in sensitivity to heat and electrical stimulation were also observed. Electrophysiological measurements across the injured spinal segments provided independent and objective measures of the change in sensory function. These data suggest that the transplanted human neural stem cells may be having a positive clinical effect in these severely injured subjects. The trial has just completed dosing of the first incomplete ASIA B subject and will continue to enroll eligible subjects until trial completion. Most human SCIs involve the cervical regions and preclinical studies are currently in progress with HuCNS-SC transplants into rodent

models of cervical cord hemi-contusions in support of advancement to clinical testing.

Retinal disorders

The retina is an integral component of the CNS with complex neural circuitry involving transmission of signals from the photoreceptors to the brain through the optic nerve. Retinal diseases have long been viewed as a prime target for consideration in transplantation approaches because of ease of access, out-patient surgical procedure, the size of the eye, and the availability of non-invasive tests for visual function assessment following cell transplantation. Photoreceptors and retinal pigmented epithelial (RPE) cells derived from pluripotent stem cells have been the lead candidates for strategies based on cell replacement [65,66].

Retinal protection using human neural stem/progenitor cells represents an alternative strategy for treating retinal diseases like dry age-related macular degeneration. The Royal College of Surgeons (RCS) rat has been used extensively as a model of retinal degeneration to assess efficacy of various cell types. The RCS rat has a mutation in the Mertk gene that causes disruption of the RPE cell's phagocytic activity, resulting in accumulation of toxic shed photoreceptor outer segments and eventual death of photoreceptors. Transplantation of human cortical neural progenitor cells into the subretinal space (between the photoreceptor and defective RPE cell layer) of the RCS rat resulted in the preservation of photoreceptors and rescue of visual function [67]. Recognizing the retinal protection conferred by the human neural progenitor cells in the study by Wang and colleagues [67], we asked whether HuCNS-SC might have a similar effect on host photoreceptors. When HuCNS-SC were transplanted into the subretinal space of RCS rats, the cells migrated within the subretinal space. Visual acuity was preserved to near normal levels and correlated to long-term protection of the photoreceptors in retinal areas adjacent to the transplanted human cells (Figure 5) [68]. Further analysis revealed that transplanted HuCNS-SC were able to phagocytose the shed outer segments, a task normally performed by healthy RPE cells. A Good Laboratory Practice safety and efficacy study was performed in RCS rats and results corroborated preservation of visual function without any safety concerns related to the transplanted cells. An Investigational New Drug was authorized by the FDA and a dose escalating phase I/II study is currently enrolling.

The study consists of two cohorts of 8 subjects (16 total). Cohort 1 will enroll subjects with best corrected visual acuity levels of ≤20/400 in the treated eye. The second cohort will enroll subjects with best corrected visual acuity of 20/200 to 20/100. The subjects will receive oral immunosuppression for 3 months after surgery and will be followed for 1 year for any adverse events. Secondary assessments for preliminary efficacy will include visual acuity testing, and other detailed evaluations of ocular function and retinal imaging. At the conclusion of the study, subjects will be asked to participate in a separate 4-year long-term follow-up study.

Targets for the future

Many CNS indications (stroke, certain forms of cerebral palsy, Alzheimer's disease, traumatic brain injury and other disorders) may benefit from the neuroprotective or neural replacement properties of human neural stem cells. One of the most challenging diseases, Alzheimer's, will have a global impact on society as the number of affected individual's increases and healthcare costs skyrocket. Moreover, the recent failure of two drugs in late stage trials, targeted toward eliminating beta amyloid plaques (bapineuzumab and solanezumab) has left a void

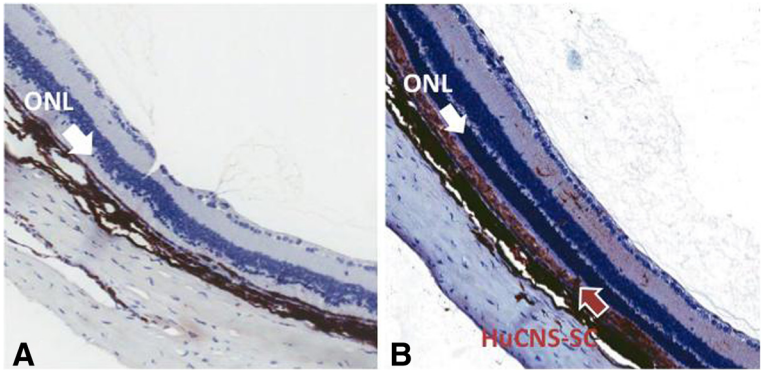

Figure 5 Human central nervous system stem cell (HuCNS-SC) subretinal transplantation preserves photoreceptors in treated Royal College of Surgeons rats. (A) Representative images of a retina cross-section showing that, at post-natal day 150 (about 4.5 months post-transplantation), only scattered photoreceptors are evident in the outer nuclear layer (ONL) of untreated eyes (the white arrow points to the remnants of the ONL, just underneath the thick inner nuclear layer). **(B)** In contrast, HuCNS-SC transplanted eyes have well preserved ONL adjacent to the HuCNS-SC. Reddish-brown outlined white arrow indicates the extent of HuCNS-SC engraftment, highlighted by SC121 staining.

in treatment options for those suffering with this devastating neurodegenerative disease and highlights the critical need to explore novel treatment paradigms. Recent studies in two mouse models relevant to Alzheimer's disease, an inducible neuronal loss model (CAM/Tet-DTA) [69] and the 3xTg-AD mice (transgenic for mutant APP, PS1, and tau) [70], have shown that transplantation of mouse neural stem cells improved cognitive function. In the case of inducible neuronal loss, cognitive improvements correlated with protection of host neurons by murine neural stem cells. In the 3xTg-AD mice, increased synaptic density was noted and, in part, mediated through production of neurotrophic factors, such as brain-derived neurotrophic factor [71,72]. Transplantation of HuCNS-SC into aged 3xTg-AD mice has been performed and shows similar rescue in hippocampal-based memory deficits [73]. APP-SCID mice, which develop heavily plaque-laden brains [74,75], were used to examine the effects of amyloid-β (Aβ) plaques on the HuCNS-SC. These studies show long-term survival of the human cells within the heavily plaque-laden brain and suggest that Aβ plaques are not toxic to the transplanted cells and that the therapeutic actions of these human cells may occur despite this pathology (G Carlson, personal communication). The observed increase in synaptic density in the 3xTg-AD mouse brain following HuCNS-SC transplantation is of particular importance because clinical disability in Alzheimer's disease patients correlates with synaptic loss. Further studies are in progress to elucidate additional effects of these transplanted cells. Preliminary data from *in vitro* studies indicate that extracellular matrix deposited by HuCNS-SC transplantation can promote neurite outgrowth from human neurons (Figure 6). Soluble Aβ was reported to decrease neurite outgrowth from neuronal cultures and this coincides with reduced synapsin staining, indicative of synaptic loss. Accordingly, promotion of neurite outgrowth and protection from Aβ-induced neuritic dystrophy is employed in phenotyping screening campaigns for Alzheimer's disease drug discovery [76]. As research progresses in the Alzheimer's disease field and more drugs targeting specific pathologies of Alzheimer's disease fail, the human neural stem cell becomes a more enticing candidate as a disease modifier by protecting host neurons and preserving synapse density. Any improvements in memory could have a significant impact on the quality of life for both patients and their caregivers and could alter current treatment paradigms for this growing health crisis.

Conclusion

The translational studies of HuCNS-SC speak to the biological activity of these cells in the brain, spinal cord and eye. To date, the preclinical studies in specific animal

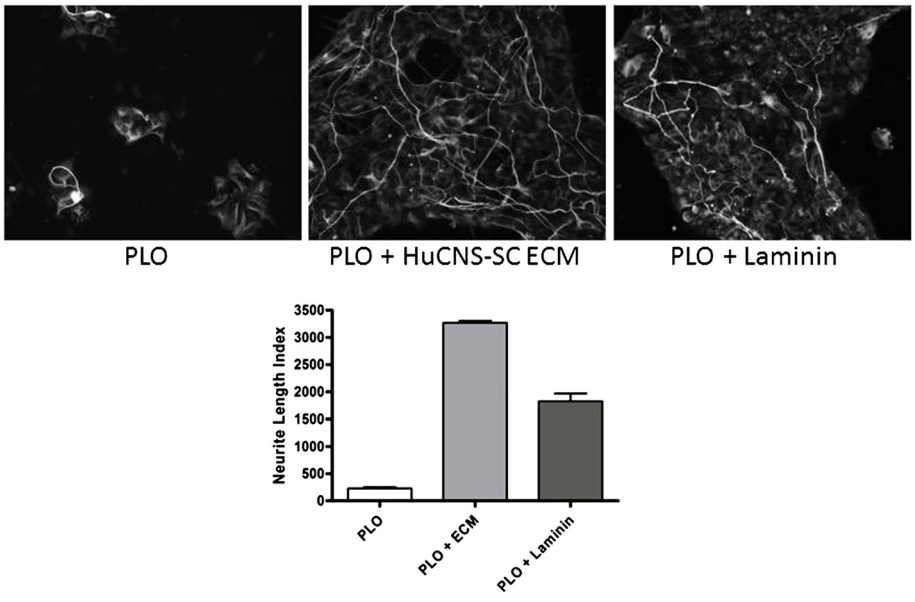

Figure 6 Human central nervous system stem cells (HuCNS-SC) are cultured adherently to confluence on poly-ornithine coated (PLO) plates and the cells removed by water lysis and repeated cell culture buffer washes, leaving only their deposited extracellular matrix (ECM) behind. Subsequently, a human neural stem cell type derived by a different protocol than the one used for the HuCNS-SC was seeded on the ECM-coated plates under neuronal differentiation-inducing conditions. Coating with PLO only and double-coating with PLO and laminin served as negative and positive controls for a neurite outgrowth-promoting substrate, respectively. The extent of neurite outgrowth was quantified with high-content assay analysis software after 96 hours. Neurite length index: total length of detected neurites in micrometers per 100 nuclei. Data represents the results of two independent experiments in five replicate wells.

models have revealed biological properties of the HuCNS-SC similar to the emerging human data in the early clinical studies. The ultimate demonstration of a confirmed effect in patients will require controlled studies but the first results on safety and preliminary effects from these trials provide justification for continued human testing. Evidence of *de novo* myelin production in a hypomyelination disorder and improved sensation in SCI as clinical endpoints, unobserved with other interventions, emphasizes the potential of neural stem cell transplantation. If neural stem cell transplantation continues to show promising clinic data in altering disease progression, this approach could provide the novel therapeutic modality sorely needed for a spectrum of challenging neurological disorders.

Note: This article is part of a thematic series on *Clinical applications of stem cells* edited by Mahendra Rao. Other articles in the series can be found online at http://stemcellres.com/series/clinical.

Abbreviations

ASIA: American Spinal Injury Association; Aβ: Amyloid-β; CNS: Central nervous system; FA: Fractional anisotropy; FDA: Food and Drug Administration; HuCNS-SC: Human central nervous system stem cells; LSD: Lysosomal storage disease; MBP: Myelin basic protein; MRI: Magnetic resonance imaging; NCL: Neuronal ceroid lipofuscinosis; PCR: Polymerase chain reaction; PLP: Proteolipid protein; PMD: Pelizaeus-Merzbacher disease; RCS: Royal College of Surgeons; RPE: Retinal pigmented epithelial; SCI: Spinal cord injury; Shi: Shiverer; Shi-id: Immunodeficient Shi mice.

Competing interests

All authors are employees of StemCells, Inc. and receive Company stock.

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