Neuron
Perspective

Translating Stem Cell Studies to the Clinic for CNS Repair: Current State of the Art and the Need for a Rosetta Stone

Karen Aboody,[1] Alexandra Capela,[2] Nilofar Niazi,[3] Jeffrey H. Stern,[4] and Sally Temple[4,*]
[1]Department of Neurosciences and Division of Neurosurgery, City of Hope National Medical Center & Beckman Research Institute, Duarte, CA 91010, USA
[2]StemCells Inc., Palo Alto, CA 94304, USA
[3]CNS Regenerative Medicine Foundation, New York, NY 10069, USA
[4]Neural Stem Cell Institute, Rensselaer, NY 12144, USA
*Correspondence: sallytemple@nynsci.org
DOI 10.1016/j.neuron.2011.05.007

Since their discovery twenty years ago and prospective isolation a decade later, neural stem cells (NSCs), their progenitors, and differentiated cell derivatives along with other stem-cell based strategies have advanced steadily toward clinical trials, spurred by the immense need to find reparative therapeutics for central nervous system (CNS) diseases and injury. Current phase I/II trials using stem cells in the CNS are the vanguard for the widely anticipated next generation of regenerative therapies and as such are pioneering the stem cell therapy process. While translation has typically been the purview of industry, academic researchers are increasingly driven to bring their findings toward treatments and face challenges in knowledge gap and resource access that are accentuated by the unique financial, manufacturing, scientific, and regulatory aspects of cell therapy. Solutions are envisioned that both address the significant unmet medical need and lead to increased funding for basic and translational research.

Introduction

Stem cell therapies are a new medical frontier. Pioneering work using hematopoietic stem cells in therapeutic settings has generated the precedent, and the recent scientific advances in stem cell biology, brain plasticity, genomics, and neuroimaging indicate that transformative changes lie ahead for repairing the CNS. These advances, supported by animal experiments that indicate some CNS damage may be preventable or reversible by stem cell-based approaches, along with the limited self-initiated reparative ability of the CNS and the enormous social burden of neurological disease and injury, make this system a prime target for regenerative therapies. Translation, by which we mean advancing scientific discoveries from the laboratory into practical applications for patient benefit, i.e., "bench to bedside," requires a comprehensive collaborative team approach: research scientists and clinicians must work closely with regulatory agencies, patient advocacy groups, ethics bodies, cell manufacturing facilities, and industry to achieve the quality of studies and necessary funding to ensure success. This requires new partnership models for research in which traditional silos are broken down, translational teams are created, and new mechanisms for effective hand-off from nonprofit to for-profit are generated. Today many researchers in the stem cell field have advanced their research far enough to attempt clinical translation but lack the knowledge and wherewithal to accomplish this arduous, expensive, and long-term task (Figure 1). The significant hurdles needed to be surmounted are illustrated in the analysis of the drug development process (Figure 2). Despite these difficulties, steady progress toward this goal is being made, spearheaded by industry, academic institutions, and nonprofit foundations in conjunction with a recent focus by the National Institutes of Health (NIH) and the Food and Drug Administration (FDA) in the U.S. on both translational research and regenerative medicine.

Here we describe the current status of, and pathways for, stem cell-based CNS therapies, analyze the landscape of current regulatory approved clinical trials, discuss the recent industry trends and regulatory developments that can catalyze further translational progress, and describe key issues and currently available resources to facilitate more efficient translation of promising research.

Stem Cell Sources for CNS Repair

Endogenous Sources

NSCs are the fundamental ancestor cells for the CNS (brain, spinal cord, and retina), defined by their ability to self-renew and produce all three major CNS cell types: neurons, astrocytes, and oligodendrocytes. NSCs can be expanded substantially, proliferating to produce cell lines that can differentiate into functional neural cells after in vivo transplantation, demonstrating tremendous promise for cell replacement and regenerative therapies. NSCs are abundant in different regions of the fetal CNS and are retained throughout life in restricted parts of the forebrain, notably the striatal subventricular zone and dentate gyrus of the hippocampus. Human NSCs have been isolated from donated fetal CNS tissue and can be defined by expression of surface markers such as CD133 (Uchida et al., 2000), enabling prospective enrichment, in vitro expansion using growth factors such as FGF2 and EGF, and in-depth characterization. NSC primary cell lines generated from human fetal CNS tissue,

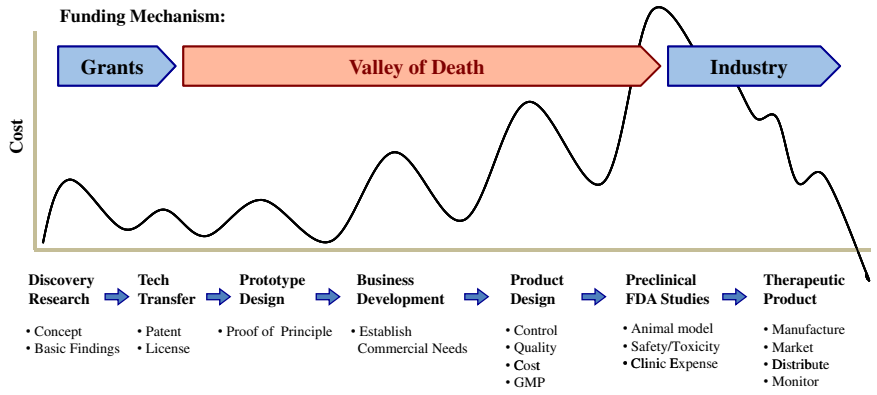

Figure 1. The Pipeline from Bench to Bedside: Costs Estimated at $1 Billion over Ten Years

typically around 8–18 weeks of gestation, are now the subject of a number of clinical studies. Progenitor cells that arise from human NSCs, such as glial-restricted progenitor cells (GRPs), which produce oligodendrocytes and new myelin, are also being advanced toward the clinic (Goldman, 2011; Sandrock et al., 2010). Other sources of neural cells showing promise in preclinical studies include cells from nasal mucosa such as olfactory ensheathing cells (Lindsay et al., 2010; Raisman and Li, 2007) and skin-derived multipotent precursors (SKPs) (Fernandes et al., 2008).

Multipotent stem and progenitor cells can also be extracted from adult CNS regions where neurogenesis is not apparent, then expanded and differentiated into neurons and glia in culture, e.g., from adult murine spinal cord (Lowry et al., 2008; Shihabuddin et al., 2000), human cortex (Schwartz et al., 2003), and retina (Giannelli et al., 2010). These observations also raise the exciting possibility that there are compartments of endogenous stem cells that could be activated in situ to promote repair. Certainly, gliogenic progenitor cells are present throughout much of the CNS and can be coaxed to replace lost oligodendrocytes or, in the case of injury or disease, can proliferate and contribute to the proteoglycan rich astrocytic scar that inhibits neuronal process regrowth. Hence controlling endogenous stem and progenitor cells to promote repair is another therapeutic avenue being actively pursued.

Pluripotent and Induced Sources

Human embryonic stem cells (hESCs) offer an abundant source of NSCs that can be further differentiated into a wide variety of functional neurons and glia. Induced pluripotent stem cell (iPSC) lines, derived by reprogramming adult somatic cells (e.g., fibroblasts) into an embryonic stem cell state, are a potential autologous source of NSCs (Hu et al., 2010), and while not yet ready for clinical use, they are being explored as preclinical disease models (Cundiff and Anderson, 2011). Another potential source still in the early stages of investigation is the directed differentiation of nonneural cells. For example, mouse fibroblasts can be transdifferentiated into neurons via addition of specific transcription factors in the neural pathway (Vierbuchen et al., 2010) and resident glia into subtype-specific neurons (Heinrich et al., 2011), which may prove valuable for CNS disease modeling and conceivably for specific repair strategies.

Controversial Nonneural Sources of Neural Cells

There has been substantial controversy over claims that neural cells can be derived from nonneural tissue such as bone marrow with just environmental manipulations, including transplantation into neural tissue. Rigorous scientific tests and lack of reproducibility have shown that such claims are unfounded, yet they continue to plague the field: they are provided as rationale for ongoing unregulated clinical trials and are used to persuade

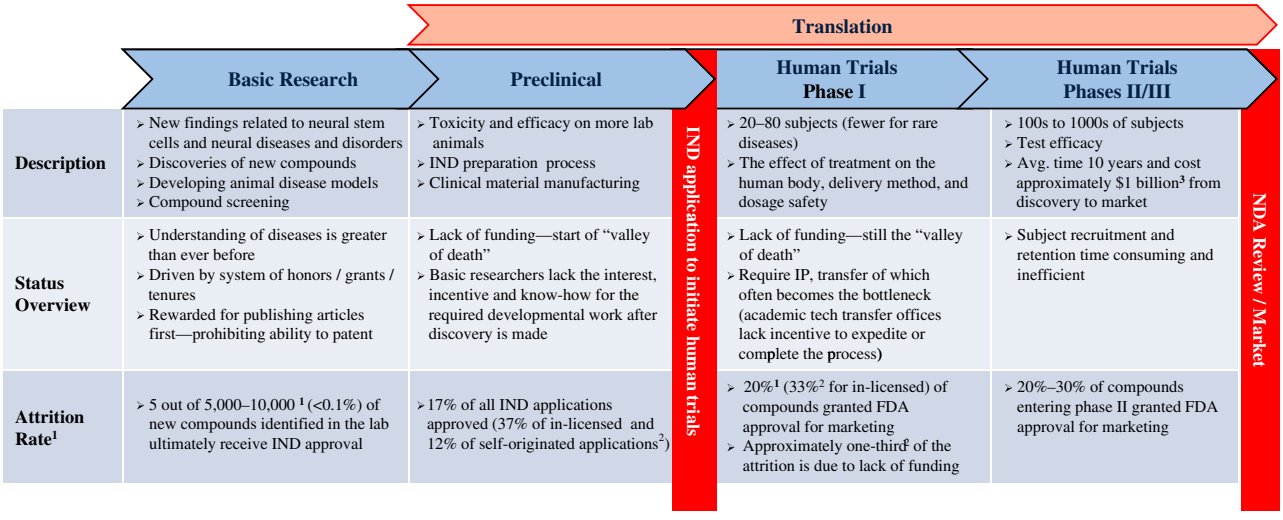

Figure 2. Drug Development Process Analysis
[1]Liu (2010). [2]DiMasi (2001). [3]PricewaterhouseCoopers (2008).

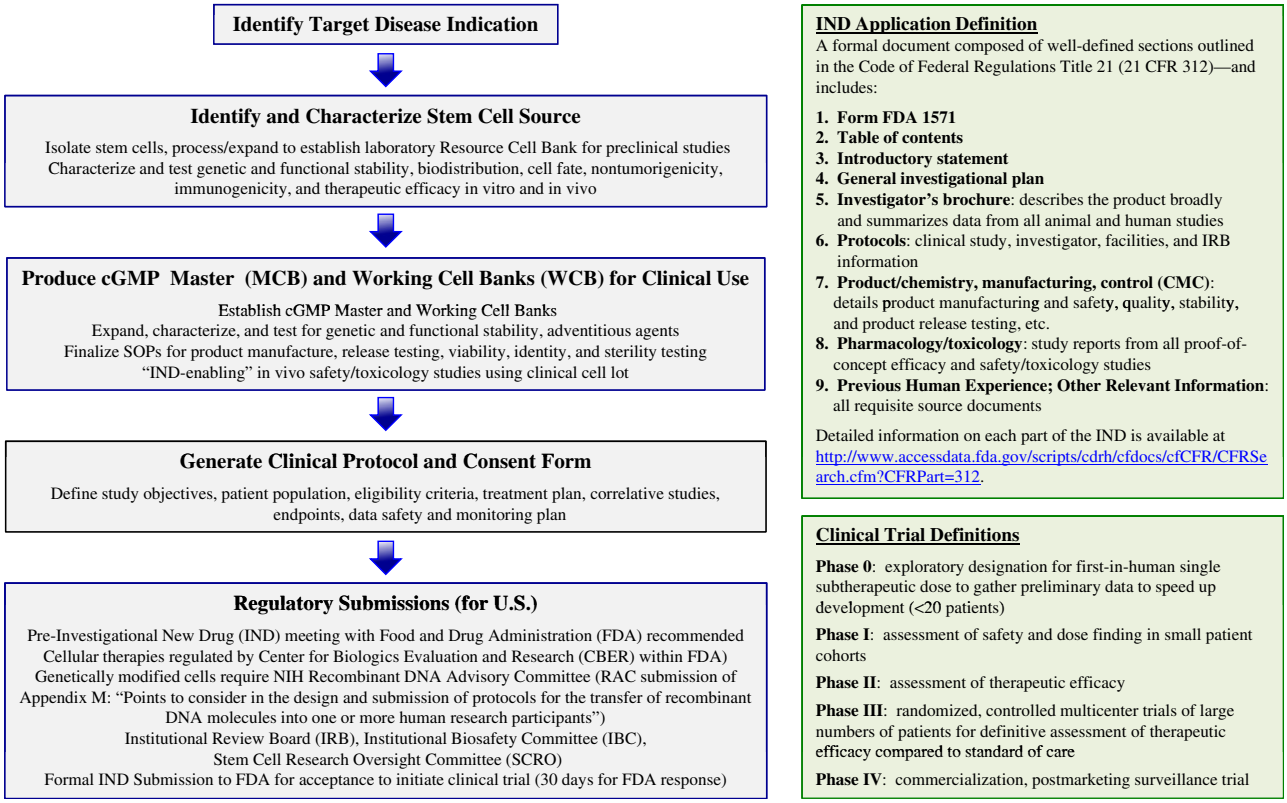

Figure 3. Bench-to-Bedside Translation of Stem Cell Therapies to CNS Clinical Trials
Regulatory information for clinical trials.

patients to pay high sums for dubious and in some cases fraudulent therapies. It is important to educate the public through avenues such as the International Society for Stem Cell Research (ISSCR) website A Closer Look at Stem Cell Treatments (http://www.closerlookatstemcells.org) to help patients make informed choices when contemplating stem cell therapies.

Pathway from Bench to Bedside

Impact

Potential CNS disease targets encompass a wide range of neurological conditions with a variety of underlying causes. These include stroke, traumatic brain injury (TBI), spinal cord injury (SCI), multiple sclerosis (MS), age-related macular degeneration (AMD), Alzheimer's disease (AD), Parkinson's disease (PD), epilepsy, brain cancer, and, perhaps further in the future, mental disorders such as depression, autism, and schizophrenia. Up to one billion people worldwide have neurological disorders, accounting for 12% of global deaths (WHO, 2006). As the population ages, the burden of age-related disorders such as dementia, AD, PD, and AMD will also increase.

Translational Challenges for the CNS

The pathway of discovery, development, and implementation of novel stem cell-based therapies for the CNS is being constructed and walked almost simultaneously. First-in-human CNS stem cell trials pose specific ethical, regulatory, and clinical challenges (Halme and Kessler, 2006). There are also numerous scientific and medical challenges that are unique to the CNS, such as the impact of cell delivery in the host tissue; the need to maintain existing connectivity and functionality while supporting new therapeutically relevant cell integration; overcoming and/or utilizing the endogenous signals that impact the proliferation, migration, and fate of implanted cells; overcoming scar formation at the site of injury; the functional and metabolic interdependence of neurons, astrocytes, and oligodendrocytes and its impact on donor cell survival and function; the complex neuroimmune axis that exists in the normal and diseased CNS; and the challenge of modeling functional CNS recovery in animals. Some examples of these challenges are discussed below.

Regulatory Considerations

Despite the specific challenges of targeting the CNS, the translation process for cellular therapies involves the same basic steps as for drug therapies: clinical investigation must follow an Investigational New Drug (IND) application in the US (Figure 3) or similar regulatory filings in other countries. Human cellular products such as stem and progenitor cells have unique requirements for characterization, manufacturing, and testing that are regulated by a specific center within the FDA: the Center for Biologics Evaluation and Research (CBER) and its Office of Cellular, Tissue, and Gene Therapies (OCTGT).

If for real estate the mantra is "location, location, location," for making regulatory contacts the mantra is "early, early, early." FDA representatives can provide guidance that represents years

of work, saving time and money. A valuable review of the FDA regulation of stem cell-based products outlines the safely issues, pointing out that the FDA has over 20 years of experience with cellular therapies to frame the work, but acknowledging that the high proliferative potential and plasticity of stem cells leads to additional concerns (Fink, 2009).

The process of submitting an IND application includes (1) a recommended pre-IND meeting with the OCTGT for guidance regarding preclinical study design, data analysis, clinical protocol schema, and necessary information for the IND application, (2) submission of the complete IND package, and (3) IND review (Figure 3). If a sponsor has not heard from the FDA after 30 days, the trial can proceed; if there are safety concerns the FDA will impose a "clinical hold" until issues are satisfactorily addressed.

Detailed information can be obtained from the FDA website: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=312.

While this process sounds straightforward, in the case of CNS stem cell therapies, the required documentation may run several thousand pages (Figure 3). This can be partially attributed to the fact that the lack of precedent for these first-in-human stem cell trials requires a higher bar for preclinical demonstration of efficacy and safety. The threshold for approval will vary depending on the disease indication and risk/benefit ratio. Additionally, if the cell product is genetically modified, separate documentation ("Appendix M") must be submitted to the NIH Recombinant DNA Advisory Committee, established for the protection of patients. Novel, unprecedented studies will probably require a public hearing by this committee, where a panel of reviewers judge data presented and make recommendations to the investigators and FDA. Finally, due to the lengthy process, members of an FDA review panel may change over time, and new issues may be raised at any time prior to trial initiation. As new data are constantly being generated in this cutting-edge field, criteria for IND acceptance are changing. Demonstration of safety and feasibility in the first round of phase I stem cell-CNS trials will probably have a great impact on facilitating future IND filings.

Initiating the clinical study also requires Institutional Review Board (IRB), Institutional Biosafety Committee (IBC), and typically Stem Cell Research Oversight Committee (SCRO) approvals. One of the barriers to the full use of NSCs in patient populations is the reluctance of some IRBs to allow children to receive transplants, although many CNS diseases are congenital and fatal in childhood. This is probably due to the deaths of several gene therapy patients under age 21, which has sensitized IRBs to the public and legal issues involved. It is possible that instating a centralized IRB, which has proved successful in oncology, with a focus on CNS regenerative medicine could facilitate the process, by providing expert guidance, e.g., on pediatric studies and other aspects of regenerative CNS approaches to local IRBs.

Preclinical Animal Testing

Support for the clinical application of NSCs or other stem/progenitor cells relies heavily on satisfactory proof of concept, efficacy, and safety in animal models of human disease. The FDA supports animal use aligned with the international commitment to the 3R concept: *reduce, refine, and replace*, ensuring that preclinical studies use reasonable numbers of animals and the optimum model and, if possible, replace animals by alternate means of testing. However, because no animal model entirely recapitulates the complexity of human pathology and anatomy, they are not always predictive of clinical outcomes. Furthermore, measuring clinically relevant endpoints related to higher neural functions such as cognition, learning, and memory is not always feasible. Large animal models are sometimes regarded as a needed second test species in which to confirm efficacy and/or safety as well as short-term surgical feasibility studies. However, these additional large animal studies are still xenogenic and very expensive and, especially in the case of nonhuman primates, require deep consideration for ethical use. Other ethical issues include humanization of the animal CNS by neural cell transplantation, which lead to additional scrutiny, for example, during SCRO review. Finally, we note that the accurate repopulation of immunodeficient rodent brains with NSCs and of the hematopoietic system with human HSCs has led to FDA-authorized clinical trials without the use of larger animals.

Characterization and Manufacture of Cell Product for Transplantation

Defining a therapeutic stem cell product is challenging as cells are not drugs with precise structures, but highly complex biological entities for which sets of key markers and attributes are still being defined. In the case of stem cell-derived RPE cells, for example, which are moving rapidly toward the clinic, signature gene expression patterns for the native tissue (Strunnikova et al., 2010) can help construct biomarker-based definitions for stem cell-derived RPE cells. While terminally differentiated cells may be most valuable for some indications, in other cases a precursor cell may be better suited for transplantation. For example, in myelination disorders, progenitors from fetal versus adult donors have distinct properties making them valuable for different applications (Goldman, 2011). Therefore, it may be necessary to define a specific stage of the lineage for optimum results, underlining the need to perform thorough developmental biology groundwork.

Once the final cell product is identified, the production of cell lots for clinical use is a complex process that starts at the donor (of cells and/or tissues) level and ends in the preparation steps for product administration to the patient. Any activity along this process may introduce elements that can pose potential risks for adverse events. Cell-based therapies thus require stringent safety assessments, particularly in relation to contamination with infectious disease agents, animal product use, instability due to extended expansion, and tumorigenicity. The FDA has created guidance documents that address the various controls and safeguards starting with donor eligibility, initial collection of the source tissue under current good tissue practice (cGTP), and subsequent manufacturing steps under current good manufacturing practice (cGMP), which include tiered testing of master and working cell banks, as well as release testing that is done on the final cell product for transplantation (e.g., sterility, purity, identity) (Burger, 2003; Rayment and Williams, 2010). Therefore, production of a cGTP/cGMP cell bank is a significant aspect of developing a cell therapy and investigators should not underestimate the complexity, the time involved, and the scientific and financial aspects of deriving cell doses for patient testing. In addition, given that pivotal preclinical studies should

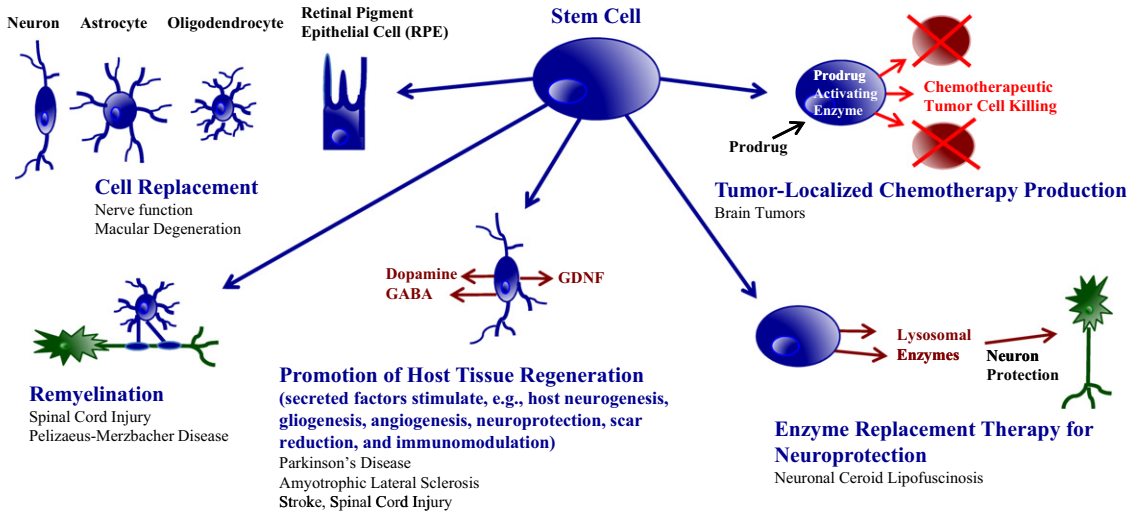

Figure 4. Stem Cell-Mediated CNS Regeneration and Therapeutic Delivery
Schematic identifying representative CNS stem cell replacement, regeneration, and therapeutic delivery strategies.

preferentially be carried out using the intended clinical cell lot, the need to implement cGMP/cGTP practices early may greatly impact timelines and development costs.

Cell Fate and Tracking In Vivo

For therapies involving cell administration to the CNS, determination of cell migration and fate in real time and long term is of major interest as it relates to dosing, efficacy, optimization, and safety concerns. Reporter genes used in preclinical studies are not intended for the final cell product used in clinical trials. Therefore, identification of donor cells in tissue at resection or autopsy can be made if there is a gender mismatch between donor and recipient or if there is a specific donor cell marker. A promising cell-tracking method for monitoring NSCs involves preloading with superparamagnetic iron oxide particles (SPIOs) just prior to administration and subsequently tracking their distribution over time by MRI. Preclinical studies in mice have demonstrated the effective use of MRI to track iron-labeled NSCs (Guzman et al., 2007; Thu et al., 2009), and the safe use of iron oxide MRI contrast agents has been demonstrated in clinical research studies for CNS tumor visualization and diagnostic MRI. K.A., R. Moats, and J. Frank et al. are currently conducting the necessary preclinical safety and toxicity studies toward achieving FDA approval of iron labeling for their current NSC-mediated glioma clinical trial, described below. These advances will probably have applications for stem cells in other CNS clinical trials.

Choice of Disease Target

First-in-human studies involving NSC transplantation have been conducted in severe diseases in which the risk/benefit ratio is favorable. The first two trials involving transplantation of human NSCs into the brain were both for fatal, rare disorders: neuronal ceroid lipofuscinosis (NCL) and Pelizeaus-Merzbacher disease (PMD). Sponsors can benefit from the expedited timeline associated with fast-track status as well as cost and marketing incentives associated with an orphan drug status to efficiently get into clinical phase I programs. The Orphan Drug Act defines orphan drugs as those used to treat rare diseases (less than 200,000 people) and it provides grant money, tax credits, and exclusive marketing rights for 7 years after drug approval. For priority drugs and biologics, the FDA has an expedited fast-track program to shorten the new drug application (NDA) review time from around 12 months to 60 days. In addition, to promote discovery of treatments for pediatric diseases, the FDA established the pediatric rule, which requires assessment of safety and efficacy in children for select products, compensated by extending market exclusivity for 6 months (Liu, 2010). Once human safety is established, both phase II dose-escalation studies and the inclusion of nonfatal diseases with larger population bases may be facilitated. Other sponsors have opted for initiating clinical testing of their cell product in more prevalent conditions such as acute spinal cord injury, stroke, ALS, and brain tumors (Tables 1, 2, and 3).

Outcome Measures

One of the important hurdles in clinical study design for cell therapy trials is defining endpoints, as this is the measure of the trial's failure or success. This is particularly challenging given the degenerative nature of many target neurological disorders under consideration and the complexity posed by the rate of progression and lack of validated surrogate markers of disease.

The overall goal of phase I studies is to assess safety and feasibility, with the primary objective typically being to determine the maximum tolerated dose and dose-limiting toxicities. Secondary objectives are usually correlative studies that will expand the knowledge gained from conducting the trial. Examples include imaging studies to determine distribution of the stem cells, assessment of possible immunogenicity, and postresection and/or postmortem histopathological evaluation. Note that in the absence of noninvasive donor cell tracking, and especially in diseases in which patients might survive for many years after transplant, histological measures of donor cell survival, migration, or differentiation may not be available for decades. In terms of assessing for toxicity, adverse events are graded using scales such as the NIH Common Terminology Criteria for

Table 1. Clinical Trials: Neural Stem Cell-Mediated CNS Regenerative Therapy

Company / Institution and Trial Site / PI	CNS Regeneration Indication Health Authority / Regulatory Agency	Stem Cell Source and Final Product	Delivery Route and Location
Advanced Cell Technology Inc., CA www.advancedcell.com Jules Stein Eye Institute at UCLA PI: S. Schwartz, MD	Phase I/II: Stargardt's macular degeneration (juvenile) Phase I/II: Dry age-related macular degeneration (AMD) U.S. Food & Drug Administration, trial initiation pending	huESC-derived retinal pigmented epithelial cells (RPEs) MA09-hRPE Allogeneic	Direct single subretinal injection of 50–200,000 RPEs.
California Stem Cell Inc., CA www.californiastemcell.com PI: K. Swoboda, MD	Phase I: Spinal muscular atrophy type I (infants age 2–6 months) U.S. Food & Drug Administration, on clinical hold	hESC-derived motor neuron progenitors (MNPs) MotorGraft™ Allogeneic	Direct multiple injections into anterior horns of thoracic spinal cord. Short-term immunosuppression.
Geron Corp., CA www.geron.com Stanford Univ,/Santa Clara Valley Med Ctr, Palo Alto, CA PI: G. Steinberg, MD, PhD Shepard Ctr, Atlanta PI: D. Apple, MD; Northwestern Univ., Chicago PI: R. Fessler, MD, PhD; Thomas Jefferson Univ Hosp, Phil PI: J. Harrop, PM	Phase I: Neurologically complete subacute, thoracic spinal cord injury ClinicalTrials.gov ID#NCT01217008 U.S. Food & Drug Administration	huESC-derived oligodendrocyte progenitor cells GRNOPC1® Allogeneic	Direct single injection of 2 million cells (50 μL) into lesioned spinal cord site (5 mm caudal of injury epicenter) between T3–T10 segments 7–14 days after injury. Short-term low-dose Tacrolimus.
Neuralstem, Inc., MD www.neuralstem.com PI: E. Feldman MD, PhD, Univ. Michigan Emory University ALS Center PI: J. Glass, MD; N. Boulis, MD	Phase I: Amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) ClinicalTrials.gov ID#NCT01348451 U.S. Food & Drug Administration	Fetal spinal-cord-derived huNSCs (8 wk gestation) expanded by epigenetic means with defined medium (polyclonal) NSI-566RSC Allogeneic	Direct multiple injections (100,000 cells per 10 μL injection) into central gray matter of lumbar spinal cord segments (L2–L4) in 5 unilateral or 10 bilateral injections (500,000 or 1 million cells total). Long-term immunosuppression.
NeuroGeneration, Inc., CA www.neurogeneration.com Cedars-Sinai Medical Ctr PI: M. Lévesque, MD Site: Los Angeles Neurosurgical Inst. PI: M. Lévesque, MD	Phase I complete: Advanced Parkinson's disease U.S. Food and Drug Administration Phase II: Advanced Parkinson's disease U.S. Food & Drug Administration, on clinical hold	huNSCs obtained by needle biopsy Dissociated cell suspension of undifferentiated huNSCs and huNSC-derived neurons Autologous	Intracerebral unilateral transplantation into putamen of 6 million cells total (6 deposits). No immunosuppression. Results reported in Lévesque et al. (2009).
ReNeuron Ltd., Surrey, UK www.reneuron.com Instit. of Neurological Sciences, Glasgow Southern General Hosp, Glasgow, UK PI: K. Muir, MD	Phase I: Stable ischemic stroke Pilot Investigation of Stem Cells in Stroke (PISCES Trial) ClinicalTrials.gov ID#NCT01151124 U.K. Medicines & Health Care Products Regulatory Agency Gene Therapy Advisory Committee	Fetal brain-derived-NSCs (12 wk gestation) conditionally immortalized with cmycER ReN001/CTX0E03 Allogeneic, genetically modified, clonal	MRI-guided intracerebral injection of NSCs directly into putamen adjacent to infarct area. No immunosuppression.
StemCells, Inc., CA www.stemcellsinc.com Univ. California San Francisco, CA PI: D. Rowitch, MD, PhD Balgrist University Hospital University of Zurich, Switzerland PI: A. Curt, MD	Phase I: Pelizaeus-Merzbacher disease (PMD) ClinicalTrials.gov ID#NCT01005004 U.S. Food & Drug Administration Phase I/II: Chronic Thoracic Spinal Cord Injury ClinicalTrials.gov ID#NCT01321333 Swissmedic	huCNS-SCs derived from donated fetal brain tissue Flow cytometry cell selection based on CD133 and CD24 expression ($CD133^{+}CD24^{-/lo}$) HuCNS-SC Allogeneic	Phase I: Direct bilateral intracerebral injections into frontal white matter (2 per hemisphere). 9 months immunosuppression. Phase I/II: Direct intraspinal injections into superior and inferior margins of thoracic spinal cord injury (3 and 12 months postinjury). 20 million cells total. 9 months immunosuppression.

Adverse Events, version 4.0. The relationship of an adverse event to study treatment (unrelated, unlikely, possibly, probably, or definitely related) is assigned based on the known side effects of the therapy and the patient's personal medical history. Long-term follow-up for assessment of late toxicity is important, particularly in patients with nonfatal conditions, such as spinal cord injury, who might survive for many years after transplant. Although we hope to see some indication of therapeutic efficacy in phase I trials, it is not a prerequisite for the initiation of phase II studies, which are designed to evaluate efficacy.

The focus of phase II studies should include clinical outcomes that can be measured and result in a benefit for the patient. Examples of primary objectives for phase II studies include assessment of response rate (for example, defined as shrinkage of tumor in brain cancer studies or improvement in neurologic function in patients with ALS), time to disease progression and overall survival. Other examples include improvement of visual acuity or visual field sensitivity for retinal disorders and transition to a different American Spinal Injury Association (ASIA) grade for spinal cord injury. A treatment that demonstrates efficacy in a phase II study will then typically move on to phase III testing. Phase III studies are randomized, controlled, multicenter trials of large numbers of patients for definitive assessment of therapeutic efficacy as compared to the standard-of-care.

In summary, defining outcome measures and endpoints is a complex and sobering exercise. Unfortunately, applicability and value of such endpoints is oftentimes only evident after the trial is completed and many millions of dollars have been spent, highlighting the importance of a thorough and realistic reflection on endpoint selection.

Survey of CNS Clinical Trials Using Stem Cell Therapy

Therapeutic approaches using NSCs and other stem cell products for the treatment of CNS injury and disease fall into two broad categories, summarized in Figure 4: (1) regenerative/cell replacement to promote host tissue repair mechanisms and/or replace missing or damaged cells, and (2) therapeutic delivery to provide therapeutic macromolecules (enzymes, cytokines, neurotrophins, drugs, etc.) for neuroprotection, drug therapy, and/or stimulation of repair. A third clinically relevant approach is drug discovery via stem cell-based disease models. In this section we focus on regulatory approved stem cell-based CNS clinical trials, summarized in Tables 1, 2, and 3, and include some preclinical studies that are considered close to IND.

1. Regenerative/Cell Replacement Strategies

Stem cell therapies for neural transplantation and repair aim to replace damaged cells and/or promote host tissue local neural repair mechanisms, including neurogenesis, gliogenesis, and angiogenesis (see Table 1). Human NSCs derived from pluripotent cells or extracted from CNS tissue can be used as undifferentiated cells, relying on the host signals to stimulate their proliferation and differentiation, or their lineage descendents can be utilized, such as GRPs. The donor cells are typically delivered via stereotactic injection into the affected regions. An alternate means of cell replacement being developed is the recruitment of endogenous neural progenitor cells from active adult germinal zones or relatively dormant progenitors elsewhere in the CNS, as demonstrated in promising animal models of PD (Androutsellis-Theotokis et al., 2010).

Spinal Cord Injury

In 2010, two trials were authorized for the use of neural cells to treat SCI. Geron Corporation (Geron) received FDA clearance to initiate a phase I trial using hESC-derived oligodendrocyte progenitors (OPCs), GRNOPC1, in subacute thoracic SCI. This landmark study represents the first huESC-derived product for clinical testing. StemCells, Inc. (StemCells) received regulatory authorization in Switzerland (SwissMedic) to conduct a phase I/II trial in chronic thoracic SCI using fetal-derived NSCs (HuCNS-SC). There are important similarities and differences in the design of each of these studies. Geron's GRNOPC1 contains hESC-derived OPCs that have demonstrated remyelinating and nerve-growth-stimulating properties leading to restoration of locomotor function in a rat model of acute contusion SCI (Keirstead et al., 2005). StemCells reported similar findings in a mouse model of spinal cord contusion injury using HuCNS-SC (Cummings et al., 2005) and demonstrated their efficacy beyond the acute injury stage (Salazar et al., 2010). The Geron phase I study will be conducted in ten patients with neurologically complete (grade A as defined using ASIA criteria) subacute thoracic (T3–T10) injuries. Enrolled patients will receive a single injection of 2 million GRNOPC1 cells into the lesioned site 1–2 weeks after injury. In contrast, StemCells' HuCNS-SC phase I/II trial is enrolling patients with complete and incomplete injury (ASIA A, B, and C) 3 to 12 months (early chronic phase) after thoracic injury (T2–T11), and patients will receive a dose of 20 million cells.

These trials should shed light on the potential for cell therapy in SCI. The Geron trial, as a first test of hESCs, is being awaited with both excitement and trepidation—if the outcome is negative, for example due to abnormal growths from infusion of cells with high proliferative potential, then this could be viewed as a blow to the hESC field. At the same time, we must remember that failures, however difficult to contemplate, are to be expected during development of a revolutionary new therapeutic, as was the case for bone marrow transplantation and the polio vaccine. Progress is most often made by going from "bench to beside" and back to the bench again with the gained clinical information applied to an improved second generation product to take back to the clinic. It is therefore important to educate the public as to the possible outcomes, both positive and negative, and the process and timeline for new stem cell therapy development.

Pelizeaus-Merzbacher Disease

StemCells is conducting a phase I study of pediatric patients with connatal PMD, a fatal congenital dysmyelination disorder, using HuCNS-SC. PMD results from a mutation in the X-linked proteolipid protein (PLP) gene, essential for myelin formation (Inoue et al., 1999). The more severe form, connatal PMD, typically presents soon after birth and severe neurological impairments with abnormal mental and physical development lead to premature death. Enrollment for this trial was completed in early 2011; its primary goal is to determine safety. The potential to measure donor-derived myelination will also be assessed by MRI as a secondary endpoint in this study. Other preclinical studies have explored human glial progenitors for the treatment

Table 2. Clinical Trials: Nonneural Stem Cell-Mediated CNS Regenerative Therapy

Company / Institution and Trial Site / PI	CNS Regeneration Indication Health Authority / Regulatory Agency	Stem Cell Source and Final Product	Delivery Route and Location
Aldagen www.aldagen.com Los Angeles Brain and Spine Insti., CA PI: G Rappard, MD	Phase II: Postacute ischemic stroke ClinicalTrials.gov ID#NCT01273337 U.S. Food & Drug Administration	Adult bone marrow SCs expressing high levels of ALDH enzyme ALD-401 Autologous	Intracarotid infusion 13 and 19 days after unilateral, predominantly cortical, ischemic nonlacunar stroke.
Athersys Inc. www.athersys.com	Phase I: Acute ischemic stroke U.S. Food & Drug Administration Trial initiation pending	Adult huBMSC-derived Multipotent Adult Progenitor Cell (MAPC) MultiStem® Allogeneic	Intravenous administration 2 days after stroke.
BrainStorm Cell Therapeutics, Ltd. www.brainstorm-cell.com Hadassah Hebrew Univ. Medical Center, Jerusalem, Israel PI: D. Karassus, MD, PhD	Phase I/ II: Amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) ClinicalTrials.gov ID#NCT01051882 Ministry of Health, Helsinki Committee of Hadassah, Israel	Adult bone-marrow-derived mesenchymal stromal cells (huMSC) differentiated into cells secreting neurotrophic factors (NTF) such as GDNF and BDNF (MSC-NTF cells) NurOwn Autologous	ALS < 6 months: Intramuscular multiple injections into 24 separate sites on biceps and triceps muscle / ALS > 6 months: Intrathecal single injection into CSF
China Medical University Hospital www.cmuh.cmu.edu.tw/cmuh/index.php China Medical University Hosp., Taiwan PI: S.Z. Lin, MD, DMSci	Phase II: Chronic ischemic stroke (age 35–70 years) ClinicalTrials.gov ID#NCT00950521 Taiwan Department of Health, China	huPBSCs - peripheral blood CD34+ SCs Autologous	Intracerebral implantation of autologous CD34+ huPBSCs into patients with chronic middle cerebral artery infarction in combination with conventional treatment.
Duke University www.dukehealth.org Duke University Med. Ctr., Durham, NC PI: J. Kurtzberg, MD	Phase I complete: Spastic cerebral palsy Phase II: Spastic cerebral palsy (children age 1–6 years) ClinicalTrials.gov ID#NCT01147653 U.S. Food & Drug Administration	huUCBs (banked at birth) Autologous	Phase I results: Sun et al. (2010) demonstrating safety and feasibility Phase II: Intravenous infusion of 10 to 50 million cells/kg of autologous UCB cells.
Johnson & Johnson Pharmaceutical Research & Development, LLC www.jnjpharmarnd.com UT Health Science Ctr., Houston Memorial Hermann Hosp., Houston TX PI: S. Savitz, MD	Phase I: Acute ischemic stroke ClinicalTrials.gov ID#NCT01273467 U.S. Food & Drug Administration	huUCB-derived cells CNTO 0007/42037788 Allogeneic	Intravenous single infusion administered within 1–5 days of stroke.
Memorial Hermann Healthcare System www.memorialhermann.org UT Health Science Center, Houston PI: J. Baumgarnter, MD	Phase I: Chronic spinal cord injury (SCI) (children age 1–15 years) ClinicalTrials.gov ID#NCT011328860 U.S. Food & Drug Administration	huBMPCs harvested from subject 6 months–4 years post SCI, processed for selection of mononuclear cells Autologous	Intravenous infusion (single dose) of autologous huBMPCs administered within 6 hr of harvest.
SanBio Inc., CA www.san-bio.com Stanford Univ. School of Med, CA PI: G. Steinberg, MD, PhD UPMC, Pittsburg, PA PI: D. Kondziolka, MD	Phase I/IIa: Chronic ischemic stroke ClinicalTrials.gov ID#NCT01287936 U.S Food & Drug Administration	Adult mesenchymal SCs transiently modified with Notch plasmid SB623 Allogeneic, transiently modified	MRI-guided stereotactic intracerebral injections (3 sites) into peri-infarct subcortical area, administered at least 6 months after stroke event.
Stemedica Cell Technologies, Inc., CA www.stemedica.com Univ. California San Diego, CA PI: M. Levy, MD, PhD FACS	Phase I/II: Chronic ischemic stroke ClinicalTrials.gov ID#NCT01297413 U.S. Food & Drug Administration	Donor mesenchymal huBMSCs Processed for mononuclear cell selection, and expansion of colonies to generate a MCB. Allogeneic	Intravenous administration (single dose), administered at least 6 months after stroke event. No immunosuppression.

Table 2. *Continued*

Company / Institution and Trial Site / PI	CNS Regeneration Indication Health Authority / Regulatory Agency	Stem Cell Source and Final Product	Delivery Route and Location
TCA Cellular Therapy, LLC www.tcacellulartherapy.com TCA Cellular Therapy, Covington, LA PI: G. Lasala, MD	Phase I: Amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) ClinicalTrials.gov ID#NCT01082653 U.S. Food & Drug Administration	BM-derived MSCs ex-vivo expanded up to passage 3 Autologous	Intrathecal single infusion of 50 million cells.
Univ. of Texas Health Science Center Houston, TX www.uthouston.edu Children's Memorial Hermann Hospital, Houston, TX PI: C.S. Cox, Jr., MD	Phase I complete: Acute traumatic brain injury (TBI) in Children (age 5–14 years) ClinicalTrials.gov ID#NCT00254722 U.S. Food & Drug Administration Phase I: Chronic TBI in children (age 18 months–17 years) ClinicalTrials.gov ID#NCT01251003 U.S. Food & Drug Administration	huBMPCs harvested from subject 12–30 hr after TBI, processed for selection of mononuclear cells (MNCs) BMMNCs Autologous huUCBs (banked at birth, Cord Blood Registry, Inc.) Autologous	Intravenous infusion of (6 million cells/kg body weight) administered within 48 hr of TBI. Results: Safety and feasibility established Cox et al., 2011. Intravenous infusion at 6–18 months after TBI event.

of such congenital dysmyelination disorders on the shiverer mouse, showing that donor cells substantially myelinate the host brain, to the point of achieving clinical rescue (Windrem et al., 2008).

Amyotrophic Lateral Sclerosis

ALS is a progressive motor neuron disease that also involves glial cell pathology. In September of 2009, NeuralStem, Inc. received FDA authorization to conduct a phase I trial in ALS using adherent cultured, fetal-derived spinal NSCs. Preclinical testing demonstrated that NSCs transplanted into the lumbar spinal cord of adult SOD1 G93A rats delayed the onset and progression of the motor neuron disease (Xu et al., 2006). The main objective of this trial is to evaluate safety of up to ten injections of NSCs in 12 patients in four groups, depending on disease severity. Q-Therapeutics is working toward an IND for ALS using a human fetal-derived GRP cell product, and if successful, transverse myelitis and MS, both involving loss of myelin, are their next anticipated targets.

Retinal Dystrophy and AMD

Retinal diseases are seen as an important point of entry for CNS cell therapy because the retina is the most accessible part of the CNS, contains a relatively small number of cells, and outcomes of visual function can be accurately monitored. Devastating blinding disorders such as retinitis pigmentosa (RP) and the highly prevalent AMD lack effective treatments. Over the past decades, replacement of the outer photoreceptor cell layer and RPE with fetal tissue has demonstrated transient visual recovery in animal models and patients leading to clinical trials of human fetal tissue transplantation for these disorders (N. Radkte, Clinicaltrials.gov NCT00346060; S. Binder, Clinicaltrials.gov NCT00401713). Retinal stem cells (RSCs) have now been isolated from retina tissue and retinal cells generated from hESCs. When transplanted, adult and ESC-derived retinal cells incorporate and rescue vision in animal models (Lamba et al., 2008; Wallace, 2011; West et al., 2009). Although retinal replacement using RCSs has promise, human trials have not yet been initiated. HuCNS-SC transplanted into the subretinal space are being moved towards an IND application by StemCells.

hESCs can be differentiated into RPE and transplantation of hESC-derived RPE cells (ESC-RPEs) preserves vision in animal models (Lu et al., 2009). Advanced Cell Technology, Inc. (ACT) received FDA authorization for studies using hESC-RPEs for Stargardt's macular dystrophy in 2010 and for AMD in early 2011. Although the primary defect in Stargardt's appears in the photoreceptors, secondary damage to the RPE underlies the rationale for replacing the RPE to improve cell function, support the photoreceptors, and delay retinal cell death. The AMD study will enroll 12 patients to address potential immunogenicity, tumorigenicity, and other safety issues for allogeneic hESC-RPE transplantation into retina. Cells will be injected as a suspension, and it remains to be seen whether they will incorporate into the existing RPE layer to form the polarized epithelium key for its normal function. Nevertheless, it is possible that a cell suspension could provide beneficial trophic factors even without epithelialization, although complications that are associated with RPE cell delamination, such as proliferative vitreoretinopathy, will be important to monitor. Related preclinicial work using hES-RPE is being developed by University College London in partnership with Pfizer's London Project, at U.C. Santa Barbara in partnership with Geron under a CIRM disease team grant and at Hadassah Medical Center, Jerusalem in partnership with CellCure Neurosciences, Ltd. Tissue-derived stem cells and adult RPE progenitor cells offer expanded quantities of standardized cells for replacement of the RPE retinal layer. The latter is being developed toward an IND for RPE replacement therapy at the Neural Stem Cell Institute.

Parkinson's Disease

PD is the most common neurodegenerative movement disorder, characterized by a loss of dopaminergic (DA) neurons in the substantia nigra, degeneration in the brainstem, and loss of other catecholaminergic neurons, which eventually leads to motor

Table 3. Clinical Trials: Stem Cell-Mediated Therapeutic Delivery to CNS

Company / Institution and Trial Site / PI	CNS Regeneration Indication Health Authority / Regulatory Agency	Stem Cell Source and Final Product	Delivery Route and Location
Biocompatibles International, PLC www.biocompatibles.com The International Neuroscience Inst. (INI), Hannover, UK	Phase I/II: Acute Hemorrhagic Stroke Paul Erlich Institute, Regulatory Institute of the Ministry of Health, Germany	Donor adult huMSCs encapsulated in alginate beads. CellBeads Programmed to deliver CM1, a proprietary version of GLP-1 protein. Allogeneic, genetically modified	Cellbeads are transplanted within a retrievable mesh device directly into injury site and retrieved after a treatment period of 14 days.
City of Hope National Medical Center www.coh.org City of Hope, Duarte, CA PI: J. Portnow, MD	Phase I: Recurrent High Grade Glioma Clinical Trials.gov ID#NSC01172964 U.S. Food & Drug Administration Recombinant DNA Advisory Committee (RAC)	Fetal brain-derived (15 wk gestation) v-*myc* immortalized, cytosine deaminase expressing NSCs HB1.F3.CD Allogeneic, genetically modified, clonal	Direct intracerebral injections (10 sites) into tumor surgical resection cavity wall followed 4 days later by 1 week of treatment with oral 5-FC. Standard postoperative dexamethasone tapered as tolerated.
StemCells Inc, CA www.stemcellsinc.com Oregon Health & Science University Portland, OR PI: N. Seiden, MD, PhD	Phase I completed: Neuronal ceroid lipofuscinosis (Batten disease) U.S. Food & Drug Administration Phase Ib: Neuronal Ceroid (suspended) Lipofuscinosis ClinicaTrials.gov ID#NCT01238315 U.S. Food & Drug Administration	huCNS-SCs derived from donated fetal brain tissue Cells selected by flow cytometry based on CD133 and CD24 expression (phenotype $CD133^{+}CD24^{-/lo}$) HuCNS-SC Allogeneic	Direct bilateral subcortical injections (3 per hemisphere) and intraventricular injections (1 per lateral ventrical). 500 million cells total (3 patients) or 1 billion cells total (3 patients). 12 months immunosuppression. Phase Ib: Direct bilateral subcortical injections (3 per hemisphere). 9 months immunosuppression.

We have made our best effort to include representative regulatory approved stem cell trials for CNS injury and disease and to fact check from original sources. We apologize for not being able to include all relevant trials. BMSCs = bone marrow stem cells; BMPCs = bone marrow progenitor cells; CNS-SCs = central nervous system stem cells; ESCs = embryonic stem cells; GDNF = glial-derived neurotrophic factor; NSCs = neural stem cells; PBSCs = peripheral blood stem cells; UCBs = umbilical cord blood cells; BDNF = brain derived neurotrophic factor; GDNF = glial-derived neurotrophic factor; and hu = human. This description pertains to Tables 1, 2, and 3.

dysfunction and multiple neurological deficits. There is a long history of fetal cell and tissue transplantation to the projection sites of these DA neurons, the caudate-putamen, which has shown some promising results, tempered by the development of disabling dyskinesias in a number of patients (Hagell et al., 2002). Concern has also been raised that engrafted cells may acquire the disease phenotype, as reflected in synuclein aggregates found at autopsy, although the significance of this observation is debated (Isacson and Mendez, 2010). Nevertheless, for some PD patients, engrafted fetal-derived cells have given long-term relief, providing a rational basis for pursuing stem cell grafts of more uniform, defined cells. Data from such studies indicate that the relevant cell type is an immature A9 type dopaminergic neuron (Grealish et al., 2010; Mendez et al., 2005). Methods are progressing to differentiate hESCs toward production of these bona fide midbrain DA neurons in sufficiently high numbers for transplantation, and this is likely to be another early indication for an hESC-derived cell product. Another approach for PD being explored by Neurogeneration, Inc. is autologous transplantation of cultured cells derived from cortical and subcortical tissue, which is reported to expand in vitro and produce some catecholaminergic and gabaergic neurons; although the current trial data are limited to a single case report, an autologous approach could be valuable as it avoids immunosuppression.

Stroke

ReNeuron is currently conducting a first-in-human trial for chronic stable stroke, administering fetal-derived allogeneic NSCs conditionally immortalized with c-mycER into the putamen adjacent to the infarct area, in order to promote surrounding host tissue regenerative responses. Preclinical studies in rats with middle cerebral artery occlusion demonstrated behavioral recovery in a dose-dependent fashion. NSCs are postulated to release factors that promote vascular growth and restoration of blood supply in damaged areas (Stroemer et al., 2009). It will be important to ascertain how long these cells survive in vivo and, given that the cell product is an immortalized line, to determine the safety profile in humans.

Nonneural CNS Stem Cell Treatments

Despite the fact that nonneural sources of stem cells do not normally generate bona fide neurons or macroglial progeny, a significant number of CNS clinical trials utilize such cells (see Table 2). In some cases there is clear rationale and evidence for nonneural cells alleviating cell loss or disease in the CNS, e.g., in

rebuilding damaged blood vessels, which can be beneficial to restore areas of ischemic damage and slow neurodegeneration, as in murine models of retinal disease (Otani et al., 2004). Another example is the treatment of Krabbe's disease (globoid cell leukodystrophy), a fatal lysosomal storage disease (LSD) in children, where clinical benefit is seen by presymptomatic treatment with allogeneic umbilical-cord blood stem cells (Escolar et al., 2005). Correction in this and similar leukodystrophies is mediated by cellular enzyme replacement therapy through long-term engraftment of donor cells in the brain. In some cases, the transplanted nonneural stem cells are present in the CNS for a very short period, perhaps weeks, but this short-term presence is envisioned to generate beneficial effectors such as cytokines to ameliorate the disease process. The use of transient nonneural cells to treat severe and progressive neurological conditions has been viewed with considerable skepticism, especially in the scientific community, and yet with considerable hope in the patient community. Now a number of clinical trials have been authorized; indeed, the regulatory hurdles for safety, e.g., using autologous stem cells, can be easier to surmount, and as they progress, efficacy for a variety of CNS indications will be determined.

Stroke

SanBio, Inc. is currently in phase I/IIa trials with a genetically modified bone marrow stromal cell product for stroke, SB623, derived by transfection with a plasmid encoding the human Notch-1 IntraCellular Domain (NICD) in order to enhance the cells' regenerative properties (Yasuhara et al., 2009), a process that may involve local delivery of soluble trophic factors, deposition of supportive extracellular matrix, and/or anti-inflammatory effects. SB623 will be delivered by direct transplantation into the brain, while other nonneural stem cell clinical trials are using intravenous infusion. Athersys, Inc. is investigating the administration of allogeneic bone marrow-derived multipotent adult progenitor cells two days after stroke. Aldagen is administering autologous bone-marrow stem cells into the carotid artery 2–3 weeks after stroke. Aldagen's cells are selected for expression of high levels of ALDH enzyme, which enriches for early hematopoietic cells (Gentry et al., 2007). A similar approach is being taken by Johnson and Johnson using umbilical-cord-derived cells. Again, multiple mechanisms have been proposed for benefit, based on expression of a complex set of factors that reduce inflammation, protect surrounding brain cells, and stimulate host angiogenesis.

Cerebral Palsy and Pediatric Traumatic Brain Injury

CP is caused by damage to brain motor areas in utero or during childbirth, often due to ischemic or hemorrhagic stroke. An ongoing study at Duke University is testing, in a randomized, placebo-controlled trial, whether an intravenous infusion of autologous cord blood, collected and banked at birth, can lessen the symptoms of children with CP between the ages of 1 and 6 years.

TBI is a major cause of death and disability in young children and adults. A phase I study completed at the University of Texas Health Science Center that used harvested bone marrow from pediatric TBI patients within 30 hr of the injury, followed by intravenous infusion of their autologous bone-marrow-derived mononuclear cells several hours later, has demonstrated safety and feasibility (Cox et al., 2011).

Stimulating Endogenous Stem/Progenitor Cells

Another CNS regenerative/cell replacement strategy utilizes drugs or cytokines, rather than stem cell transplantation, to stimulate the patient's endogenous NSCs. Stem Cell Therapeutics, for example, has treated patients with acute ischemic stroke (clinicaltrials.gov NCT00362414) with a 9 day drug regimen of Beta-hCG plus Erythropoietin (NTx-265). This drug combination is postulated to stimulate the patient's own resident NSCs to reduce brain damage and promote regenerative processes in the ischemic brain region. A phase IIb clinical trial was reported in May 2010 to have failed to show efficacy due to unexplained high-level response in the placebo group as well as the experimental group.

2. Therapeutic Delivery

Stem cells may also be used to deliver therapeutic molecules, in some cases being modified prior to transplantation for use as a delivery vehicle to target sites of pathology (see Table 3). The types of molecules delivered include (1) neurotrophic factors and cytokines that can enhance regeneration, reduce cell damage and scarring, and promote process outgrowth and connectivity, (2) enzymes that can replace lost or mutated processes, and (3) chemotherapeutic agents for novel tumor treatments (Figure 4).

Batten's Disease

The first FDA-authorized IND using prospectively purified, ex vivo-expanded NSCs derived from donated fetal human brain (HuCNS-SC) was sponsored by StemCells for enzyme replacement in the two infantile forms of (NCL; Batten's Disease), a rare and fatal lysosomal storage disease in which a genetic defect leads to abnormal accumulations in lysosomes, neuronal dysfunction, and loss. The preclinical rationale was established in the immunodeficient PPT1 knockout mouse that exhibits key hallmarks of the human disease (Gupta et al., 2001). HuCNS-SC transplanted into the mouse brain migrated widely and produced the deficient PPT1 enzyme, leading to reduced stored material, preservation of hippocampal and cortical neurons, and a delay in motor coordination loss (Tamaki et al., 2009). The NCL phase I open-label study enrolled six pediatric patients with severe infantile and late-infantile NCL in a dose escalation design: testing a total dose of 500 million cells in the first three patients and one billion in the next three patients. The surgery, which involved multiple bilateral HuCNS-SC transplants into the brain in a single-stage procedure, was well tolerated and was followed by 12 months of immunosuppression. Postmortem evidence of donor cell survival was obtained in one subject who expired from the underlying disease 11 months after transplant. This phase I study, reported in June 2009, was the first to show human safety data with a NSC product (Steiner et al., 2009). The follow-on phase 1b study was halted in April 2011 due to inability to recruit patients matching the enrollment criteria, which is one of the potential drawbacks when targeting rare diseases.

Brain Tumors: Glioma

NSCs display inherent tumor-tropic properties that can be exploited for targeted delivery of anticancer agents to tumor cells (Aboody et al., 2008). This strategy minimizes toxicity to normal tissues, potentially reducing undesirable side effects. A phase I clinical trial was initiated in September 2010 by COH for patients with recurrent high-grade gliomas, who have a median survival of 3–6 months with currently available treatments. This trial is

testing an extensively characterized allogeneic NSC line (HB1.F3.CD), derived from fetal brain telencephalon by immortalization with v-*myc*, enabling effectively unlimited in vitro clonal expansion (Kim, 2007). The line was further genetically modified to express cytosine deaminase (CD), an enzyme that converts the prodrug 5-Fluorocytosine (5-FC) to the active chemotherapeutic 5-Fluorouracil (5-FU). Safety, stability, and therapeutic efficacy studies were conducted in orthotopic glioma mouse models. Based on these and previous studies, it is postulated that after multiple injections into the tissue surrounding the tumor resection cavity at the time of surgery, the NSCs will migrate to residual and invasive brain tumor foci and convert orally administered 5-FC to 5-FU, preferentially killing surrounding tumor cells. This dose escalation safety trial will enroll 12–16 patients and is the first study to explore the safety and feasibility of a genetically modified allogeneic stem cell-based targeted cancer therapy using an enzyme/prodrug system in human patients. A second-generation strategy (funded by CIRM) is in progress with NSCs engineered to secrete a carboxylesterase that activates the prodrug CPT-11 (Irinotecan) to the topoisomerase inhibitor SN-38, a potent anticancer agent.

3. Drug Discovery and Toxicity Testing via Stem Cell-Based Disease Models

Another promising application of stem cells is in vitro models to study disease mechanisms, screen for drug candidates, and test drug toxicity. Stem cell-based "disease in a dish" models, particularly for diseases lacking good animal models, are developing rapidly and gaining recognition as proof of concept for IND applications. Improvements in stem cell-based in vitro models, and the advent of iPSCs expressing patient-specific disease characteristics, is anticipated to be an increasingly valuable component of the drug approval process.

HESCs offer an essentially unlimited supply of neural cells, enabling high-throughput drug screening, and are highly valuable for toxicology studies, given that the vast majority of early drug candidates fail at this step (Fernandes et al., 2009). HESC lines can be differentiated into specific neural cell types to recapitulate key aspects of disease. Thus, coculture models show that Super Oxide Dismutase (SOD)-deficient astrocytes secrete factors that are detrimental to hESC-derived motor neurons (Di Giorgio et al., 2008; Marchetto et al., 2008). The number of hESC lines carrying human mutations relevant to CNS disorders is building, and it would be valuable to collect these in a single, global, public-accessible registry.

With the discovery of iPSCs, an exciting avenue of research and potential therapeutic application has opened up because these cells can model the donor's disease. iPSC lines generated from patients suffering from a wide range of CNS disorders are being generated, an activity that eventually might be better centralized for banking and distribution, once the methods for iPSC generation, currently improving rapidly, reach a satisfactory threshold for standardization. Although the development of iPSC lines for autologous therapeutics has significant hurdles to overcome, such as cell instability, tumorgenicity, and expense, there is consensus that disease-specific iPSCs may have tremendous impact as drug screening platforms for efficacy testing of gene therapies and drugs (Lengerke and Daley, 2009). In the CNS arena, iPSC lines have been generated from ALS, Rett syndrome, retinal gyrate atrophy, and PD patients, allowing the derivation of cells for follow-on studies (Cundiff and Anderson, 2011; Howden et al., 2011). For example, it has been demonstrated that iPSC lines with the LRRK2 mutation show increased expression of oxidative stress response genes and increased caspase-3 activation and cell death after stress (Nguyen et al., 2011).

While the supply of tissue-derived NSC, RSC, and RPE stem and progenitor cells is more limited, the miniaturization of drug screening devices, for example to arrays of tissue printed spots of a few microns in diameter enabling 1000 point testing on a single glass slide (Fernandes et al., 2009), will allow these cell sources to be used more efficiently, and in some cases, they might better model aspects of a specific disease to accelerate drug discovery.

Recent Industry Trends and Regulatory Developments

Private industry has traditionally led the translation process, either sourced in-house or in-licensed from academia. In recent years, however, interest in and funding for early-stage R&D and translational research has dramatically declined as industry has come under increasing financial pressure. While government agencies such as the NINDS, with a budget of $1.6 billion ($139 million allocated for repair and plasticity and $77 million for translational research) (NINDS, 2011), commit resources for early stage research, the vast middle ground of work in preclinical, phase I, and phase II studies are poorly supported, hence the "valley of death" (Figures 1 and 2).

Recognizing the valley of death, several private foundations target and support translational research for specific neurological diseases (Table S1, available online). Furthermore, alternative sources of funding such as government agencies and province- and state-funded initiatives have increased their commitment to funding translational research. NIH is mobilizing new translational efforts that include a National Center for Advancing Translational Sciences (NCATS), anticipated to launch in the fall of 2011, and an NIH Center for Regenerative Medicine. NINDS continues to support translational research with a number of initiatives, including the U-grant mechanisms (Figure S1), centralized through the recently opened NINDS Office of Translational Research. An important strategic goal of NINDS is to improve connections between basic, translational, and clinical areas and to find new ways to engage the SBIR and STTR funding programs. A summary of U.S.-based resources applicable to stem-cell based CNS translation is given in Table 4, with further details in the Supplemental Resources.

To further advance translational medicine, NIH has strengthened collaborations with the FDA. In February 2010, the FDA and the NIH announced a collaborative program to accelerate the pace of drug development. The program established a Joint NIH-FDA Leadership Council to ensure that regulatory considerations are embedded in the planning of biomedical research and the regulatory review process is up to date on the latest science. In addition, $6.75 million will be made available over the next three years for research focused on improving the methods, models, and technologies to evaluate safety and efficacy of medical product development.

Table 4. Resources for CNS Translational Research

Societies/Educational
Examples of courses on drug development:
DIA: http://www.diahome.org/DIAHome/Home.aspx; International Society for Cellular Therapy: http://www.celltherapysociety.org/
PERI: http://www.peri.org/; American Society of Gene and Cell Therapy: http://www.asgct.org/
Barnett: http://www.barnettinternational.com/; European Society of Gene and Cell Therapy: http://www.esgct.eu/
ISSCR: http://www.isscr.org; NIH: http://stemcells.nih.gov
NIH Translational Programs Relevant to CNS
NIH Stem Cell Registry: http://grants.nih.gov/stem_cells/registry/current.htm
NIH Blueprint: http://neuroscienceblueprint.nih.gov/bpdrugs/index.htm
NIH-RAID: http://commonfund.nih.gov/raid/
TRND: http://trnd.nih.gov/
NIMH: http://www.nimh.nih.gov/about/organization/dnbbs/molecular-cellular-and-genomic-neuroscience-research-branch/drug-discovery-and-clinical-therapeutics-program.shtml
NIDA: http://www.nida.nih.gov/about/organization/DPMCDA/index.html
NIA: http://grants.nih.gov/grants/guide/pa-files/PAR-08-266.html
NCI: http://next.cancer.gov/ NIH Roadmap Molecular Libraries Probe Production Centers: http://mli.nih.gov/mli/mlpcn/
NINDS Office of Translational Research: http://www.ninds.nih.gov/funding/areas/technology_development/index.htm
NIH Center for Regenerative Medicine: http://commonfund.nih.gov/stemcells/
CTSA (Clinical And Translational Science Awards) and the CTSI program: http://www.ctsaweb.org/
NIH Clinical center: http://clinicalcenter.nih.gov/
DOD
Armed Forces Institute of Regenerative Medicine: http://www.afirm.mil/
Regulatory Information
http://www.fda.gov/BiologicsBloodVaccines/CellularGeneTherapyProducts/default.htm
Donor eligibility rule: http://www.fda.gov/BiologicsBloodVaccines/TissueTissueProducts/QuestionsaboutTissues/ucm102842.htm.
Centralized IRB
http://www.ncicirb.org/
Listed Trials
http://clinicaltrials.gov/ offers up-to-date information for locating federally and privately supported clinical trials for a wide range of diseases and conditions
Policy Guidelines and Translational Stem Cell Advocacy
NIH: http://stemcells.nih.gov/policy/2009guidelines.htm
ISSCR: http://www.isscr.org/meetings/index.cfm
Clinical translation guidelines: http://www.isscr.org/clinical_trans/pdfs/ISSCRGLClinicalTrans.pdf
Closer Look: http://www.closerlookatstemcells.org/
Genetics Policy Institute: http://www.genpol.org/
International Society Cell Therapy: http://www.celltherapysociety.org/
California Institute for Regenerative Medicine (CIRM) Disease Team awards: http://www.cirm.ca.gov/for-researchers/current-requests-applications
Faster Cures – Center For Accelerating Medical Solutions: http://www.fastercures.org/
Alliance for Regenerative Medicine: http://www.alliancerm.org/
Progressive IP Policy
Kauffman Institute: http://www.kauffman.org/

An example of FDA and NIH interaction to promote translational research in the area of stem cell biology was a recent workshop entitled "Pluripotent Stem Cells in Translation: Early Decisions" (http://www.cvent.com/events/pluripotent-stem-cells-in-translation-early-decisions/event-summary-942182d84b084a798f982a3c9df62678.aspx), the first of a planned series to address moving pluripotent stem cell therapies into the clinic. Topics discussed included the choice, characterization, and biology of pluripotent cells, regulatory requirements and challenges, and technologies that may facilitate the translational trajectory.

A particularly noteworthy issue to emerge from this workshop highlights the FDA Donor Eligibility and Cell Banking Requirements. The FDA donor eligibility rule, effective May 25, 2005,

requires testing tissue and cell donors for risk factors and clinical evidence of relevant communicable disease agents or diseases. It is not sufficient that the cellular or tissue-based product is tested; rather, the original donor must be screened and tested *at the time of tissue recovery*, using methods specified by the FDA (21 CFR 1271.85). The documentation of these tests must be available when the product is being evaluated by the FDA. This point cannot be stressed enough: cellular products for clinical use need to meet the FDA donor eligibility rule. For an hESC line, for example, meeting the requirements of the NIH Human Embryonic Stem Cell Registry does not ensure that the eligibility rule has been met (Table 4).

Translation in an Academic Environment

Most academic scientists are focused on discovery and creative, hypothesis-driven science and are solving problems in the lab at a remarkable pace, creating fertile ground for translation. However, funding for basic research is getting more difficult to procure, discouraging young scientists from entering the field (Rohn, 2011). Also, given that academic success is measured largely by publications and scholarly awards, there is no easy path nor career incentives for researchers to accomplish translation. Furthermore, translational research by its nature entails a high degree of risk (Figure 2) and requires milestone-based go/no-go decisions that can mean relinquishing exciting ideas, which is particularly difficult for basic researchers for whom ideas are often career identifiers. At the same time, lack of institutional funding for intellectual property (IP) investment and large lag times to generate IP, which delays publications, take a toll. When IP is generated, tech transfer is often inefficient, leaving IP to languish. Because of these inefficiencies, the number of products generated from promising basic research is disappointingly low, and researchers and academic institutions are not sharing in the benefits of productive translation. Bold solutions are needed—for example, integrating interested researchers into translational teams so that they would spend a percentage of their time on a designated translational project, with commensurate (for time spent) funding for "blue sky" research. This team-based model could work for government-led funding or within the context of private/public partnerships. Indeed, as pharmaceutical companies and biotech firms divest of in-house R&D arms, they are forming strategic academic partnerships to both capture IP and support research, and there is a growing list of companies in the stem cell space with CNS interests. Progress in such team approaches are exemplified by the NIH U-funding mechanisms and the CIRM disease-team approach (Table 4).

Looking to the Future

Stem cell research is one of the most rapidly developing areas of science and medicine. The explosive rise in discoveries and technologies that we see in the basic research labs has yet to enter the pipeline, and there is an enormous gap between what we can do at the bench and what we see in the current trials. While this is a constant source of frustration, the fact is that it means there is a lot to look forward to, as long as we can make the process of translation more efficient and affordable.

Currently, the production of specific cell types from stem cells is conducted differently in individual labs, and in some cases protocols—typically complex, multistep, and lab-idiosyncratic—can be difficult to repeat. Furthermore, cell output is measured with relatively rudimentary characterization, raising concerns that cells produced for clinical trials might not be bona fide, or stable, or as pure as reported. Developing a greater understanding of stem and progenitor cell characteristics, lineage relationships, and single-cell heterogeneity will enrich our knowledge of how NSCs generate diverse progeny and will be invaluable for cell characterization and standardization prior to transplant. It will also aid in identifying new ways to stimulate endogenous stem and progenitor cells, e.g., with small-molecule mimics of instructive factors that can lead to controlled in vivo cell expansion and differentiation.

In terms of cell transplantation for replacement, in addition to achieving routine and standardized protocols for hundreds of specific CNS cell types, we anticipate further genetic manipulation of cells prior to transplantation to correct genetically based diseases or combat the disease process. As well as directed single-gene excision or supplementation, the ability to alter networks and pathways via targeting noncoding RNAs and RNA binding proteins is another exciting avenue with great potential.

Combination therapies that take into account the specific cell-cell and cell-matrix interactions that are crucial for CNS function are an active area of research. One promising option is to employ scaffolding along with stem cells to provide a substrate and functionalized artificial niche to direct stem cell behavior (Keung et al., 2010). Expanding on this idea, CNS repair may be better achieved by transplantation of functional units that take into account the interdependence of different CNS cell types, maintaining key interactions such as endothelial cells and neural cells to improve graft vascularization, neurons, and glial cells or different neuron types to replace multiple elements of damaged circuits, perhaps in three-dimensional arrangements, as dramatically demonstrated by mouse ES-derived eye cup formation (Eiraku et al., 2011).

Medical advances require a permissive environment to reach patients, and progress in regulatory science will be critical to enable successful, efficient translation. Current regulatory paradigms are of variable stringency depending upon global region and continue to evolve with scientific progress. Failure to conduct trials under strict regulatory oversight can increase risk to patients and the stem cell field in general. Sobering examples of isolated reports of adverse events in patients exploring so-called "stem cell tourism" include a young patient with ataxia telangiectasia given multiple CNS injections of unpurified and uncharacterized mixtures of fetal-derived NSCs from multiple donors over several years that led to donor cell tumor growth (Amariglio et al., 2009). This emphasizes the need to conduct such trials under suitable regulatory and ethics oversight.

One controversial issue is that regulatory clearance can be given in the absence of peer-reviewed publication of the relevant preclinical data, which precludes full scrutiny and replication of stem cell culture protocols and results by the broader research community. It should be underscored that the IND review process provides in-depth peer-review scrutiny through ad hoc

Table 5. Ideas for More Efficient Translational Research
1. Provide a central resource for advice and guidance—a "how to" for basic researchers entering the translational domain.
2. Facilitate collaborative translational teams of clinicians, advocates, basic researchers, and business experts to promote research that is clinically needed and compatible with current or anticipated practice and to assess commercial viability.
3. Encourage investigators to consider the practical outcomes of their research and to disclose and patent protect in a timely manner that does not significantly slow publication or hinder academic freedom.
4. Encourage academic institutes to adopt progressive intellectual property (IP) policies with milestones that if not achieved allow the inventor via assignment or license to move forward with development.
5. Create centralized institutional review boards (CIRB) for CNS Regenerative Medicine, along the lines of the NCI-CIRB initiative, with access to expertise to aid and inform local IRBs.
6. Form collaborations with NINDS and patient advocacy groups to facilitate patient recruitment and retention, including pediatrics and rare diseases.
7. Encourage private foundations to provide greater financial support for translational research.
8. Develop team-based milestone-driven research plans with early go/no-go decisions to keep more funding available for viable projects.
9. Create private/public partnerships centered on CNS regeneration to bring additional funding earlier in the pipeline.
10. Increase public education and awareness of the potential impact and possible outcomes of stem cell CNS clinical trials and the timelines and costs of therapy development, and encourage public involvement.

consultants available to both regulatory and ethics bodies. Additionally, regulatory approvals by IRB, IBC, and SCRO subject the rationale and preclinical data to peer review, as does the NIH RAC for genetically modified cell products. The issue of publication is complex as the regulatory approval process has to take into account confidentiality issues to protect the sponsor, just as peer review of grant applications preserves confidentiality. Moreover, if publication were required, the wide spectrum of scientific journals would complicate distinction between meritorious preclinical data and those of lesser integrity and could cause further delays when there are many calls to speed up the regulatory approval process. It is also worth noting that a massive body of data is typically submitted in an IND application, far exceeding what can be compiled in one or two original research papers, and adding requirements would increase what is already a costly undertaking. Notably, while opinions will vary as to the scientific validity of a specific clinical trial or approach, the data most important to permit early clinical testing pertain to safety, which in the US must meet the high standards of the FDA embodied in statutes and regulations. Nevertheless, given the early stage of investigating stem cells as a source of neural therapeutics, their supreme complexity and the added challenge that they are living things that change over time and with handling and treatment, as much effort as possible toward publication and the opportunity to replicate data would greatly strengthen the overall effort by speeding knowledge exchange.

Autologous cell line production, in which a patient's own cells are cultured, expanded, and prepared for retransplantation as a patient-tailored treatment, poses another unique regulatory issue. From a biological standpoint, autologous transplantation is advantageous as it may obviate the need for immunosuppression, with its associated risks. However, the current extensive requirements for cell manufacture and testing may render such approaches cost prohibitive. Finding ways to facilitate authorization of clinical studies involving autologous transplantation will greatly benefit advances in individualized regenerative medicine.

Finally, world-wide adoption of standards for clinical trials, data collection, and data sharing would expedite the process of identifying proven treatments, which will protect patients, now growing increasingly savvy regarding regenerative medicine globally, and for whom transparency in shared information, and honest representation of risks and benefits by the scientific and medical communities is an essential public service. Efforts to find new ways to address the regulatory, cost, and funding issues, from organizations such as the FDA, EMA, NIH, ISSCR, GPI, and FasterCures (Table 4) that encourage discussion between stakeholders, are making headway.

Conclusion: Envisioning the Rosetta Stone

Stem cell research and application is opening great opportunities in CNS regenerative therapies. This survey shows that we are still at relatively early stages of defining safety for most of these studies. Nevertheless, encouraged by the progress to date, and especially by the stupendous strides being made in preclinical studies, we envision a much more concerted effort toward translation that would make the process more accessible, integrated into academic and industry settings, and efficient, therefore improving the chance that the health benefits of research reach patients (Table 5). Moreover, such integrated efforts would ensure that researchers are rewarded for their discoveries and skills, bringing more funding into the pipeline to sustain the entire research enterprise and grounding research capacity, currently expanding in an unsustainable highly leveraged model (Alberts, 2010), by linking it to revenues generated from real-world productivity. Translation is inordinately expensive and paying for this from the current NIH budget would severely hinder the basic research effort. Consequently, new funding streams, such as revenue return from successful translation, and private/public partnerships are needed. It is imperative to emphasize that the translational process—from bench to bedside—is founded at the bench, and while necessity is the mother of invention, creativity flourishes best when one is not worried about the next vial of stem cell culture medium. With the growing recognition that translation is a critical goal, and that we are on the brink of a revolution in CNS regenerative medicine, resources must continue to be amassed and

directions set that will lead toward innovative stem cell-based CNS therapies and possible solutions to the global and growing health challenge posed by neurological disorders.

SUPPLEMENTAL INFORMATION

Supplemental Information includes supplemental resources, one figure, and one table and can be found with this article online at doi:10.1016/j.neuron.2011.05.007.

ACKNOWLEDGMENTS

We thank Mahendra Rao, Steve Goldman, Stephen Huhn, Melissa Carpenter, Jana Portnow, Ann Tsukamoto, and Irv Weissman for critical reading of this manuscript, as well as Tony Jackson at NIH-RAID for helpful discussions regarding NIH resources and David Owens at NINDS for invaluable insight into the status of basic and translational neural stem cell research. A.C. is an employee of StemCells, Inc.

REFERENCES

Aboody, K.S., Najbauer, J., and Danks, M.K. (2008). Stem and progenitor cell-mediated tumor selective gene therapy. Gene Ther. *15*, 739–752.

Alberts, B. (2010). Overbuilding research capacity. Science *329*, 1257.

Amariglio, N., Hirshberg, A., Scheithauer, B.W., Cohen, Y., Loewenthal, R., Trakhtenbrot, L., Paz, N., Koren-Michowitz, M., Waldman, D., Leider-Trejo, L., et al. (2009). Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. PLoS Med. *6*, e1000029.

Androutsellis-Theotokis, A., Rueger, M.A., Park, D.M., Boyd, J.D., Padmanabhan, R., Campanati, L., Stewart, C.V., LeFranc, Y., Plenz, D., Walbridge, S., et al. (2010). Angiogenic factors stimulate growth of adult neural stem cells. PLoS ONE *5*, e9414.

Burger, S.R. (2003). Current regulatory issues in cell and tissue therapy. Cytotherapy *5*, 289–298.

Cox, C.S., Jr., Baumgartner, J.E., Harting, M.T., Worth, L.L., Walker, P.A., Shah, S.K., Ewing-Cobbs, L., Hasan, K.M., Day, M.C., Lee, D., et al. (2011). Autologous bone marrow mononuclear cell therapy for severe traumatic brain injury in children. Neurosurgery *68*, 588–600.

Cummings, B.J., Uchida, N., Tamaki, S.J., Salazar, D.L., Hooshmand, M., Summers, R., Gage, F.H., and Anderson, A.J. (2005). Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc. Natl. Acad. Sci. USA *102*, 14069–14074.

Cundiff, P.E., and Anderson, S.A. (2011). Impact of induced pluripotent stem cells on the study of central nervous system disease. Curr. Opin. Genet. Dev., in press. Published online January 27, 2011. 10.1016/j.gde.2011.01.008.

Di Giorgio, F.P., Boulting, G.L., Bobrowicz, S., and Eggan, K.C. (2008). Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. Cell Stem Cell *3*, 637–648.

DiMasi, J.A. (2001). Risks in new drug development: Approval success rates for investigational drugs. Clin. Pharmacol. Ther. *69*, 297–307.

Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T., and Sasai, Y. (2011). Self-organizing optic-cup morphogenesis in three-dimensional culture. Nature *472*, 51–56.

Escolar, M.L., Poe, M.D., Provenzale, J.M., Richards, K.C., Allison, J., Wood, S., Wenger, D.A., Pietryga, D., Wall, D., Champagne, M., et al. (2005). Transplantation of umbilical-cord blood in babies with infantile Krabbe's disease. N. Engl. J. Med. *352*, 2069–2081.

Fernandes, K.J., Toma, J.G., and Miller, F.D. (2008). Multipotent skin-derived precursors: Adult neural crest-related precursors with therapeutic potential. Philos. Trans. R. Soc. Lond. B Biol. Sci. *363*, 185–198.

Fernandes, T.G., Diogo, M.M., Clark, D.S., Dordick, J.S., and Cabral, J.M. (2009). High-throughput cellular microarray platforms: Applications in drug discovery, toxicology and stem cell research. Trends Biotechnol. *27*, 342–349.

Fink, D.W., Jr. (2009). FDA regulation of stem cell-based products. Science *324*, 1662–1663.

Gentry, T., Foster, S., Winstead, L., Deibert, E., Fiordalisi, M., and Balber, A. (2007). Simultaneous isolation of human BM hematopoietic, endothelial and mesenchymal progenitor cells by flow sorting based on aldehyde dehydrogenase activity: Implications for cell therapy. Cytotherapy *9*, 259–274.

Giannelli, S.G., Demontis, G.C., Pertile, G., Rama, P., and Broccoli, V. (2010). Adult human Muller glia cells are a highly efficient source of rod photoreceptors. Stem Cells *29*, 344–356.

Goldman, S.A. (2011). Progenitor cell-based treatment of the pediatric myelin disorders. Arch. Neurol., in press. Published online March 14, 2011. 10.1001/archneurol.2011.46.

Grealish, S., Jönsson, M.E., Li, M., Kirik, D., Björklund, A., and Thompson, L.H. (2010). The A9 dopamine neuron component in grafts of ventral mesencephalon is an important determinant for recovery of motor function in a rat model of Parkinson's disease. Brain *133*, 482–495.

Gupta, P., Soyombo, A.A., Atashband, A., Wisniewski, K.E., Shelton, J.M., Richardson, J.A., Hammer, R.E., and Hofmann, S.L. (2001). Disruption of PPT1 or PPT2 causes neuronal ceroid lipofuscinosis in knockout mice. Proc. Natl. Acad. Sci. USA *98*, 13566–13571.

Guzman, R., Uchida, N., Bliss, T.M., He, D., Christopherson, K.K., Stellwagen, D., Capela, A., Greve, J., Malenka, R.C., Moseley, M.E., et al. (2007). Long-term monitoring of transplanted human neural stem cells in developmental and pathological contexts with MRI. Proc. Natl. Acad. Sci. USA *104*, 10211–10216.

Hagell, P., Piccini, P., Björklund, A., Brundin, P., Rehncrona, S., Widner, H., Crabb, L., Pavese, N., Oertel, W.H., Quinn, N., et al. (2002). Dyskinesias following neural transplantation in Parkinson's disease. Nat. Neurosci. *5*, 627–628.

Halme, D.G., and Kessler, D.A. (2006). FDA regulation of stem-cell-based therapies. N. Engl. J. Med. *355*, 1730–1735.

Heinrich, C., Gascón, S., Masserdotti, G., Lepier, A., Sanchez, R., Simon-Ebert, T., Schroeder, T., Götz, M., and Berninger, B. (2011). Generation of subtype-specific neurons from postnatal astroglia of the mouse cerebral cortex. Nat. Protoc. *6*, 214–228.

Howden, S.E., Gore, A., Li, Z., Fung, H.L., Nisler, B.S., Nie, J., Chen, G., McIntosh, B.E., Gulbranson, D.R., Diol, N.R., et al. (2011). Genetic correction and analysis of induced pluripotent stem cells from a patient with gyrate atrophy. Proc. Natl. Acad. Sci. USA *108*, 6537–6542.

Hu, B.Y., Weick, J.P., Yu, J., Ma, L.X., Zhang, X.Q., Thomson, J.A., and Zhang, S.C. (2010). Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proc. Natl. Acad. Sci. USA *107*, 4335–4340.

Inoue, K., Osaka, H., Imaizumi, K., Nezu, A., Takanashi, J., Arii, J., Murayama, K., Ono, J., Kikawa, Y., Mito, T., et al. (1999). Proteolipid protein gene duplications causing Pelizaeus-Merzbacher disease: Molecular mechanism and phenotypic manifestations. Ann. Neurol. *45*, 624–632.

Isacson, O., and Mendez, I. (2010). Being too inclusive about synuclein inclusions. Nat. Med. *16*, 960–961, author reply 961.

Keirstead, H.S., Nistor, G., Bernal, G., Totoiu, M., Cloutier, F., Sharp, K., and Steward, O. (2005). Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J. Neurosci. *25*, 4694–4705.

Keung, A.J., Healy, K.E., Kumar, S., and Schaffer, D.V. (2010). Biophysics and dynamics of natural and engineered stem cell microenvironments. Wiley Interdiscip Rev Syst Biol Med *2*, 49–64.

Kim, S.U. (2007). Genetically engineered human neural stem cells for brain repair in neurological diseases. Brain Dev. *29*, 193–201.

Lamba, D., Karl, M., and Reh, T. (2008). Neural regeneration and cell replacement: A view from the eye. Cell Stem Cell *2*, 538–549.

Lengerke, C., and Daley, G.Q. (2009). Disease models from pluripotent stem cells. Ann. N Y Acad. Sci. *1176*, 191–196.

Lévesque, M.F., Neuman, T., and Rezak, M. (2009). Therapeutic microinjection of autologous adult human neural stem cells and differentiated neurons for Parkinson's disease: Five-year post-operative outcome. Open Stem Cell Journal *1*, 20–29.

Lindsay, S.L., Riddell, J.S., and Barnett, S.C. (2010). Olfactory mucosa for transplant-mediated repair: A complex tissue for a complex injury? Glia *58*, 125–134.

Liu M.B., ed. (2010). A Clinical Trial Manual from the Duke Research Institute (West Sussex, UK: Duke Clinical Research Institute).

Lowry, N., Goderie, S.K., Adamo, M., Lederman, P., Charniga, C., Gill, J., Silver, J., and Temple, S. (2008). Multipotent embryonic spinal cord stem cells expanded by endothelial factors and Shh/RA promote functional recovery after spinal cord injury. Exp. Neurol. *209*, 510–522.

Lu, B., Malcuit, C., Wang, S., Girman, S., Francis, P., Lemieux, L., Lanza, R., and Lund, R. (2009). Long-term safety and function of RPE from human embryonic stem cells in preclinical models of macular degeneration. Stem Cells *27*, 2126–2135.

Marchetto, M.C., Muotri, A.R., Mu, Y., Smith, A.M., Cezar, G.G., and Gage, F.H. (2008). Non-cell-autonomous effect of human SOD1 G37R astrocytes on motor neurons derived from human embryonic stem cells. Cell Stem Cell *3*, 649–657.

Mendez, I., Sanchez-Pernaute, R., Cooper, O., Viñuela, A., Ferrari, D., Björklund, L., Dagher, A., and Isacson, O. (2005). Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with Parkinson's disease. Brain *128*, 1498–1510.

NINDS (National Institute of Neurological Disorders and Stroke). (2011). 2012 Fiscal Year Budget Congressional Justification. http://www.ninds.nih.gov/news_and_events/congressional_testimony/ninds_fy_2012_cj.htm.

Nguyen, H.N., Byers, B., Cord, B., Shcheglovitov, A., Byrne, J., Gujar, P., Kee, K., Schüle, B., Dolmetsch, R.E., Langston, W., et al. (2011). LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell *8*, 267–280.

Otani, A., Dorrell, M.I., Kinder, K., Moreno, S.K., Nusinowitz, S., Banin, E., Heckenlively, J., and Friedlander, M. (2004). Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells. J. Clin. Invest. *114*, 765–774.

PricewaterhouseCoopers. (2008). Pharma 2020: Virtual R&D—Which path will you take? http://www.pwc.com/pharma2020.

Raisman, G., and Li, Y. (2007). Repair of neural pathways by olfactory ensheathing cells. Nat. Rev. Neurosci. *8*, 312–319.

Rayment, E.A., and Williams, D.J. (2010). Concise review: Mind the gap: Challenges in characterizing and quantifying cell- and tissue-based therapies for clinical translation. Stem Cells *28*, 996–1004.

Rohn, J. (2011). Give postdocs a career, not empty promises. Nature *471*, 7.

Salazar, D.L., Uchida, N., Hamers, F.P., Cummings, B.J., and Anderson, A.J. (2010). Human neural stem cells differentiate and promote locomotor recovery in an early chronic spinal cord injury NOD-scid mouse model. PLoS ONE *5*, e12272.

Sandrock, R.W., Wheatley, W., Levinthal, C., Lawson, J., Hashimoto, B., Rao, M., and Campanelli, J.T. (2010). Isolation, characterization and preclinical development of human glial-restricted progenitor cells for treatment of neurological disorders. Regen. Med. *5*, 381–394.

Schwartz, P.H., Bryant, P.J., Fuja, T.J., Su, H., O'Dowd, D.K., and Klassen, H. (2003). Isolation and characterization of neural progenitor cells from post-mortem human cortex. J. Neurosci. Res. *74*, 838–851.

Shihabuddin, L.S., Horner, P.J., Ray, J., and Gage, F.H. (2000). Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus. J. Neurosci. *20*, 8727–8735.

Steiner, R., Selden, N., Huhn, S.L., Koch, T., Al-Uzri, A., Sikora, D., Dean, S., Penfield, J., Sutcliffe, T., Bammer, R., et al. (2009). CNS transplantation of purified human neural stem cells in infantile and late-infantile neuronal ceroid lipofuscinoses: Results of the phase I trial In 12th International Congress on Neuronal Ceroid Lipofuscinoses (NCL) (Hamburg, Germany, University of Hamburg).

Stroemer, P., Patel, S., Hope, A., Oliveira, C., Pollock, K., and Sinden, J. (2009). The neural stem cell line CTX0E03 promotes behavioral recovery and endogenous neurogenesis after experimental stroke in a dose-dependent fashion. Neurorehabil. Neural Repair *23*, 895–909.

Strunnikova, N.V., Maminishkis, A., Barb, J.J., Wang, F., Zhi, C., Sergeev, Y., Chen, W., Edwards, A.O., Stambolian, D., Abecasis, G., et al. (2010). Transcriptome analysis and molecular signature of human retinal pigment epithelium. Hum. Mol. Genet. *19*, 2468–2486.

Sun, J., Allison, J., McLaughlin, C., Sledge, L., Waters-Pick, B., Wease, S., and Kurtzberg, J. (2010). Differences in quality between privately and publicly banked umbilical cord blood units: A pilot study of autologous cord blood infusion in children with acquired neurologic disorders. Transfusion *50*, 1980–1987.

Tamaki, S.J., Jacobs, Y., Dohse, M., Capela, A., Cooper, J.D., Reitsma, M., He, D., Tushinski, R., Belichenko, P.V., Salehi, A., et al. (2009). Neuroprotection of host cells by human central nervous system stem cells in a mouse model of infantile neuronal ceroid lipofuscinosis. Cell Stem Cell *5*, 310–319.

Thu, M.S., Najbauer, J., Kendall, S.E., Harutyunyan, I., Sangalang, N., Gutova, M., Metz, M.Z., Garcia, E., Frank, R.T., Kim, S.U., et al. (2009). Iron labeling and pre-clinical MRI visualization of therapeutic human neural stem cells in a murine glioma model. PLoS ONE *4*, e7218.

Uchida, N., Buck, D.W., He, D., Reitsma, M.J., Masek, M., Phan, T.V., Tsukamoto, A.S., Gage, F.H., and Weissman, I.L. (2000). Direct isolation of human central nervous system stem cells. Proc. Natl. Acad. Sci. USA *97*, 14720–14725.

Vierbuchen, T., Ostermeier, A., Pang, Z.P., Kokubu, Y., Südhof, T.C., and Wernig, M. (2010). Direct conversion of fibroblasts to functional neurons by defined factors. Nature *463*, 1035–1041.

Wallace, V.A. (2011). Concise review: Making a retina—from the building blocks to clinical applications. Stem Cells *29*, 412–417.

West, E.L., Pearson, R.A., MacLaren, R.E., Sowden, J.C., and Ali, R.R. (2009). Cell transplantation strategies for retinal repair. Prog. Brain Res. *175*, 3–21.

Windrem, M.S., Schanz, S.J., Guo, M., Tian, G.F., Washco, V., Stanwood, N., Rasband, M., Roy, N.S., Nedergaard, M., Havton, L.A., et al. (2008). Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. Cell Stem Cell *2*, 553–565.

WHO (World Health Organization). (2006). Public Health Challenges (Geneva: WHO Press), Chapter 2, p. 36, table 2.8. http://www.who.int/mental_health/neurology/neurological_disorders_report_web.pdf.

Xu, L., Yan, J., Chen, D., Welsh, A.M., Hazel, T., Johe, K., Hatfield, G., and Koliatsos, V.E. (2006). Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. Transplantation *82*, 865–875.

Yasuhara, T., Matsukawa, N., Hara, K., Maki, M., Ali, M.M., Yu, S.J., Bae, E., Yu, G., Xu, L., McGrogan, M., et al. (2009). Notch-induced rat and human bone marrow stromal cell grafts reduce ischemic cell loss and ameliorate behavioral deficits in chronic stroke animals. Stem Cells Dev. *18*, 1501–1514.