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From mice to mind: Strategies and progress in translating neuroregeneration

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\textbf{A B S T R A C T}

Decisions about what experimental therapies are advanced to clinical trials are based almost exclusively on findings in preclinical animal studies. Over the past 30 years, animal models have forecast the success of hundreds of neuroprotective pharmacological therapies for stroke, Alzheimer’s disease, spinal cord injury, traumatic brain injury and amyotrophic lateral sclerosis. Yet almost without exception, all have failed. Rapid advances in stem cell technologies have raised new hopes that these neurological diseases may one day be treatable. Still, how can neuroregenerative therapies be translated into clinical realities if available animal models are such poor surrogates of human disease? To address this question we discuss human and rodent neurogenesis, evaluate mechanisms of action for cellular therapies and describe progress in translating neuroregeneration to date. We conclude that not only are appropriate animal models critical to the development of safe and effective therapies, but that the multiple mechanisms of stem cell-mediated therapies may be particularly well suited to the mechanistically diverse nature of central nervous system diseases in mice and man.

\section{Introduction}

\subsection{The case for neuroregeneration}

Table 1: Modern medicine has made disproportionately modest progress in the management of neurological diseases. In other fields, from open-heart surgery and organ transplantation to antibiotics, vaccinations and improved management of multiple cancers, science and medicine have much to be proud of. However, the cumulative burden from neurological diseases has experienced little relief. Traumatic brain injury is still the leading cause of morbidity and mortality in young adults. Patients paralyzed by spinal cord injury during the last century have been witness to the description of human embryonic stem cells (ESCs), the sequencing of the human genome and the development of induced pluripotent stem cells (iPS)—an accomplishment deemed so important that that it earned a Nobel Prize only 6 years after its discovery. Yet, despite these accomplishments, paralyzed patients can still only dream of one day walking again. Meanwhile, Alzheimer’s disease has become the fastest growing cause of death in our aging population.

\subsection{Paralyzed, demented scientists: can their mice save them?}

The goal of restoring function to patients with neurodegenerative disease, spinal cord injury, stroke, and traumatic brain injury increasingly occupies the careers of thousands of scientists. Yet for none of these ailments is a probable cure close to being found. This is not for lack of productivity. Tens of thousands of papers have been written, many of which have described significant neurological recovery of rodent models. In contrast to human patients, previously paralyzed rats now do walk again, Alzheimer’s mice can relearn their way out of mazes, and hemi-Parkinsonian rats enjoy symmetric motor function. Based on compelling preclinical data, numerous clinical trials have been launched at momentous expense, testing the faith, hope and trust of thousands of patients. Yet, virtually without exception, all have failed. Scientists nevertheless keep breeding their mice and new cures for these vermin continue to grace the pages of high-ranking scientific journals. Are scientists dementedly performing mouse experiments again and again, having so quickly forgotten their dismal predictive value? Has the ability to recurrently cure their fury friends in novel ways paralyzed scientists’ abilities to focus on the human problem?

\subsection{Why stem cells?}

Against this bleak backdrop of failed attempts to halt degeneration, repair injury, or induce plasticity using standard pharmacotherapies, the concept of harnessing the potential of stem cells—nature’s own...
agents of regeneration and self-renewal—is appealing. One can understand the sense of optimistic anticipation in paralyzed patients who with IPS cell technology can now see their very own skin cells reprogrammed into stem cells and differentiated into spinal cord-type cells in a dish. More than just factories for making neurons and glia, however, stem cells are increasingly recognized as first responders to injury in their own right, endowed with capacity to promote angiogenesis, modulate inflammation, and secrete neurotrophic compounds. Even stem cells from within the injured spinal cord itself have been shown to play an active role in mitigating collateral injury to surrounding neurons (Sabelstrom et al., 2013).

Despite optimism for the potential stem cells hold for neurological diseases, very real challenges remain to translate stem cell-based regenerative strategies into clinical cures. Virtually all translational efforts depend upon evidence obtained from animal models. How that evidence is obtained and how it is interpreted can make or break its relevance.

2. Endogenous neurogenesis

2.1. Hippocampal neurogenesis

In his 1928 essay “Degeneration and regeneration of the nervous system,” neuroscientist Ramón y Cajal famously wrote, “In adult centers the nerve paths are something fixed, ended, immutable. Everything may die, nothing may be regenerated. It is for the science of the future to change, if possible, this harsh decree.” (Ramon y Cajal, 1928). This view stood unchallenged until 1965 when Altman and Das provided autoradiographic and histological evidence of neurogenesis in the adult rat hippocampus (Altman and Das, 1965a, 1965b). Although these findings were largely ignored for the next 2 decades, Goldman and Nottebohm (1983), reported production of new neurons in a vocal control nucleus of adult female canaries that were associated with the seasonal acquisition of song. In 1998, the Gage lab provided evidence for neurogenesis in the adult human hippocampus (Eriksson et al., 1998). To date, hippocampal neurogenesis has been evaluated in at least 71 mammalian species and found to be present in almost all, with the exception only of Cetaceans (whales, dolphins and porpoises; (Patzke et al., 2013)). While the function of hippocampal neurogenesis remains under active investigation, current evidence supports a role in memory and spatial navigation. Newly born neurons provide a cellular substrate for plasticity that manifests as they integrate into circuity several weeks after birth. Multiple magnetic resonance (MR) imaging studies have reported correlations between human hippocampal size and memory-dependent activities, with increased size in taxi drivers (Maguire et al., 2000) and medical students studying for exams (Draganski et al., 2006). Physical exercise is positively correlated with neurogenesis, while neurogenesis is decreased in association with stress, depression, neurodegeneration, and age-related cognitive decline. In rodents, hippocampal neurogenesis declines precipitously during young adult life. By contrast, hippocampal neurogenesis was recently shown by carbon-14 dating to be substantial in adult humans, supporting the idea of an active role in cognition (Spalding et al., 2013). The finding that hippocampal neurogenesis persists into adult life supports the notion that therapies to reverse age-dependent hippocampal atrophy could improve cognition (Jessenberger and Gage, 2014). Indeed, interventions known to improve hippocampal neurogenesis including exercise and cognitively demanding tasks enhance cognitive performance in aged humans (Bherer et al., 2013). In a recent exciting experiment, blood plasma from young mice or humans improved hippocampal neurogenesis, cognitive performance and several other physiological parameters of youthfulness in aged mice, offering hope that therapies to promote “rejuvenation” may soon be within reach (Villeda et al., 2014). Although the therapeutic mechanisms of young blood remain poorly understood, a clinical trial for patients with mild-to-moderate Alzheimer’s disease is underway (NCT02256306; www.clinicaltrials.gov).

2.2. Subventricular zone neurogenesis

During development, much of the forebrain brain develops from a peri-ventricular germinal region, which persists in adults as the second major site of neurogenesis—the subventricular zone (SVZ). In rodents and non-human primates (Kornack and Rakic, 2001) SVZ-derived neuroblasts migrate through the rostral migratory stream to generate new olfactory bulb (OB) neurons, which play a critical role in olfactory discrimination and memory (Alonso et al., 2012; Alonso et al., 2006; Imayoshi et al., 2008; Mouret et al., 2009). Nevertheless, the contribution of the human SVZ to olfactory neurogenesis has proven controversial (Curtis et al., 2007; Sanai et al., 2004). Most recently, a comprehensive study using carbon-14 dating, thymidine analog incorporation and immunohistochemical analysis found no evidence for human olfactory bulb neurogenesis. Unexpectedly, however, substantial numbers of newly born neurons were found in adult human striatum adjacent to the SVZ (Ernst et al., 2014). This represents a substantial departure from rodents where no evidence exists for striatal neurogenesis in healthy adults. Many questions remain...
regarding the regulation and functional importance of what now appears to be the second major site of neurogenesis in adult humans, though importantly, reduced neurogenesis was found in patients with Huntington's disease (Ernst et al., 2014).

Of particular relevance to attempts at neuroregeneration, injury stimuli such as stroke have been shown to divert rodent neuroblasts from their usual course along the RMS to the brain parenchyma towards the peri-infarct region. Although very few such cells survive or mature to become functional neurons, diversion of these newly generated cells to the infarct region continues for up to a year in rodents, and genetic ablation of such newly generated cells worsens outcomes (Jin et al. 2010; Sun et al. 2013; Sun et al. 2012; Wang et al. 2012.) Using carbon-14 dating, no new neurons were found in human cortex after stroke though the situation after human striatal stroke remains to be evaluated (Huttner et al., 2014). Interestingly, although no striatal neurogenesis occurs in healthy rodents, intraventricular delivery of adenovirus-associated viruses encoding brain-derived neurotrophic factor (BDNF) and noggin resulted in sustained mobilization of SVZ neural stem cells (NSCs) to the striatum of Huntington's mice. These neurons differentiated into functional medium spiny neurons of the type lost in Huntington's and treated mice showed improved motor function and prolonged survival (Benraiss et al., 2013). While much attention has focused on neuronal progeny of the SVZ, this region can also give rise to glia as well as progenitors that migrate to the site of injury but remain undifferentiated. The functional importance of such non-neuronal progeny remains to be fully evaluated (de Chevigny et al., 2008; Kojima et al., 2010; Menn et al., 2006).

2.3. Alternate central nervous system progenitor pools

Finally, other progenitor populations exist in the human brain outside of neurogenic regions. Oligodendrocyte progenitor cells exist throughout the brain and respond to demyelinating injuries (Franklin and Ffrench-Constant, 2008; Hartley et al., 2014). Although cell turnover in the normal adult human white matter is low, myelination appears highly dynamic and may contribute to adult central nervous system (CNS) plasticity (Yeung et al., 2014). Moreover, recent evidence in rodents suggests new oligodendrocytes may be born in response to neuronal activity and enhance pathway function (Gibson et al., 2014). Certain populations of astrocytes appear to be developmentally closely related to NSCs, and inhibition of notch signaling in mouse astrocytes can trigger in situ striatal neurogenesis (Magnusson et al., 2014). Moreover, new technologies of epigenetic reprogramming have opened the door to in vivo reprogramming of one cell type to another. Overexpression of key transcription factors such as Neurogenein2, Mash1 or Sox2 can convert pericytes, astroglia or oligodendrocyte progenitor cells into functional neurons in situ (Heinrich et al., 2014; Heinrich et al., 2010; Karow et al., 2012). Such exciting technologies offer unprecedented potential to fundamentally reconfigure the structure and function of diseased brain regions. Of course, before clinical trials can be considered for any such strategies, animal models will again find themselves indispensable for rigorous evaluations of long-term safety.

3. Exogenous stem cells

Long before endogenous neurogenesis was widely recognized, loss of specific neurons in certain diseases, such as midbrain dopaminergic neurons in Parkinson's disease, prompted hopes that the replacement of these neurons could prove therapeutic. In the 1970s, Anders Bjorklund's lab in Lund, Sweden began investigating the potential of transplanted fetal tissue to integrate into the adult CNS (Bjorklund et al., 1976). In contrast to Cajal's predictions, Bjorklund found that the CNS could accept the addition of new cells to generate functional connections. This and subsequent work ultimately paved the way for clinical trials of fetal dopamine cells for Parkinson's disease. Given the limited supply of fetal brain tissue, the discovery of neural stem cells (Reynolds et al., 1992; Reynolds and Weiss, 1992) prompted hopes of a potentially unlimited supply of new cells for transplantation. In the late 1990s to early 2000s evidence from several labs suggested that developmental boundaries restricting cells to specific lineages could be overcome, potentially freeing even bone-marrow-derived stem cells to generate neuronal cells. Although some of these reports of "transdifferentiation" proved to be due to technical artifacts (Burns et al., 2006; Wurmsner and Gage, 2002), preclinical studies with bone-marrow-derived cells, including mesenchymal stem cells (MSCs) nevertheless demonstrated functional benefits in rodent models of CNS disease (Burns and Steinberg, 2011; Burns et al., 2009); (Vu et al., 2014), suggesting the potential for functional benefits even in the absence of any new neurons.

3.1. Multifunctional stem cells

Two primary strategies of cellular therapy have emerged. "Cell replacement" strategies seek to replace the neuronal cell types lost through disease, with Parkinson's disease remaining the prototype. "Trophic" cell therapies, by contrast seek to restore function via indirect mechanisms, many of which remain ill-defined, but include modulating inflammation, promoting angiogenesis, augmenting endogenous neurogenesis, and secreting factors that support the survival, function and plasticity of remaining host neurons. Certain stem cell populations also migrate avidly toward regions of injury or neoplasia, thus helping to focus their activities at the site(s) of greatest need, and provide opportunities for focal delivery of therapeutic molecules (Lee et al., 2013; Song et al., 2010). Despite remaining mechanistic questions, the apparent multiplicity of stem cell actions, which contrasts with often highly targeted drug therapies, may prove to be a critical feature of cell-based therapies (Fig. 1). The complexity of CNS damage or degeneration, with its associated inflammation, oxidative stress, and impairment of mitochondrial, proteasomal and lysosomal function, may require a multimodal "all of the above" therapeutic approach, to which the numerous actions of stem cells may be particularly well suited.

Interestingly, such an "all of the above" approach may in fact be employed as part of the brain's natural response to neurodegeneration. We recently performed a transcriptional meta-analysis of neurodegenerative diseases, and identified a core module of neurodegeneration-associated changes conserved across amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Parkinson's disease (PD) and Alzheimer's disease (AD). Strikingly, network analysis revealed that SOD1, Huntingtin, Parkin and APP—each almost pathognomonic for changes associated with ALS, HD, PD and AD, respectively—were in fact all implicated in regulation of the observed transcriptional response across neurodegenerative diseases (Li et al., 2014). One of the challenges in the field is to distinguish disease etiologies from beneficial responses. A recent critique of translational approaches to Alzheimer's disease suggested that strategies aiming to rid transgenic Alzheimer's mice of their APP-associated amyloid-beta plaques might be analogous to attempting to prevent deployment of airbags as a strategy to prevent motor vehicle accidents (Kristic and Knuesel, 2013). While deployed airbags are found in the majority of accidents; while accidental deployment has been shown to cause accidents in rare cases; and while experimental deployment of airbags would predictably lead to more accidents, strategies to...
proposed that imperfect animal models, by activating a spectrum of defenses to promote resilience against disease and injury. Indeed, we propose that stem cell therapies may act through multiple mechanisms, potentially enhancing their efficacy once endogenous stress responses begin to fail (Burns et al., 2015). As such, the animal disease can be corrected by molecular therapies targeting the engineered origins of disease, whereas the same therapy may fail in the real-world human disease. Stem cell therapies may act through multiple mechanisms, potentially enhancing their efficacy and subsequently fail in the real-world human trials, optimized to their multiple modes of action. In contrast to highly imperfect animal models,
variety of imperfectly rendered disease models may provide better
predictive value than individual performance in even the most
“relevant” animal model.

In order for cell therapies and trial protocols to improve in the
future, thoughtful efforts must be made to maximize the data yield
from current patients. CNS tissue must be harvested and banked
from trial participants who succumb to their disease; serum
should be harvested from participants and complement imaging
studies. Although currently, we may not yet even know the right
questions to ask, banking these samples for future analysis,
possibly when new techniques emerge may prove critical. Perhaps
future evaluation of cell-free RNA (Martinez et al., 2014) from
banked serum will provide a signature of interactions between
grafted cells and the host innate immune system that will explain
why some patients showed a more dramatic improvement than
others reveal how future cells may be modified to augment their
impact. Currently, cells used in clinical trials lack identifying
transgenic markers, confounding their identification in pathologic
analyses of post-mortem tissue. This largely regulatory hurdle
must be overcome to allow surviving grafted cells to be identified
and analyzed. In vivo imaging strategies are also in their relative
infancy; though superparamagnetic iron oxide (SPIO) particles have
been used clinically to label CNS transplanted cells for detection by
MRI (Zhu et al., 2006), though it is difficult to exclude misleading
signal from phagocytic cells if engrafted cells die after transplantation
(Li et al., 2008b). Future use of appropriately engineered
 genetic constructs may permit unambiguous in vivo identification
of grafted cells by MRI or PET. Thus, while animal models are
critical in the early stages of translation, a generous cohort of
scientists must divert their gaze from mice, to the patients who
received their mouse-endorsed therapies. Only in this way will the
necessary data be gathered to optimize experimental human
neuroregenerative therapies in the future.

3.3. Primum non nocere

Among several safety-related issue surrounding cell transplanta-
tion, the greatest concern surrounds tumor formation, which has
been reported in humans receiving stem cell grafts outside of
well-controlled clinical trials (Amariglio et al., 2009; Dlouhy et al.,
2014). Tumors formation may occur for at least 3 reasons. First,
in vitro culture may lead to accumulation of tumor-promoting
genetic abnormalities (Jeong et al.; 2011; Tolar et al., 2007). This
risk is minimized by optimizing culture conditions to reduce
stress, monitoring for changes in rate of cell proliferation, and
routine evaluation of karyotype. However, even karyotypically
“normal” cells may accumulate numerous smaller “occur” genetic
abnormalities, the significance of which remains uncertain. As
such, efforts should be made to minimize duration of culture for
cells destined for clinical trials, whilst monitoring for genetic
changes with high-resolution methods (Peterson and Loring,
2014). Second, the pluripotent nature of ESCs and iPSCs permits
teratoma formation if any undifferentiated cells contaminate the
graft. Great efforts have been invested in culture, sorting and
screening strategies to ensure “pure” populations ESC-derived
cells safe for clinical use (Lukovic et al., 2014; Polanco et al.,
2013). Third, certain in vitro culture conditions have recently been
found to enable spontaneous de-differentiation of bone marrow-
derived cells into hypoblast cells that can generate embryonic yolk
sac tumors, even though cells may be derived from bone marrow
and have normal karyotype (Deten et al., 2014; Lo Nigro et al.,
2012). As such, even cell types presumed to be “safe” based on
their adult origin, lack of genetic manipulation and normal
karyotype, must be rigorously evaluated for markers of de-
differentiation such as Oct4. Moreover, in some cases, tumor
formation may be context dependent. Although no tumors were
found with system or intramuscular cell delivery (Aranguren et al.,
2008; Serafini et al., 2007), the same cells generated embryonic
yolk sac tumors in nude mice (Leten et al., 2014; Lo Nigro et al.,
2012) and after intracranial delivery. As such, there remains no
substitute for rigorous long-term safety studies in animals, to
evaluate the behavior of cells delivered at or above the passage
number and density planned for clinical trials. Animal models are
also critical for developing strategies, such as suicide constructs,
that may augment the safety of cellular therapies in the future
(Letan et al., 2014).

4. From mouse to man: translation in progress

In this section, we highlight translational steps taken to date for
cell-based treatments of Parkinson’s disease, stroke, spinal cord
injury, ALS and Alzheimer’s disease. While the reader is referred to
more comprehensive reviews as appropriate for each disease, these
sections illustrate challenges encountered and progress made in translating neuroregenerative therapies from animals to
humans.

4.1. Parkinson’s disease—the Derailed prototype for CNS cell replacement

Parkinson’s disease has widely been viewed as the “ideal”
neurodegenerative disease for cell replacement therapy, owing to
the relatively select loss of a specific neuronal subtype— midbrain
dopaminergic neurons (Bjorklund, 1992).

Animal models for cell transplantation studies have generally
involved rodents treated with neurotoxins such as 6-OHDA or MPTP, to which midbrain dopaminergic neurons are selectively
susceptible (Dauer and Przedborski, 2003). Such models are
limited in that they fail to replicate the typically insidious onset
of human neurodegeneration. Nevertheless, models of unilateral
disease, such as following stereotactic injection of 6-OHDA, have
enabled quantification of amphetamine- or apomorphine-induced
rotational behavior, which may be reversed upon restoration of
dopaminergic innervation to the striatum by fetal or stem-cell-
derived grafts (Kim et al., 2002).

Years of careful animal experimentation preceded the onset
of clinical trials of fetal cell transplantation in the 1980s (Barker et al.,
1993). Numerous open-label trials generally yielded favorable
outcomes, with patients in many cases discontinuing their use of
dopamine medications and experiencing improved motor func-
tion. A meta-analysis of 11 clinical studies including 95 patients
suggested significant improvement after cell transplantation, with
increased 18 F-fluorodopa uptake, decreased used of L-dopa,
improved response to anti-Parkinsonian medications and a 36.6% 
overall functional improvement, as measured by Motor (off)
United Parkinson’s disease rating scale (UPDRS). Improved func-
tional recovery correlated positively with younger age (<60yo),
maintained immunosuppression, bilateral grafts, and post-trans-
plantation viability of cells as measured by 18F-fluorodopa (Polgar
et al., 2003)

In 2001 and 2003, two well publicized NIH-funded randomized
double-blind placebo-controlled trials failed to meet their primary
endpoints, and some patients unexpectedly developed severe
dyskinesias (Freed et al., 2001; Olanow et al., 2003). Cell therapy
for Parkinson’s rapidly was from being the poster child of cell
based therapies for neuroregeneration, to being labeled a failure. A
moratorium on cell transplantation trials for Parkinson’s was
advised in 2003 while the field sought to understand where things
grew wrong (Robinson, 2003).

Issues investigated included immunosuppression and tissue pre-
paration protocols, as well as graft composition and distribution.
Among the differences in protocol between these trials and most successful open-label trials performed previously, tissue chunks or “noodles” were used, in place of cell suspensions, cells were stored in culture prior to administration to document dopamine production, and use of immunosuppression was minimized. Novel animal models incompletely simulating human dyskinesias were developed and analyzed in detail to try to tease apart the causes of the adverse outcomes. Leading hypotheses ultimately implicated the presence of “hot-spots” due to uneven distribution of cells, and contamination of problematic grafts with higher numbers of serotonergic neurons in the development of dyskinesias (Lane et al., 2010).

Meanwhile, in the decade following the cessation of clinical trials, it was observed that certain patients previously implanted using the original protocols remained alive and well, in some cases off all anti-Parkinson’s medications and with sustained improvement in motor performance, even up to 18 years after transplantation (Kefalopoulou et al., 2014). The favorable clinical course of such patients has thus offered hope that with optimized protocols, cellular therapy could conceivably alter the natural history of Parkinson’s disease in appropriately selected patients. As such, TRANSEURO, a randomized controlled trial of fetal cell therapies was recently launched incorporating the lessons of prior experience (Abbott, 2014). In addition, based on excellent progress in preclinical studies in rodents and primates using human ESC- and iPSC-derived cells (Ganat et al., 2012; Kriks et al., 2011; Sundberg et al., 2013), it is anticipated that trials of human stem-cell-derived dopaminergic neurons will be initiated in the near future.

### 4.2. Bone marrow for stroke

Given the clearly defined etiology of ischemic stroke (acute lack of blood supply to the brain with or without delayed reperfusion), animal models of stroke are easily generated and well defined. Despite this, neuroprotective therapies effective in preclinical studies have almost universally failed in clinical trials of stroke. Potential reasons for this have been widely reviewed (Gladstone et al., 2002; O’Collins et al., 2006; van der Worp et al., 2005; van der Worp et al., 2010; See also Table 2. Among the most frequently cited “problems” are: (1) Most preclinical studies are performed in mice and rats, rather than animals phylogenetically closer to humans. (2) Most studies include young healthy animals whereas patients suffering stroke are more frequently elderly and suffer comorbidities such as hypertension and diabetes. (3) Unrealistic time points for intervention are frequently used. Indeed, in a systematic review of animal studies of 5 neuroprotective agents tested in 21 clinical trials involving > 12,000 patients, the median time between onset of ischemia and start of treatment was 10 min. By contrast, patients very rarely arrive at the hospital within 1 h (Lacy et al., 2001; Leon-Jimenez et al., 2014). (4) Different outcome measures are used in preclinical and clinical studies. For example, outcome assessments in animal models have historically been performed just days following therapy, rather than months after therapy, as occurs in human trials (van der Worp et al., 2005). In addition, infarct size, frequently evaluated in preclinical studies, is rarely assessed in clinical trials, and appears to poorly correlate with functional outcomes. (5) Animal studies of stroke have exemplified systemic problems of lack of appropriate randomization, blinding, and publication bias (Sena et al., 2010; van der Worp et al., 2010). (6) Inappropriate extrapolation of rodent studies when calculating sample sizes for clinical trials may further result in some potentially beneficial compounds being labeled as “failures” due to insufficient power. (7) Finally, it should be considered that “failed” compounds could still be useful as a component of polytherapy, even if ineffective in adequately powered clinical trials of monotherapy (Gladstone et al., 2002).

As promising cell therapies for stroke have moved toward clinical trials, there has been strong motivation to circumvent the prior failures experienced with neuroprotective compounds. As such, the STEPS (Stem Cells as an Emerging Paradigm in Stroke) consortium has published guidelines and research priorities to facilitate successful translation of cellular stroke therapies (Savitz et al., 2011; Savitz et al., 2014; Stem Cell, 2009).

Outcomes in hundreds of preclinical studies for stroke have now been analyzed by multiple meta-analyses, revealing insights into numerous variables. Overall, findings to date suggest substantial benefits in animal models across a variety of cell types, post-infarct time periods, and delivery strategies. A recent meta-analysis by Vu et al. (2014) suggested that perhaps the greatest improvement in modified neurological severity score was seen with intracerebral, followed by intra-arterial, followed by intravenous delivery (p = 0.025). Yet, even the benefit from intravenous delivery was regarded as “very large”. Encouragingly for translational efforts, the greatest reduction in infarct size was seen in primates, followed by rats and then mice, though the number of primate studies to date has remained small. Interestingly, human cells, appeared more effective at reducing infarct volume even in rodents, than rat cells, with mouse cells proving the least effective (p = 0.036).

Poor correlation between infarct size and functional outcomes has previously been reported in both humans and animal models. Consistent with this idea, a meta-analysis of preclinical studies found autologous cells to be the most helpful in reducing infarct size, while allogeneic cells yielded the greatest benefits in functional outcome (Lees et al., 2012). Furthermore, Vu et al. suggested that infarct size was most significantly reduced with early cell delivery (0–8 h) (p = 0.038); however, the greatest improvement in rotarod performance occurred when cells were delivered > 24 h after infarct (p < 0.004). Though seemingly contradictory, these data may suggest that MSCs have the potential to both reduce damage and promote repair, and that a wide therapeutic window exists for MSCs to promote functional improvement. While concerns of under-reported negative studies cloud meta-analyses of preclinical data, the findings to date have been overwhelmingly and consistently positive.

Acknowledging the limited role for cell replacement in stroke therapy, bone-marrow-derived cells have emerged as the favored candidate for stroke therapy. Indeed, the relative accessibility of bone-marrow-derived cells, including bone marrow mononuclear cells (BMMCS) and MSCs, has perhaps contributed to the rapid proliferation of early-stage clinical trials of cell therapy for stroke (Doeppner and Hermann, 2014). While most clinical studies to date have been non-randomized safety studies, one randomized open-label observer-blinded trial of autologous MSCs showed improved survival and outcomes at 5 years following intravenous delivery of autologous MSCs at 1 and 2 months after stroke (Lee et al., 2010). These data are consistent with a preliminary meta-analysis of outcomes in single-arm studies of MSCs for stroke that suggest highly significant improvements from baseline of 5.7 points on the National Institutes of Health Stroke Scale (NIHSS) and 31.5 points on the Barthel Index.

By contrast, a multicenter randomized trial failed to demonstrate significant benefit from a single intravenous administration of autologous BMMNCs (Prasad et al., 2014). Although further studies are needed to validate these results, the negative results in this first randomized trial of IV BMMNCs for stroke are consistent with a meta-analysis of 8 clinical trials administering granulocyte colony-stimulating factor (G-CSF)—used in part to mobilize BMMNCs—which yielded non-significant improvements at early time points and no difference in outcomes at later time points (Bath et al., 2013). In a meta-analysis of preclinical studies employing IV delivery of cells for stroke, MSCs were found to be...
about twice as efficacious as BMMNCs (Janowski et al., 2010). Such differences in efficacy may not be limited to the brain. In a randomized blinded placebo-controlled trial of intra-arterially delivered cells for ischemic cardiomyopathy, culture-expanded MSCs proved more efficacious than bone marrow mononuclear cells for enhancing left ventricular ejection fraction (Heldman et al., 2014). Nevertheless, careful optimization of protocols may be needed even among MSCs. For example, Li et al. (2008a) reported greater improvement in endogenous neurogenesis and behavioral recovery in rats treated with passage 2 rather than passage 6 MSCs. All patients given Aspirin, Statins

### 4.3. Spinal cord injury

Spinal cord injury (SCI) remains one of the most frequently cited “hopes” for successful stem cell therapy. Although the need to “regrow” axons is frequently assumed for SCI, spinal cord contusion is actually much more common than spinal cord transection, leading in part to a demyelinating injury to corticospinal tracts. As such, strategies to restore myelination following SCI have been intensively investigated. Positive results in rodent models of SCI using oligodendrocyte precursor cells (OPCs) prompted the well-publicized, but investigated, therapeutic interventions. Numerous clinical trials employing MSCs for spinal cord injury are ongoing (Martinez et al., 2014). Overlapping mechanisms of hypoxic injury and neuro-inflammation, with glial and microglial activation, characterize both SCI and stroke. A resulting glial scar, which serves to restrict the scope of injury, leads to a lasting obstructive barrier to neurite outgrowth. As with stem cell therapies for stroke, MSCs have been investigated in preclinical models of SCI. A recent meta-analysis of 80 rat studies employing MSCs for SCI demonstrated a highly significant benefit across a variety of MSC sources, and immunosuppression and delivery protocols (Oliveri et al., 2014). Although the influence of under-reported negative data is again difficult to fully exclude, the combined results performed extremely well on a proposed grading scheme to objectively evaluate the strength of preclinical data for clinical translation in SCI, and higher than any drug previously evaluated on the same scale (Kwon et al., 2011). Another meta-analysis of cellular therapies including 156 studies and 5628 animals revealed an overall 27.2% improvement (95% CI 25.0–29.4%) in motor function (Antonic et al., 2013) with cell therapy. Intravenous therapy provided the greatest benefit when compared to cell therapy directly administered into the spinal cord, perhaps in part due to the challenges of cell delivery in the tiny rodent spinal cord. Interestingly, Greatest benefit was seen in animals treated with isoflurane, perhaps suggesting the potential for interactions between stem cell therapy and other neuroprotective interventions. The attitudes of patients with SCI towards participation in a stem cell clinical trial have been surveyed in detail. Over half of patients indicated they definitely or probably would be willing to participate in a stem cell trial for subacute (57%) or chronic (66%) SCI, compared to 87% who would be willing to participate in an acute drug trial. Importantly, the most common reason cited was 71–74% of patients who said they “definitely” or “probably” would not participate in a stem cell trial was “don’t want to risk getting cancer,” followed by “don’t want to have another surgery” (58–63% of patients) (Kwon et al., 2012). These findings underscore the critical obligation of the scientific community to ensure the safety of administered cellular therapeutics. To date, there is no replacement for preclinical animal models to ensure the safety of cell therapies advanced to clinical trials. In the clinical trials to date for spinal cord injury, no safety concerns have arisen, though insufficient data exist to extrapolate efficacy data from rodents to humans.

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4.4. ALS

If spinal cord injury is unique, based on the youth and longevity of its victims, ALS is unique among neurodegenerative disease for its relatively rapid loss of susceptible motor and corticospinal neurons, leading to weakness and death typically from respiratory failure.

SOD1G93A mice are widely considered the gold standard model for ALS. However, similar to animal models for other neurodegenerative diseases, this model has been criticized for poorly representing human disease. Indeed, this mutation is present in only 3% of patients with ALS, and the mice carry 23 copies of the gene. As such successful therapies in this mouse model may be inappropriately skewed towards blockade of SOD1, which may be of questionable value for human ALS (van der Worp et al., 2010). Among other problems, a high degree of noise in the SOD1G93A model has been suggested to explain the majority of successful reports describing prolonged survival with various drugs. Attempts to replicate positive findings for 8 compounds (minocycline, creatine, celcoxib, sodium phenylbutyrate, ceftriaxone, WH-P131, thalidomide, and rituximab) using optimal methods to reduce noise failed to demonstrate survival benefit with any of the 8 compounds. Such optimized methods included: (1) large group sizes of at least 24 litter-matched gender balanced mice; (2) exclusion of non-ALS deaths and low-copy transgenics and, when applicable, exclusion of the corresponding paired animal from the comparison group; (3) blinded analysis using a single uniform endpoint criterion (inability to right itself in 30 s after being placed on its side); (4) analysis of survival using appropriate multivariate statistical models (Scott et al., 2008).

It is against such elevated standards that cell therapies for ALS are now being assessed in preclinical studies. A meta-analysis of 11 studies by members of the ALS consortium revealed improved function and prolonged survival after intraspinal delivery of human or mouse NSCs into SOD1G93A mice (Teng et al., 2012). Maximal benefit was seen when NSCs were injected at multiple sites along the neuroaxis including the lumbar, thoracic and cervical spine when NSCs were injected at multiple sites along the neuroaxis—finding replicated with both mouse and human NSCs. Indeed administration of 100,000 human NSCs (mNSCs) into 4 locations resulted in improved rotor-rod performance and prolonged survival with 25% of animals surviving more than 1 year (Teng et al., 2012)—well beyond that seen in any previously tested pharmacological therapy (Scott et al., 2008).

Although the mechanisms of action remain incompletely understood, transplanted cells were found to produce trophic factors such as glial-cell-derived neurotrophic factor (GDNF), decrease astrogliosis, increase the number of surviving motor neurons, and preserve the neuromuscular junction (Teng et al., 2012), consistent with previously reported findings in ALS rats (Xu et al., 2006; Yan et al., 2007).

Collectively, these and other preclinical findings suggest that cell therapy may offer unprecedented potential to alter the natural history of ALS. Prior to translation of NSC therapy to humans, the Boullis group employed a porcine model to optimize the technical delivery of cells into the fragile spinal cord. Given that the spinal cord moves slightly during surgery with respiration, the porcine model facilitated delivery in retrievable encapsulation devices (Wahlberg et al., 2012).

Active work is additionally ongoing to investigate cellular therapies for multiple sclerosis (Ferreira et al., 2015; Martino et al., 2010), Huntington’s disease (Olson et al., 2012), traumatic brain injury (Richardson et al., 2010; Xiong et al., 2013) and epilepsy, (Roper and Steindler, 2013), and pediatric lysosomal storage diseases (Gupta et al., 2012; Selden et al., 2013), among others. Indeed, cell therapies may not be limited to CNS injuries or degeneration but are also under investigation to help combat brain tumors due to their migratory behavior, inherent anti-tumorigenic properties, and capacity to deliver genes, prodrugs or oncolytic viruses to sites of tumor infiltration (Bovenberg et al., 2013).

5. Conclusions

Despite their mechanistic limitations, animal models are indispensable for translation of neuroregenerative therapies. Failure of animal models to precisely mirror the early mechanistic changes underlying most human neurodegenerative diseases has hampered the successful translation of targeted pharmaceuticals. Real-world human neurological diseases are heterogeneous and manifest as the culmination of multiple genetic and environmental variables...
compounded by age and stress. As such, therapies likely to be successful in more than a small subset of patients must synergistically impact multiple pathways of disease. To this end, as nature’s own multipotent agents of repair, endogenous progenitor cells are now known to be working behind the scenes to mitigate CNS injury. Indeed, given their multiple modes of action, stem-cell-based therapies may be less susceptible to mechanistic inaccuracies of preclinical animal models, and more amenable to successful translation in diseases for which the underlying etiologies are heterogeneous or remain poorly understood. As understanding of human disease mechanisms improves, cell-based therapies may provide a regenerative platform upon which further enhancements can be built. Studies of rejuvenation may reveal mechanisms of youthful reliance, whose decline leaves the brain open to degeneration, but which could be replenished to help the CNS combat injury and degeneration. While success will require that the field learn from its prior and future mistakes whilst stringently adhering to evolving best practices in pre-clinical and clinical studies, neuroregenerative strategies offer substantive hope for mice, scientists and patients alike.

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References


