Chapter 2
Cell-Based Therapeutics in Stroke: An Industry Perspective

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Stroke Background

An estimated 6.8 million Americans ≥20 years of age have had a stroke [1]. Each year, ~795,000 Americans experience a new or recurrent stroke. Approximately 610,000 of these are first attacks and 185,000 are recurrent attacks. In the USA, stroke ranks No. 4 among all causes of death and is the second leading cause of death worldwide, narrowly behind ischemic heart disease [2]. Of all the leading causes of death in both the USA and worldwide, stroke is unique for not having any specific therapy approved to improve outcome post insult, save for tissue plasminogen activator (t-PA) [3]. Although t-PA thrombolytic therapy was approved for acute stroke therapy nearly 20 years ago, its use worldwide remains extremely limited and the stroke field continues to search for neuronal protection and repair strategies.

Although the 1990s were labeled the "decade of the brain," significant nihilism crept into the field of stroke and was pervasive for approximately a decade from the late 1990s into the first decade of the twenty-first century [4]. During this period, all neuroprotective strategies examined in the clinic failed, despite careful review and recommendations from the Stroke Therapy Academic Industry Roundtable [5].

Perhaps not surprisingly, many major pharmaceutical companies began to de-emphasize neuroscience as a therapeutic area [6], although the most consistent failures have come in the attempts to treat acute neurologic disease such as stroke and traumatic brain injury (TBI). Inspired, transformational strategies were needed to reinvigorate the pursuit of medicines for one of the world's leading causes of death and disability.

A significant aspect of the de-emphasis of neuroscience drug development has been and continues to be the lack of clinical translatability of animal disease models. This is particularly evident in stroke where there have been more than 1000 pub-

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D. C. Hess (ed.), *Cell Therapy for Brain Injury,* DOI 10.1007/978-3-319-15063-5_2

lished preclinical studies, yet not a single neuroprotective agent has been approved for clinical use (considering t-PA a thrombolytic, not a neuroprotectant) [7]. When properly designed to recapitulate at least some aspects of the disease (e.g., timing of the therapeutic intervention, use of functional endpoints), animal models may be seen as useful screening tools—perhaps necessary, but certainly not sufficient for de-risking therapeutic approaches for central nervous system (CNS) diseases. However, the lack of translatability has resulted in a significant conundrum as clinical development, in general, requires massive resources (patients, professional personnel, and funding), limiting both the number of approaches that can be studied in the clinic and, as a result, the overall enthusiasm for the field. There are likely other contributors to the universal failure of neuroprotective therapeutics in both stroke and TBI, perhaps most notably both the presumed limited time window afforded for neuronal rescue and the consistent strategy of pursuing potential therapeutics which possess a single mechanism of action (MOA) to treat very complex diseases in an organ which has multiple redundant systems.

Next-Gen Stroke (CNS) Strategies

The solution for CNS drug development that has begun to emerge is twofold. While neither strategy specifically targets stroke or TBI, the field could be a major beneficiary. This "new path" arises both from a paradigm shift in drug development strategy and from major scientific advances in the field.

The first part of this disruptional thinking is to limit the emphasis on animal models. It is acknowledged that significant work has recently gone into improving preclinical stroke models, including the use of more relevant species, aged animals, animals with comorbid diseases, and chronic endpoints that may possibly reflect outcome more than acute measures of biochemistry and histopathology. However, improved guidelines for stroke research have not yet provided evidence of clinical translatability. Currently, the value of animal models in defining drug toxicity and determining initial exposure limits in early clinical trials remains, although animal models continue to have very limited utility in assessing drug efficacy for most human CNS diseases. Thus, a more rational strategy may be to advance therapeutics into the clinic as soon as they have demonstrated an appropriate safety profile in rigorous animal testing. The preclinical testing should define specific organ toxicity and the appropriate exposure/dose limits where the safety findings have occurred. These promising therapeutics are then taken into clinical testing. Small, focused clinical trials would rigorously study safety and determine relevant pharmacodynamic activity in order to provide safety profiling, risk mitigation, and greater confidence in rationale for the mechanism(s). This strategy is a rather bold but necessary move, shifting from animals to humans to understand the earliest signal of efficacy. For stroke, such early, surrogate clinical endpoints are in evolution but include neuroimaging (e.g., magnetic resonance imaging (MRI) measures of water content and directional flow; blood–brain barrier (BBB) integrity) and biochemical

markers, either by employing cerebrospinal fluid (CSF) or blood-based markers and/or studying magnetic resonance spectroscopy which can combine both a biochemical signal with some indication of cell viability or activity. Although these surrogate markers lack full validation, they may be viewed as "fit for purpose" and a significant advance from relying on improvements in stroke animal models.

The second catalyst for the stroke field is the explosion of deep science on stem cells. Considering that human CNS stem cells were only identified ~15 years ago [8], the progress has been quite remarkable. Neurorestoration is an exciting alternative (and complementary) strategy to neuroprotection, removing the seemingly severe time constraints imposed by the latter. The emergence of the neurorestorative concept as applied to stroke, however, is not without its drawbacks. It seems appropriate to say that the technical capabilities of utilizing stem cells as a therapy for stroke have outstripped our understanding of how they may be efficacious in this disease setting.

Cell Therapy for Stroke: Considerations

There are multiple aspects of stem cell therapy to consider:

- Cell source. Broadly, three potential exogenous cell sources could conceivably be used for stroke therapy: embryonic, adult pluri- and multipotent stem cells, and induced pluripotent stem (iPS) cells. Additionally, although this chapter focuses on the strategy of exogenous stem cell therapy, there have also been considerations of encouraging a greater response from endogenous neural stem cells. For example, one possibility is to locally administer trophic factors that promote greater efficiency of endogenous neural stem cell proliferation, migration, differentiation, and/or survival [9].
- Cell type. If adult stem cells are considered the "gold standard," what cell type should be advanced? One could consider stem cells from a neural lineage, although non-CNS cells, especially autologous mesenchymal stem cells (MSCs), have many advantages (no/minimal immunogenicity and tumorigenic capability, no ethical issues) and also seem to improve outcome in animal models just as effectively as neural stem cells. Most of the clinical trials to date have used autologous mesenchymal stem cells derived from bone marrow [10], although adult stem cells from a wide variety of alternative sources have been used, including cells harvested from umbilical cord [11], olfactory ensheathing cells [12], adipose tissue [13], and placenta [14]. The data from animal models [15] suggest that mesenchymal stem cells are effective regardless of the route of administration. Mesenchymal stem cells also appear to modulate the local inflammatory response that contributes to the hostile environment for repair and recovery. Many of the clinical trials employing autologous mesenchymal stem cells have fulfilled the objectives of demonstrating safety and feasibility, although assessment of stroke outcome has not yet demonstrated clear evidence of efficacy, perhaps in part due

to the limited sample sizes. Autologous therapies pose challenges when it comes to large-scale manufacturing and time constraints for meaningful production, while allogeneic therapies run a higher risk of causing an immune response.

- Stem cell role (mechanism of action, MOA). There is no strong understanding of how stem cells actually would mediate neural recovery. One may think of the "3R's" when considering how stem cells may improve poststroke outcome: repair, replacement, and redirection (Fig. 2.1). Of course, these roles are not necessarily mutually exclusive:
  - Repair. In this context, there is consideration of immune response modulation, release of soluble trophic factors to create a more permissive environment either through local effects and/or niche upregulation, and instructing a specific endogenous stem cell fate. Facilitating a more permissive environment (reduced neuroinflammation, improved regional cerebral blood flow, either directly or through facilitating angiogenesis) should balance enhanced plasticity with the downregulation of inhibitory pathways that provide the CNS with the necessary feedback to maintain a homeostatic environment [16]. Mesenchymal stem cells have demonstrated experimental success in a number of pathologic scenarios. Such a broad protective/reparative effect suggests that these cells may be capable of releasing a diverse array of factors. Growth factors likely contribute to the beneficial effect [17], although much attention has recently focused on exosomes [18–20], a very heterogeneous group (both in size and content) of secreted lipid vesicles that may have therapeutic effects under a multitude of conditions. Although different exosomes may have competing actions, this is an area of medicine that provides both a rationale for the beneficial effect(s) of MSCs and is spawning a novel field of both diagnostics and therapeutics to improve the recognition of tissue injury and aid in customized tissue repair following various pathologies.
  - Replacement (including trans-differentiation). Generally, long-term functional engraftment/integration of exogenous stem cells at the site of pathology is much more the exception than the rule [12, 21, 22]. Nonetheless, even the integration of a small percentage of stem cells into the infarct area may result in a meaningful improvement. It is not clear if there is one or more critical variables exhibited by certain stroke patients that may facilitate a more permissive environment. Genetic polymorphisms, concomitant medications, or overall medical status may contribute in ways that are currently unclear. Additionally, the endogenous secretion of trophic factors directly within the stroke/penumbral region may be an important mechanism for neural repair.
  - Redirection (scaffold, bridge). This is a relatively new concept and is a hybrid between the repair and replacement mechanisms. The exogenous stem cells are necessary prerequisites at the stroke site, facilitating the directed migration of endogenous stem cells to the site of injury [23]. The exogenously placed stem cells have a limited presence at the stroke site, although it appears that the endogenously migrated stem cells may be able to integrate and improve the brain cytoarchitecture in this region.

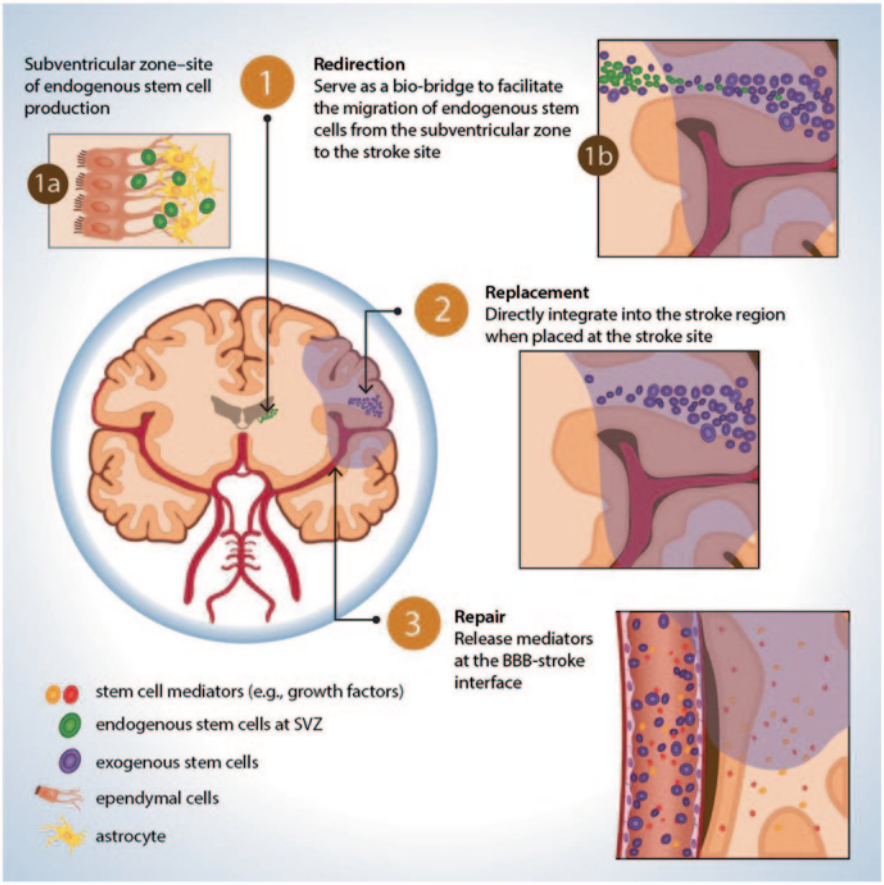

Fig. 2.1 Integrated approach to outcomes/clinical trials. The three general approaches by which stem cells could exert a beneficial effect following stroke (*1–3*). The direct placement of exogenous (autologous or allogeneic) within the stoke cavity could either serve to redirect endogenous stem cells from the subventricular zone (*SVZ*) to the infarcted region, facilitating their local engraftment and/or trophic effects or the transplanted exogenous cells could contribute more directly by replacing cells in the peri-infarct region and/or providing for a more favorable local environment. Stem cells delivered remotely (e.g., intravenously) likely exert their effect at the blood–brain barrier (*BBB*) interface through the activation of second messengers and/or the release of trophic factors, exosomes, etc. that facilitate a more permissive environment within the peri-infarct region

Even within each of the general strategies outlined above, there are multiple mechanisms by which a neurorestorative effect may be realized, just as there are multiple mechanisms that mediate the endogenous neuroplasticity that is constantly occurring. These include modulation of inhibitory circuits and facilitation of more

permissive activities. The above discussion does emphasize that the "single" approach of stem cells is actually a pleiotropic strategy that may allow the field to move beyond the single MOA approach to address a very complex insult.

Certainly, any of the approaches mentioned would need to create the appropriate permissive environment that will allow for the differentiation and expression of any/all neuronal and glial types needed to reconstitute an effective and efficient network. This is irrespective of cellular augmentation by endogenous elements or through the delivery of exogenous stem cells (or both).

There are additional critical points for discussion when considering cell therapy for stroke:

- Stem cell route of administration. To a large extent, the route of administration will be dictated by the purported role for stem cells as a poststroke therapy. Currently, many contemporary stem cell studies for stroke utilize intravenous delivery which is seen as more of a reflection of pragmatism than an understanding of their MOA. The limited ability of stem cells to cross the BBB would not be an impediment to peripheral (intravenous or intra-arterial) administration [15] if the primary role of the stem cell was to provide second messenger signaling or growth factors that would then facilitate a more permissive environment for brain repair (e.g., shed exosomes have demonstrated the ability to cross the BBB). There are animal model data to suggest that the intravenous administration of stem cells may be as efficacious as administering stem cells directly to the injured region of the brain. A direct comparison of different delivery routes within the same animal model may be of great value and would not necessarily demand translatability to the clinical scenario. Alternatively, if the pluri- or multipotent stem cells are intended to serve as replacement cells in the infarct region, or if the intent is to serve as a biobridge from internal sources of neurogenesis to the stroke region, then strategies must be developed to stereotactically deliver the cells to the appropriate brain region.
- Stem cell timing of delivery. Ischemic brain injury is accompanied by a major inflammatory response which includes both CNS intrinsic (e.g., microglial upregulation) and extrinsic (e.g., leukocyte and cytokine upregulation) activation [24]. The inflammatory response can be seen as either well orchestrated or, alternatively, well-intentioned but suboptimally regulated. A critical question is when to intervene with a stem cell therapy. If the ongoing (at least initial) inflammatory response is beneficial for self-repair (e.g., removal of necrotic debris, facilitation of a more permissive environment), then the early downregulation may be counterproductive. The ultimate answer may only be understood through direct intervention and rigorous, methodical assessment of relevant clinical (and possibly imaging/biochemical) endpoints.
- Timing of primary endpoint measurement. Traditionally, key endpoints of neurologic outcome and function have been measured at 3 months post stroke. This was the time point used in the pivotal t-PA study and continues to be used nearly two decades later for a large number of putative neuroprotective and now, neurorestorative therapies. But is the best time point for assessing improvement? Neu-

rologic recovery continues for a year or longer post stroke. For therapies that are likely to provide cellular replacement and integration or otherwise improve plasticity, significant periods of time (weeks–months) may be needed to establish these new/repaired networks, in addition to the period of time for the affected individual to actually functionally improve. This may be especially relevant for the more eloquent domains such as speech. This time frame is likely longer than for a neuroprotective agent that focuses on salvaging ischemic neurons, yet very few studies perform randomized, double-blind placebo controlled trials for more than 3 months.

- Combination therapy. This paradigm is often favorably discussed but rarely initiated. This is likely for a number of reasons, including the logistics of understanding the optimal dose of each therapeutic alone and in combination and also ensuring that the animal toxicology package will support the clinical program. The plethora of permutations typically results in therapeutics being developed independently. That said, it is of interest that in a rat model of TBI, a combination of human umbilical cord blood cells and G-CSF, administered intravenously one week post insult, provided the most significant reduction in TBI-induced behavioral deficits when compared to either agent administered alone [25]. The behavioral improvement also complemented a histologic reduction in inflammation associated with improvement in neurogenesis. This combination strategy was recently studied in a clinical trial of chronic stroke patients, enrolled 6 months to 5 years post stroke [21]. In this study, subjects were given G-CSF for 5 days prior to intracerebral implantation of autologous peripheral blood stem cells ($CD34^+$). After a 12-month follow-up period, the subjects treated with the combination G-CSF and autologous $CD34^+$ stem cells demonstrated significant improvement in neurologic and functional scales as well as MRI evidence of structural improvement when compared to the control group. Although this was a small clinical study ($N = 15$/group), the data are very encouraging.

Adding greatly to the complexity of combination therapy is a recent small study conducted in ten subjects 6 months to 20 years post stroke [12]. These subjects were treated with a combination of various stem cells including olfactory ensheathing cells, neural progenitor cells, umbilical cord mesenchymal cells and Schwann cells given through both systemic and local administration. Safety and efficacy were reported, although the extremely small sample size limits the strength of the conclusions and the multiple cell therapies and routes of administration will likely limit the use of this strategy, especially given the encouraging results with more straightforward clinical trial designs.

Noninvasive imaging will have an increasingly important role in the development of neurorestorative therapies in general, especially for stem cell and for gene therapies, where there is insufficient information on outcomes, in part due to the small sample sizes employed. In Parkinson's disease, neuroimaging is already part of the suite of modalities to understand outcomes [26]. Noninvasive imaging is a means by which promising mechanisms can be de-risked by seeking a surrogate (intermediate) biologic endpoint that can convey relevant pharmacodynamic activity

and justify larger clinical studies. Examples include diffusion tensor imaging (DTI) to assess edema and fMRI to evaluate structural (brain, BBB) integrity. Neuroimaging may also be used to visualize the stem cells as well, determining their ultimate location and viability over time in a noninvasive way [27]. These data may then be related to either surrogate biochemical markers and/or to clinical endpoints.

There are additional critical factors that need to be addressed when one is considering if a potential therapeutic will improve stroke outcome, but many of these go beyond the breadth of a chapter devoted to cell therapies. One of the topics that will be critical to any potential therapy is the stroke type. This relates not only to location vis-à-vis infarcts involving gray matter or white matter (or specific fiber tract involvement) as these different anatomic locations most certainly will have unique strategies, but also to the individual patient deficit [28]. Employing endpoints for future stroke trials should consider a weighting based both on the patient-specific deficit at baseline (or at the time of enrollment in a clinical trial) and a potentially flexible timeline to primary endpoint analysis (based on specific deficits), possibly extending this timeframe to 6–12 months post initiation of therapy.

Summary

To summarize, our understanding of the complexities of stroke pathophysiology is very incomplete and this has contributed to the dearth of therapeutic options for this devastating disease. The examination of stem cell therapies for stroke is an emerging field which is also inadequately understood. However, this should not serve to dissuade investigations from the treatment of stroke and specifically stem cell therapy. Given the data to date, intravenous delivery of autologous stem cells provides the most pragmatic strategy, in regard to both the demonstrated safety and ease of administration but also having an efficacy profile that is at least comparable to the more aggressive routes of local stem cell administration. Mesenchymal stem cells have clearly garnered the greatest interest to date, although the optimal cell type to use awaits future investigative work. What is desperately needed for the evaluation of stroke therapies (stem cells and any other potential therapeutics) are both mechanistic and general endpoints that can be quantified in early phase clinical trials to de-risk these programs, providing confidence that they can demonstrate robust efficacy in larger and less controlled clinical trial scenarios. It is encouraging that cell therapy clinical trials for stroke and TBI have moved beyond the mindset of neuronal cell replacement to the use of other stem cell types (e.g., mesenchymal) that may facilitate endogenous repair and regeneration through the creation of a more permissive environment. Multifactorial approaches are clearly needed for these complex and devastating diseases and the future of stem cell therapies may assist in achieving this goal.

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http://www.springer.com/978-3-319-15062-8

Cell Therapy for Brain Injury
Hess, D.C. (Ed.)
2015, XI, 369 p. 41 illus., 35 illus. in color., Hardcover
ISBN: 978-3-319-15062-8