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Clinical Trial Design for Stem Cell Therapies in Stroke: What Have We Learned?

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Abstract: Stem cells of various sources have been investigated in a series of small, safety and feasibility-focused studies over the past 15 years. Understanding of mechanisms of action has evolved and the trial paradigms have become focused on two different approaches – one being an early subacute delivery of cells to reduce acute tissue injury and modify the tissue environment in a direction favourable to reparative processes (for example by being anti-inflammatory, anti-apoptotic, and encouraging endogenous stem cell mobilisation); the other exploring later delivery of cells during the recovery phase after stroke to modulate the local environment in favour of angiogenesis and neurogenesis. The former approach has generally investigated intravenous or intra-arterial delivery of cells with an expected paracrine mode of action and no expected engraftment within the brain. The latter has explored direct intracerebral implantation adjacent to the infarct. Several relevant trials have been conducted, including two controlled trials of intravenously delivered bone marrow-derived cells in the early subacute stage, and two small single-arm phase 1 trials of intracerebrally implanted cells. The findings of these studies and their implications for future trial design are considered.

Introduction:

Stroke remains a major cause of mortality and disability worldwide, with very large healthcare and social costs.¹ Acute stroke care has seen major advances in recent years, with the widespread adoption of organised stroke unit care, and reperfusion therapy for the 85% with ischaemic stroke – first with intravenous thrombolysis² and more recently with endovascular mechanical thrombectomy³ for selected patients with large artery occlusion. Declining mortality and improved functional outcomes are evident in settings where these changes in care have been implemented systematically.⁴

Despite these positive developments, it remains the case that the majority of those who survive a stroke have some degree of residual disability. On a global scale, the incidence of stroke has increased and age-standardised mortality has declined, with a consequent rise in disability-adjusted life years lost to stroke, and a predominant burden in low and middle-income countries.¹ Advances in the therapeutic approaches to assist stroke recovery have notably lagged behind the gains in acute care. While reperfusion therapies offer very large treatment effects, these remain appropriate or accessible in only a minority of patients even in high-income countries. There thus remains a very substantial need for additional treatment approaches for stroke.

Mechanistic understanding of cell therapies has advanced in the four years since this topic was reviewed previously,^{5,6} and changes in our understanding have been reflected to an extent in clinical trial design. The concept of cell replacement to restore function, a paradigm that requires characterisation of cell phenotype to ensure replacement by an appropriately matched stem-cell derived population,^{7,8} may be applicable to situations where specific cell populations are lost as part of the disease process – for example dopaminergic neurones in Parkinson's Disease, or retinal epithelial cells in macular degeneration – but in stroke, ischaemia or haemorrhage results in pan-necrosis of all cellular elements, typically on a large scale, and cell replacement as a relevant mechanism seems likely to be a minor factor.⁹ Tissue infarction due to ischaemia triggers an inflammatory reaction with both local and systemic effects,^{10,11} that may be detrimental, and there is a sustained period of immunodepression that may increase liability to infection. On the other side, there is evidence of a range of responses to tissue injury to produce a facilitatory environment for repair, including upregulation of growth factor genes and promoters of angiogenesis, enhanced plasticity, as well as mobilisation of endogenous neural stem cells.¹²⁻¹⁴ The tissue outcome of stroke ultimately, however, is a cystic fluid-filled space of variable volume, surrounded by a gliotic rim. Replacement of cells within this cystic space is feasible only with some form of bioscaffold,^{15,16} and even with this, functional benefit might be limited due to the barrier of a gliotic scar impairing

connectivity. Most disabling strokes are of large volume and may entail the loss of several billion neurons, with an estimate of 1.2 billion neurons lost for a typical infarct volume of around 50ml.⁹ Cell implantation on the margins of an established stroke is also likely to yield only limited benefit, since in most cases there is either loss of neuronal cell bodies through direct injury, or degeneration of axonal connections remote from the locus of the injury. A cell replacement paradigm therefore seems likely to have limited utility.

Concepts of cell therapy for stroke have therefore evolved towards recognition of potential biological effects that are not reliant on cell replacement, and are focused on two main approaches, dictated by the mode of cell delivery and to an extent by the origin of the cells. The first relies on systemic cell delivery, usually via the intravenous route, and this has been investigated predominantly with acute or early subacute administration intended to modulate the initial injury or to reinforce early reparative tissue responses, and recognition that this strategy likely does not result in significant cell engraftment in the brain. The second strategy has delivered cells directly into the brain, predominantly into undamaged tissue close to the site of injury, and the need for clinical stability to permit anaesthesia and neurosurgery has led to administration in late subacute or chronic stages. This strategy may result in some degree of cell engraftment in the brain, but there is now general recognition that even in this situation, cell effects are likely to be mediated largely by paracrine effects on the local environment.

The Acute or Early Subacute Paradigm

Animal experimental work has predominantly followed a paradigm typical of neuroprotectant strategies, with systemic cell delivery within hours or at most a few days after induction of focal ischaemia.¹⁷⁻¹⁹ There is resulting reduction in infarct volume and functional recovery has generally been enhanced. Publication and reporting bias is evident in the animal literature, study quality has been inconsistent, and effect sizes may be overestimated as a result,^{17,19} although meta-analysis of studies of mesenchymal stem cells (MSCs) in rodent stroke suggested larger effect sizes with greater study quality.¹⁸ There is no compelling evidence favouring one type of cell over another,²⁰ with a wide range of (predominantly human) cell types having similar effects in rodent focal ischaemia models, including MSCs, mixed bone marrow mononuclear cell (BMMCs), CD34+ haematopoietic progenitor cells, umbilical cord blood cells and many others.

It has become clear that despite some degree of cell homing to the site of an ischaemic injury, cell engraftment in the brain after intravascular administration is probably minimal. Intravenous delivery does not appear to be associated with any significant retention in the brain even within the first 24

hours in biodistribution studies in animals using radiolabelled or bioluminescent cells,^{21,22} while intra-arterial delivery carries greater likelihood of cells reaching brain and persisting for up to 2 weeks.²² Intravenously delivered MSCs are of small size and are predominantly distributed to the lungs, where they may engraft with potentially useful biological actions.²³ In a human biodistribution study using larger bone marrow mononuclear cells labelled with technetium-99m (whose half-life is approximately 6 hours), no difference in brain activity was evident between intravenous and intra-arterial delivery, and brain counts for both routes of delivery at 2 hours after administration were low.²⁴

The predominant mechanism of action is therefore assumed to be paracrine, with effects on inflammation, immune modulation and stimulation of endogenous recovery processes including neurogenesis and angiogenesis. In animal models of stroke, and also in humans,²⁵ there is an acute reduction in splenic volume followed by expansion and release of cells that is postulated to mediate secondary inflammatory brain injury. Intravenous stem cell administration appears to modify this response.²⁶

Ten small clinical studies of intravenous or intra-arterial cell delivery, including 136 subjects, have reported findings.²⁷⁻³⁶ The majority of these were small, single-institution safety and tolerability studies. Only three included a control group, and the value of the control groups is questionable, since in many cases they consisted of patients deemed ineligible for the cell therapy intervention, were not randomised, and did not necessarily undergo any study related procedures. Allocation was not always concealed to either patients or investigators. Piecemeal reporting of studies^{24,27,28,34} further confuses the interpretation of these reports. Since the main focus was safety, long time windows for cell administration (between days and several months after stroke) were typically permitted. The use of autologous cells in most studies presents issues for trial design since the yield varies among individuals and cannot be controlled. The requirement for culture expansion of some cell types (especially MSCs)³⁰ introduces delay between cell harvest and administration. In addition, the invasive nature of procedures for cell harvest means that blinding is impossible, or at least difficult to achieve ethically. Allogeneic cells may therefore represent a more logical approach for acute use.

Two moderate sized multicentre trials have reported.

A phase 2 randomised, controlled trial with blinded end-point assessment undertaken at five centres in India delivered intravenous autologous bone marrow- mononuclear cells a median of 18 days after stroke onset.³⁷ Moderate to severe ischaemic stroke in the anterior circulation was required for

eligibility. Sixty patients were allocated to control and 60 to cell infusion. No differences in functional outcomes or imaging were evident over 6 months of follow-up. The mean number of cells infused was 281 million, of which around 1% were CD34+ cells, but cell dose varied widely among patients.

The Athersys MultiStem study,^{38,39} used a donor pool of allogeneic multipotent bone marrow derived cells depleted of CD45 (+)/glycophorin-A (+) cells termed multipotent adult progenitor cells (MAPCs) by the manufacturer. This trial randomised patients within 48 hours of onset of ischaemic stroke to receive an intravenous infusion of MAPCs or placebo, and followed them for 6 months. The trial included 126 subjects (65 given MAPCs and 61 placebo) and reported a trend towards better functional outcomes in the MAPC group based on a subset of control subjects recruited within 36h. Treatment effects based upon the total control group have not been reported. Some biomarkers of inflammation supported the potentially anti-inflammatory action of this cell type.

The unpublished ISIS-HERMES trial in 31 patients (including 11 controls) delivered autologous MSCs intravenously in subacute stroke patients has also been completed but data are not yet available.⁴⁰

A large multicentre academically funded European trial, RESSTORE (<http://www.resstore.eu>), uses adipose-derived donor MSCs delivered intravenously within 14 days of ischaemic stroke and will commence in late 2016 with the intention of including 400 patients.

The Prasad trial delivered far lower numbers of cells than other trials of intravenous cells (eg 0.6-1.6 x10⁸ cells in a bone marrow-derived MSC study³⁰ or 400-1200 million cells in the Athersys trial³⁹). Dosing comparisons among different studies are of uncertain validity, since cell populations are likely to differ substantially in their biological properties.

Late Subacute or Chronic Stroke

The high prevalence (an estimated 25.7 million survivors of stroke globally in 2013) and major global burden of stroke disability mean that there is a substantial unmet need for those with chronic stroke. There is considerable biological doubt, however, regarding the plausibility of modifying disability via cell engraftment, as noted above, and the justification for studying this group of patients has initially been based on stable neurological deficits offering a suitable environment for detection of any adverse effects. Older trials in this setting have used modified teratocarcinoma derived cells^{41,42} or porcine xenografts,⁴³ neither cell type being developed further. The protocols from these older studies have formed the template for recent trials of direct intracerebral delivery. Two relevant recent trials have reported findings.

The Pilot Investigation of Stem Cells for Stroke (PISCES 1) trial implanted a single dose of between 2 and 20 million cells by stereotaxic intraputaminial injection to the ipsilesional hemisphere of patients 6-60 months after disabling ischaemic stroke.⁴⁴ This was undertaken as a single centre study with no control group. Safety was the primary outcome and patients were followed for 2 years post-implant. The cells used in PISCES 1 were human foetal neural stem cells genetically modified by insertion of a c-mycER transgene that expresses the c-myc growth factor when activated by 4-hydroxytamoxifen, allowing cells to be maintained indefinitely in culture.⁴⁵ Removal of 4-hydroxytamoxifen leads cells to differentiate into neural lineages. Preclinical stroke model data reported dose dependent improvements in sensorimotor recovery over 12 weeks post-implantation when delivered 3-5 weeks after middle cerebral artery occlusive stroke in the rat.⁴⁶ In PISCES 1, eleven male patients underwent implantation a median of 22 months after stroke. No cell-related safety issues were observed, with serious adverse events being related either to the neurosurgical procedure or to long-term consequences of stroke comorbidities. Modest improvements in motor function occurred within the first 2 months after implantation and were maintained thereafter, an unexpected observation in this group of patients. A phase 2 study using the highest cell dose at late subacute stages 3-12 months after stroke is underway (PISCES 2, NCT02117635).

A similar trial design was utilised in a SanBio sponsored trial of modified donor human bone marrow-derived MSCs (transiently transfected with *Notch1* to enhance cell viability).⁴⁷ Eighteen male or female subjects were implanted with 2.5, 5 or 10 million cells between 6 and 60 months after ischaemic stroke (median 22 months). Safety issues were again limited to consequences of neurosurgery or long-term comorbidities of stroke. The study also reported modest improvements in several scales of neurological function and motor scales over the initial 2-3 months after implantation that were maintained up to 12 months in 16 of the 18 patients, two having been lost to follow-up.

Both studies reported the development of hyperintensities around the needle tracts on T2-weighted Fluid Attenuated Inversion-Recovery (FLAIR) scans in a high proportion of patients. These were most striking at 1 week after implantation in the SanBio study, a time point not investigated by this modality in PISCES 1, and had substantially resolved by 1-2 months. PISCES 1 reported more modest T2 FLAIR hyperintensities at 1 month post implantation that persisted by 12 months. In the SanBio study, greater extent of T2 hyperintensity correlated with improvement in motor impairment at 12 months by the Fugl-Meyer motor scale.

These two studies raise the possibility that there might be therapeutic potential in the population with chronic disabling stroke, although to what extent the observed modest motor changes might

lead to enhanced functional status is unclear. Considerable caution is also needed in the interpretation of the studies in the absence of a concurrent control group, although the correlation with objective MRI changes in the SanBio study makes it more plausible that this represents a biological effect. Nonetheless, lack of blinding to treatment allocation on the part of investigators and patients may confound serial functional assessments. If modest functional improvements are indeed a biological effect then the timescale for their evolution (peak improvements in both PISCES 1 and SanBio studies at around 3 months post-implantation, but change evident even at 1 month assessments) is unexpectedly early if the mechanism involves stimulation of endogenous neurogenesis or angiogenesis predominantly. The transient reaction to cell implantation evident on MRI may reflect, among other possibilities, an inflammatory response, a specific biological response to cell components, or a response to factors released by the cells, but the possibility that the location of the injection itself modifies motor function cannot be excluded either.

Animal studies using the experimental allergic encephalomyelitis model for multiple sclerosis raised concern about the potential for MSCs transplanted to the central nervous system to form mass lesions in the context of severe local inflammation.^{48,49} The relevance of this to stroke, where inflammatory responses are likely to be less intense, or indeed absent by late times after the incident, is unclear, and no clinical evidence of such a response has been observed to date.

Other Trial Paradigms

Studies have reported safety data from intrathecal delivery of cells or combined intravenous and intracerebral delivery, but characterisation of the cells involved has been limited and the number of subjects involved small.

Conclusion: Where Next for Clinical Trials?

The paradigm of acute or early subacute allogeneic cell therapy by intravascular delivery is essentially a well-trodden one, following a path laid out in numerous (albeit unsuccessful) neuroprotectant trials. Double-blind randomised controlled trials are feasible in this setting, but more invasive procedures such as intracerebral implantation represent a challenge with respect to control groups.

Use of autologous cells in either trial approach compromises blinding since invasive procedures are required to acquire cells from bone marrow or adipose tissue, and it is ethically uncertain that this could be justified if the patient is subsequently randomised to placebo. As undertaken in the trial of Prasad and colleagues,³⁷ bone marrow harvest may be undertaken a few hours prior to administration of cells in the cell therapy arm of a trial, but this permits only use of poorly

characterised cells. Ex-vivo culture expansion of more precisely characterised cell types incurs delay that both modifies the potential trial population (to survivors likely to remain hospitalised 1-2 weeks after stroke) and the possible therapeutic effect.³⁰

For intracerebral implantation, placebo surgery might maintain blinding and control for the placebo effect, but cannot control for any non-specific biological effects from “lesioning” the target site for implantation.⁵⁰ The placebo surgery approach also cannot identify overall harm from surgery itself, so there is an argument in favour of a non-operated control group in order to compare standard care against intervention, where the intervention arm will be judged on the net effect, including both benefit and harm (if present). Invasive delivery of cells carries definite procedural risk.⁵¹

The intravenous route is more feasible and acceptable, and justified on the basis of changes in our understanding of the biology of cell therapy in the acute or early subacute time window. The intra-arterial route may allow modest (although likely transient) engraftment of cells but comes at the probable expense of greater complications⁵² and more challenging recruitment unless piggy-backed on hyperacute endovascular thrombectomy. Whether this is feasible or not remains to be established.

How long should the time window be? While there may be biological rationale for time windows of up to several days after stroke onset if the proposed mechanisms of anti-inflammatory, immunomodulatory or endogenous cell mobilisation actions are correct, this group has proved very difficult to enrol in clinical trials,⁵³ and any treatment effects seem likely to be modest. Anti-inflammatory strategies using drug treatments in acute stroke have not been successful to date,⁵⁴ raising the possibility that this mechanism may be less important than proposed. The group with less severe stroke typically follow a rapid recovery trajectory and smaller proportions of patients fail to improve. The early time window strategy of the Athersys trial seems most likely to succeed both with patient recruitment, and biological credibility. Whether the current outcome measures are appropriate for recovery trials is less clear. There may be gains from adoption of more focused scales such as the Fugl-Meyer motor scale,⁵⁶ but this inevitably limits recruitment to patients with motor deficits. Restricting recruitment to specific deficits dictated by the outcome measurement chosen will inevitably restrict recruitment (eg only 6% of screened patients were eligible for an upper limb rehabilitation study⁵⁷). Even for non-invasive interventions in chronic stroke disability such as upper limb robot-assisted physiotherapy, trials have failed to recruit to target,^{55,58} so restrictive entry criteria dictated by the specificity of an outcome measure may prove counter-productive. More general scales of disability such as the modified Rankin Scale are both better characterised, arguably more relevant to patients, and likely to be necessary to persuade both regulators and clinicians of

the value of any intervention in definitive phase 3 trials. General scales of this kind are unlikely to assist in phase 2 trials where proof of concept and potential effect size estimates are sought, however, so phase 2 studies using motor scale end-points may be more feasible when combined with broad entry criteria.

More invasive delivery of cells is being further explored in PISCES 2 (NCT02117635), and the ultimate target population for these allogeneic cell therapies at present appears to be later subacute or potentially chronic stroke. The acceptability and feasibility of direct intracerebral implantation at early stages post-stroke remains to be established, but there are concerns over safety for anaesthesia and neurosurgery in the initial weeks to months after stroke, and since this is the period when most patients are experiencing their most rapid recovery, willingness to consider invasive procedures may be limited unless compelling biological selection criteria can identify with high specificity which patients will fail to improve under conventional approaches. Clinical criteria alone do not appear to be sufficient to prognosticate,⁵⁹ Whether late subacute intervention (intended to enhance endogenous recovery during the first months after stroke) has the same biological basis as the observed apparent effects in chronic patients on average two years or more after stroke is unknown.

The role of adjunctive physical therapies remains unclear, with some biological evidence favouring this as a necessary accompaniment of recovery enhancement strategies that enhance brain plasticity. In this model, the biological therapy modifies the brain environment in a more favourable direction but in itself this does not lead to recovery of function, and specific physical therapy is required to exploit the facilitative environment.⁶⁰ Since delivery of physical therapies to enhance stroke recovery varies widely across healthcare systems and the evidence base to guide specific components of physical therapies is limited, it may be necessary for trials to mandate an arbitrary minimum level of physical therapy.

Immunosuppression, although commonly given in animal models since these most often evaluate human cell xenografts, has not been administered in recent human allogeneic cell studies since immunosuppressant therapy carries significant risks to the patients, and the immunogenicity of the cells has been considered likely to be low, especially in the context of intracerebral delivery. The intravenous delivery of allogeneic cells is postulated to have a predominantly immunological effect in itself and again, immunosuppression has not been co-administered.

Neither clinical outcome criteria nor the optimal timing of measurement are yet well defined, as noted above, and proposed trials to date have sample sizes too small to detect anything other than

large effects. An apparent plateau of clinical change by 3 months after intracerebral implantation in both the PISCES and SanBio trials was observed, but the chronic and severely disabled stroke population recruited to these trials is not representative of likely future trial populations, where enhancement of endogenous recovery is likely to target earlier stages post-stroke. The rate of change of different assessment scales will also differ, and several are likely to exhibit ceiling effects. Invasive or high-cost interventions such as cell therapy may of course be justified only if treatment effects are large: however, the smaller the study, the greater the risk of a type 2 error and the abandonment of potentially valuable treatment in the face of considerable global unmet need. Imaging offers the potential for better patient selection as well as a biomarker for outcome, and may represent an important strategy when faced with moderate sample size of clinical trials. As a minimum, this may offer evidence of a biological effect in circumstances where clinical assessments alone may be confounded (for example by difficulties in blinding to treatment allocation or by marked heterogeneity of baseline prognostic markers). Imaging criteria may inform patient selection by identifying patients with unfavourable natural history of motor recovery, using predictors such as the extent of corticospinal tract integrity, although additional functional assessment (for example using transcranial magnetic stimulation) may further stratify patients.⁵⁹

Consensus recommendations on trial development largely recognise that clinical data to inform key issues such as dosing, time windows, end-points, biomarkers, and follow-up duration are very limited, and also that animal data may translate poorly to human use.⁶¹ The mechanistic understanding of cell therapies in stroke has advanced and clinical trial designs have adapted to these changes in concepts. Clinical trials have established basic safety information about a range of cell types and routes of delivery, at least in limited populations, and planned clinical trials will deliver preliminary evidence of efficacy. The two largest completed trials to date, using intravenous cell delivery of bone marrow-derived cells, have not shown evidence of significant benefit, but larger trials are required to establish effects.

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Table 1: features of recent major clinical trials in stem cell therapy for stroke.

Trial	Year	Cell Type	Window for intervention	Delivery Route	Patient number
Prasad ³⁷	2014	Autologous bone marrow mononuclear cells	7-30 days	Intravenous	120
Athersys ³⁸	2015	Allogeneic bone marrow-derived multipotent adult progenitor cells	48 hours	Intravenous	126
SanBio ⁴⁷	2016	SB623 genetically modified human bone marrow-derived mesenchymal stem cells	6 – 60 months	Intracerebral	18
PISCES ⁴⁴	2016	CTX0E03-DP genetically modified human neural stem cells	6 – 60 months	Intracerebral	11

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