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Cell Therapy in Stroke - Cautious Steps towards a Clinical Treatment

Olivier Detante^{1,2} • Keith Muir³ • Jukka Jolkkonen^{4,5}

¹Grenoble Hospital, Neurology Department, Stroke Unit, Grenoble, France

²Grenoble Institute of Neurosciences, Inserm U1216, Université Grenoble Alpes, Grenoble, France

³Institute of Neuroscience & Psychology, University of Glasgow, Queen Elizabeth University Hospital, Glasgow, Scotland, UK

⁴Neurology, University of Eastern Finland, Kuopio, Finland

⁵NeuroCenter, Kuopio University Hospital, Kuopio, Finland

Corresponding author:

Jukka Jolkkonen

Tel: +358-40-3552519

Fax: +358-17-163539

Email: jukka.jolkkonen@uef.fi

Abstract In the future, stroke patients may receive stem cell therapy as this has the potential to restore lost functions. However, the development of clinically deliverable therapy has been slower and more challenging than expected. Despite recommendations by STAIR and STEPS consortiums, there remain flaws in experimental studies such as lack of animals with comorbidities, inconsistent approaches to experimental design and concurrent rehabilitation that might lead to a bias towards positive results. Clinical studies have typically been small, lacking control groups, often without clear biological hypotheses guiding patient selection and furthermore, they have used a wide range of cell types, doses and delivery methods, and outcome measures have been inconsistent. Fortunately, some ongoing and recent trial programs offer hints that these obstacles are now being tackled. The Horizon2020 funded RESSTORE trial will be given as an example of inconsistent regulatory requirements and challenges in harmonized cell production, logistic and clinical criteria in an international multicenter study. The PISCES trials highlight the complex issues around intracerebral cell transplantation. Therefore, a better understanding of translational challenges is expected to pave the way to more successful help for stroke patients.

Keywords Cell therapy • Cerebrovascular diseases • Experimental studies • Clinical trials • Translation

Introduction

After the acute stage, the therapeutic options for stroke are limited, and despite important advances in acute reperfusion treatments, systems of care, and secondary prevention, a high proportion of patients remain disabled after their stroke (1). Thus, restorative approaches including cell therapies have been heralded as the future therapy for this devastating condition (2). Since it has been claimed that cell therapies offer a wider therapeutic time window, they might be available for a larger number of patients and allow combination with other rehabilitative strategies.

Two distinct strategies for cell therapy have emerged from animal data. The first is a neuroprotectant strategy, using systemically delivered (typically intravenously administered) cells to limit the evolution of the early brain injury (3,4). The mechanism of action appears to be reliant on “bystander” effects; these are likely to include immunomodulatory and anti-inflammatory effects mediated via the systemic release of trophic factors (5), since neither animal nor human data have found any signs of actual engraftment of intravenously delivered cells in the brain (6–8).

The second strategy utilizes cell regeneration or replacement to promote recovery during the later stages after stroke. This usually involves cell delivery to the site of injury by intraparenchymal brain implantation, usually involving stereotaxic injection into unaffected deep brain structures adjacent to the site of injury. A somewhat less invasive delivery has exploited an intra-arterial approach; this has been associated with some persistence of limited quantities of cells in the central nervous system (6,9,10). It is unclear to what extent cells survive over the long term, the differentiation fate of surviving cells, or whether survival results in functional engraftment. The putative therapeutic effect in this paradigm may also rely on the release of trophic factors, promoting endogenous stem cell mobilization, and anti-apoptotic effects in addition to the anti-inflammatory and immunomodulatory effects encountered after systemic cell delivery. The extent to which cells can migrate from their implantation site in human subjects is unclear. Placing cells within the cystic space left as a long-term consequence of ischemic damage, in the absence of some kind of bio-scaffold, will be unlikely to promote cell adherence or persistence. In addition, gliosis on the margins of

the damaged region may impede cell migration or axonal outgrowth in the same manner as encountered after spinal cord injury.

This short review will address some preclinical issues inherent with stroke models, outcome measures and cell products, which may lead to an exaggeration of experimental results and eventually contribute to translational failure. Even before they are initiated, clinical trials are confronted by methodological, regulatory and financial challenges, possibly explaining the slow progress on the clinical side. This will be discussed in the light of the RESSTORE and PISCES trials.

Promising Preclinical Evidence

A large number of different cell preparations and delivery routes have been tested in both the preclinical and clinical settings (Table 1). Surprisingly, the therapeutic effect is not clearly influenced by cell type or delivery route, again suggesting that the cells secrete beneficial factors that can have immunomodulatory or anti-inflammatory effects.

Preclinical evidence obtained with cell products appears to be extremely promising; the effects detected have included a decrease in infarct size and improved behavioral outcome (2,11). The therapeutic efficacy has been assessed in several meta-analyses (12–16). Both Lees et al. (12) and Chen et al. (14) concluded that stem cells appear to be of some benefit, but at the same time, they pointed out the poor study quality and publication bias. In contrast, another meta-analysis stated that mesenchymal stromal cells had been effective in 44 out of 46 studies (13). Frankly, one may ask whether this is too good to be true.

Nevertheless, the promising experimental data have prompted early phase studies in stroke patients, which have provided preliminary data on safety and feasibility (17), although with some major limitations. Many of these studies have been small and underpowered to reveal true statistically significant treatment effects (18). In addition, a recent survey identified only 9 studies that had included a control group of any kind to allow evaluation of treatment effects (19). Thus, larger, randomized and controlled studies are urgently warranted to prove therapeutic efficacy.

Importance of Comorbidities - Missed Lesson from Neuroprotection Studies

The experimental literature gives the impression that cell therapy should be effective in improving behavioral recovery after stroke (13,16). However, the critical evaluation of experimental research has been limited (20). Experimental cell therapy research suffers from many technical and methodological limitations as described for acute neuroprotection (21,22), deficiencies which were initially highlighted in the Stroke Therapy Academic-Industry Roundtable (STAIR) meetings. These sessions had been established when translational failures had accumulated, and subsequently, were the reason for adopting the meta-analysis approach to animal studies. Recently significant improvement has been reported in stroke study quality (23) although some basic issues such as use of anesthesia have not changed over the years (24).

Typically, experimental stroke studies are planned to ensure consistency in lesion size and location which hopefully will translate into a standardized behavioral impairment. This allows the use of minimal number of animals not only helping to keep study costs within limited budgets but also adhering to the appropriate ethical principles. However, stroke patients are a heterogeneous population with regard to pathology, mechanism, lesion size and location, and clinical background, typically having comorbidities and being treated with polypharmacy and co-interventions (18). Thus, homogeneity in experimental animals is likely to over-estimate the treatment effect size compared to that likely to be achieved in clinical trials.

Comorbidities such as ageing, hypertension and diabetes were recognized by both STAIR (25) and STEPS 2 (26) committees as potentially important confounding factors in stroke research. More importantly, ignoring common comorbidities may explain, at least partly, failures with neuroprotective drugs (27) although the extent to which these comorbidities might affect therapeutic effects is unclear. For example, if a therapeutic intervention has a different magnitude (or indeed direction) of effect in the face of these comorbidities, then this may be compensated for by increasing sample size, or by applying selection criteria in clinical phase 2 trials. However, this is only possible if the influence of potential confounders is understood. It has been argued that cell-based therapies should routinely be evaluated in animals with some comorbid disease, although this complicates study design, increases sample sizes, and prolongs the preclinical investigation phase of potential treatments. Ultimately, the clinical relevance of animal model findings may itself be uncertain, but identification of possible issues may at least allow for modification of the

clinical study design. In one example, of an unexpected interaction, bone marrow derived stem cells, which were shown to promote functional outcome after stroke in nondiabetic rats (28,29), increased mortality, blood-brain barrier leakage and incidence of hemorrhage in streptozotocin-treated rats, a model mimicking type 1 diabetes (30). Although these complications were not seen in a type 2 diabetes model (31), it emphasized the need for caution as well as the benefits of including confounding factors in study design.

In addition to comorbid conditions, non-modifiable risk factors such as age and sex should be considered for evaluation. Although highly clinically relevant, this might be challenging due to higher mortality and variability in outcomes (32). Since it is unlikely that all potentially relevant models would be available in a single laboratory, collaborative pre-clinical multicentre projects utilizing a range of models could be undertaken both as an academic collaboration and as a commercial approach that would also strengthen confidence in data and provide a more robust estimate of likely effect sizes (33). The first trials with independent academic centers have proven the feasibility of adopting such a coordinated approach (34).

Rehabilitation as a Translational Gap in Experimental Studies

Rehabilitation is another issue not often incorporated into current experimental research. Most stroke survivors receive rehabilitation in one form or another, but this is rarely included in experimental study designs. The importance of rehabilitation has been included in STEPS 3 recommendations (35).

Modelling voluntary rehabilitative training in rodents is challenging. Motivating animals without reward or imposing extra stress, which may mask the treatment effect, is challenging. Various approaches such as special rehabilitative training devices (36), forced use of a forelimb (37) and acrobatic training (38) have been introduced. The first two mimic constraint-induced movement therapy of the upper extremity in stroke patients (39). Housing in an enriched environment has also been used to model non-specific rehabilitative training with sensory, motor, social and visual stimuli (40). Compared to an enriched environment, housing in a “standard environment” means less activity and social stimuli for the experimental animals. One extremity is social isolation by single housing, which may worsen infarct size and outcome (41). The importance of housing partners is emphasized by the recent study showing improved behavioral recovery, when ischemic animals were housed

together with healthy animals (42). Since social interactions have an impact on histological and functional outcome after experimental stroke (43), the housing conditions should be carefully reported in stroke recovery studies as recommended in the ARRIVE guidelines (44). The treatment contrast between cell and control groups can be easily increased by selecting appropriate housing for the experimental animals.

Only limited evidence is available on the role of rehabilitative training in stroke recovery studies. Cell therapy seems to be more effective when combined with housing in an enriched environment. The behavioral improvement detected in the cylinder test measuring spontaneous forelimb use was associated with increased cell survival and migration and enhanced neurogenesis in cell-treated rats (45). Nonetheless, the animals' performance in the more demanding Montoya's staircase test did not show a treatment effect (46). Treadmill exercise enhanced the therapeutic potency of bone marrow-derived mesenchymal stem cells given intravenously, possibly through inhibiting apoptosis in the perilesional cortex (47). The combination of physical exercise and intravenous infusion of bone marrow derived mesenchymal stem cells exerted a synergistic effect after stroke in rats (48). The higher limb-placing scores in the combined therapy group were associated with a greater density of synaptic markers and more extensive white matter changes. Thus, it seems that rehabilitative training may augment the same brain repair mechanisms as cell therapy or other restorative therapies, leading to some degree of synergistic functional improvement.

The problem in the above studies and in the assessment of rodent behavior in general is compensatory movement strategies that stroke animals develop to complete a given task (49). Kinematic analysis is the only way to exclude compensation. For example, the pellet reaching task measures the success rate and/or number of pellets retrieved, but it also allows a more detailed analysis of movement patterns. By using kinematic analysis, Knieling et al. (50) demonstrated that although the success rate had improved in stroke animals housed in an enriched environment, this was due to compensatory movement strategies. Similarly, in stroke patients, a recent study revealed that improvement after constraint-induced movement therapy appears to be mediated through compensatory strategies rather than actual restoration of pre-stroke movement patterns (51). Kinematic analysis was also applied to study motor recovery in stroke rats treated with bone marrow derived stem cells (52). The cell-treated group showed improved forelimb functions which more closely resembled pre-stroke movement patterns.

There is an ongoing debate about whether the combination of various rehabilitative strategies has a synergistic and beneficial effect in stroke recovery but more and more evidence suggests that this is the case (45,46,48). Therefore, to mimic the clinical situation, testing of cell therapies should ideally include rehabilitation for sensorimotor function whenever possible, although the relevance of rehabilitative strategies in rodent models to the human situation is somewhat unclear.

From Proof-of-Concept Studies to Large Multicentre Trials

Despite encouraging results from cell therapy in experimental stroke, few stroke patients have been included in proof-of-concept trials or pilot phase II trials, and very few studies have been randomized or have included a control group. The trials have employed a wide range of different cell types, delivery routes, time windows and outcome measures.

Appropriate clinical populations, trial designs and end-points are likely to differ between systemic delivery of cells and intraparenchymal transplantation, although, to date, the boundaries have not been clearly demarcated in many small exploratory clinical studies (53). If one examines the 28 trials published from 2000 to 2017, only 10 studies were randomized and included a control group (a total of 261 patients and 251 controls) (Table 2). The validity of control groups is not always clear, since few studies undertook control strategies for factors such as placebo effects, or systematic differences in rehabilitation strategies. In a meta-analysis, cell therapy was associated with a positive effect independent of several explanatory variables (e.g., age, ratio of infarction/hemorrhage, delay from stroke to treatment, route of administration, cell type) (19). There was, however, substantial heterogeneity in the methodological and quality measures among these trials, and much larger, multicentre trials, will be necessary before any definitive conclusions can be made.

Intravenous cell delivery in the early subacute stages after ischemic stroke has been investigated in two moderately-sized randomized trials, one using autologous bone marrow-derived mononuclear cells (54), in 120 patients and the other administering allogeneic bone marrow-derived cells depleted of CD45 (+)/glycophorin-A (+) cells referred to as multipotent adult progenitor cells (MAPCs) (55) in 126 patients but neither of these trials detected significant impact on neurological recovery. Recruitment to the Athersys MASTERS trial was affected by requirements for cell lab processing of treatment doses, and the relaxation of

recruitment window to 48 hours instead of the initially planned 36 hours after stroke was considered to be a potential factor in failure to show efficacy (55).

Intraparenchymal brain implantation of commercially developed allogeneic cells has undergone a preliminary investigation in two recent studies involving patients with severe disability 6-60 months after stroke (56,57). The Pilot Investigation of Stem Cells for Stroke (PISCES 1) trial administered ascending doses of human foetal neural stem cells that had been genetically modified with a c-mycER transgene to allow indefinite culture (58). No safety issues were identified with doses up to 20 million cells. The 20 million cell dose was taken forward into the PISCES-2 trial, a multicentre study that enrolled patients in the subacute recovery phase of ischaemic stroke at 3-12 months after the event; this trial reported improved upper limb motor recovery in sufficient numbers of subjects to warrant further investigation, based on an interim assessment after 3-6 months. The SanBio study (57) of modified donor bone marrow-derived mesenchymal stem cells was undertaken in 18 subjects, and reported modest improvement in neurological function, as well as transient T2 hyperintensities in peri-needle track locations in brain visualized with magnetic resonance imaging. No control groups were enrolled in either PISCES 1, PISCES 2 or the SanBio study, and no conclusions regarding efficacy are therefore possible. Selection of an appropriate control group for future trials poses both practical and ethical challenges (59,60), but placebo surgery using partial thickness burr holes to blind the study participants has been considered appropriate by trialists and regulators, and appears to be acceptable to patients.

The planned phase IIb European trial of intravenous injection of allogenic adipose-derived stem cells (ADSC): Regenerative Stem Cell Therapy after Stroke in Europe (RESSTORE, www.resstore.eu) is a European multicenter translational trial (involving France, Spain, Finland, United Kingdom, Czech Republic) whose primary objective is to provide essential information on the therapeutic efficacy of allogenic ADSCs in stroke patients, following evaluation of safety and tolerability of this cell line. Several major concerns need to be taken in account in implementing a multicentre cell therapy trial i.e. standardization of cell manufacturing or processing in different sites according to regulatory agencies' requirements, acquisition of sufficient safety data to guide dose selection and delivery methods, and appropriate study design with which to assess stroke recovery.

Study Design Issues to Assess Stroke Recovery

In restorative studies, measuring recovery after stroke is challenging, and there is as yet no agreed approach (61). These problems are not unique to cell therapy, but reflect more general issues with rehabilitative or restorative approaches: cell therapy simply introduces additional constraints in terms of sample size limitations due to production issues, invasiveness of delivery, and more restrictive patient selection criteria. No clear distinction is often made between phase 2 and phase 3 studies, a failure evident from many neuroprotectant drug trials that sought to expedite development by blurring the distinctions between phase 2b and phase 3 trials, with a notable lack of success (62,63). Phase 2 studies should be concerned with exploring effect measures and finalizing dose selection, with the option of using biomarkers to provide valuable information about optimal trial design. Phase 2 studies also have the possibility of gathering more specific clinical measures to establish the biological evidence of an effect. Phase 3 studies are generally constrained by a need to establish efficacy using broader clinical measures of disability that may be less sensitive to change than more specific functional scales. They are usually undertaken in a multicenter setting where it may be difficult to obtain informative phenotypic data such as detailed imaging studies.

The primary endpoint for phase 2 and 3 cell therapy trials has usually been based on a single validated general clinical scale such as the National Institutes of Health Stroke Score (NIHSS) or disability scale such as the modified Rankin Scale, although trials have adopted a range of different clinical measures, including specific motor function or domain-specific measures. Since the neurological deficits after stroke are usually multi-faceted and recovery a dynamic process, capturing the full picture of this process may require collection and analysis of multiple complementary measures at multiple time points (64). Beyond classical methods, recovery should be considered as a latent variable based on recent statistic methods developments such as structural equation modeling which has been shown to be applicable in pre-clinical and phase 2 clinical studies (61). There is a need to balance recruitment rates with the selected measurement tools; in general, a very specific motor outcome scale, such as the Action Research Arm Test (ARAT), may be more sensitive to change in the specific aspect of function that it is designed for than a general neurological scale, such as the NIHSS. Unfortunately, adopting a more specific scale places constraints on patient recruitment (65), since far fewer patients may have demonstrable deficits on that scale, and

the means of defining an improvement of true clinical value may be less clear. For small trials – inevitable in the cell therapy field – variance of an outcome measure should ideally be minimized by having the trial population as homogeneous as possible and selecting an outcome scale that has minimum inter-observer variability. While motor function recovery has commonly been selected as a human model system for phase 2 testing of rehabilitative and regenerative therapies, such a strategy for phase 3 efficacy trials may be deemed inadequate for allowing generalizable conclusions to be drawn. The modified Rankin Scale carries advantages of regulatory acceptability as a primary endpoint, wide understanding in the stroke community, availability of measures to minimize inter-observer variability such as structured interview approaches (66–68), independent video assessment (69), and availability of utility-weighted analyses that can facilitate health economic evaluation (70), and arguably it integrates the clinical effects in a globally meaningful way: improved ability to grasp a test object in the ARAT scale is of little value unless this translates into meaningful gains of day to day function. Nonetheless, the use of more specific motor recovery assessments is favored by rehabilitation specialists (71).

Another goal of a large phase 3 trial is to validate the societal value and cost-effectiveness (72) of a cell therapy based on health economics and predictive *in silico* (virtual population) models.

As in animal studies, rehabilitation must be considered as a control “treatment” and ideally should be standardized to the extent that this is achievable. Harmonization of stroke rehabilitation is extremely difficult in practice due to inter-individual variation in patients’ individual deficits, prior functional level, and ability to participate. There have been wide variations in the delivery of “routine” rehabilitation both across and within different healthcare systems, and even documenting in a meaningful way the delivery of complex and individualized multi-dimensional rehabilitation is a major challenge. Self-directed therapy adds to the complexity. Data from diaries maintained by individual participants or their caregivers may not be very reliable. Delivery of rehabilitation is also influenced by factors that may limit participation such as intercurrent infection, injury, or other medical therapy (including drugs that affect mood or alertness). As a minimum, different stroke centers should follow relevant guidelines, and document the quality and quantity of the rehabilitation program and ideally these factors could be included in statistical analyses as explanatory co-variables. Rehabilitation literature increasingly suggests that time spent in activity matters

rather than specificity of therapy, and that there is no clear gain beyond a threshold of time spent (73). There is also a concern that more intensive therapy, at least at some stages where the brain may be vulnerable, is potentially harmful (74). In many trials of late stroke recovery, however, participants have been beyond the time frame for continued active rehabilitation provided by healthcare systems, and thus any ongoing rehabilitation may have been unstructured and personally funded. In many trials, wide variability in routine delivery of rehabilitation has led the trialists to adopt a strategy of prescribing some sessions of mandatory additional physiotherapy to all participants.

Moreover, when studying the effects of a restorative therapy, *in vivo* biomarkers are essential when assessing the initial severity of the stroke; these may be important in patient selection, and may be relevant in following the recovery process. Clinical assessments can be correlated to biological and imaging markers. Several imaging markers may be of value in predicting the potential for motor recovery, for example, task activation functional MRI (75), anatomical changes such as Wallerian degeneration (76) or thalamus damage (77), and the degree of corticospinal tract integrity (and excitability) (78–80). Nonetheless, it is far from straightforward to interpret changes over time in imaging parameters such as functional networks, task activation fMRI, or tract integrity, and furthermore there are often only limited statistical approaches to handling serial data of this kind.

Regulatory Issues for Stem Cells Approval as a Medicinal Product

Cell therapies are currently regulated as an Advanced Therapy Medicinal Product in the EU (81). This framework imposes additional regulatory requirements beyond those applied to clinical trials in general, including the requirement to have detailed information about the source of the biological agent, and to retain all documentation for a period of 30 years. Accrual of experience with cell products should ease ethical approval processes, but there remain unique issues in addition to the provenance of the cells. These include the culture media and potential for infectious agents present in cell culture to be passed on to the patient; the modification by transgene insertion of cell therapy agents, with a need to demonstrate lack of mutagenesis or other adverse effects; and the need to demonstrate the limits of cell viability after transport, storage, and any freezing or thawing procedures.

To harmonize cell manufacturing and quality controls in Europe, scientific advice from the European Medicinal Agency (EMA) is encouraged. To obtain a multi-country approval, regulatory agencies from each country involved in the trial will analyze the same dossier according to the Voluntary Harmonized Procedure (VHP). This process avoids the need for several national submissions and is intended to minimize the delay in initiating work in the clinical investigational centers in different countries. The scientific and regulation analysis concerns the cell source, culture media, cell stocks and final medicinal product being injected into the patient. A pathophysiological view including expected mechanism of action related to stroke is required, and this demands that a translational project must be linked to experimental (animal) data. Animal experiments must be conducted to provide safety/toxicity and biodistribution data and to potentially reveal mechanisms of action using appropriate biomarkers for subsequent patient follow-up (82).

Summary and Conclusions

Although experimental evidence for cell therapies is promising, an over-simplified study design without comorbid animals and a rehabilitation arm may result in the generation of too promising positive data. Multicenter studies are needed to mimic clinical reality and to take account of the heterogeneity of stroke patients; they also need to provide enough statistical power to allow the hard go/no go decision since this will eventually improve translation success. Progress on the clinical side has been slow and cautious, partly because of budgetary constraints. Now larger, randomized and controlled clinical trials are needed to reveal the efficacy of cell therapies in stroke patients. Since these will be multinational/multicenter trials, this will introduce challenges in organizing harmonized cell manufacturing in multiple sites and logistics in cell delivery. Careful patient selection, homogeneity and appropriate outcome measures might favor treatments effects (83).

Compliance with Ethical Standards

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Ethical Approval

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Table 1. Cell sources and administration routes in preclinical and clinical stroke studies.

	Preclinical	Clinical
Studies included/subjects	306/5923	26/844
Cell source		
Autologous	10/340	18/134
Allogenic	150/2673	8/710
Syngeneic	5/131	-
Xenogeneic	146/2763	-
Unspecified	2/16	-
Route of administration		
Intra-arterial	33/551	6/57
Intravenous	156/3451	11/332
Intracerebral	109/1579	9/405
Intrathecal/ventricular	20/250	2/256
Others	4/92	-

Data adapted from the recent clinical (84) and preclinical meta-analysis (Cui, in press).

Table 2. Completed clinical trials of cell therapy in stroke.

<i>Cases (age)</i>	<i>Source</i>	<i>Cell type</i>	<i>Delay</i>	<i>Dose</i>	<i>Route</i>	<i>Adverse events</i>	<i>Country</i>	<i>Ref</i>
12 IS (61 yr, 44-74)	Human embryonic terato-carcinoma cell line (NT2/D1)	LBS-Neurons (Layton BioSc.)	2.5 years (7 months - 4.5 years)	2 (60 μ L; n=8) or 6 million (180 μ L; n=4) + Cyclosporine	IC	1 single seizure at 6 months 1 remote stroke at 5 months	Pittsburgh USA	(85)
6 IS 8 ICH (58 yr) 4 controls: 3 IS / 1 ICH (46 yr)	Human embryonic terato-carcinoma cell line (NT2/D1)	LBS-Neurons (Layton BioSc.)	3.5 years (1-5 years)	5 (n=7) or 10 million (n=7) (250 μ L) + Cyclosporine	IC	1 single post-operative seizure 1 asymptomatic SDH	Pittsburgh, Stanford USA	(86)
5 IS (25-52 yr)	Primordial porcine striatum (lateral ganglionic eminence) + antiMHC1 pretreated	LGE cells (Genvec)	5 years (1.5-10 years)	1 million /cm ³ of infarct 50 (n=4) or 80 million (n=1)	IC	Adverse events: cortical vein occlusion, n=1; hyperglycemic seizures, n=1; both with MRI transient abnormalities. FDA termination	Boston USA	(87)
7 IS 3 ICH	Immature nervous and hemopoietic tissues	Immature cell (NSC)	4-24 months	1 injection (n=5) 2 injections (n=5)	IThec (LP)	transient meningeal syndrome	Novosibirsk Russia	(88)

(46 yr; 35-56) 11 controls: 6 IS / 5 ICH (55 yr)								
5 IS (63 yr; 54-72) 25 controls (59 yr)	Auto BM	MSC Expansion in fetal calf serum	4-5 and 7-9 weeks	50 million x 2	IV		Suwon S. Korea	(89)
16 IS (65 +/-14 yr) 36 controls (64 +/-12 yr) <i>including the previous trial</i>	Auto BM Expansion in fetal calf serum	MSC Expansion in fetal calf serum	<i>2.5-5 and 5- 9 weeks</i>	50 million x 2	IV	1 fever after first injection (no second injection) Safe (3-5 years follow-up)	Suwon S. Korea	(90)
1 IS (54 yr)	Auto BM	MNC	<i>4 days</i>	<i>300 million (3 mL / 10 min)</i>	IA		Rio Brazil	(91)
3 IS (53-64 yr) 2 ICH	Auto BM	MNC	3-8 years	14-55 million (115-220 µL)	IC	5 headache 2 drowsiness	Habana Cuba	(92)

(41 and 44 yr)								
6 IS (24-65 yr)	Auto BM	MNC	2-3 months	125-500 million including 20 million ^{99m} Tc-labeled	IA	Biodistribution in brain, liver, lungs, spleen, kidneys, bladder	Rio Brazil	(93) (94)
10 IS (55 +/-15 yr) 79 historical controls (63 +/-12 yr)	Auto BM	MNC	1-3 days	7 (n=1) or 8.5 (n=1) or 10 million /kg	IV	1 death from pulmonary embolism at 40 days	Houston USA	(95)
12 IS (59 yr, 41-73)	Auto BM	MSC Expansion in autologous serum	10 weeks (5- 19 weeks)	110 million (60-160 million)	IV	1 transient fever	Sapporo Japan	(96)
4 IS 2 ICH (42 yr, 20-59) 6 controls: 5 IS / 1 ICH (46 yr)	Auto BM	MSC Expansion in animal serum- free media	9 months (7- 12 months)	50-60 million	IV		New Delhi India	(97)

18 IS 2 ICH (45 +/-12 yr) 20 controls: 19 IS / 1 ICH (45 +/-10 yr) <i>including the previous trial</i>	<i>Auto BM</i>	MSC Expansion in animal serum- free media MNC	10 months 3m-2y	MSC: 50-60 million (n=6) MNC: 50-60 million (n=14)	IV		New Delhi India	(98)
11 IS (30-70 yr)	Auto BM	MNC	7-30 days	80 million	IV	Feasibility = 11/11 (target- dose = 9/11)	New Delhi India	(99)
10 IS (67 +/-14 yr) 10 controls (67 +/- 13 yr)	Auto BM	MNC	6 days (5-9 days)	159 million	IA	2 seizure at 3 months Increase of serum β NGF	Sevilla Spain	(82)
20 IS (?)	Auto BM	MNC	3-7 days	220 million	IA		Porto Alegre Brazil	(100)
60 ICH (56 yr, 39-74)	Auto BM	MNC	6 days (5-7 days)	2.4-23 million (3.5mL)	IC	5 transient fever 1 lung cancer	Shandong China	(101)

40 controls (56 yr, 35-72)								
15 IS 15 controls (35-75 yr)	Auto blood	PBSC CD34+ (GCSF prior to blood sampling) Iron cell labelling	6 months – 5 years	3-8 million (6.6 +/-1.8 in 750 µL)	IC		Taichung Taiwan	(102)
58 IS 62 controls (18-70 yr)	Auto BM	MNC	18 days (7-30)	281 million (30-500)	IV	No benefit	India	(54)
5 IS (45-75 yo)	Auto BM	HSC CD34+	7 days	1.2-2.8 million	IA	1 renal dysfunction	London United Kingdom	(103)
24 IS or ICH	Auto BM	MNC	Chronic	?	IThec		Mumbai India	(104)
12 IS (20-75 yr)	Auto BM	MNC	7-10 days	250 million (25 mL of BM, n=6) 340 million (50 mL of BM, n=6)	IV		Osaka, Kobe Japan	(105)

18 IS (61 yr, 33-75)	Allo BM	MSC transfected (Notch-1) SanBio SB623	22 months	2.5 or 5 or 10 million (n=6/ group)	IC	1 epileptic seizure 1 asymptomatic SDH 1 transient stroke 1 urinary tract infection 1 pneumopathy	Stanford USA	(57)
11 IS (69 yr, 60-82)	Human fetal neuro- epithelium	NSC CTX0E03 transfected (cMycER _{TAM})	29 +/-14 months	2 (n=3) 5 (n=3) 10 (n=3) or 20 million (n=2)	IC	1 asymptomatic extradural hematoma 1 asymptomatic SDH	Glasgow UK	(56)
67 IS (62 yo) 62 controls (63 yr)	Allo BM	MAPC Multistem	24 to 48 hours	1200 million	IV	4 fever 2 nausea	Multicentric USA / UK	(55)

Auto: autologous; Allo: allogenic; BM: bone marrow; DTI: diffusion tensor imaging; ¹⁸FDG PET: 18-fluorodeoxyglucose positron emission tomography; HSC: hematopoietic stem cells; IA: intra-arterial; IC: intracerebral; ICH: intracerebral hemorrhage; IS: ischemic stroke; IThec: intrathecal; IV: intravenous; LP: lumbar puncture; MAPC: multipotent adult progenitor cells; MNC: mononuclear cells; MSC: mesenchymal stromal/stem cells; NSC: neural stem cells; PBSC: peripheral blood stem cells