

Concise Review: Increasing the Validity of Cerebrovascular Disease Models and Experimental Methods for Translational Stem Cell Research

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ABSTRACT

Interspecies differences, anatomical and physiological aspects, as well as simplified study designs contribute to an overestimation of treatment effects and limit the transferability of experimental results into clinical applications. Confounders of cell therapies for cerebrovascular disorders (CVD) include common CVD comorbidities, frequent medications potentially affecting endogenous and transplanted stem cells, as well as age- and immune-system-related effects. All those can contribute to a substantial modeling bias, ultimately limiting the prospective quality of preclinical research programs regarding the clinical value of a particular cell therapy. In this review, we discuss the nature and impact of most relevant confounders. We provide suggestions on how they can be considered to enhance the validity of CVD models in stem cell research. Acknowledging substantial and sometimes surprising effects of housing conditions, chronobiology, and intersex differences will further augment the translational value of animal models. We finally discuss options for the implementation of high-quality functional and imaging readout protocols. Altogether, this might help to gain a more holistic picture about the therapeutic impact of a particular cell therapy for CVD, but also on potential side and off-site effects of the intervention. *STEM CELLS* 2017;35:1141–1153

SIGNIFICANCE STATEMENT

This review summarizes most important aspects that may affect stem cell and cell therapy impact in cerebrovascular disease research. We discuss relevant mechanisms potentially impacting stem cell efficacy, safety, as well as relevant options to increase the predictive value of translational research programs. Importantly, the review also covers so far underrepresented areas such as chronobiology, comorbidities, polypharmacology (effects of drugs on stem cell efficacy and behavior), as well as potential species-specific aspects of stem cell performance.

INTRODUCTION

The rising prevalence of risk factors along with a demographic change toward ageing societies steadily increases cerebrovascular diseases (CVD) incidence. In contrast, therapeutic options available can, at best, only mitigate acute CVD sequelae or decelerate chronic disease progress [1, 2]. Cell-based therapies are believed to be among the most promising options to fundamentally advance CVD treatment. Cell therapies exert numerous therapeutic mechanisms ranging from potential tissue replacement to complex paracrine or systemic effects [3]. Animal models of cerebrovascular diseases are indispensable for the understanding of these mechanisms and their translation into clinical treatments. An increasing

awareness for the necessity of bias prevention and quality increase in preclinical CVD research and result reporting [4, 5] benefits the translational process.

Nevertheless, therapeutic outcome in emerging early stage clinical investigations so far stays behind the clear and encouraging results reported from preceding animal studies [6]. Indeed, a plethora of confounding factors still impedes the execution, interpretation, and, not least, the reproducibility of preclinical work. As a consequence, too much trust and resources were spent on invalid animal studies [7]. Although great improvements have been made over the last years, a number of relevant confounders remain. Species-specific differences in the central nervous system (CNS) anatomy, in basic physiology or existing co-

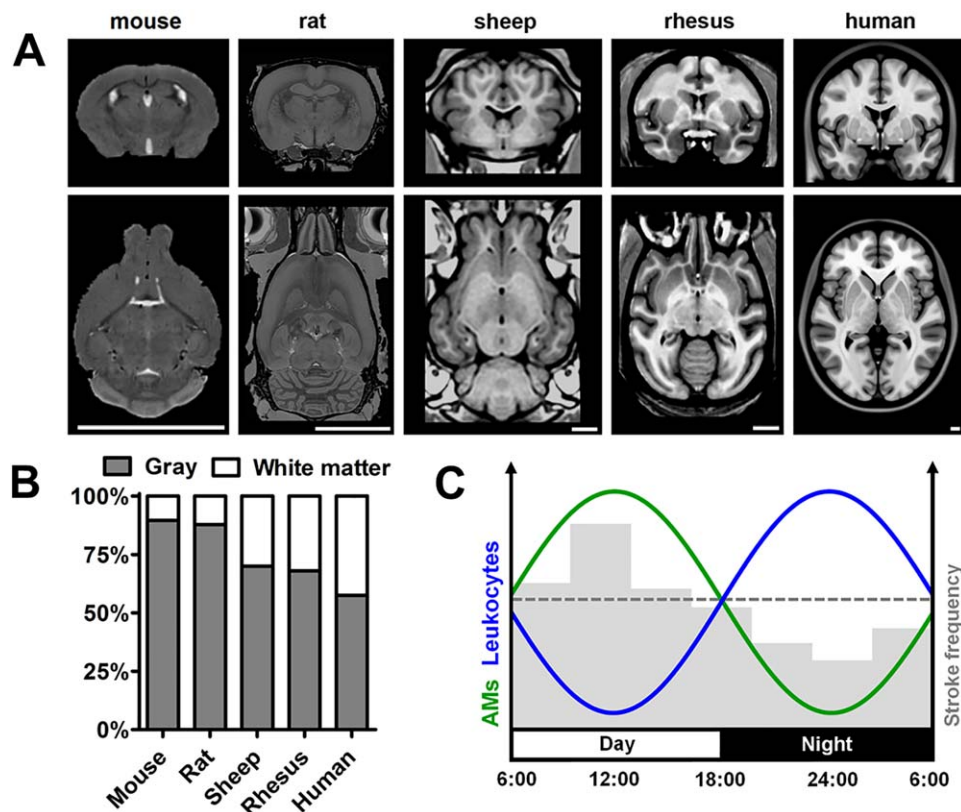


Figure 1. (A): Magnetic resonance imaging (MRI) images for a direct comparison of brain anatomy in different cerebrovascular disorder (CVD) model species. Larger animal species exhibit a much higher complexity of the brain architecture than rodents, to some extent approximating the human situation. Scale bar = 1 cm each. Please also note different proportions of gray and white matter, which are exemplified in (B). White matter structures provide guidance for cell migration in healthy and lesioned brains. There is also a close metabolic exchange between gray and white matter cells, making them important for the preservation and regeneration of brain circuits. This might favor cell-based regeneration in rodents, in which white matter is less, but not as strictly separated from gray matter areas as in higher mammals. Rodents also exhibit a completely different circadian rhythm than humans and most large animals (C). Schematic illustration of the circadian rhythm of vascular adhesion molecule (AM) expression, circulating blood leukocyte count and relative stroke incidence and severity by thromboinflammatory mechanisms. However, circadian rhythms are phase-delayed in nocturnal mice and diurnal humans whereas both animal experiments and clinical routine were typically performed during the office hours, implying the resting stage for rodents, but the active stage for humans. Considering these factors, though mostly neglected so far, is therefore important in preclinical CVD modeling. MRI images in (A) are derived from online open sources (please see Acknowledgments section).

morbidities influence the effectiveness of cell-based therapies along with using different breeds and keeping conditions. Endpoint and quality assurance check point selection can also affect study results. Next to preclinical meta-analyses [8] and multicenter preclinical trials [9], knowledge and consideration of major confounding factors is a simple and economical way to increase the predictive value of preclinical research.

The aim of this concise review is therefore an itemization and valuation of factors affecting as well as improving validity and predictability of CVD models with a focus on cell-based therapies. It also discusses the most important pathophysiological factors influencing cell therapies in CVD, including the reflection of those factors in advanced animal models and readout assays to optimize predictive value of preclinical investigations.

MODELING BIAS

Laboratory animals and particularly rodents are crucial to study the complex processes that occur during disease and

therapy. Transgenic rodent species enable the investigation of specific disease aspects and mechanisms, while a broad spectrum of functional tests, imaging technologies, and ex vivo analytical methods are available for endpoint assessment. However, animal models represent only simplified copies of the reality presenting striking and significant differences between CVD modeling species and patients.

Basic Anatomical and Physiological Aspects

The rodent brain is lissencephalic and obviously much smaller than the gyrencephalic human cerebrum. Most large animal brains are gyrencephalic, but their volumes do not reach that of the human brain (Fig. 1A). Importantly, the brain volume and the evolutionary development level might have an impact on endogenous stem cell functionality. For example, the proliferation and migration capacities of human glial progenitor cells (hGPCs) are significantly higher than those of their rodent counterparts [10]. This might be related to ontogenetic differences in brain volume growth as well as the functional

complexity of glial cells, both steeply increasing from infraprimate to primate species including humans [11]. hGPCs have a clear benefit when repopulating the smaller rodent brain and exhibit enormous therapeutic capacities in rodent models of demyelination [12]. This might be due to the fact that GPCs from species with larger brains have to travel longer distances and have to replace larger volumes of white matter during physiological remyelination, which is surprisingly effective in the healthy mammalian brain [13]. On the other hand, this likely causes an overestimation of the therapeutic impact on human patients. Hence, hypothetical inter-species differences or brain-size-related aspects in neural and neuronal stem cells behavior should be investigated in detail before clinical translation. This might be realized by testing a stem cell therapy not only in the rodent CNS but also in larger gyrencephalic brains. Brain size may not only impact cell replacement therapies but also the efficacy of cell-exerted neuroprotective and neuromodulatory effects [14]. The respective species and model should best reflect the addressed question and transplantations experiments might require tailored and species-specific immunosuppression regimens to ensure long-term survival and engraftment of xenografts [15].

Differences in gray matter (GM) and white matter (WM) volumes and architecture should also be considered. For example, cortical GM and WM volumes significantly differ among species. It is postulated that the WM content increases with brain size [16] to accommodate the increased signaling traffic. The WM fraction in the mouse and rat cortices is only 10 and 12%, respectively. In contrast, cortical WM content increases to 30% in sheep, being comparable with 32% observed in Rhesus macaques. WM in the human cortex ranges between 40% and 45% (Fig. 1B) [16, 17]. These strikingly different WM contents may have a direct translational relevance for acute CVD (e.g., stroke), often affecting GM and WM areas in humans, as well as for chronic CVD such as vascular dementia, featuring subcortical WM damage. Given the higher signaling traffic in larger brains including the human one, WM affections may also have a more severe functional impact than comparable lesions in smaller brains.

Another important difference is found in cerebral vascularization. Many rodents show an impressive sufficiency of cerebral collateralization which significantly mitigates the impact of distal cortical middle cerebral artery occlusion [18]. To some extent, this striking contrast to the human situation was also reported for larger gyrencephalic species such as ruminants [19]. A relative uniformity has been observed regarding endogenous reactions to a cerebral insult, including the mobilization but mostly imperfect migration and unsuccessful integration of endogenous progenitor cell populations in rodents and humans [20].

Large animal species might be a valuable tool to thoroughly confirm the impact of a particular CVD cell therapy as they seem to approximate the human anatomy closer than rodents, but are not suitable for exploratory research due to much higher costs and required infrastructure.

Sex and Genetic Heterogeneity

The majority of preclinical animal studies feature a single-sex design with a proven male-bias particularly in neuro- and cardiovascular research [21, 22]. Cycling female sex hormones that could unpredictably influence preclinical experiments are

an important reason for this sex preference. In fact, hormone-dependent and -independent mechanisms could not only affect CVD course but also the efficacy of cell therapies [23]. The NIH consequently pursues a “sex as a biological variable” policy [24], not least since one likely obstacle for double-sex experiments is the doubling of costs. A related subject is the use of inbred animal strains or monogenetic disease models for preclinical efficacy trials. Human diseases exhibit the greatest possible heterogeneity based not only on the genetic heterogeneity of patients and varying environmental factors but also on different stages of the disease and anatomical areas being affected. By contrast, it is rather attempted to maximize homogeneity in animal studies given that every increase in heterogeneity implies a growth of variance and, consequently, a loss of statistical power [25]. In fact, triggered acute CVD models are frequently executed in inbred mouse strains such as SV129, C57BL/6 or BALB/c [26]. However, experiments with inbred strains may obtain results applying specifically for this genotype [25] or being biased by strain-specific characteristics such as Th1 or Th2 dominance in C57BL/6 or BALB/c mice, respectively [27]. The decision to use inbred or outbred laboratory animals finally also affects cell therapies by influencing the occurrence, degree and variability of MHC mismatch which could determine therapeutic effects [28].

Standardization of Housing Conditions

Another classical way to foster the reproducibility of preclinical experiments is a standardization of animal housing conditions including light-dark rhythms, enrichment, cage changing, alimentation, and group housing—all of which can significantly affect neurological endpoints [29]. However, this instrument should be used with caution since increasing standardization can also raise the probability to detect spurious results being idiosyncratic for the specific test condition [30]. A planned heterogeneity has been suggested to solve the problem of standardization fallacy [31]. In fact, many hitherto unknown influencing factors that were disabled by standardization later turned out to be tremendously important for a possible translational process. A fascinating example is that one single commensal gut microbe, the segmented filamentous bacterium (SFB) could significantly influence the maturation and function of the immune system and thus of nearly every disease model [32]. Intriguingly, SFB are present in the microbiome of C57BL/6 mice from one vendor (Taconic Farms), but not from the other (Jackson Laboratory) which perfectly explained their different susceptibility to autoimmune diseases [33]. Who can now judge which condition is the right one and which should be subdued for standardization? A possible back door for this dilemma could be the application of factorial experimental designs [34] combining, for example, different inbred strains and different housing conditions [35].

Chronobiological Aspects

Circadian rhythms and related clock gene families orchestrate many major biological functions that are highly relevant for the risk and progression of CVD. The daily variation of blood pressure with the nadir at the end of the resting period is only one example which could, however, already explain the noticeable cumulation of stroke incidence in the time between 6 a.m. and noon [36, 37]. On the other hand, the susceptibility to pharmacological interventions is also a

function of the time of day, a fact that is being investigated in the emerging field of chronotherapy [38]. For example, in a mouse model of pressure overload cardiac hypertrophy, angiotensin-converting enzyme (ACE) inhibitors were only effective when administered during the resting phase [39]. Thus, the time of day is not only important for disease onset and initiation of therapy but also for the timing of preclinical and clinical studies. This is especially unfortunate for preclinical research since nearly all classical laboratory animals are nocturnal. Rodent experiments that typically take place during office hours thus inversely model the circadian situation of human patients (Fig. 1) which is relevant for a plethora of pathophysiological contexts.

During the activity phase, the probability for traumata or infections generally increases. It is therefore plausible that circulating white blood cell counts decrease during that phase as leukocytes are recruited to tissues to participate in defense and repair [41]. The time of day thus plays a pivotal role for the course of immune reactions during diseases [40, 42]. After myocardial infarction, not only the number of neutrophils but also of attracting cytokines and local endothelial adhesion molecules peak during the activity phase which explains the worse outcome compared with the resting phase [42]. A comparable relationship is most likely also evident in CVD and would consequently be important for the preclinical development of novel immunomodulatory approaches such as Natalizumab for acute stroke [43].

Importantly, cell therapies for CVD are particularly regulated by circadian influences. Next to the aforementioned chronobiological characteristics of the disease and chronotherapeutic aspects, one has to consider that the living cell product itself is controlled by a genome containing approximately 30% clock-related genes [44]. The time of cell sampling from bone marrow (BM) or adipose tissue thus directly determines quality and composition of the cell grafts in a therapeutically relevant manner [45, 46]. Moreover, cellular products differ in apoptosis resistance, migration, homing, and differentiation capacities as a function of time [47–49]. Finally, the risk of cell graft rejection also depends on circadian zeitgeber as both T cell function and T cell susceptibility for immunosuppressant drugs are subjected to daily variations [50]. In summary, the understanding of chronobiological aspects is crucial to both avoid modeling bias and to optimize experimental treatment regimens.

IMMUNE SYSTEM MATTERS

Immune responses are crucial for the initiation, maintenance, and recovery of nearly every known disease including stroke and vascular dementia [51, 52]. Important risk factors for stroke such as arterial hypertension or hyperlipidemia cause a chronic activation of the innate and adaptive arm of the immune system. These changes successively disrupt vascular function leading to cerebral small vessel disease and atherosclerosis [53, 54]. Moreover, a pre-activated immune system aggravates stroke severity and outcome [55]. Together, this data underpin how important the immune system of laboratory animals is for designing and interpretation of preclinical CVD research. Several important differences between the murine and human immune systems have been demonstrated

[56]. A poor correlation between the genomic response to inflammatory stressors in mouse models and human conditions has been described [57], what started an intense discussion about the general predictability of mouse models. Presumably, we have to accept that the perfect animal model to simulate human immune responses does yet not exist. Nevertheless, an understanding and consideration of the limitations could preserve their use for translational research [58].

Unfortunately, the situation even gains complexity with the presence of transplanted cells. Unpredictable immunomodulatory effects are not only probable for allogeneically or xenogeneically transplanted cells but also for cells being manufactured to induce special genes or a pluripotent state [59]. Even freshly isolated autologous cells such as BM-derived mononuclear cells (BM MNC) or adipose tissue-derived cells were influenced by centrifugation and exposition to media that cumulatively alter their phenotype and could induce immunologically relevant apoptosis [60, 61]. Aforementioned factors, leading to an undetermined extend of immunomodulation, cannot be simply categorized into beneficial or adverse. Indeed, growing evidence suggests that immunomodulation is one unifying mechanism for the efficacy of cell therapies [62–64]. On the other hand, factors including graft-versus-host disease, graft rejection, loss of function, immunosuppression, and pro-coagulative properties may be harmful and contribute to translational failure. The uncertainty concerning the validity of immune responses in CVD animal models is thus further bedeviled by the administration of therapeutic cells. Depending on the origin, passage and pre-processing, transplanted cells may interact with the host immune system in a desired or harmful way. Only the knowledge and consideration of the abovementioned factors together with rigid quality testing of cell products could help to selectively track assumed effects and exclude model specific ones.

Another fundamental unsolved but problem of preclinical testing of cell therapies is the paradox to test either the concept (e.g., syngeneic BM cells in a mouse stroke model) or the product that is developed for later clinical use (e.g., a human neural stem cell line in a mouse stroke model). The testing of concepts allows experiments complying with the immunological barriers, however, assessments of human cell products always imply a xenogeneic approach with the need for immunosuppressive treatment and/or the risk of GvHD, graft failure, and rejection. A solution for this dilemma could be the use of humanized mouse models such as the NOD/SCID/IL2r gamma (null) mouse chimerized with human CD34+ hematopoietic stem cells [65]. These mice develop human hematopoiesis ultimately allowing for the quasi-allogeneic or -autologous testing of human cell products in animal cardiovascular disease models (Fig. 2).

AGE AND FREQUENT COMORBIDITIES AFFECT CELL-BASED CVD THERAPIES

It is well known that age as well as numerous highly prevalent comorbidities represent relevant CVD risk factors and facilitate both unfavorable CVD course and outcome. Consideration of such conditions is therefore strongly recommended

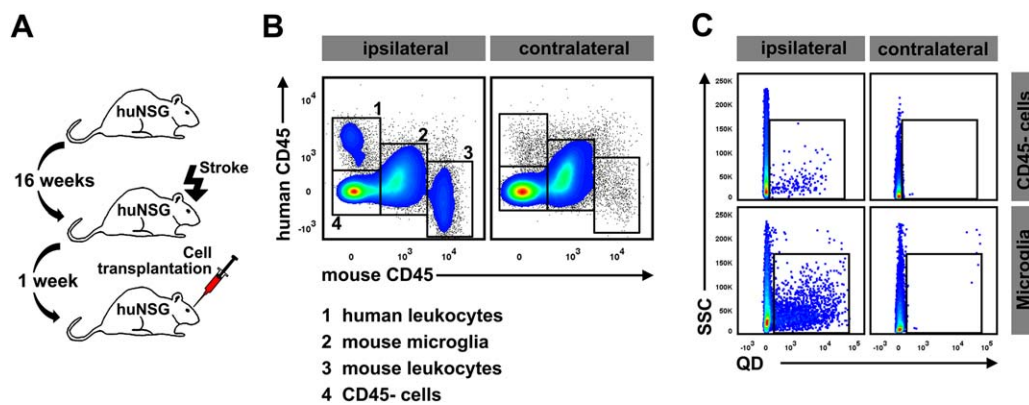


Figure 2. Humanized NOD scid gamma (huNSG) mice lacking murine lymphocytes and natural killer cells provide the possibility to study human cell products in animal models of cerebrovascular diseases in a quasi-allogeneic or -autologous setting. NSG mice were irradiated and treated with human CD34+ cells at postpartal day 1. **(A):** At the age of 16 weeks, NSG mice exhibit a high degree of hematolymphoid chimerism and could be subjected to experimental stroke and stereotactic transplantation of neural stem cells labeled with fluorescent quantum dots (QD). **(B):** Stroke caused a significant infiltration of both human (1) and murine (3) CD45 highly positive leukocytes into the ischemic ipsilateral brain hemisphere. Microglia was not affected by chimerism and thus 100% murine (2). **(C):** Few QD+ transplanted cells could be identified among the CD45⁺ cells within the ischemic hemisphere. However, numerous murine microglial cells from the ipsilateral, but not from the contralateral hemisphere were also positive for QD indicating that the transplanted cells were phagocytosed by indigenous microglia. Even though this animal model allows for mimicking human adaptive immune response to stroke and allogeneic cell transplantation, it is also biased by tissue resident murine immune cells. Abbreviations: huNSG, humanized NOD scid gamma; QD, quantum dots; SSC, sideways scatter.

in preclinical research. Importantly, recent research suggests that such conditions can also influence the impact of stem cell therapies for CVD.

Cell Donor and Recipient Age

CVD predominantly occur in the elderly. First clinical investigations on cell therapy for CVD investigated autologous transplantation paradigms [66, 67] to ensure immunological compatibility. In contrast, a potential future and widespread implementation of stem cell therapies for frequent conditions including CVD likely has to rely on allogeneic approaches of HLA-matched and/or immunomodulatory stem cells to meet the demand of cell products. Somatic stem cell populations might still be derived from volunteer donors. There is some preliminary evidence that cell donor age influences therapeutic efficacy. A study on BM MNC revealed that cells derived from younger (24 ± 1 years), but not from older donors (68 ± 1 years) induce neuroprotection in an organotypic in vitro ischemia model [68]. Aged BM cells are further believed to exert less neovascularization [69] what might be critical if used for CVD treatment. Another study showed that age- and sex-matched syngeneic transplantation of BM cells resulted in decreased, but not absent efficacy of BM cell transplantation after stroke with increasing age in otherwise healthy Wistar rats [70]. Of note, the treatment was still effective in animals close to the maximum life span with an age of up to 17 months. Consequently, cells from aged donors might not be generally impaired but other factors such as donor comorbidities or technical preparation might potentially limit the overall therapeutic efficacy of the cells.

Another translationally important aspect is the recipient's age. The aforementioned study [68] also revealed that neuroprotective BM MNC effects, previously confirmed in young and middle-aged individuals [71], cannot be observed in aged hypertensive rodents with stroke. The therapeutic refractiveness was independent from cell donor age. Indeed, the

regenerative capacity of the aging rodent brain does not match that observed in young-adult animals [72] what parallels the human situation. Spontaneously hypertensive rodents, exhibiting progressing cerebral small vessel disease [73] and spontaneous acute CVD frequency increasing with age [74] might help to reflect senescence-related aspects in translational CVD research. Moreover, the aged rodent brain limits endogenous stem cell-mediated regenerative effects or is at least less susceptible to those [75]. It is currently unclear whether this also accounts for the human brain or for exogenously administered cells.

Diabetes Mellitus

Diabetes mellitus (DM) is a common CVD comorbidity. DM sequelae can be devastating and often affect the cerebral vasculature. Moreover, DM can severely interfere with cell treatments in CVD. Mesenchymal stem cells (MSC) were shown beneficial following stroke in numerous independent experiments [3]. However, MSC exert detrimental effects in type 1 (T1) DM rodents. In one study, lesion volume and functional outcome remained unaffected, while mortality in the MSC treatment group increased [76]. Postulated reasons comprise angiogenin-mediated microvascular pathologies as well as blood brain barrier leakage increasing cerebral hemorrhage incidence. On the other hand, umbilical cord blood cells (HUCBC) and BM MSC improved functional outcome by decreasing hemorrhage frequency, by attenuating innate post-stroke inflammatory processes, and by promoting vascular and white matter remodeling in T2DM rats after stroke [77, 78]. BM MSC also mitigated long-term cognitive impairment in a T1DM rat model [79]. Hence, the interplay between DM and cell therapies may depend on the diabetes type what could be explained by the fundamental differences in T1 and T2 pathogenesis. This makes the impact of cell therapies in diabetes hardly predictable and requires careful, case- and DM type-specific investigation.

Table 1. Known effects of common pharmaceuticals on stem and progenitor cells

Drug class	Substance/substances	Affected cell type	Effect	References
Non-steroidal anti-inflammatory drugs	Aspirin	EPC	Decreased proliferation and migration	[83, 84]
		MSC	Decreased inhibition, increased apoptosis	[85]
	COX-2-inhibitors	ESC	Increased differentiation (into cardiomyocytes)	[86]
Beta blockers	Propranolol (β 1 and β 2 antagonist)	Cardiac progenitors	Increased proliferation	[87]
Catecholamines	Nebivolol (β 1 antagonist)	EPC	Increased proliferation	[88]
	Norepinephrine (β 3 stimulation)	Neural progenitor cells	Increased proliferation and neuronal differentiation	[89]
Aldosterone antagonists	Eplerenone	EPC	Increased proliferation	[90]
Statins	Simvastatin	HUCBC	Enhanced intracerebral engraftment	[91]
		EPC	Increased proliferation, migration and differentiation	[91]
	Atorvastatin	Angioblasts	Increased proliferation and differentiation	[92]
		EPC	Increased proliferation	[93]
		MSC	Increased migration and survival	[94]
	Rosuvastatin	EPC	Increased proliferation	[95]
		MSC	Increased survival	[96]
Antilipemics	Nicotinic acid	GPC	Increased oligodendrocyte progenitor proliferation and differentiation	[97]

Abbreviations: EPC, endothelial progenitor cells; GPC, glial progenitor cells; HUCBC, human umbilical cord blood cells; MSC, mesenchymal stem cells.

Hypertension

Hypertension is the most prominent single risk factor, at least contributing to all forms of chronic and acute CVD. Despite this clear relationship, not much is known on the impact of hypertension on cell-based therapeutic intervention for CVD. However, some side effects of signaling pathways mediating hypertension on stem cells have been reported in cardiovascular research. Catecholamines promote proliferation of endogenous cardiac stem cells via β 2-receptors, while β 1-mediated signaling induces apoptosis. The chronic presence of angiotensin II accelerates proliferation of hematopoietic stem cells, but induces their pre-mature differentiation and impairs homing as well as BM reconstitution abilities [80].

Hypertension aggravates the post-stroke innate immune response in the context of acute CVD [55], what might potentially influence the stem cell-mediated therapeutic effects or cell survival. Hypertension further damages the capillary system and leads to small vessel rarefaction in the brain, potentially impeding homing of systemically administered cells to the desired target areas. Interestingly, there is some evidence that hypertension can be induced by BM transplantation from hyper- to normotensive animals, what is mediated by a peripheral and central (microglia-mediated) pro-inflammatory bias [81]. This suggests an involvement of BM stem cells in the genesis of hypertension, what underpins the importance of not only considering recipient but also donor conditions in the context of cell therapies.

Interaction between Pharmacological Treatments and Cell Therapies

Pharmacotherapy as a baseline treatment is conducted in almost every CVD patient and will therefore be most likely a cotreatment in a clinical stem cell therapy scenario. It is therefore surprising that not much research has been performed so far to decipher the clinically relevant nature of drug-stem cell interactions in CVD. Indeed, some general knowledge has been collected in the related field of

cardiovascular disorders [82] for drugs also being of relevance for CVD (Table 1).

One of the rare studies in CVD reports a therapeutic synergy between simvastatin, a frequently applied statin, and HUCBC treatment in a rat model of stroke leading to increased endogenous neurogenesis, neuroblast migration, as well as enhanced axiogenesis and neurite outgrowth [91]. Simvastatin also facilitated HUCBC engraftment. This translated into reduced lesion volumes and improved functional outcome. The effect is believed to rely on a stimulation of the BDNF/TrkB pathway, but proangiogenic and vascular remodeling effects were also reported [92]. On the other hand, unfortunate timing of systemic BM MNC [61] or MSC infusion in an attempt to support a G-CSF pharmacotherapy for stroke abolished the drug's therapeutic effect, potentially due to an interference with the peripheral immune response. This illustrates the translational need of investigating the impact of a cell therapy on CVD in the context of pharmacotherapies addressing that disease, its comorbidities or risk factors. Thoroughly investigating these interactions and precisely predicting any potential beneficial or detrimental impact in future clinical trials may require new models and assessment systems, particularly featuring species in which drug effects but also pharmacodynamics and -kinetics are comparable with the human situation. Gaining more knowledge on these issues will also help to improve patient recruitment to clinical studies by considering their individual medication profiles.

IMPROVING IN VIVO ENDPOINT MEASURES

Advancing Functional Readouts

Functional recuperation is the most important therapeutic effect of a particular CVD therapy. Hence, efficacy endpoints in preclinical CVD studies preferably target improvements of cognitive or sensorimotor functions. Previous meta-analyses of experiments using stem cells and other therapeutics for

acute CVD have repeatedly revealed suboptimal study design and endpoint selection [98]. Even in studies perfectly implementing generally acknowledged quality assurance methods such as blinded model induction, randomized treatment group allocation, and blinded data recording, three major factors often limit the predictive value of functional results.

The first limitation is the structural simplicity of commonly applied functional tests. These economic assays focus on basic functions and often rely on simple score point classifications. They do not require sophisticated equipment and are optimized for time-efficient data ascertainment and analysis. On the other hand, such tests are susceptible for biases or tend to report results not correlating well with lesion extent. Moreover, it is well known that rodents have much better abilities to compensate functional deficits than humans [99]. Basic functional tests cannot discriminate between both and might therefore reflect a combination of recovery and compensation [100], overestimating or masking true treatment effects [101].

The second limitation is the preferred assessment of gross motor functions. Spontaneous gross motor function recovery is much faster in rodents as compared to humans. This may lead to treatment impact overestimations and impedes the detection of minor functional improvement in the long run [102] due to ceiling effects. On the other hand, fine motor skill impairments are a predominant disability burden for human patients. Those functions show excellent homologies among species both in health [103] and CNS disease [104]. Long-term improvement of fine motor impairments should therefore be investigated more thoroughly, particularly as this would be a desirable therapeutic effect of stem cell therapies being applied in chronic CVD stages. However, this requires application of highly specialized and more laborious functional assessment strategies such as the skilled pellet reaching tasks or the ladder rung test. Higher efforts required for their implementation into a particular research program are nevertheless believed to be outweighed by sensitivity and specificity benefits. These tests can also discriminate compensation from recovery as well as lesion-prone deficits from age-related impairments [105].

The third limitation is that CVD impact is considerably divergent between preclinical and clinical trials. Preclinical studies benefit from uniform lesions and comparable functional impairments. This allows to minimize sample sizes, and to accommodate research budget constraints. This is in sharp contrast to clinical reality, where lesion sizes, functional impairments, as well as speed and extent of recovery significantly vary among patients [106]. This variability can explain the alleged efficacy decline of experimental therapies during the translational phase. On the other hand, implementing model-inherent randomized variability in preclinical studies is challenging. As in clinical trials, this requires a tremendous increase in group sizes to detect statistically significant differences, which might be smaller and partially masked by high inter-individual variations. Variability may be best modeled by confirmative multicenter clinical trials, for example, by including different CVD models [107]. Therapeutic effects reported from such trials are indeed much less prominent than those revealed in single-center studies, but paucity of reliable funding sources for such studies [108] currently impedes their widespread implementation.

Imaging-Based Stem Cell Safety and Efficacy Surveillance

Although requiring an expensive and dedicated infrastructure, imaging technologies offer numerous significant advantages when assessing the therapeutic potential of cell therapies for CVD (Fig. 3). The size of an ischemic lesion correlates very well with functional deficits [109]. It is therefore eligible to serve as a reliable surrogate parameter for functional improvements, especially in longitudinal studies. Imaging modalities assessing brain anatomy and structure such as computed tomography (CT) or magnetic resonance imaging (MRI) can precisely assess lesion size and augment functional readout systems. Particularly MRI allows the detailed observation of secondary acute ischemic lesion effects such as focal edema or degeneration of fiber tracts and remote brain areas. Loss of gray and white matter and increasing volumes of CSF-containing compartments are hallmarks of chronic CVD and well suitable to investigate therapeutic cell therapy effects reversing those. Positron emission tomography allows the precise assessment of metabolic function and blood flow [110] and can detect pathological changes before clinical signs of neurodegenerative diseases appear [111].

A particular value of imaging technologies for the evaluation of CVD stem cell treatments is the option to track transplanted cells both systemically and in the brain, as well as to monitor cell engraftment (Table 2). Cell tracking methods have been established for MRI [123], single photon emission computed tomography (SPECT) [124], and PET [125], providing feasible tools for cell therapy safety and efficacy surveillance. Higher structural resolution of MRI allows higher sensitivity and precision of cell detection whereas some PET tracers can reveal information regarding the functional state of a cell transplant. SPECT imaging is particularly feasible to detect off-site cell effects or presence. However, all techniques require cell labeling, for example, by magnetic nanoparticles or radioactive tracers, which might affect viability and efficacy of the cells, as well as clinical applicability.

TARGETED MODELING OF PRACTICAL CELL THERAPY UTILIZATION

While numerous stem cell types were shown to exert profound therapeutic impact in many fundamental CVD models, the development of clinically deliverable therapies seems to be slow and challenging. A translationally relevant aspect of modeling cell therapies is therefore to simulate strategies and scenarios which are likely to be applied in the clinics.

The amount and time course of damage caused by CVD defines what stem cell population might be best feasible for a potential treatment. Stem cell populations with a potential neurorestorative capacity (e.g., neural stem/progenitor cells or induced pluripotent stem cells) might be the primary choice in cases of locally restricted and/or slowly emerging damage such as in vascular dementia. The targeted local delivery by stereotaxic surgery or super-selective catheter-based delivery to cerebral arteries [126] should be considered to maximize their therapeutic impact. On the other hand, these cells might be of limited efficacy after massive or swiftly evolving structural brain damage as, for example, in stroke because cytoarchitectonic cues and guidance structures required for targeted cell migration and differentiation are likely destroyed [3]. In such scenarios, adult stem cell populations with limited restorative, but profound modulatory

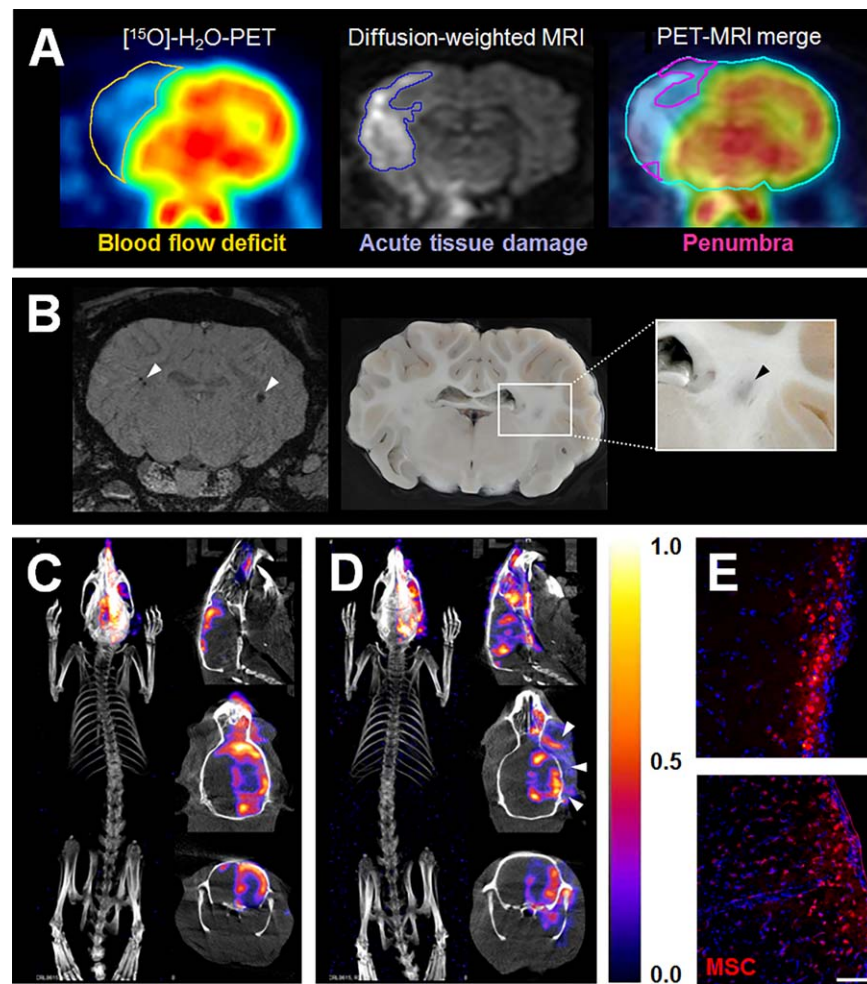


Figure 3. (A): Combined PET-MR imaging can benefit acute CVD studies. In the given example, the tissue at risk (penumbra) after middle cerebral artery occlusion in sheep is shown. These hybrid imaging techniques allow the parallel assessment of structural and metabolic information in acute and subacute stages, for example, allowing to pursue individually cell transplantation strategies tailored to the individual case. (B): The image gives an example of mesenchymal stem cell (MSC) detectability in the ovine brain (susceptibility weighted imaging). Large animal models allow to access cell traceability in the gyrencephalic brain using clinical scanning equipment. Here, three MSC depots were placed stereotactically (left panel): $2 \times 1,000$ MSCs were injected into right subcortical white matter areas whereas $1 \times 100,000$ cells were placed at the contralateral site (white arrow heads). The larger depot in the left hemisphere could be identified even macroscopically after brain preparation (right panel, black arrow head). (C): The image gives an example for cell tracking using single photon emission computed tomography in the rat. ^{111}In -oxinate-labeled mesenchymal stem cells were injected into the right external carotid artery of rats after transient (2 hours) middle cerebral artery occlusion. The image shows regular homing of the cells to the ischemic hemisphere, whereas the image (D) provides an example for off-target location of cells in the facial musculature (white arrow heads). Both images were taken 1 hour after transplantation. (E): Presence of cells in the murine brain was confirmed histologically for cases shown in (C). All images were derived from in-house experiments. Scale bar = 100 μm . Abbreviations: MRI, magnetic resonance imaging; MSC, mesenchymal stem cell.

capabilities such as HUCBC or BM-derived populations might be advantageous. These cells also exhibit interesting systemic treatment effects such as immunomodulatory activities in the spleen [127]. Importantly, the effects could be fully utilized by systemic administration.

Other relevant aspects are potential complications and safety concerns arising from the chosen cell type and transplantation route. Hence, both must be weighed against the optimized therapeutic effect and overall clinical applicability. For example, systemic administration might be clinically feasible, but can cause filtering of applied cells in the lung after intravenous [128] and even in the brain after intraarterial injections [129]. The resulting risk of secondary embolism could even outweigh the benefits of systemic administration. A detailed overview on

potential concerns arising from particular cell populations and administration strategies can be found elsewhere [130]. It must be recognized that carefully looking for potential complications and the impact of manufacturing or administration procedures rather than for optimized efficacy only, as well as to simulate potential strategies to circumvent such risks is pivotal for high-quality and valid modeling of cell therapies for CVD.

CONCLUSIONS AND IMPLICATIONS FOR CVD CELL TREATMENT RESEARCH

The prospective value of animal models for the investigation and translation of cell therapies for CVD is limited by a

Table 2. Preclinical and clinical imaging techniques for tracking of transplanted cells in the brain

Imaging modality	Cell labeling	Clinical applicability	Acquired information	Sensitivity/specificity	Tracking examples	Temporal detection limit	Spatial resolution (μm)/depth (mm)	References
X-ray	Encapsulated sulfate, metalloalgininate (BaCl_2 , Au-alginate)	Yes	Anatomical	Low/moderate	Monocytes, MSC	Seconds to minutes	C-arm CT: 2-D (0.15 mm per pixel)/unlimited	[112]
μCT , animal		No	Anatomical	Low/moderate	MSC	Seconds to weeks	~ 0.001 /unlimited	[113]
CT, clinical	So far not applied for cell tracking in the brain							
MRI, animal (3.0–17.4 T)	Gadolinium rhodamine dextran (GRID), (ultrasmall) superparamagnetic iron oxide (USPIO), iron platinum particles (SIPP)	No	Anatomical, functional, molecular	Moderate/moderate	BM MNC, MSC, NPC, NSC, T-cells	Seconds to hours	1×10^{-3} /Unlimited	[114]
MRI, clinical (1.5–7.0T)		Yes	Anatomical, functional, molecular	Moderate/moderate	MSC, NSC	Seconds to hours (?)	~ 0.1 /Unlimited	[115]
μSPECT , animal	Radionuclide (e.g., $^{99\text{m}}\text{Tc}$, ^{111}In -oxyquinoline)	No	Molecular, metabolic	High/high	EPC, HSC, HUCBC, MSC	Seconds to weeks	2-D (~ 1 mm per pixel)/unlimited	[116]
SPECT, clinical		Yes	Molecular, metabolic	High/high	BM MNC, HUTC, MSC	Seconds to weeks	2-D (~ 1 mm per pixel)/unlimited	[117]
μPET , animal	Radionuclide labeled enzymes, receptors, transporters (e.g., ^{51}Mn , $^{124/131}\text{I}$ -FAU, ^{18}F -FDG, ^{18}F -HFB)	No	Molecular, metabolic	High/high	MAPS, MSC, NPC	Seconds to weeks	~ 1 /Unlimited	[118]
PET, clinical		Yes	Functional, molecular/metabolic	High/high	BM MNC	Seconds to weeks	~ 5 /Unlimited	[119]
Fluorescence	Quantum dots, antibody-complex (e.g., [NHS]-biotin-streptavidin/SA-Alexa647)	No	Molecular	High/high	MSC, T-cells	Seconds to weeks	1×10^{-5} /10	[120]
Bio-luminescence	Luciferin	No	Molecular	High/moderate	ECFC, ESC, NSC	Minutes to hours	~ 10 /30	[121]
Photoacoustic imaging	Gold nanoparticle	No	Anatomical, molecular	High/high	MSC	Seconds to minutes	Yet unknown	[122]

Abbreviations: BM MNC, bone marrow-derived mononuclear cells; CT, computed tomography; ECFC, endothelial colony-forming cell; EPC, endothelial progenitor cells; ESC, embryonic stem cells; HUCBC, human umbilical cord blood cells; HUTC, human umbilical tissue-derived cells; MAPS, multipotent adult progenitor cells; MRI, magnetic resonance imaging; MSC, mesenchymal stem cells; NPC, neuronal progenitor cells; NSC, neural stem cells; SPECT, single photon emission computed tomography.

number of confounding factors. Anatomical differences also need to be considered. Large animal models exhibiting higher similarities with human patients are available, but are laborious and require complex infrastructures. These models should therefore be limited to confirmative research or studies that cannot be performed in small animal CVD models. On the other hand, many confounding factors can be modeled in rodent species although this requires intelligent and slightly more complex study designs. Since it will be impossible to model all confounding factors, a careful discussion regarding which ones will be of highest impact for a particular cell treatment is warranted before initiating an experiment. So far under-recognized influences such as chronobiology and interaction with pharmacotherapies are expected to be of increasing importance over the next years. Readout parameters should be carefully chosen and go beyond simple and economic, but potentially biased functional readout assays. Finally, late-stage preclinical research should investigate optimal practical implementation of the cell treatment. This includes potentially limiting factors and side effects, while the optimal route of cell administration should be tailored to the targeted disease scenario and stage.

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REFERENCES

- O'Brien JT, Thomas A. Vascular dementia. *Lancet* 2015;386:1698–1706.
- Ramee SR, White CJ. Acute stroke intervention. *Curr Probl Cardiol* 2014;39:59–76.
- Janowski M, Wagner DC, Boltze J. Stem cell-based tissue replacement after stroke: Factual necessity or notorious fiction? *Stroke* 2015;46:2354–2363.
- Stem cell therapies as an emerging paradigm in stroke (STEPS): Bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. *Stroke* 2009;40:510–515.
- Glasziou P, Altman DG, Bossuyt P et al. Reducing waste from incomplete or unusable reports of biomedical research. *Lancet* 2014;383:267–276.
- Bang OY. Clinical trials of adult stem cell therapy in patients with ischemic stroke. *J Clin Neurol* 2016;12:14–20.
- Prinz F, Schlange T, Asadullah K. Believe it or not: How much can we rely on published data on potential drug targets? *Nat Rev Drug Discov* 2011;10:712.
- Kleikers PW, Hooijmans C, Gob E et al. A combined pre-clinical meta-analysis and randomized confirmatory trial approach to improve data validity for therapeutic target validation. *Sci Rep* 2015;5:13428.
- Llovera G, Liesz A. The next step in translational research: Lessons learned from the first preclinical randomized controlled trial. *J Neurochem* 2016;139:271–279.
- Windrem MS, Schanz SJ, Morrow C et al. A competitive advantage by neonatally engrafted human glial progenitors yields mice whose brains are chimeric for human glia. *J Neurosci* 2014;34:16153–16161.
- Oberheim NA, Takano T, Han X et al. Uniquely hominid features of adult human astrocytes. *J Neurosci* 2009;29:3276–3287.
- Windrem MS, Schanz SJ, Guo M et al. Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiver mouse. *Cell Stem Cell* 2008;2:553–565.
- McTigue DM, Tripathi RB. The life, death, and replacement of oligodendrocytes in the adult CNS. *J Neurochem* 2008;107:1–19.
- Boltze J, Forschler A, Nitzsche B et al. Permanent middle cerebral artery occlusion in sheep: A novel large animal model of focal cerebral ischemia. *J Cereb Blood Flow Metab* 2008;28:1951–1964.
- Diehl R, Ferrara F, Muller C et al. Immunosuppression for in vivo research: State-of-the-art protocols and experimental approaches. *Cell Mol Immunol* 2016;
- Zhang K, Sejnowski TJ. A universal scaling law between gray matter and white matter of cerebral cortex. *Proc Natl Acad Sci USA* 2000;97:5621–5626.
- Nitzsche B, Frey S, Collins LD et al. A stereotaxic, population-averaged T1w ovine brain atlas including cerebral morphology and tissue volumes. *Front Neuroanat* 2015;9:69.
- Coyle P, Jokelainen PT. Differential outcome to middle cerebral artery occlusion in spontaneously hypertensive stroke-prone rats (SHRSP) and Wistar Kyoto (WKY) rats. *Stroke* 1983;14:605–611.
- Ashwini CA, Shubha A, Jayanthi KS. Comparative anatomy of the circle of Willis in man, cow, sheep, goat, and pig. *Neuroanatomy* 2008;7:54–65.
- Marti-Fabregas J, Romaguera-Ros M, Gomez-Pinedo U et al. Proliferation in the human ipsilateral subventricular zone after ischemic stroke. *Neurology* 2010;74:357–365.
- Beery AK, Zucker I. Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev* 2011;35:565–572.
- Florez-Vargas O, Brass A, Karystianis G et al. Bias in the reporting of sex and age in biomedical research on mouse models. *Elife* 2016;5.
- Bai Y, Sun T, Ye P. Age, gender and diabetic status are associated with effects of bone marrow cell therapy on recovery of left ventricular function after acute myocardial infarction: A systematic review and meta-analysis. *Ageing Res Rev* 2010;9:418–423.
- Clayton JA. Studying both sexes: A guiding principle for biomedicine. *FASEB J* 2016;30:519–524.
- Chia R, Achilli F, Festing MF et al. The origins and uses of mouse outbred stocks. *Nat Genet* 2005;37:1181–1186.
- Carmichael ST. Rodent models of focal stroke: Size, mechanism, and purpose. *NeuroRx* 2005;2:396–409.
- Watanabe H, Numata K, Ito T et al. Innate immune response in Th1- and Th2-dominant mouse strains. *Shock* 2004;22:460–466.

Representative magnetic resonance brain images in Figure 1 were derived from the following open sources: mouse: http://www.bioeng.nus.edu.sg/cfa/mouse_atlas.html; rat: <http://software.incf.org/software/waxholm-space-atlas-of-the-sprague-dawley-rat-brain>; sheep: <https://www.mcgill.ca/bic/resources/brain-atlases/ovine-brain-atlas>; rhesus macaque: <https://www.mcgill.ca/bic/resources/brain-atlases/non-human-primate>; human brain: <https://www.mcgill.ca/bic/resources/brain-atlases/human>. The authors are grateful to the people behind this work and for sharing their data with the scientific community.

AUTHOR CONTRIBUTIONS

J.B. and D.-C.W.: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; F.N.: performed experiments contributing to Figure 3, design of Figure 3, critical discussion of the manuscript, final approval of the manuscript; J.J.: supervised experiments contributing to Figure 3, detailed input on behavioral testing, critical revision of the manuscript, final approval of the manuscript; G.W. and C.P.: data acquisition and analysis, data discussion; B.N.: design of Figures 1 and 3, partial writing and critical discussion of the manuscript, final approval of the manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

- 28 Weinger JG, Weist BM, Plaisted WC et al. MHC mismatch results in neural progenitor cell rejection following spinal cord transplantation in a model of viral-induced demyelination. *STEM CELLS* 2012;30:2584–2595.
- 29 Prager EM, Bergstrom HC, Grunberg NE et al. The importance of reporting housing and husbandry in rat research. *Front Behav Neurosci* 2011;5:38.
- 30 Wurbel H. Behaviour and the standardization fallacy. *Nat Genet* 2000;26:263.
- 31 Richter SH, Garner JP, Zipser B et al. Effect of population heterogenization on the reproducibility of mouse behavior: A multi-laboratory study. *PLoS One* 2011;6:e16461.
- 32 Ericsson AC, Hagan CE, Davis DJ et al. Segmented filamentous bacteria: Commensal microbes with potential effects on research. *Comp Med* 2014;64:90–98.
- 33 Ivanov II, Atarashi K, Manel N et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009;139:485–498.
- 34 Festing MF. Principles: The need for better experimental design. *Trends Pharmacol Sci* 2003;24:341–345.
- 35 Walker M, Fureix C, Palme R et al. Mixed-strain housing for female C57BL/6, DBA/2, and BALB/c mice: Validating a split-plot design that promotes refinement and reduction. *BMC Med Res Methodol* 2016;16:11.
- 36 Chen L, Yang G. Recent advances in circadian rhythms in cardiovascular system. *Front Pharmacol* 2015;6:71.
- 37 Elliott WJ. Circadian variation in the timing of stroke onset: A meta-analysis. *Stroke* 1998;29:992–996.
- 38 Tsimakouridze EV, Alibhai FJ, Martino TA. Therapeutic applications of circadian rhythms for the cardiovascular system. *Front Pharmacol* 2015;6:77.
- 39 Martino TA, Tata N, Simpson JA et al. The primary benefits of angiotensin-converting enzyme inhibition on cardiac remodeling occur during sleep time in murine pressure overload hypertrophy. *J Am Coll Cardiol* 2011;57:2020–2028.
- 40 Scheiermann C, Kunisaki Y, Frenette PS. Circadian control of the immune system. *Nat Rev Immunol* 2013;13:190–198.
- 41 Scheiermann C, Kunisaki Y, Lucas D et al. Adrenergic nerves govern circadian leukocyte recruitment to tissues. *Immunity* 2012;37:290–301.
- 42 Schloss MJ, Horckmans M, Nitz K et al. The time-of-day of myocardial infarction onset affects healing through oscillations in cardiac neutrophil recruitment. *EMBO Mol Med* 2016;
- 43 Llovera G, Hofmann K, Roth S et al. Results of a preclinical randomized controlled multicenter trial (pRCT): Anti-CD49d treatment for acute brain ischemia. *Sci Transl Med* 2015;7:299ra121.
- 44 Curtis AM, Bellet MM, Sassone-Corsi P et al. Circadian clock proteins and immunity. *Immunity* 2014;40:178–186.
- 45 Shostak A, Husse J, Oster H. Circadian regulation of adipose function. *Adipocyte* 2013;2:201–206.
- 46 Casanova-Acebes M, Pitaval C, Weiss LA et al. Rhythmic modulation of the hematopoietic niche through neutrophil clearance. *Cell* 2013;153:1025–1035.
- 47 Boucher H, Vanneaux V, Domet T et al. Circadian clock genes modulate human bone marrow mesenchymal stem cell differentiation, migration and cell cycle. *PLoS One* 2016;11:e0146674.
- 48 Rolls A, Pang WW, Ibarra I et al. Sleep disruption impairs haematopoietic stem cell transplantation in mice. *Nat Commun* 2015;6:8516.
- 49 Granda TG, Liu XH, Smaaland R et al. Circadian regulation of cell cycle and apoptosis proteins in mouse bone marrow and tumor. *FASEB J* 2005;19:304–306.
- 50 Leyking S, Budich K, van BK et al. Calcineurin inhibitors differentially alter the circadian rhythm of T-cell functionality in transplant recipients. *J Transl Med* 2015;13:51.
- 51 Fu Y, Liu Q, Anrather J et al. Immune interventions in stroke. *Nat Rev Neurol* 2015;11:524–535.
- 52 Iadecola C. The pathobiology of vascular dementia. *Neuron* 2013;80:844–866.
- 53 Schiffrin EL. Immune mechanisms in hypertension and vascular injury. *Clin Sci (Lond)* 2014;126:267–274.
- 54 Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis (*). *Annu Rev Immunol* 2009;27:165–197.
- 55 Moller K, Posel C, Kranz A et al. Arterial hypertension aggravates innate immune responses after experimental stroke. *Front Cell Neurosci* 2015;9:461.
- 56 Mestas J, Hughes CC. Of mice and not men: Differences between mouse and human immunology. *J Immunol* 2004;172:2731–2738.
- 57 Seok J, Warren HS, Cuenca AG et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci USA* 2013;110:3507–3512.
- 58 Drake AC. Of mice and men: What rodent models don't tell us. *Cell Mol Immunol* 2013;10:284–285.
- 59 Zhao T, Zhang ZN, Rong Z et al. Immunogenicity of induced pluripotent stem cells. *Nature* 2011;474:212–215.
- 60 Morelli AE, Larregina AT. Concise review: Mechanisms behind apoptotic cell-based therapies against transplant rejection and graft versus host disease. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor 2016;34:1142–1150.
- 61 Posel C, Scheibe J, Kranz A et al. Bone marrow cell transplantation time-dependently abolishes efficacy of granulocyte colony-stimulating factor after stroke in hypertensive rats. *Stroke* 2014;45:2431–2437.
- 62 Einstein O, Feinstein N, Vaknin I et al. Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. *Ann Neurol* 2007;61:209–218.
- 63 Pluchino S, Zanotti L, Rossi B et al. Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. *Nature* 2005;436:266–271.
- 64 Thum T, Bauersachs J, Poole-Wilson PA et al. The dying stem cell hypothesis: Immune modulation as a novel mechanism for progenitor cell therapy in cardiac muscle. *J Am Coll Cardiol* 2005;46:1799–1802.
- 65 Ishikawa F, Yasukawa M, Lyons B et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor γ chain(null) mice. *Blood* 2005;106:1565–1573.
- 66 Savitz SI, Misra V, Kasam M et al. Intravenous autologous bone marrow mononuclear cells for ischemic stroke. *Ann Neurol* 2011;70:59–69.
- 67 Lee JS, Hong JM, Moon GJ et al. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. *STEM CELLS* 2010;28:1099–1106.
- 68 Wagner DC, Bojko M, Peters M et al. Impact of age on the efficacy of bone marrow mononuclear cell transplantation in experimental stroke. *Exp Transl Stroke Med* 2012;4:17.
- 69 Sugihara S, Yamamoto Y, Matsuura T et al. Age-related BM-MNC dysfunction hampers neovascularization. *Mech Ageing Dev* 2007;128:511–516.
- 70 Coelho BP, Giraldo-Guimaraes A. Effect of age and gender on recovery after stroke in rats treated with bone marrow mononuclear cells. *Neurosci Res* 2014;88:67–73.
- 71 Brenneman M, Sharma S, Harting M et al. Autologous bone marrow mononuclear cells enhance recovery after acute ischemic stroke in young and middle-aged rats. *J Cereb Blood Flow Metab* 2010;30:140–149.
- 72 Buchhold B, Mogoanta L, Suofu Y et al. Environmental enrichment improves functional and neuropathological indices following stroke in young and aged rats. *Restor Neurol Neurosci* 2007;25:467–484.
- 73 Kaiser D, Weise G, Moller K et al. Spontaneous white matter damage, cognitive decline and neuroinflammation in middle-aged hypertensive rats: An animal model of early-stage cerebral small vessel disease. *Acta Neuropathol Commun* 2014;2:169.
- 74 Bailey EL, Smith C, Sudlow CL et al. Is the spontaneously hypertensive stroke prone rat a pertinent model of sub cortical ischemic stroke? A systematic review. *Int J Stroke* 2011;6:434–444.
- 75 Artegiani B, Calegari F. Age-related cognitive decline: Can neural stem cells help us? *Aging (Albany NY)* 2012;4:176–186.
- 76 Chen J, Ye X, Yan T et al. Adverse effects of bone marrow stromal cell treatment of stroke in diabetic rats. *Stroke* 2011;42:3551–3558.
- 77 Yan T, Venkat P, Chopp M et al. Neurorestorative therapy of stroke in type 2 diabetes mellitus rats treated with human umbilical cord blood cells. *Stroke* 2015;46:2599–2606.
- 78 Yan T, Venkat P, Chopp M et al. Neurorestorative responses to delayed human mesenchymal stromal cells treatment of stroke in type 2 diabetic rats. *Stroke* 2016;47:2850–2858.
- 79 Nakano M, Nagaishi K, Konari N et al. Bone marrow-derived mesenchymal stem cells improve diabetes-induced cognitive impairment by exosome transfer into damaged neurons and astrocytes. *Sci Rep* 2016;6:24805.
- 80 Kim S, Zingler M, Harrison JK et al. Angiotensin II regulation of proliferation, differentiation, and engraftment of hematopoietic stem cells. *Hypertension* 2016;67:574–584.

- 81 Santisteban MM, Ahmari N, Carvajal JM et al. Involvement of bone marrow cells and neuroinflammation in hypertension. *Circ Res* 2015;117:178–191.
- 82 Finan A, Richard S. Stimulating endogenous cardiac repair. *Front Cell Dev Biol* 2015; 3:57.
- 83 de Boer HC, Hovens MM, van Oeveren-Rietdijk AM et al. Human CD34+/KDR+ cells are generated from circulating CD34+ cells after immobilization on activated platelets. *Arterioscler Thromb Vasc Biol* 2011;31:408–415.
- 84 Sato T, Amano H, Ito Y et al. NSAID, aspirin delays gastric ulcer healing with reduced accumulation of CXCR4(+)-VEGFR1(+) cells to the ulcer granulation tissues. *Biomed Pharmacother* 2013;67:607–613.
- 85 Deng L, Hu S, Baydoun AR et al. Aspirin induces apoptosis in mesenchymal stem cells requiring Wnt/beta-catenin pathway. *Cell Prolif* 2009;42:721–730.
- 86 Chillar A, So SP, Ruan CH et al. A profile of NSAID-targeted arachidonic acid metabolisms in human embryonic stem cells (hESCs): Implication of the negative effects of NSAIDs on heart tissue regeneration. *Int J Cardiol* 2011;150:253–259.
- 87 Musumeci M, Maccari S, Sestili P et al. Propranolol enhances cell cycle-related gene expression in pressure overloaded hearts. *Br J Pharmacol* 2011;164:1917–1928.
- 88 Sorrentino SA, Doerries C, Manes C et al. Nebivolol exerts beneficial effects on endothelial function, early endothelial progenitor cells, myocardial neovascularization, and left ventricular dysfunction early after myocardial infarction beyond conventional β 1-blockade. *J Am Coll Cardiol* 2011;57:601–611.
- 89 Jhaveri DJ, Mackay EW, Hamlin AS et al. Norepinephrine directly activates adult hippocampal precursors via beta3-adrenergic receptors. *J Neurosci* 2010;30:2795–2806.
- 90 Jung C, Florvaag A, Oberle V et al. Positive effect of eplerenone treatment on endothelial progenitor cells in patients with chronic heart failure. *J Renin Angiotensin Aldosterone Syst* 2012;13:401–406.
- 91 Cui X, Chopp M, Shehadah A et al. Therapeutic benefit of treatment of stroke with simvastatin and human umbilical cord blood cells: Neurogenesis, synaptic plasticity, and axon growth. *Cell Transplant* 2012;21:845–856.
- 92 Cui X, Chopp M, Zacharek A et al. Combination treatment of stroke with subtherapeutic doses of Simvastatin and human umbilical cord blood cells enhances vascular remodeling and improves functional outcome. *Neuroscience* 2012;227:223–231.
- 93 Baran C, Durdu S, Dalva K et al. Effects of preoperative short term use of atorvastatin on endothelial progenitor cells after coronary surgery: A randomized, controlled trial. *Stem Cell Rev* 2012;8:963–971.
- 94 Cai A, Qiu R, Li L et al. Atorvastatin treatment of rats with ischemia-reperfusion injury improves adipose-derived mesenchymal stem cell migration and survival via the SDF-1alpha/CXCR-4 axis. *PLoS One* 2013;8: e79100.
- 95 Tousoulis D, Andreou I, Tsiatas M et al. Effects of rosuvastatin and allopurinol on circulating endothelial progenitor cells in patients with congestive heart failure: The impact of inflammatory process and oxidative stress. *Atherosclerosis* 2011;214:151–157.
- 96 Yang YJ, Qian HY, Huang J et al. Atorvastatin treatment improves survival and effects of implanted mesenchymal stem cells in post-infarct swine hearts. *Eur Heart J* 2008;29:1578–1590.
- 97 Ye X, Yan T, Chopp M et al. Combination BMSC and Niaspan treatment of stroke enhances white matter remodeling and synaptic protein expression in diabetic rats. *Int J Mol Sci* 2013;14:22221–22232.
- 98 Lees JS, Sena ES, Egan KJ et al. Stem cell-based therapy for experimental stroke: A systematic review and meta-analysis. *Int J Stroke* 2012;7:582–588.
- 99 Metz GA, Antonow-Schlorke I, Witte OW. Motor improvements after focal cortical ischemia in adult rats are mediated by compensatory mechanisms. *Behav Brain Res* 2005;162:71–82.
- 100 Boltze J, Lukomska B, Jolkonen J. Mesenchymal stromal cells in stroke: Improvement of motor recovery or functional compensation? *J Cereb Blood Flow Metab* 2014;34:1420–1421.
- 101 Hicks A, Schallert T, Jolkonen J. Cell-based therapies and functional outcome in experimental stroke. *Cell Stem Cell* 2009;5: 139–140.
- 102 Girard S, Murray KN, Rothwell NJ et al. Long-term functional recovery and compensation after cerebral ischemia in rats. *Behav Brain Res* 2014;270:18–28.
- 103 Sacrey LA, Alaverdashvili M, Whishaw IQ. Similar hand shaping in reaching-for-food (skilled reaching) in rats and humans provides evidence of homology in release, collection, and manipulation movements. *Behav Brain Res* 2009;204:153–161.
- 104 Whishaw IQ, Suchowsky O, Davis L et al. Impairment of pronation, supination, and body co-ordination in reach-to-grasp tasks in human Parkinson's disease (PD) reveals homology to deficits in animal models. *Behav Brain Res* 2002;133:165–176.
- 105 Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: A new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *J Neurosci Methods* 2002; 115:169–179.
- 106 Sutherland BA, Minnerup J, Balami JS et al. Neuroprotection for ischaemic stroke: Translation from the bench to the bedside. *Int J Stroke* 2012;7:407–418.
- 107 Dirnagl U, Hakim A, Macleod M et al. A concerted appeal for international cooperation in preclinical stroke research. *Stroke* 2013;44:1754–1760.
- 108 Boltze J, Wagner DC, Henninger N et al. Phase III preclinical trials in translational stroke research: Community Response on framework and guidelines. *Transl Stroke Res* 2016;7:241–247.
- 109 Rogers DC, Campbell CA, Stretton JL et al. Correlation between motor impairment and infarct volume after permanent and transient middle cerebral artery occlusion in the rat. *Stroke* 1997;28:2060–2065.
- 110 Heiss WD, Zimmermann-Meinzingen S. PET imaging in the differential diagnosis of vascular dementia. *J Neurol Sci* 2012;322: 268–273.
- 111 Teipel S, Drzezga A, Grothe MJ et al. Multimodal imaging in Alzheimer's disease: Validity and usefulness for early detection. *Lancet Neurol* 2015;14:1037–1053.
- 112 Chhour P, Naha PC, O'Neill SM et al. Labeling monocytes with gold nanoparticles to track their recruitment in atherosclerosis with computed tomography. *Biomaterials* 2016;87:93–103.
- 113 Astolfo A, Schultke E, Menk RH et al. In vivo visualization of gold-loaded cells in mice using x-ray computed tomography. *Nanomedicine* 2013;9:284–292.
- 114 Watanabe T, Frahm J, Michaelis T. In Vivo Brain MR Imaging at Subnanoliter Resolution: Contrast and Histology. *Magn Reson Med* 2016;15:11–25.
- 115 Lobsien D, Dreyer AV, Stroh A et al. Imaging of VSOP labeled stem cells in agarose phantoms with susceptibility weighted and T2* weighted MR Imaging at 3T: Determination of the detection limit. *PLoS One* 2013;8:e62644.
- 116 Arbab AS, Thiffault C, Navia B et al. Tracking of In-111-labeled human umbilical tissue-derived cells (hUTC) in a rat model of cerebral ischemia using SPECT imaging. *BMC Med Imaging* 2012;12:33.
- 117 Huang J, Lee CC, Sutcliffe JL et al. Radiolabeling rhesus monkey CD34+ hematopoietic and mesenchymal stem cells with ^{64}Cu -pyruvaldehyde-bis(N4-methylthiosemicarbazone) for microPET imaging. *Mol Imaging* 2008;7:1–11.
- 118 Lewis CM, Graves SA, Hernandez R et al. ^{52}Mn production for PET/MRI tracking of human stem cells expressing divalent metal transporter 1 (DMT1). *Theranostics* 2015;5: 227–239.
- 119 Sood V, Mittal BR, Bhansali A et al. Biodistribution of ^{18}F -FDG-labeled autologous bone marrow-derived stem cells in patients with type 2 diabetes mellitus: Exploring targeted and intravenous routes of delivery. *Clin Nucl Med* 2015;40:697–700.
- 120 Park JH, Sun W, Cui M. High-resolution in vivo imaging of mouse brain through the intact skull. *Proc Natl Acad Sci USA* 2015; 112:9236–9241.
- 121 Ding J, Zhao Z, Wang C et al. Bioluminescence imaging of transplanted human endothelial colony-forming cells in an ischemic mouse model. *Brain Res* 2016;1642:209–218.
- 122 Nam SY, Ricles LM, Suggs LJ et al. In vivo ultrasound and photoacoustic monitoring of mesenchymal stem cells labeled with gold nanotracers. *PLoS One* 2012;7:e37267.
- 123 Stroh A, Boltze J, Sieland K et al. Impact of magnetic labeling on human and mouse stem cells and their long-term magnetic resonance tracking in a rat model of Parkinson disease. *Mol Imaging* 2009;8:166–178.
- 124 Mitkari B, Kerkela E, Nystedt J et al. Intra-arterial infusion of human bone marrow-derived mesenchymal stem cells results in transient localization in the brain after cerebral ischemia in rats. *Exp Neurol* 2013;239:158–162.
- 125 Stojanov K, de Vries EF, Hoekstra D et al. [^{18}F]FDG labeling of neural stem cells

for in vivo cell tracking with positron emission tomography: Inhibition of tracer release by phloretin. *Mol Imaging* 2012;11: 1–12.

126 Walczak P, Wojtkiewicz J, Nowakowski A et al. Real-time MRI for precise and predictable intra-arterial stem cell delivery to the central nervous system. *J Cereb Blood Flow Metab* 2016. doi:10.1177/0271678X16665853.

127 Acosta SA, Tajiri N, Hoover J et al. Intravenous bone marrow stem cell grafts preferentially migrate to spleen and abrogate chronic inflammation in stroke. *Stroke* 2015;46:2616–2627.

128 Fischer UM, Harting MT, Jimenez F et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: The pulmonary first-pass effect. *STEM CELLS DEV* 2009; 18:683–692.

129 Cui LL, Kerkela E, Bakrean A et al. The cerebral embolism evoked by intra-arterial delivery of allogeneic bone marrow mesenchymal stem cells in rats is related to cell dose and infusion velocity. *Stem Cell Res Ther* 2015;6:11.

130 Boltze J, Arnold A, Walczak P et al. The dark side of the force—Constraints and complications of cell therapies for stroke. *Front Neurol* 2015;6:155.