Cell therapy for ischemic stroke: are differences in preclinical and clinical study design responsible for the translational loss of efficacy?

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ABSTRACT

Cell therapy is an attractive strategy for enhancing post-stroke recovery. Different cell types and several treatment strategies have been successfully applied in animal models, but efficacy in stroke patients has not yet been confirmed. We hypothesize that the significant design differences between preclinical and clinical trials may account for this situation. Using a meta-analysis approach and comparing preclinical with clinical trials, we reveal and discuss preliminary evidence for such design differences. While available datasets are not yet numerous enough to draw definitive conclusions, these findings may represent signposts on the route to efficacy by harmonizing preclinical and clinical study designs.

Abbreviations

ART	adhesive removal test
BBB	blood-brain barrier
CI	confidence intervals
FMS	Fugl-Meyer Scale
mBI	modified Barthel Index
MSC	mesenchymal stem/stromal cell
MNC	mononuclear cell
mNSS	modified neurological severity score
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
NRCT	non-randomized controlled trial
NSC	neural stem cell
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	randomized controlled trial
SMD	standardized mean differences
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus

Cell-based therapy has been proposed as a promising paradigm for ischemic stroke for almost two decades[1]. Different types of cells, particularly mesenchymal stem/stromal cells (MSCs), neural stem cells (NSCs) and mononuclear cells (MNCs), have been successfully tested in animal models. Several mechanisms, such as (limited) cell replacement, immunomodulation, as well as promotion of angiogenesis and neurogenesis, have been claimed be responsible for the observed improvements[2-4].

Although a number of early-phase clinical studies investigating cell therapy for ischemic stroke have been conducted, convincing evidence of efficacy is still lacking[5-8]. Inadequate quality of preclinical tests has been previously considered as a major reason for unsuccessful translation of experimental stroke therapies into the clinic, but the quality of preclinical stroke studies is clearly improving[9]. However, significant design differences between preclinical and clinical trials may hinder translation. Hence, it is essential to explore how well the currently described preclinical and clinical trial designs correspond to each other in order to devise innovative ways for advancing clinical translation of cell therapies in stroke.

An objective way to approach such problems is to conduct a systematic meta-analysis. Therefore, we have adopted this approach to: 1) estimate the current cell therapy efficacies in both animal stroke models and stroke patients; 2) explore the sources of heterogeneity in preclinical and clinical studies; 3) investigate the hypothesis of poorly-corresponding preclinical and clinical trial designs; and 4) identify the potential gaps in clinical translation to be bridged in future approaches**.**

Methodological approach

Studies on cell therapy for ischemic stroke published before April 3rd 2018 were identified from PubMed, Web of Science, and Scopus according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The detailed protocol is available on PROSPERO (CRD42018093214 and CRD42018096257) or in Supplemental Material. Briefly, preclinical studies describing cell transplantation in animal models of focal cerebral ischemia, and controlled clinical studies were gathered. We included the four most frequently examined outcome measures: 1) infarct size; 2) modified Neurological Severity Score (mNSS); 3) rotarod test; and 4) adhesive removal test (ART) performance. A quality score was also assigned[10,11]. In clinical studies, functional outcomes were measured by National Institutes of Health Stroke Scale (NIHSS), modified Barthel index (mBI), modified Rankin scale (mRS), and Fugl-Meyer scale (FMS). Standardized mean differences (SMD), 95% confidence intervals (95% CI) and statistical significances were examined using the inverse-variance method[12]. For each outcome, the effect size was calculated using *Hedges' g* and heterogeneity was calculated as I^2. In view of the substantial heterogeneity in the included studies, a random effects model was applied to estimate the pooled effect size. Univariate meta-regression was conducted on preclinical studies and subgroup analysis was conducted on clinical studies to explore the sources of heterogeneity. $P<0.05$ was considered as statistically significant and $0.05 \leqq P<0.10$ as a trend. Data were analyzed using Stata version 14.0 (Stata-Corp).

A total of 3868 records from PubMed, 5041 records from Scopus, and 4073 records from Web of Science were identified. Ultimately, 355 preclinical studies with 10830 animals, and 10 controlled clinical studies (phase I/II) with 460 patients were included (Fig 1).

Preclinical and clinical study qualities

The median quality score of the preclinical studies was 5 out of 10 (interquartile range: 4-6) (Online Table 1). Randomization and blinded outcome assessment had been applied in 222 (62.5%) and 203 (57.2%) of the 355 included studies, respectively. Only 23 (6.5%) studies reported allocation concealment and 10 (2.8%) studies provided a *priori* sample size

calculation. Animal models with comorbidities such as hypertension or diabetes were utilized in only 24 (6.8%) studies.

Nine out of the ten clinical trials had at least one source of bias according to the Cochrane Risk of Bias Tool[13]. Four studies were non-randomized controlled trials (NRCT), five studies did not report allocation concealment, and five studies featured a non-blinded outcome assessment. Only one trial was double-blinded. All studies reported complete outcome data, but only two provided details regarding power calculation.

Therapeutic effect differences in cell therapy

Robust efficacy of cell therapy in stroke animals

Analysis of published data confirmed previous findings that cell therapy significantly improved both structural and functional outcomes in experimental stroke[14-18]. Although substantial inter-study heterogeneities (I^2) were observed (65.7%-75.2%), the effect size was consistently large for each outcome measure (1.35 for infarct size reduction, 1.69 for mNSS, 1.56 for rotarod test, and 1.56 for ART; $P < 0.001$, Fig 2).

Funnel plotting suggested that there was a significant left-sided bias, meaning that studies with effect sizes smaller than mean values were under-reported (Fig 3). Egger's regression test revealed a significant publication bias for each outcome ($P<0.01$). Nonetheless, after adjusting for publication bias by trim and fill, the mean effect sizes remained large (0.79 for infarct size, 1.09 for mNSS, 1.00 for rotarod test and 1.07 for ART).

Potential reasons for therapeutic effect heterogeneity in preclinical studies

The immunogenicity of injected cells accounted for 5.3%-16.0% of the observed heterogeneity in outcome ($P<0.05$). Based on the results of infarct size and ART, autologous cells had the largest effect, followed by allogeneic and xenogeneic cells; syngeneic cells did not display any significant efficacy in comparison with control treatment (Fig 4Ai, Ci, Di).

Freeze-thawing procedure accounted for up to 9.9% of the observed heterogeneity, e.g. in ART performance ($P<0.01$). Freshly-isolated cells were consistently associated with larger effects than frozen-thawed cells (Fig 4Aii, Bi, Dii).

Cell type accounted for 7.6% of the observed heterogeneity in functional outcome (rotarod test) ($P=0.0075$). Treatment with NSCs showed the largest effect, followed by MSCs, MNCs, and other cells (Fig 4Ci). Cell origin, cell stemness and manipulation also contributed to the heterogeneity (Supplemental Fig 1B-E).

Comorbidities also influenced outcome and explained 1.3%-5.8% of the observed heterogeneity ($P<0.05$). Cell treatment of comorbid animals consistently induced smaller effects than those obtained in healthy animals (Fig 4Aiii, Bii, Diii).

The stroke model accounted for 2.3% of the observed heterogeneity in infarct size ($P=0.0407$). The intraluminal filament model was associated with the largest therapeutic effects (Fig 4Aiv). Moreover, an earlier assessment of infarct size (within one month) revealed larger effects as compared to a later assessment, suggesting that the therapeutic effect of injected cells may decline with time (Supplemental Fig 1A).

The cell delivery route accounted for 8.1% of the observed heterogeneity in infarct size ($P=0.0001$). Intraventricular cell delivery achieved the largest effects on infarct size (Fig 4Av), but intracortical cell transplantation induced the greatest impact on mNSS performance, followed by intraventricular cell delivery (Fig 4Biii).

A higher quality score tended to result in a smaller effect size ($P=0.09$) (Fig 4Biv). A higher impact factor of the published journal also tended to associate with a smaller effect size ($P=0.0961$) (Supplemental Fig 1F).

Moderate cell therapy efficacy in patients

Despite the small number of clinical trials, cell therapy induced a statistically significant beneficial effect in mBI (SMD=0.32, 95% CI: 0.03-0.61, P=0.032) as well as a trend in mRS (SMD=0.30, 95% CI: -0.03-0.64, P=0.078), but not in NIHSS (P=0.298) or FMS (P=0.112). The heterogeneity varied considerably with the various outcome measures (24.3%-85.0%) (Fig 5).

Potential reasons for therapeutic effect heterogeneity in clinical trials

We performed further subgroup analyses to clarify the effects of different clinical study design characteristics (Fig 6). MSCs showed a larger effect size than MNCs in mRS (0.20 vs. 0.07; Fig 6Ai), mBI (0.94 vs. 0.13; Fig 6Bi), and FMS (1.77 vs. 0.35; Fig 6Di). Allogeneic/cryopreserved cells had been administered in only one study. Similar to the preclinical findings, studies using autologous/freshly-harvested cell therapy achieved better outcomes than those utilizing allogeneic/cryopreserved cells in mRS (0.37 vs. 0.17; Fig 6Aii, 6Aiii), mBI (0.39 vs. 0.23; Fig 6Bii, 6Biii), and NIHSS (0.88 vs. -0.02; Fig 6Cii, 6Ciii). Furthermore, a trial using intracortical cell delivery reported better outcomes as compared to intravascular delivery in mRS (Fig 6Aiv) and NIHSS (Fig 6Civ). Randomized clinical studies revealed larger effect sizes than non-randomized ones in mRS (0.37 vs. 0.22; Fig 6Av) and NIHSS (0.78 vs. 0.08; Fig 6Cv), but smaller effect sizes in mBI (0.28 vs. 0.42; Fig 6Bv) and FMS (0.52 vs. 0.80; Fig 6Dv).

Preclinical and clinical study design differences: current situation and potential impact

In contrast to the very positive results in animal models, therapeutic effects in clinical studies have been less impressive. It is noteworthy that current clinical studies on cell therapy for stroke are early stage clinical trials and are often underpowered to reveal all but the most prominent therapeutic effects. Nevertheless, remarkable design differences between preclinical and clinical studies were detected (Fig 7), which may affect clinical translation. Interestingly, we already identified cell immunogenicity, cryopreservation, cell type, comorbidity profiles and occlusion modality (i.e., the stroke model) as sources of effect size heterogeneity in preclinical studies. Basic preclinical study design characteristics are described in Online Table 2, and those of clinical studies are in Online Table 3.

Cell immunogenicity

The majority of the cells used in preclinical studies were allogeneic (47.9%) or xenogeneic (46.2%), but most of the clinical studies have utilized autologous cell transplants (70.9%) (Fig 7A). In line with another analysis[16], autologous cells have achieved better outcomes than their allogeneic counterparts in preclinical and clinical studies. Therefore, cell immunogenicity may not be a major reason for the current translational loss of efficacy. However, autologous cells were used in 25 preclinical studies, while allogeneic cells were used in only one clinical trial, and thus these results need to be interpreted with caution. Interestingly, syngeneic cells only demonstrated minor treatment effects. This contradictory result may be due to the small number of these studies and their high quality scores (median: 9 as compared to 5 for the entire data set).

Autologous cell therapy avoids adverse immunological side effects after transplantation, which is clinically relevant. The effect of systemic xenogeneic cell transplantations in preclinical experiments may theoretically be related to the immunosuppressive effects of apoptotic cells diminishing secondary inflammatory brain damage. This idea was proposed more than two decades ago[19], but has never been truly investigated in a stroke paradigm. If this concept is true, its clinical impact would be substantial and clearly warrants future investigation.

Cryopreservation

In preclinical studies, 33.1% of stroke animals received freshly harvested cells, 10.3% received cryopreserved cells, while no clear details on cryopreservation were provided in the other studies. In clinical trials, freshly harvested cells were used in 70.9% of patients with cryopreserved cells being used in the remainder (Fig 7B). The exploitation of cryopreserved cells as off-the-shelf products allows cell delivery at acute time points. However, our results indicate that frozen-thawed cells were associated with significantly reduced efficacy when compared to their freshly-harvested counterparts in preclinical and clinical trials. Cryopreserved cells may indeed exhibit lower viability, compromise immunomodulatory effects, and induce more intense or immediate blood-mediated inflammatory reactions[20-23], particularly in MNC populations[23]. Nonetheless, some recent studies have also described comparable therapeutic effects between cryopreserved and fresh cells[24-26]. The exact conditions compromising the efficacy of cryopreserved cells may be complex but should be clarified as soon as possible.

Cell type

MSCs were the most frequently investigated cells in preclinical studies (51.5% of stroke animals), followed by NSCs (21.8%), and MNCs (14.9%) (Fig 7C). Other cells such as endothelial progenitors, induced pluripotent or embryonic stem cells, had been administered in 11.9% of stroke animals. In contrast, MNCs were much more frequently given to stroke patients (55.2%). Six out of ten included clinical studies used heterogeneous MNC populations, three administered MSCs, and one utilized $CD34^+$ cells.

The optimal cell type for ischemic stroke treatment remains unclear. Our analysis pointed to a superior effect with MSCs as compared to MNCs in both preclinical and clinical trials. The predominant use of MNCs in the clinic is likely due to practical issues such as ease of isolation and availability without time-consuming and sensitive in vitro cultivation, as well as the excellent safety profile of MNCs, compensating for their potentially lower efficacy. However, one meta-analysis of bone marrow-derived MNCs[17] showed an effect size larger than that achieved with either MSCs[16] or NSCs[18], perhaps due to the different data searching and inclusion criteria. Analysis of preclinical studies also revealed that the trial with the overall most significant improvement involved NSCs (Fig 4Ciii), but unfortunately no clinical study on NSC transplantation could be included here due to the lack of appropriate control groups[7,27].

Recipient comorbidities

More than 90% of animals were healthy before stroke induction whereas many stroke patients suffered from comorbidities such as hypertension (38.3%), diabetes (25.2%), and heart disease (30.7%). Only 5.4% of the patients were reported as being healthy before the stroke event (Fig 7D). Comorbidities themselves may exert a detrimental impact on treatment efficacy[28], as confirmed also in our study. Furthermore, stroke patients often take medications such as anti-diabetics to counter comorbidities, and these compounds may interact with injected cells[29,30]. In addition, stroke patients are usually prescribed anti-platelet drugs for secondary stroke prevention and undergo post-stroke rehabilitation. However, few preclinical studies have investigated these potential interactions, leaving a clear knowledge gap in translational stroke research that likely affects the results obtained in subsequent clinical trials.

Mimicking the complex reality of clinical patient populations in preclinical studies is expensive and time-consuming. A potential solution might be to focus on patients with a particular, more specific risk profile and to mimic that experimentally. The disadvantage of this approach will be a slower patient recruitment as only a subgroup would receive treatment, but comparability will be higher.

Recipient sex and age

Cell therapy has been mainly provided to young (93.7%) male (85.1%) rodents (99.3%). In contrast, stroke patients were often middle-aged (40-60 years old, 54.8%) or elderly (>60 years old, 45.2%), consisting of both males and females, and were always enrolled in clinical trials without selection (Fig 7E-F). We did not find sex and age to significantly affect the efficacy of cell therapy in stroke animals. However, this kind of influence cannot be excluded simply due to the very limited number of studies assessing female and aged individuals. Indeed, gender-related differences are a well-known and frequently discussed confounder for outcome in translational stroke research[31]. While simulating a clinically realistic sex distribution pattern in preclinical studies requires significant additional resources, neglecting sex differences introduces one more bias into experimental results [32]. Moreover, there is experimental evidence that cell donor's and recipient's ages can influence cell treatment efficacy, particularly in commonly applied MNC populations[33].

Cerebral vessel occlusion modalities

The intraluminal filament stroke model was used in 80.5% of the studied animals. As shown above, the use of this model is associated with a larger effect size. It is also characterized by an extremely large infarct size limiting recovery processes, which is similar to the situation after large territorial infarction in humans. About 10-20% of ipsilateral corticospinal fibers do not cross in rodents, cats or monkeys, but it remains rather uncertain whether there are functionally relevant non-crossing corticospinal fibers in adult humans[34]. Hence, the large effect size in preclinical studies employing the filament model may be partly due to the rapid spontaneous stroke recovery mediated via activation and strengthening of the ipsilateral corticospinal pathway[35], a process that can be further enhanced by cell therapy in rodents – but possibly not in humans.

Moreover, 74.1% of the stroke modelling in preclinical studies was transient, which mimics the recanalization in patients achieved by thrombectomy[36], thrombolysis, or that occurring spontaneously. However, only a limited number of patients experience prompt recanalization, thus the permanent occlusion models might better reflect the present-day clinical situation except for intra-arterial cell transplantation[5,37,38]. The current advances in recanalization approaches may lead to increasing numbers of patients receiving cell therapy immediately after recanalization in the future, warranting the use of a transient stroke model for preclinical evaluation regardless of the targeted time point of cell administration, and would allow cell transplantation in a more acute stage after stroke-onset in clinical trials.

Time window of cell transplantation

In the preclinical studies, cells were transplanted within 24h after stroke onset in 67.5%, and between 24h and 1 week in 28.2% of experiments. However, in clinical trials, cells were more often transplanted at later time points, i.e. one week or even one month after stroke-onset (67.3%). No study had transplanted cells within 24h post-stroke (Fig 7G). Cell delivery in acute phase post-stroke is considered to result primarily in neuroprotection via enhanced blood-brain barrier (BBB) integrity and modulation of post-ischemic immune responses, but it is challenging to administer cells so early in the clinic. Post-acute cell delivery potentially increases angiogenesis, neurogenesis, and axonal plasticity, offering a wider time window for cell therapies[39].

Although the time window was not found here to affect efficacy significantly, many preclinical studies claimed that earlier transplantation of cells could result in a better outcome[40-42], although no conclusive evidence has been reported[16,43,44]. Successful clinical stroke care involves several strictly timed therapeutic interventions, dramatically restricting the time available for complex cell treatments. For this reason in clinical trials, cells have usually been

transplanted in either the subacute or chronic post-stroke stages. A good example is the MASTERS trial that evaluated the MultiStem cell product[8], which is one of the best characterized cell products in the field. The preclinical research on the MultiStem cell product revealed an excellent outcome (effect size=3.98 for infarct size and 3.00 for mNSS)[45], but only modest results were observed in the clinical MASTERS trial[8]. It is noteworthy that the time window for patient inclusion was expanded from 36h to 48h due to logistical requirements beyond the investigators' and sponsor's control. Although the safety of cell transplantation was demonstrated in the final MASTERS clinical trial, no significant therapeutic effect was detected after intravenous cell infusion at 24-48h post-stroke. However, a *post-hoc* subgroup analysis revealed beneficial effects of cell transplantation at earlier time points (<36h), which is exactly the patient population assumed to benefit most according to the preclinical data. The currently ongoing MASTERS-2 trial will take this aspect into account. This supports our proposal that understanding the timing and mechanisms of improvement following cell therapy is essential for successful clinical translation.

Delivery route

Intravenous cell delivery had been performed in 51.6% of the preclinical studies, and was also predominantly chosen in clinical trials (74.9%). While intravenous cell delivery is a straightforward approach in both preclinical and clinical arenas, the optimal cell delivery route remains unclear and is likely related to the cell type used. Vu et al.[16] reported better mNSS performance after intracortical MSC delivery as compared to intra-arterial and intravenous delivery, whereas Lee et al.[14] found no significant effect of cell delivery route on outcome. Both preclinical and clinical data revealed better outcome of intracortical cell delivery compared to intravascular delivery. However, the utility of intracortical delivery is limited clinically due to its invasiveness[46]. Proof-of-concept studies may be required to assess whether the advantage of intracortical delivery outweighs the safety concerns. Indeed, the recent phase 2b study (NCT02448641) of SB623 (transiently transfected MSCs overexpressing Notch-1) showed safety, but failed to show efficacy in chronic stroke patients, in contrast to the preliminary signs of efficacy from the preceding animal study[47] and phase 1/2b clinical trial[48]. Another sham controlled trial, the recently-started PISCES 3 trial (NCT03629275) is utilizing intracranial transplantation as well, but with a different cell product, cell dose and primary outcome measures; its results are keenly anticipated. The preclinical analysis also revealed superior effects of intraventricular over intracortical cell delivery. The intraventricular route is also slightly easier to perform than intracortical administration while still bypassing the BBB, and thus may be a promising option for the future. However, the potential risk of adverse effects such as hydrocephalus must be considered. No significant difference in therapeutic effect was found between studies using intra-arterial and intravenous delivery routes, but improper intra-arterial cell administration can trigger complications[20, 49, 50].

Methodological limitations

We included studies using different cell types, administration modes, etc., which increased the sample size for data analysis, particularly for clinical studies, and also enabled us to explore the sources of heterogeneity. As expected, substantial inter-study heterogeneity was observed. We addressed this in several ways: first, the heterogeneous outcome measures were standardized; second, a random-effects model was used, assuming that the treatment effect can vary across studies because of differences in study characteristics rather than by chance[51]; third, meta-regressions or subgroup analyses were performed to identify the sources of heterogeneity[52,53]. However, unexplained heterogeneity from sources that were not considered in our analysis may remain. It is noteworthy that heterogeneity cannot be avoided, and

considerable heterogeneity was also observed in previous studies using more strict inclusion criteria[16-18].

Moreover, the number of included clinical studies is still small. In some cases, there was only one study (e.g. allogeneic/cryopreserved) available for subgroup analysis. Thus, the validity of these results remains uncertain, and more clinical results are needed.

Conclusions and outlook

Although the considerable heterogeneity in preclinical data and the so-far small number of available clinical datasets make it difficult to draw any definitive conclusions, we identified substantial design differences between preclinical and clinical trials, which may contribute to the modest efficacy of cell therapy in stroke patients and have important implications for future translational projects.

We propose several suggestions for preclinical studies which may prevent translational failure. First, in confirmatory preclinical studies, there should be greater similarity to patient populations likely to be treated in clinical trials. For example, use of aged male and female animals with comorbidities such as hypertension and diabetes would better reflect the clinical reality. Second, drug-cell interactions require further investigation. Third, permanent, instead of transient, stroke models might represent a more clinically relevant strategy when evaluating the efficacy of cell therapies[54,55], particularly when targeting patient populations that cannot benefit from recanalization. Fourth, wider therapeutic time windows would benefit more patients. Hence, it would be beneficial to conduct more experimental studies testing cell transplantation in the subacute and chronic stroke stages. Fifth, it would be advantageous to devise a battery of sensory-motor function tests sensitive at detecting long-term impairment and conducive to repeated testing without developing compensatory strategies[56]. Last but not least, the therapeutic mechanisms of cell therapy still need to be clarified.

Several recommendations for clinical studies also emerge from our meta-analysis. First, freshly-harvested, autologous cells are recommended for future clinical trials to ensure maximal effects, if logistical challenges can be overcome. Second, studies of cell transplantation with more acute time windows (within 24h or 1 week) after stroke should be conducted, as these better resemble the situation in successful preclinical studies. Third, due to the extensive heterogeneity of the stroke patients, it will be crucial to identify the optimal recipients that are most likely to benefit from cell treatments, and to devise biomarkers that pinpoint such patients[57]. Last, multi-centered, randomized, double blinded clinical trials with larger sample sizes are urgently needed to evaluate the effect of cell therapy in stroke patients.

Finally, an illustration of comparability between preclinical and clinical studies by a similarity check list might help when translating a specific cell product from bench to clinic: 1) same time window (acute, subacute or chronic); 2) same delivery route; 3) same cell dose (number of cells per kg/body surface area); 4) same cell immunogenicity; 5) same preparation procedure before transplantation (e.g. fresh vs. cryopreservation); 6) same target infarcts (e.g., hemispherical infarcts of middle cerebral artery territory only, with or without reperfusion); 7) matched sex profile; 8) matched age; 9) same comorbidity; 10) same concomitant treatment.

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Author Contributions

LC, AT, JB and JJ contributed to the conception and design of the study; LC, DG, AT, JB and JJ contributed to the acquisition and analysis of data; LC, DG, AT, JB and JJ contributed to drafting the text and preparing the figures.

Potential Conflict of Interest

None.

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Figure Legends

Figure 1. PRISMA flowchart.

Figure 2. Effect sizes of (A) infarct size reduction, (B) mNSS, (C) rotarod test performance, and (D) ART performance in preclinical studies.

Figure 3. Funnel plot of (A) infarct size reduction, (B) mNSS, (C) rotarod test performance, and (D) ART performance in preclinical studies.

Figure 4. Study characteristics that significantly accounted for effect size heterogeneity in different outcome measures. (A) infarct size reduction: (i) cell immunogenicity; (ii) cell cryopreservation; (iii) use of animals with comorbidity; (iv) stroke model; (v) delivery time relative to stroke-onset. (B) mNSS: (i) cell cryopreservation; (ii) use of animals with comorbidity; (iii) delivery route; (iv) quality score of studies. (C) rotarod test: (i) cell immunogenicity; (ii) cell type. (D) ART: (i) cell immunogenicity; (ii) cell cryopreservation; (iii) use of animals with comorbidity. The dotted line indicates the pooled effect size of all studies.

Figure 5. Effect sizes of different outcome measures (mRS, mBI, NIHSS, and FMS) in clinical studies. Y: yes, N: no, Auto: autologous, Allo: allogeneic, Cryo: cryopreservation, N (T/C): number of patients (treated/control)

Figure 6. Subgroup analysis of mRS (A), mBI (B), NIHSS (C) and FMS (D) in clinical studies. (A-D): (i) cell type; (ii) cell immunogenicity; (iii) cell cryopreservation; (iv) delivery route; (v) randomization. The dotted line indicates the pooled effect size of all studies.

Figure 7. Study design discrepancies between preclinical and clinical studies: (A) immunogenicity of transplanted cells; (B) cell cryopreservation; (C) cell type; (D) comorbidities of stroke individuals; (E) age of stroke individuals; (F) sex profile; (G) time of cell transplantation.