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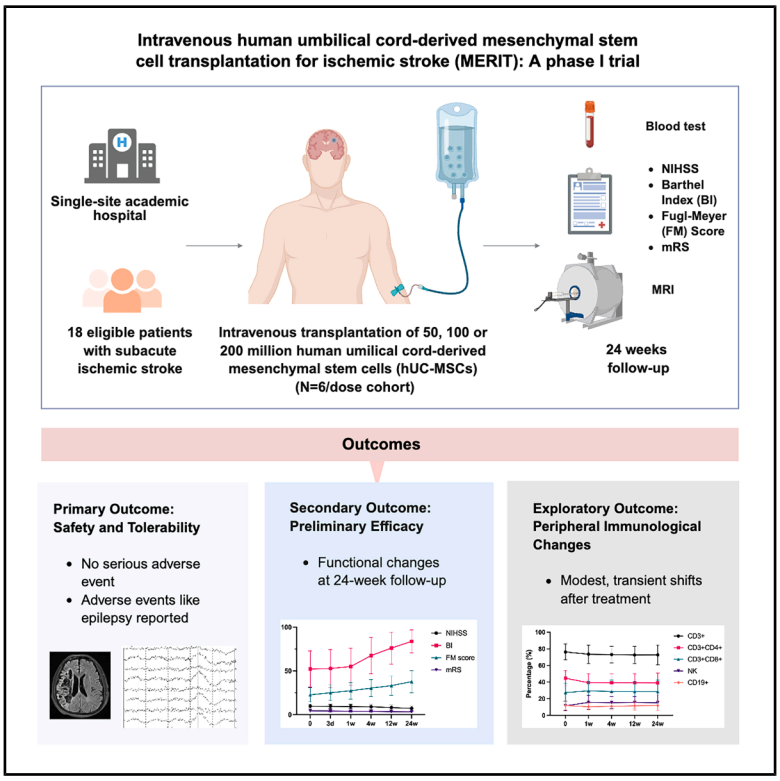
Intravenous human umbilical cord-derived mesenchymal stem cell transplantation for ischemic stroke (MERIT): A phase 1 trial

Graphical abstract



Highlights

- UC-MSC intravenous transplantation was well tolerated in subacute ischemic stroke patients
- No serious adverse events occurred
- Possible treatment-related adverse events like epilepsy were reported
- Patients showed functional improvement during follow-up

Authors

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In brief

Umbilical cord derived-mesenchymal stem cell (UC-MSC) transplantation is a promising therapy for ischemic stroke. Cui et al. report the results of a phase 1 clinical trial that enrolled 18 patients with subacute ischemic stroke and received intravenous UC-MSC administration.

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Intravenous human umbilical cord-derived mesenchymal stem cell transplantation for ischemic stroke (MERIT): A phase 1 trial

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SUMMARY

Umbilical cord-derived mesenchymal stem cells (UC-MSCs) hold promise as a potential therapy for ischemic stroke, but the optimal transplantation strategy remains to be explored. This open-label, single-arm, phase 1 study enrolls 18 patients with subacute ischemic stroke and National Institutes of Health Stroke Scale (NIHSS) scores of 6–20 (median, 9; interquartile range, 8–12). The patients receive a single intravenous transplantation of 50, 100, or 200 million UC-MSCs (n = 6 for each dose) and are followed for 24 weeks. The primary outcome is safety and tolerability, with secondary assessment of preliminary efficacy and exploratory analyses of immunological changes. Possible treatment-related adverse events include dizziness, nausea, sweating, fatigue, and epilepsy. No serious adverse event occurs. A reduction in NIHSS score by 1–5 points is observed in 14 patients during follow-up. In conclusion, intravenous administration of UC-MSCs is well-tolerated in subacute ischemic stroke. The trial is registered on ClinicalTrials.gov (NCT05697718).

INTRODUCTION

Stroke is one of the leading causes of death and a major cause of disabilities in the world. Ischemic stroke accounted for 62.4% of all global stroke cases in 2019,[1] and its global age-standardized incidence rate is estimated to increase to 89.32 per 100,000 in 2030.[2] Effective treatments for ischemic stroke are currently limited. The therapeutic time window of reperfusion therapies (intravenous thrombolysis and mechanical thrombectomy) in the acute stage is relatively narrow, leaving many patients untreated, while rehabilitation is the only established treatment option during the recovery period.[3] Therefore, there is an urgent need for new effective therapeutic strategies to mitigate the patients' neurological deficits after ischemic stroke and enable them to return to their premorbid societal role.

Cell-based therapies are one of the most promising innovative strategies for the treatment of ischemic stroke. Positive effects of cell transplantation after stroke, including mesenchymal stem cells (MSCs), have been shown in numerous animal studies.[4,5] Potentially underlying therapeutic mechanisms of cell therapy comprise immune modulation, neuroprotection, promotion of angiogenesis as well as (limited) neurogenesis, and restoration of neural circuits in the injured brain via paracrine or cell-to-cell contact mechanisms.[4,6,7] However, results from current clinical studies are less conclusive, although preliminary evidence of efficacy was seen in some studies.[5,8–18] Therefore, the appropriate cell transplantation strategy for stroke patients remains to be determined.

MSCs are the most used cell type with significant clinical potential due to their ease of isolation; lack of significant immunogenicity, which allows allogeneic transplantation without immunosuppression; and less ethical restriction.[5] MSCs can be isolated from multiple sources such as bone marrow, umbilical cord, and adipose tissue. MSCs from perinatal tissues or infants

are considered to exert stronger immunosuppressive effects and proliferative abilities than bone marrow- or adipose tissue-derived MSCs from adults, probably due to aging of the cells.[19,20] Moreover, the isolation of MSCs from perinatal tissues such as umbilical cords is easier compared to isolation from bone marrow or adipose tissue and comes with fewer ethical concerns due to the lack of invasiveness of the sampling. Nevertheless, most clinical studies used MSCs from bone marrow[8–10,17,18] or adipose tissue,[11,21] while umbilical cord-derived MSCs (UC-MSCs) have been rarely reported.[22]

Most clinical trials investigating cell therapies for ischemic stroke focus on either the acute (e.g., within 48 h)[11–14] or the chronic stage (>6 months)[8,15,16,18]; only few studies tested the safety and efficacy of MSCs during the subacute stage of ischemic stroke.[9,10] Moreover, the underlying immunological changes after MSC treatment for stroke patients are rarely reported.[12] An interesting time window for cell therapies to explore would be the late subacute period (around 3–6 months after the initial event), during which the functional recovery of the patients often reaches a plateau despite intensive rehabilitation. MSCs may further promote the patients' recovery through their proposed mechanisms, and cell transplantation would be less challenging at that time because the patients are usually clinically stable and may be more willing to accept cell therapy as an additional treatment strategy.

Another undetermined aspect of MSC therapy for ischemic stroke is the optimal delivery route. MSCs have been administered via different routes such as intra-arterial, intravenous, and intralesional delivery in preclinical and clinical trials.[5] Intralesional transplantation can bypass the systemic distribution of the cells, but the invasive procedure limits its application. Intra-arterial delivery can increase the homing of infused cells to the brain, but it also carries the risk for secondary vessel occlusion caused by the infused cells and the procedure is also relatively invasive.[23,24] Intrathecal cell delivery has been rarely used in stroke patients. Intravenous cell delivery is the most frequently used both in animal experiments and in clinical trials due to its minimal invasiveness, simplicity of the procedure, and an excellent safety profile. Preliminary evidence for modest efficacy of intravenous MSC transplantation in stroke patients has also been reported.[8–11,17]

We conducted this prospective phase 1 clinical study to primarily evaluate the safety and tolerability of intravenous administration of an off-the-shelf, allogeneic UC-MSC product in patients with subacute ischemic stroke. A total of 18 patients were treated across three dose cohorts, enabling assessment of safety across escalating dose levels. Secondary assessments included changes in functional outcomes and infarct volume, while exploratory analyses examined immunological profiles such as cytokines, lymphocyte subsets, and immunoglobulins following treatment.

During the study period, no serious adverse event (SAE) was observed. One patient experienced epileptic seizures after treatment, which were conservatively classified as possibly related and highlight the need for careful safety monitoring in future studies. Improvements in functional scores were observed during follow-up in patients who completed the final assessment; however, these findings should be interpreted cautiously given the open-label, single-arm design.

RESULTS

Patient characteristics

This open-label, single-arm, single-center, phase 1 study (www.clinicaltrials.gov, NCT 05697718) was conducted at Xuanwu Hospital of Capital Medical University. A total of 128 patients with ischemic stroke were initially screened, of whom 22 patients were assessed for eligibility. Patients were eligible for inclusion in the trial if they were 18–80 years of age, experienced an anterior circulation ischemic stroke confirmed by brain computed tomography or MRI 12–24 weeks prior to enrollment, and had a National Institutes of Health Stroke Scale (NIHSS)[25] score of 6–20 at baseline, with NIHSS item 1a <2. A complete list of the inclusion and exclusion criteria is provided in the supplemental information.

Eighteen patients (12 males and 6 females, mean age 52.5 ± 9.0 years) were enrolled between April 12, 2023, and January 26, 2024, and received a single intravenous infusion of 50, 100, or 200 million UC-MSCs in a sequential dose-escalation manner ($n = 6$ for each dose). The study flow chart is shown in Figure 1. All participants were at 12–24 weeks post-stroke at enrollment, with a median modified Rankin scale (mRS) score of 4.5 (interquartile range [IQR], 4–5) and a median NIHSS score of 9 (IQR, 8–12). The mean interval from stroke onset to UC-MSC administration was 122.2 ± 22.7 days. Baseline demographic and clinical characteristics of all the eligible patients are summarized in Table 1. A detailed description of each patient's demographic data upon enrollment is provided in Table S1.

Primary outcome: Safety and tolerability

All enrolled patients were followed for 24 weeks to assess the safety and tolerability of UC-MSC treatment, which was the primary endpoint of this study. Dose-limiting toxicity (DLT) was defined as any grade 3 or higher adverse event (AE) according to Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v.5.0) that was deemed at least possibly related to UC-MSC administration. During the whole course of the study, a total of 48 AEs were reported, including 30 evaluated as grade 1 and 18 as grade 2 (see Table 2). All patients recovered spontaneously or after short-term intervention. No SAE or DLT was observed after UC-MSC administration. The reported AEs included pain and swelling of the paralyzed limb, alanine aminotransferase elevation, accidental falls, hypoglycemia, constipation, and other events, most of which were assessed as unrelated or unlikely related to UC-MSC treatment.

Two patients experienced 5 AEs that were classified as possibly treatment related. One patient (patient 8) developed transient infusion-related symptoms, including dizziness, nausea, sweating, and fatigue, which occurred near the end of infusion and resolved spontaneously within approximately 10 min. Another patient (patient 17), who had a large right hemispheric cortical infarction 103 days prior to treatment, experienced a generalized tonic-clonic seizure 1 day after receiving 200 million UC-MSCs. He was treated with a single intramuscular injection of phenobarbital sodium followed by oral levetiracetam. The patient experienced two additional generalized tonic-clonic seizures 81 and 140 days after cell infusion, respectively. Given the temporal proximity to UC-MSC administration,

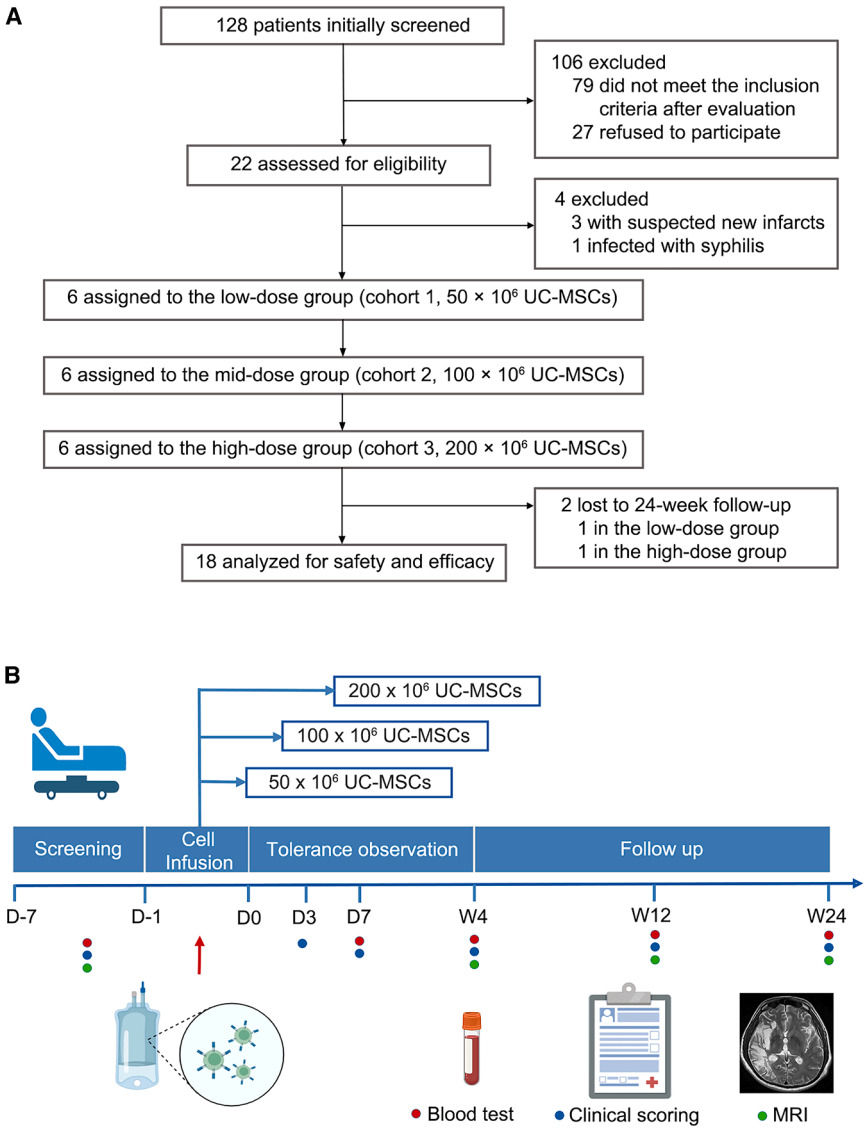


Figure 1. Trial profile

(A) The trial flow chart. A total of 128 patients with ischemic stroke were initially screened, and 22 patients were assessed for eligibility. Eighteen patients were finally recruited with 6 patients enrolled for each dose group.

(B) The diagram of the study procedure. After assessment of eligibility, the patients received cell infusion. They were followed up for 24 weeks and observed for tolerance. Clinical scoring, blood tests, and MRI were performed before and after cell infusion. Some icons of the figure were created with BioRender. UC-MSCs, umbilical cord-derived mesenchymal stem cells; D, days; W, weeks. See also Figure S9.

this event was conservatively classified as possibly related after internal review and independent evaluation by the institutional ethics committee, although cortical infarction is a well-established risk factor for post-stroke epilepsy. Corresponding brain MRI and electroencephalogram findings are shown in Figure S1. A complete summary of all AEs is provided in Table S2.

Changes in hemograms, coagulation function, and biochemical parameters were also investigated and analyzed. No significant changes were observed in any hemograms after cell treatment except for a decrease in the percentage of monocytes ($p < 0.05$ at 12 weeks vs. baseline) (see Figure S2). No significant changes in coagulation function were observed after cell treatment either. A minor, transient elevation in D-dimer level was noticed in some patients after cell infusion (see Figure S3), but it was not associated with any clinical symptoms or evidence of thrombosis. There was a significant increase in serum total protein and albumin after UC-MSC infusion ($p < 0.01$), which lasted until the 24-week follow-up, whereas no clinically meaningful changes were observed in liver or kidney function, glucose, uric acid, or lipid profiles (Figure S4). Additional evaluations including electrocardiography, brain MRI, and physical examinations revealed no clinically significant abnormalities following treatment.

Secondary outcome: Preliminary efficacy

As the secondary endpoints, functional outcomes including the mRS, Barthel index (BI), NIHSS, and Fugl-Meyer (FM) scores, as well as the infarct volume, were assessed at 12 and 24 weeks after UC-MSC infusion and compared with baseline values. The proportions of patients with BI ≧ 95 at 12 and 24 weeks post-treatment were also recorded. All the patients completed the 12-week follow-up, and 16 patients completed the 24-week follow-up. One patient (patient 16) completed the 24-week follow-up after cell infusion with only mRS, BI, part of the laboratory tests, as well as brain MRI being performed. Concomitant pharmaceutical and other treatments received by the patients are presented in Tables S3 and S4.

Numerical changes in functional scores were observed during follow-up. At 12 weeks, mRS scores decreased by 1–2 points (mean ± SD, 0.9 ± 0.6) from baseline in 14 of 18 patients, and at 24 weeks (1.0 ± 0.6), in 13 of 16 patients. NIHSS scores showed absolute decreases of 1–6 (1.6 ± 1.7) points at 12 weeks and 1–5 (2.4 ± 1.5) points at 24 weeks compared to baseline. FM scores increased by absolute changes of 2–23 (10.5 ± 6.4) points at 12 weeks and 1–31 (14.7 ± 8.0) points at 24 weeks. BI scores increased by absolute changes of 5–55 (23.9 ± 15.0) points at 12 weeks and 5–55 (31.6 ± 15.1) points at 24 weeks, with 3 of 18 patients achieving a BI ≧ 95 at 12 weeks and 5 of 16 at 24 weeks (Table 3). Individual patient trajectories by dose cohort

Table 1. Baseline characteristics of the patients

	Total (*n* = 18)	Low-dose group (*n* = 6)	Mid-dose group (*n* = 6)	High-dose group (*n* = 6)
Age (years), mean ± SD	52.5 ± 9.0	53.0 ± 9.1	54.3 ± 5.6	50.2 ± 12.3
Gender, *n* (%)				
Female	6 (33.3)	3 (50)	2 (33.3)	1 (16.7)
Male	12 (66.7)	3 (50)	4 (66.7)	5 (83.3)
Medical history, *n* (%)				
Atrial fibrillation	5 (27.8)	2 (33.3)	1 (16.7)	2 (33.3)
Diabetes mellitus	5 (27.8)	2 (33.3)	2 (33.3)	1 (16.7)
Hypertension	13 (72.2)	2 (33.3)	5 (83.3)	6 (100)
Hyperlipidemia	13 (72.2)	5 (83.3)	4 (66.7)	4 (66.7)
Infarct hemisphere, *n* (%)				
Left	9 (50)	4 (66.7)	5 (83.3)	0 (0)
Right	9 (50)	2 (33.3)	1 (16.7)	6 (100)
Occlusion site, *n* (%)				
MCA	15 (83.3)	4 (66.7)	6 (100)	5 (83.3)
ICA	3 (16.7)	2 (33.3)	0 (0)	1 (16.7)
Etiology, *n* (%)				
Atherosclerosis (large artery)	11 (61.1)	2 (33.3)	5 (83.3)	4 (66.7)
Cardiogenic embolism	2 (11.1)	1 (16.7)	0 (0)	1 (16.7)
Others	3 (16.7)	1 (16.7)	1 (16.7)	1 (16.7)
Unclear	2 (11.1)	2 (33.3)	0 (0)	0 (0)
Infarct volume (mL), mean ± SD	91.6 ± 82.3	110.5 ± 93.8	66.8 ± 75.3	97.7 ± 85.6
mRS score				
Median (IQR)	4.5 (4–5)	5 (4–5)	5 (3.8–5)	4 (3.8–4.3)
Mean ± SD	4.4 ± 0.7	4.7 ± 0.5	4.5 ± 0.8	4.0 ± 0.6
NIHSS score				
Median (IQR)	9 (8–12)	12 (9–13.3)	10 (6.8–12.3)	8 (8–8.3)
Mean ± SD	9.7 ± 2.5	11.2 ± 2.9	9.7 ± 2.8	8.2 ± 0.4
Barthel index, mean ± SD	52.2 ± 20.7	43.3 ± 18.6	43.3 ± 17.2	70 ± 15.8
Fugl-Meyer score, mean ± SD	22.7 ± 8.1	19.2 ± 6.0	20.5 ± 9.6	28.3 ± 6.1
Duration from stroke onset to cell infusion (days), mean ± SD	122.2 ± 22.7	140.0 ± 21.2	119.8 ± 22.6	106.8 ± 10.8[a]

MCA, middle cerebral artery; ICA, internal carotid artery; mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; IQR, interquartile range; SD, standard deviation.
See also Tables S1, S3, and S4.
[a]$p < 0.05$: low-dose vs. high-dose group.

and pooled analyses are shown in Figures 2 and S5. No significant differences in functional score changes were observed among dose cohorts, and no time-by-dose interactions were detected.

Infarct volumes were measured by a trained, blinded rater using ITK-SNAP software based on the fluid-attenuated inversion recovery sequence of MRI (Figure S6). Absolute infarct volumes showed small numerical increase (0.5 ± 3.7 mL) at 12 weeks, while minor decrease (−1.4 ± 7.1 mL) at 24 weeks post-treatment compared to baseline without statistically significant change (Figures S6C and S6D). In the exploratory post hoc analyses stratified by baseline infarct size (≦50 mL vs. >50 mL, Table S5), patients with smaller infarcts exhibited greater numerical reductions in infarct volume; however, no corresponding differences in functional outcomes were observed (Tables S6–S8).

Exploratory outcome: Peripheral immunological changes after treatment

Peripheral lymphocyte subsets and circulating inflammatory mediators were assessed as exploratory endpoints to investigate potential immunological changes following UC-MSC infusion. Peripheral lymphocyte subpopulation analysis by flow cytometry revealed modest, transient shifts in immune cell composition after treatment, including numerically lower proportions of $CD3^+$ cells, $CD4^+$ T cells, $CD19^+$ B cells, as well as CD4/CD8 ratio, concomitantly with higher proportions of $CD8^+$ T cells and natural killer cells. These changes appeared most pronounced at early post-infusion time points. However, no statistically significant differences were observed across time points or dose cohorts (Figure 3A; absolute values are shown in Figure S7).

Table 2. Adverse events

	Total (*n* = 18)	Low-dose group (*n* = 6)	Mid-dose group (*n* = 6)	High-dose group (*n* = 6)
Adverse event, n (%)	48 (100)	7 (14.6)	31 (64.6)	10 (20.8)
Grade 1	30 (62.5)	6 (12.5)	21 (43.7)	3 (6.3)
Grade 2	18 (37.5)	1 (2.1)	10 (20.8)	7 (14.6)
Relationship with UC-MSC treatment, *n* (%)				
Not related	18 (37.5)	1 (2.1)	13 (27.1)	4 (8.3)
Possibly not related	25 (52.1)	6 (12.5)	14 (29.2)	5 (10.4)
Possibly related	5 (10.4)	0 (0.0)	4 (8.3)	1 (2.1)
Dizziness (grade 1)	1 (2.1)	–	1 (2.1)	–
Nausea (grade 1)	1 (2.1)	–	1 (2.1)	–
Excessive perspiration (grade 1)	1 (2.1)	–	1 (2.1)	–
Fatigue (grade 1)	1 (2.1)	–	1 (2.1)	–
Epilepsy (grade 2)	1 (2.1)	–	–	1 (2.1)

UC-MSC, umbilical cord-derived mesenchymal stem cell.
See also Figures S1–S4 and Table S2.

Quantitative analysis of plasma cytokines showed modest numerical trends toward lower levels of several proinflammatory cytokines such as IL-5, IL-1β, and IL-17A and higher levels of anti-inflammatory cytokines such as IL-4 and IL-10 after treatment (Figure 3B). Complement components (C3 and C4) and immunoglobulins (IgA, IgG, and IgM) exhibited small numerical increases, whereas C-reactive protein levels showed a decreasing trend after treatment (Figure S8).

Table 3. Secondary outcomes of the patients

Endpoint	Pooled (*n* = 18)[a]	Low dose (*n* = 6)[b]	Mid dose (*n* = 6)	High dose (*n* = 6)[c]
mRS score change vs. baseline at 12 w, mean ± SD	0.9 ± 0.6	1.2 ± 0.8	0.8 ± 0.4	0.7 ± 0.5
mRS score change vs. baseline at 24 w, mean ± SD	1.0 ± 0.6	1.2 ± 0.4	1.2 ± 0.8	0.6 ± 0.5
NIHSS score change vs. baseline at 12 w, mean ± SD	1.6 ± 1.7	1.2 ± 1.3	2.0 ± 2.2	1.5 ± 1.8
NIHSS score change vs. baseline at 24 w, mean ± SD	2.4 ± 1.5	1.4 ± 1.1	2.7 ± 1.6	3.3 ± 1.5
Barthel index change vs. baseline at 12 w, mean ± SD	23.9 ± 15.0	28.3 ± 18.9	25.8 ± 17.2	17.5 ± 6.1
Barthel index change vs. baseline at 24 w, mean ± SD	31.6 ± 15.1	34.0 ± 16.4	40.0 ± 12.2	19.0 ± 9.6
Barthel index ≥95 at 12 w, *n* (%)	3 (16.7)	0 (0)	0 (0)	3 (50.0)
Barthel index ≥95 at 24 w, *n* (%)	5 (31.3)	1 (20.0)	2 (33.3)	2 (40.0)
FM score change vs. baseline at 12 w, mean ± SD	10.5 ± 6.4	10.2 ± 7.9	7.8 ± 5.3	13.5 ± 5.2
Upper limb	5.9 ± 4.0	5.5 ± 4.1	3.8 ± 2.9	8.3 ± 3.9
Lower limb	4.6 ± 3.4	4.7 ± 5.0	4.0 ± 3.0	5.2 ± 1.7
FM score change vs. baseline at 24 w, mean ± SD	14.7 ± 8.0	13.0 ± 9.0	13.8 ± 7.3	18.3 ± 8.8
Upper limb	8.2 ± 5.0	6.2 ± 4.9	8.2 ± 4.2	10.8 ± 6.4
Lower limb	6.5 ± 4.0	6.8 ± 5.3	5.7 ± 4.0	7.5 ± 2.6
Infarct volume change vs. baseline (mL) at 12 w, mean ± SD	0.5 ± 3.7	0.7 ± 4.2	2.0 ± 4.0	−1.1 ± 2.8
Infarct volume change vs. baseline (mL) at 24 w, mean ± SD	−1.4 ± 7.1	−0.5 ± 3.8	1.5 ± 4.0	−5.7 ± 10.9

mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; FM, Fugl-Meyer; SD, standard deviation.
[a]*n* = 16 for mRS, Barthel index, and infarct volume, *n* = 15 for NIHSS and FM scores at 24 weeks.
[b]*n* = 5 at 24 weeks.
[c]*n* = 5 for mRS, Barthel index, and infarct volume; *n* = 4 for NIHSS and FM scores at 24 weeks. See also Figures S5 and S6, Tables S5–S8.

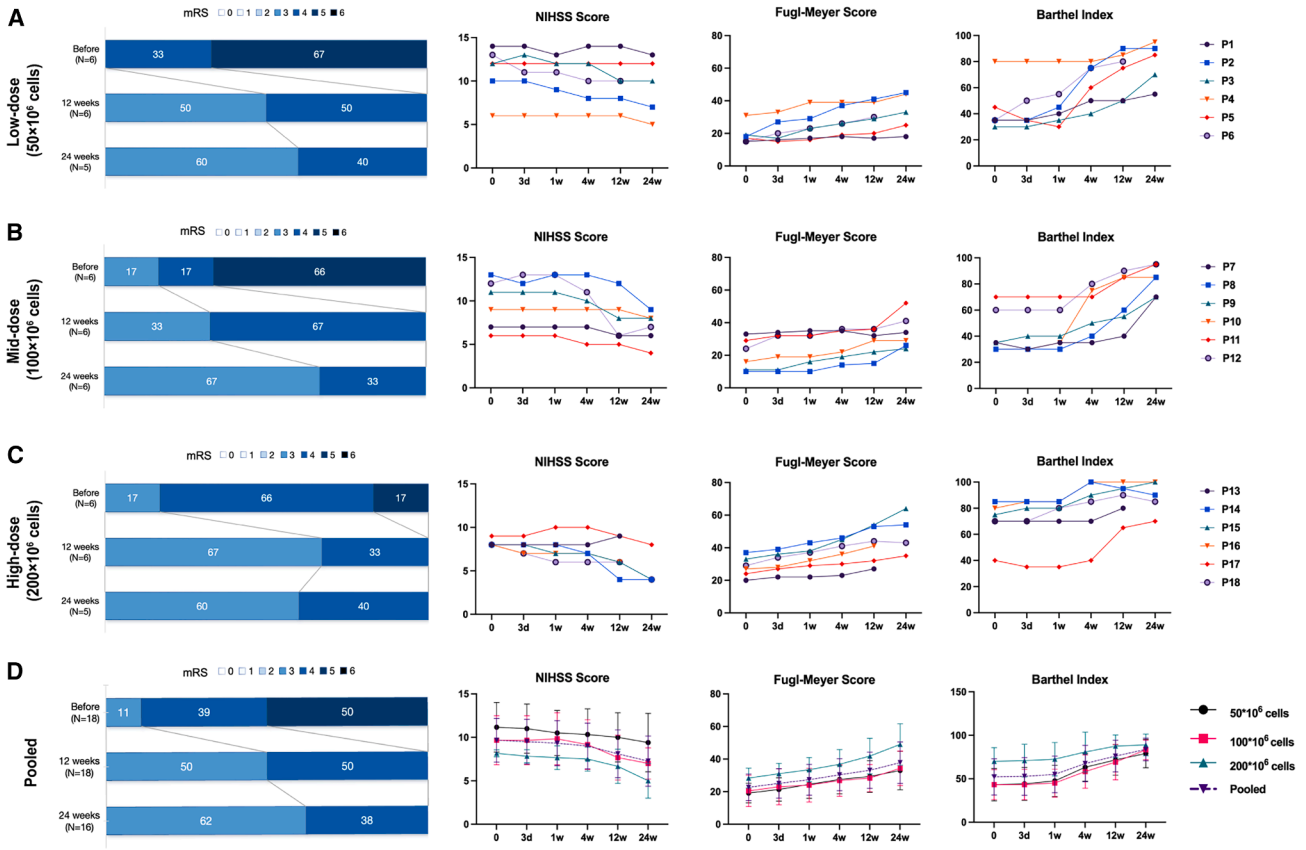


Figure 2. Functional outcome of the patients

Distribution of mRS scores at 12 and 24 weeks, as well as NIHSS, Fugl-Meyer scores, and Barthel index at each time point, in (A) low-dose (50 × 10^6 cells), (B) mid-dose (100 × 10^6 cells), and (C) high-dose (200 × 10^6 cells) groups and (D) the pooled results (data are presented as mean ± SD for NIHSS, Fugl-Meyer scores, and Barthel index). mRS, modified Rankin scale; NIHSS, National Institutes of Health Stroke Scale; P, patient. See also Figure S5.

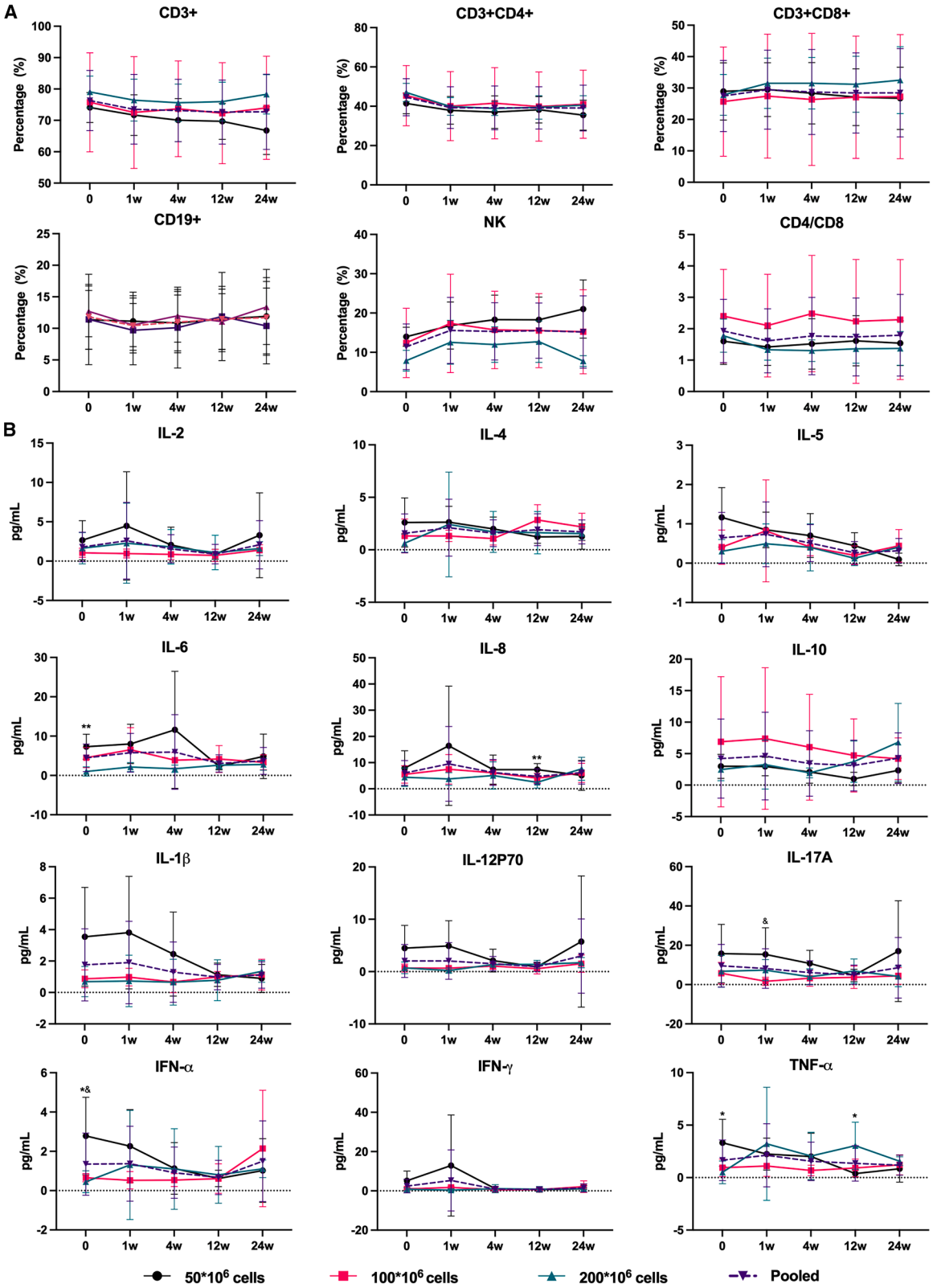
Post hoc correlation analyses were conducted to explore the potential associations between immunological parameters and clinical or imaging outcomes. In these exploratory analyses, changes in IL-4 and IL-10 levels were positively correlated with changes in FM scores at 24 weeks, whereas changes in immunoglobulin levels (IgA, IgG, and IgE) showed inverse correlations with functional score changes (Figure 4).

DISCUSSION

This phase 1 clinical trial provides preliminary evidence regarding the safety and tolerability of intravenous administration of the off-the-shelf cryopreserved allogeneic UC-MSCs in patients with subacute ischemic stroke. No SAE or DLT occurred throughout the follow-up period, and treatment-related AEs were infrequent. Changes in functional outcomes were observed during follow-up in many patients, and peripheral immunological parameters following treatment were also explored.

MSCs derived from various tissues such as the bone marrow and adipose tissue have shown satisfying safety profiles in the reported clinical studies.[5,8–13,21,22] The potentially infusion-related AEs observed in our study were mainly unspecific, including dizziness, nausea, sweating, and fatigue. Those are also common in other clinical trials and are usually mild and transient.[23] Potential embolic events have been occasionally reported in other clinical trials, including pulmonary embolism after intravenous and cerebral microinfarcts after intra-arterial cell delivery.[24,26] We did not observe any embolic events after cell infusion in our study. However, transient and mild increases of D-dimer were observed in some patients without accompanying clinical symptoms or evidence of thrombosis. A similar phenomenon was reported in a previous study, accompanied by increased levels of thrombin-antithrombin complex.[27] The culture-expanded MSCs have been shown to elicit an instant blood-mediated inflammatory reaction (IBMIR) upon exposure to human blood, attributable to prothrombotic tissue and stromal factors expressed on the cell surface. The magnitude of IBMIR appears to depend on both cell dose and cell-passage number. Under the low-passage and moderate-dose conditions applied in the present study, IBMIR is expected to be limited. However, higher cell doses and particularly higher passage numbers may increase this risk and should, therefore, be approached with caution.

One safety issue warranting careful consideration is the occurrence of epileptic seizures after cell infusion. Seizures have also been reported in prior studies investigating cell therapy in stroke



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patients.[8,10,15,23] It has been speculated that the injected cells may alter the systemic immune response or activate the brain repair mechanisms, which modifies the excitability in the perilesional areas and may lead to seizures.[28] In the present study, one patient exhibited a generalized tonic-clonic seizure shortly after UC-MSC infusion. Given the temporal proximity to treatment, a potential association with cell infusion cannot be excluded; however, the patient had a large cortical infarction, which is a well-established risk factor for post-stroke epilepsy. Importantly, the observed seizure frequency in this cohort falls within the range reported for post-stroke epilepsy in patients not receiving cell therapy.[29,30] Moreover, available randomized controlled trials have not demonstrated a higher incidence of seizures in MSC-treated patients compared with controls.[10,11] Nonetheless, these observations are based on limited sample sizes, and careful monitoring for seizure activity remains warranted in future studies, particularly in patients with cortical involvement or other known epilepsy risk factors.

Another important safety issue of cell therapy is tumorigenesis. Although cumulative clinical and preclinical evidence suggests that MSCs do not directly cause tumors, it remains unclear whether transplanted MSCs could indirectly accelerate the growth of pre-existing or undetected tumors in the human body. In this study, we will follow-up the patients up to 30 years (or until death) to monitor for tumorigenesis and other potential delayed AEs.

Substantial preclinical evidence indicates that MSCs can improve neurological function after ischemic stroke via various pathways involving immune responses, endogenous neurogenesis, angiogenesis, and neuronal plasticity.[4–7] Functional improvement in stroke patients after MSC transplantation, particularly motor function, has been reported in some clinical studies.[8–10] A recent meta-analysis reported that ischemic stroke patients who received MSC therapy at 3 months or later after stroke onset showed significant improvement in the mRS and NIHSS scores, while patients receiving MSC therapy within 2 weeks after stroke onset did not. MSC therapy during 2 weeks–3 months after stroke onset also resulted in significant improvement in the NIHSS score and BI.[31] However, heterogeneity in study design, timing, cell source, and outcome measures limits direct comparisons across studies. In the present phase 1 trial, numerical changes in functional outcome measures (mRS, NIHSS, FM, and BI) were observed during follow-up. However, rehabilitation was not restricted or standardized for ethical and practical reasons, and spontaneous recovery cannot be excluded, particularly given the variability in baseline stroke severity and lesion characteristics. Although functional recovery is often thought to plateau by the late subacute stage, inter-individual differences remain substantial. Accordingly, the functional findings in this open-label, single-arm study should be interpreted cautiously and considered as hypothesis-generating only, requiring confirmation in adequately powered randomized controlled trials.

No significant changes in infarct volume were detected in this study, which is expected during the subacute phase when lesion size has largely stabilized and gliosis is evolving. Small numerical changes in infarct volume may also reflect lesion atrophy or technical variability in image acquisition rather than treatment-related effects. These imaging findings are, therefore, presented mainly descriptively and should not be overinterpreted.

Dose-response relationships represent an important but unresolved question in cell therapy for stroke. Animal studies indicate that a higher cell number may result in better therapeutic effect after stroke.[32–34] However, so far, no clear evidence of such dose-dependent effect has been found in stroke patients receiving cell therapy.[35,36] In this study, no significant dose-dependent difference in functional outcome was observed, likely reflecting the small sample size and substantial inter-individual variability. Exploratory subgroup analyses based on infarct size were underpowered and did not provide sufficient evidence to guide dose selection. Future studies with larger cohorts will be required to determine appropriate dosing strategies.

Exploratory immune profiling revealed modest, transient shifts in peripheral immune parameters following UC-MSC infusion. These findings are broadly consistent with prior studies suggesting that MSCs exert immunomodulatory effects primarily through interactions with T cells and inflammatory mediators.[12,22,37–39] Post hoc correlation analyses suggested associations between selected immune markers and functional outcomes. It is interesting to notice that the increase of immunoglobulins was negatively correlated with functional improvement. One hypothesis is that the low immunogenicity of the allogeneic MSCs and the induced weak IBMIR after systemic infusion may compromise the survival, engraftment, and function of the transplanted cells.[27,40] However, these analyses do not establish any causality given the exploratory nature and small sample size of this study and lack of further mechanistic proof. The immunological findings should, therefore, be regarded as hypothesis generating only, and more studies are needed to find the appropriate transplantation strategy that could induce more benefits while reducing the negative effects.

To date, clinical data on UC-MSC transplantation for ischemic stroke remain limited, despite several ongoing trials. UC-MSCs offer practical advantages including ease of isolation, high proliferative capacity, and immunomodulatory properties, but differences between MSCs derived from various tissue sources are not fully understood. Further comparative studies are needed to elucidate source-specific characteristics, therapeutic potential, and mechanisms of action.

In conclusion, single intravenous transplantation of UC-MSCs in patients with subacute ischemic stroke is feasible and well tolerated across three dose levels in this phase 1 clinical trial, with no SAE observed during follow-up. Functional and immunological changes observed in this study are exploratory and

Figure 3. Changes of lymphocyte subsets and cytokines in the peripheral blood of the enrolled patients

(A) Changes of lymphocyte subsets in the peripheral blood of the enrolled patients.

(B) Changes of cytokine levels in the peripheral blood of the enrolled patients.

$n = 6$ in 50×10^6 cells group at each time point except for $n = 5$ at the 24-week follow-up; $n = 6$ in 100×10^6 cells group at each time point; $n = 5$ in 200×10^6 cells group at each time point except for $n = 4$ at the 24-week follow-up. Data are presented as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$: 50×10^6 vs. 200×10^6 cells group, $^{\&}p < 0.05$: 50×10^6 vs. 100×10^6 cells group. See also Figures S7 and S8.

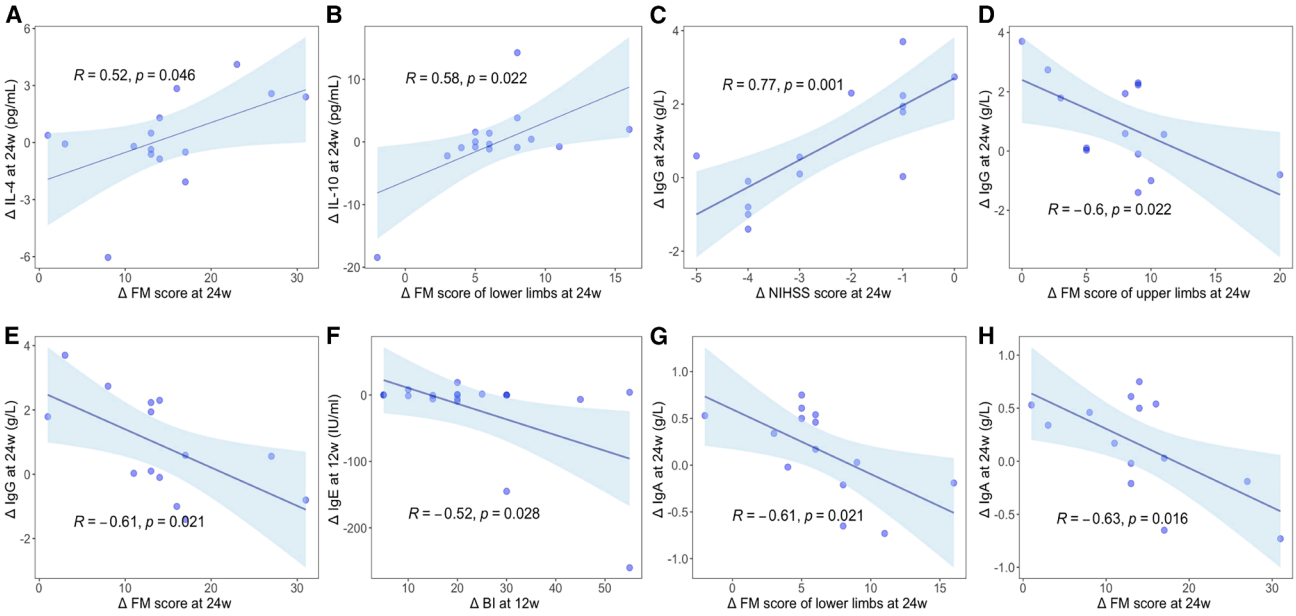

Figure 4. Post hoc correlation analysis between immunological parameters and functional outcome

BI, Barthel index; NIHSS, National Institutes of Health Stroke Scale; FM, Fugl-Meyer; IgE, immunoglobulin E; IgG, immunoglobulin G; IgA, immunoglobulin A.

should be interpreted cautiously. These findings support the conduction of future large-scale, randomized controlled trials to further evaluate the safety, dosing strategies, and potential therapeutic role of UC-MSCs in ischemic stroke.

Limitations of the study

This study has several limitations. First, it was an open-label, single-arm, phase 1 trial with a small sample size and heterogeneous baseline characteristics, limiting data interpretation. Second, the follow-up duration was relatively short for assessing long-term outcomes, although extended surveillance is ongoing. Third, pharmacokinetics and biodistribution of infused cells were not evaluated. Fourth, only selected immunological parameters were examined, and other relevant paracrine mediators were not measured. Finally, UC-MSCs were administered as a single infusion. The potential impact of repeated dosing warrants future investigation. Subsequent large-scale, randomized, placebo-controlled, multicenter trials with stratification by key prognostic factors will be essential to further assess safety, dosing, and potential efficacy of UC-MSCs for ischemic stroke.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Junwei Hao (haojunwei@vip.163.com).

Materials availability

Requests for reagents should be directed to and will be fulfilled by the lead contact after signing a materials transfer agreement. Cell source requests should be directed to Shanghai IxCell Biotech Co. Ltd, Shanghai 201203, China.

Data and code availability

- The data that support the findings of this paper are available from the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this work paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Study design, J.H., G.G., M.X., L.C., and G.M.; patient recruitment, follow-up, and data collection, L.C., Q.Z., H.L., Y.R., D.Z., D.G., Q.M., L.Y., M.L., L. Zhang, and L. Zhong; cell infusion, H.Y., R.W., and Y.W.; statistical analysis of the data, L.C. and M.X.; drafting manuscript, L.C.; data interpretation and manuscript revision, J.H., G.G., M.X., J.C., J.J., and J.B.; all authors reviewed and approved the final version of this manuscript.

DECLARATION OF INTERESTS

G.G. is a co-founder and the chief executive officer of Shanghai IxCell Biotech Co. Ltd. M.X. is an employee of Shanghai IxCell Biotech Co. Ltd. G.G. holds a

patent registration (CN114164171A) covering the cell product related to this work.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Cell lines		
Umbilical cord derived-mesenchymal stem cell	Shanghai IxCell Biotech Co. Ltd.	IxCell hUC-MSC-S
Software and algorithms		
ITK-SNAP	ITK-SNAP	ITK-SNAP 4.0
SAS	SAS Institute	SAS 9.4

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study design

This open-label, single-arm, single-center, dose-escalation (50, 100, 200 million UC-MSCs), phase 1 study was conducted in Xuanwu Hospital of Capital Medical University between April 12, 2023, and July 29, 2024. A total of 18 patients (12 males and 6 females, mean age 52.5 ± 9.0 years) were enrolled, with 6 patients for each dosage group. Each patient was followed up for 24 weeks to explore the safety and tolerability of MSC treatment. DLT was defined as any Grade $\geq$3 AE (CTCAE v5.0) deemed at least possibly related to UC-MSC infusion, with a 28-day post-infusion observation window. A target toxicity threshold of <33% was applied, such that escalation to the next dose level was permitted only if fewer than two of six participants experienced DLT event in the preceding cohort. The study was approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University ([2022]043), and has been performed in accordance with the Declaration of Helsinki. Informed consents were obtained from all participants or their legally authorized representatives before enrollment.

Participants

Patients were eligible for inclusion in the trial if they were 18–80 years old, and experienced ischemic stroke in the anterior circulation as determined by brain CT or MRI 12–24 weeks prior to enrollment, and had a NIHSS score of 6–20 upon enrollment with NIHSS 1a <2. Patients were recruited from our discharged patients or identified through referrals from rehabilitation services. We also allowed self-referral triggered by media awareness. Patients with a history of other neurological or psychological diseases such as intracranial hemorrhage, neoplasms, Parkinson's disease, severe depression and other conditions that could affect the ability to participate in the clinical trial, or with severe cardiovascular, respiratory, renal, hepatic or immunological diseases, as well as inability to undergo an MRI scan were excluded.

MSC preparation

Off-the-shelf Good Manufacturing Practice (GMP)-grade human UC-MSCs (IxCell hUC-MSCs) were provided by Shanghai IxCell Biotech Co. Ltd., which have been approved by the China National Medical Products Administration (NMPA) to be used in clinical trials for knee osteoarthritis and interstitial lung disease. The umbilical cord of a healthy full-term fetus delivered by cesarean section was collected and stored in sterile cell culture medium- Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Waltham MA, USA) containing 10×gentamicin/amphotericin B. After transportation to the GMP laboratory, the umbilical cord was washed twice with normal saline containing 10×gentamicin/amphotericin B, cut into 3cm segments, and thoroughly cleaned again. Wharton's jelly was separated and minced into 1-2mm^3 pieces in a 150 mm culture dish before being transferred into a 50 mL centrifuge tube. The sample was digested using DMEM supplemented with 0.06U/mL collagenase NB 6 (SERVA, Heidelberg, Germany) and placed in a 37°C, 5% CO_2 incubator for 2 h. The cell suspension was filtered through a mesh with a pore size of 0.425mm, washed with normal saline containing 10×gentamicin/amphotericin B, centrifuged at 2000rpm for 15 min at 20°C–25°C, resuspended in MSC NutriStem in XF complete culture medium (IxCell, Shanghai, China) with 1×gentamicin/amphotericin B, and then seeded into a 100mm culture dish. On the fifth day, half of the medium was replaced with fresh medium, and complete medium replacement was conducted every 2 days thereafter. After 14–16 days of culture, cells were detached and isolated using TrypLE Select CTS (ThermoFisher, Waltham MA, USA). The cells were passaged and cultured until ~90% confluency and then collected and cryopreserved as a seed cell bank. Prior to transplantation, the cryopreserved cells were thawed, expanded, and harvested at passage 4. A total of 25 million IxCell hUC-MSCs per vial were cryopreserved in CryoStor CS5, a serum-free, animal component-free and defined cryopreservation medium containing 5% dimethyl sulfoxide (BioLife Solutions, USA) and stored in liquid nitrogen.

Sterility, viability and genetic stability of the cells were assessed routinely. Cell sterility testing was performed using membrane filtration method in accordance with the rule 1101 of Chinese Pharmacopoeia. Absence of pathogenic microorganisms such as

mycoplasms, treponema pallidum, HIV-1, HBV, HCV, cytomegalovirus, Epstein-Barr virus, parvovirus B19, human T-lymphotropic virus I (HTLV-1) and HTLV-2, were confirmed by fluorescence real-time quantitative PCR (qPCR). Chromosome karyotyping was performed using the G-band colorimetric method. The cells expressed CD105, CD73, and CD90 surface markers and showed potency of osteogenesis, chondrogenesis and adipogenesis (see cell characterization in Figure S9), consistent with the International Society for Cellular Therapy definitions.[41]

METHOD DETAILS

MSC transplantation

Before infusion, MSCs were thawed using an automated thawing device (BioLife Solutions, USA, Serial. No. CFT205108), transferred to 50 mL normal saline supplemented with 0.25% human albumin using 5 mL syringes under sterile conditions at room temperature. A cell viability above 90% was confirmed by AO-DAPI staining. Eligible patients were enrolled into three different dosage groups sequentially (50, 100, 200 million UC-MSCs), with 6 patients enrolled for each group. A single dose of UC-MSC suspension was intravenously transplanted within two hours after preparation at a speed of 1–2 mL/min using a blood transfusion set with a filter of 170 μm pore size (see Figure S10 for illustration).

Endpoints

The primary endpoint of this study was the safety and tolerability of UC-MSCs, which were reflected by the occurrence of AEs and SAEs, changes in laboratory tests (blood routine, biochemical test, etc.) and others such as electrocardiography during the 24-week follow-up. AEs after cell transplantation were recorded and evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 guidelines. The grading of AEs and their correlation with UC-MSC treatment were evaluated by the investigators.

The secondary endpoint was the preliminary efficacy of UC-MSC treatment, which was evaluated by changes in mRS, BI, NIHSS, FM scores and infarct volume at 12 and 24 weeks after UC-MSC transplantation, comparing to those at baseline. The percentages of patients with BI $\geq$ 95 at 12 and 24 weeks post-treatment were also recorded. The mRS score ranged from 0 (no symptom) to 6 (death), with higher scores indicating worse outcome. Exploratory endpoints were the peripheral immunological changes after UC-MSC treatment.

Imaging

Brain MRI was performed before and 4, 12, 24 weeks after UC-MSC transplantation using a 3.0 T MRI scanner (SIGNA Premier, GE Healthcare, Milwaukee, WI, USA) equipped with a 32-channel head phased-array coil with the following parameters: T1-weighted imaging (T1WI): repetition time (TR)/time to echo (TE) = 120/2ms, flip angle = 70°, field of view (FOV) = 240 mm × 240 mm, matrix = 260 × 260, slice thickness/gap = 5/1mm, acceleration factor = 1; T2-weighted imaging (T2WI): TR/TE = 4500/90ms, flip angle = 140°, FOV = 240 mm × 240 mm, matrix = 320 × 320, slice thickness/gap = 5/1mm, acceleration factor = 1.5; FLAIR: TR/TE = 8500/90ms, inversion time (TI) = 2400ms, flip angle = 160°, FOV = 240 mm × 240 mm, matrix = 256 × 256, slice thickness/gap = 5/1 mm, acceleration factor = 1; diffusion-weighted imaging (DWI): TR/TE = 2000/60ms, flip angle = 90°, FOV = 240 mm × 240 mm, matrix = 160 × 160, slice thickness/gap = 5/1mm, acceleration factor = 1, b values = 0, 1000s/mm2. Apparent diffusion coefficient (ADC) was calculated using the monoexponential model: Sb/S0 = exp (-b*ADC), where Sb and S0 represent the signal intensity with (b = 1000s/mm2) and without (b = 0s/mm2) the application of diffusion gradient, respectively. A total of 20 slices were acquired for each sequence. Infarct volume was measured on fluid attenuated inversion recovery (FLAIR) sequence using ITK-SNAP by a blinded, experienced neuroradiologist.[42] The results were validated by a second, independent rater, showing excellent inter-rater reproducibility (intraclass correlation coefficient = 0.995, 95% CI: 0.992–0.997).

Immunological tests

Lymphocyte subpopulations and inflammatory cytokines from the patients' peripheral blood before and after UC-MSC treatment were measured to investigate the peripheral immunological changes. Briefly, blood samples were collected from the patients at each visiting point, stained with BD Multi-test 6-color TBNK reagent for 15 min and then treated with lysing solution (BD Biosciences, USA) for 15 min. The stained cells were analyzed by FACS Canto II cytometry (BD Biosciences, USA) for lymphocyte subpopulation analysis. The concentrations of 12 cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12P70, IL-17, IFN-α, IFN-γ and TNF-α) in the patients' peripheral blood plasma were quantitatively analyzed by flow cytometry-based immunofluorescence using a 12-plex assay kit (Jiangxi CellGene Biotech Co. Ltd, China). Complements (C3, C4), immunoglobulins (IgA, IgE, IgG, IgM), and C-reaction protein levels were quantified using immunoturbidimetry (Beckman Coulter, USA).

QUANTIFICATION AND STATISTICAL ANALYSIS

Given the exploratory nature of this phase I study, a conventional sample size was selected to evaluate safety and feasibility without formal power calculation. Statistical analyses were mainly descriptive and performed using SAS 9.4 (SAS Institute). Quantitative data were presented as mean ± standard deviation (SD) and/or median (IQR). The data were compared between groups using the

Student's *t* test, one-way ANOVA, Mann-Whitney U-test, or Kruskal-Wallis test, when appropriate. Categorical data were presented as n (percentages) and compared between groups using chi-square test or Fisher's Exact test. A general linear model or binary logistic regression was used to examine the treatment effects on the secondary endpoints, with adjustment for baseline NIHSS score. Bonferroni correction was performed to adjust for post hoc multiple comparisons. Time-group interactions were investigated using repeated measures ANOVA. Post hoc Pearson or Spearman correlation analyses were performed to investigate the relationship between immunological parameters and secondary outcomes. All analyses were two-tailed, and $p < 0.05$ was considered statistically significant.

ADDITIONAL RESOURCES

The trial was registered on ClinicalTrials.gov as NCT 05697718.