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A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-161, allogeneic human dental Pulp stem cells, in patients with Acute Ischemic stRoke (J-REPAIR)

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Abstract

Introduction: JTR-161 is a novel allogeneic human cell product consisting of dental pulp stem cells isolated from the extracted teeth of healthy adults. It is currently under development as a cell-based therapy for ischemic stroke. The aim of this study is to evaluate the safety and efficacy of JTR-161 in patients with acute ischemic stroke when given as a single intravenous administration within 48 hours of symptom onset.

Methods and analysis: This is the first-in-human, randomized, double-blind, placebo-controlled, multicenter clinical trial to be conducted in Japan (from December 2018 to July 2021). Patients with a clinical diagnosis of anterior circulation ischemic stroke with a National Institutes of Health Stroke Scale (NIHSS） score of 5–20 at baseline were enrolled. Patients previously treated with recombinant tissue-type plasminogen activator and/or endovascular thrombectomy were allowed to be enrolled. The study consists of three cohorts: cohorts 1 and 2 (each eight patients), and cohort 3 (60 patients). Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and 2, and in a 1:1 ratio in cohort 3. The number of cells administered was increased sequentially from 1 x 10^8 (cohort 1) to 3 x 10^8 (cohort 2). In cohort 3, the higher tolerated dose among the two cohorts was administered. The primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all of the following criteria at day 91 in cohort 3: modified Rankin Scale (mRS) ≤ 1, NIHSS ≤ 1, and Barthel Index (BI) ≥ 95.

Ethics and dissemination: The study protocol and informed consent form were approved by the institutional review board at each participating study site. A manuscript with the results of the primary study will be published in a peer-reviewed journal.

Trial registration: Clinical Trials.gov: NCT04608838

Strengths and limitations of this study

- This study is the first-in-human, randomized, double-blind, placebo-controlled clinical trial of a cell-based therapy for ischemic stroke using JTR-161, a novel allogeneic human cell product consisting of dental pulp stem cells.
- The study consists of three cohorts; patients received 1 x 10^8 cells in cohort 1, 3 x 10^8 cells in cohort 2, and the higher tolerated dose among the two cohorts (either 1 x 10^8 cells or 3 x 10^8 cells) in cohort 3.
- The results of this study will be used to determine the safe dose of JTR-161 administered as a single intravenous dose within 48 hours of symptom onset.
- Primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all of the following criteria at day 91 at the optimized dose: modified Rankin Scale ≤ 1, NIHSS ≤ 1, and Barthel Index ≥ 95.
- This is a proof-of-concept study; therefore, further study will be required.

INTRODUCTION

Stroke is the most prevalent cerebrovascular disease worldwide, and still one of the leading causes of death and severe disability. Ischemic stroke accounts for about 80% of all stroke events.[1] The recent advances in reperfusion therapy using endovascular thrombectomy have allowed its benefits to be expanded to a larger population of patients with large vessel occlusion. However, the rate of favorable clinical outcomes remains low[2,3], underscoring an unmet clinical need for adjunctive neuroprotective treatments. Among them, cell-based therapies using human somatic stem cells have been attracting attention, and there are ongoing clinical studies investigating the use of intravenous or intracerebral human somatic stem cells, mainly using bone marrow-derived mesenchymal stem cells (BM-MSCs), in patients with ischemic stroke from the acute to the chronic phase.[4-7]

In 2000, human dental pulp stem cells (DPSCs) were discovered in impacted molar teeth.[8] DPSCs are thought to originate from the cranial neural crest derived from the neuroectoderm, thus they express early markers for both mesenchymal and neuroectodermal stem cells.[9,10] DPSCs can secrete various neurotrophic factors such as neurotrophin-3, brain-derived neurotrophic factor, and vascular endothelial growth factor, which promote neuronal survival, proliferation, differentiation, and migration.[10] Furthermore, compared to BM-MSCs, DPSCs can be obtained by a less invasive process, are more easily expanded, and exert more potent immunosuppressive effects via the inhibition of activated T cell responses[11], which makes them attractive for use in allogeneic transplantation. Some studies have shown the beneficial effects of human DPSC transplantation in animal models of neurological disease[12,13].

JTR-161 is an allogeneic cell-based product consisting of human DPSCs isolated from the extracted teeth of healthy adults. In the preclinical study, intravenous administration of DPSCs decreased ischemic damage and promoted functional improvement in a rodent model of focal cerebral ischemia by modulating neuroinflammatory reactions.[14,15] Here, we report the protocol of the first-in-human clinical trial of JTR-161 in patients with acute ischemic stroke.

METHODS AND ANALYSIS

Study design

This is A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-

161, allogeneic human DPSCs, in patients with Acute Ischemic stRoke (J-REPAIR study). The aims of the study are to evaluate the efficacy and safety of JTR-161 in Japanese patients with acute ischemic stroke when given as a single intravenous administration. Patients received 1 x 10^8 cells in cohort 1, and 3 x 10^8 cells in cohort 2, sequentially. In cohort 3, the higher tolerated dose among the two cohorts (either 1 x 10^8 cells or 3 x 10^8 cells), determined according to the recommendation by the Data and Safety Monitoring Board (DSMB) (figure 1), was administered. The DSMB consists of three independent external experts and recommends advancing to the next cohort only when no product-related serious adverse events (AEs) are observed. The DSMB does not recommend advancing to the next cohort when two or more deaths occur in the same cohort or any other serious safety concerns are reported. Death due to cerebral infarction itself including concomitant symptoms, pretreatment with intravenous recombinant tissue-type plasminogen activator (rt-PA) or endovascular treatment, and combination treatment for the primary disease are excluded as causes of death in this study. The study schedule and assessments are shown in table 1.

Each cohort consists of a 91-day observation period and a 275-day follow-up period (total study period: 366 days). Patients were recruited from 29 stroke centers in Japan between December 2018 and July 2021. The study has been registered in Clinical Trials.gov: NCT04608838 prior to study patient enrollment.

Patient population

Inclusion criteria

Patients who met all the following criteria were included:

- Japanese male or female patients 20 years of age or older;
- Clinical diagnosis of anterior circulation ischemic stroke based on the results of brain magnetic resonance imaging (MRI) or computed tomography (CT);
- National Institutes of Health Stroke Scale (NIHSS) score of ≥5 to ≤20 at screening;
- Onset of ischemic stroke had to have occurred within 48 hours prior to the start of administration of the study product; and
- A modified Rankin Scale (mRS) of 0 or 1, by either self-report or family report, prior to ischemic stroke onset.

Table 1 Schedule for assessments

		Assessment period															Follow-up period		Discharge	Termination
		Pre-observation period				Observation period														
						Day 1														
			Pre-enrolment	Qualification	Pre-dosing	0 h	1 h	2 h	4 h	6 h	12 h	Day 2	Day 3	Day 8	Day 31	Day 91	Day 181	Day 366		
Informed consent		x																		
Patient characteristics			x[5]																	
Administration of study product						x														
Ability assessment	mRS		x[6]												x	x		x	x	
	Barthel Index														x	x		x		
Function assessment	NIHSS		x[7]	x[8]								x		x	x	x			x	
QOL assessment	EQ-5D-5L														x	x		x		
Clinical laboratory tests	Hematology		x[7]		x							x	x	x	x	x	x	x		x
	Biochemistry		x[7]		x							x	x	x	x	x	x	x		x
	Blood coagulation test		x[7]		x							x	x	x	x	x	x	x		x
	Biomarker[1]				x								x	x						
	Urinalysis		x[7]		x							x	x	x	x	x	x	x		x
Imaging examinations	Safety assessment		x[7]									x		x[10]	x					
	Infarct volume[2]				x[9]									x[10]	x					
	Penumbra region volume[2, 3]				x															
Body measurements	Height, weight		x[7]																	
Vital signs	Blood pressure, pulse		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Body temperature		x		x			x	x	x		x	x	x	x	x	x	x	x	x
Oxygen saturation	SpO_2[4]		x		x		x	x	x	x	x	x	x	x	x	x	x	x		x
Medical examination	Medical examination and interview		x		x							x	x	x	x	x	x	x	x	x

1. Assessed in the cohort 3 only.
2. Assessed at the central imaging analysis organization
3. Performed at some study sites.
4. In addition to the scheduled period in the table, SpO_2 is assessed at 15 min , 30 min, 45 min, 1h 15 min, 1h 30 min, 1 h 45 min, 2 h 15 min, 2 h 30 min, 2 h 45 min, 3 h 15 min, 3 h, 3 h 15 min, 3 h 30 min, 3 h 45 min, 4 h 30 min, 5 h, and 5 h 30 min post-dose.
5. Pregnancy test is performed in premenopausal women or unknown women whether menopause
6. The mRS before ischemic stroke onset is assessed based on interview from patients or their family.
7. Data before obtaining consent are acceptable.
8. Assessed at least 4 hours after enrolment.
9. Imaging data after standard treatment are accepted for patients who have undergone standard treatment (rt-PA intravenous or endovascular treatment).
10. Assessed once during Day 5 to Day 8.

mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; QOL, quality of life; ; SpO2, oxygen salutation of peripheral artery

Exclusion criteria

Patients who met one or more of the following criteria were excluded:

- Presence of a new ischemic lesion in the cerebellum or brainstem at screening;
- A marked decline in level of consciousness (NIHSS 1a. evaluation of consciousness level is score of 3) at screening;
- Patients who had an extensive infarct and for whom maintaining life was expected to be difficult, or who were expected to undergo cranial decompression at screening;
- Presence of intracranial hemorrhagic change diagnosed by brain imaging which was judged to be clinically important by the investigator at screening;
- Convulsions after onset of ischemic stroke;
- History of neurological events such as stroke or clinically significant head trauma within 180 days prior to informed consent (IC);
- Systolic blood pressure >220 mmHg or diastolic blood pressure >120 mmHg, with or without antihypertensive treatment at screening;
- Blood glucose level <50 mg/dL or >400 mg/dL at screening;
- Patients who had any of the serious complication(s) listed below at screening:
  - End stage kidney disease for which dialysis was required;
  - Progressive liver disease such as hepatitis, cirrhosis with Child-Pugh classification class B or C, or liver dysfunction with aspartate aminotransferase or alanine aminotransferase over three times the upper limit of the standard value of the study site;
  - Severe congestive heart failure rated as New York Heart Association class III or IV, active unstable angina, or ventricular dysfunction with left ventricular ejection fraction (LVEF) <30%; or
  - Severe pulmonary dysfunction requiring home oxygen therapy.
- Human immunodeficiency virus infection, ongoing systemic infection, severe local infection, or immunocompromised condition at screening;
- Alzheimer's disease or other dementias, or any other neurological disorder that was judged to affect their ability to give consent to participate in the trial or could confound study assessments performed by the investigator at screening;

- Malignant tumor(s) or history of malignant tumor(s) prior to 2 years of ischemic stroke onset at screening;
- Contraindications for MRI such as implanted pacemakers or other metallic prosthesis incompatible with MRI, or claustrophobia;
- Thrombocytopenia (platelet count <100,000/mm^3) or heparin-induced thrombocytopenia at screening;
- History of allergies to human tissues, bovine or porcine preparations;
- History of allergy to streptomycin;
- Patients who participated in other clinical trials within 12 weeks prior to IC, or planned to participate in other clinical trials during this trial, or participated in clinical trials of other cell products in the past;
- History of splenectomy;
- Patients who might have a transient ischemic attack;
- Patients who were scheduled to undergo revascularization treatment including carotid endarterectomy, stenting, etc. by the end of the evaluation (day 91);
- Patients who were pregnant or lactating at screening, or who wished to become pregnant during the study;
- Patients who could not use extremely effective contraception including intrauterine device, intrauterine system, oral contraception (low dose pill), surgical sterilization, double barrier method (condom with spermicide, or combination of condom with pessary) under the guidance of the investigator from the time of IC to one year post-dose (day 366), or who had a partner who could not take similar contraceptive measures; or
- Patients who the investigator considered to be inappropriate for inclusion in the study.

Exclusion criteria on eligibility confirmation assessment

After eligibility assessment at screening, the investigator assessed NIHSS again ≥4 h after the assessment at screening to confirm patient eligibility. Patients who met one or more of the following criteria were excluded:

- NIHSS score ≤4 or ≥21;

- Change in NIHSS score from screening ≥5;
- Administration of the study product could not be started within 48 h of symptom onset; or
- Patients who the investigator considered to be inappropriate for inclusion in the study.

Randomization and blinding

Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and 2. In cohort 3, subjects were randomly assigned in a 1:1 ratio to receive either JTR-161 or placebo. Randomization was performed by the minimization method, which was adjusted centrally by dynamic assignment with NIHSS at the time of eligibility assessment, with / without standard treatment including intravenous rt-PA or endovascular treatment, and age at the time of IC as the allocation factors. The randomization sequence was generated by an organization independent of the study sponsors. Allocation of treatment to subjects was randomized via a website. The investigators, patients, and the sponsor are masked to the treatment assignment until the observation period is completed. After the final subject in cohort 3 completes the day 91 assessment, the database will be fixed, and the key will be opened. After that, the sponsor, statistical analysts, and unblinded personnel will be placed under open blind, and patients and assessors will be blinded until the end of the follow-up period (day 366). JTR-161 and placebo can be identified by the vial appearance; therefore, to ensure masking is maintained, only unblinded persons appointed by the investigator prepared the administration solution, intravenously injected the study product into the patient, and cleaned up any spilled administration solution.

Procedure

JTR-161 was manufactured in accordance with good manufacturing practice by JCR Pharmaceuticals Co., Ltd. The JTR-161 vial (5.0 mL) contained 1.0 x 10^8 cells of DPSC isolated from the extracted teeth of healthy adults, and was stored in the gas space of a liquid nitrogen refrigerator.

The frozen study product was thawed in a constant temperature bath at 37 ± 1 ° C for about five minutes, then the required number of cells (one or three vials) was diluted in 100 mL of saline. The solution was intravenously administered once at a rate of 4 mL/min but ≤ 6 mL/min within 48 h of

symptom onset. Number of cells administered in each cohort and flow chart of the cohorts are shown in figure 1. The DSMB was primarily involved in deciding whether or not to advance to the next cohort, as well as the dose (number of cells) for cohort 3. Surgical revascularization such as carotid endarterectomy and carotid artery stenting was prohibited during the observation period, and attending any clinical trials other than this study was prohibited until the end of the study. In cohorts 1 and 2, the administration interval between subjects was ≥ 72 hours.

Baseline assessments were carried out at day 0 prior to administration, including (1) primary disease: initial or recurrent, type of cerebral infarction, infarcted blood vessels, onset time, and diffusion-weighted imaging (DWI) -Alberta Stroke Program Early Computed Tomography Score, (2) with/without standard treatment with intravenous rt-PA or endovascular treatment. If yes, treatment start time (endovascular treatment only), degree of recanalization (modified thrombolysis in cerebral infarction classification), recanalization time, and number of passes. If no, reasons for not implementing standard treatment, (3) NIHSS at time of arrival, pre-registration, and eligibility tests, (4) mRS before the onset of cerebral infarction reported by patients or her/his family, (5) disease history related to the exclusion criteria and, where relevant, the time of complete cure of any malignant condition, effected at least 2 years before IC and still considered cured at the start of administration of the study product. In addition, a medical history deemed necessary for considering AEs was taken. After administration of the study product, mRS and Barthel Index (BI) were assessed at days 31, 91, and 366. NIHSS was assessed at days 2, 8, 31, and 91, and on the day of discharge. Patients were asked to answer the EuroQOL 5 dimensions 5-level scores (EQ-5D-5L) questionnaire at days 31, 91, and 336. Laboratory tests were performed pre-registration, pre-administration, and on days 2, 3, 8, 31, 91, 181, and 366 after administration. Blood pressures including systolic and diastolic blood pressures and pulse rates were measured pre-registration, pre-administration, 1, 2, 4, 6, 12, and 24 hours after administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Body temperature was measured pre-registration, pre-administration, 2, 4, 6, and 24 hours after administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Saturated oxygen was measured pre-registration, pre-administration, every 15 minutes between one and four hours after administration, every 30 minutes between four and six hours after administration, 12 and 24 hours after administration, and on days 3, 8, 31, 91, 181, and 366 after administration.

Imaging tests were performed pre-registration, and on days 2, 8, and 31 after administration. Serum cytokines and growth factors including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-10, IL-17, IL-23, and angiopoietin-1 (Ang-1) were measured pre-administration, and on days 3 and 8 after administration in cohort 3. Infarct volumes were measured on DWI and/or fluid-attenuated inversion recovery using MRI pre-administration, and on days 8 and 31 after administration. Ischemic penumbra was measured using MRI as the mismatch between the hypoperfused area on perfusion-weighted imaging and the abnormal area on DWI pre-administration, if available. Assessment of imaging was performed at the central assessment organization. Discontinuance criteria for individual subjects were (1) AEs, worsening of complications, and other safety concerns, (2) no visit to the study site due to inconvenience to patients, (3) termination of the study by the sponsor, and (4) termination of the study by the investigator due to safety concerns regarding the study product.

Outcome measures

The primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all of the following criteria at day 91 in cohort 3: mRS ≤ 1, NIHSS ≤ 1, and BI ≥ 95. Secondary endpoints were (1) proportion of patients who achieve mRS ≤ 1 or mRS ≤ 2 at days 91 and 366, (2) proportion of patients who achieve BI ≥ 95 at days 91 and 366, (3) proportion of patients who achieve NIHSS ≤ 1, who achieve improvement of ≥ 75%, and who achieve improvement of ≥ 10 points at day 91, (4) changes in EQ-5D-5L scores at day 366, (5) proportion of patients who achieve an excellent outcome (mRS ≤ 1, NIHSS ≤ 1, and BI ≥ 95) at day 91. EQ-5D-5L consists of two parts: the EQ-5D descriptive system and the EQ visual analogue scale (VAS). The descriptive system consists of five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has five levels: 1 = "no problems", 2 = "slight problems", 3 = "moderate problems", 4 = "severe problems", and 5 = "extreme problems". The EQ VAS was recorded during the patient's self-rated health assessment on a vertical VAS, where the endpoints were labelled 'The best health you can imagine' and 'The worst health you can imagine', (6) proportion of patients who achieve an excellent outcome (mRS ≤ 2, improvement in NIHSS ≥ 75% , and BI ≥ 95) at day 91. Safety was assessed based on AEs, laboratory tests, vital signs, transcutaneous oxygen saturation, and imaging test including MRI or CT. The investigator assessed

the intensity, severity, and relatedness of an AE. All serious AEs were reported using a standardized SAE report form. Exploratory assessments were (1) cytokines and growth factors such as TNF-α, IL-1β, IL-6, IL-10, IL-17, IL-23, and Ang-1 as biomarkers in cohort 3, (2) infarct volumes, and (3) penumbra area volume if available.

Data monitoring body

All data were collected via an electronic case report form prepared using Rave® (Medidata Solutions Japan, Tokyo, Japan). Periodic monitoring was performed independently by the sponsor during the trial in order to confirm that the trial was conducted in accordance with the study protocol.

Sample size estimates

In cohorts 1 and 2, eight subjects per cohort (JTR-161, n = 6; placebo, n = 2) were set as the appropriate number of subjects for the safety evaluation. In cohort 3, 60 subjects (JTR-161, n = 30; placebo, n = 30) were set as the number sufficient for designing a future clinical trial based on the safety and efficacy data even if a subpopulation analysis is performed.

Statistical analyses

Efficacy analyses will be performed in the full analysis set (FAS); the population of enrolled patients who will have received the study product at least once and have had a post-dose efficacy assessment, and secondary endpoints will be assessed in the per protocol set (PPS); the FAS population excluding those patients with a significant protocol violation. The safety analysis will be performed for patients in the safety analysis set (SAF); the population of all enrolled patients who will receive the study product and have a post-dose safety assessment. Categorical variables of patient characteristics and baseline parameters will be aggregated for each treatment group and cohort, and descriptive statistics will be calculated for continuous variables. Comparison analysis will be performed between the JTR-161 and placebo groups in cohort 3, and between the merged JTR-161 groups of cohort 3 and the cohort receiving the same dose as cohort 3, and the merged placebo groups of cohorts 1, 2, and 3. As for the primary endpoint, the proportions and their confidence intervals will be calculated for each administration group. Also, the point estimates of difference in the proportion and its confidence

interval will be calculated and compared between the JTR-161 and placebo groups. As for secondary endpoints, the proportions and their confidence intervals for mRS, BI, and NIHSS will be calculated for each administration group, and point estimates of the difference in the proportions and its confidence interval will be calculated. The common odds ratio of the mRS will be calculated for each administration group, and the distribution in each category will be shown. Descriptive statistics of mRS, BI, EQ-5D-5L, biomarkers, infarct volumes, and penumbra area volume at the time of assessments will be calculated for each treatment group.

For AEs and adverse drug reactions for each administration group, the number of patients, the number of cases, and the rate of occurrence will be tabulated according to degree of seriousness, severity, and time of onset. AEs will be listed according to MedDRA as lowest level term, and are similarly aggregated using the system organ class and preferred term. For laboratory tests, vital signs, and oxygen saturation, descriptive statistics will be calculated or tabulated for each administration group and each test time point. The presence or absence of abnormal fluctuations for each test item in individual cases will be summarized. No adjustment for multiplicity will be performed. The two-sided significance level will be set at 5%. Interval estimation will be calculated with a confidence coefficient of 95%.

Study organization and funding

The study was designed and conducted by the sponsor, Teijin Pharma Ltd., Tokyo, Japan in collaboration with the principal investigators. The sponsor monitored study conduct, collected the data, and performed the statistical analyses. This study is funded by Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.

Patient and public involvement

No patients and/or public were involved in setting the research questions nor they were involved in developing plans for the design (or implementation) of this study protocol.

Ethics and dissemination

The study protocol and IC form were approved by the institutional review board at each participating

study site. All patients gave written IC before initiation of any study-specific procedures. IC from proxies was also allowed due to the pathophysiology of patients with acute cerebral infarction. The study was conducted in accordance with the ethical principles originating in or derived from the Declaration of Helsinki and Good Clinical Practice guidelines. A manuscript with the results of the primary study will be published in a peer-reviewed journal. On completion of the trial, and after publication of the primary manuscript, data requests can be submitted to the corresponding author.

DISCUSSION

Bone marrow is a major source of stem cells and systemic delivery of BM-MSCs after cerebral ischemia has been widely studied.[4-7] While collection of BM-MSCs requires invasive bone marrow puncture, DPSCs can be obtained easily and less invasively from the extracted teeth of healthy adults. They exhibit better plasticity and proliferation capability, and have more potent immunoregulatory effects.[11,16,17] This J-REPAIR study is the first-in-human, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of JTR-161 in patients with acute ischemic stroke. Patients were selected as participants in this first-in-human study from the viewpoint of invasiveness and unknown risk of DPSCs to the subjects, referring to the "Guidance on quality, and technical guidance on conducting non-clinical trials and clinical trials of regenerative medicine products (human cell processed products)".[18] The eligible patients were restricted to those with anterior circulation ischemic stroke because the severity of their symptoms can be assessed using NIHSS[19], one of the key criteria for assessing eligibility and efficacy in our study. It is difficult to confirm the accurate etiology of stroke on admission; therefore, there is no limitation regarding stroke subtype such as lacuna, atherothrombotic, cardioembolic, and others. Our study did not limit the use of standard treatment including intravenous rt-PA and/or endovascular thrombectomy for recruitment. In addition, available treatments for acute ischemic stroke except revascularization treatment such as carotid endarterectomy and stenting in routine clinical practice were allowed to be used as a combination therapy. Patients to whom standard treatment could not be given, and patients who received standard treatment but had a NIHSS $\geq$ 5 were allowed to be enrolled. However, these pretreatment and combination therapies may make it difficult to evaluate the safety and efficacy of JTR-161 accurately; therefore, a placebo arm was established as a control group. The study is conducted in a double-blinded manner during the

observation period. The keys were opened to the sponsor, statistical analysts, and unblinded personnel, but patients and assessors continued under blind conditions until the end of the follow-up period, since EQ-5D-5L was assessed at day 366. In order to explore the therapeutic time window, timing of administration was set to be within 48 h of symptom onset.

The proportion of subjects who achieve an excellent outcome defined as mRS ≤ 1, NIHSS ≤ 1, and BI ≥ 95 was set as the primary endpoint because we considered this clinical outcome was the most accurate way of detecting any difference in effectiveness between the subjects receiving JTR-161 and the placebo group. As secondary endpoints, the efficacy of JTR-161 was also evaluated using mRS and BI for disability assessments, and NIHSS for function assessment, all of which are widely accepted for use as endpoints in clinical trials of acute ischemic stroke.[20] In recent clinical trials of intravenous rt-PA and endovascular treatment, clinical outcomes as per mRS were evaluated 90 days after the start of treatment.[21,22] Similarly, period during which the efficacy of JTR-161 was evaluated was set to 90 days after administration of the study product. EQ-5D-5L was used as a patient-reported outcome for evaluating patient health status. It is reported that there was a significant correlation between stroke type and severity, and EQ-5D-5L scores; reproducibility and validity have been verified in stroke patients.[23] We measured a variety of serum cytokines and growth factors before and after transplantation of JTR-161 to investigate the mechanism of human DPSCs on acute ischemic stroke.

In a pre-clinical study, the distribution of JTR-161 labelled with a radioactive tracer was highest in the lung two hours after a single intravenous administration (in-house data), as reported in other types of stem cells.[24] The onset of symptoms such as respiratory distress and decreased oxygen saturation should be carefully followed immediately after administration of JTR-161. Oxygen saturation was measured every 15 minutes for up to 4 hours and every 30 minutes for up to 6 hours after administration. Imaging tests were performed to assess infarct lesions and the presence or absence of significant hemorrhagic changes. On the other hand, time of disappearance of JTR-161 from the body has not been elucidated. Therefore, we established a follow-up period of up to one year after administration (day 366).

In conclusion, JTR-161 will provide a novel therapeutic option for the treatment of patients with ischemic stroke due to the wider therapeutic time window for human DPSC transplantation.

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Authors' contributions

All authors were involved in the study design, protocol preparation, and acquisition of funding. SS and CN were responsible for the first draft. All authors have reviewed and approved the final manuscript. The work is funded by Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.

Declaration of conflicts of interest

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Expert Witness from Teijin Pharma Ltd. (SS, CN, KK). Research funding from Teijin Pharma Ltd. (KK). Lecture fee from Teijin Pharma Ltd. (YI). The other authors report no conflicts.

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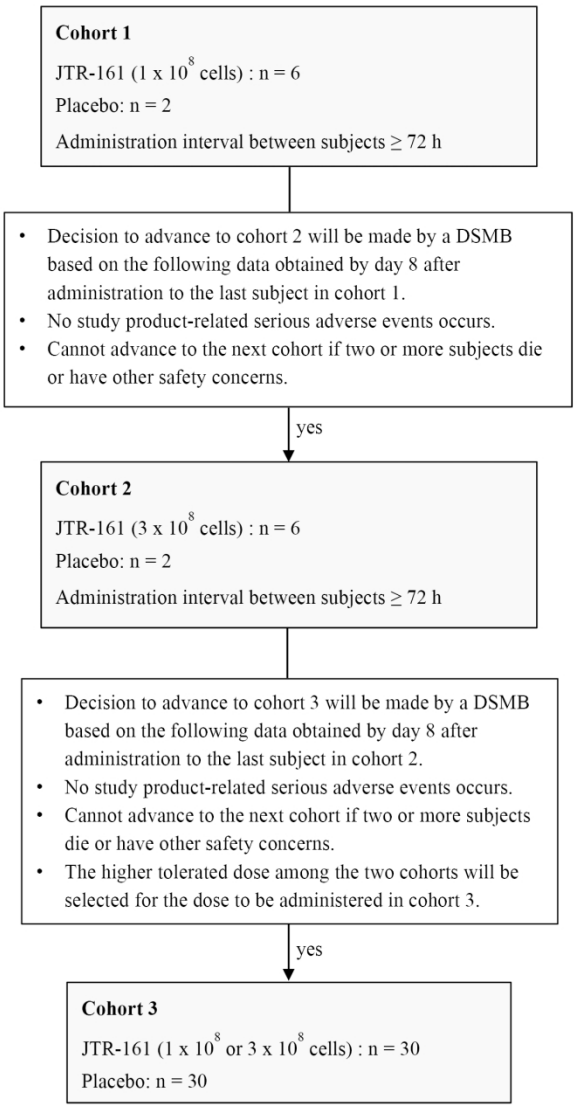

Figure 1

Figure 1

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	3	Date and version identifier (Issue date: 9 Jul 2019, Protocol amendment number: 04)	-
Funding	4	Sources and types of financial, material, and other support	13
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 16
	5b	Name and contact information for the trial sponsor	13
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	13
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	9-10, Figure 1

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
	6b	Explanation for choice of comparators	14
Objectives	7	Specific objectives or hypotheses	5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5-6, Figure1
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10, Figure1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	9-11, Figure1
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10,14
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6, Table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	9-11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	-
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12-13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	12
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6, Figure 1
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	-
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	-
	31b	Authorship eligibility guidelines and any intended use of professional writers	15-16
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-161, allogeneic human dental Pulp stem cells, in patients with Acute Ischemic stRoke (J-REPAIR)

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A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-161, allogeneic human dental Pulp stem cells, in patients with Acute Ischemic stRoke (J-REPAIR)

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Abstract

Introduction: JTR-161 is a novel allogeneic human cell product consisting of dental pulp stem cells isolated from the extracted teeth of healthy adults. It is currently under development as a cell-based therapy for ischemic stroke. The aim of this study is to evaluate the safety and efficacy of JTR-161 in patients with acute ischemic stroke when given as a single intravenous administration within 48 hours of symptom onset.

Methods and analysis: This is a first-in-human, randomized, double-blind, placebo-controlled, multicenter, phase 1/2 clinical trial to be conducted in Japan (from January 2019 to July 2021). Patients with a clinical diagnosis of anterior circulation ischemic stroke with a National Institutes of Health Stroke Scale (NIHSS） score of 5–20 at baseline were enrolled. Patients previously treated with recombinant tissue-type plasminogen activator and/or endovascular thrombectomy were allowed to be enrolled. The study consists of three cohorts: cohorts 1 and 2 (each eight patients), and cohort 3 (60 patients). Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and 2, and in a 1:1 ratio in cohort 3. The number of cells administered was increased sequentially from 1 x 10^8 (cohort 1) to 3 x 10^8 (cohort 2). In cohort 3, the higher tolerated dose among the two cohorts was administered. The primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all of the following criteria at day 91 in cohort 3: modified Rankin Scale ≤ 1, NIHSS ≤ 1, and Barthel Index ≥ 95.

Ethics and dissemination: The protocol and informed consent form were approved by the institutional review board at each participating study site. A manuscript with the results of the primary study will be published in a peer-reviewed journal.

Trial registration: JapicCTI-194570 and Clinical Trials. gov: NCT04608838

Strengths and limitations of this study

- This study is a first-in-human, randomized, double-blind, placebo-controlled phase 1/2 clinical trial of a cell-based therapy for ischemic stroke using JTR-161, a novel allogeneic human cell product consisting of dental pulp stem cells.
- The study consists of three cohorts; patients received 1 x 10^8 cells in cohort 1, 3 x 10^8 cells in cohort 2, and the higher tolerated dose among the two cohorts (either 1 x 10^8 cells or 3 x 10^8 cells) in cohort 3.
- The results of this study will be used to determine the safe dose of JTR-161 administered as a single intravenous dose within 48 hours of symptom onset.
- Primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all of the following criteria at day 91 at the optimized dose: modified Rankin Scale ≤ 1, NIHSS ≤ 1, and Barthel Index ≥ 95.
- This is a proof-of-concept study; therefore, further study will be required.

INTRODUCTION

Stroke is the most prevalent cerebrovascular disease worldwide, and still one of the leading causes of death and severe disability. Ischemic stroke accounts for about 80% of all stroke events.[1,2] The recent advances in reperfusion therapy using endovascular thrombectomy have allowed its benefits to be expanded to a larger population of patients with large vessel occlusion. However, the rate of favorable clinical outcomes remains low[3,4], underscoring an unmet clinical need for adjunctive neuroprotective treatments. Among them, cell-based therapies using human somatic stem cells have been attracting attention, and there are ongoing clinical studies investigating the use of intravenous or intracerebral human somatic stem cells, mainly using bone marrow-derived mesenchymal stem cells (BM-MSCs), in patients with ischemic stroke from the acute to the chronic phase.[5-8] Administration of human BM-MSCs was safe and well tolerated in patients with acute ischemic stroke, but no significant clinical improvement was observed.[7,8]

In 2000, human dental pulp stem cells (DPSCs) were discovered in impacted molar teeth.[9] DPSCs are thought to originate from the cranial neural crest derived from the neuroectoderm, thus they express early markers for both mesenchymal and neuroectodermal stem cells.[10,11] DPSCs can secrete various neurotrophic factors such as neurotrophin-3, brain-derived neurotrophic factor, and vascular endothelial growth factor, which promote neuronal survival, proliferation, differentiation, and migration.[11] Furthermore, compared to BM-MSCs, DPSCs can be obtained by a less invasive process, are more easily expanded, and exert more potent immunosuppressive effects via the inhibition of activated T cell responses[12], which makes them attractive for use in allogeneic transplantation. Several reports have shown the beneficial effects of human DPSC transplantation in animal models of neurological disease. [13,14] Sakai et al.[14] reported that human DPSC transplantation into the completely transected spinal cord of adult rats resulted in marked recovery of hind limb locomotor functions, whereas transplantation of human BM-MSC or skin-derived fibroblasts led to substantially less recovery of locomotor function. Based on a rat stroke model and an *in vitro* model of ischemia[15], human DPSCs are reported to be a better source of cell therapy for ischemic stroke than human BM-MSCs.

JTR-161 is an allogeneic cell-based product consisting of human DPSCs isolated from the extracted teeth of healthy adults. In the preclinical study, intravenous administration of DPSCs decreased

ischemic damage and promoted functional improvement in a rodent model of focal cerebral ischemia by modulating neuroinflammatory reactions.[16,17] Preclinical toxicological study of a single intravenous administration of JTR-161 to male and female nude rats showed no notable toxicological findings two weeks after administration (In house data). There were no notable findings regarding tumorigenicity 16 weeks after administration. Furthermore, no scaffold-independent proliferation ability was observed. Regarding non-cellular components of the study product and impurities derived from the manufacturing process, because the amount of residual impurities was low, there were negligible concerns regarding safety. Here, we report the protocol of the first-in-human clinical trial of JTR-161 in patients with acute ischemic stroke.

METHODS AND ANALYSIS

Study design

This is A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-161, allogeneic human DPSCs, in patients with Acute Ischemic stRoke (J-REPAIR study). The aims of this phase 1/2 study are to evaluate the efficacy and safety of JTR-161 in Japanese patients with acute ischemic stroke when given as a single intravenous administration. Patients received 1 x 10^8 cells in cohort 1, and 3 x 10^8 cells in cohort 2, sequentially. In cohort 3, the higher tolerated dose among the two cohorts (either 1 x 10^8 cells or 3 x 10^8 cells), determined according to the recommendation by the Data and Safety Monitoring Board (DSMB) (figure 1), was administered. The DSMB consists of three independent external experts and recommends advancing to the next cohort only when no product-related serious adverse events (AEs) are observed. The DSMB does not recommend advancing to the next cohort when two or more deaths occur in the same cohort or any other serious safety concerns are reported. Death due to cerebral infarction itself and concomitant disorders including transtentorial herniation, followed in frequency by pneumonia, cardiac causes, and pulmonary embolism, pretreatment with intravenous recombinant tissue-type plasminogen activator (rt-PA) or endovascular treatment, and combination treatment for the primary disease are excluded as causes of death in this study. The study schedule and assessments are shown in table 1.

Each cohort consists of a 91-day observation period and a 275-day follow-up period (total study period: 366 days). Patients were recruited from 29 stroke centers in Japan between January 2019 and July

2021. The study was registered as JapicCTI-194570, prior to study patient enrollment, and subsequently on Clinical Trials.gov: NCT04608838.

Patient population

Inclusion criteria

Patients who met all the following criteria were included:

- Japanese male or female patients 20 years of age or older;
- Clinical diagnosis of anterior circulation ischemic stroke based on the results of brain magnetic resonance imaging (MRI) or computed tomography (CT);
- National Institutes of Health Stroke Scale (NIHSS) score of ≥5 to ≤20 at screening;
- Onset of ischemic stroke had to have occurred within 48 hours prior to the start of administration of the study product; and
- A modified Rankin Scale (mRS) of 0 or 1, by either self-report or family report, prior to ischemic stroke onset.

Table 1 Schedule for assessments

		Assessment period															Follow-up period		Dis-charge	Termi-nation
		Pre-observation period				Observation period														
			Pre-enrolment	Qualification	Pre-dosing	Day 1						Day 2	Day 3	Day 8	Day 31	Day 91	Day 181	Day 366		
						0 h	1 h	2 h	4 h	6 h	12 h									
Informed consent		x																		
Patient characteristics			x[5]																	
Administration of study product						x														
Ability assessment	mRS		x[6]												x	x		x	x	
	Barthel Index														x	x		x		
Function assessment	NIHSS		x[7]	x[8]								x		x	x	x			x	
QOL assessment	EQ-5D-5L														x	x		x		
Clinical laboratory tests	Hematology		x[7]		x							x	x	x	x	x	x	x		x
	Biochemistry		x[7]		x							x	x	x	x	x	x	x		x
	Blood coagulation test		x[7]		x							x	x	x	x	x	x	x		x
	Biomarker[1]				x								x	x						
	Urinalysis		x[7]		x							x	x	x	x	x	x	x		x
Imaging examinations	Safety assessment		x[7]									x		x[10]	x					
	Infarct volume[2]				x[9]									x[10]	x					
	Penumbra region volume[2, 3]				x															
Body measurements	Height, weight		x[7]																	
Vital signs	Blood pressure, pulse		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Body temperature		x		x			x	x	x		x	x	x	x	x	x	x	x	x
Oxygen saturation	SpO_2[4]		x		x		x	x	x	x	x	x	x	x	x	x	x	x		x
Medical examination	Medical examination and interview		x		x							x	x	x	x	x	x	x	x	x

1. Assessed in the cohort 3 only.

2. Assessed at the central imaging analysis organization.

3. Performed at some study sites.

4. In addition to the scheduled period in the table, SpO_2 is assessed at 15 min , 30 min, 45 min, 1h 15 min, 1h 30 min, 1 h 45 min, 2 h 15 min, 2 h 30 min, 2 h 45 min, 3 h 15 min, 3 h, 3 h 15 min, 3 h 30 min, 3 h 45 min, 4 h 30 min, 5 h, and 5 h 30 min post-dose.

5. Pregnancy test is performed in premenopausal women or unknown women whether menopause.

6. The mRS before ischemic stroke onset is assessed based on interview from patients or their family.

7. Data before obtaining consent are acceptable.

8. Assessed at least 4 hours after enrolment.

9. Imaging data after standard treatment are accepted for patients who have undergone standard treatment (rt-PA intravenous or endovascular treatment).

10. Assessed once during Day 5 to Day 8.

mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; QOL, quality of life; SpO2, oxygen salutation of peripheral artery

Exclusion criteria

Patients who met one or more of the following criteria were excluded:

- Presence of a new ischemic lesion in the cerebellum or brainstem at screening;
- A marked decline in level of consciousness (NIHSS 1a. evaluation of consciousness level is score of 3) at screening;
- Patients who had an extensive infarct and for whom maintaining life was expected to be difficult, or who were expected to undergo cranial decompression at screening;
- Presence of intracranial hemorrhagic change diagnosed by brain imaging which was judged to be clinically important by the investigator at screening;
- Convulsions after onset of ischemic stroke;
- History of neurological events such as stroke or clinically significant head trauma within 180 days prior to informed consent (IC);
- Systolic blood pressure >220 mmHg or diastolic blood pressure >120 mmHg, with or without antihypertensive treatment at screening;
- Blood glucose level <50 mg/dL or >400 mg/dL at screening;
- Patients who had any of the serious complication(s) listed below at screening:
  - End stage kidney disease for which dialysis was required;
  - Progressive liver disease such as hepatitis, cirrhosis with Child-Pugh classification class B or C, or liver dysfunction with aspartate aminotransferase or alanine aminotransferase over three times the upper limit of the standard value of the study site;
  - Severe congestive heart failure rated as New York Heart Association class III or IV, active unstable angina, or ventricular dysfunction with left ventricular ejection fraction (LVEF) <30%; or
  - Severe pulmonary dysfunction requiring home oxygen therapy.
- Human immunodeficiency virus infection, ongoing systemic infection, severe local infection, or immunocompromised condition at screening;
- Alzheimer's disease or other dementias, or any other neurological disorder that was judged to affect their ability to give consent to participate in the trial or could confound study assessments performed by the investigator at screening;

- Malignant tumor(s) or history of malignant tumor(s) prior to 2 years of ischemic stroke onset at screening;
- Contraindications for MRI such as implanted pacemakers or other metallic prosthesis incompatible with MRI, or claustrophobia;
- Thrombocytopenia (platelet count <100,000/mm^3) or heparin-induced thrombocytopenia at screening;
- History of allergies to human tissues, bovine or porcine preparations;
- History of allergy to streptomycin;
- Patients who participated in other clinical trials within 12 weeks prior to IC, or planned to participate in other clinical trials during this trial, or participated in clinical trials of other cell products in the past;
- History of splenectomy;
- Patients who might have a transient ischemic attack;
- Patients who were scheduled to undergo revascularization treatment including carotid endarterectomy, stenting, etc. by the end of the evaluation (day 91);
- Patients who were pregnant or lactating at screening, or who wished to become pregnant during the study;
- Patients who could not use extremely effective contraception including intrauterine device, intrauterine system, oral contraception (low dose pill), surgical sterilization, double barrier method (condom with spermicide, or combination of condom with pessary) under the guidance of the investigator from the time of IC to one year post-dose (day 366), or who had a partner who could not take similar contraceptive measures; or
- Patients who the investigator considered to be inappropriate for inclusion in the study.

Exclusion criteria on eligibility confirmation assessment

After eligibility assessment at screening, the investigator assessed NIHSS again ≥4 h after the assessment at screening to confirm patient eligibility. Patients who met one or more of the following criteria were excluded:

- NIHSS score ≤4 or ≥21;

- Change in NIHSS score from screening ≥5;
- Administration of the study product could not be started within 48 h of symptom onset; or
- Patients who the investigator considered to be inappropriate for inclusion in the study.

Randomization and blinding

Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and 2. In cohort 3, subjects were randomly assigned in a 1:1 ratio to receive either JTR-161 or placebo. Randomization was performed by the minimization method, which was adjusted centrally by dynamic assignment with NIHSS at the time of eligibility assessment, with / without standard treatment including intravenous rt-PA or endovascular treatment, and age at the time of IC as the allocation factors. The randomization sequence was generated by an organization independent of the study sponsors. Allocation of treatment to subjects was randomized via a website. The investigators, patients, and the sponsor are masked to the treatment assignment until the observation period is completed. After the final subject in cohort 3 completes the day 91 assessment, the database will be fixed, and the key will be opened. After that, the sponsor, statistical analysts, and unblinded personnel will be placed under open blind, and patients and assessors will be blinded until the end of the follow-up period (day 366). JTR-161 and placebo can be identified by the vial appearance; therefore, to ensure masking is maintained, only unblinded persons appointed by the investigator prepared the administration solution, intravenously injected the study product into the patient, and cleaned up any spilled administration solution.

Procedure

JTR-161 was manufactured in accordance with good manufacturing practice by JCR Pharmaceuticals Co., Ltd. The JTR-161 vial (5.0 mL) contained 1.0 x 10^8 cells of DPSC isolated from the extracted teeth of healthy adults, and was stored in the gas space of a liquid nitrogen refrigerator.

The frozen study product was thawed in a constant temperature bath at 37 ± 1 ° C for about five minutes, then the required number of cells (one or three vials) was diluted in 100 mL of saline. The solution was intravenously administered once at a rate of 4 mL/min but ≤ 6 mL/min within 48 h of symptom onset. Number of cells administered in each cohort and flow chart of the cohorts are shown in figure

1. The DSMB was primarily involved in deciding whether or not to advance to the next cohort, as well as the dose (number of cells) for cohort 3. Surgical revascularization such as carotid endarterectomy and carotid artery stenting was prohibited during the observation period, and attending any clinical trials other than this study was prohibited until the end of the study. In cohorts 1 and 2, the administration interval between subjects was ≥ 72 hours.

Baseline assessments were carried out at day 0 prior to administration, including (1) primary disease: initial or recurrent, type of cerebral infarction, infarcted blood vessels, onset time, and diffusion-weighted imaging (DWI) -Alberta Stroke Program Early Computed Tomography Score, (2) with/without standard treatment with intravenous rt-PA or endovascular treatment. If yes, treatment start time (endovascular treatment only), degree of recanalization (modified thrombolysis in cerebral infarction classification), recanalization time, and number of passes. If no, reasons for not implementing standard treatment, (3) NIHSS at time of arrival, pre-registration, and eligibility tests, (4) mRS before the onset of cerebral infarction reported by patients or her/his family, (5) disease history related to the exclusion criteria and, where relevant, the time of complete cure of any malignant condition, effected at least 2 years before IC and still considered cured at the start of administration of the study product. In addition, a medical history deemed necessary for considering AEs was taken. After administration of the study product, mRS and Barthel Index (BI) were assessed at days 31, 91, and 366. NIHSS was assessed at days 2, 8, 31, and 91, and on the day of discharge. Patients were asked to answer the EuroQOL 5 dimensions 5-level scores (EQ-5D-5L) questionnaire at days 31, 91, and 336. Laboratory tests were performed pre-registration, pre-administration, and on days 2, 3, 8, 31, 91, 181, and 366 after administration. Blood pressures including systolic and diastolic blood pressures and pulse rates were measured pre-registration, pre-administration, 1, 2, 4, 6, 12, and 24 hours after administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Body temperature was measured pre-registration, pre-administration, 2, 4, 6, and 24 hours after administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Saturated oxygen was measured pre-registration, pre-administration, every 15 minutes between one and four hours after administration, every 30 minutes between four and six hours after administration, 12 and 24 hours after administration, and on days 3, 8, 31, 91, 181, and 366 after administration. Imaging tests were performed pre-registration, and on days 2, 8, and 31 after administration. Serum

cytokines and growth factors including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-10, IL-17, IL-23, and angiopoietin-1 (Ang-1) were measured pre-administration, and on days 3 and 8 after administration in cohort 3. Infarct volumes were measured on DWI and/or fluid-attenuated inversion recovery using MRI pre-administration, and on days 8 and 31 after administration. Ischemic penumbra was measured using MRI as the mismatch between the hypoperfused area on perfusion-weighted imaging and the abnormal area on DWI pre-administration, if available. Assessment of imaging was performed at the central assessment organization. Discontinuance criteria for individual subjects were (1) AEs, worsening of complications, and other safety concerns, (2) no visit to the study site due to inconvenience to patients, (3) termination of the study by the sponsor, and (4) termination of the study by the investigator due to safety concerns regarding the study product.

Outcome measures

The primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all of the following criteria at day 91 in cohort 3: mRS ≤ 1, NIHSS ≤ 1, and BI ≥ 95. Secondary endpoints were (1) proportion of patients who achieve mRS ≤ 1 or mRS ≤ 2 at days 91 and 366, (2) proportion of patients who achieve BI ≥ 95 at days 91 and 366, (3) proportion of patients who achieve NIHSS ≤ 1, who achieve improvement of ≥ 75%, and who achieve improvement of ≥ 10 points at day 91, (4) changes in EQ-5D-5L scores at day 366, (5) proportion of patients who achieve an excellent outcome (mRS ≤ 1, NIHSS ≤ 1, and BI ≥ 95) at day 91. EQ-5D-5L consists of two parts: the EQ-5D descriptive system and the EQ visual analogue scale (VAS). The descriptive system consists of five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has five levels: 1 = "no problems", 2 = "slight problems", 3 = "moderate problems", 4 = "severe problems", and 5 = "extreme problems". The EQ VAS was recorded during the patient's self-rated health assessment on a vertical VAS, where the endpoints were labelled 'The best health you can imagine' and 'The worst health you can imagine', (6) proportion of patients who achieve overall improvement (mRS ≤ 2, improvement in NIHSS ≥ 75% , and BI ≥ 95) at day 91. Safety was assessed based on AEs, laboratory tests, vital signs, transcutaneous oxygen saturation, and imaging test including MRI or CT. The investigator assessed the intensity, severity, and relatedness of an AE. All serious AEs were reported using a standardized SAE report form. Exploratory assessments were (1) cytokines and

growth factors such as TNF-α, IL-1β, IL-6, IL-10, IL-17, IL-23, and Ang-1 as biomarkers in cohort 3, (2) infarct volumes, and (3) penumbra area volume if available.

Data monitoring body

All data were collected via an electronic case report form prepared using Rave® (Medidata Solutions Japan, Tokyo, Japan). Periodic monitoring was performed independently by the sponsor during the trial in order to confirm that the trial was conducted in accordance with the study protocol.

Sample size estimates

In cohorts 1 and 2, eight subjects per cohort (JTR-161, n = 6; placebo, n = 2) were set as the appropriate number of subjects for the safety evaluation. In cohort 3, 60 subjects (JTR-161, n = 30; placebo, n = 30) were set as the number sufficient for designing a future clinical trial based on the safety and efficacy data even if a subpopulation analysis is performed.

Statistical analyses

Efficacy analyses will be performed in the full analysis set (FAS); the population of enrolled patients who will have received the study product at least once and have had a post-dose efficacy assessment, and secondary endpoints will be assessed in the per protocol set (PPS); the FAS population excluding those patients with a significant protocol violation. The safety analysis will be performed for patients in the safety analysis set (SAF); the population of all enrolled patients who will receive the study product and have a post-dose safety assessment. Categorical variables of patient characteristics and baseline parameters will be aggregated for each treatment group and cohort, and descriptive statistics will be calculated for continuous variables. Comparison analysis will be performed between the JTR-161 and placebo groups in cohort 3, and between the merged JTR-161 groups of cohort 3 and the cohort receiving the same dose as cohort 3, and the merged placebo groups of cohorts 1, 2, and 3. As for the primary endpoint, the proportions and their confidence intervals will be calculated for each administration group. Also, the point estimates of difference in the proportion and its confidence interval will be calculated and compared between the JTR-161 and placebo groups. As for secondary endpoints, the proportions and their confidence intervals for mRS, BI, and NIHSS will be calculated

for each administration group, and point estimates of the difference in the proportions and its confidence interval will be calculated. The common odds ratio of the mRS will be calculated for each administration group, and the distribution in each category will be shown. Descriptive statistics of mRS, BI, EQ-5D-5L, biomarkers, infarct volumes, and penumbra area volume at the time of assessments will be calculated for each treatment group.

For AEs and adverse drug reactions for each administration group, the number of patients, the number of cases, and the rate of occurrence will be tabulated according to degree of seriousness, severity, and time of onset. AEs will be listed according to MedDRA as lowest level term, and are similarly aggregated using the system organ class and preferred term. For laboratory tests, vital signs, and oxygen saturation, descriptive statistics will be calculated or tabulated for each administration group and each test time point. The presence or absence of abnormal fluctuations for each test item in individual cases will be summarized. No adjustment for multiplicity will be performed. The two-sided significance level will be set at 5%. Interval estimation will be calculated with a confidence coefficient of 95%.

Study organization and funding

Teijin Pharma Ltd., Tokyo, Japan and JCR Pharmaceuticals Co., Ltd., Kobe, Japan were involved in study design, data collection, data analysis, data interpretation, writing of the clinical study report, and made the decision to submit the study results for publication. The delegates of the sponsor are Ken-ichi Umino, Teijin Pharma Limited, Clinical Development Department, Research, Development & Technology Unit, 2-1 Kasumigaseki 3-chome, Chiyoda-ku, Tokyo 100-8585, Japan and Kiwamu Imagawa, JCR Pharmaceuticals Co., Ltd., Research Division, Drug Discovery Research Institute, 2-2-9 Murotani, Nishi-ku, Kobe, Hyogo, 651-2241 Japan. This study and its publication are funded by Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.

Patient and public involvement

No patients and/or public were involved in setting the research questions nor they were involved in developing plans for the design (or implementation) of this study protocol.

Ethics and dissemination

The study protocol and IC form were approved by the institutional review board at each participating study site. First approval was obtained from the institutional review board of Nippon Medical School on 20 December 2018. The protocol version 02 issued on 2 November 2018 was reviewed there. All patients gave written IC before initiation of any study-specific procedures. IC from proxies was also allowed due to the pathophysiology of patients with acute cerebral infarction. The study was conducted in accordance with the ethical principles originating in or derived from the Declaration of Helsinki and Good Clinical Practice guidelines. A manuscript with the results of the primary study will be published in a peer-reviewed journal. On completion of the trial, and after publication of the primary manuscript, data requests can be submitted to the corresponding author.

DISCUSSION

Bone marrow is a major source of stem cells and systemic delivery of BM-MSCs after cerebral ischemia has been widely studied. [5-8] While collection of BM-MSCs requires invasive bone marrow puncture, DPSCs can be obtained easily and less invasively from the extracted teeth of healthy adults. They exhibit better plasticity and proliferation capability, and have more potent immunoregulatory effects.[12,18,19] This J-REPAIR study is the first-in-human, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of JTR-161 in patients with acute ischemic stroke. Patients were selected as participants in this first-in-human study from the viewpoint of invasiveness and unknown risk of DPSCs to the subjects, referring to the "Guidance on quality, and technical guidance on conducting non-clinical trials and clinical trials of regenerative medicine products (human cell processed products)".[20] The eligible patients were restricted to those with anterior circulation ischemic stroke because the severity of their symptoms can be assessed using NIHSS[21], one of the key criteria for assessing eligibility and efficacy in our study. It is difficult to confirm the accurate etiology of stroke on admission; therefore, there is no limitation regarding stroke subtype such as lacuna, atherothrombotic, cardioembolic, and others. Our study did not limit the use of standard treatment including intravenous rt-PA and/or endovascular thrombectomy for recruitment. In addition, available treatments for acute ischemic stroke except revascularization treatment such as carotid endarterectomy and stenting in routine clinical practice were allowed to be used as a combination therapy. Patients to

whom standard treatment could not be given, and patients who received standard treatment but had a NIHSS ≥ 5 were allowed to be enrolled. However, these pretreatment and combination therapies may make it difficult to evaluate the safety and efficacy of JTR-161 accurately; therefore, a placebo arm was established as a control group. The study is conducted in a double-blinded manner during the observation period. The keys were opened to the sponsor, statistical analysts, and unblinded personnel, but patients and assessors continued under blind conditions until the end of the follow-up period, since EQ-5D-5L was assessed at day 366. In order to explore the therapeutic time window, timing of administration was set to be within 48 h of symptom onset.

The proportion of subjects who achieve an excellent outcome defined as mRS ≤ 1, NIHSS ≤ 1, and BI ≥ 95 was set as the primary endpoint because we considered this clinical outcome was the most accurate way of detecting any difference in effectiveness between the subjects receiving JTR-161 and the placebo group. As secondary endpoints, the efficacy of JTR-161 was also evaluated using mRS and BI for disability assessments, and NIHSS for function assessment, all of which are widely accepted for use as endpoints in clinical trials of acute ischemic stroke.[22] In recent clinical trials of intravenous rt-PA and endovascular treatment, clinical outcomes as per mRS were evaluated 90 days after the start of treatment.[23,24] Similarly, period during which the efficacy of JTR-161 was evaluated was set to 90 days after administration of the study product. EQ-5D-5L was used as a patient-reported outcome for evaluating patient health status. It is reported that there was a significant correlation between stroke type and severity, and EQ-5D-5L scores; reproducibility and validity have been verified in stroke patients.[25] We measured a variety of serum cytokines and growth factors before and after transplantation of JTR-161 to investigate the mechanism of human DPSCs on acute ischemic stroke.

In a pre-clinical study, the distribution of JTR-161 labelled with a radioactive tracer was highest in the lung two hours after a single intravenous administration (in-house data), as reported in other types of stem cells.[26] The onset of symptoms such as respiratory distress and decreased oxygen saturation should be carefully followed immediately after administration of JTR-161. Oxygen saturation was measured every 15 minutes for up to 4 hours and every 30 minutes for up to 6 hours after administration. Imaging tests were performed to assess infarct lesions and the presence or absence of significant hemorrhagic changes. On the other hand, time of disappearance of JTR-161 from the body has not been elucidated. Therefore, we established a follow-up period of up to one year after administration

(day 366).

In conclusion, JTR-161 will provide a novel therapeutic option for the treatment of patients with ischemic stroke due to the wider therapeutic time window for human DPSC transplantation.

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Authors' contributions

All authors were involved in the study design, protocol preparation, and acquisition of funding. SS and CN were responsible for the first draft. All authors have reviewed and approved the final manuscript. The work is funded by Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.

Declaration of conflicts of interest

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Expert Witness from Teijin Pharma Ltd. (SS, CN, KK). Research funding from Teijin Pharma Ltd. (KK). Lecture fee from Teijin Pharma Ltd. (YI). The other authors report no conflicts.

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Figure legend

Figure 1 Flow chart of the cohorts

DSMB, Data and Safety Monitoring Board

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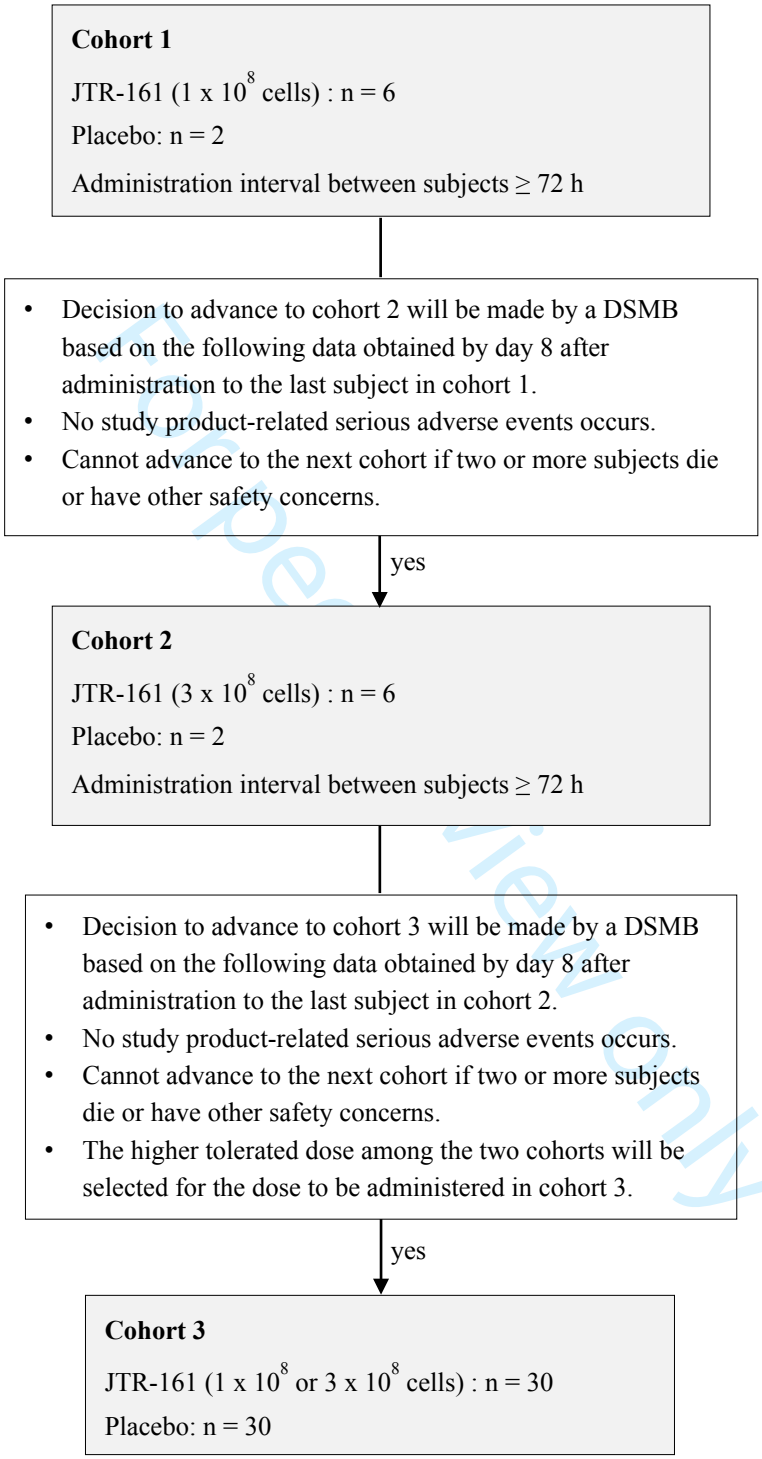

Figure 1 Flow chart of the cohorts
DSMB, Data and Safety Monitoring Board

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3 ,7
	2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	3	Date and version identifier (Issue date: 9 Jul 2019, Protocol amendment number: 04)	-
Funding	4	Sources and types of financial, material, and other support	15
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 18
	5b	Name and contact information for the trial sponsor	15
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	6, Figure 1

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-6
	6b	Explanation for choice of comparators	16,17
Objectives	7	Specific objectives or hypotheses	5-6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6-7, Figure1
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6-7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-11
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	11-12, Figure1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	11-13, Figure1
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	14
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12,16
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13,14
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6, Table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	11-13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	-
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14-15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	14
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6, Figure 1
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	16
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	-
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15,18
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	-
	31b	Authorship eligibility guidelines and any intended use of professional writers	18
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplemental material
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-161, allogeneic human dental Pulp stem cells, in patients with Acute Ischemic stRoke (J-REPAIR)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-054269.R2
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A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-161, allogeneic human dental Pulp stem cells, in patients with Acute Ischemic stRoke (J-REPAIR)

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Abstract

Introduction: JTR-161 is a novel allogeneic human cell product consisting of dental pulp stem cells isolated from the extracted teeth of healthy adults. It is currently under development as a cell-based therapy for ischemic stroke. The aim of this study is to evaluate the safety and efficacy of JTR-161 in patients with acute ischemic stroke when given as a single intravenous administration within 48 hours of symptom onset.

Methods and analysis: This is a first-in-human, randomized, double-blind, placebo-controlled, multicenter, phase 1/2 clinical trial to be conducted in Japan (from January 2019 to July 2021). Patients with a clinical diagnosis of anterior circulation ischemic stroke with a National Institutes of Health Stroke Scale (NIHSS） score of 5–20 at baseline were enrolled. Patients previously treated with recombinant tissue-type plasminogen activator and/or endovascular thrombectomy were allowed to be enrolled. The study consists of three cohorts: cohorts 1 and 2 (each eight patients), and cohort 3 (60 patients). Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and 2, and in a 1:1 ratio in cohort 3. The number of cells administered was increased sequentially from 1 x 10^8 (cohort 1) to 3 x 10^8 (cohort 2). In cohort 3, the higher tolerated dose among the two cohorts was administered. The primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all of the following criteria at day 91 in cohort 3: modified Rankin Scale ≤ 1, NIHSS ≤ 1, and Barthel Index ≥ 95.

Ethics and dissemination: The protocol and informed consent form were approved by the institutional review board at each participating study site. A manuscript with the results of the primary study will be published in a peer-reviewed journal.

Trial registration: JapicCTI-194570 and Clinical Trials. gov: NCT04608838

Strengths and limitations of this study

- This study is a first-in-human, randomized, double-blind, placebo-controlled phase 1/2 clinical trial of a cell-based therapy for ischemic stroke using JTR-161, a novel allogeneic human cell product consisting of dental pulp stem cells.
- The study consists of three cohorts; patients received 1 x 10^8 cells in cohort 1, 3 x 10^8 cells in cohort 2, and the higher tolerated dose among the two cohorts (either 1 x 10^8 cells or 3 x 10^8 cells) in cohort 3.
- The results of this study will be used to determine the safe dose of JTR-161 administered as a single intravenous dose within 48 hours of symptom onset.
- Primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all of the following criteria at day 91 at the optimized dose: modified Rankin Scale ≤ 1, NIHSS ≤ 1, and Barthel Index ≥ 95.
- This is a proof-of-concept study; therefore, further study will be required.

INTRODUCTION

Stroke is the most prevalent cerebrovascular disease worldwide, and still one of the leading causes of death and severe disability. Ischemic stroke accounts for about 80% of all stroke events.[1,2] The recent advances in reperfusion therapy using endovascular thrombectomy have allowed its benefits to be expanded to a larger population of patients with large vessel occlusion. However, the rate of favorable clinical outcomes remains low[3,4], underscoring an unmet clinical need for adjunctive neuroprotective treatments. Among them, cell-based therapies using human somatic stem cells have been attracting attention, and there are ongoing clinical studies investigating the use of intravenous or intracerebral human somatic stem cells, mainly using bone marrow-derived mesenchymal stem cells (BM-MSCs), in patients with ischemic stroke from the acute to the chronic phase.[5-8] Administration of human BM-MSCs was safe and well tolerated in patients with acute ischemic stroke, but no significant clinical improvement was observed.[7,8]

In 2000, human dental pulp stem cells (DPSCs) were discovered in impacted molar teeth.[9] DPSCs are thought to originate from the cranial neural crest derived from the neuroectoderm, thus they express early markers for both mesenchymal and neuroectodermal stem cells.[10,11] DPSCs can secrete various neurotrophic factors such as neurotrophin-3, brain-derived neurotrophic factor, and vascular endothelial growth factor, which promote neuronal survival, proliferation, differentiation, and migration.[11] Furthermore, compared to BM-MSCs, DPSCs can be obtained by a less invasive process, are more easily expanded, and exert more potent immunosuppressive effects via the inhibition of activated T cell responses[12], which makes them attractive for use in allogeneic transplantation. Several reports have shown the beneficial effects of human DPSC transplantation in animal models of neurological disease. [13,14] Sakai et al.[14] reported that human DPSC transplantation into the completely transected spinal cord of adult rats resulted in marked recovery of hind limb locomotor functions, whereas transplantation of human BM-MSC or skin-derived fibroblasts led to substantially less recovery of locomotor function. Based on a rat stroke model and an *in vitro* model of ischemia[15], human DPSCs are reported to be a better source of cell therapy for ischemic stroke than human BM-MSCs.

JTR-161 is an allogeneic cell-based product consisting of human DPSCs isolated from the extracted teeth of healthy adults. In the preclinical study, intravenous administration of DPSCs decreased

ischemic damage and promoted functional improvement in a rodent model of focal cerebral ischemia by modulating neuroinflammatory reactions.[16,17] Preclinical toxicological study of a single intravenous administration of JTR-161 to male and female nude rats showed no notable toxicological findings two weeks after administration (In house data). There were no notable findings regarding tumorigenicity 16 weeks after administration. Furthermore, no scaffold-independent proliferation ability was observed. Regarding non-cellular components of the study product and impurities derived from the manufacturing process, because the amount of residual impurities was low, there were negligible concerns regarding safety. Here, we report the protocol of the first-in-human clinical trial of JTR-161 in patients with acute ischemic stroke.

METHODS AND ANALYSIS

Study design

This is A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-161, allogeneic human DPSCs, in patients with Acute Ischemic stRoke (J-REPAIR study). The aims of this phase 1/2 study are to evaluate the efficacy and safety of JTR-161 in Japanese patients with acute ischemic stroke when given as a single intravenous administration. Patients received 1 x 10^8 cells in cohort 1, and 3 x 10^8 cells in cohort 2, sequentially. In cohort 3, the higher tolerated dose among the two cohorts (either 1 x 10^8 cells or 3 x 10^8 cells), determined according to the recommendation by the Data and Safety Monitoring Board (DSMB) (figure 1), was administered. The DSMB consists of three independent external experts. The DSMB does not recommend advancing to the next cohort when two or more product-related death or death for which a causal relationship cannot be ruled out occur in the same cohort, or any other serious safety concerns are reported. Death due to cerebral infarction itself and concomitant disorders including pneumonia and transtentorial herniation, followed in frequency by cardiac causes and pulmonary embolism, pretreatment with intravenous recombinant tissue-type plasminogen activator (rt-PA) or endovascular treatment, and combination treatment for the primary disease are excluded as causes of death in this study. The study schedule and assessments are shown in table 1.

Each cohort consists of a 91-day observation period and a 275-day follow-up period (total study period: 366 days). Patients were recruited from 29 stroke centers in Japan between January 2019 and July

2021. The study was registered as JapicCTI-194570, prior to study patient enrollment, and subsequently on Clinical Trials.gov: NCT04608838.

Patient population

Inclusion criteria

Patients who met all the following criteria were included:

- Japanese male or female patients 20 years of age or older;
- Clinical diagnosis of anterior circulation ischemic stroke based on the results of brain magnetic resonance imaging (MRI) or computed tomography (CT);
- National Institutes of Health Stroke Scale (NIHSS) score of ≥5 to ≤20 at screening;
- Onset of ischemic stroke had to have occurred within 48 hours prior to the start of administration of the study product; and
- A modified Rankin Scale (mRS) of 0 or 1, by either self-report or family report, prior to ischemic stroke onset.

Table 1 Schedule for assessments

		Assessment period															Follow-up period		Dis-charge	Termi-nation
		Pre-observation period				Observation period														
			Pre-enrolment	Qualification	Pre-dosing	Day 1						Day 2	Day 3	Day 8	Day 31	Day 91	Day 181	Day 366		
						0 h	1 h	2 h	4 h	6 h	12 h									
Informed consent		x																		
Patient characteristics			x[5]																	
Administration of study product						x														
Ability assessment	mRS		x[6]												x	x		x	x	
	Barthel Index														x	x		x		
Function assessment	NIHSS		x[7]	x[8]								x		x	x	x			x	
QOL assessment	EQ-5D-5L														x	x		x		
Clinical laboratory tests	Hematology		x[7]		x							x	x	x	x	x	x	x		x
	Biochemistry		x[7]		x							x	x	x	x	x	x	x		x
	Blood coagulation test		x[7]		x							x	x	x	x	x	x	x		x
	Biomarker[1]				x								x	x						
	Urinalysis		x[7]		x							x	x	x	x	x	x	x		x
Imaging examinations	Safety assessment		x[7]									x		x[10]	x					
	Infarct volume[2]				x[9]									x[10]	x					
	Penumbra region volume[2, 3]				x															
Body measurements	Height, weight		x[7]																	
Vital signs	Blood pressure, pulse		x		x		x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Body temperature		x		x			x	x	x		x	x	x	x	x	x	x	x	x
Oxygen saturation	SpO_2[4]		x		x		x	x	x	x	x	x	x	x	x	x	x	x		x
Medical examination	Medical examination and interview		x		x							x	x	x	x	x	x	x	x	x

1. Assessed in the cohort 3 only.

2. Assessed at the central imaging analysis organization.

3. Performed at some study sites.

4. In addition to the scheduled period in the table, SpO_2 is assessed at 15 min , 30 min, 45 min, 1h 15 min, 1h 30 min, 1 h 45 min, 2 h 15 min, 2 h 30 min, 2 h 45 min, 3 h 15 min, 3 h, 3 h 15 min, 3 h 30 min, 3 h 45 min, 4 h 30 min, 5 h, and 5 h 30 min post-dose.

5. Pregnancy test is performed in premenopausal women or unknown women whether menopause.

6. The mRS before ischemic stroke onset is assessed based on interview from patients or their family.

7. Data before obtaining consent are acceptable.

8. Assessed at least 4 hours after enrolment.

9. Imaging data after standard treatment are accepted for patients who have undergone standard treatment (rt-PA intravenous or endovascular treatment).

10. Assessed once during Day 5 to Day 8.

mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; QOL, quality of life; SpO2, oxygen salutation of peripheral artery

Exclusion criteria

Patients who met one or more of the following criteria were excluded:

- Presence of a new ischemic lesion in the cerebellum or brainstem at screening;
- A marked decline in level of consciousness (NIHSS 1a. evaluation of consciousness level is score of 3) at screening;
- Patients who had an extensive infarct and for whom maintaining life was expected to be difficult, or who were expected to undergo cranial decompression at screening;
- Presence of intracranial hemorrhagic change diagnosed by brain imaging which was judged to be clinically important by the investigator at screening;
- Convulsions after onset of ischemic stroke;
- History of neurological events such as stroke or clinically significant head trauma within 180 days prior to informed consent (IC);
- Systolic blood pressure >220 mmHg or diastolic blood pressure >120 mmHg, with or without antihypertensive treatment at screening;
- Blood glucose level <50 mg/dL or >400 mg/dL at screening;
- Patients who had any of the serious complication(s) listed below at screening:
  - End stage kidney disease for which dialysis was required;
  - Progressive liver disease such as hepatitis, cirrhosis with Child-Pugh classification class B or C, or liver dysfunction with aspartate aminotransferase or alanine aminotransferase over three times the upper limit of the standard value of the study site;
  - Severe congestive heart failure rated as New York Heart Association class III or IV, active unstable angina, or ventricular dysfunction with left ventricular ejection fraction (LVEF) <30%; or
  - Severe pulmonary dysfunction requiring home oxygen therapy.
- Human immunodeficiency virus infection, ongoing systemic infection, severe local infection, or immunocompromised condition at screening;
- Alzheimer's disease or other dementias, or any other neurological disorder that was judged to affect their ability to give consent to participate in the trial or could confound study assessments performed by the investigator at screening;

- Malignant tumor(s) or history of malignant tumor(s) prior to 2 years of ischemic stroke onset at screening;
- Contraindications for MRI such as implanted pacemakers or other metallic prosthesis incompatible with MRI, or claustrophobia;
- Thrombocytopenia (platelet count <100,000/mm^3) or heparin-induced thrombocytopenia at screening;
- History of allergies to human tissues, bovine or porcine preparations;
- History of allergy to streptomycin;
- Patients who participated in other clinical trials within 12 weeks prior to IC, or planned to participate in other clinical trials during this trial, or participated in clinical trials of other cell products in the past;
- History of splenectomy;
- Patients who might have a transient ischemic attack;
- Patients who were scheduled to undergo revascularization treatment including carotid endarterectomy, stenting, etc. by the end of the evaluation (day 91);
- Patients who were pregnant or lactating at screening, or who wished to become pregnant during the study;
- Patients who could not use extremely effective contraception including intrauterine device, intrauterine system, oral contraception (low dose pill), surgical sterilization, double barrier method (condom with spermicide, or combination of condom with pessary) under the guidance of the investigator from the time of IC to one year post-dose (day 366), or who had a partner who could not take similar contraceptive measures; or
- Patients who the investigator considered to be inappropriate for inclusion in the study.

Exclusion criteria on eligibility confirmation assessment

After eligibility assessment at screening, the investigator assessed NIHSS again ≥4 h after the assessment at screening to confirm patient eligibility. Patients who met one or more of the following criteria were excluded:

- NIHSS score ≤4 or ≥21;

- Change in NIHSS score from screening ≥5;
- Administration of the study product could not be started within 48 h of symptom onset; or
- Patients who the investigator considered to be inappropriate for inclusion in the study.

Randomization and blinding

Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and 2. In cohort 3, subjects were randomly assigned in a 1:1 ratio to receive either JTR-161 or placebo. Randomization was performed by the minimization method, which was adjusted centrally by dynamic assignment with NIHSS at the time of eligibility assessment, with / without standard treatment including intravenous rt-PA or endovascular treatment, and age at the time of IC as the allocation factors. The randomization sequence was generated by an organization independent of the study sponsors. Allocation of treatment to subjects was randomized via a website. The investigators, patients, and the sponsor are masked to the treatment assignment until the observation period is completed. After the final subject in cohort 3 completes the day 91 assessment, the database will be fixed, and the key will be opened. After that, the sponsor, statistical analysts, and unblinded personnel will be placed under open blind, and patients and assessors will be blinded until the end of the follow-up period (day 366). JTR-161 and placebo can be identified by the vial appearance; therefore, to ensure masking is maintained, only unblinded persons appointed by the investigator prepared the administration solution, intravenously injected the study product into the patient, and cleaned up any spilled administration solution.

Procedure

JTR-161 was manufactured in accordance with good manufacturing practice by JCR Pharmaceuticals Co., Ltd. The JTR-161 vial (5.0 mL) contained 1.0 x 10^8 cells of DPSC isolated from the extracted teeth of healthy adults, and was stored in the gas space of a liquid nitrogen refrigerator.

The frozen study product was thawed in a constant temperature bath at 37 ± 1 ° C for about five minutes, then the required number of cells (one or three vials) was diluted in 100 mL of saline. The solution was intravenously administered once at a rate of 4 mL/min but ≤ 6 mL/min within 48 h of symptom onset. Number of cells administered in each cohort and flow chart of the cohorts are shown in figure

1. The DSMB was primarily involved in deciding whether or not to advance to the next cohort, as well as the dose (number of cells) for cohort 3. Surgical revascularization such as carotid endarterectomy and carotid artery stenting was prohibited during the observation period, and attending any clinical trials other than this study was prohibited until the end of the study. In cohorts 1 and 2, the administration interval between subjects was ≥ 72 hours.

Baseline assessments were carried out at day 0 prior to administration, including (1) primary disease: initial or recurrent, type of cerebral infarction, infarcted blood vessels, onset time, and diffusion-weighted imaging (DWI) -Alberta Stroke Program Early Computed Tomography Score, (2) with/without standard treatment with intravenous rt-PA or endovascular treatment. If yes, treatment start time, degree of recanalization (modified thrombolysis in cerebral infarction classification), recanalization time, and number of passes. If no, reasons for not implementing standard treatment, (3) NIHSS at time of arrival, pre-registration, and eligibility tests, (4) mRS before the onset of cerebral infarction reported by patients or her/his family, (5) disease history related to the exclusion criteria and, where relevant, the time of complete cure of any malignant condition, effected at least 2 years before IC and still considered cured at the start of administration of the study product. In addition, a medical history deemed necessary for considering adverse events (AEs) was taken. After administration of the study product, mRS and Barthel Index (BI) were assessed at days 31, 91, and 366. NIHSS was assessed at days 2, 8, 31, and 91, and on the day of discharge. Patients were asked to answer the EuroQOL 5 dimensions 5-level scores (EQ-5D-5L) questionnaire at days 31, 91, and 336. Laboratory tests were performed pre-registration, pre-administration, and on days 2, 3, 8, 31, 91, 181, and 366 after administration. Blood pressures including systolic and diastolic blood pressures and pulse rates were measured pre-registration, pre-administration, 1, 2, 4, 6, 12, and 24 hours after administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Body temperature was measured pre-registration, pre-administration, 2, 4, 6, and 24 hours after administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Saturated oxygen was measured pre-registration, pre-administration, every 15 minutes between one and four hours after administration, every 30 minutes between four and six hours after administration, 12 and 24 hours after administration, and on days 3, 8, 31, 91, 181, and 366 after administration. Imaging tests were performed pre-registration, and on days 2, 8, and 31 after administration. Serum

cytokines and growth factors including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-10, IL-17, IL-23, and angiopoietin-1 (Ang-1) were measured pre-administration, and on days 3 and 8 after administration in cohort 3. Infarct volumes were measured on DWI and/or fluid-attenuated inversion recovery using MRI pre-administration, and on days 8 and 31 after administration. Ischemic penumbra was measured using MRI as the mismatch between the hypoperfused area on perfusion-weighted imaging and the abnormal area on DWI pre-administration, if available. Assessment of imaging was performed at the central assessment organization. Discontinuance criteria for individual subjects were (1) AEs, worsening of complications, and other safety concerns, (2) no visit to the study site due to inconvenience to patients, (3) termination of the study by the sponsor, and (4) termination of the study by the investigator due to safety concerns regarding the study product.

Outcome measures

The primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all of the following criteria at day 91 in cohort 3: mRS ≤ 1, NIHSS ≤ 1, and BI ≥ 95. Secondary endpoints were (1) proportion of patients who achieve mRS ≤ 1 or mRS ≤ 2 at days 91 and 366, (2) proportion of patients who achieve BI ≥ 95 at days 91 and 366, (3) proportion of patients who achieve NIHSS ≤ 1, who achieve improvement of ≥ 75%, and who achieve improvement of ≥ 10 points at day 91, (4) changes in EQ-5D-5L scores at day 366, (5) proportion of patients who achieve an excellent outcome (mRS ≤ 1, NIHSS ≤ 1, and BI ≥ 95) at day 91, (6) proportion of patients who achieve overall improvement (mRS ≤ 2, improvement in NIHSS ≥ 75% , and BI ≥ 95) at day 91. EQ-5D-5L consists of two parts: the EQ-5D descriptive system and the EQ visual analogue scale (VAS). The descriptive system consists of five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has five levels: 1 = "no problems", 2 = "slight problems", 3 = "moderate problems", 4 = "severe problems", and 5 = "extreme problems". The EQ VAS was recorded during the patient's self-rated health assessment on a vertical VAS, where the endpoints were labelled 'The best health you can imagine' and 'The worst health you can imagine'. Safety was assessed based on AEs, laboratory tests, vital signs, transcutaneous oxygen saturation, and imaging test including MRI or CT. The investigator assessed the intensity, severity, and relatedness of an AE. All serious AEs were reported using a standardized SAE report form. Exploratory assessments were (1) cytokines and

growth factors such as TNF-α, IL-1β, IL-6, IL-10, IL-17, IL-23, and Ang-1 as biomarkers in cohort 3, (2) infarct volumes, and (3) penumbra area volume if available.

Data monitoring body

All data were collected via an electronic case report form prepared using Rave® (Medidata Solutions Japan, Tokyo, Japan). Periodic monitoring was performed independently by the sponsor during the trial in order to confirm that the trial was conducted in accordance with the study protocol.

Sample size estimates

In cohorts 1 and 2, eight subjects per cohort (JTR-161, n = 6; placebo, n = 2) were set as the appropriate number of subjects for the safety evaluation. In cohort 3, 60 subjects (JTR-161, n = 30; placebo, n = 30) were set as the number sufficient for designing a future clinical trial based on the safety and efficacy data even if a subpopulation analysis is performed.

Statistical analyses

Efficacy analyses will be performed in the full analysis set (FAS); the population of enrolled patients who will have received the study product once and have had a post-dose efficacy assessment, and secondary endpoints will be assessed in the per protocol set (PPS); the FAS population excluding those patients with a significant protocol violation. The safety analysis will be performed for patients in the safety analysis set (SAF); the population of all enrolled patients who will receive the study product and have a post-dose safety assessment. Categorical variables of patient characteristics and baseline parameters will be aggregated for each treatment group and cohort, and descriptive statistics will be calculated for continuous variables. Comparison analysis will be performed between the JTR-161 and placebo groups in cohort 3, and between the merged JTR-161 groups of cohort 3 and the cohort receiving the same dose as cohort 3, and the merged placebo groups of cohorts 1, 2, and 3. As for the primary endpoint, the proportions and their confidence intervals will be calculated for each administration group. Also, the point estimates of difference in the proportion and its confidence interval will be calculated and compared between the JTR-161 and placebo groups. As for secondary endpoints, the proportions and their confidence intervals for mRS, BI, and NIHSS will be calculated

for each administration group, and point estimates of the difference in the proportions and its confidence interval will be calculated. The common odds ratio of the mRS will be calculated for each administration group, and the distribution in each category will be shown. Descriptive statistics of mRS, BI, EQ-5D-5L, biomarkers, infarct volumes, and penumbra area volume at the time of assessments will be calculated for each treatment group.

For AEs and adverse drug reactions for each administration group, the number of patients, the number of cases, and the rate of occurrence will be tabulated according to degree of seriousness, severity, and time of onset. AEs will be listed according to MedDRA as lowest level term, and are similarly aggregated using the system organ class and preferred term. For laboratory tests, vital signs, and oxygen saturation, descriptive statistics will be calculated or tabulated for each administration group and each test time point. The presence or absence of abnormal fluctuations for each test item in individual cases will be summarized. No adjustment for multiplicity will be performed. The two-sided significance level will be set at 5%. Interval estimation will be calculated with a confidence coefficient of 95%.

Study organization and funding

Teijin Pharma Ltd., Tokyo, Japan and JCR Pharmaceuticals Co., Ltd., Kobe, Japan were involved in study design, data collection, data analysis, data interpretation, writing of the clinical study report, and made the decision to submit the study results for publication. The delegates of the sponsor are Ken-ichi Umino, Teijin Pharma Limited, Clinical Development Department, Research, Development & Technology Unit, 2-1 Kasumigaseki 3-chome, Chiyoda-ku, Tokyo 100-8585, Japan and Kiwamu Imagawa, JCR Pharmaceuticals Co., Ltd., Research Division, Drug Discovery Research Institute, 2-2-9 Murotani, Nishi-ku, Kobe, Hyogo, 651-2241 Japan. This study and its publication are funded by Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.

Patient and public involvement

No patients and/or public were involved in setting the research questions nor they were involved in developing plans for the design (or implementation) of this study protocol.

Ethics and dissemination

The study protocol and IC form were approved by the institutional review board at each participating study site. First approval was obtained from the institutional review board of Nippon Medical School on 20 December 2018. The protocol version 02 issued on 2 November 2018 was reviewed there. All patients gave written IC before initiation of any study-specific procedures. IC from proxies was also allowed due to the pathophysiology of patients with acute cerebral infarction. The study was conducted in accordance with the ethical principles originating in or derived from the Declaration of Helsinki and Good Clinical Practice guidelines. A manuscript with the results of the primary study will be published in a peer-reviewed journal. On completion of the trial, and after publication of the primary manuscript, data requests can be submitted to the corresponding author.

DISCUSSION

Bone marrow is a major source of stem cells and systemic delivery of BM-MSCs after cerebral ischemia has been widely studied. [5-8] While collection of BM-MSCs requires invasive bone marrow puncture, DPSCs can be obtained easily and less invasively from the extracted teeth of healthy adults. They exhibit better plasticity and proliferation capability, and have more potent immunoregulatory effects.[12,18,19] This J-REPAIR study is the first-in-human, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of JTR-161 in patients with acute ischemic stroke. Patients were selected as participants in this first-in-human study from the viewpoint of invasiveness and unknown risk of DPSCs to the subjects, referring to the "Guidance on quality, and technical guidance on conducting non-clinical trials and clinical trials of regenerative medicine products (human cell processed products)".[20] The eligible patients were restricted to those with anterior circulation ischemic stroke because the severity of their symptoms can be assessed using NIHSS[21], one of the key criteria for assessing eligibility and efficacy in our study. It is difficult to confirm the accurate etiology of stroke on admission; therefore, there is no limitation regarding stroke subtype such as lacuna, atherothrombotic, cardioembolic, and others. Our study did not limit the use of standard treatment including intravenous rt-PA and/or endovascular thrombectomy for recruitment. In addition, available treatments for acute ischemic stroke except revascularization treatment such as carotid endarterectomy and stenting in routine clinical practice were allowed to be used as a combination therapy. Patients to

whom standard treatment could not be given, and patients who received standard treatment but had a NIHSS ≥ 5 were allowed to be enrolled. However, these pretreatment and combination therapies may make it difficult to evaluate the safety and efficacy of JTR-161 accurately; therefore, a placebo arm was established as a control group. The study is conducted in a double-blinded manner during the observation period. The keys were opened to the sponsor, statistical analysts, and unblinded personnel, but patients and assessors continued under blind conditions until the end of the follow-up period, since EQ-5D-5L was assessed at day 366. In order to explore the therapeutic time window, timing of administration was set to be within 48 h of symptom onset.

The proportion of subjects who achieve an excellent outcome defined as mRS ≤ 1, NIHSS ≤ 1, and BI ≥ 95 was set as the primary endpoint because we considered this clinical outcome was the most accurate way of detecting any difference in effectiveness between the subjects receiving JTR-161 and the placebo group. As secondary endpoints, the efficacy of JTR-161 was also evaluated using mRS and BI for disability assessments, and NIHSS for function assessment, all of which are widely accepted for use as endpoints in clinical trials of acute ischemic stroke.[22] In recent clinical trials of intravenous rt-PA and endovascular treatment, clinical outcomes as per mRS were evaluated 90 days after the start of treatment.[23,24] Similarly, period during which the efficacy of JTR-161 was evaluated was set to 90 days after administration of the study product. EQ-5D-5L was used as a patient-reported outcome for evaluating patient health status. It is reported that there was a significant correlation between stroke type and severity, and EQ-5D-5L scores; reproducibility and validity have been verified in stroke patients.[25] We measured a variety of serum cytokines and growth factors before and after transplantation of JTR-161 to investigate the mechanism of human DPSCs on acute ischemic stroke.

In a pre-clinical study, the distribution of JTR-161 labelled with a radioactive tracer was highest in the lung two hours after a single intravenous administration (in-house data), as reported in other types of stem cells.[26] The onset of symptoms such as respiratory distress and decreased oxygen saturation should be carefully followed immediately after administration of JTR-161. Oxygen saturation was measured every 15 minutes for up to 4 hours and every 30 minutes for up to 6 hours after administration. Imaging tests were performed to assess infarct lesions and the presence or absence of significant hemorrhagic changes. On the other hand, time of disappearance of JTR-161 from the body has not been elucidated. Therefore, we established a follow-up period of up to one year after administration

(day 366).

In conclusion, JTR-161 will provide a novel therapeutic option for the treatment of patients with ischemic stroke due to the wider therapeutic time window for human DPSC transplantation.

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Authors' contributions

CN, MI, YI, TU, YM, NS and KK were involved in the study design, protocol preparation, and acquisition of funding. SS, CN and KK will be responsible for directly accessing and verifying all data. SS and CN were responsible for the first draft. All authors have reviewed and approved the final manuscript. The work is funded by Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.

Declaration of conflicts of interest

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Expert Witness from Teijin Pharma Ltd. (SS, CN, KK). Research funding from Teijin Pharma Ltd. (KK). Lecture fee from Teijin Pharma Ltd. (YI). The other authors report no conflicts.

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Figure legend

Figure 1 Flow chart of the cohorts

DSMB, Data and Safety Monitoring Board

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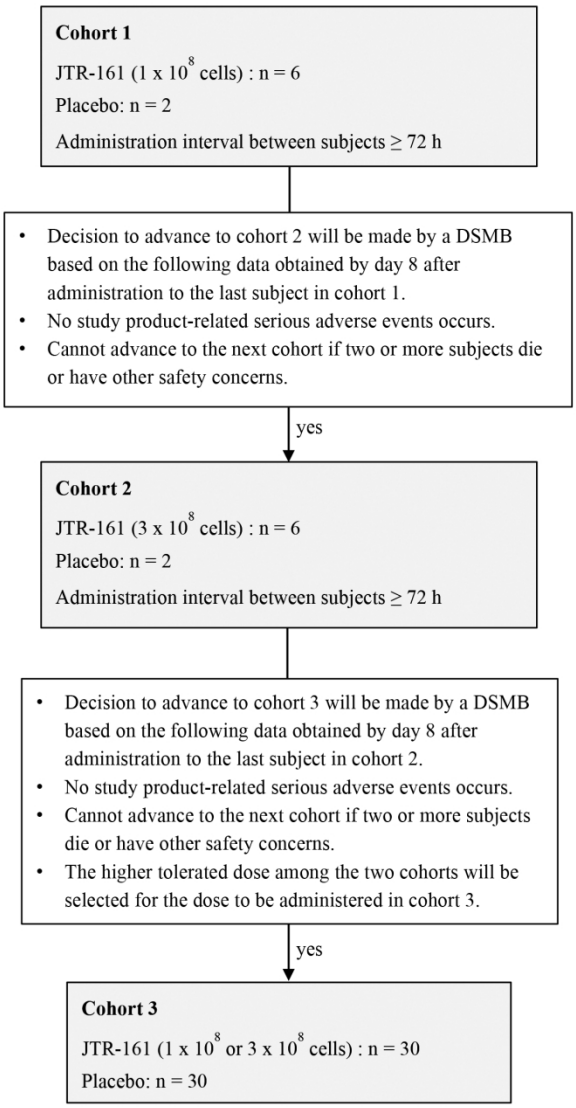

Figure 1

Figure 1. Flow chart of the cohorts

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3 ,7
	2b	All items from the World Health Organization Trial Registration Data Set	-
Protocol version	3	Date and version identifier (Issue date: 9 Jul 2019, Protocol amendment number: 04)	-
Funding	4	Sources and types of financial, material, and other support	16-17
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 19
	5b	Name and contact information for the trial sponsor	16
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	6, Figure 1

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-6
	6b	Explanation for choice of comparators	17,18
Objectives	7	Specific objectives or hypotheses	5-6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6-7, Figure1
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7,10,11
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12-13, Figure1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	12-13, Figure1
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	15
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	13,18
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14,15
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6, Table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	15
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	12
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-14
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	-

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	-
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15-16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	6, Figure 1
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	15
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	-

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	17
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	-
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	19
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	19
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	-
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	-
	31b	Authorship eligibility guidelines and any intended use of professional writers	19
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplemental material
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.