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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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Manuscripts

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4 1 **A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-**
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6 2 **161, allogeneic human dental Pulp stem cells, in patients with Acute Ischemic stRoke**
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8 3 **(J-REPAIR)**
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12 5 Satoshi Suda^{1*}; Chikako Nito^{1*}; Masafumi Ihara²; Yasuyuki Iguchi³; Takao Urabe⁴; Yuji
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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 1×10^8 (cohort 1) to 3×10^8 (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 1×10^8 cells in cohort 1, 3×10^8 cells in cohort 2, and the higher tolerated dose among the two cohorts (either 1×10^8 cells or 3×10^8 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 responses¹¹, 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-

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4 104 161, allogeneic human DPSCs, in patients with Acute Ischemic stRoke (J-REPAIR study). The aims
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6 105 of the study are to evaluate the efficacy and safety of JTR-161 in Japanese patients with acute ischemic
7
8 106 stroke when given as a single intravenous administration. Patients received 1×10^8 cells in cohort 1,
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10107 and 3×10^8 cells in cohort 2, sequentially. In cohort 3, the higher tolerated dose among the two cohorts
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12108 (either 1×10^8 cells or 3×10^8 cells), determined according to the recommendation by the Data and
13
14109 Safety Monitoring Board (DSMB) (figure 1), was administered. The DSMB consists of three
15
16110 independent external experts and recommends advancing to the next cohort only when no product-
17
18111 related serious adverse events (AEs) are observed. The DSMB does not recommend advancing to the
19
20112 next cohort when two or more deaths occur in the same cohort or any other serious safety concerns are
21
22113 reported. Death due to cerebral infarction itself including concomitant symptoms, pretreatment with
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24114 intravenous recombinant tissue-type plasminogen activator (rt-PA) or endovascular treatment, and
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26115 combination treatment for the primary disease are excluded as causes of death in this study. The study
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28116 schedule and assessments are shown in table 1.

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30117 Each cohort consists of a 91-day observation period and a 275-day follow-up period (total study period:
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32118 366 days). Patients were recruited from 29 stroke centers in Japan between December 2018 and July
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34119 2021. The study has been registered in Clinical Trials.gov: NCT04608838 prior to study patient
35
36120 enrollment.

40122 **Patient population**

42123 **Inclusion criteria**

44124 Patients who met all the following criteria were included:

- 46125 ➤ Japanese male or female patients 20 years of age or older;
 - 48126 ➤ Clinical diagnosis of anterior circulation ischemic stroke based on the results of brain magnetic
49 resonance imaging (MRI) or computed tomography (CT);
 - 52128 ➤ National Institutes of Health Stroke Scale (NIHSS) score of ≥ 5 to ≤ 20 at screening;
 - 54129 ➤ Onset of ischemic stroke had to have occurred within 48 hours prior to the start of administration
55 of the study product; and
 - 58131 ➤ A modified Rankin Scale (mRS) of 0 or 1, by either self-report or family report, prior to
59 ischemic stroke onset.
- 60132

133 **Table 1** Schedule for assessments

	Assessment period															Discharge	Termination		
	Pre-observation period				Observation period													Follow-up 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	
	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							
Imaging examinations	Urinalysis	x ⁷		x						x	x	x	x	x	x	x		x	
	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	
	Body temperature	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	
Medical examination	Medical examination and interview	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, SpO₂ 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 saturation of peripheral artery

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4 135 Exclusion criteria

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6 136 Patients who met one or more of the following criteria were excluded:

- 7
8 137 ➤ Presence of a new ischemic lesion in the cerebellum or brainstem at screening;
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10 138 ➤ A marked decline in level of consciousness (NIHSS 1a. evaluation of consciousness level is
11
12 139 score of 3) at screening;
- 13
14 140 ➤ Patients who had an extensive infarct and for whom maintaining life was expected to be
15
16 141 difficult, or who were expected to undergo cranial decompression at screening;
- 17
18 142 ➤ Presence of intracranial hemorrhagic change diagnosed by brain imaging which was judged to
19
20 143 be clinically important by the investigator at screening;
- 21
22 144 ➤ Convulsions after onset of ischemic stroke;
- 23
24 145 ➤ History of neurological events such as stroke or clinically significant head trauma within 180
25
26 146 days prior to informed consent (IC);
- 27
28 147 ➤ Systolic blood pressure >220 mmHg or diastolic blood pressure >120 mmHg, with or without
29
30 148 antihypertensive treatment at screening;
- 31
32 149 ➤ Blood glucose level <50 mg/dL or >400 mg/dL at screening;
- 33
34 150 ➤ Patients who had any of the serious complication(s) listed below at screening:
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36 151 · End stage kidney disease for which dialysis was required;
- 37
38 152 · Progressive liver disease such as hepatitis, cirrhosis with Child-Pugh classification class B
39
40 153 or C, or liver dysfunction with aspartate aminotransferase or alanine aminotransferase over
41
42 154 three times the upper limit of the standard value of the study site;
- 43
44 155 · Severe congestive heart failure rated as New York Heart Association class III or IV, active
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46 156 unstable angina, or ventricular dysfunction with left ventricular ejection fraction (LVEF)
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48 157 <30%; or
- 49
50 158 · Severe pulmonary dysfunction requiring home oxygen therapy.
- 51
52 159 ➤ Human immunodeficiency virus infection, ongoing systemic infection, severe local infection, or
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54 160 immunocompromised condition at screening;
- 55
56 161 ➤ Alzheimer's disease or other dementias, or any other neurological disorder that was judged to
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58 162 affect their ability to give consent to participate in the trial or could confound study assessments
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60 163 performed by the investigator at screening;

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4 164 ➤ Malignant tumor(s) or history of malignant tumor(s) prior to 2 years of ischemic stroke onset at
5 screening;

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8 166 ➤ Contraindications for MRI such as implanted pacemakers or other metallic prosthesis
9 incompatible with MRI, or claustrophobia;

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12 168 ➤ Thrombocytopenia (platelet count $<100,000/\text{mm}^3$) or heparin-induced thrombocytopenia at
13 screening;

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16 170 ➤ History of allergies to human tissues, bovine or porcine preparations;

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18 171 ➤ History of allergy to streptomycin;

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20 172 ➤ Patients who participated in other clinical trials within 12 weeks prior to IC, or planned to

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22 173 participate in other clinical trials during this trial, or participated in clinical trials of other cell

23

24 174 products in the past;

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26 175 ➤ History of splenectomy;

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28 176 ➤ Patients who might have a transient ischemic attack;

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30 177 ➤ Patients who were scheduled to undergo revascularization treatment including carotid

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32 178 endarterectomy, stenting, etc. by the end of the evaluation (day 91);

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34 179 ➤ Patients who were pregnant or lactating at screening, or who wished to become pregnant during

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36 180 the study;

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38 181 ➤ Patients who could not use extremely effective contraception including intrauterine device,

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40 182 intrauterine system, oral contraception (low dose pill), surgical sterilization, double barrier

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42 183 method (condom with spermicide, or combination of condom with pessary) under the guidance

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44 184 of the investigator from the time of IC to one year post-dose (day 366), or who had a partner

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46 185 who could not take similar contraceptive measures; or

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48 186 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.

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52 188 Exclusion criteria on eligibility confirmation assessment

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54 189 After eligibility assessment at screening, the investigator assessed NIHSS again ≥ 4 h after the

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56 190 assessment at screening to confirm patient eligibility. Patients who met one or more of the following

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58 191 criteria were excluded:

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60 192 ➤ NIHSS score ≤ 4 or ≥ 21 ;

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4 193 ➤ Change in NIHSS score from screening ≥ 5 ;
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6 194 ➤ Administration of the study product could not be started within 48 h of symptom onset; or
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8 195 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.
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10196 11 12197 **Randomization and blinding**

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14198 Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and
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16199 2. In cohort 3, subjects were randomly assigned in a 1:1 ratio to receive either JTR-161 or placebo.
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18200 Randomization was performed by the minimization method, which was adjusted centrally by
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20201 dynamic assignment with NIHSS at the time of eligibility assessment, with / without standard
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22202 treatment including intravenous rt-PA or endovascular treatment, and age at the time of IC as the
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24203 allocation factors. The randomization sequence was generated by an organization independent of the
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26204 study sponsors. Allocation of treatment to subjects was randomized via a website. The investigators,
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28205 patients, and the sponsor are masked to the treatment assignment until the observation period is
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30206 completed. After the final subject in cohort 3 completes the day 91 assessment, the database will be
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32207 fixed, and the key will be opened. After that, the sponsor, statistical analysts, and unblinded
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34208 personnel will be placed under open blind, and patients and assessors will be blinded until the end of
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36209 the follow-up period (day 366). JTR-161 and placebo can be identified by the vial appearance;
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38210 therefore, to ensure masking is maintained, only unblinded persons appointed by the investigator
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40211 prepared the administration solution, intravenously injected the study product into the patient, and
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42212 cleaned up any spilled administration solution.
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46214 **Procedure**

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48215 JTR-161 was manufactured in accordance with good manufacturing practice by JCR
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50216 Pharmaceuticals Co., Ltd. The JTR-161 vial (5.0 mL) contained 1.0×10^8 cells of DPSC isolated
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52217 from the extracted teeth of healthy adults, and was stored in the gas space of a liquid nitrogen
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54218 refrigerator.
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56219 The frozen study product was thawed in a constant temperature bath at 37 ± 1 °C for about five
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58220 minutes, then the required number of cells (one or three vials) was diluted in 100 mL of saline. The
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60221 solution was intravenously administered once at a rate of 4 mL/min but ≤ 6 mL/min within 48 h of

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4 222 symptom onset. Number of cells administered in each cohort and flow chart of the cohorts are shown
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6 223 in figure 1. The DSMB was primarily involved in deciding whether or not to advance to the next
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8 224 cohort, as well as the dose (number of cells) for cohort 3. Surgical revascularization such as carotid
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10 225 endarterectomy and carotid artery stenting was prohibited during the observation period, and
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12 226 attending any clinical trials other than this study was prohibited until the end of the study. In cohorts
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14 227 1 and 2, the administration interval between subjects was ≥ 72 hours.

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16 228 Baseline assessments were carried out at day 0 prior to administration, including (1) primary disease:
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18 229 initial or recurrent, type of cerebral infarction, infarcted blood vessels, onset time, and diffusion-
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20 230 weighted imaging (DWI) -Alberta Stroke Program Early Computed Tomography Score, (2)
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22 231 with/without standard treatment with intravenous rt-PA or endovascular treatment. If yes, treatment
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24 232 start time (endovascular treatment only), degree of recanalization (modified thrombolysis in cerebral
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26 233 infarction classification), recanalization time, and number of passes. If no, reasons for not
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28 234 implementing standard treatment, (3) NIHSS at time of arrival, pre-registration, and eligibility tests,
29
30 235 (4) mRS before the onset of cerebral infarction reported by patients or her/his family, (5) disease
31
32 236 history related to the exclusion criteria and, where relevant, the time of complete cure of any malignant
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34 237 condition, effected at least 2 years before IC and still considered cured at the start of administration of
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36 238 the study product. In addition, a medical history deemed necessary for considering AEs was taken.
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38 239 After administration of the study product, mRS and Barthel Index (BI) were assessed at days 31, 91,
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40 240 and 366. NIHSS was assessed at days 2, 8, 31, and 91, and on the day of discharge. Patients were asked
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42 241 to answer the EuroQOL 5 dimensions 5-level scores (EQ-5D-5L) questionnaire at days 31, 91, and
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44 242 336. Laboratory tests were performed pre-registration, pre-administration, and on days 2, 3, 8, 31, 91,
45
46 243 181, and 366 after administration. Blood pressures including systolic and diastolic blood pressures and
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48 244 pulse rates were measured pre-registration, pre-administration, 1, 2, 4, 6, 12, and 24 hours after
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50 245 administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Body
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52 246 temperature was measured pre-registration, pre-administration, 2, 4, 6, and 24 hours after
53
54 247 administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge.
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56 248 Saturated oxygen was measured pre-registration, pre-administration, every 15 minutes between one
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58 249 and four hours after administration, every 30 minutes between four and six hours after administration,
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60 250 12 and 24 hours after administration, and on days 3, 8, 31, 91, 181, and 366 after administration.

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4 251 Imaging tests were performed pre-registration, and on days 2, 8, and 31 after administration. Serum
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6 252 cytokines and growth factors including tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, IL-
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8 253 10, IL-17, IL-23, and angiopoietin-1 (Ang-1) were measured pre-administration, and on days 3 and 8
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10254 after administration in cohort 3. Infarct volumes were measured on DWI and/or fluid-attenuated
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12255 inversion recovery using MRI pre-administration, and on days 8 and 31 after administration. Ischemic
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14256 penumbra was measured using MRI as the mismatch between the hypoperfused area on perfusion-
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16257 weighted imaging and the abnormal area on DWI pre-administration, if available. Assessment of
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18258 imaging was performed at the central assessment organization. Discontinuance criteria for individual
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20259 subjects were (1) AEs, worsening of complications, and other safety concerns, (2) no visit to the study
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22260 site due to inconvenience to patients, (3) termination of the study by the sponsor, and (4) termination
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24261 of the study by the investigator due to safety concerns regarding the study product.
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28263 **Outcome measures**

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30264 The primary endpoint is the proportion of patients who achieve an excellent outcome as defined by
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32265 all of the following criteria at day 91 in cohort 3: mRS ≤ 1 , NIHSS ≤ 1 , and BI ≥ 95 . Secondary
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34266 endpoints were (1) proportion of patients who achieve mRS ≤ 1 or mRS ≤ 2 at days 91 and 366, (2)
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36267 proportion of patients who achieve BI ≥ 95 at days 91 and 366, (3) proportion of patients who
37
38268 achieve NIHSS ≤ 1 , who achieve improvement of $\geq 75\%$, and who achieve improvement of ≥ 10
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40269 points at day 91, (4) changes in EQ-5D-5L scores at day 366, (5) proportion of patients who achieve
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42270 an excellent outcome (mRS ≤ 1 , NIHSS ≤ 1 , and BI ≥ 95) at day 91. EQ-5D-5L consists of two
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44271 parts: the EQ-5D descriptive system and the EQ visual analogue scale (VAS). The descriptive
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46272 system consists of five dimensions: mobility, self-care, usual activities, pain/discomfort, and
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48273 anxiety/depression. Each dimension has five levels: 1 = "no problems", 2 = "slight problems", 3 =
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50274 "moderate problems", 4 = "severe problems", and 5 = "extreme problems". The EQ VAS was
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52275 recorded during the patient's self-rated health assessment on a vertical VAS, where the endpoints
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54276 were labelled 'The best health you can imagine' and 'The worst health you can imagine', (6)
55
56277 proportion of patients who achieve an excellent outcome (mRS ≤ 2 , improvement in NIHSS $\geq 75\%$,
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58278 and BI ≥ 95) at day 91. Safety was assessed based on AEs, laboratory tests, vital signs,
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60279 transcutaneous oxygen saturation, and imaging test including MRI or CT. The investigator assessed

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4 280 the intensity, severity, and relatedness of an AE. All serious AEs were reported using a standardized
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6 281 SAE report form. Exploratory assessments were (1) cytokines and growth factors such as TNF- α , IL-
7
8 282 1 β , IL-6, IL-10, IL-17, IL-23, and Ang-1 as biomarkers in cohort 3, (2) infarct volumes, and (3)
9
10283 penumbra area volume if available.
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14285 **Data monitoring body**

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16286 All data were collected via an electronic case report form prepared using Rave® (Medidata Solutions
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18287 Japan, Tokyo, Japan). Periodic monitoring was performed independently by the sponsor during the
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20288 trial in order to confirm that the trial was conducted in accordance with the study protocol.
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24290 **Sample size estimates**

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26291 In cohorts 1 and 2, eight subjects per cohort (JTR-161, n = 6; placebo, n = 2) were set as the
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28292 appropriate number of subjects for the safety evaluation. In cohort 3, 60 subjects (JTR-161, n = 30;
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30293 placebo, n = 30) were set as the number sufficient for designing a future clinical trial based on the
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32294 safety and efficacy data even if a subpopulation analysis is performed.
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36296 **Statistical analyses**

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38297 Efficacy analyses will be performed in the full analysis set (FAS); the population of enrolled patients
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40298 who will have received the study product at least once and have had a post-dose efficacy assessment,
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42299 and secondary endpoints will be assessed in the per protocol set (PPS); the FAS population excluding
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44300 those patients with a significant protocol violation. The safety analysis will be performed for patients
45
46301 in the safety analysis set (SAF); the population of all enrolled patients who will receive the study
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48302 product and have a post-dose safety assessment. Categorical variables of patient characteristics and
49
50303 baseline parameters will be aggregated for each treatment group and cohort, and descriptive statistics
51
52304 will be calculated for continuous variables. Comparison analysis will be performed between the JTR-
53
54305 161 and placebo groups in cohort 3, and between the merged JTR-161 groups of cohort 3 and the
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56306 cohort receiving the same dose as cohort 3, and the merged placebo groups of cohorts 1, 2, and 3. As
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58307 for the primary endpoint, the proportions and their confidence intervals will be calculated for each
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60308 administration group. Also, the point estimates of difference in the proportion and its confidence

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4 309 interval will be calculated and compared between the JTR-161 and placebo groups. As for secondary
5
6 310 endpoints, the proportions and their confidence intervals for mRS, BI, and NIHSS will be calculated
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8 311 for each administration group, and point estimates of the difference in the proportions and its
9
10312 confidence interval will be calculated. The common odds ratio of the mRS will be calculated for each
11
12313 administration group, and the distribution in each category will be shown. Descriptive statistics of
13
14314 mRS, BI, EQ-5D-5L, biomarkers, infarct volumes, and penumbra area volume at the time of
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16315 assessments will be calculated for each treatment group.

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18316 For AEs and adverse drug reactions for each administration group, the number of patients, the
19
20317 number of cases, and the rate of occurrence will be tabulated according to degree of seriousness,
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22318 severity, and time of onset. AEs will be listed according to MedDRA as lowest level term, and are
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24319 similarly aggregated using the system organ class and preferred term. For laboratory tests, vital
25
26320 signs, and oxygen saturation, descriptive statistics will be calculated or tabulated for each
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28321 administration group and each test time point. The presence or absence of abnormal fluctuations for
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30322 each test item in individual cases will be summarized. No adjustment for multiplicity will be
31
32323 performed. The two-sided significance level will be set at 5%. Interval estimation will be calculated
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34324 with a confidence coefficient of 95%.

35 36325 37 38326 **Study organization and funding**

39
40327 The study was designed and conducted by the sponsor, Teijin Pharma Ltd., Tokyo, Japan in
41
42328 collaboration with the principal investigators. The sponsor monitored study conduct, collected the
43
44329 data, and performed the statistical analyses. This study is funded by Teijin Pharma Ltd. and JCR
45
46330 Pharmaceuticals Co., Ltd.

47 48331 49 50332 **Patient and public involvement**

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52333 No patients and/or public were involved in setting the research questions nor they were involved in
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54334 developing plans for the design (or implementation) of this study protocol.

55 56335 57 58336 **Ethics and dissemination**

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60337 The study protocol and IC form were approved by the institutional review board at each participating

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4 338 study site. All patients gave written IC before initiation of any study-specific procedures. IC from
5
6 339 proxies was also allowed due to the pathophysiology of patients with acute cerebral infarction. The
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8 340 study was conducted in accordance with the ethical principles originating in or derived from the
9
10341 Declaration of Helsinki and Good Clinical Practice guidelines. A manuscript with the results of the
11
12342 primary study will be published in a peer-reviewed journal. On completion of the trial, and after
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14343 publication of the primary manuscript, data requests can be submitted to the corresponding author.
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16344

17 **DISCUSSION**

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20346 Bone marrow is a major source of stem cells and systemic delivery of BM-MSCs after cerebral
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22347 ischemia has been widely studied.⁴⁻⁷ While collection of BM-MSCs requires invasive bone marrow
23
24348 puncture, DPSCs can be obtained easily and less invasively from the extracted teeth of healthy adults.
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26349 They exhibit better plasticity and proliferation capability, and have more potent immunoregulatory
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28350 effects.^{11,16,17} This J-REPAIR study is the first-in-human, randomized, double-blind, placebo-
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30351 controlled study to evaluate the efficacy and safety of JTR-161 in patients with acute ischemic stroke.
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32352 Patients were selected as participants in this first-in-human study from the viewpoint of invasiveness
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34353 and unknown risk of DPSCs to the subjects, referring to the "Guidance on quality, and technical
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36354 guidance on conducting non-clinical trials and clinical trials of regenerative medicine products (human
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38355 cell processed products)".¹⁸ The eligible patients were restricted to those with anterior circulation
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40356 ischemic stroke because the severity of their symptoms can be assessed using NIHSS¹⁹, one of the key
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42357 criteria for assessing eligibility and efficacy in our study. It is difficult to confirm the accurate etiology
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44358 of stroke on admission; therefore, there is no limitation regarding stroke subtype such as lacuna,
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46359 atherothrombotic, cardioembolic, and others. Our study did not limit the use of standard treatment
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48360 including intravenous rt-PA and/or endovascular thrombectomy for recruitment. In addition, available
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50361 treatments for acute ischemic stroke except revascularization treatment such as carotid endarterectomy
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52362 and stenting in routine clinical practice were allowed to be used as a combination therapy. Patients to
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54363 whom standard treatment could not be given, and patients who received standard treatment but had a
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56364 NIHSS ≥ 5 were allowed to be enrolled. However, these pretreatment and combination therapies may
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58365 make it difficult to evaluate the safety and efficacy of JTR-161 accurately; therefore, a placebo arm
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60366 was established as a control group. The study is conducted in a double-blinded manner during the

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4 367 observation period. The keys were opened to the sponsor, statistical analysts, and unblinded personnel,
5
6 368 but patients and assessors continued under blind conditions until the end of the follow-up period, since
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8 369 EQ-5D-5L was assessed at day 366. In order to explore the therapeutic time window, timing of
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10370 administration was set to be within 48 h of symptom onset.

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12371 The proportion of subjects who achieve an excellent outcome defined as mRS ≤ 1 , NIHSS ≤ 1 , and
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14372 BI ≥ 95 was set as the primary endpoint because we considered this clinical outcome was the most
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16373 accurate way of detecting any difference in effectiveness between the subjects receiving JTR-161
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18374 and the placebo group. As secondary endpoints, the efficacy of JTR-161 was also evaluated using
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20375 mRS and BI for disability assessments, and NIHSS for function assessment, all of which are widely
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22376 accepted for use as endpoints in clinical trials of acute ischemic stroke.²⁰ In recent clinical trials of
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24377 intravenous rt-PA and endovascular treatment, clinical outcomes as per mRS were evaluated 90 days
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26378 after the start of treatment.^{21,22} Similarly, period during which the efficacy of JTR-161 was evaluated
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28379 was set to 90 days after administration of the study product. EQ-5D-5L was used as a patient-
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30380 reported outcome for evaluating patient health status. It is reported that there was a significant
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32381 correlation between stroke type and severity, and EQ-5D-5L scores; reproducibility and validity have
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34382 been verified in stroke patients.²³ We measured a variety of serum cytokines and growth factors
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36383 before and after transplantation of JTR-161 to investigate the mechanism of human DPSCs on acute
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38384 ischemic stroke.

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40385 In a pre-clinical study, the distribution of JTR-161 labelled with a radioactive tracer was highest in
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42386 the lung two hours after a single intravenous administration (in-house data), as reported in other
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44387 types of stem cells.²⁴ The onset of symptoms such as respiratory distress and decreased oxygen
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46388 saturation should be carefully followed immediately after administration of JTR-161. Oxygen
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48389 saturation was measured every 15 minutes for up to 4 hours and every 30 minutes for up to 6 hours
49
50390 after administration. Imaging tests were performed to assess infarct lesions and the presence or
51
52391 absence of significant hemorrhagic changes. On the other hand, time of disappearance of JTR-161
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54392 from the body has not been elucidated. Therefore, we established a follow-up period of up to one
55
56393 year after administration (day 366).

57
58394 In conclusion, JTR-161 will provide a novel therapeutic option for the treatment of patients with
59
60395 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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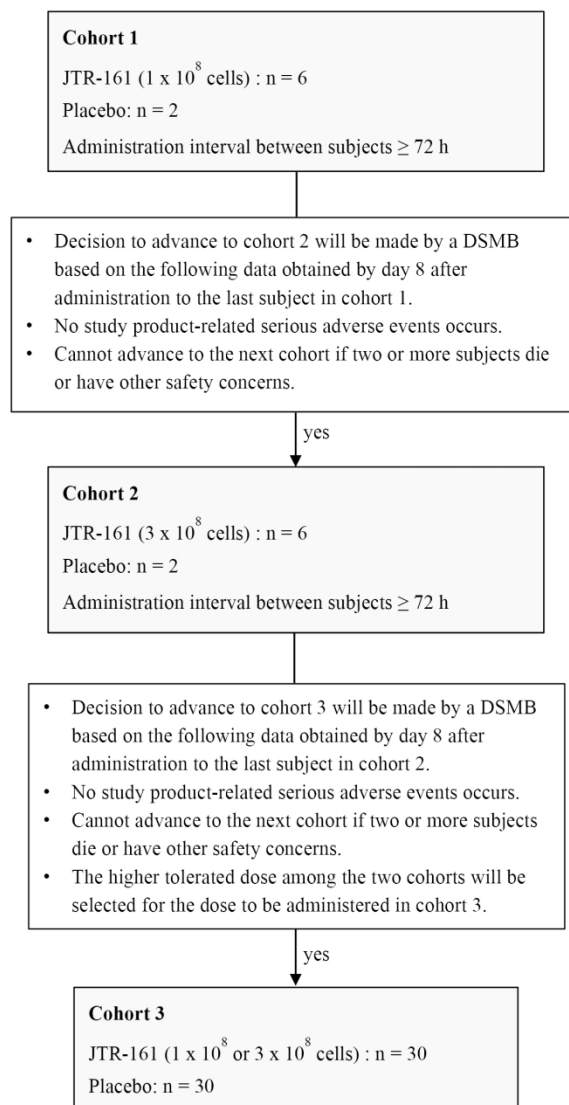


Figure 1

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

1	Introduction						
2							
3	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			
4							
5							
6		6b	Explanation for choice of comparators	14			
7							
8	Objectives	7	Specific objectives or hypotheses	5			
9							
10	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			
11							
12							
13							
14	Methods: Participants, interventions, and outcomes						
15							
16	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			
17							
18							
19	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			
20							
21							
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10, Figure1			
23							
24							
25							
26		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			
27							
28							
29		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12			
30							
31							
32		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10,14			
33							
34	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			
35							
36							
37							
38	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			
39							
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42							

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
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-
5				
6	Methods: Assignment of interventions (for controlled trials)			
7				
8	Allocation:			
9				
10	Sequence	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
11	generation			
12				
13				
14				
15				
16	Allocation	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
17	concealment			
18	mechanism			
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
28				
29				
30				
31	Methods: Data collection, management, and analysis			
32				
33	Data collection	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
34	methods			
35				
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39		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	-
40				
41				
42				

1	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	-
2				
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4				
5	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
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
9				
10		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
11				
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13				
14	Methods: Monitoring			
15				
16	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
17				
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22		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
23				
24				
25	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
26				
27				
28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	12
29				
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32	Ethics and dissemination			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13
35				
36				
37	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)	-
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	13
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
5				
6				
7	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	-
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
11				
12				
13	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
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16	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	-
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20	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	-
21				
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23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	15-16
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
27				
28				
29	Appendices			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	-
32				
33				
34	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
35				
36				

*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”](https://creativecommons.org/licenses/by-nc-nd/3.0/) 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:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-054269.R1
Article Type:	Protocol
Date Submitted by the Author:	06-Jan-2022
Complete List of Authors:	Suda, Satoshi; Nippon Medical School, Department of Neurology Nito, Chikako ; Nippon Medical School, Department of Neurology Ihara, Masafumi; Kokuritsu Junkankibyō Kenkyū Center, Neurology Iguchi, Yasuyuki ; Jikei University School of Medicine, Department of Neurology Urabe, Takao; Juntendo University Urayasu Hospital, Department of Neurology Matsumaru, Yuji; University of Tsukuba Faculty of Medicine, Department of Neurosurgery Sakai, Nobuyuki; Kobe City Medical Center General Hospital, Department of Neurosurgery Kimura, Kazumi ; Nippon Medical School, Department of Neurology
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	NEUROLOGY, INTERNAL MEDICINE, Neurology < INTERNAL MEDICINE, Stroke < NEUROLOGY

SCHOLARONE™
Manuscripts

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4 1 **A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-**
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6 2 **161, allogeneic human dental Pulp stem cells, in patients with Acute Ischemic stRoke**
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8 3 **(J-REPAIR)**
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12 5 Satoshi Suda^{1*}; Chikako Nito^{1*}; Masafumi Ihara²; Yasuyuki Iguchi³; Takao Urabe⁴; Yuji
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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 1×10^8 (cohort 1) to 3×10^8 (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 1×10^8 cells in cohort 1, 3×10^8 cells in cohort 2, and the higher tolerated dose among the two cohorts (either 1×10^8 cells or 3×10^8 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 responses¹², 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 ischemia¹⁵, 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

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4 104 ischemic damage and promoted functional improvement in a rodent model of focal cerebral ischemia
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6 105 by modulating neuroinflammatory reactions.^{16,17} Preclinical toxicological study of a single intravenous
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8 106 administration of JTR-161 to male and female nude rats showed no notable toxicological findings two
9
10 107 weeks after administration (In house data). There were no notable findings regarding tumorigenicity
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12 108 16 weeks after administration. Furthermore, no scaffold-independent proliferation ability was
13
14 109 observed. Regarding non-cellular components of the study product and impurities derived from the
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16 110 manufacturing process, because the amount of residual impurities was low, there were negligible
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18 111 concerns regarding safety. Here, we report the protocol of the first-in-human clinical trial of JTR-161
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20 112 in patients with acute ischemic stroke.
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24 114 **METHODS AND ANALYSIS**

26 115 **Study design**

28 116 This is A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-
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30 117 161, allogeneic human DPSCs, in patients with Acute Ischemic stRoke (J-REPAIR study). The aims
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32 118 of this phase 1/2 study are to evaluate the efficacy and safety of JTR-161 in Japanese patients with
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34 119 acute ischemic stroke when given as a single intravenous administration. Patients received 1×10^8 cells
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36 120 in cohort 1, and 3×10^8 cells in cohort 2, sequentially. In cohort 3, the higher tolerated dose among the
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38 121 two cohorts (either 1×10^8 cells or 3×10^8 cells), determined according to the recommendation by the
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40 122 Data and Safety Monitoring Board (DSMB) (figure 1), was administered. The DSMB consists of three
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42 123 independent external experts and recommends advancing to the next cohort only when no product-
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44 124 related serious adverse events (AEs) are observed. The DSMB does not recommend advancing to the
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46 125 next cohort when two or more deaths occur in the same cohort or any other serious safety concerns are
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48 126 reported. Death due to cerebral infarction itself and concomitant disorders including transtentorial
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50 127 herniation, followed in frequency by pneumonia, cardiac causes, and pulmonary embolism,
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52 128 pretreatment with intravenous recombinant tissue-type plasminogen activator (rt-PA) or endovascular
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54 129 treatment, and combination treatment for the primary disease are excluded as causes of death in this
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56 130 study. The study schedule and assessments are shown in table 1.

58 131 Each cohort consists of a 91-day observation period and a 275-day follow-up period (total study period:
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60 132 366 days). Patients were recruited from 29 stroke centers in Japan between January 2019 and July

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4 133 2021. The study was registered as JapicCTI-194570, prior to study patient enrollment, and
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6 134 subsequently on Clinical Trials.gov: NCT04608838.
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10 136 **Patient population**

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12 137 Inclusion criteria

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14 138 Patients who met all the following criteria were included:

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16 139 ➤ Japanese male or female patients 20 years of age or older;
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18 140 ➤ Clinical diagnosis of anterior circulation ischemic stroke based on the results of brain magnetic
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20 141 resonance imaging (MRI) or computed tomography (CT);
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22 142 ➤ National Institutes of Health Stroke Scale (NIHSS) score of ≥ 5 to ≤ 20 at screening;
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24 143 ➤ Onset of ischemic stroke had to have occurred within 48 hours prior to the start of administration
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26 144 of the study product; and
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28 145 ➤ A modified Rankin Scale (mRS) of 0 or 1, by either self-report or family report, prior to ischemic
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30 146 stroke onset.
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Table 1 Schedule for assessments

		Assessment period														Follow-up period		Dis-charge	Termination					
		Pre-observation period			Observation period											Day 181	Day 366							
		Pre-enrolment	Qualification	Pre-dosing	Day 1						Day 2	Day 3	Day 8	Day 31	Day 91									
					0 h	1 h	2 h	4 h	6 h	12 h														
8	Informed consent	x																						
9	Patient characteristics		x ⁵																					
11	Administration of study product				x																			
13	Ability assessment	mRS	x ⁶											x	x			x	x					
14		Barthel Index													x	x			x					
16	Function assessment	NIHSS	x ⁷	x ⁸									x		x	x	x			x				
17	QOL assessment	EQ-5D-5L													x	x				x				
19	Clinical laboratory tests	Hematology	x ⁷		x								x	x	x	x	x	x			x			
20		Biochemistry	x ⁷		x								x	x	x	x	x	x				x		
22		Blood coagulation test	x ⁷		x								x	x	x	x	x	x				x		
23		Biomarker ¹			x									x	x									
25		Urinalysis	x ⁷		x								x	x	x	x	x	x					x	
27	Imaging examinations	Safety assessment	x ⁷										x		x ¹⁰	x								
28		Infarct volume ²			x ⁹										x ¹⁰	x								
29		Penumbra region volume ^{2,3}			x																			
31	Body measurements	Height, weight	x ⁷																					
33	Vital signs	Blood pressure, pulse	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x			x	x	
34		Body temperature	x		x		x	x	x			x	x	x	x	x	x	x	x			x	x	
36	Oxygen saturation	SpO ₂ ⁴	x		x		x	x	x	x	x	x	x	x	x	x	x	x					x	
38	Medical examination	Medical examination and interview	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.

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3 3. Performed at some study sites.

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5 4. In addition to the scheduled period in the table, SpO₂ 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

6 30 min, 3 h 45 min, 4 h 30 min, 5 h, and 5 h 30 min post-dose.

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

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

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11 7. Data before obtaining consent are acceptable.

12 8. Assessed at least 4 hours after enrolment.

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

15 10. Assessed once during Day 5 to Day 8.

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

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4 149 Exclusion criteria

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6 150 Patients who met one or more of the following criteria were excluded:

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8 151 ➤ Presence of a new ischemic lesion in the cerebellum or brainstem at screening;
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10 152 ➤ A marked decline in level of consciousness (NIHSS 1a. evaluation of consciousness level is score
11 of 3) at screening;
- 12 153
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14 154 ➤ Patients who had an extensive infarct and for whom maintaining life was expected to be difficult,
15 or who were expected to undergo cranial decompression at screening;
- 16 155
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18 156 ➤ Presence of intracranial hemorrhagic change diagnosed by brain imaging which was judged to be
19 clinically important by the investigator at screening;
- 20 157
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22 158 ➤ Convulsions after onset of ischemic stroke;
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24 159 ➤ History of neurological events such as stroke or clinically significant head trauma within 180 days
25 prior to informed consent (IC);
- 26 160
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28 161 ➤ Systolic blood pressure >220 mmHg or diastolic blood pressure >120 mmHg, with or without
29 antihypertensive treatment at screening;
- 30 162
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32 163 ➤ Blood glucose level <50 mg/dL or >400 mg/dL at screening;
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34 164 ➤ Patients who had any of the serious complication(s) listed below at screening:
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36 165 · End stage kidney disease for which dialysis was required;
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38 166 · Progressive liver disease such as hepatitis, cirrhosis with Child-Pugh classification class B
39 or C, or liver dysfunction with aspartate aminotransferase or alanine aminotransferase over
40 167 three times the upper limit of the standard value of the study site;
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42 168 · Severe congestive heart failure rated as New York Heart Association class III or IV, active
43 unstable angina, or ventricular dysfunction with left ventricular ejection fraction (LVEF)
44 169 <30%; or
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46 170 · Severe pulmonary dysfunction requiring home oxygen therapy.
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50 172 ➤ Human immunodeficiency virus infection, ongoing systemic infection, severe local infection, or
51 immunocompromised condition at screening;
- 52 173
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54 174 ➤ Alzheimer's disease or other dementias, or any other neurological disorder that was judged to
55 affect their ability to give consent to participate in the trial or could confound study assessments
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58 176 performed by the investigator at screening;
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- 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 $<100,000/\text{mm}^3$) 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 ;

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4 207 ➤ Change in NIHSS score from screening ≥ 5 ;
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6 208 ➤ Administration of the study product could not be started within 48 h of symptom onset; or
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8 209 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.
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10210 11 **Randomization and blinding**

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14212 Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and
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16213 2. In cohort 3, subjects were randomly assigned in a 1:1 ratio to receive either JTR-161 or placebo.
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18214 Randomization was performed by the minimization method, which was adjusted centrally by dynamic
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20215 assignment with NIHSS at the time of eligibility assessment, with / without standard treatment
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22216 including intravenous rt-PA or endovascular treatment, and age at the time of IC as the allocation
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24217 factors. The randomization sequence was generated by an organization independent of the study
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26218 sponsors. Allocation of treatment to subjects was randomized via a website. The investigators, patients,
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28219 and the sponsor are masked to the treatment assignment until the observation period is completed.
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30220 After the final subject in cohort 3 completes the day 91 assessment, the database will be fixed, and the
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32221 key will be opened. After that, the sponsor, statistical analysts, and unblinded personnel will be placed
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34222 under open blind, and patients and assessors will be blinded until the end of the follow-up period (day
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36223 366). JTR-161 and placebo can be identified by the vial appearance; therefore, to ensure masking is
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38224 maintained, only unblinded persons appointed by the investigator prepared the administration solution,
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40225 intravenously injected the study product into the patient, and cleaned up any spilled administration
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42226 solution.
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46228 **Procedure**

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48229 JTR-161 was manufactured in accordance with good manufacturing practice by JCR Pharmaceuticals
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50230 Co., Ltd. The JTR-161 vial (5.0 mL) contained 1.0×10^8 cells of DPSC isolated from the extracted
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52231 teeth of healthy adults, and was stored in the gas space of a liquid nitrogen refrigerator.
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54232 The frozen study product was thawed in a constant temperature bath at $37 \pm 1^\circ \text{C}$ for about five minutes,
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56233 then the required number of cells (one or three vials) was diluted in 100 mL of saline. The solution
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58234 was intravenously administered once at a rate of 4 mL/min but ≤ 6 mL/min within 48 h of symptom
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60235 onset. Number of cells administered in each cohort and flow chart of the cohorts are shown in figure

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4 236 1. The DSMB was primarily involved in deciding whether or not to advance to the next cohort, as well
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6 237 as the dose (number of cells) for cohort 3. Surgical revascularization such as carotid endarterectomy
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8 238 and carotid artery stenting was prohibited during the observation period, and attending any clinical
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10239 trials other than this study was prohibited until the end of the study. In cohorts 1 and 2, the
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12240 administration interval between subjects was ≥ 72 hours.

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14241 Baseline assessments were carried out at day 0 prior to administration, including (1) primary disease:
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16242 initial or recurrent, type of cerebral infarction, infarcted blood vessels, onset time, and diffusion-
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18243 weighted imaging (DWI) -Alberta Stroke Program Early Computed Tomography Score, (2)
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20244 with/without standard treatment with intravenous rt-PA or endovascular treatment. If yes, treatment
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22245 start time (endovascular treatment only), degree of recanalization (modified thrombolysis in cerebral
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24246 infarction classification), recanalization time, and number of passes. If no, reasons for not
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26247 implementing standard treatment, (3) NIHSS at time of arrival, pre-registration, and eligibility tests,
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28248 (4) mRS before the onset of cerebral infarction reported by patients or her/his family, (5) disease
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30249 history related to the exclusion criteria and, where relevant, the time of complete cure of any malignant
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32250 condition, effected at least 2 years before IC and still considered cured at the start of administration of
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34251 the study product. In addition, a medical history deemed necessary for considering AEs was taken.
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36252 After administration of the study product, mRS and Barthel Index (BI) were assessed at days 31, 91,
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38253 and 366. NIHSS was assessed at days 2, 8, 31, and 91, and on the day of discharge. Patients were asked
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40254 to answer the EuroQOL 5 dimensions 5-level scores (EQ-5D-5L) questionnaire at days 31, 91, and
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42255 336. Laboratory tests were performed pre-registration, pre-administration, and on days 2, 3, 8, 31, 91,
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44256 181, and 366 after administration. Blood pressures including systolic and diastolic blood pressures and
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46257 pulse rates were measured pre-registration, pre-administration, 1, 2, 4, 6, 12, and 24 hours after
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48258 administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Body
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50259 temperature was measured pre-registration, pre-administration, 2, 4, 6, and 24 hours after
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52260 administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge.
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54261 Saturated oxygen was measured pre-registration, pre-administration, every 15 minutes between one
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56262 and four hours after administration, every 30 minutes between four and six hours after administration,
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58263 12 and 24 hours after administration, and on days 3, 8, 31, 91, 181, and 366 after administration.
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60264 Imaging tests were performed pre-registration, and on days 2, 8, and 31 after administration. Serum

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4 265 cytokines and growth factors including tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, IL-
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6 266 10, IL-17, IL-23, and angiopoietin-1 (Ang-1) were measured pre-administration, and on days 3 and 8
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8 267 after administration in cohort 3. Infarct volumes were measured on DWI and/or fluid-attenuated
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10268 inversion recovery using MRI pre-administration, and on days 8 and 31 after administration. Ischemic
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12269 penumbra was measured using MRI as the mismatch between the hypoperfused area on perfusion-
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14270 weighted imaging and the abnormal area on DWI pre-administration, if available. Assessment of
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16271 imaging was performed at the central assessment organization. Discontinuance criteria for individual
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18272 subjects were (1) AEs, worsening of complications, and other safety concerns, (2) no visit to the study
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20273 site due to inconvenience to patients, (3) termination of the study by the sponsor, and (4) termination
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22274 of the study by the investigator due to safety concerns regarding the study product.
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26276 **Outcome measures**

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28277 The primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all
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30278 of the following criteria at day 91 in cohort 3: mRS \leq 1, NIHSS \leq 1, and BI \geq 95. Secondary endpoints
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32279 were (1) proportion of patients who achieve mRS \leq 1 or mRS \leq 2 at days 91 and 366, (2) proportion
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34280 of patients who achieve BI \geq 95 at days 91 and 366, (3) proportion of patients who achieve NIHSS \leq
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36281 1, who achieve improvement of \geq 75%, and who achieve improvement of \geq 10 points at day 91, (4)
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38282 changes in EQ-5D-5L scores at day 366, (5) proportion of patients who achieve an excellent outcome
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40283 (mRS \leq 1, NIHSS \leq 1, and BI \geq 95) at day 91. EQ-5D-5L consists of two parts: the EQ-5D descriptive
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42284 system and the EQ visual analogue scale (VAS). The descriptive system consists of five dimensions:
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44285 mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has five
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46286 levels: 1 = "no problems", 2 = "slight problems", 3 = "moderate problems", 4 = "severe problems",
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48287 and 5 = "extreme problems". The EQ VAS was recorded during the patient's self-rated health
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50288 assessment on a vertical VAS, where the endpoints were labelled 'The best health you can imagine'
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52289 and 'The worst health you can imagine', (6) proportion of patients who achieve overall improvement
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54290 (mRS \leq 2, improvement in NIHSS \geq 75% , and BI \geq 95) at day 91. Safety was assessed based on AEs,
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56291 laboratory tests, vital signs, transcutaneous oxygen saturation, and imaging test including MRI or CT.
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58292 The investigator assessed the intensity, severity, and relatedness of an AE. All serious AEs were
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60293 reported using a standardized SAE report form. Exploratory assessments were (1) cytokines and

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4 294 growth factors such as TNF- α , IL-1 β , IL-6, IL-10, IL-17, IL-23, and Ang-1 as biomarkers in cohort 3,
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6 295 (2) infarct volumes, and (3) penumbra area volume if available.
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10297 **Data monitoring body**

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12298 All data were collected via an electronic case report form prepared using Rave® (Medidata Solutions
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14299 Japan, Tokyo, Japan). Periodic monitoring was performed independently by the sponsor during the
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16300 trial in order to confirm that the trial was conducted in accordance with the study protocol.
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20302 **Sample size estimates**

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22303 In cohorts 1 and 2, eight subjects per cohort (JTR-161, n = 6; placebo, n = 2) were set as the appropriate
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24304 number of subjects for the safety evaluation. In cohort 3, 60 subjects (JTR-161, n = 30; placebo, n =
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26305 30) were set as the number sufficient for designing a future clinical trial based on the safety and
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28306 efficacy data even if a subpopulation analysis is performed.
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32308 **Statistical analyses**

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34309 Efficacy analyses will be performed in the full analysis set (FAS); the population of enrolled patients
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36310 who will have received the study product at least once and have had a post-dose efficacy assessment,
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38311 and secondary endpoints will be assessed in the per protocol set (PPS); the FAS population excluding
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40312 those patients with a significant protocol violation. The safety analysis will be performed for patients
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42313 in the safety analysis set (SAF); the population of all enrolled patients who will receive the study
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44314 product and have a post-dose safety assessment. Categorical variables of patient characteristics and
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46315 baseline parameters will be aggregated for each treatment group and cohort, and descriptive statistics
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48316 will be calculated for continuous variables. Comparison analysis will be performed between the JTR-
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50317 161 and placebo groups in cohort 3, and between the merged JTR-161 groups of cohort 3 and the
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52318 cohort receiving the same dose as cohort 3, and the merged placebo groups of cohorts 1, 2, and 3. As
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54319 for the primary endpoint, the proportions and their confidence intervals will be calculated for each
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56320 administration group. Also, the point estimates of difference in the proportion and its confidence
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58321 interval will be calculated and compared between the JTR-161 and placebo groups. As for secondary
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60322 endpoints, the proportions and their confidence intervals for mRS, BI, and NIHSS will be calculated

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4 323 for each administration group, and point estimates of the difference in the proportions and its
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6 324 confidence interval will be calculated. The common odds ratio of the mRS will be calculated for each
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8 325 administration group, and the distribution in each category will be shown. Descriptive statistics of
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10326 mRS, BI, EQ-5D-5L, biomarkers, infarct volumes, and penumbra area volume at the time of
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12327 assessments will be calculated for each treatment group.

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14328 For AEs and adverse drug reactions for each administration group, the number of patients, the number
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16329 of cases, and the rate of occurrence will be tabulated according to degree of seriousness, severity, and
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18330 time of onset. AEs will be listed according to MedDRA as lowest level term, and are similarly
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20331 aggregated using the system organ class and preferred term. For laboratory tests, vital signs, and
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22332 oxygen saturation, descriptive statistics will be calculated or tabulated for each administration group
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24333 and each test time point. The presence or absence of abnormal fluctuations for each test item in
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26334 individual cases will be summarized. No adjustment for multiplicity will be performed. The two-sided
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28335 significance level will be set at 5%. Interval estimation will be calculated with a confidence coefficient
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30336 of 95%.

31 32337 33 34338 **Study organization and funding**

35
36339 Teijin Pharma Ltd., Tokyo, Japan and JCR Pharmaceuticals Co., Ltd., Kobe, Japan were involved in
37
38340 study design, data collection, data analysis, data interpretation, writing of the clinical study report, and
39
40341 made the decision to submit the study results for publication. The delegates of the sponsor are Ken-
41
42342 ichi Umino, Teijin Pharma Limited, Clinical Development Department, Research, Development &
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44343 Technology Unit, 2-1 Kasumigaseki 3-chome, Chiyoda-ku, Tokyo 100-8585, Japan and Kiwamu
45
46344 Imagawa, JCR Pharmaceuticals Co., Ltd., Research Division, Drug Discovery Research Institute, 2-2-
47
48345 9 Murotani, Nishi-ku, Kobe, Hyogo, 651-2241 Japan. This study and its publication are funded by
49
50346 Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.

51 52347 53 54348 **Patient and public involvement**

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56349 No patients and/or public were involved in setting the research questions nor they were involved in
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58350 developing plans for the design (or implementation) of this study protocol.
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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 NIHSS²¹, 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

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6 411 In conclusion, JTR-161 will provide a novel therapeutic option for the treatment of patients with
7
8 412 ischemic stroke due to the wider therapeutic time window for human DPSC transplantation.
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10413 11 12414 **Acknowledgments**

13
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15
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20418 Co., Ltd.) on behalf of the authors and all authors have authorized the submission of this manuscript
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24420 25 26421 **Authors' contributions**

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28422 All authors were involved in the study design, protocol preparation, and acquisition of funding. SS and
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29
30423 CN were responsible for the first draft. All authors have reviewed and approved the final manuscript.
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31
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34425 35 36426 **Declaration of conflicts of interest**

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37
38427 The authors declared the following potential conflicts of interest with respect to the research,
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39
40428 authorship, and/or publication of this article: Expert Witness from Teijin Pharma Ltd. (SS, CN, KK).
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42429 Research funding from Teijin Pharma Ltd. (KK). Lecture fee from Teijin Pharma Ltd. (YI). The other
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44430 authors report no conflicts.
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56436 57 58437 **Figure legend**

58
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60438 Figure 1 Flow chart of the cohorts

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4 439 DSMB, Data and Safety Monitoring Board
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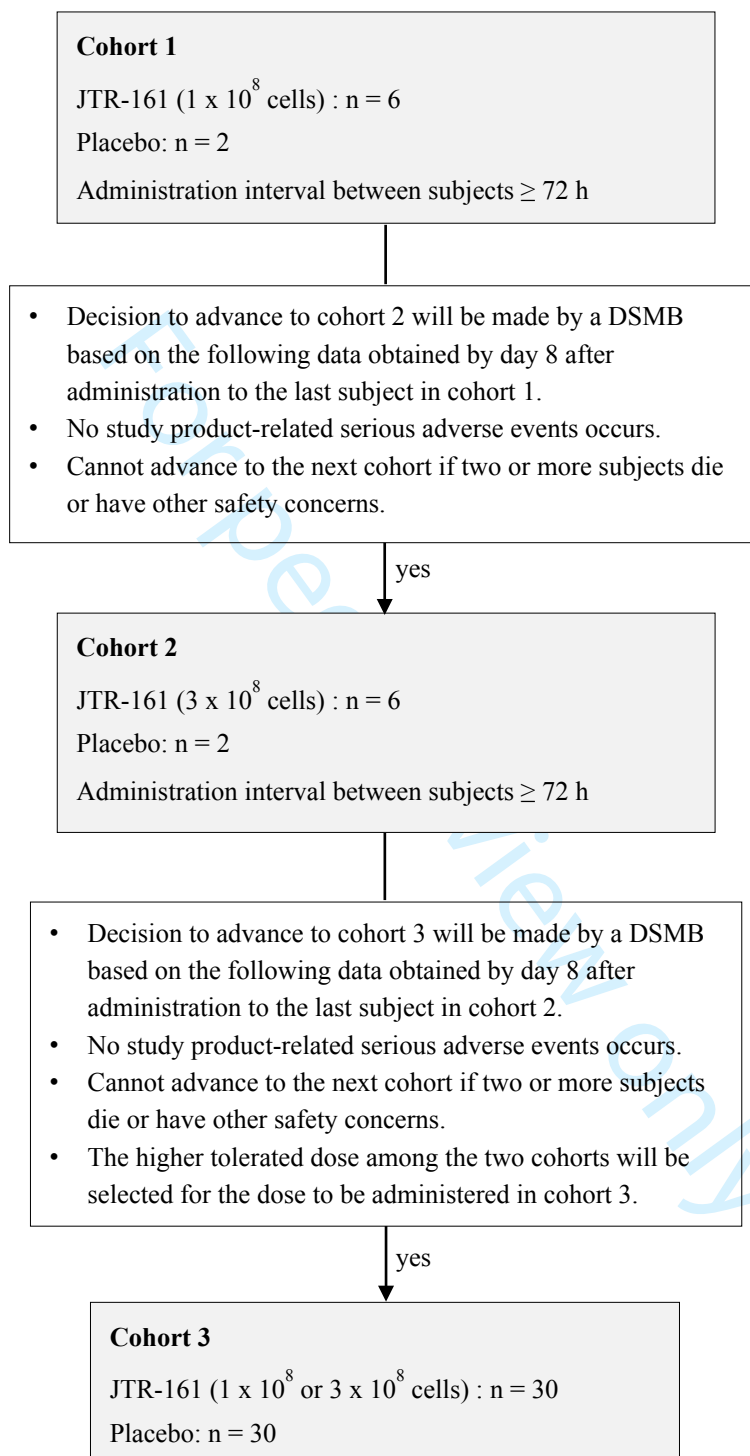


Figure 1 Flow chart of the cohorts
DSMB, Data and Safety Monitoring Board



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

1	Introduction			
2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	5-6
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	16,17
7				
8	Objectives	7	Specific objectives or hypotheses	5-6
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	6-7, Figure1
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	
12				
13				
14	Methods: Participants, interventions, and outcomes			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	6-7
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	7-11
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	11-12, Figure1
23			administered	
24				
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	11-13, Figure1
26			change in response to harms, participant request, or improving/worsening disease)	
27				
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	14
29			(eg, drug tablet return, laboratory tests)	
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12,16
32				
33	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	13,14
34			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	
35			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
36			efficacy and harm outcomes is strongly recommended	
37				
38	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	6, Table 1
39			participants. A schematic diagram is highly recommended (see Figure)	
40				
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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
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-
5				

6 **Methods: Assignment of interventions (for controlled trials)**

7 Allocation:

8				
9				
10	Sequence	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
11	generation			
12				
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16	Allocation	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
17	concealment			
18	mechanism			
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
25				
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27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11
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31 **Methods: Data collection, management, and analysis**

32				
33	Data collection	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
34	methods			
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39		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	-
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1	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	-
2				
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5	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
6				
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8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
9				
10		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
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14	Methods: Monitoring			
15				
16	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
17				
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22		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
23				
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25	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
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28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
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32	Ethics and dissemination			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
35				
36				
37	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)	-
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	16
2				
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
5				
6				
7	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	-
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
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13	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
14				
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16				
17	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	-
18				
19				
20	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	-
21				
22				
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	18
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
27				
28				
29	Appendices			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplemental material
32				
33				
34	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
35				
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*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”](https://creativecommons.org/licenses/by-nc-nd/3.0/) 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:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-054269.R2
Article Type:	Protocol
Date Submitted by the Author:	04-Apr-2022
Complete List of Authors:	Suda, Satoshi; Nippon Medical School, Department of Neurology Nito, Chikako ; Nippon Medical School, Department of Neurology Ihara, Masafumi; Kokuritsu Junkankibyō Kenkyū Center, Neurology Iguchi, Yasuyuki ; Jikei University School of Medicine, Department of Neurology Urabe, Takao; Juntendo University Urayasu Hospital, Department of Neurology Matsumaru, Yuji; University of Tsukuba Faculty of Medicine, Department of Neurosurgery Sakai, Nobuyuki; Kobe City Medical Center General Hospital, Department of Neurosurgery Kimura, Kazumi ; Nippon Medical School, Department of Neurology
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	NEUROLOGY, INTERNAL MEDICINE, Neurology < INTERNAL MEDICINE, Stroke < NEUROLOGY

SCHOLARONE™
Manuscripts

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4 1 **A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-**
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6 2 **161, allogeneic human dental Pulp stem cells, in patients with Acute Ischemic stRoke**
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8 3 **(J-REPAIR)**
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12 5 Satoshi Suda^{1*}; Chikako Nito^{1*}; Masafumi Ihara²; Yasuyuki Iguchi³; Takao Urabe⁴; Yuji
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14 6 Matsumaru⁵; Nobuyuki Sakai⁶; Kazumi Kimura¹; on behalf of the J- REPAIR trial group
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6 31

7
8 32 **Keywords**

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10 33 Cell-based therapy, clinical trial, dental pulp stem cells, ischemic stroke
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14 35 **Word count**

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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 1×10^8 (cohort 1) to 3×10^8 (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 1×10^8 cells in cohort 1, 3×10^8 cells in cohort 2, and the higher tolerated dose among the two cohorts (either 1×10^8 cells or 3×10^8 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 responses¹², 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 ischemia¹⁵, 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

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4 104 ischemic damage and promoted functional improvement in a rodent model of focal cerebral ischemia
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6 105 by modulating neuroinflammatory reactions.^{16,17} Preclinical toxicological study of a single intravenous
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8 106 administration of JTR-161 to male and female nude rats showed no notable toxicological findings two
9
10 107 weeks after administration (In house data). There were no notable findings regarding tumorigenicity
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12 108 16 weeks after administration. Furthermore, no scaffold-independent proliferation ability was
13
14 109 observed. Regarding non-cellular components of the study product and impurities derived from the
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16 110 manufacturing process, because the amount of residual impurities was low, there were negligible
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18 111 concerns regarding safety. Here, we report the protocol of the first-in-human clinical trial of JTR-161
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20 112 in patients with acute ischemic stroke.
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24 114 **METHODS AND ANALYSIS**

26 115 **Study design**

28 116 This is A Randomized placebo-controlled multicenter trial to Evaluate the efficacy and safety of JTR-
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30 117 161, allogeneic human DPSCs, in patients with Acute Ischemic stRoke (J-REPAIR study). The aims
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32 118 of this phase 1/2 study are to evaluate the efficacy and safety of JTR-161 in Japanese patients with
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34 119 acute ischemic stroke when given as a single intravenous administration. Patients received 1×10^8 cells
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36 120 in cohort 1, and 3×10^8 cells in cohort 2, sequentially. In cohort 3, the higher tolerated dose among the
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38 121 two cohorts (either 1×10^8 cells or 3×10^8 cells), determined according to the recommendation by the
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40 122 Data and Safety Monitoring Board (DSMB) (figure 1), was administered. The DSMB consists of three
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42 123 independent external experts. The DSMB does not recommend advancing to the next cohort when two
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44 124 or more product-related death or death for which a causal relationship cannot be ruled out occur in the
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46 125 same cohort, or any other serious safety concerns are reported. Death due to cerebral infarction itself
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48 126 and concomitant disorders including pneumonia and transtentorial herniation, followed in frequency
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50 127 by cardiac causes and pulmonary embolism, pretreatment with intravenous recombinant tissue-type
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52 128 plasminogen activator (rt-PA) or endovascular treatment, and combination treatment for the primary
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54 129 disease are excluded as causes of death in this study. The study schedule and assessments are shown
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56 130 in table 1.

58 131 Each cohort consists of a 91-day observation period and a 275-day follow-up period (total study period:
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60 132 366 days). Patients were recruited from 29 stroke centers in Japan between January 2019 and July

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4 133 2021. The study was registered as JapicCTI-194570, prior to study patient enrollment, and
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6 134 subsequently on Clinical Trials.gov: NCT04608838.
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10 136 **Patient population**

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12 137 Inclusion criteria

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14 138 Patients who met all the following criteria were included:

- 15
16 139 ➤ Japanese male or female patients 20 years of age or older;
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18 140 ➤ Clinical diagnosis of anterior circulation ischemic stroke based on the results of brain magnetic
19
20 141 resonance imaging (MRI) or computed tomography (CT);
21
22 142 ➤ National Institutes of Health Stroke Scale (NIHSS) score of ≥ 5 to ≤ 20 at screening;
23
24 143 ➤ Onset of ischemic stroke had to have occurred within 48 hours prior to the start of administration
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26 144 of the study product; and
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28 145 ➤ A modified Rankin Scale (mRS) of 0 or 1, by either self-report or family report, prior to ischemic
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30 146 stroke onset.
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Table 1 Schedule for assessments

		Assessment period														Follow-up period		Dis-charge	Termination					
		Pre-observation period			Observation period											Day 181	Day 366							
		Pre-enrolment	Qualification	Pre-dosing	Day 1						Day 2	Day 3	Day 8	Day 31	Day 91									
					0 h	1 h	2 h	4 h	6 h	12 h														
8	Informed consent	x																						
9	Patient characteristics		x ⁵																					
11	Administration of study product				x																			
13	Ability assessment	mRS	x ⁶											x	x			x	x					
14		Barthel Index													x	x			x					
16	Function assessment	NIHSS	x ⁷	x ⁸									x		x	x	x			x				
17	QOL assessment	EQ-5D-5L													x	x				x				
19	Clinical laboratory tests	Hematology	x ⁷		x								x	x	x	x	x	x			x			
20		Biochemistry	x ⁷		x								x	x	x	x	x	x				x		
22		Blood coagulation test	x ⁷		x								x	x	x	x	x	x					x	
23		Biomarker ¹			x									x	x									
25		Urinalysis	x ⁷		x								x	x	x	x	x	x					x	
27	Imaging examinations	Safety assessment	x ⁷									x		x ¹⁰	x									
28		Infarct volume ²			x ⁹										x ¹⁰	x								
29		Penumbra region volume ^{2,3}			x																			
31	Body measurements	Height, weight	x ⁷																					
33	Vital signs	Blood pressure, pulse	x		x		x	x	x	x	x	x	x	x	x	x	x	x	x			x	x	
34		Body temperature	x		x		x	x	x			x	x	x	x	x	x	x				x	x	
36	Oxygen saturation	SpO ₂ ⁴	x		x		x	x	x	x	x	x	x	x	x	x	x	x					x	
38	Medical examination	Medical examination and interview	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.

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2

3 3. Performed at some study sites.

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5 4. In addition to the scheduled period in the table, SpO₂ 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

6 30 min, 3 h 45 min, 4 h 30 min, 5 h, and 5 h 30 min post-dose.

7

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

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

10

11 7. Data before obtaining consent are acceptable.

12 8. Assessed at least 4 hours after enrolment.

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

15 10. Assessed once during Day 5 to Day 8.

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

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4 149 Exclusion criteria

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6 150 Patients who met one or more of the following criteria were excluded:

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8 151 ➤ Presence of a new ischemic lesion in the cerebellum or brainstem at screening;
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10 152 ➤ A marked decline in level of consciousness (NIHSS 1a. evaluation of consciousness level is score
11 of 3) at screening;
- 12 153
13
14 154 ➤ Patients who had an extensive infarct and for whom maintaining life was expected to be difficult,
15 or who were expected to undergo cranial decompression at screening;
- 16 155
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18 156 ➤ Presence of intracranial hemorrhagic change diagnosed by brain imaging which was judged to be
19 clinically important by the investigator at screening;
- 20 157
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22 158 ➤ Convulsions after onset of ischemic stroke;
- 23
24 159 ➤ History of neurological events such as stroke or clinically significant head trauma within 180 days
25 prior to informed consent (IC);
- 26 160
27
28 161 ➤ Systolic blood pressure >220 mmHg or diastolic blood pressure >120 mmHg, with or without
29 antihypertensive treatment at screening;
- 30 162
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32 163 ➤ Blood glucose level <50 mg/dL or >400 mg/dL at screening;
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34 164 ➤ Patients who had any of the serious complication(s) listed below at screening:
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36 165 · End stage kidney disease for which dialysis was required;
 - 37
38 166 · Progressive liver disease such as hepatitis, cirrhosis with Child-Pugh classification class B
39 or C, or liver dysfunction with aspartate aminotransferase or alanine aminotransferase over
40 167 three times the upper limit of the standard value of the study site;
 - 41
42 168 · Severe congestive heart failure rated as New York Heart Association class III or IV, active
43 unstable angina, or ventricular dysfunction with left ventricular ejection fraction (LVEF)
44 169 <30%; or
 - 45
46 170 · Severe pulmonary dysfunction requiring home oxygen therapy.
- 47
48 171
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50 172 ➤ Human immunodeficiency virus infection, ongoing systemic infection, severe local infection, or
51 immunocompromised condition at screening;
- 52 173
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54 174 ➤ Alzheimer's disease or other dementias, or any other neurological disorder that was judged to
55 affect their ability to give consent to participate in the trial or could confound study assessments
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58 176 performed by the investigator at screening;
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- 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 $<100,000/\text{mm}^3$) 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 ;

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4 207 ➤ Change in NIHSS score from screening ≥ 5 ;
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6 208 ➤ Administration of the study product could not be started within 48 h of symptom onset; or
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8 209 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.
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10210 11 12211 **Randomization and blinding**

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14212 Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and
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16213 2. In cohort 3, subjects were randomly assigned in a 1:1 ratio to receive either JTR-161 or placebo.
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18214 Randomization was performed by the minimization method, which was adjusted centrally by dynamic
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20215 assignment with NIHSS at the time of eligibility assessment, with / without standard treatment
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22216 including intravenous rt-PA or endovascular treatment, and age at the time of IC as the allocation
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24217 factors. The randomization sequence was generated by an organization independent of the study
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26218 sponsors. Allocation of treatment to subjects was randomized via a website. The investigators, patients,
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28219 and the sponsor are masked to the treatment assignment until the observation period is completed.
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30220 After the final subject in cohort 3 completes the day 91 assessment, the database will be fixed, and the
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32221 key will be opened. After that, the sponsor, statistical analysts, and unblinded personnel will be placed
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34222 under open blind, and patients and assessors will be blinded until the end of the follow-up period (day
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36223 366). JTR-161 and placebo can be identified by the vial appearance; therefore, to ensure masking is
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38224 maintained, only unblinded persons appointed by the investigator prepared the administration solution,
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40225 intravenously injected the study product into the patient, and cleaned up any spilled administration
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42226 solution.
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46228 **Procedure**

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48229 JTR-161 was manufactured in accordance with good manufacturing practice by JCR Pharmaceuticals
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50230 Co., Ltd. The JTR-161 vial (5.0 mL) contained 1.0×10^8 cells of DPSC isolated from the extracted
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52231 teeth of healthy adults, and was stored in the gas space of a liquid nitrogen refrigerator.
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54232 The frozen study product was thawed in a constant temperature bath at 37 ± 1 °C for about five minutes,
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56233 then the required number of cells (one or three vials) was diluted in 100 mL of saline. The solution
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58234 was intravenously administered once at a rate of 4 mL/min but ≤ 6 mL/min within 48 h of symptom
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60235 onset. Number of cells administered in each cohort and flow chart of the cohorts are shown in figure

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4 236 1. The DSMB was primarily involved in deciding whether or not to advance to the next cohort, as well
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6 237 as the dose (number of cells) for cohort 3. Surgical revascularization such as carotid endarterectomy
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8 238 and carotid artery stenting was prohibited during the observation period, and attending any clinical
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10239 trials other than this study was prohibited until the end of the study. In cohorts 1 and 2, the
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12240 administration interval between subjects was ≥ 72 hours.

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14241 Baseline assessments were carried out at day 0 prior to administration, including (1) primary disease:
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16242 initial or recurrent, type of cerebral infarction, infarcted blood vessels, onset time, and diffusion-
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18243 weighted imaging (DWI) -Alberta Stroke Program Early Computed Tomography Score, (2)
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20244 with/without standard treatment with intravenous rt-PA or endovascular treatment. If yes, treatment
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22245 start time, degree of recanalization (modified thrombolysis in cerebral infarction classification),
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24246 recanalization time, and number of passes. If no, reasons for not implementing standard treatment, (3)
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26247 NIHSS at time of arrival, pre-registration, and eligibility tests, (4) mRS before the onset of cerebral
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28248 infarction reported by patients or her/his family, (5) disease history related to the exclusion criteria
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30249 and, where relevant, the time of complete cure of any malignant condition, effected at least 2 years
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32250 before IC and still considered cured at the start of administration of the study product. In addition, a
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34251 medical history deemed necessary for considering adverse events (AEs) was taken. After
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36252 administration of the study product, mRS and Barthel Index (BI) were assessed at days 31, 91, and
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38253 366. NIHSS was assessed at days 2, 8, 31, and 91, and on the day of discharge. Patients were asked to
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40254 answer the EuroQOL 5 dimensions 5-level scores (EQ-5D-5L) questionnaire at days 31, 91, and 336.
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42255 Laboratory tests were performed pre-registration, pre-administration, and on days 2, 3, 8, 31, 91, 181,
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44256 and 366 after administration. Blood pressures including systolic and diastolic blood pressures and
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46257 pulse rates were measured pre-registration, pre-administration, 1, 2, 4, 6, 12, and 24 hours after
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48258 administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Body
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50259 temperature was measured pre-registration, pre-administration, 2, 4, 6, and 24 hours after
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52260 administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge.
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54261 Saturated oxygen was measured pre-registration, pre-administration, every 15 minutes between one
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56262 and four hours after administration, every 30 minutes between four and six hours after administration,
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58263 12 and 24 hours after administration, and on days 3, 8, 31, 91, 181, and 366 after administration.
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60264 Imaging tests were performed pre-registration, and on days 2, 8, and 31 after administration. Serum

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4 265 cytokines and growth factors including tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6, IL-
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6 266 10, IL-17, IL-23, and angiopoietin-1 (Ang-1) were measured pre-administration, and on days 3 and 8
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8 267 after administration in cohort 3. Infarct volumes were measured on DWI and/or fluid-attenuated
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10268 inversion recovery using MRI pre-administration, and on days 8 and 31 after administration. Ischemic
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12269 penumbra was measured using MRI as the mismatch between the hypoperfused area on perfusion-
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14270 weighted imaging and the abnormal area on DWI pre-administration, if available. Assessment of
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16271 imaging was performed at the central assessment organization. Discontinuance criteria for individual
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18272 subjects were (1) AEs, worsening of complications, and other safety concerns, (2) no visit to the study
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20273 site due to inconvenience to patients, (3) termination of the study by the sponsor, and (4) termination
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22274 of the study by the investigator due to safety concerns regarding the study product.
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26276 **Outcome measures**

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28277 The primary endpoint is the proportion of patients who achieve an excellent outcome as defined by all
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30278 of the following criteria at day 91 in cohort 3: mRS \leq 1, NIHSS \leq 1, and BI \geq 95. Secondary endpoints
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32279 were (1) proportion of patients who achieve mRS \leq 1 or mRS \leq 2 at days 91 and 366, (2) proportion
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34280 of patients who achieve BI \geq 95 at days 91 and 366, (3) proportion of patients who achieve NIHSS \leq
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36281 1, who achieve improvement of \geq 75%, and who achieve improvement of \geq 10 points at day 91, (4)
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38282 changes in EQ-5D-5L scores at day 366, (5) proportion of patients who achieve an excellent outcome
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40283 (mRS \leq 1, NIHSS \leq 1, and BI \geq 95) at day 91, (6) proportion of patients who achieve overall
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42284 improvement (mRS \leq 2, improvement in NIHSS \geq 75% , and BI \geq 95) at day 91. EQ-5D-5L consists
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44285 of two parts: the EQ-5D descriptive system and the EQ visual analogue scale (VAS). The descriptive
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46286 system consists of five dimensions: mobility, self-care, usual activities, pain/discomfort, and
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48287 anxiety/depression. Each dimension has five levels: 1 = "no problems", 2 = "slight problems", 3 =
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50288 "moderate problems", 4 = "severe problems", and 5 = "extreme problems". The EQ VAS was recorded
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52289 during the patient's self-rated health assessment on a vertical VAS, where the endpoints were labelled
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54290 'The best health you can imagine' and 'The worst health you can imagine'. Safety was assessed based
55
56291 on AEs, laboratory tests, vital signs, transcutaneous oxygen saturation, and imaging test including MRI
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58292 or CT. The investigator assessed the intensity, severity, and relatedness of an AE. All serious AEs
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60293 were reported using a standardized SAE report form. Exploratory assessments were (1) cytokines and

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4 294 growth factors such as TNF- α , IL-1 β , IL-6, IL-10, IL-17, IL-23, and Ang-1 as biomarkers in cohort 3,
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6 295 (2) infarct volumes, and (3) penumbra area volume if available.
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10297 **Data monitoring body**

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12298 All data were collected via an electronic case report form prepared using Rave® (Medidata Solutions
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14299 Japan, Tokyo, Japan). Periodic monitoring was performed independently by the sponsor during the
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16300 trial in order to confirm that the trial was conducted in accordance with the study protocol.
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20302 **Sample size estimates**

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22303 In cohorts 1 and 2, eight subjects per cohort (JTR-161, n = 6; placebo, n = 2) were set as the appropriate
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24304 number of subjects for the safety evaluation. In cohort 3, 60 subjects (JTR-161, n = 30; placebo, n =
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26305 30) were set as the number sufficient for designing a future clinical trial based on the safety and
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28306 efficacy data even if a subpopulation analysis is performed.
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32308 **Statistical analyses**

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34309 Efficacy analyses will be performed in the full analysis set (FAS); the population of enrolled patients
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36310 who will have received the study product once and have had a post-dose efficacy assessment, and
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38311 secondary endpoints will be assessed in the per protocol set (PPS); the FAS population excluding those
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40312 patients with a significant protocol violation. The safety analysis will be performed for patients in the
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42313 safety analysis set (SAF); the population of all enrolled patients who will receive the study product
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44314 and have a post-dose safety assessment. Categorical variables of patient characteristics and baseline
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46315 parameters will be aggregated for each treatment group and cohort, and descriptive statistics will be
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48316 calculated for continuous variables. Comparison analysis will be performed between the JTR-161 and
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50317 placebo groups in cohort 3, and between the merged JTR-161 groups of cohort 3 and the cohort
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52318 receiving the same dose as cohort 3, and the merged placebo groups of cohorts 1, 2, and 3. As for the
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54319 primary endpoint, the proportions and their confidence intervals will be calculated for each
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56320 administration group. Also, the point estimates of difference in the proportion and its confidence
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58321 interval will be calculated and compared between the JTR-161 and placebo groups. As for secondary
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60322 endpoints, the proportions and their confidence intervals for mRS, BI, and NIHSS will be calculated

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4 323 for each administration group, and point estimates of the difference in the proportions and its
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6 324 confidence interval will be calculated. The common odds ratio of the mRS will be calculated for each
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8 325 administration group, and the distribution in each category will be shown. Descriptive statistics of
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10326 mRS, BI, EQ-5D-5L, biomarkers, infarct volumes, and penumbra area volume at the time of
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12327 assessments will be calculated for each treatment group.

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14328 For AEs and adverse drug reactions for each administration group, the number of patients, the number
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16329 of cases, and the rate of occurrence will be tabulated according to degree of seriousness, severity, and
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18330 time of onset. AEs will be listed according to MedDRA as lowest level term, and are similarly
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20331 aggregated using the system organ class and preferred term. For laboratory tests, vital signs, and
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22332 oxygen saturation, descriptive statistics will be calculated or tabulated for each administration group
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24333 and each test time point. The presence or absence of abnormal fluctuations for each test item in
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26334 individual cases will be summarized. No adjustment for multiplicity will be performed. The two-sided
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28335 significance level will be set at 5%. Interval estimation will be calculated with a confidence coefficient
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30336 of 95%.

31 32337 33 34338 **Study organization and funding**

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36339 Teijin Pharma Ltd., Tokyo, Japan and JCR Pharmaceuticals Co., Ltd., Kobe, Japan were involved in
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38340 study design, data collection, data analysis, data interpretation, writing of the clinical study report, and
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40341 made the decision to submit the study results for publication. The delegates of the sponsor are Ken-
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42342 ichi Umino, Teijin Pharma Limited, Clinical Development Department, Research, Development &
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44343 Technology Unit, 2-1 Kasumigaseki 3-chome, Chiyoda-ku, Tokyo 100-8585, Japan and Kiwamu
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46344 Imagawa, JCR Pharmaceuticals Co., Ltd., Research Division, Drug Discovery Research Institute, 2-2-
47
48345 9 Murotani, Nishi-ku, Kobe, Hyogo, 651-2241 Japan. This study and its publication are funded by
49
50346 Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.

51 52347 53 54348 **Patient and public involvement**

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56349 No patients and/or public were involved in setting the research questions nor they were involved in
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58350 developing plans for the design (or implementation) of this study protocol.
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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 NIHSS²¹, 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

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4 410 (day 366).

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6 411 In conclusion, JTR-161 will provide a novel therapeutic option for the treatment of patients with
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8 412 ischemic stroke due to the wider therapeutic time window for human DPSC transplantation.
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10413 11 12414 **Acknowledgments**

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15
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20418 Co., Ltd.) on behalf of the authors and all authors have authorized the submission of this manuscript
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26421 **Authors' contributions**

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28422 CN, MI, YI, TU, YM, NS and KK were involved in the study design, protocol preparation, and
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30423 acquisition of funding. SS, CN and KK will be responsible for directly accessing and verifying all data.
29
30
31
32424 SS and CN were responsible for the first draft. All authors have reviewed and approved the final
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33425 manuscript. The work is funded by Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.
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38427 **Declaration of conflicts of interest**

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40428 The authors declared the following potential conflicts of interest with respect to the research,
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42429 authorship, and/or publication of this article: Expert Witness from Teijin Pharma Ltd. (SS, CN, KK).
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44430 Research funding from Teijin Pharma Ltd. (KK). Lecture fee from Teijin Pharma Ltd. (YI). The other
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46431 authors report no conflicts.
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60438 **Figure legend**

1
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4 439 Figure 1 Flow chart of the cohorts

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6 440 DSMB, Data and Safety Monitoring Board
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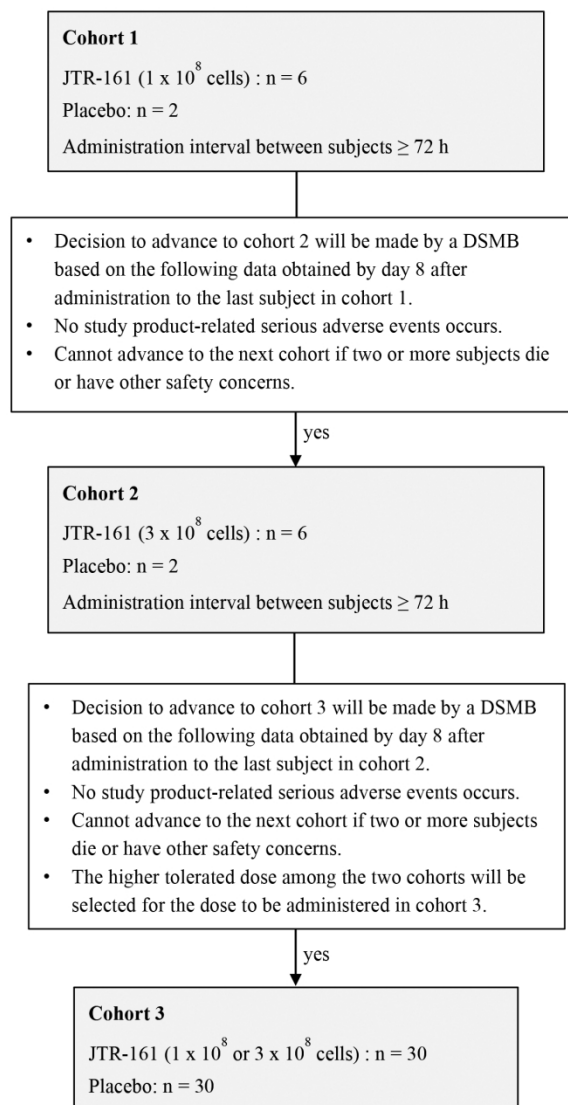


Figure 1

Figure 1. Flow chart of the cohorts



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

1	Introduction			
2				
3	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
4		6b	Explanation for choice of comparators	17,18
5				
6	Objectives	7	Specific objectives or hypotheses	5-6
7				
8	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
9				
10				
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13				
14	Methods: Participants, interventions, and outcomes			
15				
16	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
17				
18				
19	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
20				
21	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	12-13, Figure1
22		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
23		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	15
24		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	13,18
25				
26	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
27				
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29	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
30				
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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
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	-
5				

6 **Methods: Assignment of interventions (for controlled trials)**

7 Allocation:

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9				
10	Sequence	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
11	generation			
12				
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16	Allocation	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
17	concealment			
18	mechanism			
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
21				
22				
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24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
25				
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27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12
28				
29				
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31 **Methods: Data collection, management, and analysis**

32				
33	Data collection	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
34	methods			
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39		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	-
40				
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1	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	-
2				
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5	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
6				
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8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	NA
9				
10		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
11				
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14	Methods: Monitoring			
15				
16	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
17				
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22		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
23				
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25	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
26				
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28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
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32	Ethics and dissemination			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
35				
36				
37	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)	-
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	17
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
5				
6				
7	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	-
8				
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	19
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13	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
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17	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	-
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20	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	-
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	19
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
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29	Appendices			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplemental material
32				
33				
34	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
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*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”](https://creativecommons.org/licenses/by-nc-nd/3.0/) license.