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# 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)

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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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## 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  $1 \times 10^8$  (cohort 1) to  $3 \times 10^8$  (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)  $\leq 1$ , NIHSS  $\leq 1$ , and Barthel Index (BI)  $\geq 95$ .

**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  $1 \times 10^8$  cells in cohort 1,  $3 \times 10^8$  cells in cohort 2, and the higher tolerated dose among the two cohorts (either  $1 \times 10^8$  cells or  $3 \times 10^8$  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  $\leq 1$ , NIHSS  $\leq 1$ , and Barthel Index  $\geq 95$ .
- 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.<sup>1</sup> 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<sup>2,3</sup>, 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.<sup>4-7</sup> In 2000, human dental pulp stem cells (DPSCs) were discovered in impacted molar teeth.<sup>8</sup> 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.<sup>9,10</sup> 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.<sup>10</sup> Furthermore, compared to BM-MSCs, DPSCs can be obtained by a less invasive process, are more easily expanded, and exert more potent immunosuppressive effects via the inhibition of activated T cell responses<sup>11</sup>, 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<sup>12,13</sup>.

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.<sup>14,15</sup> 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  $1 \times 10^8$  cells in cohort 1,  
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10107 and  $3 \times 10^8$  cells in cohort 2, sequentially. In cohort 3, the higher tolerated dose among the two cohorts  
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12108 (either  $1 \times 10^8$  cells or  $3 \times 10^8$  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  
23  
24114 intravenous recombinant tissue-type plasminogen activator (rt-PA) or endovascular treatment, and  
25  
26115 combination treatment for the primary disease are excluded as causes of death in this study. The study  
27  
28116 schedule and assessments are shown in table 1.

29  
30117 Each cohort consists of a 91-day observation period and a 275-day follow-up period (total study period:  
31  
32118 366 days). Patients were recruited from 29 stroke centers in Japan between December 2018 and July  
33  
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  $\geq 5$  to  $\leq 20$  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 |
|                                 |                                       |                |                | 0 h            | 1 h                | 2 h | 4 h | 6 h | 12 h |   |       |       |                 |        |                  |           |             |         |
| Informed consent                | x                                     |                |                |                |                    |     |     |     |      |   |       |       |                 |        |                  |           |             |         |
| Patient characteristics         |                                       | x <sup>5</sup> |                |                |                    |     |     |     |      |   |       |       |                 |        |                  |           |             |         |
| Administration of study product |                                       |                |                |                | x                  |     |     |     |      |   |       |       |                 |        |                  |           |             |         |
| Ability assessment              | mRS                                   | x <sup>6</sup> |                |                |                    |     |     |     |      |   |       |       |                 | x      | x                |           |             | x       |
|                                 | Barthel Index                         |                |                |                |                    |     |     |     |      |   |       |       |                 | x      | x                |           |             | x       |
| Function assessment             | NIHSS                                 | x <sup>7</sup> | x <sup>8</sup> |                |                    |     |     |     |      | x |       |       | x               | x      | x                |           |             | x       |
| QOL assessment                  | EQ-5D-5L                              |                |                |                |                    |     |     |     |      |   |       |       |                 | x      | x                |           |             | x       |
| Clinical laboratory tests       | Hematology                            | x <sup>7</sup> |                | x              |                    |     |     |     |      | x | x     | x     | x               | x      | x                | x         |             | x       |
|                                 | Biochemistry                          | x <sup>7</sup> |                | x              |                    |     |     |     |      | x | x     | x     | x               | x      | x                | x         |             | x       |
|                                 | Blood coagulation test                | x <sup>7</sup> |                | x              |                    |     |     |     |      | x | x     | x     | x               | x      | x                | x         |             | x       |
|                                 | Biomarker <sup>1</sup>                |                |                | x              |                    |     |     |     |      |   | x     | x     |                 |        |                  |           |             |         |
|                                 | Urinalysis                            | x <sup>7</sup> |                | x              |                    |     |     |     |      | x | x     | x     | x               | x      | x                | x         |             | x       |
| Imaging examinations            | Safety assessment                     | x <sup>7</sup> |                |                |                    |     |     |     |      | x |       |       | x <sup>10</sup> | x      |                  |           |             |         |
|                                 | Infarct volume <sup>2</sup>           |                |                | x <sup>9</sup> |                    |     |     |     |      |   |       |       | x <sup>10</sup> | x      |                  |           |             |         |
|                                 | Penumbra region volume <sup>2,3</sup> |                |                | x              |                    |     |     |     |      |   |       |       |                 |        |                  |           |             |         |
| Body measurements               | Height, weight                        | x <sup>7</sup> |                |                |                    |     |     |     |      |   |       |       |                 |        |                  |           |             |         |
| Vital signs                     | Blood pressure, pulse                 | x              |                | x              |                    | x   | x   | x   | x    | x | x     | x     | x               | x      | x                | x         | x           | x       |
|                                 | Body temperature                      | x              |                | x              |                    |     | x   | x   | x    | x | x     | x     | x               | x      | x                | x         | x           | x       |
| Oxygen saturation               | SpO <sub>2</sub> <sup>4</sup>         | x              |                | x              |                    | x   | x   | x   | x    | x | x     | x     | x               | x      | x                | x         | x           | x       |
| Medical examination             | Medical examination and interview     | x              |                | x              |                    |     |     |     |      | x | x     | x     | x               | x      | x                | x         | x           | x       |

1. Assessed in the cohort 3 only.

2. Assessed at the central imaging analysis organization

3. Performed at some study sites.

4. In addition to the scheduled period in the table, SpO<sub>2</sub> 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 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; ; SpO<sub>2</sub>, 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;
- 9  
10 138 ➤ A marked decline in level of consciousness (NIHSS 1a. evaluation of consciousness level is  
11 score of 3) at screening;
- 12 139  
13  
14 140 ➤ Patients who had an extensive infarct and for whom maintaining life was expected to be  
15 difficult, or who were expected to undergo cranial decompression at screening;
- 16 141  
17  
18 142 ➤ Presence of intracranial hemorrhagic change diagnosed by brain imaging which was judged to  
19 be clinically important by the investigator at screening;
- 20 143  
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 days prior to informed consent (IC);
- 26 146  
27  
28 147 ➤ Systolic blood pressure >220 mmHg or diastolic blood pressure >120 mmHg, with or without  
29 antihypertensive treatment at screening;
- 30 148  
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:
- 35  
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 or C, or liver dysfunction with aspartate aminotransferase or alanine aminotransferase over  
40 153 three times the upper limit of the standard value of the study site;
  - 41  
42 154 · 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)  
44 155 <30%; or
  - 45  
46 156 · Severe pulmonary dysfunction requiring home oxygen therapy.
- 47  
48 157  
49  
50 158 ➤ Human immunodeficiency virus infection, ongoing systemic infection, severe local infection, or  
51 immunocompromised condition at screening;
- 52 159  
53  
54 160 ➤ 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  
56 161  
57  
58 162 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  
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6 165 screening;
- 7  
8 166 ➤ Contraindications for MRI such as implanted pacemakers or other metallic prosthesis  
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10 167 incompatible with MRI, or claustrophobia;
- 11  
12 168 ➤ Thrombocytopenia (platelet count  $<100,000/\text{mm}^3$ ) or heparin-induced thrombocytopenia at  
13  
14 169 screening;
- 15  
16 170 ➤ History of allergies to human tissues, bovine or porcine preparations;
- 17  
18 171 ➤ History of allergy to streptomycin;
- 19  
20 172 ➤ Patients who participated in other clinical trials within 12 weeks prior to IC, or planned to  
21  
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;
- 25  
26 175 ➤ History of splenectomy;
- 27  
28 176 ➤ Patients who might have a transient ischemic attack;
- 29  
30 177 ➤ Patients who were scheduled to undergo revascularization treatment including carotid  
31  
32 178 endarterectomy, stenting, etc. by the end of the evaluation (day 91);
- 33  
34 179 ➤ Patients who were pregnant or lactating at screening, or who wished to become pregnant during  
35  
36 180 the study;
- 37  
38 181 ➤ Patients who could not use extremely effective contraception including intrauterine device,  
39  
40 182 intrauterine system, oral contraception (low dose pill), surgical sterilization, double barrier  
41  
42 183 method (condom with spermicide, or combination of condom with pessary) under the guidance  
43  
44 184 of the investigator from the time of IC to one year post-dose (day 366), or who had a partner  
45  
46 185 who could not take similar contraceptive measures; or
- 47  
48 186 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.
- 49  
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52188 Exclusion criteria on eligibility confirmation assessment

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54189 After eligibility assessment at screening, the investigator assessed NIHSS again  $\geq 4$  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:

- 59  
60 192 ➤ NIHSS score  $\leq 4$  or  $\geq 21$ ;

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4 193 ➤ Change in NIHSS score from screening  $\geq 5$ ;  
5  
6 194 ➤ Administration of the study product could not be started within 48 h of symptom onset; or  
7  
8 195 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.  
9

### 10196 11 **Randomization and blinding**

12197 Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and  
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14198 2. In cohort 3, subjects were randomly assigned in a 1:1 ratio to receive either JTR-161 or placebo.  
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16199 Randomization was performed by the minimization method, which was adjusted centrally by  
17  
18200 dynamic assignment with NIHSS at the time of eligibility assessment, with / without standard  
19  
20201 treatment including intravenous rt-PA or endovascular treatment, and age at the time of IC as the  
21  
22202 allocation factors. The randomization sequence was generated by an organization independent of the  
23  
24203 study sponsors. Allocation of treatment to subjects was randomized via a website. The investigators,  
25  
26204 patients, and the sponsor are masked to the treatment assignment until the observation period is  
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28205 completed. After the final subject in cohort 3 completes the day 91 assessment, the database will be  
29  
30206 fixed, and the key will be opened. After that, the sponsor, statistical analysts, and unblinded  
31  
32207 personnel will be placed under open blind, and patients and assessors will be blinded until the end of  
33  
34208 the follow-up period (day 366). JTR-161 and placebo can be identified by the vial appearance;  
35  
36209 therefore, to ensure masking is maintained, only unblinded persons appointed by the investigator  
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38210 prepared the administration solution, intravenously injected the study product into the patient, and  
39  
40211 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  $1.0 \times 10^8$  cells of DPSC isolated  
51  
52217 from the extracted teeth of healthy adults, and was stored in the gas space of a liquid nitrogen  
53  
54218 refrigerator.  
55  
56219 The frozen study product was thawed in a constant temperature bath at  $37 \pm 1$  °C for about five  
57  
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  $\leq 6$  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  
5  
6 223 in figure 1. The DSMB was primarily involved in deciding whether or not to advance to the next  
7  
8 224 cohort, as well as the dose (number of cells) for cohort 3. Surgical revascularization such as carotid  
9  
10 225 endarterectomy and carotid artery stenting was prohibited during the observation period, and  
11  
12 226 attending any clinical trials other than this study was prohibited until the end of the study. In cohorts  
13  
14 227 1 and 2, the administration interval between subjects was  $\geq 72$  hours.

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16 228 Baseline assessments were carried out at day 0 prior to administration, including (1) primary disease:  
17  
18 229 initial or recurrent, type of cerebral infarction, infarcted blood vessels, onset time, and diffusion-  
19  
20 230 weighted imaging (DWI) -Alberta Stroke Program Early Computed Tomography Score, (2)  
21  
22 231 with/without standard treatment with intravenous rt-PA or endovascular treatment. If yes, treatment  
23  
24 232 start time (endovascular treatment only), degree of recanalization (modified thrombolysis in cerebral  
25  
26 233 infarction classification), recanalization time, and number of passes. If no, reasons for not  
27  
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  
33  
34 237 condition, effected at least 2 years before IC and still considered cured at the start of administration of  
35  
36 238 the study product. In addition, a medical history deemed necessary for considering AEs was taken.  
37  
38 239 After administration of the study product, mRS and Barthel Index (BI) were assessed at days 31, 91,  
39  
40 240 and 366. NIHSS was assessed at days 2, 8, 31, and 91, and on the day of discharge. Patients were asked  
41  
42 241 to answer the EuroQOL 5 dimensions 5-level scores (EQ-5D-5L) questionnaire at days 31, 91, and  
43  
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  
47  
48 244 pulse rates were measured pre-registration, pre-administration, 1, 2, 4, 6, 12, and 24 hours after  
49  
50 245 administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Body  
51  
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.  
55  
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)- $\alpha$ , interleukin (IL)-1 $\beta$ , IL-6, IL-  
7  
8 253 10, IL-17, IL-23, and angiopoietin-1 (Ang-1) were measured pre-administration, and on days 3 and 8  
9  
10254 after administration in cohort 3. Infarct volumes were measured on DWI and/or fluid-attenuated  
11  
12255 inversion recovery using MRI pre-administration, and on days 8 and 31 after administration. Ischemic  
13  
14256 penumbra was measured using MRI as the mismatch between the hypoperfused area on perfusion-  
15  
16257 weighted imaging and the abnormal area on DWI pre-administration, if available. Assessment of  
17  
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  $\leq$  1, NIHSS  $\leq$  1, and BI  $\geq$  95. Secondary  
33  
34266 endpoints were (1) proportion of patients who achieve mRS  $\leq$  1 or mRS  $\leq$  2 at days 91 and 366, (2)  
35  
36267 proportion of patients who achieve BI  $\geq$  95 at days 91 and 366, (3) proportion of patients who  
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38268 achieve NIHSS  $\leq$  1, who achieve improvement of  $\geq$  75%, and who achieve improvement of  $\geq$  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  $\leq$  1, NIHSS  $\leq$  1, and BI  $\geq$  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 =  
49  
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  
53  
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  $\leq$  2, improvement in NIHSS  $\geq$  75% ,  
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58278 and BI  $\geq$  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- $\alpha$ , IL-  
7  
8 282 1 $\beta$ , 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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12284  
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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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22289  
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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  
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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-  
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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  
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6 310 endpoints, the proportions and their confidence intervals for mRS, BI, and NIHSS will be calculated  
7  
8 311 for each administration group, and point estimates of the difference in the proportions and its  
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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  
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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  
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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  
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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  
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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**

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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  
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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  
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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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## 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.<sup>4-7</sup> While collection of BM-MSCs requires invasive bone marrow  
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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.<sup>11,16,17</sup> 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)".<sup>18</sup> 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 NIHSS<sup>19</sup>, 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  $\geq 5$  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,  
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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  $\leq 1$ , NIHSS  $\leq 1$ , and  
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14372 BI  $\geq 95$  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.<sup>20</sup> 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.<sup>21,22</sup> 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.<sup>23</sup> 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.<sup>24</sup> 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  
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50390 after administration. Imaging tests were performed to assess infarct lesions and the presence or  
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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  
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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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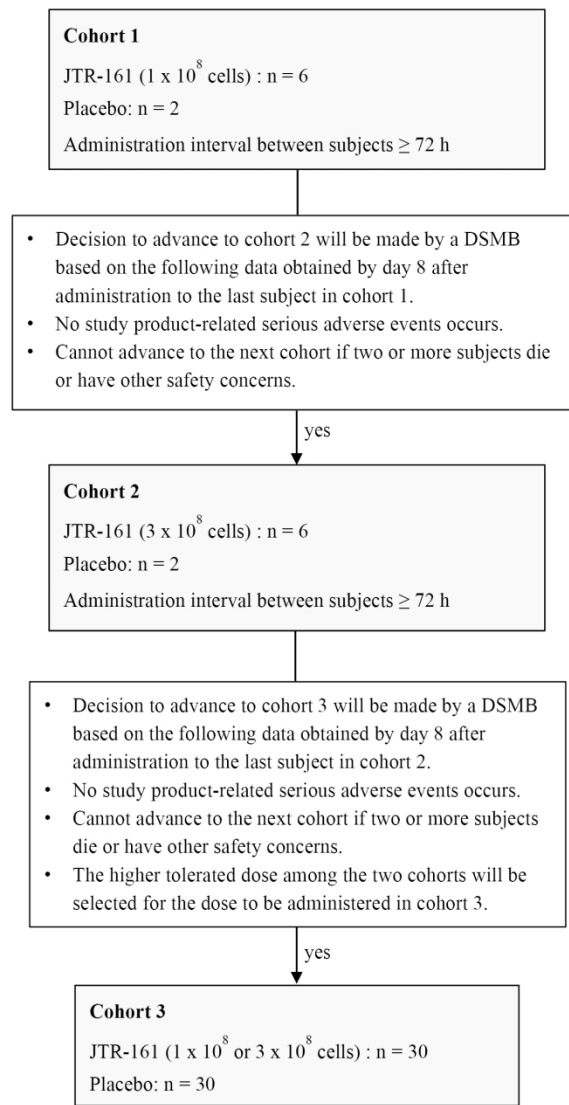


Figure 1

Figure 1



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

| Section/item                      | Item No | Description  | Addressed on page number |
|-----------------------------------|---------|--|--------------------------|
| <b>Administrative information</b> |         |  |                          |
| 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<br>(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  | <b>Introduction</b>                                       |     |  |               |
| 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 | <b>Methods: Participants, interventions, and outcomes</b> |     |  |               |
| 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 |   | 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 |
| 25 |   |     |  |               |
| 26 |   | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)  | 12            |
| 27 |   |     |  |               |
| 28 |   | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial  | 10,14         |
| 29 |   |     |  |               |
| 30 | 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            |
| 31 |   |     |  |               |
| 32 |   |     |  |               |
| 33 |   |     |  |               |
| 34 | 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    |
| 35 |   |     |  |               |
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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  | 12   |
| 2  |   |     |  |      |
| 3  |   |     |  |      |
| 4  | Recruitment   | 15  | Strategies for achieving adequate participant enrolment to reach target sample size  | -    |
| 5  |   |     |  |      |
| 6  | <b>Methods: Assignment of interventions (for controlled trials)</b> |     |  |      |
| 7  | <b>Allocation:</b>  |     |  |      |
| 8  |   |     |  |      |
| 9  |   |     |  |      |
| 10 | Sequence generation   | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions   | 9    |
| 11 |   |     |  |      |
| 12 |   |     |  |      |
| 13 |   |     |  |      |
| 14 |   |     |  |      |
| 15 |   |     |  |      |
| 16 | Allocation concealment mechanism                                    | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned  | 9    |
| 17 |   |     |  |      |
| 18 |   |     |  |      |
| 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 | <b>Methods: Data collection, management, and analysis</b>           |     |  |      |
| 32 |   |     |  |      |
| 33 | Data collection methods   | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 9-11 |
| 34 |   |     |  |      |
| 35 |   |     |  |      |
| 36 |   |     |  |      |
| 37 |   |     |  |      |
| 38 |   |     |  |      |
| 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 |   |     |  |      |
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| 45 |   |     |  |      |
| 46 |   |     |  |      |

|    |                                 |     |   |             |
|----|---------------------------------|-----|---|-------------|
| 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  |                                 |     |   |             |
| 3  |                                 |     |   |             |
| 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 |                                 |     |   |             |
| 12 |                                 |     |   |             |
| 13 |                                 |     |   |             |
| 14 | <b>Methods: Monitoring</b>      |     |   |             |
| 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 |                                 |     |   |             |
| 18 |                                 |     |   |             |
| 19 |                                 |     |   |             |
| 20 |                                 |     |   |             |
| 21 |                                 |     |   |             |
| 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 |                                 |     |   |             |
| 30 |                                 |     |   |             |
| 31 |                                 |     |   |             |
| 32 | <b>Ethics and dissemination</b> |     |   |             |
| 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)  | -           |
| 38 |                                 |     |   |             |
| 39 |                                 |     |   |             |
| 40 |                                 |     |   |             |
| 41 |                                 |     |   |             |
| 42 |                                 |     |   |             |
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| 46 |                                 |     |   |             |

|    |                               |     |   |       |
|----|-------------------------------|-----|---|-------|
| 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    |
| 14 |                               |     |   |       |
| 15 |                               |     |   |       |
| 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   | -     |
| 17 |                               |     |   |       |
| 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 |                               |     |   |       |
| 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 | <b>Appendices</b>             |     |   |       |
| 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/4.0/)" 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)

|                                 |   |
|---------------------------------|---|
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| Keywords:                       | NEUROLOGY, INTERNAL MEDICINE, Neurology < INTERNAL MEDICINE, Stroke < NEUROLOGY   |
|                                 |   |

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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<sup>1\*</sup>; Chikako Nito<sup>1\*</sup>; Masafumi Ihara<sup>2</sup>; Yasuyuki Iguchi<sup>3</sup>; Takao Urabe<sup>4</sup>; Yuji  
13  
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7  
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9  
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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  $1 \times 10^8$  (cohort 1) to  $3 \times 10^8$  (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  $\leq 1$ , NIHSS  $\leq 1$ , and Barthel Index  $\geq 95$ .

**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  $1 \times 10^8$  cells in cohort 1,  $3 \times 10^8$  cells in cohort 2, and the higher tolerated dose among the two cohorts (either  $1 \times 10^8$  cells or  $3 \times 10^8$  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  $\leq 1$ , NIHSS  $\leq 1$ , and Barthel Index  $\geq 95$ .
- 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.<sup>1,2</sup> 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<sup>3,4</sup>, 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.<sup>5-8</sup> Administration of human BM-MSCs was safe and well tolerated in patients with acute ischemic stroke, but no significant clinical improvement was observed.<sup>7,8</sup>

In 2000, human dental pulp stem cells (DPSCs) were discovered in impacted molar teeth.<sup>9</sup> 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.<sup>10,11</sup> 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.<sup>11</sup> Furthermore, compared to BM-MSCs, DPSCs can be obtained by a less invasive process, are more easily expanded, and exert more potent immunosuppressive effects via the inhibition of activated T cell responses<sup>12</sup>, 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.<sup>13,14</sup> Sakai et al.<sup>14</sup> reported that human DPSC transplantation into the completely transected spinal cord of adult rats resulted in marked recovery of hind limb locomotor functions, whereas transplantation of human BM-MSC or skin-derived fibroblasts led to substantially less recovery of locomotor function. Based on a rat stroke model and an *in vitro* model of ischemia<sup>15</sup>, 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.<sup>16,17</sup> 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  
19  
20 112 in patients with acute ischemic stroke.  
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22 113

## 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-  
29  
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  $1 \times 10^8$  cells  
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36 120 in cohort 1, and  $3 \times 10^8$  cells in cohort 2, sequentially. In cohort 3, the higher tolerated dose among the  
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38 121 two cohorts (either  $1 \times 10^8$  cells or  $3 \times 10^8$  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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8 135  
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10 136 **Patient population**

11  
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  
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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  $\geq 5$  to  $\leq 20$  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 |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
|    |                                   | 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 |       |       |       |        |                 |                  |         |            |             |   |
| 8  | Informed consent                  | x                                     |                |                |                    |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 9  | Patient characteristics           |                                       | x <sup>5</sup> |                |                    |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 11 | Administration of study product   |                                       |                |                | x                  |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 13 | Ability assessment                | mRS                                   | x <sup>6</sup> |                |                    |                |     |     |     |      |       |       |       | x      | x               |                  |         | x          | x           |   |
|    |                                   | Barthel Index                         |                |                |                    |                |     |     |     |      |       |       |       |        | x               | x                |         |            | x           |   |
| 16 | Function assessment               |                                       | x <sup>7</sup> | x <sup>8</sup> |                    |                |     |     |     |      |       |       | x     |        | x               | x                |         |            | x           |   |
| 17 | QOL assessment                    |                                       |                |                |                    |                |     |     |     |      |       |       |       |        | x               | x                |         |            | x           |   |
| 22 | Clinical laboratory tests         | Hematology                            | x <sup>7</sup> |                | x                  |                |     |     |     |      |       |       | x     | x      | x               | x                | x       | x          | x           | x |
|    |                                   | Biochemistry                          |                | x <sup>7</sup> |                    | x              |     |     |     |      |       |       | x     | x      | x               | x                | x       | x          | x           | x |
|    |                                   | Blood coagulation test                |                | x <sup>7</sup> |                    | x              |     |     |     |      |       |       | x     | x      | x               | x                | x       | x          | x           | x |
|    |                                   | Biomarker <sup>1</sup>                |                |                |                    | x              |     |     |     |      |       |       |       | x      |                 |                  |         |            |             |   |
|    |                                   | Urinalysis                            |                | x <sup>7</sup> |                    | x              |     |     |     |      |       |       | x     | x      | x               | x                | x       | x          | x           | x |
| 28 | Imaging examinations              | Safety assessment                     |                | x <sup>7</sup> |                    |                |     |     |     |      |       |       | x     |        | x <sup>10</sup> | x                |         |            |             |   |
|    |                                   | Infarct volume <sup>2</sup>           |                |                |                    | x <sup>9</sup> |     |     |     |      |       |       |       |        | x <sup>10</sup> | x                |         |            |             |   |
|    |                                   | Penumbra region volume <sup>2,3</sup> |                |                |                    | x              |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 31 | Body measurements                 |                                       |                | x <sup>7</sup> |                    |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 33 | Vital signs                       | Blood pressure, pulse                 |                | x              |                    | x              | x   | x   | x   | x    | x     | x     | x     | x      | x               | x                | x       | x          | x           |   |
|    |                                   | Body temperature                      |                | x              |                    | x              |     | x   | x   | x    |       | x     | x     | x      | x               | x                | x       | x          | x           |   |
| 36 | Oxygen saturation                 |                                       |                | x              |                    | x              | x   | x   | x   | x    | x     | x     | x     | x      | x               | x                | x       | x          | x           |   |
| 38 | 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. Performed at some study sites.

4. In addition to the scheduled period in the table, SpO<sub>2</sub> 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 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; SpO<sub>2</sub>, 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:

- 7  
8 151 ➤ Presence of a new ischemic lesion in the cerebellum or brainstem at screening;
- 9  
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  
17  
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  
21  
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  
31  
32 163 ➤ Blood glucose level <50 mg/dL or >400 mg/dL at screening;
- 33  
34 164 ➤ Patients who had any of the serious complication(s) listed below at screening:
- 35  
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  
49  
50 172 ➤ Human immunodeficiency virus infection, ongoing systemic infection, severe local infection, or  
51 immunocompromised condition at screening;
- 52 173  
53  
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  
56 175  
57  
58 176 performed by the investigator at screening;
- 59  
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4 178 ➤ Malignant tumor(s) or history of malignant tumor(s) prior to 2 years of ischemic stroke onset at  
5 screening;

6 179

7

8 180 ➤ Contraindications for MRI such as implanted pacemakers or other metallic prosthesis  
9 incompatible with MRI, or claustrophobia;

10 181

11

12 182 ➤ Thrombocytopenia (platelet count  $<100,000/\text{mm}^3$ ) or heparin-induced thrombocytopenia at  
13 screening;

14 183

15

16 184 ➤ History of allergies to human tissues, bovine or porcine preparations;

17

18 185 ➤ History of allergy to streptomycin;

19

20 186 ➤ Patients who participated in other clinical trials within 12 weeks prior to IC, or planned to  
21 participate in other clinical trials during this trial, or participated in clinical trials of other cell  
22 187 products in the past;

24 188

25

26 189 ➤ History of splenectomy;

27

28 190 ➤ Patients who might have a transient ischemic attack;

29

30 191 ➤ Patients who were scheduled to undergo revascularization treatment including carotid  
31 endarterectomy, stenting, etc. by the end of the evaluation (day 91);

32 192

33

34 193 ➤ Patients who were pregnant or lactating at screening, or who wished to become pregnant during  
35 the study;

36 194

37

38 195 ➤ Patients who could not use extremely effective contraception including intrauterine device,  
39 intrauterine system, oral contraception (low dose pill), surgical sterilization, double barrier method  
40 196 (condom with spermicide, or combination of condom with pessary) under the guidance of the  
41 investigator from the time of IC to one year post-dose (day 366), or who had a partner who could  
42 197 not take similar contraceptive measures; or

44 198

45

46 199 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.

47

48 200 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.

49

50 201

51

52 202 Exclusion criteria on eligibility confirmation assessment

53

54 203 After eligibility assessment at screening, the investigator assessed NIHSS again  $\geq 4$  h after the  
55 assessment at screening to confirm patient eligibility. Patients who met one or more of the following  
56 204 criteria were excluded:

58 205

59

60 206 ➤ NIHSS score  $\leq 4$  or  $\geq 21$ ;



- 1  
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3  
4 207 ➤ Change in NIHSS score from screening  $\geq 5$ ;  
5  
6 208 ➤ Administration of the study product could not be started within 48 h of symptom onset; or  
7  
8 209 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.  
9

### 10210 11 **Randomization and blinding**

1212 Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and  
15  
16213 2. In cohort 3, subjects were randomly assigned in a 1:1 ratio to receive either JTR-161 or placebo.  
17  
18214 Randomization was performed by the minimization method, which was adjusted centrally by dynamic  
19  
20215 assignment with NIHSS at the time of eligibility assessment, with / without standard treatment  
21  
22216 including intravenous rt-PA or endovascular treatment, and age at the time of IC as the allocation  
23  
24217 factors. The randomization sequence was generated by an organization independent of the study  
25  
26218 sponsors. Allocation of treatment to subjects was randomized via a website. The investigators, patients,  
27  
28219 and the sponsor are masked to the treatment assignment until the observation period is completed.  
29  
30220 After the final subject in cohort 3 completes the day 91 assessment, the database will be fixed, and the  
31  
32221 key will be opened. After that, the sponsor, statistical analysts, and unblinded personnel will be placed  
33  
34222 under open blind, and patients and assessors will be blinded until the end of the follow-up period (day  
35  
36223 366). JTR-161 and placebo can be identified by the vial appearance; therefore, to ensure masking is  
37  
38224 maintained, only unblinded persons appointed by the investigator prepared the administration solution,  
39  
40225 intravenously injected the study product into the patient, and cleaned up any spilled administration  
41  
42226 solution.  
43  
44227  
45

### 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  $1.0 \times 10^8$  cells of DPSC isolated from the extracted  
51  
52231 teeth of healthy adults, and was stored in the gas space of a liquid nitrogen refrigerator.  
53  
54232 The frozen study product was thawed in a constant temperature bath at  $37 \pm 1$  °C for about five minutes,  
55  
56233 then the required number of cells (one or three vials) was diluted in 100 mL of saline. The solution  
57  
58234 was intravenously administered once at a rate of 4 mL/min but  $\leq 6$  mL/min within 48 h of symptom  
59  
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  
5  
6 237 as the dose (number of cells) for cohort 3. Surgical revascularization such as carotid endarterectomy  
7  
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  
11  
12240 administration interval between subjects was  $\geq 72$  hours.

13  
14241 Baseline assessments were carried out at day 0 prior to administration, including (1) primary disease:  
15  
16242 initial or recurrent, type of cerebral infarction, infarcted blood vessels, onset time, and diffusion-  
17  
18243 weighted imaging (DWI) -Alberta Stroke Program Early Computed Tomography Score, (2)  
19  
20244 with/without standard treatment with intravenous rt-PA or endovascular treatment. If yes, treatment  
21  
22245 start time (endovascular treatment only), degree of recanalization (modified thrombolysis in cerebral  
23  
24246 infarction classification), recanalization time, and number of passes. If no, reasons for not  
25  
26247 implementing standard treatment, (3) NIHSS at time of arrival, pre-registration, and eligibility tests,  
27  
28248 (4) mRS before the onset of cerebral infarction reported by patients or her/his family, (5) disease  
29  
30249 history related to the exclusion criteria and, where relevant, the time of complete cure of any malignant  
31  
32250 condition, effected at least 2 years before IC and still considered cured at the start of administration of  
33  
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,  
37  
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  
41  
42255 336. Laboratory tests were performed pre-registration, pre-administration, and on days 2, 3, 8, 31, 91,  
43  
44256 181, and 366 after administration. Blood pressures including systolic and diastolic blood pressures and  
45  
46257 pulse rates were measured pre-registration, pre-administration, 1, 2, 4, 6, 12, and 24 hours after  
47  
48258 administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge. Body  
49  
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.  
53  
54261 Saturated oxygen was measured pre-registration, pre-administration, every 15 minutes between one  
55  
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)- $\alpha$ , interleukin (IL)-1 $\beta$ , 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  
7  
8 267 after administration in cohort 3. Infarct volumes were measured on DWI and/or fluid-attenuated  
9  
10268 inversion recovery using MRI pre-administration, and on days 8 and 31 after administration. Ischemic  
11  
12269 penumbra was measured using MRI as the mismatch between the hypoperfused area on perfusion-  
13  
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  
19  
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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24275  
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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  
31  
32279 were (1) proportion of patients who achieve mRS  $\leq$  1 or mRS  $\leq$  2 at days 91 and 366, (2) proportion  
33  
34280 of patients who achieve BI  $\geq$  95 at days 91 and 366, (3) proportion of patients who achieve NIHSS  $\leq$   
35  
36281 1, who achieve improvement of  $\geq$  75%, and who achieve improvement of  $\geq$  10 points at day 91, (4)  
37  
38282 changes in EQ-5D-5L scores at day 366, (5) proportion of patients who achieve an excellent outcome  
39  
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  
41  
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'  
51  
52289 and 'The worst health you can imagine', (6) proportion of patients who achieve overall improvement  
53  
54290 (mRS  $\leq$  2, improvement in NIHSS  $\geq$  75% , and BI  $\geq$  95) at day 91. Safety was assessed based on AEs,  
55  
56291 laboratory tests, vital signs, transcutaneous oxygen saturation, and imaging test including MRI or CT.  
57  
58292 The investigator assessed the intensity, severity, and relatedness of an AE. All serious AEs were  
59  
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- $\alpha$ , IL-1 $\beta$ , 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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8 296  
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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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18301  
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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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30307  
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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  
39  
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  
45  
46315 baseline parameters will be aggregated for each treatment group and cohort, and descriptive statistics  
47  
48316 will be calculated for continuous variables. Comparison analysis will be performed between the JTR-  
49  
50317 161 and placebo groups in cohort 3, and between the merged JTR-161 groups of cohort 3 and the  
51  
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  
55  
56320 administration group. Also, the point estimates of difference in the proportion and its confidence  
57  
58321 interval will be calculated and compared between the JTR-161 and placebo groups. As for secondary  
59  
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  
5  
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  
9  
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  
15  
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  
19  
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  
23  
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  
27  
28335 significance level will be set at 5%. Interval estimation will be calculated with a confidence coefficient  
29  
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 &  
43  
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**

55  
56349 No patients and/or public were involved in setting the research questions nor they were involved in  
57  
58350 developing plans for the design (or implementation) of this study protocol.  
59  
60351

## 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.<sup>5-8</sup> 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.<sup>12,18,19</sup> 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)".<sup>20</sup> The eligible patients were restricted to those with anterior circulation ischemic stroke because the severity of their symptoms can be assessed using NIHSS<sup>21</sup>, one of the key criteria for assessing eligibility and efficacy in our study. It is difficult to confirm the accurate etiology of stroke on admission; therefore, there is no limitation regarding stroke subtype such as lacuna, atherothrombotic, cardioembolic, and others. Our study did not limit the use of standard treatment including intravenous rt-PA and/or endovascular thrombectomy for recruitment. In addition, available treatments for acute ischemic stroke except revascularization treatment such as carotid endarterectomy and stenting in routine clinical practice were allowed to be used as a combination therapy. Patients to



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

The proportion of subjects who achieve an excellent outcome defined as mRS  $\leq 1$ , NIHSS  $\leq 1$ , and BI  $\geq 95$  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.<sup>22</sup> 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.<sup>23,24</sup> 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.<sup>25</sup> 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.<sup>26</sup> 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).

5  
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.  
9

### 10413 11 12414 **Acknowledgments**

13  
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15  
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17  
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19  
20418 Co., Ltd.) on behalf of the authors and all authors have authorized the submission of this manuscript  
21  
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23  
24420  
25

### 26421 **Authors' contributions**

27  
28422 All authors were involved in the study design, protocol preparation, and acquisition of funding. SS and  
29  
30423 CN were responsible for the first draft. All authors have reviewed and approved the final manuscript.  
31  
32424 The work is funded by Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.  
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34425  
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### 36426 **Declaration of conflicts of interest**

37  
38427 The authors declared the following potential conflicts of interest with respect to the research,  
39  
40428 authorship, and/or publication of this article: Expert Witness from Teijin Pharma Ltd. (SS, CN, KK).  
41  
42429 Research funding from Teijin Pharma Ltd. (KK). Lecture fee from Teijin Pharma Ltd. (YI). The other  
43  
44430 authors report no conflicts.  
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46431  
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### 58437 **Figure legend**

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60438 Figure 1 Flow chart of the cohorts



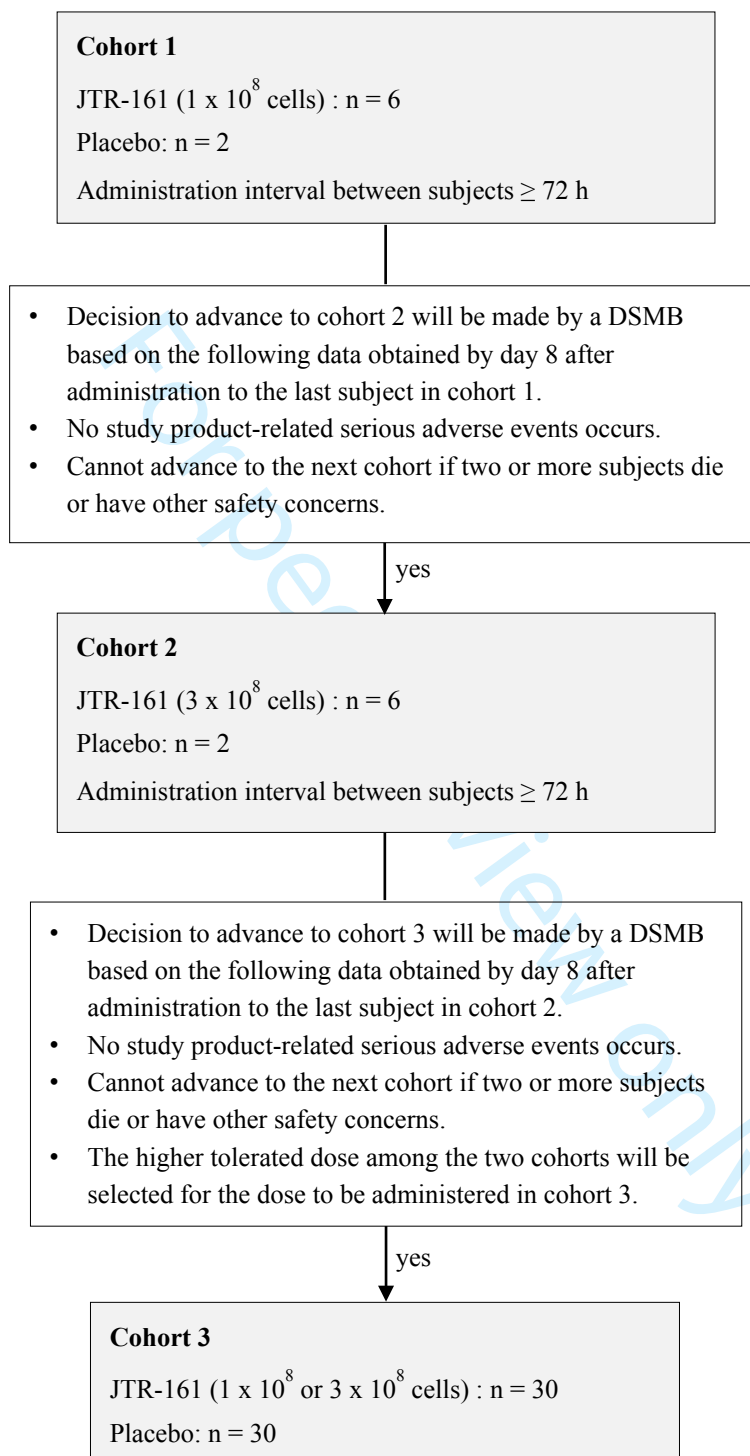
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4 439 DSMB, Data and Safety Monitoring Board  
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6 440  
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**Figure 1** Flow chart of the cohorts  
DSMB, Data and Safety Monitoring Board



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

| Section/item                      | Item No | Description  | Addressed on page number |
|-----------------------------------|---------|--|--------------------------|
| <b>Administrative information</b> |         |  |                          |
| 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<br>(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  | <b>Introduction</b>                                       |     |  |                |
| 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  |   |     |  |                |
| 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), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)  | 6-7, Figure1   |
| 11 |   |     |  |                |
| 12 |   |     |  |                |
| 13 |   |     |  |                |
| 14 | <b>Methods: Participants, interventions, and outcomes</b> |     |  |                |
| 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   | 6-7            |
| 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)   | 7-11           |
| 20 |   |     |  |                |
| 21 |   |     |  |                |
| 22 | Interventions   | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered   | 11-12, Figure1 |
| 23 |   |     |  |                |
| 24 |   | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)   | 11-13, Figure1 |
| 25 |   |     |  |                |
| 26 |   | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)  | 14             |
| 27 |   |     |  |                |
| 28 |   | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial  | 12,16          |
| 29 |   |     |  |                |
| 30 | Outcomes  | 12  | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 13,14          |
| 31 |   |     |  |                |
| 32 |   |     |  |                |
| 33 |   |     |  |                |
| 34 | 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     |
| 35 |   |     |  |                |
| 36 |   |     |  |                |
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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  |   |     |  |       |
| 7  | <b>Methods: Assignment of interventions (for controlled trials)</b> |     |  |       |
| 8  | Allocation:   |     |  |       |
| 9  |   |     |  |       |
| 10 | Sequence generation   | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions   | 11    |
| 11 |   |     |  |       |
| 12 |   |     |  |       |
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| 14 |   |     |  |       |
| 15 |   |     |  |       |
| 16 | Allocation concealment mechanism                                    | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned  | 11    |
| 17 |   |     |  |       |
| 18 |   |     |  |       |
| 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 |   |     |  |       |
| 26 |   |     |  |       |
| 27 |   | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial   | 11    |
| 28 |   |     |  |       |
| 29 |   |     |  |       |
| 30 |   |     |  |       |
| 31 | <b>Methods: Data collection, management, and analysis</b>           |     |  |       |
| 32 |   |     |  |       |
| 33 | Data collection methods   | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 11-13 |
| 34 |   |     |  |       |
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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  |                                 |     |   |             |
| 3  |                                 |     |   |             |
| 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  | 14-15       |
| 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)   | 14          |
| 11 |                                 |     |   |             |
| 12 |                                 |     |   |             |
| 13 |                                 |     |   |             |
| 14 | <b>Methods: Monitoring</b>      |     |   |             |
| 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 |                                 |     |   |             |
| 18 |                                 |     |   |             |
| 19 |                                 |     |   |             |
| 20 |                                 |     |   |             |
| 21 |                                 |     |   |             |
| 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   | 13          |
| 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   | 14          |
| 29 |                                 |     |   |             |
| 30 |                                 |     |   |             |
| 31 |                                 |     |   |             |
| 32 | <b>Ethics and dissemination</b> |     |   |             |
| 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)  | -           |
| 38 |                                 |     |   |             |
| 39 |                                 |     |   |             |
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| 46 |                                 |     |   |             |



|    |                               |     |   |                       |
|----|-------------------------------|-----|---|-----------------------|
| 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  |                               |     |   |                       |
| 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   | 18                    |
| 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   | 15,18                 |
| 14 |                               |     |   |                       |
| 15 |                               |     |   |                       |
| 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   | -                     |
| 17 |                               |     |   |                       |
| 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 |                               |     |   |                       |
| 23 |                               |     |   |                       |
| 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 | <b>Appendices</b>             |     |   |                       |
| 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 |                               |     |   |                       |
| 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/4.0/)" 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<br>Nito, Chikako ; Nippon Medical School, Department of Neurology<br>Ihara, Masafumi; Kokuritsu Junkankibyō Kenkyū Center, Neurology<br>Iguchi, Yasuyuki ; Jikei University School of Medicine, Department of Neurology<br>Urabe, Takao; Juntendo University Urayasu Hospital, Department of Neurology<br>Matsumaru, Yuji; University of Tsukuba Faculty of Medicine, Department of Neurosurgery<br>Sakai, Nobuyuki; Kobe City Medical Center General Hospital, Department of Neurosurgery<br>Kimura, Kazumi ; Nippon Medical School, Department of Neurology |
| <b>Primary Subject Heading</b>: | Neurology   |
| Secondary Subject Heading:      | Pharmacology and therapeutics   |
| Keywords:                       | NEUROLOGY, INTERNAL MEDICINE, Neurology < INTERNAL MEDICINE, Stroke < NEUROLOGY   |
|                                 |   |

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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<sup>1\*</sup>; Chikako Nito<sup>1\*</sup>; Masafumi Ihara<sup>2</sup>; Yasuyuki Iguchi<sup>3</sup>; Takao Urabe<sup>4</sup>; Yuji  
13  
14 6 Matsumaru<sup>5</sup>; Nobuyuki Sakai<sup>6</sup>; Kazumi Kimura<sup>1</sup>; on behalf of the J- REPAIR trial group  
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52 25 **list below the title nor registered as an official author in the ScholarOne system**

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7  
8 32 **Keywords**

9  
10 33 Cell-based therapy, clinical trial, dental pulp stem cells, ischemic stroke  
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12 34

13  
14 35 **Word count**

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16 36 Abstract word count: 300 (journal limit  $\leq 300$ )  
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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  $1 \times 10^8$  (cohort 1) to  $3 \times 10^8$  (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  $\leq 1$ , NIHSS  $\leq 1$ , and Barthel Index  $\geq 95$ .

**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  $1 \times 10^8$  cells in cohort 1,  $3 \times 10^8$  cells in cohort 2, and the higher tolerated dose among the two cohorts (either  $1 \times 10^8$  cells or  $3 \times 10^8$  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  $\leq 1$ , NIHSS  $\leq 1$ , and Barthel Index  $\geq 95$ .
- 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.<sup>1,2</sup> 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<sup>3,4</sup>, 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.<sup>5-8</sup> Administration of human BM-MSCs was safe and well tolerated in patients with acute ischemic stroke, but no significant clinical improvement was observed.<sup>7,8</sup>

In 2000, human dental pulp stem cells (DPSCs) were discovered in impacted molar teeth.<sup>9</sup> 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.<sup>10,11</sup> 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.<sup>11</sup> Furthermore, compared to BM-MSCs, DPSCs can be obtained by a less invasive process, are more easily expanded, and exert more potent immunosuppressive effects via the inhibition of activated T cell responses<sup>12</sup>, 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.<sup>13,14</sup> Sakai et al.<sup>14</sup> reported that human DPSC transplantation into the completely transected spinal cord of adult rats resulted in marked recovery of hind limb locomotor functions, whereas transplantation of human BM-MSC or skin-derived fibroblasts led to substantially less recovery of locomotor function. Based on a rat stroke model and an *in vitro* model of ischemia<sup>15</sup>, 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.<sup>16,17</sup> 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  
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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  $1 \times 10^8$  cells  
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36 120 in cohort 1, and  $3 \times 10^8$  cells in cohort 2, sequentially. In cohort 3, the higher tolerated dose among the  
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38 121 two cohorts (either  $1 \times 10^8$  cells or  $3 \times 10^8$  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  
45  
46 125 same cohort, or any other serious safety concerns are reported. Death due to cerebral infarction itself  
47  
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:

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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  $\geq 5$  to  $\leq 20$  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 |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
|    |                                   | 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 |       |       |       |        |                 |                  |         |            |             |   |
| 8  | Informed consent                  | x                                     |                |                |                    |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 9  | Patient characteristics           |                                       | x <sup>5</sup> |                |                    |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 11 | Administration of study product   |                                       |                |                | x                  |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 13 | Ability assessment                | mRS                                   | x <sup>6</sup> |                |                    |                |     |     |     |      |       |       |       | x      | x               |                  |         | x          | x           |   |
|    |                                   | Barthel Index                         |                |                |                    |                |     |     |     |      |       |       |       |        | x               | x                |         |            | x           |   |
| 16 | Function assessment               |                                       | x <sup>7</sup> | x <sup>8</sup> |                    |                |     |     |     |      |       |       | x     |        | x               | x                |         |            | x           |   |
| 17 | QOL assessment                    |                                       |                |                |                    |                |     |     |     |      |       |       |       |        | x               | x                |         |            | x           |   |
| 22 | Clinical laboratory tests         | Hematology                            | x <sup>7</sup> |                | x                  |                |     |     |     |      |       |       | x     | x      | x               | x                | x       | x          | x           | x |
|    |                                   | Biochemistry                          |                | x <sup>7</sup> |                    | x              |     |     |     |      |       |       | x     | x      | x               | x                | x       | x          | x           | x |
|    |                                   | Blood coagulation test                |                | x <sup>7</sup> |                    | x              |     |     |     |      |       |       | x     | x      | x               | x                | x       | x          | x           | x |
|    |                                   | Biomarker <sup>1</sup>                |                |                |                    | x              |     |     |     |      |       |       |       | x      |                 |                  |         |            |             |   |
|    |                                   | Urinalysis                            |                | x <sup>7</sup> |                    | x              |     |     |     |      |       |       | x     | x      | x               | x                | x       | x          | x           | x |
| 28 | Imaging examinations              | Safety assessment                     |                | x <sup>7</sup> |                    |                |     |     |     |      |       |       | x     |        | x <sup>10</sup> | x                |         |            |             |   |
|    |                                   | Infarct volume <sup>2</sup>           |                |                |                    | x <sup>9</sup> |     |     |     |      |       |       |       |        | x <sup>10</sup> | x                |         |            |             |   |
|    |                                   | Penumbra region volume <sup>2,3</sup> |                |                |                    | x              |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 31 | Body measurements                 |                                       |                | x <sup>7</sup> |                    |                |     |     |     |      |       |       |       |        |                 |                  |         |            |             |   |
| 33 | Vital signs                       | Blood pressure, pulse                 |                | x              |                    | x              | x   | x   | x   | x    | x     | x     | x     | x      | x               | x                | x       | x          | x           | x |
|    |                                   | Body temperature                      |                | x              |                    | x              |     | x   | x   | x    |       |       | x     | x      | x               | x                | x       | x          | x           | x |
| 36 | Oxygen saturation                 |                                       |                | x              |                    | x              | x   | x   | x   | x    | x     | x     | x     | x      | x               | x                | x       | x          | x           |   |
| 38 | Medical examination and interview |                                       |                | 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. Performed at some study sites.

4. In addition to the scheduled period in the table, SpO<sub>2</sub> 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 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; SpO<sub>2</sub>, 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;
- 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  
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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;
- 33  
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  
56 175  
57  
58 176 performed by the investigator at screening;
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4 178 ➤ Malignant tumor(s) or history of malignant tumor(s) prior to 2 years of ischemic stroke onset at  
5 screening;

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7  
8 180 ➤ Contraindications for MRI such as implanted pacemakers or other metallic prosthesis  
9 incompatible with MRI, or claustrophobia;

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11

12 182 ➤ Thrombocytopenia (platelet count  $<100,000/\text{mm}^3$ ) or heparin-induced thrombocytopenia at  
13 screening;

14 183

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16 184 ➤ History of allergies to human tissues, bovine or porcine preparations;

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18 185 ➤ History of allergy to streptomycin;

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20 186 ➤ Patients who participated in other clinical trials within 12 weeks prior to IC, or planned to  
21 participate in other clinical trials during this trial, or participated in clinical trials of other cell  
22 187 products in the past;

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26 189 ➤ History of splenectomy;

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28 190 ➤ Patients who might have a transient ischemic attack;

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30 191 ➤ Patients who were scheduled to undergo revascularization treatment including carotid  
31 endarterectomy, stenting, etc. by the end of the evaluation (day 91);

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34 193 ➤ Patients who were pregnant or lactating at screening, or who wished to become pregnant during  
35 the study;

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38 195 ➤ Patients who could not use extremely effective contraception including intrauterine device,  
39 intrauterine system, oral contraception (low dose pill), surgical sterilization, double barrier method  
40 196 (condom with spermicide, or combination of condom with pessary) under the guidance of the  
41 investigator from the time of IC to one year post-dose (day 366), or who had a partner who could  
42 197 not take similar contraceptive measures; or

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46 199 ➤ Patients who the investigator considered to be inappropriate for inclusion in the study.

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52 202 Exclusion criteria on eligibility confirmation assessment

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54 203 After eligibility assessment at screening, the investigator assessed NIHSS again  $\geq 4$  h after the  
55 assessment at screening to confirm patient eligibility. Patients who met one or more of the following  
56 204 criteria were excluded:

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60 206 ➤ NIHSS score  $\leq 4$  or  $\geq 21$ ;

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4 207 ➤ Change in NIHSS score from screening  $\geq 5$ ;  
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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**

1212 Subjects were randomly assigned to receive either JTR-161 or placebo in a 3:1 ratio in cohorts 1 and  
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14212 2. In cohort 3, subjects were randomly assigned in a 1:1 ratio to receive either JTR-161 or placebo.  
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16213 Randomization was performed by the minimization method, which was adjusted centrally by dynamic  
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18214 assignment with NIHSS at the time of eligibility assessment, with / without standard treatment  
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20215 including intravenous rt-PA or endovascular treatment, and age at the time of IC as the allocation  
21  
22216 factors. The randomization sequence was generated by an organization independent of the study  
23  
24217 sponsors. Allocation of treatment to subjects was randomized via a website. The investigators, patients,  
25  
26218 and the sponsor are masked to the treatment assignment until the observation period is completed.  
27  
28219 After the final subject in cohort 3 completes the day 91 assessment, the database will be fixed, and the  
29  
30220 key will be opened. After that, the sponsor, statistical analysts, and unblinded personnel will be placed  
31  
32221 under open blind, and patients and assessors will be blinded until the end of the follow-up period (day  
33  
34222 366). JTR-161 and placebo can be identified by the vial appearance; therefore, to ensure masking is  
35  
36223 maintained, only unblinded persons appointed by the investigator prepared the administration solution,  
37  
38224 intravenously injected the study product into the patient, and cleaned up any spilled administration  
39  
40225 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  $1.0 \times 10^8$  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  $37 \pm 1$  °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  $\leq 6$  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  
5  
6 237 as the dose (number of cells) for cohort 3. Surgical revascularization such as carotid endarterectomy  
7  
8 238 and carotid artery stenting was prohibited during the observation period, and attending any clinical  
9  
10239 trials other than this study was prohibited until the end of the study. In cohorts 1 and 2, the  
11  
12240 administration interval between subjects was  $\geq 72$  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)  
19  
20244 with/without standard treatment with intravenous rt-PA or endovascular treatment. If yes, treatment  
21  
22245 start time, degree of recanalization (modified thrombolysis in cerebral infarction classification),  
23  
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  
31  
32250 before IC and still considered cured at the start of administration of the study product. In addition, a  
33  
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  
37  
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,  
43  
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  
49  
50259 temperature was measured pre-registration, pre-administration, 2, 4, 6, and 24 hours after  
51  
52260 administration, days 3, 8, 31, 91, 181, and 366 after administration, and on the day of discharge.  
53  
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)- $\alpha$ , interleukin (IL)-1 $\beta$ , 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-  
13  
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  
33  
34280 of patients who achieve BI  $\geq$  95 at days 91 and 366, (3) proportion of patients who achieve NIHSS  $\leq$   
35  
36281 1, who achieve improvement of  $\geq$  75%, and who achieve improvement of  $\geq$  10 points at day 91, (4)  
37  
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  
45  
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  
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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- $\alpha$ , IL-1 $\beta$ , 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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8 296

### 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  
15  
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  
19  
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  
23  
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  
27  
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 &  
43  
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**

55  
56349 No patients and/or public were involved in setting the research questions nor they were involved in  
57  
58350 developing plans for the design (or implementation) of this study protocol.  
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60351

## 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.<sup>5-8</sup> 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.<sup>12,18,19</sup> 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)".<sup>20</sup> The eligible patients were restricted to those with anterior circulation ischemic stroke because the severity of their symptoms can be assessed using NIHSS<sup>21</sup>, 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

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4 381 whom standard treatment could not be given, and patients who received standard treatment but had a  
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6 382 NIHSS  $\geq 5$  were allowed to be enrolled. However, these pretreatment and combination therapies may  
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8 383 make it difficult to evaluate the safety and efficacy of JTR-161 accurately; therefore, a placebo arm  
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10384 was established as a control group. The study is conducted in a double-blinded manner during the  
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12385 observation period. The keys were opened to the sponsor, statistical analysts, and unblinded personnel,  
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14386 but patients and assessors continued under blind conditions until the end of the follow-up period, since  
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16387 EQ-5D-5L was assessed at day 366. In order to explore the therapeutic time window, timing of  
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18388 administration was set to be within 48 h of symptom onset.

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20389 The proportion of subjects who achieve an excellent outcome defined as mRS  $\leq 1$ , NIHSS  $\leq 1$ , and BI  
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22390  $\geq 95$  was set as the primary endpoint because we considered this clinical outcome was the most  
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24391 accurate way of detecting any difference in effectiveness between the subjects receiving JTR-161 and  
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26392 the placebo group. As secondary endpoints, the efficacy of JTR-161 was also evaluated using mRS  
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28393 and BI for disability assessments, and NIHSS for function assessment, all of which are widely accepted  
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30394 for use as endpoints in clinical trials of acute ischemic stroke.<sup>22</sup> In recent clinical trials of intravenous  
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32395 rt-PA and endovascular treatment, clinical outcomes as per mRS were evaluated 90 days after the start  
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34396 of treatment.<sup>23,24</sup> Similarly, period during which the efficacy of JTR-161 was evaluated was set to 90  
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36397 days after administration of the study product. EQ-5D-5L was used as a patient-reported outcome for  
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38398 evaluating patient health status. It is reported that there was a significant correlation between stroke  
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40399 type and severity, and EQ-5D-5L scores; reproducibility and validity have been verified in stroke  
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42400 patients.<sup>25</sup> We measured a variety of serum cytokines and growth factors before and after  
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44401 transplantation of JTR-161 to investigate the mechanism of human DPSCs on acute ischemic stroke.  
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46402 In a pre-clinical study, the distribution of JTR-161 labelled with a radioactive tracer was highest in the  
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48403 lung two hours after a single intravenous administration (in-house data), as reported in other types of  
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50404 stem cells.<sup>26</sup> The onset of symptoms such as respiratory distress and decreased oxygen saturation  
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52405 should be carefully followed immediately after administration of JTR-161. Oxygen saturation was  
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54406 measured every 15 minutes for up to 4 hours and every 30 minutes for up to 6 hours after administration.  
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56407 Imaging tests were performed to assess infarct lesions and the presence or absence of significant  
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58408 hemorrhagic changes. On the other hand, time of disappearance of JTR-161 from the body has not  
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60409 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.  
9

### 10413 11 12414 **Acknowledgments**

13  
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17  
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19  
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21  
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24420  
25

### 26421 **Authors' contributions**

27  
28422 CN, MI, YI, TU, YM, NS and KK were involved in the study design, protocol preparation, and  
29  
30423 acquisition of funding. SS, CN and KK will be responsible for directly accessing and verifying all data.  
31  
32424 SS and CN were responsible for the first draft. All authors have reviewed and approved the final  
33  
34425 manuscript. The work is funded by Teijin Pharma Ltd. and JCR Pharmaceuticals Co., Ltd.  
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36426  
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### 38427 **Declaration of conflicts of interest**

39  
40428 The authors declared the following potential conflicts of interest with respect to the research,  
41  
42429 authorship, and/or publication of this article: Expert Witness from Teijin Pharma Ltd. (SS, CN, KK).  
43  
44430 Research funding from Teijin Pharma Ltd. (KK). Lecture fee from Teijin Pharma Ltd. (YI). The other  
45  
46431 authors report no conflicts.  
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48432  
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### 60438 **Figure legend**

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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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8 441  
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10442 **References**

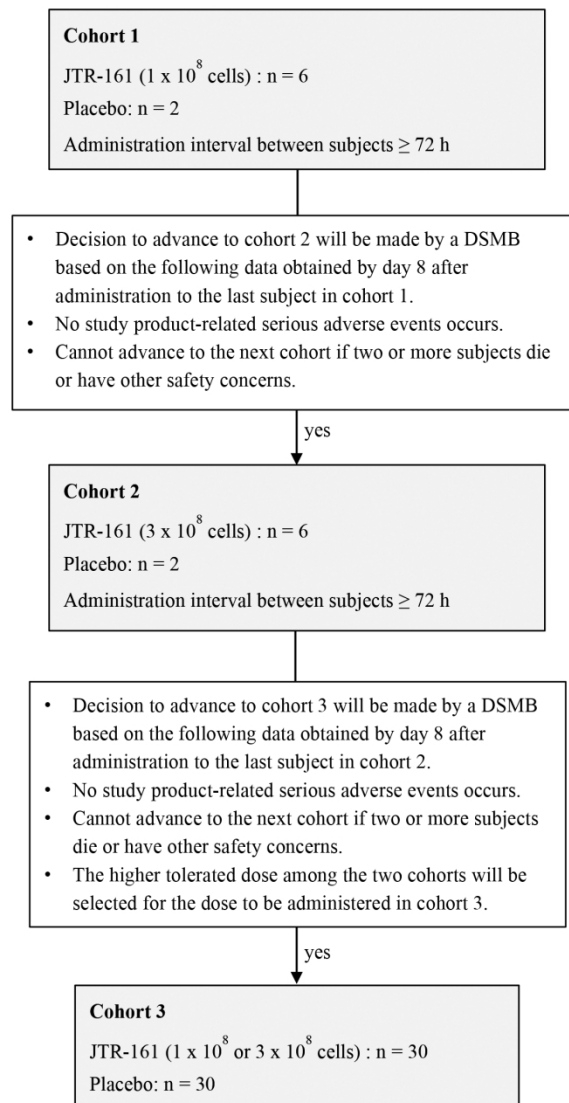
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**Figure 1**

Figure 1. Flow chart of the cohorts



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

| Section/item                      | Item No | Description  | Addressed on page number |
|-----------------------------------|---------|--|--------------------------|
| <b>Administrative information</b> |         |  |                          |
| 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<br>(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  | <b>Introduction</b>                                       |     |  |                |
| 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  |   |     |  |                |
| 5  |   |     |  |                |
| 6  |   | 6b  | Explanation for choice of comparators  | 17,18          |
| 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), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)  | 6-7, Figure1   |
| 11 |   |     |  |                |
| 12 |   |     |  |                |
| 13 |   |     |  |                |
| 14 | <b>Methods: Participants, interventions, and outcomes</b> |     |  |                |
| 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 |   |     |  |                |
| 22 | Interventions   | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered   | 12-13, Figure1 |
| 23 |   |     |  |                |
| 24 |   | 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 |
| 25 |   |     |  |                |
| 26 |   | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)  | 15             |
| 27 |   |     |  |                |
| 28 |   | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial  | 13,18          |
| 29 |   |     |  |                |
| 30 | 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          |
| 31 |   |     |  |                |
| 32 |   |     |  |                |
| 33 |   |     |  |                |
| 34 | 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     |
| 35 |   |     |  |                |
| 36 |   |     |  |                |
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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  | <b>Methods: Assignment of interventions (for controlled trials)</b> |     |  |       |
| 7  | <b>Allocation:</b>  |     |  |       |
| 8  |   |     |  |       |
| 9  |   |     |  |       |
| 10 | Sequence generation   | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions   | 12    |
| 11 |   |     |  |       |
| 12 |   |     |  |       |
| 13 |   |     |  |       |
| 14 |   |     |  |       |
| 15 |   |     |  |       |
| 16 | Allocation concealment mechanism                                    | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned  | 12    |
| 17 |   |     |  |       |
| 18 |   |     |  |       |
| 19 |   |     |  |       |
| 20 | Implementation  | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions  | 12    |
| 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  | 12    |
| 25 |   |     |  |       |
| 26 |   |     |  |       |
| 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 |   |     |  |       |
| 30 |   |     |  |       |
| 31 | <b>Methods: Data collection, management, and analysis</b>           |     |  |       |
| 32 |   |     |  |       |
| 33 | Data collection methods   | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 12-14 |
| 34 |   |     |  |       |
| 35 |   |     |  |       |
| 36 |   |     |  |       |
| 37 |   |     |  |       |
| 38 |   |     |  |       |
| 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 |   |     |  |       |
| 43 |   |     |  |       |
| 44 |   |     |  |       |
| 45 |   |     |  |       |
| 46 |   |     |  |       |

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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  |                                 |     |   |             |
| 3  |                                 |     |   |             |
| 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  | 15-16       |
| 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)   | 15          |
| 11 |                                 |     |   |             |
| 12 |                                 |     |   |             |
| 13 |                                 |     |   |             |
| 14 | <b>Methods: Monitoring</b>      |     |   |             |
| 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 |                                 |     |   |             |
| 18 |                                 |     |   |             |
| 19 |                                 |     |   |             |
| 20 |                                 |     |   |             |
| 21 |                                 |     |   |             |
| 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   | 15          |
| 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   | 15          |
| 29 |                                 |     |   |             |
| 30 |                                 |     |   |             |
| 31 |                                 |     |   |             |
| 32 | <b>Ethics and dissemination</b> |     |   |             |
| 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)  | -           |
| 38 |                                 |     |   |             |
| 39 |                                 |     |   |             |
| 40 |                                 |     |   |             |
| 41 |                                 |     |   |             |
| 42 |                                 |     |   |             |
| 43 |                                 |     |   |             |
| 44 |                                 |     |   |             |
| 45 |                                 |     |   |             |
| 46 |                                 |     |   |             |

|    |                               |     |   |                       |
|----|-------------------------------|-----|---|-----------------------|
| 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  |                               |     |   |                       |
| 9  |                               |     |   |                       |
| 10 | Declaration of interests      | 28  | Financial and other competing interests for principal investigators for the overall trial and each study site   | 19                    |
| 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   | 19                    |
| 14 |                               |     |   |                       |
| 15 |                               |     |   |                       |
| 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   | -                     |
| 17 |                               |     |   |                       |
| 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 |                               |     |   |                       |
| 23 |                               |     |   |                       |
| 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                    |
| 27 |                               |     |   |                       |
| 28 |                               |     |   |                       |
| 29 | <b>Appendices</b>             |     |   |                       |
| 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 |                               |     |   |                       |
| 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/4.0/)" license.