Clinical Trial Protocol

Placebo-Controlled, Double-Blind, Phase 2/3 Efficacy and Safety Trial of HLCM051 (MultiStem®) in Patients with Ischemic Stroke

Protocol Number: B01-03

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Confidentiality Statement

Protocol Synopsis

Title:
Placebo-Controlled, Double-Blind, Phase 2/3 Efficacy and Safety Trial of HLCM051 (MultiStem®) in Patients with Ischemic Stroke

Protocol Number: B01-03

Investigational Product: HLCM051

Phase: 2/3

Subjects: Patients with acute ischemic stroke (within 36 hours of onset)

Objectives:
The objectives of this trial are the following:

<Primary objectives>

- To evaluate the efficacy of HLCM051 on functional outcome in subjects with acute ischemic stroke.
- To evaluate the safety of HLCM051 in subjects with acute ischemic stroke.

<Secondary objectives>

- To examine changes in function and severity of subjects with ischemic stroke treated with either HLCM051 or Placebo.

<Exploratory objectives>

- To evaluate changes in biomarkers in subjects with ischemic stroke treated with either HLCM051 or Placebo.

Inclusion/Exclusion Criteria:

<Inclusion Criteria>
Patients will be determined to be eligible to participate in the trial if they meet all of the following criteria:

1. Japanese male or female patients between 20 and 84 years of age, inclusive;
2. Clinical diagnosis of cerebral cortical ischemic stroke;
3. Occurrence of an ischemic stroke with clear motor or speech deficit documented by National Institutes of Health Stroke Scale (NIHSS) score of 8 to 20 (at the baseline assessment) that did not change by ≥4 points from the screening to the baseline assessment;

   NOTE: The NIHSS assessment must be performed by the trial doctor who is trained for the NIHSS assessment.

   NOTE: There must be ≥6 hours between the baseline and screening NIHSS scores.

   NOTE: NIHSS screening score must be collected within 4 hours following reperfusion therapy procedure for those who received tPA treatment or underwent mechanical reperfusion.

   NOTE: The baseline NIHSS assessment must be confirmed within 16 to 34 hours after the onset of ischemic stroke.

4. Onset of ischemic stroke must have occurred within 18 to 36 hours prior to the

start of administration of the investigational product;

NOTE: Time of onset is defined as the time point when symptoms first began. For ischemic stroke that occurred during sleep, time of onset is defined as the time point when the patient was last observed to be normal or as the latest time point when the patient was self-reported to be normal.

5. Confirmation of hemispheric cortical infarct with brain magnetic resonance imaging (MRI) including diffusion-weighted imaging with b-value of 1,000 demonstrating an acute lesion measuring ≥ 2.0 cm of longest diameter;
6. A modified Rankin Scale (mRS) of 0 or 1, by either self-report or family report, prior to the onset of ischemic stroke;
7. Female patients who meet either:
    a. Not pregnant, not breastfeeding/ interrupting breastfeeding, and not planning on becoming pregnant during the trial;
    b. Not of childbearing potential, defined as one who has been postmenopausal for at least 1 year, or has been surgically sterilized, or has had a hysterectomy at least 3 months prior to the start of this trial; or
    c. If of childbearing potential, one who has agreed to follow investigator's advice and use an effective contraceptive method up to 1st year of the trial. Effective contraceptive methods include contraceptive methods used consistently and correctly (oral contraceptives, intrauterine devices, diaphragm, or male or female condoms), abstinence, and a sterile sexual partner;
8. Male patients with female partners of childbearing potential must agree to follow investigator's advice and use adequate contraceptive methods (a combination of a condom and another form of contraception) up to 1st year of the trial if engaging in sexual intercourse;
9. Patients or legal representatives must freely sign the informed consent form after the nature of the trial and the disclosure of his/her data have been explained;
10. Willing and able to comply with all aspects of the treatment and testing schedule; and
11. Willing and able to return to the trial site for the post-treatment evaluations.

<Exclusion criteria>

Patients will not be eligible to participate in the trial if they meet any of the following:

1. Presence of a lacunar, a lesion of ≤ 2.0 cm of longest diameter, or a brainstem infarct on MRI as the etiology of symptoms of ischemic stroke;
2. Reduced level of consciousness (score of 3 for item 1a of NIHSS);
3. Occurrence of a hemorrhagic transformation as evidenced by computerized tomography (CT) or brain MRI scan that is clinically significant in the opinion of the investigator;

4. Ipsilateral focal neurological deficits from prior lesions in the brain that would complicate evaluation;
5. Experienced seizures since the onset of ischemic stroke;
6. History of a neurological event such as stroke or clinically significant head trauma within 6 months prior to the start of screening;
7. Patients who both received tPA treatment and underwent mechanical reperfusion (patients are eligible for the trial if they had only one of them, tPA treatment or mechanical reperfusion);
8. Uncontrolled hypertension, defined as persistent systolic blood pressure >220 mmHg or diastolic blood pressure >120 mmHg, despite antihypertensive therapy;
9. Blood glucose level <50 mg/dL or >350 mg/dL at baseline;
10. Patients who have a significant comorbid medical condition(s), including, but not limited to:
    a. Severe kidney disease requiring hemodialysis or peritoneal dialysis;
    b. Advanced liver disease such as hepatitis or liver cirrhosis;
    c. Severe congestive heart failure or history of ejection fraction <30%;
    d. Severe lung disease requiring home oxygen; or
    e. Active unstable angina requiring daily treatment with nitrates or other medications;
11. Known human immunodeficiency virus infection, ongoing systemic infection, severe local infection or who are immunocompromised;
12. Alzheimer's disease or other dementias, Parkinson's disease, or any other neurological disorder that in the opinion of the trial doctor would affect their ability to participate in the trial or confound study assessments;
13. History of malignant tumor(s) within 2 years of the onset of ischemic stroke, with the exception of adequately treated basal or squamous cell carcinoma of the skin;
14. Contraindication for MRI such as implanted pacemakers or other metallic prosthesis incompatible with MRI, body weight, or claustrophobia;
15. Thrombocytopenia (platelet count <100,000/mm^3) or heparin-induced thrombocytopenia;
16. Known allergy to human tissue or bovine or porcine products, or religious objections to biological products;
17. Prior participation in another clinical trial involving investigational pharmacological agents or devices within 30 days prior to providing consent to receive the investigational product, or participation in investigational pharmacological agents, devices, or rehabilitation stroke recovery program is planned during the trial;

18. Other serious medical or psychiatric illness that is not adequately controlled and, in the investigator's opinion, would not permit the subject to be managed according to the protocol;
19. Previous surgical removal of the spleen;
20. Major fluctuation in neurological status since the onset of ischemic stroke indicating progression or expansion of ischemic stroke, or possible transient ischemic attack;
21. Plan to have a neurovascular procedure (e.g., carotid endarterectomy, stent placement, etc.) within the first year following ischemic stroke; or
22. Abnormal laboratory test results which investigators consider clinically significant and inappropriate for the trial.

Trial Design and Duration:

This is a randomized, placebo-controlled, double-blind, multicenter, phase 2/3 trial to evaluate the efficacy and safety of intravenous administration of HLCM051 compared with placebo in subjects with acute ischemic stroke (within 36 hours of onset). Japanese subjects who developed a subcortical ischemic stroke and are eligible to participate in the trial will be evaluated. In addition, the safety survey will be conducted 2 years after administration.

Approximately 220 subjects will be randomized in a 1:1 ratio (HLCM051 group [n=110] or placebo group [n=110]) to receive a single infusion of HLCM051 or placebo. Randomization will be stratified by Baseline NIHSS score (≤12 and ≥13), concomitant reperfusion therapy (Yes or No), and age (18 to 64 and ≥65). All subjects will be enrolled continuously into the trial.

A Data and Safety Monitoring Board (DSMB) will be established beforehand to assess the data after approximately 30% and 60% of the planned number of subjects complete the 7-day follow-up. The DSMB will assess the continuity of the trial if any of the following is encountered:

- Occurrence of serious adverse events (SAEs) that are unexpected and related to investigational product within 7 days after treatment;
- Occurrence of death that is related to investigational product;
- Occurrence of infusion-related allergic reactions whose severity are Grade 3 or 4 according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 within 24 hours after start of treatment; or
- Worsening of neurological symptoms (increase of ≥4 points in the NIHSS score) related to the investigational product within 7 days after treatment.

This study consists of four phases, Screening, Baseline (Day of treatment: Day 0), Post-treatment up to Day 365 and 2-year safety survey (Day 730) after administration.

Subjects will be evaluated at screening, baseline (Day 0: before and after treatment), Day 1, Day 2, Day 7, Day 30, Day 90, and Day 365, or Early Termination visit. In addition, an additional safety survey will be conducted in subjects who can be investigated on Day 730.

To prevent subjects from being lost to follow-up, trial sites will contact subjects by telephone

at Day 60, Day 150, Day 210, Day 270, and Day 330 to update the information on disease status and health conditions.

At screening, subjects will undergo standard procedures including medical history, physical examination, 12-lead electrocardiogram (ECG), clinical laboratory tests, vital signs, NIHSS assessment, mRS assessment prior to the onset of ischemic stroke, and brain MRI. At baseline (Day 0: day of treatment), subjects will undergo physical examination, vital signs, pulse oximetry, adverse event and concomitant treatment assessments, allogeneic antibody and exploratory biomarker testing, and the NIHSS assessment. During the follow-up (post-treatment) period (from Day 1 to Day 365 or Early Termination), adverse events (AEs), laboratory test results, vital signs, pulse oximetry, ECGs, and physical examination results will be followed for the safety assessment, and mRS, NIHSS, and Barthel Index (BI) scores will be assessed for the efficacy assessment. Biomarker testing will be assessed at baseline and each assessment. In addition, the survey of survival, development of malignancy,serious adverse event and other information will be conducted as an additional safety survey in subjects who can be investigated on Day 730.

Dosage Forms and Route of Administration:

Within 18 to 36 hours after the onset of ischemic stroke, subjects will receive a single dose of 1.2 billion HLCM051 cells or placebo to be intravenously administered at the rate of up to 10 mL/minute over a period of 30 to 60 minutes.

If a concomitant drug is intravenously administered, simultaneously with HLCM051, the drug should be administered from another line.

Efficacy Endpoints:

Primary endpoint

- The proportion of subjects with an excellent outcome at Day 90 defined by the functional assessments

  <Excellent outcome>
  mRS score of ≤1 (scale, 0 to 6), NIHSS score of ≤1 (scale, 0 to 42), and BI score of ≥95 (scale, 0 to 100)

Secondary endpoints

- The proportion of subjects with an excellent outcome defined by the functional assessments at Day 365
- The proportion of subjects exhibiting functional outcome throughout the range of mRS scores by shift analysis at Day 90
- The proportion of subjects exhibiting functional outcome throughout the range of mRS scores by shift analysis at Day 365
- The proportion of subjects with a mRS score of ≤2, NIHSS score improvement of ≥75% from baseline and a BI score of ≥95 at Day 90
- The proportion of subjects with a mRS score of ≤1 and a mRS score of ≤2 at Day 90
- The proportion of subjects with a NIHSS score of ≤1 at Day 90

- The proportion of subjects with a favorable outcome (NIHSS score improvement of ≥75% from baseline) in neurological symptoms at Day 90
- The proportion of subjects with a BI score of ≥95 at Day 90
- The proportion of subjects who survived without life-threatening adverse events (AEs) at Day 90.
- The proportion of subjects who survived without secondary infections at Day 90.
- Global recovery (i.e., GEE) and dichotomous assessment at Day 90. Integrated assessment using three component efficacy endpoints (mRS score of ≤2, NIHSS score improvement of ≥75% from baseline, and BI score of ≥95) and two component safety endpoints (survival without life-threatening AEs and secondary infections) together.

Exploratory endpoints

- Changes in biomarkers (white blood cell populations and inflammatory markers) from baseline to Day 2, Day 7, and Day 30.

Safety Endpoints:

The primary safety endpoints to be compared between the HLCM051 and the placebo groups will be defined as any of the following:

- Grade 3 or 4 infusion-related allergic reactions related to the investigational product. Abnormality of cardiovascular system and abnormality or allergic reactions (e.g., rash, erythema) of/to respiratory function that occurred within 24 hours after treatment;
- SAEs that occurred within 7 days after treatment and are related to the investigational product;
- Worsening of neurological symptoms that are related to the investigational product. Worsening is defined as an increase of ≥4 points in the NIHSS score from baseline assessed through 7 days after treatment; and
- Death, life-threatening AEs and incidence of secondary infections at Day 90.

The secondary safety endpoints are as follows:

- Comparison of the incidence of secondary infections (local and systemic), AEs, death, vital signs (blood pressure, heart rate, respiration rate, temperature, and oxygen saturation), and laboratory parameters through Day 365 between the HLCM051 and the placebo groups.

Statistical Analyses:

A primary objective of the trial is to generate additional safety data and evidence of efficacy of HLCM051 in the Japanese patient population, building on the experience from the phase 2 study (B01-02) conducted outside Japan.

Analysis of efficacy

For Full Analysis Set (FAS), the proportion of subjects with an excellent outcome at Day 90

defined by the functional assessments, as a primary efficacy endpoint, will be compared between the HLCM051 and the placebo groups and superiority of HLCM051 to placebo groups will be assessed by Cochran–Mantel–Haenszel (CMH) test (the two-tailed significance level of 0.05), with adjustment factor of Baseline NIHSS score (≤12 or ≥13), concomitant reperfusion therapy (Yes or No), and age (≤74 and ≥75).

Unblinding and analysis of Day 90 and Day 365 data is scheduled after the Day 365 data of all patients are fixed.

Analysis of efficacy

For the analysis of the safety, the frequency and its percentage for each endpoint will be calculated and compared between HLCM051 and placebo groups. Details of statistical analysis will be described in a Statistical Analysis Plan.

Target Sample Size:

As the number of evaluable subjects, 110 subjects per group; 220 subjects in total

Trial Period:

October 2016 to April 2023 (planned, 2-year safety survey included)

Trial Schedule

Period	Screening [1]	Baseline (Day 0)		Post-treatment period								Additional 2-year safety survey
Visiting Timing	(0 to 28 hours after the onset of ischemic stroke)	Before the start of administration	Start of administration (18 to 36 hours after the onset of ischemic stroke)	Day 1 (24 ±6 hr) [2]	Day 2 (48 ±6 hr) [2]	Day 7 [3] (±2 days)	Day 30 (±3 days)[18]	Day 60 (±7 days)	Day 90 (±7 days)	Day 150, Day 210, Day 270, Day 330 (±7 days)	Day 365 (±7 days)/ Early Termination (+28 days)	Day 730 (±56 days)
Obtainment of consent[4]	X											
Inclusion/exclusion criteria		X										
Medical history and subject demographics	X											
Physical examination	X	X		X			X[19]				X[19]	
Vital signs	X	X[5]	X[6]	X	X	X	X		X		X	
Pulse oximetry		X[5]	X[7]	X	X							
Chemistry and hematology testing, and urinalysis	X[8]			X	X	X	X				X	
Pregnancy test[9]	X								X		X (Only ET prior to Day 90)	
Allogeneic antibody testing		X[10]					X[11]					
12-lead ECG	X			X	X							
Assessment of AEs and investigation of concomitant medications	X	X	X	X	X	X	X[19]	X	X[19]	X	X[19]	X[20]
NIHSS assessment[12]	X[13]	X[14]		X		X	X[19]		X[19]		X[19]	
modified Rankin Scale (mRS) assessment[12]	X[15]					X	X[19]		X[19]		X[19]	
Barthel Index (BI) assessment[12]						X	X[19]		X[19]		X[19]	
Brain MRI	X											
Exploratory biomarker testing		X[10]			X[11]	X[11]	X[11]					
Administration of HLCM051 or placebo		X										
Telephone call[16]								X		X		
Subject randomization		X[14]										
Inquiry of rehabilitation activities[17]						X	X[19]	X	X[19]		X[19]	
Additional 2-year safety survey												X[20]

1. Screening procedures are the procedures for standard of care to be performed for subjects at each trial site during initial assessment of subjects with the exception of pregnancy test and obtainment of consent.
2. Day 1 and Day 2 assessment times are calculated from the start time for administration at Day 0.
3. If hospital discharge occurs before Day 7, Day 7 assessment can be performed in the hospital at discharge.
4. Consent can be obtained beyond 28 hours after the onset of ischemic stroke if data collected prior to obtaining consent within 28 hours after the onset can be used and the baseline examinations to be performed after obtaining consent and the start of administration of HLCM051 are done within 36 hours after the onset.
5. Pulse oximetry and vital signs including blood pressure, heart rate, body temperature, and respiration rate are measured 1 hour ±30 minutes before the start of administration.
6. Vital signs including blood pressure, heart rate, body temperature, and respiration rate are measured every 15 ±5 minutes for the first 2 hours after the start of administration, and then at 4 hours ±30 minutes and 6 hours ±30 minutes after administration.
7. Pulse oximetry is measured every 15 ±5 minutes for the first hour after the start of administration.
8. Chemistry and hematology testing and urinalysis are performed per standard of care at each trial site at according to the regulations at screening only. Subsequently, the analytes listed in Section 5.2.1 are measured. For urinalysis, the urine collection and test can be performed beyond 28 hours after the onset of ischemic stroke as long as it is before the baseline examinations shown in 5.1.2.
9. Pregnancy tests are performed on females of childbearing potential.
10. Blood collection for the test will be performed wherever possible.
11. Blood collection for the test will be performed wherever possible. If it is not performed at baseline, it will not need to be performed.

12. Scores will be assessed ideally by one investigator from Screening through Day 365/ Early Termination.
13. NIHSS screening score must be collected within 4 hours following reperfusion therapy procedure for those who received tPA treatment or underwent mechanical reperfusion.
14. Baseline NIHSS assessment should occur within 16 to 34 hours from the time of the onset of ischemic stroke and ≥6 hours after the last NIHSS assessment at screening. The baseline NIHSS score should not have changed by ≥4 points from the screening NIHSS. The baseline NIHSS score should be assessed prior to randomization
15. The modified Rankin Scale (mRS) score determined at screening is used to assess the condition of a subject prior to the onset of ischemic stroke based on either self-report or family report, and scores at Day 7 and thereafter are used to assess the condition of the subject at each visit.
16. Trial sites will contact subjects (or representatives) via telephone to obtain updated information on disease status and health conditions and inquire about rehabilitation activities, adverse events, and concomitant medications.
17. Trial sites will ask subjects about any rehabilitation activities that have taken place (e.g., inpatient physical therapy, outpatient physical therapy, etc.).
18. It allows at discharge between Day14-26 unless patient can visit Day30.
19. It allows the Physical examination (only at Day30 and Day365 or Early Termination), assessment of AEs and investigation of concomitant medications, NIHSS assessment, mRS assessment, BI assessment and inquiry of rehabilitation by visit examination unless patient can visit trial sites.
20. The survey of survival, development of malignancy, serious adverse event and the investigation of concomitant medications for serious adverse event will be conducted as an additional safety survey in subjects who can be investigated on 2 years after administration.

MRI = magnetic resonance imaging, NIHSS = National Institutes of Health Stroke Scale

List of Abbreviation

ALT	Alanine aminotransferase
AMI	Acute myocardial infarction
ARDS	Acute respiratory distress syndrome
ASPECTS	Alberta Stroke Program Early CT Score
AST	Aspartate aminotransferase
BI	Barthel Index
CRA	Clinical research associate
CRO	Contract Research Organization
CT	Computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
FAS	Full Analysis Set
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GvHD	Graft versus host disease
HSCT	Hematopoietic stem cell transplantation
ICH	International Conference for Harmonisation
IRB	Institutional Review Board
IWRS	Interactive web response system
MAPC	Multipotent adult progenitor cells
MCAO	Middle cerebral artery occlusion
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
mRS	modified Rankin Scale
MSC	Mesenchymal stem cells
NCI	National Cancer Institute
NIHSS	National Institutes of Health Stroke Scale
NOAEL	No-Observable-Adverse-Effect Level
NOD/SCID	Non-obese diabetic/severe combined immunodeficiency
PPS	Per-protocol Set

PT	Preferred term
qPCR	Quantitative polymerase chain reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SOC	System organ class
SOT	Solid organ transplant
tPA	Tissue plasminogen activator
UC	Ulcerative colitis

1 Introduction

1.1 Background Information on Disease

Stroke is one of the leading causes of death and physical disabilities in the world, including Japan and the United States.

According to the "2014 Patient Survey" prepared by the Ministry of Health, Labour and Welfare, the total number of patients with cerebrovascular disease was 1,179,000 in Japan[1]. In recent years, cerebrovascular diseases have been the fourth leading cause of death, and in 2014, 114,207 people died of cerebrovascular disease, and of these, 57.8% (66,058 people) suffered ischemic stroke (Vital statistics in Japan, 2014) [2]. Cerebrovascular disease is also the leading cause of disease in need of care (Comprehensive Survey of Living Conditions 2010) [3].

Current therapy for ischemic stroke is limited. Tissue plasminogen activator (tPA) is the well-established and widely used current treatment and must be given within 3 to 4.5 hours after the onset of ischemic stroke. According to the Japan Stroke Databank 2015, 76,376 patients were registered in the Japan Standard Stroke Registry Study Group between 1999 and February 2013 and only 2.5% of those (1,892 patients) were given tPA within 3 hours after the onset[4].

In the latest treatment guidelines in Japan (Japanese Guidelines for the Management of Stroke 2015[5], Japanese Guidelines for Thrombectomy of Stroke Version 3 Mar 2018[6]) intravenous administration of recombinant tissue plasminogen activator (rtPA, alteplase) within 4.5 hours after stroke onset is strongly recommended as thrombolytic therapy (the recommendation grade A: strongly recommended). In addition, due to advances in technology of catheter-based interventions, mechanical reperfusion therapy in the blood vessels is rapidly evolving in Japan, the United States, and Europe. In Japan, endovascular therapy using clot retrievers in the brain within 6 hours of stroke onset is recommended (the grade A). Although local recanalization therapy for acute stroke using devices is recommended for patients who satisfy the conditions within 16 hours (the grade A) or 24 hours (the grade B: recommended) from the time confirmed normal even if it exceeds 6 hours from stroke onset in the treatment guidelines, the treatment, similar to tPA treatment, is available only for patients who are transferred to hospital in a timely manner. Moreover, edaravone (free radical scavenger) whose activity is thought to protect the brain after an acute stroke was approved in 2001 as the treatment to be administered within 24 hours after stroke onset in Japan, and its use is recommended as the grade B; however, patients are required to undergo frequent liver and kidney function tests and blood tests during treatment. Although the administrations of aspirin (an anti-platelet drug) after early onset, argatroban (an anti-coagulant) within 48 hours after onset and ozagrel (an anti-platelet drug) within 5 days after onset are recommended as the grade A, grade B and grade B respectively, these drugs have no immediate benefit following an acute attack.

In consideration of the numbers of affected individuals, the costs necessary to facilitate their care and rehabilitation, coupled with the lack of current therapies, ischemic stroke is a disease with unmet medical needs.

1.2 Stem Cell Therapy for Ischemic Stroke

Cellular therapy for the treatment of ischemic stroke is aimed at developing new treatments for subjects who have no other therapeutic options. Cell therapeutics hold the promise of treating ischemic stroke through protection or support of tissue at risk after the initial ischemic event, thereby improving function and preventing or ameliorating permanent neurological deficits.

Stem cells have demonstrated efficacy when transplanted into animal models of ischemic stroke, although the exact mechanisms through which cells provide benefit in these studies have not been established.

Bone marrow-derived cells were used in the experiments where their direct transplantation into the brain was performed. Direct transplantation of bone marrow into the margins of the ischemic zone of the brains of rats appeared to improve functional recovery following middle cerebral artery occlusion (MCAO) [7]. Similarly, transplantation of mesenchymal stem cells (MSCs) into the striatum in mice improved function following ischemic stroke[8]. Mouse bone marrow grafts in the brain also restored cerebral blood flow and blood-brain barrier following ischemic stroke in rats[9].

The positive effects of systemic administration (intravenous or intra-arterial injection) of stem cells in animal models of ischemic stroke were also observed. Intra-carotid artery administration of MSCs following MCAO in rats improved functional outcome[10]. Similarly, intravenous administration of cord blood stem cells following ischemic stroke in rats improved motor and neurological deficits[11]. For functional improvement following ischemic stroke in rats, intravenous administration of cord blood was reported to have a higher efficacy than intra-striatal administration[12]. Intravenous administration of MSCs was found to induce angiogenesis at the margins of the ischemic zone following ischemic stroke in rats[13]. Those studies demonstrated the putative mechanisms of cell type, route of administration, and efficacy that may contribute to the recovery in animal models of ischemic stroke.

1.3 Summary of HLCM051

HLCM051 is a mass produced stem cell product originating from adult adherent stem cells taken from the bone marrow of healthy, consenting, non-related donors and expanded ex vivo. HLCM051 was discovered by Athersys, Inc. and is being developed in the US and EU. It is now developed by Healios K.K. (Healios) in Japan.

HLCM051 is considered to provide benefit through various mechanisms of action such as reduction of inflammation, control of immune function, protection of damaged or injured cells and tissues, promotion of angiogenesis, repair of tissues, and promotion of healing[14].

Based on those characteristics of pluripotency and immune regulation, HLCM051 has been studied for development of various indications, including treatment for acute myocardial infarction (AMI), prevention of graft versus host disease (GvHD), treatment for ischemic stroke, treatment for ulcerative colitis (UC), adjuvant immunotherapy for solid organ transplantation (SOT), and treatment for acute respiratory distress syndrome (ARDS).

The hypothesis for the HLCM051 treatment for ischemic stroke is based on the improvement of motor function observed when HLCM051 was directly transplanted into the adult rat brains[15] or into the hippocampus of newborn rats[16,17,18] following the induced hypoxic-ischemic disorders. Intravenous administration of homogeneous or human HLCM051 (without immunosuppressant drugs) in rat models of ischemic stroke resulted in statistically significant and continuous effects in a dose-dependent manner[19,20].

The data suggest that HLCM051 provides benefit through various biological mechanisms, such as prevention of apoptosis, inhibition of inflammatory injury, and production of homing factors that recruit endogenous stem cells or progenitor cells followed by further improvement of CNS

functions and recovery; therefore, the effects of HLCM051 has been examined in patients with ischemic stroke.

1.3.1 Results of preclinical studies

The assessment of HLCM051 for its clinical use is performed based on preclinical pharmacological studies, tissue distribution studies, and safety assessment studies in animal models. In vitro pharmacological studies of rat and human HLCM051 cells demonstrated that HLCM051 inhibits the proliferation of T cells, indicating that HLCM051 has immunomodulatory activity. These conclusions were supported by the results of in vivo pharmacological studies in pig models of AMI, mouse and rat models of GvHD and those animal models of ischemic disorders, and rat models of heart transplantation.

Following intravenous (IV) infusion of the HLCM051 cells, the cells are first localized in the lungs. Safety pharmacology studies on the lungs and cardiovascular system demonstrated that a single infusion or repeated infusions of rat or human HLCM051 cells were not associated with adverse effects on pulmonary function or any effects on the cardiovascular system.

Preclinical pharmacokinetics of the HLCM051 cells were performed by focusing on tissue distribution and retention after infusion. After HLCM051 was intravenously administered in mouse models of GvHD, its tissue distribution was assessed using the live imaging of the cells marked with the luciferase reporter gene. The imaging in model animals revealed that the HLCM051 cells first accumulate in the lungs for a few hours immediately after infusion followed by its redistribution in the gastrointestinal tract over the next 24 to 48 hours. The bioluminescent signals from the reporter gene in the majority of the samples measured 10 days after infusion were below the limit of detection. The results suggest that most of the administered cells disappear from the tissue by then.

Tissue distribution and retention of the multipotent adult progenitor cells (MAPCs®)/the HLCM051 cells were also assessed in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice, rodent models of disease, and pig models of disease. Tumorigenicity studies in nude mice after subcutaneous (SC) and IV infusion and other preclinical studies for tissue assessment showed no evidence of tumorigenicity. In rat studies, most of detectable homogeneous rat MAPCs disappeared from the tissue within 2 to 3 days after infusion. In tissue distribution studies conducted 2 and 4 weeks after infusion, the results of the measurements of cerebral distribution in rats of ischemic stroke injury using the quantitative PCR (qPCR) method showed no signals of the human cells at any time points.

In vitro and in vivo studies in rats after a single infusion or repeated infusions of cells showed no infusion-induced immune responses to HLCM051 cells when measured based on allogeneic antibodies or activation responses of T cells.

Single-dose toxicity studies of human and rat HLCM051 cells were performed in mice and rats. The tolerability of the HLCM051 cells was favorable up to 10 million cells/dose (500 million cells/kg: SC infusion of human HLCM051 cells in mice) and up to 40 million cells/dose (200 million/kg: IV administration of rat MAPCs in rats). Homogeneous rat HLCM051 cells were administered in rats (one infusion weekly) in an IV infusion study of HLCM051 administered up to 5 weeks. The no observed adverse effect level (NOAEL) in this study was 2.5 million cells/dose (12.5 million cells/kg). No adverse effects were observed in rats when 10 million homogeneous rat cells/dose (50 million cells/kg) were intravenously administered twice at one week intervals. In another study where 200 million HLCM051 cells were directly administered

in the outer membrane of the coronary arteries in pigs, HLCM051 had a favorable tolerability profile.

See the Investigator's Brochure for further information on the results of preclinical studies.

1.3.2 Results of clinical studies

For clinical experience in HLCM051, there are 7 completed or discontinued and 5 ongoing studies. In these studies, the safety, tolerability, and preliminary efficacy of HLCM051 have been evaluated for various indications including treatment for AMI (AMI-07-001 Study, B02-02 Study), prevention of GvHD during and after hematopoietic stem cell transplantation (HSCT) (GVHD-2007-001 Study), treatment of acute ischemic stroke (B01-02 Study, B01-03 Study and B01-04 Study), treatment of UC (B3041001 Study), support during and after liver transplantation (MiSOT-I Study), treatment of ARDS (B04-01 Study, B04-02 Study and B04-03 Study), and Trauma Study (B06-01 Study). Although various types of HLCM051 with different drug product and cell concentration are used in the studies, those cells have the same components.

As of November 25, 2020, 413 subjects are treated with HLCM051. The disposition of the subjects of the completed or discontinued studies is as follows: 36 AMI subjects who received treatment with MultiStem via a coronary arterial line; 36 GvHD subjects who received IV infusions of MultiStem via a central venous catheter; 84 UC subjects who received IV infusions of MultiStem via a peripheral venous catheter; 71 subjects with acute ischemic stroke; 26 subjects with ARDS; 1 subject with SCI and 1 subject with GvHD who received treatment from a humanist perspective; and 3 liver transplanted subjects who received treatment with MultiStem via the portal circulation and via a peripheral venous catheter. The disposition of subjects of the ongoing studies is as follows: estimated 123 subject with ischemic stroke who received IV infusions of MultiStem via a peripheral venous catheter, estimated 32 subjects with ARDS who received infusions of MultiStem via a coronary arterial line. The ARDS trial in Japan had a report of a serious adverse event of chills possibly related to HLCM051. In the ARDS oversea study, there was a serious adverse event of hypotension reported by the investigator as related to MultiStem; however, the sponsor deemed this SAE related to the underlying disease and not related to MultiStem. In the completed study in patients with UC, one case each of hypersensitivity and pancytopenia were reported as SAEs, which were determined possibly related to HLCM051 or the product used to dilute HLCM051. In the ongoing international stroke study (B01-04), there has been one report (HLCM051 or Placebo unknown) of a non-serious, Grade 2 infusion related reaction that resolved after treatment. In the ongoing stroke study (B01-03) in Japan, there has been 3 reports (HLCM051 or Placebo unknown) of a non-serious, Grade 2 infusion related reaction that resolved after treatment. The cumulative summary of the serious adverse drug reactions that have occurred in clinical studies of HLCM051 up to November 25, 2020 is shown in the table below:

Table: Cumulative Summary of Serious Adverse Drug Reactions

System Organ Class (SOC) Preferred Term (PT)	**HLCM051**	**Blinded - HLCM051/Placebo**
Total of serious adverse drug reactions	8	4
Immune system disorders		
Hypersensitivity	1	0
Blood and lymphatic system disorders		

Table: Cumulative Summary of Serious Adverse Drug Reactions		
System Organ Class (SOC) Preferred Term (PT)	**HLCM051**	**Blinded - HLCM051/Placebo**
Pancytopenia	1	0
Injury, poisoning and procedural complications		
Procedural complication	0	1**
Procedural intestinal perforation	0	1**
Nervous system disorders		
Convulsion	1*	0
Dyskinesia	0	1
Cardiac disorders		
Atrial fibrillation	1	0
Coronary artery dissection	0	1***
Hepatobiliary disorders		
Hyperbilirubinaemia	1	0
Vascular disorders		
Hypotension	1*	0
Neoplasms benign, malignant and unspecified		
Acute myeloid leukemia	1	0
General disorders and administration site conditions		
Chills	1	0

* Although this case was possibly related in the opinion of the trial doctor, it was downgraded by the sponsor as the onset of symptoms of underlying disease.
** These cases occurred before administration of the investigational product after enrollment and obtainment of consent and were reported to be related to the study procedure.
*** This case was reported by the study doctor to be unrelated to the investigational product and definitely related to the microcatheterization in the study.

The safety of HLCM051 in subjects with ischemic stroke was evaluated in the phase 2 double-blind, randomized, placebo-controlled, safety and efficacy trial in adult patients with acute ischemic stroke (B01-02 Study, also called MASTERS-1 Study) among completed studies. The total trial duration for safety and efficacy follow-up will be 12 months. The B01-02 Study has been completed and the final data have been reported. In 24-48 hours after the onset of ischemic stroke, a total of 71 subjects received a single-infusion of HLCM051 (6 subjects received 4.0×10^8 cells and 65 subjects received 1.2×10^9 cells) and 63 subjects received a single infusion of placebo. The results showed that the safety was confirmed in all dose cohorts and the tolerability was mostly favorable. Particularly at the highest dose (1.2×10^9 cells in total) investigated, the safety was confirmed and the tolerability was favorable throughout the study period. There were neither subjects who had treatment-induced allergic reaction or neurological worsening nor subjects who developed any adverse event requiring dose limitation which corresponds to a discontinuation criterion in the protocol. Many of the adverse events were mild or moderate in severity and matched the target disease conditions in the study. No clinically significant differences were seen in laboratory test values and vital signs among the treatment groups. There were no SAEs that are related to the use of HLCM051 in the opinion of the sponsor.

See the current Investigator's Brochure for further information on the results of clinical studies (B01-02 Study).

1.4 Risk-Benefit Assessment

1.4.1 Risk Assessment

The cell components of HLCM051 originate from the bone marrow collected from a human donor. Accordingly, there are risks of viral or non-viral contaminations and infections when HLCM051 is administered to a patient. The manufacturing process used for cell proliferation and harvesting involve various steps and during these steps there are risks associated with taking in exogenous viral and non-viral substances. For that reason, the quality monitoring procedures (current GMP, verification and testing of raw materials in contact with cells, multi-step sterility test of products) to minimize and specify those risks are established for the manufacturing process.

The major risks associated with the HLCM051 treatment are generally comparable to those associated with cell-based treatment and include immunogenicity and treatment-induced toxicities. Retention of HLCM051 cells is extremely low in animals and there is no evidence of toxicity or tumorigenicity in many animal experiments that have been conducted so far; however, those risks cannot be removed. Mitigation of those risks must be planned by carefully assessing the safety of subjects in conducting the trial. In addition, vital signs must be measured during and after the HLCM051 treatment.

Treatment-induced toxicities were assessed in many animal experiments. The results showed that the tolerability of IV infusion of homogeneous HLCM051 was favorable with no evidence of respiratory distress or biologically significant changes in body weight after single-dose administration, as well as no cumulative adverse drug reactions after repeated doses of 5 times. In clinical studies, to date, there has been only one case of allergic reaction occurred after treatment with HLCM051 and considered as a SAE. The event was observed after the second dose in a subject in the UC Study and characterized by fever and chills. The subject with this reaction recovered following the in-hospital treatment with IV steroids. There has been one serious adverse event of chills possibly related to HLCM051 administration in the patients exposed. This occurred in an open-label ARDS trial patient and was characterized as shivering about 20 minutes following the end of HLCM051 administration with the event being reported as severe in severity and resolved about 20 minutes after onset. In the completed AMI, GvHD, ARDS and ischemic stroke studies, there were no allergic reactions or SAEs that were definitely related to HLCM051. The safety profiles in the AMI, GvHD, ARDS and ischemic stroke studies matched the events expected to occur in the target high-risk population in the study.

1.4.2 Benefit Assessment

The 90-day data from the ischemic stroke study (B01-02) were analyzed to evaluate the efficacy endpoints.

The B01-02 Study was designed for the purpose of evaluation of intravenous administration of HLCM051 in subjects who developed significant and long-term disability due to ischemic stroke (NIHSS score of 8 to 20 at the baseline assessment). At first, the B01-02 Study was designed to administer HLCM051 within 24 to 36 hours after the onset of ischemic stroke. This time frame was selected to identify patients who had recovered sufficiently (improvement

within 24 to 30 hours after the onset of ischemic stroke) by a physician and to exclude them from the study. Subsequently, the study design was revised to be able to enroll patients who had developed ischemic stroke within 48 hours (since it was revised to promote the subject enrollment in the study based on the time restriction at the Cell Processing Center, the revision will not be applied for future studies) and allowed patients who both received tPA treatment and underwent mechanical reperfusion to enroll, however, no adjustment of the sample size was made.

The efficacy of HLCM051 in subjects who developed ischemic stroke within 36 hours was evaluated by comparing the proportion of subjects who achieved good or excellent outcome (Global Recovery assessed by each improvement of 3 verified endpoints; i.e., mRS [assessment of overall physical disabilities], NIHSS [assessment of cognitive and motor skill], and BI [assessment of the ability to engage in activities of daily living]) with that of subjects treated with placebo. The results of the analysis are shown in the table below:

Table: Comparison between HLCM051 Group (MS) and the Placebo Group (P) in B01-02 Study

Assessment at 90 days	Intent-to-Treat 65 MS v 61 P	Early MS treatment (i.e. w/in 36 hrs of stroke) 31 MS v 61 P	Post-hoc Analysis (excludes tPA + MR) 27 MS v 52 P
Excellent Outcome (patients achieving mRS≤1, and NIHSS≤1 and BI≥95)	MS: 15.4% vs P: 6.6% Δ = 8.8%*	MS: 16.1% vs P: 6.6% Δ = 9.5%	MS: 18.5% vs P: 3.8% Δ = 14.7%**
Global Recovery (patients achieving mRS≤2, and NIHSSΔ≥75% and BI≥95)	MS: 30.8% vs P: 24.6% Δ = 6.2%	MS: 41.9% vs P: 24.6% Δ = 17.3%*	MS: 44.4% vs P: 17.3% Δ = 27.1%***
% of Patients Achieving Global Recovery without Secondary Infections, Life Threatening AE's, or Death	MS: 23.1% vs P:19.7% Δ = 3.4%	MS: 38.7% vs P:19.7% Δ = 19.0%*	MS: 40.7% vs P:11.5% Δ = 29.2%***
Life threatening AEs / death	MS: 10.8% vs P: 24.6% Δ = (13.8%)**	MS: 9.7% vs P: 24.6% Δ = (14.9%)*	MS: 11.1% vs P: 26.9% Δ = (15.8%)
Secondary infections	MS: 36.9% vs P: 47.5% Δ = (10.6%)	MS: 16.1% vs P: 47.5% Δ = (31.4%)***	MS: 14.8% vs P: 53.8% Δ = (39.0%)***
Hospitalization days	MS: 7.9 d vs P: 9.8 d Δ = (1.9 d, 19.4%)	MS: 6.8 d vs P: 9.8 d Δ = (3.0 d, 30.6%)**	MS: 6.7 d vs P: 10.3 d Δ = (3.6 d, 35.0%)***

* p-value ≤ 0.10; ** p-value ≤ 0.05 ***p-value<0.01
mRS = modified Rankin Scale; NIHSS = NIH Stroke Scale; and BI = Barthel Index

At Day 90, the proportion of subjects who obtained an excellent outcome (mRS score of ≤1 [scale, 0 to 6], NIHSS score of ≤1 [scale, 0 to 42], and BI score of ≥95 [scale, 0 to 100]) and the proportion of subjects who obtained Global Recovery (mRS of ≤2, NIHSS score improvement of ≥75% from baseline, and BI score of ≥95) for 3 endpoints were higher in HLCM051 group than in the placebo group. Treatment with HLCM051 was also associated with lower patient mortality and frequency of life-threatening AEs, as well as statistically significant evidence of accelerated patient recovery at 7 and 30 days after the onset of ischemic stroke. This was also reflected in reduced hospitalization time among subjects who received HLCM051 treatment, and a statistically significant reduction in hospitalization days among subjects who received treatment with HLCM051 within 36 hours after the onset of ischemic stroke ($p < 0.05$).

In a post hoc analysis, for multiple endpoints (Global Recovery's test statistic, distribution of mRS, Excellent Outcome, etc.), the treatment effect of HLCM051 was particularly seen in subjects whose treatment time was within 36 hours, and the differences in the treatment effect between the HLCM051 and the placebo groups were greater, when subjects who received both tPA treatment and mechanical reperfusion (MR) were excluded. Also, this effect was more markedly observed after one year[21].

Since there are not enough effective treatments for acute ischemic stroke after the time to treatment with tPA (within 3 to 4.5 hours after the onset of ischemic stroke), HLCM051 has the potential to provide patients with ischemic stroke with a new treatment.

2 Trial Objectives

The following objectives will be examined in subjects with moderate to moderately severe ischemic stroke within 36 hours after the onset of ischemic stroke:

<Primary objectives>

- To evaluate the efficacy of HLCM051 on functional outcome in subjects with acute ischemic stroke.
- To evaluate the safety of HLCM051 in subjects with acute ischemic stroke.

<Secondary objectives>

- To examine changes in function and disease severity of subjects with ischemic stroke treated with either HLCM051 or placebo.

<Exploratory objectives>

- To evaluate the changes in biomarkers in subjects with ischemic stroke treated with either HLCM051 or placebo.

3 Trial Design

3.1 Summary of Trial Design

This is a randomized, placebo-controlled, double-blind, multicenter, phase 2/3 trial to evaluate the efficacy and safety of intravenous administration of HLCM051 compared with placebo in subjects with acute ischemic stroke (within 36 hours of onset). Japanese subjects who developed a subcortical ischemic stroke and are eligible to participate in the trial will be evaluated. In addition, the safety survey will be conducted 2 years after administration.

Approximately 220 subjects will be randomized in a 1:1 ratio (HLCM051 group [n=110] or placebo group [110 subjects]) to receive a single infusion of HLCM051 or placebo. Randomization will be stratified by baseline NIHSS score (≤12 and ≥13), concomitant reperfusion therapy (Yes or No), and age (18 to 64 and ≥65).

All subjects will be enrolled continuously into the trial. A Data and Safety Monitoring Board (DSMB) will be established beforehand to assess the data after approximately 30% and 60% of the planned number of subjects complete the 7-day follow-up. DSMB will also assess whether to continue or discontinue the trial if any of the following is encountered:

- Occurrence of serious adverse events (SAEs) that are unexpected and related to investigational product within 7 days after treatment;
- Occurrence of death that is related to investigational product;
- Occurrence of infusion-related allergic reactions whose severity are Grade 3 or 4 according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 within 24 hours after start of treatment; or
- Worsening of neurological symptoms (increase of ≥4 points in the NIHSS score) related to the investigational product within 7 days after treatment.

This study consists of four different phases; screening, baseline (Day 0: before and after treatment), post-treatment up to Day 365 and 2-year safety survey (Day 730) after administration.

Subjects will be evaluated at screening, baseline (Day 0: before and after treatment), Day 1, Day 2, Day 7, Day 30, Day 90, and Day 365, or Early Termination visit. In addition, an additional safety survey will be conducted in subjects who can be investigated on Day 730.

To prevent subjects from being lost to follow-up, trial sites will contact subjects (or representatives) by telephone at Day 60, Day 150, Day 210, Day 270, and Day 330 to update the information on disease status and health conditions.

At screening, subjects will undergo standard procedures including medical history, physical examination, 12-lead electrocardiogram (ECG), clinical laboratory tests, vital signs, NIHSS assessment, mRS assessment prior to the onset of ischemic stroke, and brain MRI (Magnetic resonance imaging). At baseline (Day 0: day of treatment), subjects will undergo physical examination, vital signs, pulse oximetry, adverse event and concomitant treatment assessments, allogeneic antibody and exploratory biomarker testing, and the NIHSS assessment. During the follow-up (post-treatment) period (from Day 1 to Day 365 or Early Termination), adverse events (AEs), laboratory test results, vital signs, pulse oximetry, ECGs, and physical examination results will be followed for the safety assessment, and mRS, NIHSS, and BI scores

will be assessed for the efficacy assessment. Biomarker testing will be assessed at baseline and each assessment. In addition, the survey of survival, development of malignancy, serious adverse event and other information will be conducted as an additional safety survey in subjects who can be investigated on Day 730.

3.2 Planned Period of the Clinical Trial

October 2016 to April2023 (planned, 2-year safety survey included)

4 Target Population

4.1 Selection of Target Population

Patients with acute ischemic stroke (within 36 hours of onset).

And investigators and sponsor should discuss with sharing sufficient information regarding the eligibility of patients before theenrollment.

4.2 Inclusion criteria

Patients will be determined to be eligible to participate in the trial if they meet all of the following criteria:

1. Japanese male or female patients between 20 and 84 years of age, inclusive;
2. Clinical diagnosis of cerebral cortical ischemic stroke;
3. Occurrence of an ischemic stroke with clear motor or speech deficit documented by National Institutes of Health Stroke Scale (NIHSS) score of 8 to 20 (at the baseline assessment) that did not change by ≥4 points from the screening to the baseline assessment;

   NOTE: The NIHSS assessment must be performed by the trial doctor who is trained for the NIHSS assessment.

   NOTE: There must be ≥6 hours between the baseline and screening NIHSS scores.

   NOTE: NIHSS screening score must be collected within 4 hours following reperfusion therapy procedure for those who received tPA treatment or underwent mechanical reperfusion.

   NOTE: The baseline NIHSS assessment must be confirmed within 16 to 34 hours after the onset of ischemic stroke.
4. Onset of ischemic stroke must have occurred within 18 to 36 hours prior to the start of administration of the investigational product;

   NOTE: Time of onset is defined as the time point when symptoms first began. For ischemic stroke that occurred during sleep, time of onset is defined as the time point when the patient was last observed to be normal or as the latest time point when the patient was self-reported to be normal.
5. Confirmation of hemispheric cortical infarct with brain magnetic resonance imaging (MRI) including diffusion-weighted imaging with b-value of 1,000 demonstrating an acute lesion measuring ≥ 2.0 cm of longest diameter;
6. A modified Rankin Scale (mRS) of 0 or 1, by either self-report or family report, prior to the onset of ischemic stroke;
7. Female patients who meet either:
   a. Not pregnant, not breastfeeding/ interrupting breastfeeding, and not planning on becoming pregnant during the trial;

b. Not of childbearing potential, defined as one who has been postmenopausal for at least 1 year, or has been surgically sterilized, or has had a hysterectomy at least 3 months prior to the start of this trial; or

c. If of childbearing potential, one who has agreed to follow investigator's advice and use an effective contraceptive method up to 1st year of the trial. Effective contraceptive methods include contraceptive methods used consistently and correctly (oral contraceptives, intrauterine devices, diaphragm, or male or female condoms), abstinence, and a sterile sexual partner;

8. Male patients with female partners of childbearing potential must agree to follow investigator's advice and use adequate contraceptive methods (a combination of a condom and another form of contraception) up to 1st year of the trial if engaging in sexual intercourse;
9. Patients or legal representatives must freely sign the informed consent form after the nature of the trial and the disclosure of his/her data have been explained;
10. Willing and able to comply with all aspects of the treatment and testing schedule; and
11. Willing and able to return to the trial site for the post-treatment evaluations.

[Rationale for Selection of Inclusion Criteria]

1. Selected to conduct the study in a patient population representing males and females with a wide range of ages, excluding the non-adult age group (<20 years of age). Since patients aged 85 years or older had a high incidence of comorbid medical condition such as pneumonia in addition to the primary disease, and many of them die due to factors other than ischemic stroke. Upper age limit shall be 84 years of old or younger.
2. Selected since the efficacy data obtained from preclinical studies of HLCM051 are currently limited to those on cerebral cortical ischemic stroke and the risk to benefit ratio of HLCM051 on patients with ischemic stroke other than cortical type is unknown.
3. Selected to enroll patients with a homogeneous type of ischemic stroke who have stable disease (to be determined when the baseline NIHSS score has not changed by ≥4 points).
4. Selected to enroll patients who had the onset within a time period in which the efficacy was confirmed in clinical studies conducted outside Japan.
5. Selected to maintain uniformity within a population to be enrolled in the trial by confirming that the infarct volume is not too big or too small.
6. Selected to enroll patients with significant deficits caused by the current ischemic stroke only, and not those caused by the previous disease or conditions.
7. Since reproduction studies of HLCM051 have not been completed at this point, #7 was selected to prevent pregnant female patients from being enrolled in the trial and make sure to prevent patients from becoming pregnant during 1st year of the trial.

8. Since reproduction studies of HLCM051 have not been completed at this point, #8 was selected to prevent partners of male patients from being pregnant.
9. Selected to perform the informed consent process completely.

10, 11. Selected to make sure to perform the complete patient follow-ups.

4.3 Exclusion Criteria

Patients will not be eligible to participate in the trial if they meet any of the following:

1. Presence of a lacunar, a lesion of ≤ 2.0 cm of longest diameter, or a brainstem infarct on MRI as the etiology of symptoms of ischemic stroke;
2. Reduced level of consciousness (score of 3 for item 1a of NIHSS);
3. Occurrence of a hemorrhagic transformation as evidenced by computerized tomography (CT) or brain MRI scan that is clinically significant in the opinion of the investigator;
4. Ipsilateral focal neurological deficits from prior lesions in the brain that would complicate evaluation;
5. Experienced seizures since the onset of ischemic stroke;
6. History of a neurological event such as stroke or clinically significant head trauma within 6 months prior to the start of screening;
7. Patients who both received tPA treatment and underwent mechanical reperfusion (patients are eligible for the trial if they had only one of them, tPA treatment or mechanical reperfusion);
8. Uncontrolled hypertension, defined as persistent systolic blood pressure >220 mmHg or diastolic blood pressure >120 mmHg, despite antihypertensive therapy;
9. Blood glucose level <50 mg/dL or >350 mg/dL at baseline;
10. Patients who have a significant comorbid medical condition(s), including, but not limited to:
    a. Severe kidney disease requiring hemodialysis or peritoneal dialysis;
    b. Advanced liver disease such as hepatitis or liver cirrhosis;
    c. Severe congestive heart failure or history of ejection fraction <30%;
    d. Severe lung disease requiring home oxygen; or
    e. Active unstable angina requiring daily treatment with nitrates or other medications;
11. Known human immunodeficiency virus infection, ongoing systemic infection, severe local infection or who are immunocompromised;
12. Alzheimer's disease or other dementias, Parkinson's disease, or any other neurological disorder that in the opinion of the trial doctor would affect their ability to participate in the trial or confound study assessments;

13. History of malignant tumor(s) within 2 years of the onset of ischemic stroke, with the exception of adequately treated basal or squamous cell carcinoma of the skin;
14. Contraindication for MRI such as implanted pacemakers or other metallic prosthesis incompatible with MRI, body weight, or claustrophobia;
15. Thrombocytopenia (platelet count <100,000/mm^3) or heparin-induced thrombocytopenia;
16. Known allergy to human tissue or bovine or porcine products, or religious objections to biological products;
17. Prior participation in another clinical trial involving investigational pharmacological agents or devices within 30 days prior to providing consent to receive the investigational product, or participation in pharmacological agents, devices, or investigational rehabilitation stroke recovery program is planned during the trial;
18. Other serious medical or psychiatric illness that is not adequately controlled and, in the investigator's opinion, would not permit the subject to be managed according to the protocol;
19. Previous surgical removal of the spleen;
20. Major fluctuation in neurological status since the onset of ischemic stroke indicating progression or expansion of ischemic stroke, or possible transient ischemic attack;
21. Plan to have a neurovascular procedure (e.g., carotid endarterectomy, stent placement, etc.) within the first year following ischemic stroke; or
22. Abnormal laboratory test results which investigators consider clinically significant and inappropriate for the trial.

[Rationale for Selection of Exclusion Criteria]

1. Since there are no preclinical study data supporting the enrollment of patients with small infarction or injured tissues limited to the brainstem.
2. Since patients with reduced level of consciousness are considered to have poor long-term prognosis that may affect the study results, they will be excluded from the enrollment.
3. Since patients, who had an exacerbation of the original ischemic stroke followed by a hemorrhagic transformation, are expected to have poor long-term prognosis that may affect the study results, they will be excluded from the enrollment.
4. Since patients with a history of stroke in the same anatomical region who currently have additional neurological deficit may become a confounding factor in the interpretation of the clinical data, they will be excluded from the enrollment.
5. Patients with a history of any types of seizures including partial seizures will be excluded from the trial. Generalized or partial seizures cause the changes in weakness and affect recovery rates in patients. This exclusion criterion is commonly used in clinical studies in stroke.

6. Since patients with the latest stroke or other significant neurological event may become a confounding factor in the interpretation of the clinical data, they will be excluded from the trial.
7. Patients with acute ischemic stroke who both received tPA treatment and underwent mechanical reperfusion will be excluded from the trial in consideration of the patient safety.

8~10, 15, 18. Selected to exclude patients with serious illness who are unable to complete the follow-up period in the trial.

11. Selected to exclude patients who may have a confounding factor in the clinical interpretation of the HLCM051 treatment since they are likely to be immunosuppressed.
12. Selected to exclude patients with neurological deficit that may interrupt the identification of the expected effects of HLCM051 on improvement in neurological and functional deficits caused by ischemic stroke.
13. Selected to exclude patients with a history of the latest malignant tumor until sufficient safety information on HLCM051 in this patient group is obtained.
14. Selected to exclude patients who are unable to undergo the procedures required in the trial.
16. At the time of manufacturing HLCM051, an extremely slight amount of bovine or porcine product is used during the process of cell culture prior to administration. Accordingly, #16 was selected to exclude patients with known allergy or religious objections to those products.
17. Since patients participating in other research studies may have a confusing or confounding factor in the interpretation of the trial of HLCM051, they will be excluded from the trial.
19. Selected to exclude patients without the spleen based on the hypothesis that the mechanism of action of HLCM051 is linked to the coordination of secondary lymphatic organs, mainly that of the spleen.
20. Selected to assure that all patients enrolled in the trial have no hemorrhagic transformation following ischemic stroke or have appropriately controlled and stable ischemic stroke without significant changes in their neurological conditions.
21. Selected to exclude patients who are unlikely to complete the follow-up period in the trial since they require large-scale procedures due to the status of co-existing disease.
22. Selected to exclude inappropriate patients showing clinically significant laboratory test results from the trial.

5 Trial Procedures and Assessment Methods

5.1 Assessment Procedures

The trial schedule is shown in “Trial Schedule”.

5.1.1 Screening Period (0 to 28 hours after the onset of ischemic stroke)

The following procedures will be performed. Other than Pregnancy test and obtaining consent of this study, treatments and tests are standard of cares at each clinical site and therefore clinical data collected prior to obtaining written consent will also be accepted as data of screening:

- Obtain written consent from subjects or legal representatives;

  Note: Consent can be obtained beyond 28 hours after the onset of ischemic stroke if data collected prior to obtaining consent within 28 hours after the onset can be used and the baseline examinations to be performed after obtaining consent and the start of administration of HLCM051 are done within 36 hours after the onset.

- Confirm medical history and subject demographics;
- Perform a physical examination (medical examination by a physician);
- Measure vital signs (blood pressure, pulse, body temperature, and respiration rate);
- Collect blood samples for chemistry and hematology testing;
- Collect urine samples for urinalysis;

  Note: For urinalysis, the urine collection and test can be performed beyond 28 hours after the onset of ischemic stroke as long as it is before the baseline examinations shown in 5.1.2.

- Perform pregnancy test on women of childbearing potential;
- Perform an ECG;
- Assess AEs and investigate concomitant treatments;
- Assess NIHSS score;

  NOTE: The NIHSS assessment must be completed by a qualified physician who is trained to assess the NIHSS. The NIHSS assessment at screening must be completed within 4 hours following reperfusion therapy procedure for those who received tPA treatment or underwent mechanical reperfusion.

- Perform mRS assessment, prior to the onset of symptoms of ischemic stroke by either self-report or family report; and
- Perform brain MRI.

5.1.2 Baseline (18 to 36 hours after the onset of ischemic stroke)

The following procedures will be performed as the assessment at Day 0 before the start of the administration (infusion). And investigators and sponsor should discuss with sharing sufficient information regarding the eligibility of patients before theenrollment:

- Evaluate inclusion/exclusion criteria;

- Assess NIHSS score 16 to 34 hours after the onset of ischemic stroke (final eligibility assessment);

  NOTE: The NIHSS screening score used for eligibility should be ≥6 hours between baseline and the NIHSS assessment at screening.

- Perform a physical examination (medical examination by a physician);
- Measure vital signs 1 hour ±30 minutes prior to the start of administration;
- Perform pulse oximetry 1 hour ±30 minutes prior to the start of administration;
- Collect blood samples for allogeneic antibody testing (wherever possible);
- Assess AEs and investigate concomitant medications; and
- Collect blood samples for exploratory biomarker testing (wherever possible).

See Section 6.1.2.2 for randomization procedures. Initiate infusion of HLCM051 or placebo within 18 to 36 hours after the onset of ischemic stroke. Perform infusion at the rate of up to 10 mL/minute over a period of 30 to 60 minutes. See the investigational product handling procedures for details of the treatment procedures.

The following procedures will be performed as the assessment at Day 0 after the start of administration (infusion):

- Measure vital signs every 15 ±5 minutes for the first 2 hours after the start of administration and then at 4 hours ±30 minutes and 6 hours ±30 minutes after treatment;
- Perform pulse oximetry every 15 ±5 minutes for the first 1 hour after the start of administration; and
- Assess AEs and investigate concomitant treatments.

5.1.3 Post-treatment – From Day 1 to Day 365

Following the administration of the investigational product, subjects will be monitored. At Day 1, Day 2, and Day 7 (or by the time of discharge if a subject is discharged before Day 7), the following assessments for each study visit will be performed in the hospital. Then, subjects will return for outpatient study visits according to the assessment schedule and the following assessment procedures for each study visit will be performed:

Day 1 (24±6 hours: visit time ± window calculated from the start of infusion)

The following procedures will be performed at Day 1:

- Perform a physical examination (medical examination by a physician);
- Measure vital signs;
- Perform pulse oximetry;
- Collect blood samples for chemistry and hematology testing;
- Collect urine samples for urinalysis;
- Perform an ECG;

- Assess AEs and investigate concomitant treatments; and
- Assess NIHSS score.

Day 2 (48±6 hours: visit time ± window calculated from the start of infusion)

The following procedures will be performed at Day 2:

- Measure vital signs;
- Perform pulse oximetry;
- Collect blood samples for chemistry and hematology testing;
- Collect urine samples for urinalysis;
- Perform an ECG;
- Assess AEs and investigate concomitant treatments; and
- Collect blood samples for exploratory biomarker testing (wherever possible; if it is not performed at the baseline, it will not need to be performed).

Day 7 (±2 days) or Day of discharge if earlier than Day 7

The following procedures will be performed on Day 7 or by the time of discharge if a subject is discharged before Day 7:

- Measure vital signs;
- Collect blood samples for chemistry and hematology testing;
- Collect urine samples for urinalysis;
- Assess AEs and investigate concomitant treatments;
- Assess NIHSS, mRS, and BI scores;
- Collect blood samples for exploratory biomarker testing (wherever possible; if it is not performed at the baseline, it will not need to be performed); and
- Inquire about rehabilitation activities (e.g., inpatient physical therapy, outpatient physical therapy, etc.).

Day 30 (±3 days) or discharge between Day 14-26 unless patient can visit Day 30

The following procedures will be performed at Day 30 or discharge between Day 14-26:

- Perform a physical examination (medical examination by a physician[*)]);
- Measure vital signs;
- Collect blood samples for chemistry and hematology testing;
- Collect urine samples for urinalysis;
- Collect blood samples for allogeneic antibody testing (wherever possible; if it is not performed at the baseline, it will not need to be performed);
- Assess AEs and investigate concomitant treatments[*)];

- Assess NIHSS, mRS, and BI scores[*)];
- Collect blood samples for exploratory biomarker testing (wherever possible; if it is not performed at the baseline, it will not need to be performed); and
- Inquire about rehabilitation activities[*)].

Day 60 (±7 days)

Trial sites will contact subjects (or representatives) by telephone to obtain updated information on disease status and in-hospital and discharge status, and inquire about rehabilitation activities, adverse events, and concomitant treatments.

Day 90 (±7 days)

The following procedures will be performed at Day 90[*)]:

- Measure vital signs;
- Perform pregnancy test on women of childbearing potential, by either urinalysis or serologic test;
- Assess AEs and investigate concomitant treatments[*)];
- Assess NIHSS, mRS, and BI scores[*)];
- Inquire about rehabilitation activities[*)];

Days 150, 210, 270, and 330 (±7 days)

Trial sites will contact subjects (or representatives) via telephone to obtain updated information on disease status and in-hospital and discharge status, and inquire about rehabilitation activities, adverse events, and concomitant treatments.

Day 365 (±7 days)/ Early Termination (±28 days)

The following procedures will be performed at Day 365 or Early Termination prior to Day 365[*)]:

- Perform a physical examination (medical examination by a physician) [*)];
- Measure vital signs;
- Collect blood samples for chemistry and hematology testing;
- Collect urine samples for urinalysis;
- Perform pregnancy test on women of childbearing potential, by either urinalysis or serologic test;
- Assess AEs and investigate concomitant treatments[*)];
- Assess NIHSS, mRS, and BI scores[*)];
- Inquire about rehabilitation activities[*)].

*）It allows the Physical examination (only at Day30 and Day365 or Early Termination), assessment of AEs and investigation of concomitant medications, NIHSS assessment, mRS assessment, BI assessment and inquiry of rehabilitation by visit examination unless patient can visit trial site.
For details, see the SOP of the patient who can not visit trial site.

5.1.4 Additional 2-year safety survey (Day730 ±56 days)

Trial site shall conduct the safety survey on Day 730 as far as possible in accordance with the "Procedures for additional 2-year safety survey" stigated separately.

- Survival
- Development of malignancy
- Development of serious adverse event
- Investigation of concomitant medications for serious adverse event

5.2 Assessment Schedule

5.2.1 Laboratory Procedures

In standard blood tests and urinalysis, laboratory certification (including expiration date) and normal reference ranges for all laboratories to be used during the trial will be provided to the sponsor prior to trial initiation. Blood and urine samples will be collected and processed per individual institutional processes.

The trial doctor must review all the reported laboratory test data and sign the reports. At screening, subjects who have laboratory values that are outside the normal reference ranges specified in Section 4.3, Exclusion Criteria, may not proceed to randomization.

Following the administration of the investigational product, the trial doctor needs to check if there are laboratory values outside of normal range, and takes appropriate measures if needed. In case of the presence of out-of-range laboratory values, the trial doctor will assess the clinical significance.

The laboratory tests will be performed at screening, Day 1, Day 2, Day 7, Day 30, and Day 365 or Early Termination. The following analytes will be measured:

Chemistry: Alkaline phosphatase, ALT, AST, total bilirubin, blood urea blood urea nitrogen, creatinine, sodium, potassium, glucose, albumin, total protein, calcium, and chloride

Hematology: Hemoglobin, hematocrit, red blood cell count, white blood cell count, and platelets

Note: At screening, clinical data collected prior to obtaining written consent will be accepted. If there are some analytes missing from standard of cares, these analytes need to be additionally analyzed to determine eligibility per Section 4.3, Exclusion Criteria. After screening, the analytes listed above will be measured.

Urinalysis: protein, glucose, occult blood, and high power field.

Note: Urinalysis will be conducted only to those from whom urine passed by oneself can be collected or those with indwelled urinary catheter.

A blood sample for exploratory biomarker testing (white blood cell populations and inflammatory markers) will be drawn at baseline (Day 0 before the start of infusion), Day 2, Day 7, and Day 30 (wherever possible; if it cannot be performed at baseline, it will not need to be performed at Day 2 and thereafter).

A blood sample for allogeneic antibody testing to HLCM051 will also be drawn at baseline (Day 0 before the start of infusion) and at Day 30 (wherever possible; if it cannot be performed at baseline, it will not need to be performed at Day 30). The following analytes will be measured:

White blood cell populations: CD3-positive cell, and regulatory T cell

Inflammatory markers: IL-1b、IL-2、IL-6、IL-12、IL-17、IL-23、IFN ɤ、TNF-α、MCP-1

For information on blood volume, collection tubes, sample processing, storage, and shipping procedures, see the Laboratory Manual for this trial.

5.2.2 Medical and Surgical History

Medical history will be collected from all subjects at screening. Medical history should include details and dates of onset of all illnesses and allergies, current conditions, and smoking and drinking habits. Additionally, information such as past surgical and medical procedures and medication history is to be collected.

5.2.3 Subject Demographics

Demographic information including day, month, and year of birth, race, and gender will be collected from all subjects at screening.

5.2.4 Vital signs (blood pressure, pulse, body temperature, and respiration rate)

Vital signs will be measured at screening, baseline (Day 0 before and after the start of infusion), Day 1, Day 2, Day 7, Day 30, Day 90, and Day 365 or Early Termination.

5.2.5 Physical Examination

A physical examination will be performed at screening, baseline (Day 0 before the start of infusion), Day 1, Day 30, and Day 365 or Early Termination.

5.2.6 Pulse Oximetry

Pulse oximetry will be performed at baseline (Day 0 of before and after the start of infusion), Day 1, and Day 2.

5.2.7 Electrocardiogram (ECG)

A 12-lead ECG will be performed at screening, Day 1, and Day 2. Site personnel should make every attempt to perform a subject’s ECG using the same equipment at each visit.

5.2.8 Brain MRI

An MRI scan of the brain will be performed at screening. The sponsor collects brain MRI images obtained at screening and evaluates the infarct volume and ASPECTS at the central institution.

5.2.9 Pregnancy Test

Pregnancy test will be performed at screening, Day 90 or Early Termination if the withdrawn is prior to Day 90.

6 Treatment with Investigational Product and Restrictions

6.1 Treatment Regimen

6.1.1 Dosage Forms and Route of Administration

Subjects will receive a single dose of 1.2 billion HLCM051 cells or placebo within 18 to 36 hours after the onset of ischemic stroke. Perform infusion at the rate of up to 10 mL/minute over a period of 30 to 60 minutes. See the handling procedures for details of the preparation of the investigational product and the treatment regimen.

In the first 2 hours after the start of HLCM051 administration, patients should be carefully monitored during and after administration according to the rules specified in Section 5.1.2 Baseline, such as vital signs (blood pressure, pulse rate, body temperature, and respiratory rate) measured every 15 minutes.

If a concomitant drug is intravenously administered, simultaneously with HLCM051, the drug should be administered from another line.

[Rationale for Selection of Dosage Forms and Route of Administration]

The number of cells to be administered and the route of administration in the trial are same as those in the high-dose group in the phase 2 clinical study in ischemic stroke conducted outside Japan (B01-02 Study). The dose according to body weight in the trial has an approximately 8-fold (calculating body weight in humans to be 50 kg) margin of safety compared to that in preclinical safety studies in rats and mice and the tolerability of the dose has been confirmed in the completed B01-02 Study.

Since this trial is the first study of HLCM051 to be administered to Japanese patients, there are no safety data in Japanese patients. Among the clinical studies that have been conducted so far, HLCM051 was administered to 8 Asian subjects in the phase 2 clinical studies in patients with ulcerative colitis and those with ischemic stroke conducted outside Japan, and no AEs that were different from commonly observed events have been reported when compared to non-Asian subjects. It is considered that there are no clinically significant differences in the possible mechanism of action and pharmacokinetics of HLCM051 between the ethnic groups. Moreover, there may be no differences in medical environments between in the United States where the B01-02 Study was conducted and in Japan that can impact the assessment of the efficacy and safety of HLCM051.

6.1.2 Treatment Assignment and Blinding

6.1.2.1 Assignment of Subject Identification Number

Each subject who provides consent to participate in the trial will be assigned a specific identification number.

The subject identification number will be used to identify the subject throughout the trial and will be entered on all trial documents.

A same subject identification number will not be assigned to more than 1 subject. If a subject is found not eligible to receive the investigational product or if the subject discontinues from the trial, the subject identification number cannot be reassigned to another subject.

6.1.2.2 Randomization

A total of 220 subjects will be randomly assigned to receive HLCM051 or placebo in a 1:1 ratio. Randomization will be performed by the use of an Interactive Web Response System (IWRS). A stratification factor will be the baseline NIHSS scores (≤12 and ≥13).

An IWRS will assign a specific code to each subject and generate the subject to a treatment group and its corresponding investigational product.

6.1.2.3 Blinding

This is a double-blind trial. The investigational product label on the container will indicate the study number.

The assignment code will not be provided to the trial doctor. The assignment code will be maintained in an IWRS. In case of an emergency unblinding, the trial doctor can unblind individual subjects using the function of an IWRS.

While the double-blind trial is ongoing, the subjects, trial doctor, and trial personnel at the site will be blinded to subject treatment assignments.

Although the designated investigational product processing personnel at the trial site will be unblinded, the treatment assignment will not to be provided to other site personnel, including the trial doctor, at any time, except in the case of a true emergency.

DSMB is in the position where they can know the treatment assignments for the routine reviews on unblinded data.

6.1.2.4 Emergency Unblinding of Subjects

In an emergency, when knowledge of the assignment of the blinded investigational product is essential for the clinical management or welfare of the subject, the trial doctor should make every effort to contact the sponsor (or designee) prior to the unblinding process. Prior to unblinding, the trial doctor should assess the relationship of an adverse event to the use of the investigational product (definitely, probably, possibly, unlikely, unrelated) and discuss the need to unblind with the medical expert. The trial doctor then proceeds with the unblinding process.

The trial doctor must record the date and the reason for unblinding on the appropriate electronic Case Report Form (eCRF) and source documents.

6.2 Trial Restrictions

6.2.1 Concomitant Treatments

Any medications and treatments prescribed from the time of hospital admission through the Day 365 Visit /Early Termination before Day 365 must be recorded on the Concomitant Treatment eCRF. From Day 365 to Day730, any medications and treatments for serious adverse event must be recorded on the eCRF. Subjects must not have taken any investigational agent or product within 30 days prior to screening. Subjects cannot participate in any other investigational medication trial up to 1st year of this trial.

6.2.2 Concomitant Medications and Therapies

Subjects who either received tPA treatment or underwent mechanical reperfusion after admission will be eligible for the trial.

7 Investigational Product

See the handling procedures for details of the storage, preparation, administration, and disposal of the investigational product.

7.1 HLCM051 and Placebo

The investigational product, both HLCM051 and placebo, will be supplied by Healios and sent to each trial site. The pharmacy or appropriate facility department will store the investigational product under appropriate conditions.

Healios will supply sufficient quantities of HLCM051 and placebo to allow for completion of the trial. The lot numbers will be recorded in the final trial report.

7.2 Dosage Forms

Test product: HLCM051 will be provided in one unit of 1.2 billion (± 20%) cells. The components of cryopreservation medium are Plasma-Lyte A (solution used for dilution), dimethyl sulfoxide, and human serum albumin.

Control product: Placebo contains no cells, but rather a cryopreservation medium, consisting of Plasma-Lyte A, dimethyl sulfoxide, and human serum albumin.

HLCM051 and placebo will be separately provided after being filled in a 6-mL vial and sealed.

7.3 Distribution and Storage Conditions

7.3.1 Control, Accountability, and Return

The investigational product will be provided frozen to the pharmacy or appropriate facility department at each trial site. The head of the trial site or the investigational product manager appointed by the head of the trial site must store and control the investigational product under appropriate conditions according to the procedures. Prior to the conduct of the trial, the sponsor will provide appropriate trial personnel with training on the handling of the investigational product, and prior to use, appropriate trial personnel will thaw the investigational product and prepare it for infusion, and then deliver it to the department responsible for its administration according to the procedures.

The head of the trial site or the investigational product manager must record the number of all the investigational product received, dispensed, and stored on the investigational product accountability log prepared by the sponsor or the trial site, and store the records. At the conclusion of the trial, all unused investigational product will be returned to the sponsor (or its appointed designee). In case there is no remaining investigational product, the fact should be also recorded on the investigational product accountability log.

7.3.2 Storage Conditions

The investigational product must be stored at below minus 140 degrees Celsius in secure storage facility accessible only to those who authorized by the head of the trial site or Healios to process and distribute the investigational product. At the conclusion of the trial, the clinical research associate (CRA) will account for all used and unused investigational product.

8 Efficacy Assessments

8.1 Efficacy Endpoints

8.1.1 Primary Endpoint

- The proportion of subjects with an excellent outcome at Day 90 defined by the functional assessments

  <Excellent outcome>

  mRS score of ≤1 (scale, 0 to 6), NIHSS score of ≤1 (scale, 0 to 42), and BI score of ≥95 (scale, 0 to 100)

8.1.2 Secondary Endpoints

- The proportion of subjects with an excellent outcome defined by the functional assessments at Day 365
- The proportion of subjects exhibiting functional outcome throughout the range of mRS scores by shift analysis at Day 90
- The proportion of subjects exhibiting functional outcome throughout the range of mRS scores by shift analysis at Day 365
- The proportion of subjects with a mRS score of ≤2, NIHSS score improvement of ≥75% from baseline and a BI score of ≥95 at Day 90
- The proportion of subjects with a mRS score of ≤1 and a mRS score of ≤2 at Day 90
- The proportion of subjects with a NIHSS score of ≤1 at Day 90
- The proportion of subjects with a favorable outcome (NIHSS score improvement of ≥75% from baseline) in neurological symptoms at Day 90
- The proportion of subjects with a BI score of ≥95 at Day 90
- The proportion of subjects who survived without life-threatening adverse events (AEs) at Day 90.
- The proportion of subjects who survived without secondary infections at Day 90 Day.
- Global recovery (i.e., GEE) and dichotomous assessment at Day 90. Integrated assessment using three component efficacy endpoints (mRS score of ≤2, NIHSS score improvement of ≥75% from baseline, and BI score of ≥95) and two component safety endpoints (survival without life-threatening AEs and secondary infections) together.

8.1.3 Exploratory Endpoints

- Changes in biomarkers (white blood cell populations and inflammatory markers) from baseline to Day 2, Day 7, and Day 30.

8.2 Assessment Methods

For scores of NIHSS, mRS, and BI, one subject will be assessed ideally by one investigator from Screening through Day 365/ Early Termination.

8.2.1 National Institutes of Health Stroke Scale (NIHSS)

The NIHSS assessment is a standardized method to assess the level of impairment caused by a stroke using the scales from 0 to 42 and must be performed by the trial doctor. The several aspects of brain function, including consciousness, vision, sensation, movement, dysarthria, and aphasia (0 = no stroke symptoms, 42 = severe stroke) will be assessed.

The NIHSS score will be assessed at screening (0 to 28 hours after the onset of ischemic stroke), baseline (Day 0, prior to the start of infusion), Day 1, Day 7, Day 30, Day 90, and Day 365 or Early Termination.

For the final eligibility assessment, the NIHSS score must be confirmed at baseline within 16 to 34 hours after the onset of ischemic stroke. There should be ≥6 hours between baseline and the NIHSS assessment at screening.

8.2.2 modified Rankin Scale (mRS)

The mRS is used to assess functional independence rather than performance of specific tasks. This tool incorporates mental symptoms as well as physiological adaptations to the neurological deficits. The scale consists of 7 grades, from 0 to 6, with 0 corresponding to no symptoms and 6 corresponding to death.

The mRS score will be assessed at screening, Day 7, Day 30, Day 90, and Day 365 or Early Termination.

8.2.3 Barthel Index (BI)

The BI is an assessment method to measure a subject's performance in 10 activities of daily life. The items can be divided into a group that is related to self-care (feeding, grooming, bathing, dressing, bowel and bladder care, and toilet use) and a group related to mobility (ambulation, transfers, and stair climbing). The score becomes 100 (the maximum score) with 5-point increments, if the subject is fully independent in physical functioning. The lowest score is 0, representing a totally bedridden state.

The BI score will be assessed at Day 7, Day 30, Day 90, and Day 365 or Early Termination.

9 Safety Assessments

Safety assessments up to Day365 after the administration of the investigational product shall be conducted in accordance with the Sections 9.1 through 9.7. Additional 2-year safety survey shall be conducted in accordance with the Section 9.8.

9.1 Safety Endpoints

The safety endpoints include adverse events and changes in laboratory parameters, vital signs, ECG, allogeneic antibody, and incidence of infection (local and systemic).

The primary safety endpoints are as follows:

- Grade 3 or 4 infusion-related allergic reactions that are related to the investigational product. Perturbation of cardiovascular system and respiratory function, and allergic reactions (e.g., rash, erythema) that occurred within 24 hours after treatment;
- SAEs that occurred within 7 days after treatment and are related to the investigational product;
- Worsening of neurological symptoms that are related to the investigational product. Worsening of neurological symptoms is defined as an increase of ≥4 points in the NIHSS score from baseline assessed through 7 days after treatment; and
- Death, life-threatening AEs and incidence of secondary infections at Day 90.

The secondary safety endpoints are as follows:

- Comparison of the incidence of secondary infections (local and systemic), AEs, death, vital signs (blood pressure, pulse, respiration rate, body temperature), and oxygen saturation, and laboratory parameters through Day 365.

9.2 Adverse Events

An adverse event (AE) is defined as any untoward medical events occurred in subjects who have received investigational products, and does not have to be causally related to the use of the investigational product. Therefore, an AE indicates any unintended sign (including an abnormal laboratory finding), symptom, or disease that occurs when an investigational product is used, whether or not related to the investigational product. All AEs, including observed or reported problems, complaints, and symptoms, are to be recorded on the eCRF.

AEs, which include clinical laboratory test variables, will be monitored from the time of obtaining informed consent until Day 365 or Early Termination. From Day 365 to Day 730, development of malignancy and serious adverse event including death will be monitored. The outcomes of those AEs and the occurrence of any new events will be followed by telephone up to additional one year following Day 365 to the maximum extent possible. Subjects should be instructed to report any AE that they experience to the trial doctor. Beginning at the signing of informed consent by subjects or legal representatives, the trial doctor should assess for AEs at each visit or telephone interview and record the event on the AE page of the eCRF.

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the trial doctor and recorded on the eCRF. However, if an

observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the trial doctor, it should be recorded as a separate AE on the eCRF.

Any medical condition that is present when a subject is screened or present at baseline that does not deteriorate should not be reported as an adverse event. However, medical conditions or signs or symptoms present at baseline that changes in severity or seriousness at any time during the period above should be reported as an AE.

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the period above or are present at baseline and significantly worsen will be reported as AEs or serious AEs (SAEs). The trial doctor will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. All abnormal laboratory values considered clinically significant by the trial doctor must be recorded on the AE page of the eCRF. Significant abnormal laboratory values occurring during the period above will be re-tested and followed until they return to normal, stabilize, or are no longer clinically significant.

Severity of an Adverse Event

For infusion-related reaction events, the trial doctor will rate the severity (intensity) of each event according to Appendix 1 and Common Terminology Criteria for Adverse Events (CTCAE) v4.0 as described below:

- Mild (CTCAE v4.0 Grade 1): asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated;
- Moderate (CTCAE v4.0 Grade 2): minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living;
- Severe (CTCAE v4.0 Grade 3): medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care Activities of Daily Living;
- Life-threatening (CTCAE v4.0 Grade 4): life-threatening consequences; urgent intervention indicated;
- Death (CTCAE v4.0 Grade 5): death-related AE.

For all other AEs, the following guidelines will be used to assess severity:

- Mild - awareness of sign or symptom but easily tolerated, with no interference with subject's usual activity.
- Moderate - enough discomfort to cause some interference with usual activity.
- Severe - incapacitating with inability to work or significant interference with usual activity.

Causal Relationship

The causal relationship of an adverse event to the administration of the investigational product is to be assessed according to the following definitions:

- Definitely related: Follows a reasonable temporal sequence from investigational product administration; abates upon discontinuation of the investigational product

(dechallenge); and is confirmed by reappearance of the reaction on repeat exposure (rechallenge);

- Probably related: Follows a reasonable temporal sequence from investigational product administration; abates upon discontinuation of the investigational product (dechallenge); and cannot be reasonably explained by the known characteristics of the subject's clinical state;
- Possibly related: Follows a reasonable temporal sequence from investigational product administration and could have been produced by the subject's state or by other modes of therapy administered to the subject;
- Unlikely related: The temporal sequence between the adverse event and the investigational product administration is such that the drug is not likely to have had any reasonable association with the observed event and the adverse event could have been produced by the subject's clinical condition or by other modes of therapy administered to the subject; or
- Unrelated: The adverse event is definitely produced by the subject's clinical condition or by other modes of therapy administered to the subject and the adverse event does not follow a temporal sequence from investigational product administration.

The following factors should also be considered:

- The temporal sequence from trial medication administration

  The event should occur after the investigational product is given. The length of time from investigational product exposure to event should be evaluated in the clinical context of the event.

- Underlying, concomitant, and intercurrent diseases

  Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.

- Concomitant medication

  The other medications the subject is taking or the treatment the subject receives should be examined to determine whether any of them might be recognized to cause the event in question.

- Known response pattern for this class of investigational product

  Clinical and/or preclinical data may indicate whether a particular response is likely to be a class effect.

- Exposure to physical and/or mental stresses

  The exposure to stress might induce adverse changes in the recipient and provide a logical and better explanation for the event.

- The pharmacology and pharmacokinetics of the investigational product

The known pharmacological properties (absorption, distribution, metabolism, and excretion) of the investigational product should be considered.

Unexpected Adverse Events

An adverse event not listed in the Investigator's Brochure or not listed at the specificity or severity that has been observed. Additionally, unexpected adverse events include those that are mentioned in the Investigator's Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the investigational product, but not specifically mentioned as occurring with the particular product under investigation.

9.2.1 Serious Adverse Events

An adverse event or suspected adverse event is considered serious if, in the view of either the trial doctor or sponsor, it results in any of the following outcomes:

- Lethal;
- Life-threatening; or

  NOTE: An event determined to be "life-threatening" by the trial doctor or the sponsor that actually posed an urgent risk of death upon a subject. It does not include an event that, had it occurred in a more severe form, might have caused death.

- Requires hospitalization or prolongation of existing hospitalization;

  NOTE: In general, hospitalization for treatment of a pre-existing condition that did not worsen from baseline is not considered an AE and should not be reported as an SAE.

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- A congenital anomaly/birth defect; or
- An important medical event.

  NOTE: Important medical events that may not be immediate life threatening, result in death, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalizations, or the development of drug dependency.

9.3 Procedures for Serious Adverse Event Reporting

Initial Reports

All SAEs occurring from the obtainment of informed consent until Day 730 or Early Termination must be entered into Electron Data Capture (EDC) and reported to the sponsor or designee (Healios, Athersys, or CRO) **within 24 hours** of the knowledge of the occurrence. Once a SAE information is entered in EDC the sponsor or designee will be notified electronically from EDC. If the PI cannot reach to EDC due to system's technical issues a paper

SAE form must be used instead and reported to the sponsor or designee by fax or e-mail. The sponsor or designee (Healios, Athersys, or CRO) will concurrently receive SAE reports at anytime 24 hours even on a bank holiday. Healios and Athersys will conduct medical review of SAE reports within 48 hours of the knowledge of the occurrence and share results of medical review with CRO. Based on the results of medical review, Healios will report to PMDA. CRO will then provide safety information to investigators.

The PI is required to submit SAE reports to the head of the trial site. The sponsor will report SAEs to the regulatory authorities and notify the investigator at each trial site of them in compliance with the regulations. All reports sent to each investigator will be blinded.

Follow-up Reports

The PI must continue to follow the subject until the SAE has subsided, or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the subject dies. Within 24 hours of receipt of follow-up information, the investigator must update the SAE form electronically in the EDC system for the trial and submit any supporting documentation (e.g., laboratory test results, subject discharge summary, or autopsy reports) to the sponsor (or designee) by fax or email. When the SAE form is updated, the sponsor (or designee) will be notified electronically and will retrieve the form. If it is not possible to access the form, refer to the procedures outlined above for initial reporting of SAEs.

9.4 Measures Taken in Case of Pregnancy

If a subject or the partner of a subject participating in the trial becomes pregnant from signing informed consent through Day 365 or Early Termination, or within 28 days of discontinuing from the study, the investigator should report the pregnancy to the sponsor (or designee) within 24 hours of being notified, by entering data into EDC system.

The subject/partner should be followed by the investigator until completion of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the investigator should notify the sponsor. At the completion of the duration of pregnancy, the investigator will document the outcome of the pregnancy in Pregnancy Investigation form. If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the investigator should follow the procedures for reporting a SAE.

9.5 Adverse Device Effect

An Adverse Device Effect is defined as any untoward and unintended response, for example malfunction of investigational product and a compound kit for infusion of investigational product and any reactions cells affect to a human body, regardless of whether they are occurred in a phase of manufacturing, distribution, storage, or infusion. A compound kit for infusion of investigational product includes transfer pack, blood set with filter, etc. All adverse device effects, but excluding AEs and SAEs, have to be reported to the sponsor (or designee) by FAX .

A Serious Adverse Device Effect is defined as an Adverse Device Effect that has resulted in any of the consequences characteristic of a Serious Adverse Event or that might have led to any of these consequences if suitable action had not been taken or intervention had not been made or if circumstances had been less opportune.

Information of Serious Adverse Device Effects have to be reported to the sponsor (or designee) within one (1) business day. Investigator should follow the procedures for reporting a SAE

when a SAE occurs.

9.6 Investigational Product-related Events

If any of the following events occurs during the administration of the investigational product, the investigator must follow the procedures described in Section 9.3 for reporting as a SAE:

- When an overdose of the investigational product occurs;
- When the investigational product is incorrectly administered; or
- When a Grade 3 or 4 infusion-related allergic reaction occurs.

9.7 Report of Worsening of Neurological Symptoms at Early Post-infusion

If the following worsening of neurological symptoms occurs, as it possibly meets a criterion described in Section 11.5 Enrollment Suspending Rules, the investigator must follow the procedures described in Section 9.3 for reporting the information to the sponsor or designee (Healios, Athersys, or CRO) **within 24 hours** of the knowledge of the occurrence.

- Occurrence of neurological worsening (increase of ≥4 points in the NIHSS score) related to the investigational product within 7 days after treatment with the investigational product.

9.8 Additional 2-year safety survey

When the investigator recognizes the occurrence of death or serious adverse event in 2-year safety survey of death, malignancy and serious adverse event, the investigator shall promptly report to the sponsor or contract research organization (CRO) as a SAE with reference to the procedures in Section 9.3.

10 Data and Safety Monitoring Board (DSMB)

A Data and Safety Monitoring Board (DSMB) with multidisciplinary representation will be established to evaluate accumulating trial data and to assess the safety of the conduct of the trial for the subjects enrolled and to be enrolled. Assessments will be performed after approximately 30% and 60% of the planned number of subjects complete Day 7 assessment. As a result, following each data review, the DSMB will make a recommendation to the sponsor regarding continuation, revision of dosage, or necessity to terminate the trial. When any of an Enrollment Suspending Rules described in Section 11.5 is met, the DSMB will also review the safety of the trial and determine whether or not to continue the further trial enrollment. Details related to the DSMB responsibilities, authorities, and procedures will be documented in the DSMB charter that is separately prepared. The charter will be finalized prior to the first data review meeting.

11 Discontinuation of the Trial/Withdrawal of Consent

Subjects may be discontinued from their participation in the trial at any time, at the discretion of the investigator. The investigator or his/her designee must contact the sponsor (or designee) and the head of the trial site, and report that randomized subjects will be prematurely discontinued from the trial and the reasons of it.

11.1 Withdrawal of Consent/Reasons for Withdrawal

The sponsor (or designee) should be notified if a subject withdraws consent or is discontinued because of an adverse event. Subjects must be followed until the adverse event or laboratory abnormality is resolved, or the outcome is determined.

Participation of a subject in this clinical trial may be discontinued for any of the following reasons:

- Subject withdraws consent to participate in the trial or requests discontinuation from the trial for any reason;
- Sponsor discontinues the trial;
- Occurrence of a clinical or laboratory value-related adverse event, either serious or non serious, at the discretion of the trial doctor;
- Any medical condition or personal circumstance that, in the opinion of the trial doctor, exposes the subject to risk by continuing in the trial or precludes adherence to the stipulations in the protocol.

11.2 Withdrawal Procedures

Subjects may withdraw from trial participation at any time at their own request. They may be withdrawn at any time at the discretion of the trial doctor for safety reasons.

11.3 Data Collection through Follow-up for Withdrawn Subjects

Every effort should be made by the trial personnel at the site to complete the trial procedures as per the stipulations in the protocol. All withdrawals are recorded in the appropriate eCRF. If a subject is withdrawn, the trial doctor should request that the subject complete the procedures for Early Termination.

11.4 Lost to Follow-up

The trial doctor should document all efforts to get a subject to return to complete the trial procedures. A file will be treated as lost to follow-up after the trial sites complete 3 phone calls to the subject (or representatives) with no response obtained.

11.5 Enrollment Suspending Rules

If any of the following is met, trial enrollment should be stopped until DSMB determines that it is safe for the trial to continue enrollment:

- Occurrence of serious adverse events (SAEs) that are unexpected and related to the investigational product within 7 days after treatment with the investigational product;
- Occurrence of death that is related to the investigational product;

- Occurrence of any Grade 3 or 4 infusion-related allergic reaction in the first 24 hours after treatment with the investigational product (see Appendix 1 for definitions and management of infusion-related allergic reactions); or
- Occurrence of neurological worsening (increase of ≥4 points in the NIHSS score) related to the investigational product within 7 days after treatment with the investigational product.

11.6 Discontinuation of the Trial

If the trial is to be discontinued or suspended, the sponsor will promptly notify all the heads of the trial sites that are related to the trial and the regulatory authorities of the trial discontinuation or suspension and detailed reasons in writing.

If the investigator is notified of the trial discontinuation or suspension decided by the sponsor from the head of the trial site, he or her should obtain the document explaining the discontinuation or suspension in details from the head of the trial site and promptly inform subjects of the sponsor's decision for the post-procedures.

If the development of the test drug is to be discontinued, the sponsor will promptly notify all the heads of the trial sites that are related to the trial, the investigators, and the regulatory authorities of the discontinuation of the development and detailed reasons in writing.

12 Statistics

The sponsor (or designee) will perform statistical analysis. In this trial, a separate Statistical Analysis Plan (SAP) will be prepared to provide additional details on the approach to analyses and data displays. The SAP will be finalized before the database is locked. By the time of unblinding, the sponsor determines analysis sets, data management criteria of the analysis sets, and criteria of management of issues which are not scheduled. The details of statistical analysis of the additional 2-year safety survey will be specified in the statistical additional analysis plan prepared separately from the SAP.

12.1 Analysis Set

The primary efficacy analysis set will be Full Analysis Set (FAS), and defined as the subjects who are randomized, treated with the investigational product, and have the results of the mRS, NIHSS, and BI scores assessed after Day 7 at least once. Per-protocol population (PPT) will also be analyzed to assure the results between two analysis sets are not different.

For the safety analysis set, subjects who are randomized and treated with the investigational product will be included.

12.2 Statistical Methods:

The aim of this study is to evaluate efficacy and safety data of HLCM051 in Japanese patients with Ischemic Stroke. The following items will be analyzed and compared between HLCM051 group and Placebo group; the number of cases and proportion for frequency, the number of cases, mean, standard deviation, minimum, median, and maximum for summary statistics.

See SAP for further information.

12.2.1 Analysis of Efficacy

12.2.1.1 Primary Efficacy Endpoint

For Full Analysis Set (FAS), the proportion of subjects with an excellent outcome at Day 90 defined by the functional assessments, as a primary efficacy endpoint, will be compared between the HLCM051 and the placebo groups and superiority of HLCM051 to placebo groups will be assessed by Cochran–Mantel–Haenszel (CMH) test (the two-tailed significance level of 0.05), with adjustment factor of Baseline NIHSS score (≤12 or ≥13), concomitant reperfusion therapy (Yes or No), and age (≤74 and ≥75).

As a sensitivity analysis, the primary efficacy analysis will also be conducted in PPT. Similarly, as a sensitivity analysis of the primary analysis, an analysis for FAS will be conducted with age (≤64 and ≥65), instead of the age of adjustment factor (≤74 and ≥75) in the primary analysis.

<Excellent outcome>

Defined as mRS score of ≤1 (scale, 0 to 6), NIHSS score of ≤1 (scale, 0 to 42), and BI score of ≥95 (scale, 0 to 100)

12.2.1.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are as follows:

- The proportion of subjects with an excellent outcome defined by the functional assessments at Day 365

- The proportion of subjects exhibiting functional outcome throughout the range of mRS scores by shift analysis at Day 90
- The proportion of subjects exhibiting functional outcome throughout the range of mRS scores by shift analysis at Day 365
- The proportion of subjects with mRS score of ≤2, NIHSS score improvement of ≥75% from baseline, BI score of ≥95 at Day 90
- The proportion of subjects with a mRS score of ≤1 and a mRS score of ≤2 at Day 90
- The proportion of subjects with a NIHSS score of ≤1 at Day 90
- The proportion of subjects with a favorable outcome (NIHSS score improvement of ≥75% from baseline) in neurological symptoms at Day 90
- The proportion of subjects with a BI score of ≥95 at Day 90
- The proportion of subjects who survived without life-threatening adverse events (AEs) at Day 90
- The proportion of subjects who survived without secondary infections at Day 90
- Global recovery (mRS score of ≤2, NIHSS score improvement of ≥75% from baseline, and BI score of ≥95) and two components (survival without life-threatening AEs and secondary infections) together

12.2.1.3 Exploratory Efficacy Endpoints

The exploratory efficacy endpoints are as follows:

- Changes in biomarkers (white blood cell populations and inflammatory markers) from baseline to Day 2, Day 7, and Day 30.

12.2.1.4 Subgroup Analysis

Subgroup analysis will be adopted by stratifying subjects into the following groups: the group to include subjects with no tPA treatment or mechanical reperfusion; the group to exclude subjects with tPA treatment; and the group to exclude subjects with mechanical reperfusion. There is also intention to evaluate the impact of factors including the severity of ischemic stroke, subject age, gender, and/or other factors.

Details will be described in the SAP.

12.2.2 Analysis of Safety

The primary safety endpoints are as follows:

- The proportion of subjects with Grade 3 or 4 infusion-related allergic reactions that are related to the investigational product. Perturbation of cardiovascular system and respiratory function, and allergic reactions (e.g., rash, erythema) that occurred within 24 hours after treatment;
- The proportion of subjects with SAEs that occurred within 7 days after treatment and are related to the investigational product;

- The proportion of subjects with worsening of neurological symptoms that are related to the investigational product. Worsening is defined as an increase of ≥4 points in the NIHSS score from baseline assessed through 7 days after treatment; and
- The proportion of subjects with death, and incidence of life-threatening AEs and secondary infections at Day 90.

The secondary safety endpoints are as follows:

- The incidence of secondary infections (local and systemic), AEs, mortality, vital signs (blood pressure, heart rate, respiration rate, temperature, and oxygen saturation), and laboratory parameters through Day 365.

The safety parameters included in the secondary endpoints above will be assessed by subgroup analysis, as required.

12.2.3 Characteristics of Demographics and Other Parameters

For FAS, PPT, and analysis set for safety endpoints, frequencies and summary statistics with respect to demographics and other parameters will be shown in each group. Also homogeneity in terms of demographics will be assessed between two groups

12.3 Analysis Schedule

Unblinding and analysis of Day 90 and Day 365 data is scheduled after the Day 365 data of all patients are fixed. Efficacy and safety analysis of data at the time of the fixation of Day 365 data will be described in SAP. The data of additional 2-year safety survey will be analyzed at the time when the data can be evaluated according to the additional statistical analysis plan.

12.4 Significance Level and Confidence Index

Since the primary efficacy endpoints will be analyzed with all data collected by the time the last patient of the study completes Day 90 and multiplicity of tests is not necessarily taken into consideration, significance level is not adjusted. For analysis of individual endpoint, two-tailed hypothesis test will be conducted with significance level of 5% and estimated confidence interval of 95%, unless SAP defines.

12.5 Deviation from Statistic Analysis Plan

- Analysis plan is modified from that originally defined in the protocol by the time when SAP is fixed, contents and reasons of modification shall be described in SAP and Clinical Study Report (CSR).
- When analysis plan is modified after unblinding, the modified analysis and the analysis originally planned shall be clarified and assessed how its modification affects conclusion of the study results. These are also described in CSR, together with contents and reasons of modification.

12.6 Sample Size Determination

The number of evaluable subjects in the trial is 220 subjects (HLCM051 group: 110 subjects; placebo group: 110 subjects).

[Rationale for Selection of Sample Size]

According to the results of the completed B01-02 Study obtained in 27 subjects in the HLCM051 group and 52 subjects in the placebo group excluding subjects who received both

t-PA and MR from those treated within 36 hours after the onset of ischemic stroke, the proportions of subject with an excellent outcome at Day 90 defined by the functional assessment were 18.5% and 3.8% in the HLCM051 and the placebo groups, respectively. Under the setting that the results of this trial are the same as those observed in the B01-02 Study, the number of subjects is calculated as 100 subjects per group with a significance level of 5% and a power of 90%. After considering that there may be some subjects to be excluded/dropouts, 110 subjects per group will be enrolled in the trial.

13 Data Management and Record Keeping

13.1 Data Management

13.1.1 Data handling

Data will be recorded at the trial site on eCRFs and reviewed by the CRA during monitoring visits. The CRAs will verify data recorded in the EDC system with source documents. All corrections or changes made to any trial data must be appropriately tracked in the EDC system. Electronic CRFs (eCRFs) will be considered complete when all missing, incorrect, and/or inconsistent data has been accounted for.

13.1.2 Computer Systems

Data will be processed using a validated computer system conforming to regulatory requirements.

13.1.3 Data Entry

Data must be recorded using the EDC system during the conduct of the trial. All trial personnel at the site must log into the system using their secure user name and password in order to enter, review, or correct trial data. These procedures must comply with the Title 21 Code of Federal Regulations (21 CFR Part 11) and other appropriate international regulations. All passwords will be strictly confidential.

13.1.4 Medical Information Coding

For medical information, the following glossaries will be used:

- Coding of AEs: Latest version of the Medical Dictionary for Regulatory Activities (MedDRA)
- Coding of concomitant medications: World Health Organization Drug Dictionary

13.1.5 Data Validation

Validation checks programmed within the EDC system, as well as supplemental validation performed via review of the downloaded data, will be applied to the data in order to ensure accurate, consistent, and reliable data. Data identified as erroneous, or data that are missing, will be referred to the trial site for resolution through data queries.

eCRFs must be reviewed and electronically signed by an investigator who signed the protocol.

13.2 Record Keeping

The investigator must keep the records of subjects, source documents, monitoring visit logs, eCRFs, inventory of investigational product, regulatory documents and other sponsors correspondence pertaining to the trial in the appropriate trial files at the trial site. Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the evaluation and reconstruction of the clinical trial. Source data are the information contained in source documents (original records or certified copies of original records). These records will be retained in a secure file for the period as set forth in the Clinical Trial Agreement. Prior to transfer or destruction of these records, the sponsor must be notified in writing and be given the opportunity to further store such records.

Records to be retained by the trial site are those listed in GCP as the essential documents. These records must be retained by the head of the trial site or a record keeping manager appointed by the head of the trial site until the completion of retention period is notified by the sponsor. These documents are to be reviewed through source document verification and must be readily available to the requests made by the sponsor and regulatory authorities.

The head of the trial site will keep the essential documents to be retained by the trial site in an appropriate manner according to the regulations prepared by the site. The retention period will last up to the date described in 1 or 2 below, whichever occurs later:

1. The date of obtainment of the marketing approval (excluding approval with conditions and that for a limited period set forth in Article 23-26, Paragraph 1 of the Pharmaceuticals and Medical Devices Law, as well as in Article 45, Article 53, and Article 61, Paragraph 2) for regenerative medicines related to the investigational product (or until the day three years after the notification of the decision on the discontinuation if the development is discontinued), or
2. The day three years after discontinuation or completion of the trial

In case that retention of essential documents to be retained by the head of the trial site is no longer necessary, the sponsor will notify the head of the trial site of the fact.

14 Quality Control and Quality Assurance

The sponsor will perform quality control and quality assurance checks of all clinical trials that he/she requested for. Prior to the conduct of the trial, the representative of the sponsor (or designee) will review with the investigator and the trial personnel at the site the following documents: protocol, Investigator's Brochure, eCRFs and the procedures for their completion, the informed consent process, and the procedure for reporting SAEs. The representative of the sponsor (or designee) will visit the trial sites regularly to verify the information recorded on the eCRFs against source documents and request for clarification or correction as necessary. After the data is entered into the eCRF by the investigator (or designee), the sponsor (or designee) will review the safety information, completeness, accuracy, and logical consistency. Computer programs that identify data inconsistencies can be used to help monitor the clinical trial. If necessary, requests for clarification or correction will be sent to trial doctors.

By signing the protocol, the sponsor (or designee) assures that he/she is responsible for establishing and maintaining quality control and quality assurance systems with written standard operating procedures to ensure that trials are conducted and data are generated, documented, and reported in compliance with the protocol and GCP.

In order to assure that the conduct of the trial, data generation, records, and reporting are performed in compliance with the protocol and GCP, the auditor of the sponsor (or designee) will perform the audit internally and, if necessary, at the trial sites to assure that quality control is appropriately performed.

15 Ethical Consideration

15.1 Ethical Conduct of the Trial

This trial must be conducted in compliance with the ICH guidelines, Ministerial Ordinance for the Good Clinical Practice (GCP) for Regenerative Medicines, Article 23-25, Paragraph 3, and Article 80-2 of the Pharmaceuticals and Medical Devices Law, and ethical principles that have their origin in the Declaration of Helsinki. Compliance with this standard provides public assurance that the rights, safety, and well-being of trial subjects are protected, and that the clinical trial data are credible.

15.2 Institutional Review Board (IRB)

Prior to the conduct of the trial, the protocol, written information and informed consent form, and all other explanatory documents that are provided to subjects must be approved or positively agreed by the institutional review board (IRB) of each trial site.

15.3 Obtainment of Consent

Prior to the start of screening, the trial doctor must give an applicable subject or his/her legal representative an explanation of the objectives of the trial, any potential risks, and other important matters clearly and sufficiently after providing the written information and informed consent form approved by the IRB, and must obtain written consent voluntarily given by the subject or his/her legal representative.

The trial doctor is responsible for the following activities:

- A subject should be provided with a copy of the informed consent form and written trial-related information prepared in the language that he/she is most proficient in prior to participation in the trial. Terminology to be used in those documents must not be technical terms but words that are easily understood.
- The subject or his/her legal representative should be provided with sufficient time for asking questions on the details of the trial.
- The written information and informed consent form signed and dated by the subject or his/her legal representative and the personnel who gives an explanation for obtainment of consent must be obtained.
- The written information and informed consent form, as well as the information to be provided to subjects, must be approved or positively agreed in writing by the IRB prior to the start of trial or following the revision of those documents due to obtainment of new information.
- If the first consent was obtained from a subject's legal representative and then the subject became able to indicate his/her consent during the trial, consent from the subject must be also obtained.
- If any important information that may have an impact on the subject's consent is newly obtained, the written information and informed consent form should be revised as needed and approved by the IRB.
- If any important information that may have an impact on the subject's consent is newly obtained, the investigator or his/her designee will give the subject or his/her legal representative a sufficient explanation on new information that may have an

impact on the subject's willingness, and obtain reconsent to accept all trial-related aspects and continue his/her participation in the trial.

- If the event concerned is orally delivered to a subject before the revised written information and informed consent form is provided to him/her, the contents of explanation given to the subject should be documented and the subject must be later given an explanation again with the revised written information and informed consent form. The investigator obtains consent from the subject.

15.4 Protection of Privacy of Subjects

All the trial-related individuals and organizations must exercise extreme caution to protect the privacy of subjects (e.g., prohibiting the use of personal information (name, address, and others) that could identify subjects). Details of the privacy protection must conform to the applicable Japanese laws or regulatory requirements.

Although the trial-related personnel, including the monitor and the auditor, will be able to know the information on the privacy of subjects with direct access to source documents, they must not divulge the privacy to any third party even if they acquired the information.

16 Publication Policy

Unpublished trial-related information and unpublished information provided to the sponsor (or designee) by the trial doctor must be handled as set forth in the Clinical Trial Agreement.

Publication by submitting a paper to a journal or presentation at a scientific meeting will be proceeded under the agreement between Coordinating Investigator and Healios.

This trial will be registered to *ClinicalTrials.gov* and details of the trial will be open.

17 Trial Administrative Matters

17.1 Protocol Amendments

This trial must be conducted according to the contents of the protocol approved by the IRB. All protocol amendments will be provided in written forms to the investigators by Healios or its appointed Contract Research Organization. Protocol amendments will undergo the same review and approval process as the original protocol. A protocol amendment may be implemented after it has been approved by the IRB, unless immediate implementation of the change is necessary for subject safety. When immediate implementation of the change is necessary, the situation must be documented and reported to the head of the trial sites and the sponsor within 5 working days.

17.2 Implementation System of Trial

See Attachment

18 References

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APPENDIX 1: Definitions and Management of Infusion-Related Allergic Reactions

In the event of allergic/hypersensitivity reactions, trial doctors should institute treatment measures according to best medical process.

Any event of infusion-related reaction or allergic response, generally defined as clinically significant deviations in blood pressure, pulse, respiratory rate and oxygen saturation, will be recorded.

In the event of infusion-related allergic reaction and if flushing, sudden rash, or difficulty breathing occur, the infusion will be stopped immediately and affected subjects will be monitored until the infusion-related allergic reaction has resolved.

The following treatment guidelines may be employed at the discretion of the treating physician:

Grade 1 infusion-related reaction (NCI-CTCAE v4.0):

Mild transient reaction; infusion interruption not indicated; intervention not indicated.

Grade 2 infusion-related reaction (NCI-CTCAE v4.0):

Therapy or infusion interruption indicated, but responds promptly to symptomatic treatment (e.g., antihistamines, non-steroidal anti-inflammatory drugs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hours.

1. Decrease infusion rate by 50%, treat with antihistamines, corticosteroids, etc. as medically indicated and monitor for worsening condition in subjects.
2. Stop infusion if infusion-related symptoms continue despite #1.
3. Treat with bronchodilators, oxygen, antihistamines, corticosteroids etc., as needed.
4. Resume infusion at 50% of the initial rate once symptoms have decreased to Grade 1 in severity. Monitor subjects closely for any worsening.
5. If symptoms reoccur, stop the infusion. The use of the investigational product is to be discontinued.

Grade 3 infusion-related reaction (NCI-CTCAE v4.0):

Characterized as prolonged symptoms (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae.

Grade 4 infusion-related reaction (NCI-CTCAE v4.0):

Characterized as life-threatening symptoms; urgent intervention indicated.

Treatment of Grade 3 or Grade 4 infusion-related reactions:

1. Stop the infusion immediately and disconnect infusion tubing from the subject.
2. Treat with epinephrine, bronchodilators, antihistamines, glucocorticoids, intravenous fluids, vasopressor agents, oxygen, etc. as needed.
3. Immediately contact the CRO Medical Expert and report as a SAE. See Section 9.3 for the reporting procedures.
4. The use of the investigational product is to be discontinued.

APPENDIX 2：NIH Stroke Scale（NIHSS）（1994）

Item	Name	Response	
1A	Level of consciousness	0 = Alert 1 = Alert with mild stimulation	2 = Alert with repeatitive or strong stimulation 3 = Unresponsive (except reflexive position of limbs)
1B	Level of consciousness Questions	0 = Answers both correctly 1 = Answers one correctly	2 = Answers neither correctly
1C	Level of consciousness Commands	0 = Performs both tasks correctly 1 = Perform one task correctly	2 = Perform neither task
2	Gaze	0 = Normal 1 = Partial gaze palsy	2 = Total gaze palsy
3	Visual fields	0 = No visual loss 1 = Partial hemianopsia (including quadranopsia)	2 = Complete hemianopsia (including homonymous hemianopsia) 3 = Bilateral hemianopsia (total blindness including cortical blindness)
4	Facial palsy	0 = Normal 1 = Minor paralysis	2 = Partial paralysis 3 = Complete paralysis
5a	Left motor arm	0 = No drift (for 10 seconds) 1 = Drift before 10 seconds 2 =Resist gravity but falls before 10 seconds	3 = No effort against gravity 4 = No movement
5b	Right motor arm	0 = No drift (for 10 seconds) 1 = Drift before 10 seconds 2 =Resist gravity but falls before 10 seconds	3 = No effort against gravity 4 = No movement
6a	Left motor leg	0 = No drift (for 5 seconds) 1 = Drift before 5 seconds 2 =Resist gravity but falls before 5 seconds	3 = No effort against gravity 4 = No movement
6b	Right motor leg	0 = No drift (for 5 seconds) 1 = Drift before 5 seconds 2 =Resist gravity but falls before 5 seconds	3 = No effort against gravity 4 = No movement
7	Ataxia	0 = Absent 1 = One limb	2 = Two limbs
8	Sensory	0 = Normal 1 = Mild to moderate loss	2 = Severe loss
9	Language	0 = Normal 1 = Mild aphasia	2 = Severe aphasia 3 = Mute or global aphasia
10	Dysarthria	0 = Normal 1 = Mild to moderate	2 = Severe
11	Extinction / inattention	0 = Normal 1 = Mild to moderate	2 = Severe

Total＝ ／42

Lyden P, Brott T, Tilleu B, Welch KM, Mascha EJ, Levine S, Haley EC, Grotta J, Mrler J, Improved reliability of the NIH Stroke Scale using video training. NINDS TPA Stroke Study Group., Stroke, 1994; 25:2220-2226, American Heart Association, Inc. and Wolters Kluwer Health.

APPENDIX 3：modified Rankin Scale（mRS）in Japanese Version

modified Rankin Scale		Notes
0	No symptoms at all	No subjective symptoms and objective signs
1	No significant disability despite symptoms; able to carry out all usual duties and activities	Despite subjective symptoms or objective signs, there has been no change in the person's ability to work and activities of daily living compared to before the stroke
2	Slight disability; unable to carry out all previous activities but able to look after own affairs without assistance	Despite some limitations in the person's ability to carry out his/her usual duties and activities compared to before the stroke, he/she can lead an independent life
3	Moderate disability; requiring some help, but able to walk without assistance	Assistancea is essential for using public transport to get around, but is not essential for walkingb, eating, maintaining routine daily hygiene, using the toilet, etc.
4	Moderately severe disability; unable to walk without assistance, and unable to attend to own bodily needs without assistance	Assistancea is essential for walkingb, eating, maintaining routine daily hygiene, using the toilet, etc., but constant care is not required
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention	Assistance is necessary at all times
6	Death	

a Assistance includes physical assistance, verbal instruction, or supervision by another person.
b Ability to walk on a fl at surface is mainly checked. In addition, assistance does not include the use of any aid (e.g. stick/cane, or walking frame/walker).

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Appendix 4：Barthel Index

Item	Definition		Score
1	Feeding		
	＊Independent. The patient can feed himself a meal. He must put on an assistive device. He must accomplish this in a reasonable time.	10	
	＊Some help is necessary (with cutting up food, etc.).	5	
	＊Whole help is neceaary.	0	
2	Moving from wheelchair to bed		
	＊Independent in all phases of this activity. Patient can lock brakes and lift footrests.	15	
	＊Either some minimal help is needed or the patient needs to be supervised.	10	
	＊Patient can come to a sitting position without the help of a second person but needs a great deal of help.	5	
	＊ Whole help is neceaary or being impossible to do them.	0	
3	Doing personal toilet		
	＊Patient can wash hands and face, comb hair, clean teeth, and shave.	5	
	＊Partial help is neceaary or being impossible to do them.	0	
4	Getting on and off toilet		
	＊Patient is able to get on and off toilet, fasten and unfasten clothes. If it is necessary to use a bed pan instead of a toilet, he must be able to place it on a chair, empty it, and clean it.	10	
	＊Partial help is neceaary in any of above.	5	
	＊Whole help is neceaary or being impossible to do them.	0	
5	Bathing self		
	＊Independent in all phases of this activity.	5	
	＊ Partial help is neceaary or being impossible to do them	0	
6	Walking		
	＊Patient can walk at least 45 meters without help or supervision. He may wear braces or prostheses and use crutches, or canes but not a rolling walker or a wheelchair.	15	
	＊Patient needs help or supervision in any of the above but can walk at least 45 meters with a little help.	10	
	＊If a patient cannot ambulate but can propel a wheelchair independently. He must be able to push a chair at least 45 meters.	5	
	＊ Other than them above	0	
7	Ascending and descending stairs		
	＊Independent in all phases of this activity. He may and should use handrails when needed.	10	
	＊Patient needs help with or supervision of any one.	5	
	＊Being impossible to do them	0	
8	Dressing and undressing		
	＊Patient is able to put on and remove and fasten all clothing, and tie shoe laces.	10	
	＊Patient needs help in putting on and removing or fastening any clothing. He must do at least half the work himself. He must accomplish this in a reasonable time.	5	
	＊Other than them above	0	
9	Continence of bowels		
	＊Patient is able to control his bowels and have no accidents. He can use a suppository or take an enema when necessary.	10	

	＊Patient needs help in using a suppository or taking an enema or has occasional accidents. ＊Other than them above	5 0	
10	Controlling bladder ＊Patient is able to control his bladder day and night and does not need help with an external device. ＊Patient has occasional accidents or can not wait for the bed pan or get to the toilet in time or needs help with an external device. ＊ Other than them above	10 5 0	
Total			／100

Remark) This is a representative ADL rating scale. One hundred perfect score does not mean being able to live alone.

Mahoney FI, Barthe DW, Functional Evaluation: The Barthel Index., Maryland State Medical Journal, 1965; 14:61-65

STATISTICAL ANALYSIS PLAN

PROTOCOL NUMBER: B01-03

PLACEBO-CONTROLLED, DOUBLE-BLIND, PHASE 2/3 EFFICACY AND SAFETY TRIAL OF HLCM051 (MULTISTEM®) IN PATIENTS WITH ISCHEMIC STROKE

AUTHOR: YOHEI TERASAKI

VERSION NUMBER AND DATE: V1.0, 25APR2022

STATISTICAL ANALYSIS PLAN SIGNATURE PAGE

Statistical Analysis Plan V1.0 (Dated 25APR2022) for Protocol <<B01-03>>.

	Name	Signature	Date
Author:	Yohei Terasaki	DocuSigned by Yohei Terasaki Yohei Terasaki E5AD5E93D2AA4E7AA9B69A9081A1EBBC	April 26, 2022 I am the author of this document 2022年4月26日 | 2:36:22 午後 JST
Position:	Statistical Team Lead		
Company:	IQVIA Services Japan K.K		

Upon review of this document, the undersigned approves this version of the Statistical Analysis Plan, authorizing that the content is acceptable for the reporting of this study.

	Name	Signature	Date
Approved By:	Toshiyuki Osawa	DocuSigned by Toshiyuki Osawa Toshiyuki Osawa 7AE41F76EBEF4099B0F05ABB509D99F5	2022年4月26日 この文書を承認する April 26, 2022 | 2:42:44 PM JST
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MODIFICATION HISTORY

Unique Identifier for this Version	Date of the Document Version	Author	Significant Changes from Previous Authorized Version
1.0	25APR2022	Yohei Terasaki	Not Applicable – First Version

TABLE OF CONTENTS

1. INTRODUCTION

This document describes the rules and conventions to be used in the presentation and analysis of efficacy and safety data for Protocol B01-03. It describes the data to be summarized and analyzed, including specifics of the statistical analyses to be performed for clinical study report (CSR).
This statistical analysis plan (SAP) is based on protocol version 5.3, dated 18NOV2021.

2. STUDY OBJECTIVES

2.1. PRIMARY OBJECTIVE

The primary objectives are the following:

- To evaluate the efficacy of HLCM051 on functional outcome in subjects with acute ischemic stroke.
- To evaluate the safety of HLCM051 in subjects with acute ischemic stroke.

2.2. SECONDARY OBJECTIVES

The secondary study objective is to examine changes in function and severity of subjects with ischemic stroke treated with either HLCM051 or Placebo.

2.3. EXPLORATORY OBJECTIVES

The exploratory objective is to evaluate changes in biomarkers in subjects with ischemic stroke treated with either HLCM051 or Placebo.

3. STUDY DESIGN

3.1. GENERAL DESCRIPTION

This is a randomized, placebo-controlled, double-blind, multicenter, phase 2/3 trial to evaluate the efficacy and safety of intravenous administration of HLCM051 compared with placebo in subjects with acute ischemic stroke (within 36 hours of onset). Japanese subjects who developed a cortical ischemic stroke and are eligible to participate in the trial will be evaluated. In addition, the safety survey will be conducted 2-years after administration.

Approximately 220 subjects will be randomized in a 1:1 ratio (HLCM051 group [n=110] or placebo group [110 subjects]) to receive a single infusion of HLCM051 or placebo. Randomization will be stratified by baseline National Institutes of Health Stroke Scale (NIHSS) score (≤12 and ≥13), concomitant reperfusion therapy (Yes or No), and age (20 to 64 and ≥65).

All subjects will be enrolled continuously into the trial. A Data and Safety Monitoring Board (DSMB) will be established beforehand to assess the safety data after approximately 30% and 60% of the planned number of subjects complete the 7-day follow-up. DSMB will also assess whether to continue or discontinue the trial if any of the following is encountered:

・Occurrence of serious adverse events (SAEs) that are unexpected and related to investigational product within 7 days after treatment;

・Occurrence of death that is related to investigational product;

・Occurrence of infusion-related allergic reactions whose severity are Grade 3 or 4 according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 within 24 hours after start of treatment; or

・Worsening of neurological symptoms (increase of ≥4 points in the NIHSS score) related to the investigational product within 7 days after treatment.

This study consists of four different phases; screening, baseline (Day 0: before and after treatment), and post-treatment up to Day 365 and 2-year (Day 730) safety survey after administration.

Subjects will be evaluated at screening, baseline (Day 0: before and after treatment), Day 1, Day 2, Day 7, Day 30, Day 90, and Day 365, or Early Termination visit. In addition, an additional safety survey will be conducted in subjects who can be investigated on Day 730.

To prevent subjects from being lost to follow-up, trial sites will contact subjects by telephone at Day 60, Day 150, Day 210, Day 270, and Day 330 to update the information on disease status and health conditions.

At screening, subjects will undergo standard procedures including medical history, physical examination, 12-lead electrocardiogram (ECG), clinical laboratory tests, vital signs, NIHSS assessment, Modified Rankin Scale (mRS) assessment prior to the onset of ischemic stroke, and brain MRI (Magnetic resonance imaging).

At baseline (Day 0: day of treatment), subjects will undergo physical examination, vital signs, pulse oximetry, adverse event and concomitant treatment assessments, allogeneic antibody and exploratory

biomarker testing, and the NIHSS assessment. During the follow-up (post-treatment) period (from Day 1 to Day 365 or Early Termination), adverse events (AEs), laboratory test results, vital signs, pulse oximetry, ECGs, and physical examination results will be followed for the safety assessment, and mRS, NIHSS, and Barthel Index (BI) scores will be assessed for the efficacy assessment. Biomarker testing will be assessed at baseline and each assessment. In addition, the survey of survival and development of malignancy will be conducted as an additional safety survey in subjects who can be investigated on Day 730.

3.2. SCHEDULE OF EVENTS

The schedule of events can be found in Pages 7 and 8 of the protocol.

3.3. CHANGES TO ANALYSIS FROM PROTOCOL

3.3.1. CHANGES TERM OF ENDPOINT FROM PROTOCOL

The following terms of endpoint will be used to clear meaning in the SAP:

The term of endpoint in the protocol version 5.3	The term of endpoint in the SAP version 1.0
The proportion of subjects exhibiting functional outcome throughout the range of mRS scores by shift analysis at Day 90	The distribution of mRS score at Day 90
The proportion of subjects exhibiting functional outcome throughout the range of mRS scores by shift analysis at Day 365	The distribution of mRS score at Day 365

4. PLANNED ANALYSES

The following analyses will be performed for this study:

- Analyses for DSMB Meeting
- CSR Analysis with Unblinding (Included Primary Efficacy Analysis)
- Analysis of additional 2-year safety survey

4.1. ANALYSES FOR DSMB MEETING

A DSMB will be established beforehand to assess the data after approximately 30% and 60% of the planned number of subjects complete the 7-day follow-up. A DSMB SAP, describing the methodology and presentation of results and access to results will be provided by IQVIA as a separate SAP from the CSR analyses described here.

The DSMB will assess the continuity of the trial if any of the following is encountered:
・Occurrence of serious adverse events (SAEs) that are unexpected and related to investigational product within 7 days after treatment;
・Occurrence of death that is related to investigational product;
・Occurrence of infusion-related allergic reactions whose severity are Grade 3 or 4 according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0 within 24 hours after start of treatment; or
・Worsening of neurological symptoms (increase of ≥4 points in the NIHSS score) related to the investigational product within 7 days after treatment.

Derivations and definitions for the DSMB analyses will be based on those required for the CSR analysis contained in this final statistical analysis plan unless deviations are stated within the text.
The IQVIA Biostatistics study team, including those responsible for creating the programs to produce the outputs for the DSMB analyses, will remain blinded. Once the programs have been produced by the IQVIA study team, these programs will be sent to an IQVIA independent statistician, who will use the randomization schedule and provide DSMB Lead (PAREXEL) with a set of unblinded outputs.
DSMB Analyses will be conducted by using assessment data before planned cut-off date.

4.2. CSR ANALYSIS WITH UNBLINDING

This final statistical analysis plan (Final SAP) including contents of CSR analysis, describing the purpose, timing, presentation of results and access to results will be provided by IQVIA Biostatistics as a separate document from DSMB SAP.
All final, planned analyses identified in Final SAP will be performed by IQVIA Biostatistics following Sponsor Authorization of Final SAP, Interim Database Lock, Sponsor Authorization of Analysis Sets and Unblinding of Treatment.
Unblinding is scheduled after the Interim Database Lock of Day 365 data of all patients are fixed.

One CSR analysis will take place for this study once Day 365 assessments of the last subjects who are treated with the investigational product have been completed or early termination in the study, the results of which will be based on the unblinded treatment groups. See section 12.3 of the protocol for more details.

4.3. ANALYSIS OF ADDITIONAL 2-YEAR SAFETY SURVEY

An Additional Statistical Analysis Plan (Additional SAP) for additional 2-year safety survey, describing the purpose, timing, presentation of results and access to results will be provided by IQVIA Biostatistics as a separate document from DSMB SAP and Final SAP for CSR analysis.
All planned analyses identified in Additional SAP for additional 2-year safety survey will be performed by IQVIA Biostatistics following Sponsor Authorization of this Additional SAP.
The data of additional 2-year safety survey will be analyzed at the time when the data can be evaluated following this Additional SAP.

5. ANALYSIS SETS FOR CSR ANALYSIS

Agreement and authorization of subjects included/ excluded from each below analysis set will be conducted prior to the Database Lock for CSR analysis with unblinding. When any subjects included/ excluded from each analysis set are changed between first agreement and the Database Lock for CSR analysis, the agreement and authorization will be conducted again prior to the Database Lock for CSR analysis.

5.1. ALL SUBJECTS ENROLLED SET [ENR]

The all subjects enrolled set (ENR) will contain all subjects who provide informed consent for this study.

5.2. ALL SUBJECTS RANDOMIZED SET [RND]

The all subjects randomized set (RND) will contain all subjects in the ENR set who were randomized to investigational product.
For analyses and displays based on the RND, subjects will be classified according to randomized treatment.

5.3. FULL ANALYSIS SET [FAS]

The full analysis set (FAS) will contain all subjects who are randomized and treated with the investigational product and have the results of the mRS, NIHSS, and BI scores assessed after Day 7 at least once.

5.4. PER PROTOCOL SET [PPS]

The per protocol set (PPS) will contain all subjects in the FAS who do not have any violations or entry criteria or protocol deviations that could significantly impact the assessment or interpretation of efficacy data.

5.5. SAFETY ANALYSIS SET [SAF]

The safety analysis set (SAF) will contain all subjects who are randomized and treated with the investigational product.

6. GENERAL CONSIDERATIONS

6.1. REFERENCE START DATE AND STUDY DAY

Study Day will be calculated from the reference start date and will be used to show start/ stop day of assessments and events.
Reference start date is defined as the day of investigational product (Day 1 is the day of the investigational product infusion) and will appear in every listing where an assessment date or event date appears.

- If the date of the assessment or event is on or after the reference date, then:

Study Day = (date of assessment or event – reference date) + 1.

- If the date of the assessment or event is prior to the reference date, then:

Study Day = (date of assessment or event – reference date).

In the situation where the assessment or event date is partial or missing, Study Day, and any corresponding durations will appear partial or missing in the listings.

6.2. BASELINE FOR ANALYSIS

Unless otherwise specified, baseline is defined as the last non-missing measurement taken prior to reference start date (including unscheduled assessments). In the case where the last non-missing measurement and the reference start date coincide, that measurement will be considered pre-baseline, but Adverse Events (AEs) and medications commencing on the reference start date will be considered post-baseline.

6.3. DERIVED TIMEPOINTS

Derived timepoints will be considered EOS/ET (End of Study/Early Termination) in each analysis for this study.

6.4. RETESTS, UNSCHEDULED VISITS AND EARLY TERMINATION DATA

In general, for by-visit summaries, data recorded at the nominal visit will be presented considering windowing conventions for analysis in section 6.5. Unscheduled measurements will be included in by-visit summaries and will contribute to the EOS/ET, LOCF (last observation carried forward) value, or best/ worst case value where required.
Early termination data will be mapped to the EOS/ET analysis visit number for by-visit summaries.
Listings will include scheduled, unscheduled, retest and early termination data.

6.5. WINDOWING CONVENTIONS FOR ANALYSIS

For analysis visit, subject data will be associated with an analysis window based upon the actual date the assessment took place as outlined the following table. Where multiple measurements for a same parameter appear within an analysis window, the rules outlined in the table below will be used to determine which observations to use for the summary measure. Though all measures may not be used in the data summaries (e.g., two lab measures within the same analysis visit window), all measurements will appear in the datasets and listings. For efficacy assessments where the event date is missing, the study day and analysis window

will also be missing. Windowing will be applied to the data prior to any missing data calculations.

Analysis Visit	Study Day[1)]	Analysis Timepoint	Analysis Visit Window[1)]	
			Between Start and End Day	Between Start and End Time
Pre-dose at Baseline	≤ 1		-	-
Post-dose at Baseline	-	15 minutes	-	-
	-	30 minutes 45 minutes	-	-
	-	60 minutes	-	-
	-	75 minutes	-	-
	-	90 minutes	-	-
	-	105 minutes	-	-
	-	2 hours	-	-
		4 hours	-	-
		6 hours	-	-
Day 1 (24 hours after investigational product infusion start)		24 hours	-	Between 18 and 30 hours after investigational product infusion start
Day 2 (48 hours after investigational product infusion start)		48 hours	-	Between 42 and 54 hours after investigational product infusion start
Day 7[2)]	8	-	(1) If hospital discharge occurs before Day 7, assigned CRF visit of Day 7 as analysis visit Day 7 (not setting analysis visit window) (2) Otherwise, between 6 and 11	-
Day 30[3)]	31	-	(1) If hospital discharge occurs between Day 14 and Day 26, between 15 and 38 (2) Otherwise, between 28 and 38	-
Day 90	91	-	Between 84 and 112	-
Day 365	366	-	Between 359 and 394	-
EOS/ET[4)]	Day of last assessment visit after investigational product infusion start	-	-	-

1) The day when the study treatment is started will be Day 1.
2) If hospital discharge occurs before Day 7, Day 7 assessment can be performed in the hospital at discharge.
3) It allows at discharge between Day14-26 unless patient can visit Day30.
4) If a subject died before assessments of early termination, the analysis visit "EOS/ET" is defined as last observed assessment visit.

6.6. HANDLING OF DATA AFTER IMPORTANT PROTOCOL DEVIATIONS

For the individual assessed efficacy data after important protocol deviation such as receiving contraindicated drugs and therapies, the data will not be included in efficacy analysis for the PPS. Also, the important protocol deviation will be identified in case review meeting before unblinding.

6.7. STATISTICAL TESTS AND ESTIMATIONS

For analysis of individual endpoint, two-tailed hypothesis test will be conducted with significance level of 5% and estimated confidence interval of 95%, unless specified in the SAP.

6.8. COMMON CALCULATIONS

For quantitative measurements, change from baseline will be calculated as:

- Test Value at Visit X – Baseline Value

6.9. SOFTWARE VERSION

All analyses will be performed using SAS version 9.4.

7. STATISTICAL CONSIDERATIONS

7.1. ADJUSTMENTS FOR COVARIATES AND FACTORS TO BE INCLUDED IN ANALYSES

The primary analysis will be performed adjusting the following stratification factors: Baseline NIHSS score (≤12 or ≥13), concomitant reperfusion therapy (Yes or No), age (20 to 74 or ≥75).
We will not perform adjusted analysis with covariates and factors other than stratification factors (baseline NIHSS, concomitant reperfusion therapy and age) because these covariates and factors don't have any evidence to affect the prognosis of ischemic stroke.

7.2. MULTICENTER STUDIES

This study will be conducted by multiple investigators at multiple centers.
Center pooling will not be carried out for use in the analyses for this study.

7.3. MISSING DATA

Missing safety data will not be imputed for the analyses in this study.
Missing efficacy data will be imputed for the analyses in this study. Refer to each section of 16. Efficacy Outcomes in this SAP.

7.4. MULTIPLE COMPARISONS/ MULTIPLICITY

Multiple comparisons will not be performed in the analyses for this study.

7.5. ACTIVE-CONTROL STUDIES INTENDED TO SHOW NON-INFERIORITY OR EQUIVALENCE

The one of the primary study objectives is to confirm superiority of HLCM051 to placebo groups. So non-

inferiority or equivalence will not be considered in this study.

7.6. EXAMINATION OF SUBGROUPS

"Excellent Outcome at Day 90 and Day 365, "the distribution of mRS scores at Day 90 and Day 365" and "mRS score of ≤1 at Day 90" and "mRS score of ≤2 at Day 90" will be summarized over time for the following subgroups of the FAS:

- Baseline NIHSS score (≤12 or ≥13)
- Age group 1 (20 to 64 or ≥65)
- Age group 2 (20 to 74 or ≥75)
- Received only tPA (Yes, No)
- Received only mechanical reperfusion (Yes, No)
- Concomitant reperfusion therapy (Yes, No)
- Concomitant reperfusion therapy type (Received Only tPA, Received Only mechanical reperfusion or Neither)
- Gender (Male, Female)
- Infarct volume group 1 (<25 or ≥25)
- Infarct volume group 2 (<50 or ≥50)
- Infarct volume group 3 (<70 or ≥70)
- ASPECTS group 1 (<6 or ≥6)
- ASPECTS group 2 (<7 or ≥7)

8. OUTPUT PRESENTATIONS

Appendix 1 shows conventions for presentation of data in outputs.
The templates provided with this SAP describe the presentations for this study and therefore the format and content of the summary tables and listings to be provided by IQVIA Biostatistics.

9. DISPOSITION, EARLY TERMINATION AND ANALYSIS SETS

9.1. SUBJECT DISPOSITION

All subjects who provide informed consent will be accounted for in this study.

- Subject disposition and withdrawals will be summarized with counts and percentages by treatment group and overall, for all subjects (Total) in the ENR. Disposition and categories will include:
- Informed Consent
- Screening Failure (Pre-Randomization Discontinuation) along with the reasons below
    - Did not meet inclusion /exclusion criteria
    - Subject withdrew consent
    - Termination of the trial
    - Pregnancy
    - Disease of interest not present
    - Other
- Randomized
- Not Treated
- Treated
- Early termination until Day 365 and along with the reasons below
    - Subject withdrew consent
    - Sponsor discontinued the trial
    - Subject lost to follow-up
    - Death
    - Adverse event
    - Investigator decision
    - Other
- Completed Day 365
- Proceeded Day 730

An analysis listing of all randomized subjects who withdraw early will be provided by treatment group that will specify reason for withdrawal.

9.2. ANALYSIS SETS

- Inclusion or exclusion of each analysis set will be summarized with counts and percentages by treatment group and overall, for all subjects (Total) in the RND. The categories of analysis sets will include:
- Exclusion of SAF
- Inclusion of SAF
- Exclusion of FAS
- Inclusion of FAS
- Exclusion of PPS
- Inclusion of PPS

An analysis listing of all randomized subjects who inclusion or exclusion of each analysis set will be provided by treatment group that will specify reason for exclusion.

9.3. PROTOCOL DEVIATION

All protocol deviations are recorded in the CTMS. Important protocol deviations will be summarized with counts and percentages each type of deviation by treatment group and overall, for all subjects (Total) in the RND.
An analysis listing of all randomized subjects who has protocol deviations will be provided by treatment group.

10. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Demographics and baseline characteristics will be summarized by treatment group for the FAS, PPS and SAF.
Continuous variables, including those assessed on a discrete scale, will be summarized using descriptive statistics including number of subjects, mean, median, standard deviation, maximum, and minimum. For categorical variables, descriptive statistical summaries will include number of subjects and percentages. Also, stratification factors will be tabulated to confirm discrepancy between IWRS and eCRF source by treatment group and total for the FAS.

The following demographic and other baseline characteristics will be reported for this study.

- Age (years) - calculated relative to date of informed consent

- Age category 1 (≤ 64 years or ≥ 65 years) - calculated relative to date of informed consent
- Age category 2 (≤ 74 years or ≥ 75 years) - calculated relative to date of informed consent
- Gender
- Ethnicity
- Race
- Time from symptom onset until investigational product infusion (hours)
- Time from hospitalization until investigational product infusion (hours)
- Primary location
- Hemisphere
- Axis of lesion (cm)
- Subcortical structures involved
- Receive only tPA
- Primary reason for tPA
- Receive only mechanical reperfusion
- Reperfusion achieved
- Concomitant reperfusion therapy
- Concomitant reperfusion therapy type (Receive only tPA, Receive only mechanical reperfusion or Neither)
- Other procedures completed after presentation to the hospital prior to IP infusion
- Baseline NIHSS score
- Baseline NIHSS score category 1 (≤12 or ≥13)
- Baseline NIHSS score category 2 (8-10, 11-12, 13-14, 15-16, 17-18, or 19-20)
- Infarct volume
- Infarct volume group 1 (<25 or ≥25)
- Infarct volume group 2 (<50 or ≥50)
- Infarct volume group 3 (<70 or ≥70)
- ASPECTS
- ASPECTS group 1 (<6 or ≥6)
- ASPECTS group 2 (<7 or ≥7)
- pc-ASPECTS

Demographic and other baseline characteristics will be listed for the RND.

10.1. DERIVATIONS

- Time from symptom onset until investigational product infusion (hours)
    - = (datetime of investigational product infusion – datetime of symptom onset).
- Time from hospitalization until investigational product infusion (hours)
    - = (datetime of investigational product infusion – datetime of hospitalization).

11. RISK FACTORS

Risk factors will be summarized with number of subjects and percentages by treatment group for the FAS. Continuous variables, including those assessed on a discrete scale, will be summarized using descriptive statistics including number of subjects, mean, median, standard deviation, maximum, and minimum. For categorical variables, descriptive statistical summaries will include number of subjects and percentages. No statistical testing will be carried out for risk factors.

The following risk factors will be reported for this study.

- Received previous biological therapy
- Any clinically significant Medical History
- Any history of autoimmune diseases
- Hypertension
- Prior TIA
- Prior CVA
- Diagnosed Vascular Heart Disease
- Diabetes
- Hypercholesterolemia
- Hypercoagulable States
- Smoking status
- Duration of smoking
- Average Cigarettes Per Day
- Alcohol Use
- Illicit drug status

Risk factors will be listed for the RND.

12. MEDICAL HISTORY AND COMPLICATION

Medical History and complication will be listed for the RND.

- Medical History and complication will be coded using Medical Dictionary for Regulatory Activities (MedDRA).
- Medical History are defined as those conditions which stop prior to or at Screening.
    - Complications are defined as conditions which started prior to or at Screening and are ongoing at the date of Screening.
- Data captured on the Medical History page of the CRF will be presented by SOC (System Organ Class) and PT (Preferred Term).

13. PRIOR AND CONCOMITANT MEDICATIONS

Medications will be listed for the RND and coded using the World Health Organization Drug Dictionary. Medications will be summarized with number of subjects and percentages by treatment group for the FAS and SAF.

- 'Prior medications' are medications which started and stopped prior to the investigational product infusion.
- 'Concomitant medications' are medications which:
    - started prior to, on or after investigational product,
    - and ended on or after the date of investigational product infusion or were ongoing at the end of the study.
- If stopped date of medications is partial date, then impute stop date as latest possible date (i.e. last day of month if day unknown). And then following procedure:
    - if imputed stop date of medications < start date of investigational product infusion, assign as prior
    - if imputed stop date of medications >= start date of investigational product infusion, assign as

concomitant

- Data captured on the Prior and Concomitant Medications Before 90 days and After 90 Days page of the CRF will be presented by ATC (Anatomic Therapeutic Class) level 4 and Preferred Name.

14. REHABILITATION ACTIVITIES AND THERAPIES

Rehabilitation activities and therapies except rehabilitation recorded on the "Rehabilitation Activities", "Therapies Except Rehabilitation Before 90 Days" and "Therapies Except Rehabilitation After 90 Days" page of the eCRF will be listed for the RND.

15. INVESTIGATIONAL PRODUCT INFUSION AND COMPLIANCE

Investigational product infusion recorded on the "IP Infusion" page of the eCRF will be listed for the RND. Compliance for investigational product will not be presented in the study.

16. EFFICACY OUTCOMES

16.1. PRIMARY EFFICACY

16.1.1. PRIMARY EFFICACY VARIABLE & DERIVATION

The primary efficacy variable is the proportion of subjects with an excellent outcome at Day 90 defined by the functional assessments. Excellent outcome is defined as mRS score of ≤1 (scale, 0 to 6), NIHSS score of ≤1 (scale, 0 to 42), and BI score of ≥95 (scale, 0 to 100).

16.1.2. MISSING DATA METHODS FOR PRIMARY EFFICACY VARIABLE

The primary time point for efficacy assessments is Day 90. Subjects missing the Day 90 evaluation will have their last post-randomization primary efficacy assessment (Day 7 or later) carried forward (LOCF) to impute the missing Day 90 assessment. For this LOCF, primary efficacy assessment will not be imputed using per

value for components of excellent outcome.
A subject who died during the study will be scored as follows for all subsequent analysis time points after the subject dead: 6 for mRS, and failures for binary NIHSS (NIHSS score of >1) and BI score (BI score of <95).

16.1.3. PRIMARY ANALYSIS OF PRIMARY EFFICACY VARIABLE

For the FAS, the proportion of subjects with an excellent outcome at Day 90 defined by the functional assessments, as a primary efficacy endpoint, will be compared between the HLCM051 and the placebo groups and superiority of HLCM051 to placebo groups will be assessed by Cochran–Mantel–Haenszel (CMH) test (the two-tailed significance level of 0.05), with adjustment factor of baseline NIHSS score (≤12 or ≥13), concomitant reperfusion therapy (Yes or No), and age (20 to 74 or ≥75).
The risk difference and the corresponding two-sided 95% CI of between HLCM051 and placebo groups adjusted with the same adjustment factor will be calculated using the Mantel–Haenszel (MH) method with Sato T (1989).
Also, the excellent outcomes will be summarized with number of subjects and percentages at Day 7, Day 30, Day 90, Day 365 and EOS/ET and each treatment groups.
Excellent outcomes will be listed for the RND.

16.1.4. SUBGROUP ANALYSIS OF PRIMARY EFFICACY VARIABLE

For primary efficacy endpoint, same primary analysis will be performed by subgroups. Refer more details in section 7.6 and 16.1.3.

16.1.5. SENSITIVITY ANALYSIS OF PRIMARY EFFICACY VARIABLE

16.1.5.1. Sensitivity to Analysis Sets

As a sensitivity analysis, the primary efficacy analysis will be conducted in the PPS same as the FAS. Also refer to section 16.1.3 in this SAP.

16.1.5.2. Sensitivity to Statistical Methods for Missing Data

For primary efficacy variable without imputation (Observed Cases), the primary efficacy analysis, i.e., the complete case analysis, will also be conducted in the FAS as a sensitivity analysis. Also refer to section

16.1.3 in this SAP.

Additionally, generalized linear mixed model will be analyzed in the FAS where missing data are assumed to be missing at random (MAR). The model will include treatment group (HLCM051, Placebo), baseline NIHSS score (≤12 or ≥13), concomitant reperfusion therapy (Yes or No), age (20 to 74 or ≥75), time point (planned visit as a categorical variable) and interaction between time point and treatment group as fixed factors, where this model will assume response variable including primary endpoint, the "excellent outcome" status at baseline, Day 7, Day 30, Day 90, and Day 365, to follow correlated Bernoulli distribution and the link function to be logit. The Kenward–Roger method will be used to determine the degrees of freedom. The G side covariance structure for the random effects will be unstructured.

Also, multiple imputation (MI) will be performed, where post-baseline missing primary efficacy variable at each visit will be imputed based on appropriate logistic regression models including treatment, baseline NIHSS score, reperfusion category, age, gender. Twenty imputed complete datasets will be generated from MI procedure. These imputed datasets are analyzed by using the same method for the primary efficacy analysis each imputed dataset. The results from each imputed complete dataset will be combined using Rubin's rule (1987).

16.1.5.3. Sensitivity to Discrepancy of Stratification Factors at Randomization

The distribution of primary efficacy variable will be compared between the two treatment groups by using the CMH test adjusted for the three stratification factors (baseline NIHSS score, concomitant reperfusion therapy and Age group from eCRF source), if there exist more than 10% discrepancy at least one factor between stratification factors (IWRS source) and stratification factors (eCRF source) in Total.

16.1.5.4. Sensitivity to Adjustments for Covariates and Factors

As a sensitivity analysis, the distribution of primary efficacy variable will be compared between the two treatment groups by using the CMH test adjusted by the three factors (Age (20 to 64 or ≥65) from eCRF source, baseline NIHSS score (≤12 or ≥13) and concomitant reperfusion therapy (Yes or No) from IWRS source).

Additionally, for primary efficacy variable with imputation (LOCF), logistic regression model will be analyzed in the FAS. The model will include treatment group (HLCM051, Placebo) and stratification factors at randomization (baseline NIHSS score (≤12 or ≥13), concomitant reperfusion therapy (Yes or No)) as fixed factors and age as covariate.

16.2. SECONDARY EFFICACY

The secondary efficacy analyses will be performed for the FAS and PPS.

16.2.1. SECONDARY EFFICACY VARIABLES & DERIVATIONS

16.2.1.1. The Proportion of Subjects With an Excellent Outcome Defined by the Functional Assessments at Day 365

The excellent outcome is derived same as primary efficacy variable. Refer to section 16.1.1 in this SAP.

16.2.1.2. The Distribution of mRS Scores at Day 90

The distribution of mRS scores at Day 90 consists of proportion of subjects with a mRS score at each fixed level.

16.2.1.3. The Distribution of mRS Scores at Day 365

The distribution of mRS scores at Day 365 consists of proportion of subjects with a mRS score at each fixed level.

16.2.1.4. The Proportion of Subjects With a Global Recovery at Day 90

The global recovery is defined as integrated assessment using three component efficacy endpoints that satisfy all of the following: mRS score of ≤2, NIHSS score improvement of ≥75% from baseline, and BI score of ≥95.

16.2.1.5. The Proportion of Subjects With an mRS Score of ≤1 at Day 90

The endpoint is defined as the percentage of subjects with an mRS score of "0" or "1" at Day 90 on the "Modified Rankin Scale" page of the eCRF.

16.2.1.6. The Proportion of Subjects With an mRS Score of ≤2 at Day 90

The endpoint is defined as the percentage of subjects with an mRS score of "0", "1" or "2" at Day 90 on the "Modified Rankin Scale" page of the eCRF.

16.2.1.7. The Proportion of Subjects With an NIHSS Score of ≤1 at Day 90

The endpoint is defined as the percentage of subjects with an NIHSS score "0" or "1" at Day 90 on the "NIH Stroke Scale" page of the eCRF.

16.2.1.8. The Proportion of Subjects With a Favorable Outcome in Neurological Symptoms at Day 90

The endpoint is defined as the percentage of subjects with a favorable outcome at Day 90. The favorable outcome is defined as NIHSS score improvement of ≥75% from baseline.

16.2.1.9. The Proportion of Subjects With a BI Score of ≥95 at Day 90

The endpoint is defined as the percentage of subjects with a BI score "95 or more" at Day 90 on the "Barthel Index" page of the eCRF.

16.2.1.10. The Proportion of Subjects Who Survived Without Life-Threatening Adverse Events (AEs) at Day 90

The endpoint is defined as the percentage of subjects who survive at Day 90 and do not experience life-threatening adverse events (AEs). The life-threatening AEs are those events recorded as "Death" or "Life threatening event" in the question "Is the adverse event serious?" on the "Adverse Events Before 90 Days" page of the eCRF.

16.2.1.11. The Proportion of Subjects Who Survived Without Secondary Infections at Day 90

The endpoint is defined as the percentage of subjects who survive at Day 90 and do not experience secondary infections. The secondary infections are defined as coded SOC "Infections and infestations" recorded on the "Adverse Event Before 90 Days" page of the eCRF.

16.2.1.12. The Proportion of Subjects With a Global Recovery and Survival Without Life-Threatening AEs and Secondary Infections at Day 90.

The endpoint is defined as the percentage of subjects with a global recovery and survival without life-threatening AEs and secondary infections. For definition of the global recovery, refer to section 16.2.1.4. For

definition of the life-threatening AEs, refer to section 16.2.1.10. For definition of the secondary infections, refer to section 16.2.1.11.

16.2.2. MISSING DATA METHODS FOR SECONDARY EFFICACY VARIABLE(S)

For "Excellent Outcome at Day 365", "the distribution of mRS scores at Day 90 and Day 365", missing data of these secondary efficacy data will be imputed with similar manner at primary efficacy end point. Refer to section 16.1.2. in this SAP. Other secondary efficacy data will not be imputed in secondary efficacy analyses for this study.
Also, a subject who died during the study will be scored as follows for all subsequent analysis time points after the subject dead: 6 for mRS, and failures for binary NIHSS (NIHSS score of >1) and BI score (BI score of <95).

16.2.3. ANALYSIS OF SECONDARY EFFICACY VARIABLES

16.2.3.1. Analysis of the Proportion of Subjects With an Excellent Outcome Defined by the Functional Assessments at Day 365

Excellent outcome at Day 365 will be summarized and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP.

16.2.3.2. Analysis of the Distribution of mRS Scores at Day 90

To compare the distributions of the mRS scores at Day 90 between the HLCM051 group and placebo group. The proportion of subjects in each category will be summarized with counts and percentages at Day 7, Day 30, Day 90, Day 365 and EOS/ET, and compared between the HLCM051 group and placebo group. Specifically, Cochran-Mantel-Haenszel test using modified ridit scores, an extension of the two-sample Wilcoxon rank sum test, stratified by baseline NIHSS score (≤12 or ≥13), concomitant reperfusion therapy (Yes or No), and age (20 to 74 or ≥75) will be used for this comparison. Also, see Howard et al. (2012) in section 20 to refer more detail of this analysis.
Also, mRS Score will be listed for the RND.

16.2.3.3. Analysis of the Distribution of mRS Scores at Day 365

The distribution of mRS score at Day 365 will be summarized and analyzed in a similar manner at the same

time as the variable at Day 90. Refer to section 16.2.3.2. in this SAP.

16.2.3.4. Analysis of the Proportion of Subjects With a Global Recovery at Day 90

The global recovery at Day 90 will be summarized and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP.

16.2.3.5. Analysis of the Proportion of Subjects With a mRS Score of ≤1 at Day 90

This proportion will be summarized by planned visit and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP.

16.2.3.6. Analysis of the Proportion of Subjects With a mRS Score of ≤2 at Day 90

This proportion will be summarized by planned visit and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP.

16.2.3.7. Analysis of the Proportion of Subjects With a NIHSS Score of ≤1 at Day 90

This proportion will be summarized by planned visit and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP. Also, NIHSS Score will be listed for the RND.

16.2.3.8. Analysis of the Proportion of Subjects With a Favorable Outcome in Neurological Symptoms at Day 90

This proportion will be summarized by planned visit and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP.

16.2.3.9. Analysis of the Proportion of Subjects With a BI Score of ≥95 at Day 90

This proportion will be summarized by planned visit and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP. Also, BI Score will be listed for the RND.

16.2.3.10. Analysis of the Proportion of Subjects Who Survived Without Life-Threatening Adverse Events (AEs) at Day 90

This proportion will be summarized and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP.

16.2.3.11. Analysis of the Proportion of Subjects Who Survived Without Secondary Infections at Day 90

This proportion will be summarized and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP.

16.2.3.12. Analysis of the Proportion of Subjects With a Global Recovery and Survival Without Life-Threatening AEs and Secondary Infections at Day 90

This proportion will be summarized and analyzed in a similar manner at primary efficacy end point. Refer to section 16.1.3. in this SAP.

16.2.4. SUBGROUP ANALYSIS OF SECONDARY EFFICACY VARIABLE

16.2.4.1. Subgroup Analysis of the Proportion of Subjects With an Excellent Outcome Defined by the Functional Assessments at Day 365

For “Excellent Outcome at Day 365” of secondary efficacy endpoint, same primary analysis will be performed by subgroups. Refer more details in section 7.6 and 16.1.3.

16.2.4.2. Subgroup Analysis of the Distribution of mRS Scores at Day 90

For “the distribution of mRS scores at Day 90” of secondary efficacy endpoint, same the analysis in section 16.2.3.2 will be performed by subgroups. Refer more detail of subgroups in section 7.6.

16.2.4.3. Subgroup Analysis of the Distribution of mRS Scores at Day 365

For “the distribution of mRS scores at Day 365” of secondary efficacy endpoint, same the analysis in section 16.2.3.3 will be performed by subgroups. Refer more detail of subgroups in section 7.6.

16.2.4.4. Subgroup Analysis of the Proportion of Subjects With a mRS Score of ≤1 at Day 90

For "mRS score of ≤1 at Day 90" of secondary efficacy endpoint, same the analysis in the section 16.2.3.5 will be performed by subgroups. Refer more detail of subgroups in section 7.6.

16.2.4.5. Subgroup Analysis of the Proportion of Subjects With a mRS Score of ≤2 at Day 90

For "mRS score of ≤2 at Day 90" of secondary efficacy endpoint, same the analysis in the section 16.2.3.6 will be performed by subgroups. Refer more detail of subgroups in section 7.6.

16.2.5. SENSITIVITY ANALYSIS OF SECONDARY EFFICACY VARIABLE

16.2.5.1. Sensitivity Analysis of the Proportion of Subjects With an Excellent Outcome Defined by the Functional Assessments at Day 365

For "Excellent Outcome at Day 365" of secondary efficacy endpoint, same sensitivity analysis of primary endpoint will be performed. Refer more detail of sensitivity analysis in section 16.1.5.2.

16.2.5.2. Sensitivity Analysis of the Distribution of mRS Scores at Day 90

For "the distribution of mRS score at Day 90" without imputation (Observed Cases), the Cochran-Mantel-Haenszel test using modified ridit scores will also be conducted in the FAS as a sensitivity analysis. Additionally, generalized linear mixed model will be analyzed in the FAS where missing data are assumed to be missing at random (MAR). The model will include treatment group (HLCM051, Placebo), baseline NIHSS score (≤12 or ≥13), concomitant reperfusion therapy (Yes or No), age (20 to 74 or ≥75), time point (planned visit as a categorical variable) and interaction between time point and treatment group as fixed factors, where this model will assume response variable, the "distribution of mRS" status at baseline, Day 7, Day 30, Day 90, and Day 365, to follow correlated multinomial distribution and the link function to be cumulative logit. The Kenward–Roger method will be used to determine the degrees of freedom. The G side covariance structure for the random effects will be unstructured.
Also, multiple imputation (MI) will be performed, where post-baseline missing this secondary efficacy variable at each visit will be imputed based on appropriate linear multiple regression models including treatment, baseline NIHSS score, reperfusion category, age, gender. Twenty imputed complete datasets will be generated from MI procedure. These imputed complete datasets are analyzed each imputed complete dataset by using the Cochran-Mantel-Haenszel test using modified ridit scores. The results from each imputed complete dataset will be combined using Rubin's rule (1987).

16.2.5.3. Sensitivity Analysis of the Distribution of mRS Scores at Day 365

For “the distribution of mRS score at Day 365” of secondary efficacy endpoint, same sensitivity analysis method of “the distribution of mRS score at Day 90” will be performed. Refer more detail of sensitivity analysis in section 16.2.5.2.

16.3. EXPLORATORY EFFICACY

The exploratory efficacy analyses will be performed for the FAS.

16.3.1. EXPLORATORY EFFICACY VARIABLES & DERIVATIONS

16.3.1.1. Changes in Biomarkers (White Blood Cell Populations and Inflammatory Markers) From Baseline to Day 2, Day 7, and Day 30.

Specific derivations of biomarkers will not be considered for this study.

16.3.2. MISSING DATA METHODS FOR EXPLORATORY EFFICACY VARIABLE(S)

Missing exploratory data will not be imputed in exploratory efficacy analyses for this study.

16.3.3. ANALYSIS OF EXPLORATORY EFFICACY VARIABLES

The values and changes of Blood markers will be analyzed using descriptive statistics including number of subjects, mean, median, standard deviation, maximum, and minimum at each visit.
Blood markers will be listed for the RND.

17. PRIMARY SAFETY OUTCOMES

All outputs for primary safety outcomes will be based on safety data through 7 days after investigational product infusion.
The primary safety outcomes to be compared between the HLCM051 and the placebo groups will be defined as any of the following:

- Grade 3 or 4 infusion-related allergic reactions related to the investigational product. Abnormality of cardiovascular system and abnormality or allergic reactions (e.g., rash, erythema) of/to respiratory function that occurred within 24 hours after treatment;
- SAEs that occurred within 7 days after treatment and are related to the investigational product;
- Worsening of neurological symptoms that are related to the investigational product. Worsening is defined as an increase of ≥4 points in the NIHSS score from baseline assessed through 7 days after treatment;
- Incidence rates of death or life-threatening AEs until Day 90;
- Incidence rate of secondary infections until Day 90.

17.1. DERIVATIONS FOR PRIMARY SAFETY VARIABLES

17.1.1. GRADE 3 OR 4 INFUSION-RELATED ALLERGIC REACTIONS RELATED TO THE INVESTIGATIONAL PRODUCT.

The adverse events are those events recorded as Grade 3 or 4 infusion-related allergic adverse event related to the investigational product on the “Adverse Events Before 90 Days” page of the eCRF and occurring in the first 24 hours post-infusion of investigational product.

17.1.2. WORSENING OF NEUROLOGICAL SYMPTOMS THAT ARE RELATED TO THE INVESTIGATIONAL PRODUCT

The adverse events are those events related to the investigational product, also recorded as “Yes” in the question “Increase of >= 4 points in the NIHSS score within 7 days after treatment with the investigational product; Occurrence of neurological worsening?” on the “Adverse Events Before 90 Days” page of the eCRF.

17.1.3. DEATH OR LIFE-THREATENING AES UNTIL DAY 90

The adverse events are those events recorded as “Death” or “Life threatening event” in the question “Is the adverse event serious?” on the “Adverse Events Before 90 Days” page of the eCRF.

17.1.4. SECONDARY INFECTIONS UNTIL DAY 90

The variable is defined as coded SOC “Infections and infestations” recorded on the “Adverse Event Before 90 Days” page of the eCRF.

17.2. MISSING DATA METHODS FOR PRIMARY SAFETY VARIABLES

Missing safety data will not be imputed in safety analyses for this study.

17.3. ANALYSIS OF PRIMARY SAFETY VARIABLES

For primary safety outcomes, descriptive statistical summaries will include number of subjects and percentages by treatment group and overall, for all subjects (Total) in the SAF.
There will be no statistical comparisons between the treatment groups for safety data.

18. SECONDARY SAFETY OUTCOMES

All outputs for secondary safety outcomes will be summarized by treatment group for the SAF and listed for the RND.
There will be no statistical comparisons between the treatment groups for safety data.

18.1. ADVERSE EVENTS

Adverse Events (AEs) will be coded using Medical Dictionary for Regulatory Activities (MedDRA).

Treatment emergent adverse events (TEAEs) are defined as AEs that started or worsened in severity on or after the investigational product infusion.

An overall summary of number of subjects within each of the TEAE categories (related, infusion-related allergic reaction, neurological worsening, secondary infection, leading to permanent discontinuation of investigational product infusion, serious, leading to death, maximum severity of infusion-related allergic and other), will be provided as specified in the templates.
Data captured on the "Adverse Event Before 90 Days" and "Adverse Event After 90 Days" page of the CRF will be presented by SOC (System Organ Class) and PT (Preferred Term).
Adverse Events within seven days of treatment infusion are also summarized same as TEAEs.
Listings will include TEAEs and Non-TEAEs.

18.1.1. ALL TEAEs

Incidence of TEAEs will be presented by SOC and PT and will also be broken down further by maximum severity and relationship to investigational product.

18.1.1.1. Severity

Severity of infusion-related allergic adverse event is classed as mild/ moderate/ severe/ life-threatening or disabling AE/ death related to AE (increasing severity). Severity of other than infusion-related allergic adverse event is classed as mild/ moderate/ severe (increasing severity). TEAEs starting after investigational product with a missing severity will be classified as severe. If a subject reports a TEAE more than once within the same SOC/ PT, the AE with the worst-case severity will be used in the corresponding severity summaries.

18.1.1.2. Relationship to Investigational Product

Relationship, as indicated by the Investigator, is classed as "Definitely related", "Probably related", "Possibly related", "Unlikely related", "Unrelated" (decreasing severity of relationship). A "related" TEAE is defined as a TEAE with a relationship to investigational product as "Definitely related", "Probably related" or "Possibly related" to investigational product. TEAEs with a missing assessment for relationship to investigational product will be regarded as "related" to investigational product. If subject reports the same AE more than once within that SOC/ PT, the AE with the worst-case relationship to investigational product will be used in the corresponding relationship summaries.

18.1.2. TEAEs Leading to Discontinuation of Investigational Product Infusion

TEAEs leading to permanent discontinuation of investigational product infusion will be identified with "Discontinued" in "Study medication action taken" on the "Adverse Events Before 90 Days" and "Adverse Event After 90 Days" page of the eCRF.
For TEAEs leading to discontinuation of investigational product, summaries of incidence rates (frequencies and percentages) by SOC and PT will be prepared.

18.1.3. Serious Adverse Events

Serious adverse events (SAEs) are those events recorded as "Serious" on the "Adverse Events Before 90 Days" and "Adverse Event After 90 Days" page of the eCRF. A summary of serious TEAEs by SOC and PT will be prepared.

18.1.4. Adverse Events Leading to Death

TEAEs leading to Death are those events which are recorded as "Death" on the "Adverse Events Before 90 Days" and "Adverse Event After 90 Days" page of the eCRF. A summary of TEAEs leading to death by SOC and PT will be prepared.

18.1.5. Adverse Events Occurring in >= 5% of Subjects

TEAEs occurring in 5 % or more of the subjects in either treatment group will be separately presented by SOC and PT.
Also, serious TEAEs, investigational product-related TEAEs, TEAEs leading to discontinuation of investigational product infusion and TEAEs leading to death are also summarized same as TEAEs.

18.2. Laboratory Evaluations

Results from the local laboratory will be included in the reporting of this study for hematology, chemistry and urinalysis. A list of laboratory assessments to be included in the outputs is included in the protocol, Section 5.2.1.

The presentations for these results will use SI Units.
Quantitative laboratory measurements reported as "< X", i.e. below the lower limit of quantification (BLQ), or "> X", i.e. above the upper limit of quantification (ULQ), will be converted to X for the purpose of quantitative summaries, but will be presented as recorded, i.e. as "< X" or "> X" in the listings.
The following summaries will be provided for laboratory data by treatment group:

- Actual and change from baseline by planned visit (for quantitative measurements)
- Incidence of abnormal values according to normal range criteria (for quantitative and categorical measurements)
- Shift from baseline according to normal range criteria (for quantitative and categorical measurements)

Results and reference ranges from the local laboratory will be listed for the RND.

18.2.1. LABORATORY SPECIFIC DERIVATIONS

Laboratory specific derivations will not be considered for this study.

18.2.2. LABORATORY REFERENCE RANGES

Results of local laboratory will not be compared with laboratory refence ranges and categorized in analyses.
Quantitative laboratory measurements will be compared with the relevant laboratory reference ranges in SI units and categorized as:

- Low: Below the lower limit of the laboratory reference range.
- Normal: Within the laboratory reference range (upper and lower limit included).
- High: Above the upper limit of the laboratory reference range.

Categorical laboratory measurements will be compared with the relevant laboratory reference ranges and categorized as:

- Normal: Within the laboratory reference range (upper and lower limit included).
- Abnormal: Above the upper limit of the laboratory reference range.

18.3. ECG EVALUATIONS

Results from the central ECG (Electrocardiogram) Reading Centre will be included in the reporting of this study.
The following ECG parameters will be reported for this study:

- PR Interval (msec)
- QRS Interval (msec)
- QTc Interval (msec) by using Fridericia's Correction
- Results (Overall assessment of ECG (Investigator's judgment)):
    - Normal
    - Abnormal, Not Clinically Significant (ANCS)
    - Abnormal, Clinically Significant (ACS)

The following summaries will be provided for ECG data by treatment group:

- Actual and change from baseline by planned visit (for quantitative measurements)
- Shift from baseline according to markedly abnormal criteria (for quantitative measurements and categorical measurements)
- Incidence of markedly abnormal criteria QTc interval and Result by visit
- Listing of subjects meeting markedly abnormal criteria

ECG parameters will be listed for the RND.

18.3.1. ECG SPECIFIC DERIVATIONS

ECG specific derivations will not be considered for this study.

18.3.2. ECG MARKEDLY ABNORMAL CRITERIA

Markedly abnormal quantitative ECG measurements will be identified in accordance with the following predefined markedly abnormal criteria:

- Absolute values for QTc interval will be classified as:

- > 450 msec
- > 480 msec
- > 500 msec

- Change from Baseline for QTc interval will be classified as:
  - >30 msec increase from baseline
  - >60 msec increase from baseline

18.4. VITAL SIGNS

The following Vital Signs measurements will be reported for this study:

- Systolic Blood Pressure (mmHg)
- Diastolic Blood Pressure (mmHg)
- Heart rate (bpm)
- Temperature (^{0}C)
- Respiration (breath/min)
- Pulse oximetry (%)

The following summaries will be provided for vital signs data by treatment group:

- Actual and change from baseline by planned visit

Vital signs will be listed for the RND.

18.5. PHYSICAL EXAMINATION

The following summaries will be provided for physical examination data by treatment group:

- Incidence of abnormalities at baseline
- Incidence of abnormalities post baseline

Physical examination will be listed for the RND.

18.6. OTHER SAFETY ASSESSMENTS

The presence of a Human Leukocyte Antigen (HLA) antibody will be measured and results will be summarized as being positive or negative (number and percentage of patients) for the antibody reactive against HLA Class I or Class II epitopes by planned visit. Where indicated, the specificity of antibody reactivity will be reported.

Human Leukocyte Antigen (HLA) antibody will be listed for the RND.

19. DATA NOT SUMMARIZED OR PRESENTED

The other variables and/or eCRF pages not summarized or presented are:

- System Screening
- Safety Follow-up
- Questionnaire Of Adverse Events Before 90 Days Visit
- Questionnaire Of CM And CT Before 90 Days Visit
- Questionnaire Of Adverse Events After 90 Days Visit
- Questionnaire Of CM And CT After 90 Days Visit
- Questionnaire of Local Laboratory Reference Ranges (SPONSOR USE ONLY)
- Signature For 90 Days Visit Cut Off
- Signature For 365 Days Visit Cut Off
- Signature For Book

These domains and/or variables will not be summarized or presented, but will be available in the clinical study database, SDTM and/or ADaM datasets.

20. REFERENCES

Sato T. (1989). On the variance estimator for the Mantel-Haenszel risk difference. (Letter). Biometrics, 45: 1323-1324.

Rubin DB. (1987). Multiple Imputation for Nonresponse in Surveys. New York, NY: John Wiley & Sons, Inc, 1987.

Howard G, W. J. (2012). A Simple, Assumption-Free, and Clinically Interpretable Approach for Analysis of Modified Rankin Outcomes. Stroke, 43: 664-669.

APPENDIX 1. PROGRAMMING CONVENTIONS FOR OUTPUTS

IQVIA OUTPUT CONVENTIONS

Outputs will be presented according to the following Global Bios > Processes > GBIOS Processes - Implementation Guidelines and Templates > General Guidelines and Templates > Output Conventions.

DATES & TIMES

Depending on data available, dates and times will take the form yyyy-mm-ddThh:mm:ss.

SPELLING FORMAT

English US.

PRESENTATION OF TREATMENT GROUPS

For outputs, treatment groups will be represented as follows and in that order:

Treatment Group	For Tables and Graphs	For Listings (include if different to tables)
HLCM051 (MultiStem)	MultiStem	MultiStem
Placebo	Placebo	Placebo
Total	Total	Not applicable
Not Randomized	Not applicable	Not Randomized

PRESENTATION OF VISITS

For outputs, visits will be represented as follows and in that order:

Long Name (default)	Short Name
Not applicable	

LISTINGS

All listings will be ordered by the following (unless otherwise indicated in the TLFs shell template):

- randomized treatment group, first by active dose and then placebo,
- subject ID,
- date (where applicable).