Cerebrolysin as an Early Add-on to Reperfusion Therapy: Risk of Hemorrhagic Transformation after Ischemic Stroke (CEREHETIS). A Prospective, Randomized, Active-Control, Multicenter Pilot Study

Clinical Trial Protocol

Date: 30 April 2018

PROTOCOL SYNOPSYS

Sponsor
None

Final Product
Cerebrolysin

Active Ingredient
Cerebrolysin concentrate

Clinical Trial Title
Cerebrolysin as an Early Add-on to Reperfusion Therapy: Risk of Hemorrhagic Transformation after Ischemic Stroke (CEREHETIS). A Prospective, Randomized, Active-Control, Multicenter Pilot Study

Primary Investigator
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Clinical Trial Centers

1. Interregional Clinical Diagnostic Center (Kazan, Russia), tertiary stroke center
2. Municipal Clinical Hospital #7 (Kazan, Russia), primary stroke center
3. Federal University Hospital (Kazan, Russia), primary stroke center
4. Perm Territorial Clinical Hospital (Perm, Russia), primary stroke center
5. Emergency Medical Center (Naberezhnye Chelny, Russia), primary stroke center
6. Leninogorsk District Hospital (Leninogorsk, Russia), primary stroke center
7. Nizhnekamsk District Hospital (Nizhnekamsk, Russia), primary stroke center
8. Arsk District Hospital (Arsk, Russia), primary stroke center

Clinical Trial Schedule
First subject's first visit: April 2018
Last subject's last visit: August 2020 (extension possible)
Study report completion: September 2020

Clinical Trial Phase
IIIb

Goals of the Study

Primary objectives:

To test the hypothesis that the patients in the Cerebrolysin group would achieve reduced incidence of intracranial haemorrhagic events compared to the patients randomised to the control group

Secondary objectives:

To test the hypothesis that more patients in the Cerebrolysin group would achieve short- (day 14) and long-term (day 90) favorable functional outcome after stroke.

To evaluate hypothesis that the use of Cerebrolysin with thrombolytic therapy would be safe.

Additional objectives:

To test the hypothesis that Cerebrolysin would ameliorate ischemic injury to the brain tissue (neuroprotective effect) and stabilize blood brain barrier (BBB) permeability in stroke patients.

Methodology
A prospective, randomized, open-label, active control, multicenter, parallel-group phase IIIb pilot study.

Sample Size Calculation

Sample size calculation was performed by means of power analysis for matched case-control studies using STATA v.14.2.

The minimum detectable odds ratio (OR) for any hemorrhagic transformation (HT) was assumed as low as 0.5. The probability of any HT among controls was expected as high as 0.2. The significance level, power, and correlation of any HT between the arms were set at the values recommended by the software manual, which were 0.05, 0.8, and 0.3, respectively. As it was a pilot study, any HT was chosen for the calculation because it encompassed all types of HT. The drop-out rate is expected as low as 0.1. A 1:2 design was chosen to reduce the sample size by approximately 30%.

Thus, the expected sample size is 264: 88 patients in the Cerebrolysin group and 176 subjects in the control arms.

Inclusion Criteria:

- Confirmed diagnosis of acute ischemic stroke
- Age ≥18 years
- Onset of stroke symptoms within 4.5 h before initiation of rtPA administration

Exclusion Criteria:

- Current or previous intracranial hemorrhage
- Symptoms suggestive of subarachnoid hemorrhage, even if CT scan was normal
- Imaging data on admission suggestive of a brain tumor, arteriovenous malformation, brain abscess or intracerebral aneurism
- Previous history of brain tumor, intracranial aneurism or arteriovenous malformation
- Previous history of brain or spine surgery
- Acute myocardial infarction within the previous 3 months
- Major bleeding, current or within the previous 6 months
- Gastrointestinal or genitourinary bleeding within the previous 3 months
- Confirmed relapse of gastric or duodenal ulcer
- Unknown time of symptom onset
- Minor (NIHSS score <4) or severe stroke (NIHSS score >25) on admission
- Seizure at stroke onset
- Stroke or serious head trauma within the previous 3 months
- Administration of heparin within the 48 h preceding the stroke onset, with an activated partial thromboplastin time at presentation exceeding the upper limit of the normal range
- Platelet count $<100\times10^9$/L
- Systolic blood pressure >185 mm Hg or diastolic blood pressure >110 mm Hg
- Blood glucose <50 mg/dL (2.8 mmol/L) or >400 mg/dL (22.2 mmol/L)
- Oral anticoagulant treatment
- Major surgery or severe trauma within the previous 3 months
- Other major disorders associated with an increased risk of bleeding (neoplasm, bleeding diathesis, acute pancreatitis, infective endocarditis, pericarditis, severe liver and kidney failure)
- Known allergic reactions to rtPA, Cerebrolysin and its components
- Pregnancy and lactation
- Endovascular treatment

Additional Inclusion Criteria for Advanced Brain Imaging:

- No contraindications to magnetic resonance imaging (MRI) and computed tomography (CT) perfusion (CTP) study
- Stroke in the middle cerebral artery territory with a minimum diffusion-weighted imaging (DWI) lesion diameter of 20 mm on admission

Study product, dose and method of administration:
Both groups would receive a standard dose of 0.9 mg/kg recombinant tissue plasminogen activator (rtPA; alteplase) administered by IV infusion within 4.5 h after symptom onset. In addition, measures of standard care for stroke patients would be applied for both groups. Patients in the Cerebrolysin group would additionally receive 30 mL of Cerebrolysin diluted in 100 mL of normal saline administered intravenously through a separate IV line over 20 min. No treatment with other neuroprotective agents would be allowed.

Length of the Study:
Cerebrolysin treatment would be initiated simultaneously with IVT and continued once daily for 14 consecutive days. A follow-up period would be 90 days for each patient. The visits would be scheduled as follows: screening (on admission), in 24 h (visit 1), on day 7, 14, and 90 (visit 2, 3, and 4, respectively).

Endpoint evaluation:

Primary endpoint evaluation:

The study primary endpoints would be any and symptomatic HT verified on a follow-up CT scan. Symptomatic HT would be defined according to the ECASS III trial: any apparently extravascular blood in the brain or within the cranium that is associated with clinical deterioration, as defined by an increase of 4 points or more in the score on the NIHSS, or that lead to death and that is identified as the predominant cause of the neurologic deterioration.

Secondary endpoint evaluation:

Secondary endpoints would be the functional outcome measured with the National Institutes of Health Stroke Scale (NIHSS) and modified Rankin scale (mRS) as well as drug safety.

Favorable functional outcome would be defined as the mRS score of ≤2 at V4 (day 90).

The NIHSS score at V3 (day 14) would be considered as a marker of short-term neurological recovery.

Patients in both arms would be monitored for any adverse events (AE) at V1-3, including changes in vital signs, general and neurological condition, electrocardiogram, and routine laboratory tests (liver and kidney function tests, complete blood count).

Additional evaluation (advanced brain imaging):

At V1 and V3, a routine brain MRI on a 1.5T scanner would be acquired followed by an axial DTI scan. The DTI sequence parameters are as follows: spin-echo echo-planar imaging, repetition time = 6000 ms, echo time = 102.9 ms, field of view = 260 mm, b-value = 0 and 1000 s/mm2, matrix = 256 × 256; slice thickness = 4.5 mm, interslice gap = 1 mm, total number of slices = 24, diffusion directions = 25, scan time = 5 min 10 s.

The maps of fractional anisotropy (FA), axial (AD), radial (RD) and mean (MD) diffusivity would be derived from the raw DTI scans.

At V3, a brain CT perfusion (CTP) scan would be obtained using the Dankbaar's approach. It involves a cine mode CT acquisition, with a temporal sampling rate of one image every 2 s for the first 60 s. Additional gantry rotations would be performed at 90, 120, 150, 180, 210 and 240 s. Acquisition parameters are 80 kVp and 100 mA. A bolus of 40 mL iohexol (Omnipaque, GE Healthcare, USA; 300 mg/mL of iodine) would be injected into an antecubital vein at an injection rate of 5 mL/s. CT scanning would be initiated 5 s after start of the injection of the contrast bolus. A series of CT scans covered the whole brain would be obtained with 5 mm slice thickness.

The CTP data would be processed to obtain a series of the permeability surface-area product (PS) maps.

The most representative slice would be chosen for analysis in each set of images. On that slice, the infarcted area is outlined and mirrored to the contralateral hemisphere. The values of FA, AD, RD, MD, and PS would be assessed within each region of interest.

To cope with the heterogeneity of the ischemic lesions due to different locations, absolute values of the laterality index for each parameter would be calculated using the formula:

|Laterality index| = (Affected side – Unaffected side) / (Affected side + Unaffected side) × 100%.

The infarct volume was calculated on DWI (at V1) and CT (at V3) scans according to the ABC/2 method [10].

Statistical methods

This statistical plan describes biostatistical analysis scheduled at the time of development of this study design. Detailed statistical analysis plan will be developed after study completion. Analysis report with final statistical analysis plan will be integral part of the clinical trial report.

Statistical analysis would be performed using statistical software like STATA, IBM SPSS Statistics.

In this study, statistical analysis will be exploratory as the study is designed to develop a true hypothesis of efficacy and safety for the future clinical trials rather than a confirmation of any pre-specified claims. All p-values and confidence intervals may be only interpreted given exploratory nature of the analysis in consideration.

LIST OF ABBREVIATIONS

AE Adverse Event
ATC Anatomic Therapeutic Category
ASPECTS The Alberta Stroke Program Early CT Score
BBB Blood-brain barrier
BP Blood Pressure
CI Confidence Interval
CT Computed Tomography
CTP Computer tomography perfusion
CRF Case Report Form
GCP Good Clinical Practice
IEC Independent Ethics Committee
DWI Diffusion-Weighted MR Imaging
HT Haemorrhagic transformations
IRB Institutional Review Board
IV Intravenous
IVT intravenous thrombolytic therapy
Forward) MAP-2 Microtubuli-associated Protein 2
MCA Middle Cerebral Artery
MedDRA Medical Dictionary for Drug Regulatory Affairs
mL Milliliter
MRI Magnetic Resonance Imaging
mRS Modified Rankin Scale
NIHSS National Institutes of Health Stroke Scale
PS - permeability-surface area product
ROI - Region of interest
rtPA Recombinant Tissue Plasminogen Activator

SAE Serious Adverse Event
SAP Statistical Analysis Plan
SHT Symptomatic haemorrhagic transformations
SUSAR Serious Unexpected Severe Adverse Reaction
WHO World Health Organisation
WHO-ATC World Health Organisation: Anatomic Therapeutic Category (System of Coding Recorded Terms)
WHO-DD World Health Organisation: Drug Dictionary

BACKGROUND

1. STUDY PRODUCT

Cerebrolysin is a solution for injection.

Cerebrolysin is a peptide product derived biotechnologically by standardised fermentative splitting of purified and lipid-free cerebral proteins. It consists of neuropeptides with low molecular weight (< 10 kDa) and free amino acids. Cerebrolysin has been authorised and approved for commercial distribution for the treatment of stroke and dementia in numerous European and Asian countries.

2. BACKGROUND DATA

Generally, cerebrolysin imitates effect of neurotrophic factors and exhibits neuroprotective (Wronski et al 2000), neurotrophic (Akai et al. 1992, Satou et al. 1994), and neuroplastic effects. Pharmacological data obtained in numerous clinical studies *in vitro* including biochemical experiments and cell culture models definitely revealed these neurotrophic and neuroprotective properties of the product. Cerebrolysin protects cerebral neurons against stroke-induced hypoxic and ischemic damage addressing various states of the ischemic cascade. The product provides positive effect on cerebral metabolism by increasing efficiency of the cascade of aerobic reactions associated with significant lactate level decrease. It neutralises glutamate-induced excitotoxicity by binding with the receptors of inhibitory neurotransmitters resulting in increased neuronal viability. In addition, cerebrolysin was found to prevent cell death by suppressing production of free acid radicals and affecting expression of antioxidant enzyme genes. Cytoskeletal proteins such as MAP2 resulting from ischemic damage tend to be stabilised by cerebrolysin accounting for preservation of the structural cell integrity and, probably, blood-brain barrier (BBB). Most importantly, hypoxia model on animals confirmed penetration of cerebrolysin neuropeptides through BBB into cerebral parenchyma after intravenous administration unlike natural neurotrophic factors. Hypoxia and ischemia models in animals also revealed cerebrolysin potential to prevent cytoskeletal degeneration, reduce swelling and decrease lethality. Furthermore, the model of acute focal ischemia in rats demonstrated that administration of cerebrolysin within 2 hours post ischemic episode was able to reduce infarction area and improve neurological outcome (Hanson, 2009).

Cerebrolysin was found to be effective as neuroprotective support to thrombolytic therapy in terms of stroke outcomes, especially at long-term administration of the product against step-wise neurorehabilitation (Wolf-Dieter Heiss et al., 2012). The peculiarities of multimodal effect of cerebrolysin demonstrated on the experimental models suggested a hypothesis of efficacy of this product during intravenous thrombolytic therapy. The clinical study carried out by W. Lang et al. 2012 evidence that the patients receiving cerebrolysin as add-on to thrombolytic therapy had significantly faster treatment response compared to those receiving thrombolytic therapy only.

This clinical study is designed to evaluate efficacy of drug protection of cerebral tissue, predominantly against reperfusion damage during reperfusion therapy.

3. POTENTIAL RISKS AND BENEFITS FOR HUMAN

Currently, the results of 60 controlled clinical studies of cerebrolysin have been published. In these clinical studies, 2,905 patients received cerebrolysin; these patients reported 835 adverse events (28.74 event per 100 patients). For comparison, 2,594 placebo-treated patients reported 629 adverse events (24.25 events per 100 patients). These results suggest that in cerebrolysin group average adverse event rate of 12.1 % was slightly higher compared to placebo group (9.6 %). Summarising, we may conclude that the safety data from both controlled and uncontrolled clinical studies of cerebrolysin demonstrate excellent benefit/risk ratio.

4. DESCRIPTION OF THERAPY

Study product cerebrolysin is expected to be administered at a daily dose of 30 mL diluted in 0.9 % sodium chloride up to 100 mL as a 20-minute intravenous infusion. The infusion of the study product will be initiated simultaneously with bolus administration of rt-PA, the products will be injected using different access routes (e.g. using a cubital catheter of right and left cubital veins). Where indicated baseline therapy (e.g. BP-controlling products) will be administered prior to or after the first infusion of the study product and thrombolytic infusion. Subsequent infusions of cerebrolysin will be made on a daily basis at the same time for 14 days.

5. CLINICAL STUDY COMPLIANCE

The clinical study will be carried out in accordance with this protocol, ICH GCP and current requirements of the national regulatory authorities. The study was approved by the Local Ethics Committee of the Interregional Clinical Diagnostic Center, Kazan, Russia (Protocol #81 of 04/24/2018). All recruited subjects or their legal representatives would sign written informed consent. The study methods would be performed in accordance with the Declarations of Helsinki.

6. REFERENCE INFORMATION

Stroke is a severe disease and one of the leading causes of disability and lethality globally. Ischemic stroke (IS) accounts for the most part of stroke cases overall, i.e. up to 85 %. Reperfusion therapy is currently one of the verified effective IS treatment. IV TLT is deemed to be effective in improving neurological symptoms while reducing NIHSS by ≥ 4 within one day. One of the factors of reperfusion therapy failure may be reperfusion damage developing after recanalisation of a cerebral vessel and generally is considered as a consequence and progression of abnormal ischemic effects. Therefore, recanalisation of a clotted or embolised cerebral vessel within "therapeutic window" promotes preservation of the most part of inactive though viable neurons; meanwhile recovery of blood flow in ischemic area may cause reperfusion-induced cerebral tissue damage of various grades, thus aggravating peracute IS.

One of manifestations of reperfusion damage is BBB failure with increased permeability or impaired integrity resulting in exacerbation of edema of ischemic area or in hemorrhagic transformation (HT) of the ischemic area. Meanwhile, HTs are also reported without recanalisation effect and outside of the ischemia area; this is due to both cerebral tissue damage in hypoperfusion area and due to toxic effect of the thrombolytic agent. Thus, TLT increases the risk of HT 4-10-fold by activating matrix metalloproteinase 9, 2, 3 in neutrophils, endothelium, astrocytes, and platelet-derived growth factor-CC, however the pathogenesis of early (up to 24 hours) HTs and late HTs varies. While early HTs are associated with acute processes of BBB damage, the late ones are due to cerebral tissue inflammation and vascular remodelling. Clinically, the most relevant are symptomatic HTs (SHT) associated with aggravated neurological symptoms ≥ 4 according to NIHSS scale or lethality (according to ECASS III 2008, 2016). Generally, SHTs have the nature of parenchymatous haematomas (hemorrhages, PH2 according to neuroimaging HT classification) exceeding 30 % at ischemic area. Smaller haematomas and petechia-like HTs are asymptomatic (HI-1, HI-2, PH-1).

Development of the principles of pharmacological protection of cerebral tissue against reperfusion damage, one of the modifiable factors defining severity of cerebral damage and, therefore, neurological deficit and rehabilitation potential, is a promising trend in the treatment of acute cerebral ischemia.

Cerebrolysin has been approved for the treatment of stroke in more than 45 countries globally.

Significant progress has been made in stroke therapy since approval of cerebrolysin, including improved general care and condition of stroke units, rehabilitation became more targeted, fibrinolytic therapy (rt-PA, Actilise) - more commonly available in global specialised centers.

CLINICAL STUDY PURPOSES AND OBJECTIVES

The purpose of this study is to investigate the effect of cerebrolysin on post-reperfusion period in patients with ischemic stroke during thrombolytic therapy with decreased frequency of HTs. In addition, it is planned to study the hypothesis that cerebrolysin group will show better ischemic area and BBB changes, better general neurological status and functional outcomes.

CLINICAL STUDY DESIGN

1. PRIMARY EFFICACY CRITERION

Evaluation of frequency of any and symptomatic hemorrhagic transformations of ischemic area, detection of intracranial haemorrhages outside the ischemic area. Neuroimaging techniques will be used for evaluation of early and late haemorrhagic transformations in ischemic area and outside. Efficacy endpoint will be assessed in 24 hours, on day 7 and 14 after reperfusion therapy.

2. SECONDARY EFFICACY CRITERIA

The study will use common assessments found to be sensitive to therapeutic effect in NINDS clinical study investigating intravenous administration of rt-PA (Huber, 1999). These include National Institutes of Health Stroke Scale (NIHSS) and modified Rankin scale during hospital follow-up period and on day 90.

In addition, advanced brain imaging will evaluate changes in ischemic area and BBB permeability.

3. CLINICAL STUDY DESIGN

The study is designed to investigate efficacy of cerebrolysin 30 mL for patients with ischemic stroke during IV TLT and standard baseline therapy and early neurorehabilitation.

Therefore, the study design provides for two study groups:

Study group 1 (Cerebrolysin): 30 mL of cerebrolysin in 100.0 mL of 0.9 % NaCl during IVT, baseline therapy, early neurorehabilitation according to standards

Study group 2 (Control): IVT, baseline therapy, early neurorehabilitation according to standards

The clinical study will be carried out in patients with ischemic stroke meeting IVT inclusion and exclusion criteria (Clinical Guideline for Thrombolytic Therapy During Ischemic Stroke (National Clinical Guidelines:
http://193.232.7.120/feml/clinical_ref/0001410674S/HTML/)

The diagnosis of ischemic stroke and its localisation will be determined clinically based on neuroimaging findings (TOAST criteria).

The clinical study stipulates for follow-up period of 90 days, with 4 scheduled visits for clinical assessment at the following time points: screening (at admission), in 24 hours, on day 7, 14, and day 90.

The clinical study is expected to begin in April 2018 (first patient enrollment) and to end (final study report) by September 2020.

The clinical study is expected to be performed from 2018 till 2020.

Schedule of the clinical study procedures

Patient visits	**Screening**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**
Time since treatment initiation	**Admission**	**24 hours after initiation of therapy**	**Day 7 after treatment initiation**	**Day 14 after treatment initiation**	**Day 90 after treatment initiation**
Baseline patient information (demographic data, medical history)	+				
Inclusion/exclusion criteria review	+				
Randomisation, number assignment	+				
Non-contrast CT of the brain	+	+	+	+	
CTP* (PS)				+	
MRI* of the brain (DWI, FLAIR, 3D TOF)	+				
MRI* of the brain (DWI, DTI, 3D TOF, ROI)		+			
MRI* of the brain (DTI)				+	
Assessment of neurological impairment using to the NIHSS scale	+	+	+	+	
Assessment of disabilities using the Rankin scale (attachment)	+			+	+
Assessment of vital functions (BP, RR, pulse, T)	+			+	
Haematology	+			+	
Serum biochemistry	+			+	
CRP		+			
ECG	+				
Assessment of the presence and severity of adverse events		+	+	+	

* For patients, eligible for advanced brain imaging at the tertiary stroke center.

4. MEASURES TO MINIMISE/PREVENT SYSTEMIC BIAS

Randomisation

Each eligible patient would be randomly assigned into either the Cerebrolysin or control group by simple randomization procedure. One randomization list for all centers would be issued

by generating Bernoulli variates with the probability parameter of 0.333. The Mersenne twister would be used as an active generator and the starting point was set at random.

Allocation instructions would be sealed in opaque envelopes, mixed and distributed between the centers by an independent statistician. Each envelop would be randomly picked by the investigators and would be opened after the subject's recruitment.

Investigators would enroll participants, assign them to the intervention, and assess clinically the primary and secondary endpoints. Imaging data would be evaluated locally by radiologists who would be blinded to the intervention. However, investigators and participants would not be blinded to the treatment assignment because Cerebrolysin has its particular yellowish color and it is impossible to conceal it properly.

5. DESCRIPTION OF THERAPY

Study product

Cerebrolysin

Dosage Form

Cerebrolysin 10 mL/ampule

Study product(s) - Introduction

Cerebrolysin will be administered on a daily basis at a single dose of 30 mL diluted in 0.9 % sodium chloride solution up to 100 mL in a 20-minute intravenous infusion. The control group will not receive cerebrolysin.

The study product will be administered once daily for 14 days starting on the day of baseline assessment.

6. STUDIES

This clinical study will be pilot multicenter clinical trial. The primary endpoint will be testing hypothesis that the patients randomized to the cerebrolysin group will achieve reduced incidence of hemorrhagic transformation compared to the patients randomized to the control group. The secondary endpoints will include overall evaluation of disability and neurological status. Overall number of patients to be enrolled to both groups (1:2 ratio) will be 264.

Eight study centers will recruit patients. The treatment will be initiated within 45-270 min after stroke onset and include 14 daily intravenous infusions. The Cerebrolysin infusions would be associated with the standard baseline and rehabilitation therapy. The result will be assessed at baseline and at three visits, with the final assessment 90 days post stroke.

On admission (the screening), the patients will be evaluated for compliance with inclusion/exclusion criteria. The patients found eligible for the clinical study will be enrolled. The data to be collected at the screening will be provided in table "Schedule of the Clinical Study Procedures". These data include date and time of the stroke, modified Rankin scale rating, evaluation of medicinal products and allergic reactions, collection of medical, neurological history; physical examination, NIHSS rating, evaluation of X-ray findings and evaluation of the results of laboratory tests. Clinical diagnosis of ischemic stroke will be made. Inclusion/exclusion criteria should be further checked. Brain MRI and CT should be made at enrollment and used to rule out hemorrhagic stroke or intracranial tumor. The patients meeting these criteria and providing their informed consent will be enrolled and randomized to the Cerebrolysin group or to the control group.

By the end of the evaluation procedures the patient will receive the first dose of the study product along with thrombolytic therapy.

24-hour monitoring will be performed based on IVT protocol.

Treatment and rehabilitation diary will also be initiated as well as monitoring of adverse events and administration of co-medications.

All patients assessed by inclusion criteria will be entered in the registration log.

The list of baseline evaluations also includes NIHSS rating and modified Rankin scale rating.

During the first 24 hours the patients should be monitored based on IVT protocol.

The treatment period will be 14 days. During this period which will always begin 45-270 minutes after stroke onset the patient will receive daily intravenous dose of the study product. The treatment will be assigned by randomization. In 24 hours (visit 1), on days 7 (visit 2), 14 (visit 3) of the treatment period further evaluation procedures will be made according to table "Schedule of the Clinical Study Procedures". Throughout the clinical study all adverse events regardless of their association with cerebrolysin will be collected and recorded in standardised adverse event and serious adverse event forms. Serious adverse events are defined as any medically unfavourable event developing after administration of any dose of the product and resulting in death, being life-threatening, requiring hospitalisation or its prolongation, resulting in long-term or expressed disability/incapacity. Diagnosis rather than the signs, symptoms and/or other clinical information should be recorded as possible. Severity of each adverse event should be qualified as mild, moderate or severe. The events are evaluated medically including testing and referral to medical specialists. All the current requirements of local regulatory authorities concerning notification of regulatory authorities and local ethics committee on serious adverse events will be followed.

In case a significant adverse event is detected, discontinuation or planned continuation of the study therapy should be discussed immediately. The key factor in this discussion should be the severity of the adverse event and the probability that this adverse event or its exacerbation may be induced by cerebrolysin. The study therapy should be suspended if the patient reports an adverse event which, according to the investigator, may result in discontinuation of further of therapy or if the patient or his/her relatives apply for discontinuation of further therapy.

At the final visit (visit 4, day 90), Rankin scale rating is scheduled.

7. WITHDRAWAL CRITERIA

Patients would be withdrawn from further intervention in case neurosurgery is performed or a life-threatening medical (non-neurological) condition occurs. The study would be also discontinued in case of patient’s death. The participants have the opportunity to exit the study at any time. The intention-to-treat (ITT) population comprises all recruited patients, subjects completed the study would be included in the per-protocol (PP) analysis. The study ended once the required number of patients would be reached and the protocol would be accomplished by the participants.

Any patient may withdraw from the clinical study without specifying the reason for such decision. If possible, the patient should discuss his/her decision with the investigator. The reasons as well as date and time of withdrawal should be documented in CRF. All the information on the patients deciding to withdraw should be fully documented in CRF until the time of such withdrawal. Where possible, condition of such patients should be monitored for 90 days. Need in final examination (this is a safety visit).

Every effort should be made to make sure the patients randomized remain in the clinical trial for 90 days. This will guarantee that the deviations will not affect the treatment outcome.

Medical conditions developing during the clinical study which are considered as exclusion criteria will require withdrawal of the patient only if clinically needed or at the patient's decision (ITT principle).

The patients withdrawing from the clinical study due to AE will receive therapy in accordance with the current clinical standard, and these patients should be monitored until recovery or stabilisation or until discovery of the plausible cause of the event. All the related information on AE outcome should be documented in CRF.

RANDOMISATION CODES

Preservation of randomisation codes

Patient randomisation codes will be assigned by a person who is not involved in any clinical trial procedures. The envelopes with randomisation codes in each study center will be kept by this person in both electronic and paper format.

8. SOURCE DATA

The following data will be added directly to CRF and considered as source data:

- vital signs
- data on compliance with thrombolytic therapy criterion including CT scans
- physical examination findings
- efficacy variables including modified Rankin scale, NIHSS scale

Any other data added to CRF (e.g. AEs, medical history, co-medications, laboratory tests, advanced CT and MRI scans, etc.) should be added to primary documents for each patient.

TREATMENT OF PATIENTS

At admission department two cubital peripheral venous catheters should be inserted to left and right arms of the patient. After evaluation of inclusion and exclusion criteria infusion of rt-PA and study product should be initiated.

Dosing of rt-PA will be determined on an individual basis as 0.9 mg/kg body weight, maximum rt-PA is 90 mg. The product will be diluted with water for injections as 50 mg of rt-PA in 50 mL of solvent. 10 % of rt-PA dose will be administered as a bolus intravenous injection within one minute, while the other 90 % dose will be administered intravenously for 60 min (according to patient information leaflet and IVT protocol: Clinical Guidelines for Thrombolytic Therapy During Ischemic Stroke (National Clinical Guidelines).
http://193.232.7.120/feml/clinical_ref/0001410674S/HTML/

The study product will be administered simultaneously with rt-PA infusion.

After the completion of rt-PA infusion, the patient should receive standard medication in accordance with the Standard of Medical Care for Patients with Cerebral Infarction (Order of the Ministry of Health of the Russian Federation dated December 29, 2012 No.1740n).

1. STUDY PRODUCT

The study product cerebrolysin is expected to be administered as a single daily dose of 30 mL diluted in 0.9 % sodium chloride up to 100 mL in a 20-min intravenous infusion.
Study group 1: cerebrolysin 30 mL
Study group 2: no cerebrolysin

2 CO-MEDICATIONS

All previously used and concomitant medications must be recorded in the CRF, including the start date and the date of discontinuation, if possible, the maximum total daily dose and route of administration. Then the reason (diagnosis) on the basis of which this special concomitant therapy was prescribed should be specified. Finally, if a concomitant drug is used in preventive regimen, this should be reported on the appropriate CRF page.

3 Acceptable co-medication

Appropriate concomitant medications may be prescribed if the investigator believes there is clinical need:

- Baseline stroke therapy within the general management of the patient can be used without restriction if necessary. At each visit, it is envisaged to register co-medications received by the patient (including dosage and frequency).
- Medications to compensate for abnormality in water and acid-base balance.
- Medications required for adequate treatment of secondary symptoms, including, but not limited to, antihypertensive agents, cardiovascular therapy, antidiabetic agents, if necessary, drugs to normalise sleep disorders (excluding benzodiazepines), antibiotics, and antipyretics.

4. Prohibited co-medications

The use of the following drugs in a clinical study is limited and every effort should be made to avoid taking the following:

- Concomitant use of other neuroprotective or nootropic drugs (e.g., citicoline, memantine, amantadine, erythropoietin, diazepam, investigational neuroprotective drugs; piracetam, pramiracetam, pyritinol, meclosulfonate, glycine)
- Concomitant use of drugs with a vasodilatory effect, such as naftidrofuryl, cinnarizine, flunarizine, nimodipine, nicergoline, pentoxifylline, dihydroergotoxin (codergocrine), vinpocetine, vincamine, or ginkgo biloba
- Antioxidant drugs (e.g., but not limited to lipoic acid, ethylmethylhydroxypyridine succinate)
- Levodopa and dopamine agonists
- Statins within the first 7 days from the disease onset.

5. Previous medications

If necessary, constant dosage of previously used medications can be taken throughout the clinical study.
Any changes to previously used medications should be recorded in the CRF.

6. Drug interactions

To date, no drug interactions for the study product have been reported.

REHABILITATION PROGRAM

Rehabilitation program will be in accordance with ischemic stroke standard of care starting no later than 48 hours post stroke.

EFFICACY EVALUATION

1. DESCRIPTION OF EFFICACY CRITERIA

1. Development of hemorrhagic transformations
Development of hemorrhagic transformations (HT) in ischemic area should be determined based on the results of follow-up brain CT. According ECASS 3 criteria, HT is defined as symptomatic (SHT) when neurological symptom exacerbation according to NIHSS is ≥ 4 NIHSS or results in death.

2. NIHSS scale
Neurological deficit is assessed using NIH scale. It is a 15-item scale evaluating level of consciousness, gaze, visual field, facial palsy, motor functions, limb ataxia, aphasia, dysarthria as well as extinction, and inattention (Brott et al, 1989).

Each item has a 3-5 score from 0 to 4, with the highest score being consistent with the most severe disability. The worst score is assigned in case of death.

Drastic improvement - cases of improvement with reduced NIHSS score ≥ 4 one day after the disease debut is considered as "drastic improvement".

3. Modified Rankin Scale
Modified Rankin Scale (Van Swieten et al, 1988) is the scale evaluating general functional outcome. It is used to grade the stroke outcome and post-stroke disability. Modified Rankin scale is the ordinal 7-point scale scoring from 0 (lack of residual symptoms) to 6 (worst) which corresponds to patient death.

4. Neuroimaging techniques
Brain non-contrast CT will be performed at admission, in 24 h, on day 7 and 14 after IVT using standard technique; helical scanning is preferred; minimum section width (post reconstruction) is 5 mm; scanning area - the whole brain; the sections are made without gantry slope.

The sections obtained at admission will be assessed using ASPECTS scale indicating the presence of the symptom of hyperdense middle cerebral artery (MCA) sign.
Hyperdense MCA sing is determined based on the density of affected and normal vessels. Absolute density of affected MCA > 43 Hounsfield units and affected/normal MCA > 1.2 on non-contrast CT is considered as a positive symptom.

24 hours, on day 7 and 14 after IVT non-contrast CT is performed to evaluate presence of haemorrhagic transformation.

Advance brain imaging protocol:

Non-contrast MRI will be performed at admission. Axial DWI, FLAIR, MR-angiography are performed using standard techniques.
In 24 h after IVT non-contrast brain MRI is carried out in DWI, DTI and MR-angiography.
DWI is used to determine infarction volume using ABC/2 formula.
Axial DTI is performed using standard technique. The section with the largest changes will be selected from the whole DTI scan collection at postprocessing step. Region of interest (ROI) should include the whole infarction area at this section, the reference area being the contralateral hemisphere mirror image. The protocol will specify the number of the section selected, mean

values of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD) both in ROI and in reference area.

On day 14 non-contrast brain MRI will be performed in DTI.
DTI will be made and processed in axial methods using the technique described above. Quantitative parameters are evaluated at the level of the pre-selected section (see DTI scans 24 h after IVT).

Also on day 14, CT-perfusion will be performed.
The patients head should be fixed using a restrainer to reduce motor artefacts; 40 mL of contrast (Omnipaque, 300 mg/mL of iodine) should be administered as a bolus injection into median cubital vein using an injector at 5 mL/s rate, further 40 mL of 0.9 % sodium chloride was administered at the same rate. The scanning will begin in 5 seconds; 80 kV, 100 mA; time resolution - 1 scan every 2 second for the first 60 seconds, then 1 scan every 30 seconds during the next 3 min. Overall scanning time is 4 min. At such scanning mode radiation dose should not exceed 3.2-3.5 mSv (J. Hom at al., 2011). The sections will be made without gantry slope.
Post-processing will be made at GE Workstation. The scan with the largest changes will be chosen out of the scans obtained. The first phase will be processed using deconvolution algorithms insensitive to delay. Region of interest (ROI) is the whole hypodense area, reference area is the contralateral hemisphere (mirror image). The BBB permeability maps (PS) will be processed using Johnson and Wilson kinetic model. The pre-selected section will be used. The protocol will specify PS values (permeability-surface area product) both in ROI and in reference area.

In addition to standard contraindications to non-contrast CT, CT-perfusion and MRI, no additional contraindications are in place in this study.

2. EFFICACY CRITERIA

NIHSS scoring should be made at screening, at baseline visit before the first administration of the study product, within 24 hours, according to the protocol of thrombolytic therapy, in 24 hours (visit 1), on day 7 (visit 2), on day 14 (visit 3).

Modified Rankin Scale scoring will be made at the baseline visit before the first administration of the study product on day 14 (visit 3) and on day 90 (visit 4).

Brain imaging findings will be reported by radiologists.

Evaluation of efficacy criteria will be analysed in accordance with the provisions of the statistical analysis plan.

SAFETY EVALUATION

1. SAFETY DATA

Safety data should include AEs, vital signs and laboratory tests including haematology, serum chemistry, and urinalysis.

All the AEs developing after signing informed consent should be recorded regardless of whether they are considered as related or unrelated to the study product. At each visit, the investigator should detect AEs and evaluate them. AEs should be monitored until reduction of intensity, classification as medically stable or until loss to follow-up.
AEs should be recorded directly in CRF. Analysis will be performed in accordance with statistical analysis plan.

2. ADVERSE EVENT REPORTING

The investigator will be responsible to document all the adverse events occurring during the study. The list of AEs includes any unfavourable, abnormal or unexpected changes in anatomical, physiological or metabolic function manifested as physical signs, symptoms and/or

laboratory changes developing at any phase of the clinical study regardless of whether the event is related to the study product or placebo, and will be considered as related to the study product. This list includes exacerbation of pre-existing conditions or events, co-morbidities, cases of drug interaction or significant aggravation of the study disease. Note that surgeries (e.g. appendectomy) are not considered as adverse events as these are medical procedures. In case of surgery, the underlying disease should be recorded as the adverse event (e.g. "appendicitis").

In addition to spontaneous reports, the patient should be asked concerning AEs by general questions.

1. Severity assessment

AE severity should be evaluated using the following criteria:

- Mild: AEs easily tolerated by the patient, causing minimum discomfort and not interfering with daily activities.
- Moderate: AEs causing significant discomfort and interfering with daily activities.
- Severe: AEs preventing from normal daily activities.

The term "severe" is used to describe severity grade of a specific event; however the event itself may have relatively low medical significance. This term is a synonym of the term "serious" which is defined according to the criteria based on the event outcome, outcome for the patient, measures taken; it was generally associated with the life-threatening or disabling events. Seriousness (but not severity) is a criterion to define the responsibilities on expedited reporting.

Serious adverse events

SAEs denotes any medically unfavourable event developing after administration of any dose of the product and resulting in death, being life-threatening, requiring hospitalisation or its prolongation, resulting in long-term or expressed disability/incapacity.

Early withdrawal

Early withdrawal from the study can occur due to the following reasons:

- development of exclusion criteria during the study; serious study protocol deviation;
- surgery concerning the underlying disease
- patient death,
- patient's will to withdraw from the study.
- serious adverse events or major abnormal laboratory values requiring discontinuation of the study therapy.

STATISTICAL METHODS

The data will be summarised and analyzed using tables and diagrams for demographic/baseline characteristics and observations and efficacy/safety evaluations. Standard parameters of descriptive summary statistics (e.g. calculation of arithmetic mean, standard deviation, median, minimum/maximum - quartiles if applicable) will be calculated for continuous variables. Categorical data will be presented in frequency tables showing numbers and proportions. Tabulated summaries will be presented for each treatment group and in general for all patients. For individual parameters, lists of data for specific patients sorted by treatment group, center, patient number and visit will be complied. For OR calculation logistic regression analysis will be used (both univariate and multivariate) using Firth method due to relatively small sample size and rare nature of haemorrhagic events. Missing data will be analysed for patterns and where possible will be processed using multiple imputation.

ETHICAL PRINCIPLES

INDEPENDENT ETHICS COMMITTEE (IEC)/INSTITUTIONAL REVIEW BOARD (IRB)

Recruitment of patients will not be allowed before IEC/IRB written authorisation/positive decision on the study performance is obtained.

No protocol deviations or changes are allowed without written approval/positive decision of IEC/IRB concerning the relevant amendment to the protocol except for the cases when the risk for the patient should be eliminated immediately or when the change(s) deal with logistic or administrative aspect(s) of the study (e.g. change of monitor, phone number, etc.).

ETHICS PRINCIPLES

The clinical study will be carried out in accordance with the protocol, ethics principles outlined in Helsinki Declaration, applicable GCP regulations and requirements of regulatory authorities.

DATA PROCESSING AND RECORD-KEEPING

The data recorded in CRF taken from source documents should be consistent with the source documents while discrepancies should be explained.