CLINICAL TRIAL PROTOCOL

Multicenter open-label randomized comparative trial of the efficacy and safety of single bolus injection of Fortelyzin® (Supergene, LLC) and bolus-infusion of Actilyse® (Boehringer Ingelheim Pharma GmbH) in patients with ischemic stroke

Study drug:	Fortelyzin® (substance Forteplase®)
Trial Phase:	III b
Trial design:	Multicenter, open-label, randomized, comparative, non-inferiority trial of efficacy and safety
Sponsor:	The Russian Academy of Sciences, Supergene, LLC, Russia
Version:	1.0, approved by the Ministry of Health of the Russian Federation as of July 15, 2016

INFORMATION ABOUT THE PRINCIPAL INVESTIGATOR

The National Investigator will coordinate the research activities of all research centers participating in the multicenter clinical trial.

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I read all the pages of the Clinical Trial Protocol «Multicenter open-label randomized comparative trial of the efficacy and safety of single bolus injection of Fortelyzin® (Supergene, LLC) and bolus-infusion of Actilyse® (Boehringer Ingelheim Pharma GmbH) in patients with ischemic stroke», and I agree that it contains all the information necessary to conduct this trial. I will conduct the trial as described in this Protocol and comply with all terms and conditions specified therein. I confirm that I will conduct the trial in accordance with the current legislation, the Declaration of Helsinki, and the principles of Good Clinical Practice approved at the International Conference on Harmonization (ICH GCP). I also guarantee that the co-investigator(s) and the other subjects involved will conduct the trial in accordance with these documents.

Principal Investigator's signature:

Signature: _____ Date: _____/_____/_____
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LIST OF ABBREVIATIONS

AG	– angiography
AIS	– acute ischemic stroke
BP	– blood pressure
ALAT	– alanine aminotransferase
AST	– aspartate aminotransferase
APPT	– activated partial thromboplastin time
RSC	– Russian Cardiology Society
WHO	– World Health Organization
DBP	– diastolic blood pressure
DWI	– diffusion-weighted image
DS MAH	– Duplex scanning of major arteries of the head
IPIC	– Individual Patient Identification Code
CRF	– Case Report Form
K^+	– blood serum potassium
CT	– computed tomography
CPK	– creatine phosphokinase
MP	– medicinal product
INR	– international normalized ratio
MRI	– magnetic resonance imaging
mRS	– modified Rankin scale
UAR	– unforeseen adverse reaction
AE	– adverse event
AMI	– acute myocardial infarction
ADCC	– acute disorders of cerebral circulation
SBP	– systolic blood pressure
SH	– subarachnoid hemorrhage
MA	– middlecerebral artery
SAE	– serious adverse event

SAK	– staphylokinase
TPA	– tissue plasminogen activator
TLT	– thrombolytic therapy
HDL-C	– high-density lipoprotein cholesterol
RR	– respiratory rate
HR	– heart rate
EC	– Ethics Committee
ECG	– electrocardiography
BI	– Barthel Index for activities of daily living
ECASS	– European Cooperative Acute Stroke Study
Hb	– hemoglobin
Ht	– hematocrit
ICH	– International Conference on Harmonisation
GCP	– Good Clinical Practice
GUSTO	– Global Use of Strategies to Open Occluded Coronary Arteries
Na^+	– Sodium in blood
NIHSS	– National Institutes of Health Stroke Scale
NYHA	– New York Heart Association
TIMI	– Thrombolysis in Myocardial Infarction
TFC	– TIMI Frame Count
TOAST	– Trial of ORG 10172 in Acute Stroke Treatment

PROTOCOL SUMMARY

Official Title:	Multicenter open-label randomized comparative trial of the efficacy and safety of single-bolus intravenous injection of Fortelyzin® (manufactured by Supergene, LLC) and bolus infusion of Actilyse® (manufactured by Boehringer Ingelheim Pharma GmbH) in patients with ischemic stroke
Short title:	FRIDA
Trial phase:	III b
Trial aim:	Assessment of the efficacy and safety of single-bolus intravenous injection of Fortelyzin® in comparison with bolus infusion of Actilyse® in patients with ischemic stroke
Trial objectives:	• To assess the efficacy of single-bolus intravenous injection of Fortelyzin® in comparison with bolus infusion of Actilyse® in patients with ischemic stroke • To assess the safety and possible adverse events of single-bolus intravenous injection of Fortelyzin® in comparison with bolus-infusion of Actilyse® in patients with ischemic stroke
Trial design:	A multicenter, open-label, randomized, comparative, parallel-group non-inferiority trial of efficacy and safety. In clinical centers, patients will be randomized using the "envelope method" into two equal groups of 168 patients each (a total of 336 people, including 10 % of those who may have dropped out) and assigned to receive either Fortelyzin® or Actilyse®. The drugs will be administered no later than 4,5 hours after ischemic stroke onset and after signing the informed consent. Fortelyzin® will be given as single i.v. bolus over

	5-10 seconds. Actilyse® will be administered in accordance with the instruction for use. Patients will be monitored for 90 days: 1-2 days in the Neurological Intensive Care Unit and the Department of Neurology in the remaining days before discharge (on average 14 days), with an outpatient visit on the 90th day. Enrollment of patients into the trial will be conducted on a competitive basis.
Trial duration:	The trial completion date is the 90th day after the final trial patient was included in the trial. All patients will be examined for 90 days
Trial population:	Men and women aged 18 years and older with a diagnosis of ischemic stroke no later than 4.5 hours after the onset of symptoms.
Sample size:	The total number of patients included in the trial – 336 people, including 10 % of possible dropouts
Study drug:	**Fortelyzin®**, registration number – 001941 as of December 18, 2012. **Pharmaceutical form:** Lyophilizate for preparation of a solution for intravenous administration, 5 mg (745,000 IU), supplied with a solvent **Composition:** Active substance: Forteplase® (recombinant protein containing the amino acid sequence of staphylokinase) 5 mg (745,000 IU). Excipients: L-arginine - 15,0 mg, L-histidine – 2,0 mg, glycine – 30,0 mg, povidone-17: 20,0 mg, polysorbate-20: 0,4 mg. Solvent: 5 mL ampule of sodium chloride 0.9%, solution for injection

Inclusion criteria:	• Men and women aged 18-80 (inclusive) years old. • Verified diagnosis of ischemic stroke (neuroimaging (CT/MRI) signs of ischemic stroke, from 5 to 25 points on the NIHSS scale). • Duration of symptoms of ischemic stroke is not more than 4.5 hours before starting thrombolytic therapy. • The patient's consent to use reliable contraceptive methods throughout the trial and within 3 weeks after: – women who have a negative pregnancy test and use the following means of contraception: intrauterine devices, oral contraceptives, a contraceptive patch, long-acting injectable contraceptives, a double-barrier method of contraception. Women who are not at risk of pregnancy for health-related reasons (documented conditions: hysterectomy, tubal ligation, infertility, menopause for more than 1 year) may also be involved in the trial); – men who use barrier contraception. Men who unable to fertilize (documented conditions: vasectomy, infertility) may also be involved in the trial. • A participant provided signed and dated informed consent of participation in the trial.
Exclusion criteria:	• The time of the onset of the first symptoms is more than 4.5 hours from the onset of the disease or the time of the onset of the first symptoms of a stroke is not known (for example, the development of a stroke during sleeping - the so-called "night stroke") • Increased sensitivity to alteplase, gentamicin (residual traces from the production process). • Systolic blood pressure greater than 185 mm Hg or diastolic blood pressure greater than 110 mm Hg or the need for

	intravenous administration of drugs to reduce the blood pressure to these boundaries. • Neuroimaging (CT/MRI) signs of intracranial hemorrhage, brain tumors, arteriovenous malformation, brain abscess, aneurysm of cerebral vessels. • Surgery on the brain or spinal cord. • Suspicion of subarachnoid hemorrhage. • Signs of severe stroke: clinical signs (stroke scale NIH> 25), neuroimaging (according to CT of the brain and/or MRI of the brain in the DWI, the ischemia focuses on the territory of more than 1/3 of the MA pool). • Simultaneous administration of oral anticoagulants, for example, warfarin with INR> 1.3. • The use of direct anticoagulants (heparin, heparinoids) in the 48 hours preceding the stroke with APTT values above the norm. • Prior stroke or severe head injury within 3 months. • Significant regression of neurological symptoms during the observation of the patient. • Mild neurological symptoms (NIH <4 points). • Hemorrhagic stroke or stroke unspecified in medical history. • Strokes of any genesis in the history of a patient with diabetes mellitus. • Gastrointestinal bleeding or bleeding from the genitourinary system in the last 3 weeks. Confirmed exacerbations of gastric ulcer and duodenal ulcer during the last 3 months. • Extensive bleeding, present or during the previous 6 months.

	• Severe liver disease, including liver failure, cirrhosis, portal hypertension (with varicose veins of the esophagus), active hepatitis. • Acute pancreatitis. • Bacterial endocarditis, pericarditis. • Aneurysms of arteries, malformations of arteries and veins. Suspicion of exfoliating aortic aneurysm. • Neoplasms with an increased risk of bleeding. • Large operations or severe injuries within the last 14 days, minor surgery or invasive manipulations in the last 10 days. • Puncture of uncompensated arteries and veins during the last 7 days. • Prolonged or traumatic cardiopulmonary resuscitation (more than 2 min). • Pregnancy, obstetrics, 10 days after birth. • The number of platelets is less than 100,000/μL. • Blood glucose less than 2.8 mmol/l or more than 22.5 mmol/l. • Hemorrhagic diathesis, including renal and hepatic insufficiency. • Data on bleeding or acute trauma at the time of examination. • Seizures in the onset of the disease, if there is an uncertainty that the seizure is a clinical manifestation of ischemic stroke with a postictal residual deficiency.
Trial procedures:	• collection of anamnesis, physical examination, measurement of vital signs (respiratory rate, heart rate, blood pressure)

	• assessment of neurological status according to generally accepted methods with the trial of cerebral, meningeal, and focal symptoms • assessment of neurological status using NIHSS, which allows to quantify the severity of neurological deficits • Brain CT or MRI performed within 40 minutes of the patient's admission to the hospital • angiography of cerebral vessels (if necessary) • duplex scanning of the major arteries of the head (if necessary) • venous blood sampling for complete blood count (with platelet count) and biochemical blood analysis (with glucose level determination), coagulogram (INR, APTT, fibrinogen). The results of the tests should be obtained within 20 minutes from the moment the patient admitted to the hospital • common urine analysis
Efficacy criteria:	• The primary efficacy endpoint is a good functional recovery on day 90 (modified Rankin scale, mRS, 0-1 score). • Secondary combined efficacy endpoint on day 90 – mRS (0-1) + NIHSS (0-1 score) + Barthel Index (BI) - 95 points or higher. • Additional efficacy criterion – NIHSS score after 24 hours and after 90 days.
Safety criteria:	• Safety endpoint - overall mortality on day 90. • Safety endpoint - hemorrhagic transformation (all cases). • Safety endpoint - symptomatic hemorrhagic transformation (as defined by ECASS III). • The number of SAE and AE by organs and systems.

Finally:	Each research center prepares a report, which is signed by the Principal Investigator and approved by the head of the medical institution, where the center is based. The submission deadline is up to 1 month after the completion of the trial. The statistical processing is carried out by an independent statistical expert according to the Case Report Form (CRF).

1. BACKGRAUND FOR THE TRIAL

Ischemic stroke is one of the leading causes of death and disability worldwide. In 2004, the World Health Organization declared stroke a global epidemic. The annual mortality rate from stroke in Russia is one of the highest in the world (175 per 100,000 people). The early 30-day mortality after stroke is 34.6%, and about half of the cases die within a year. Stroke is the leading cause of disability in the population. According to the National Stroke Registry, 31 % of stroke survivors require assistance, and 20% are not able to walk on their own. Only 8% people stroke after can return to their previous work. Stroke imposes special obligations on the family members of the patient, significantly reducing their labor potential.

International experience shows that the decline in mortality from cardiovascular diseases is achieved by a coordinated set of measures, including raising public awareness about the risk factors and prevention of cardiovascular diseases, the introduction of effective prevention programs, and improvement of the medical care system in stroke.

New approaches to the treatment of ischemic stroke include the use of modern and highly effective brain substance reperfusion techniques in the first hours after a stroke. These methods are aimed at restoring blood flow in the damaged vessel, which allows to prevent the development of irreversible damage to the brain substance or reduce its amount, i.e. minimize the severity of residual neurological deficit.

According to the Decrees of the RF Government as of December 27, 2007, No. 1012 and No. 186 as of March 2, 2009, Russia has begun the implementation of measures aimed at improving medical care for cardiovascular patients. Regional Vascular Centers and Primary Vascular Departments have established units for the treatment of patients with acute disorders of cerebral circulation (ADCC). The procedure for providing medical care to patients with ADCC is developed and approved by the orders of the RF Ministry of Health and Social Development of July 6, 2009, No. 389n and No. 44n of February 2, 2010. Within the framework of these measures, modern methods of diagnosis, treatment, rehabilitation, and secondary prevention of stroke are being introduced in the departments for the treatment of

patients with ADCC, including thrombolytic therapy, which is aimed at restoring blood flow in the damaged vessel.

The safety and efficacy of systemic thrombolysis therapy using Actilyse® in patients with ischemic stroke have been proven in a number of large randomized placebo-controlled clinical trials (NINDS, ECASS I, II, III, ATLANTIS), as well as the SITS-MOST and SITS-ISTR registers.

In addition, a high frequency of hemorrhagic transformation (6-23.8%) and a high 3-month mortality rate (18.2%) were noted in some studies with alteplase, which, according to the researchers, is associated with an initially high neurological deficit.

In this regard, the search for new thrombolytic drugs that can improve the efficacy and safety of thrombolytic therapy is an important scientific and economic task.

2. GENERAL INFORMATION ABOUT THE STUDY DRUG

Brand name: Fortelyzin®, valid registration number - 001941 as of December 18, 2012.

Chemical name: recombinant protein, which contains the amino acid sequence of staphylokinase.

Pharmacology and pharmacokinetics

Fortelyzin® refers to the so-called group of fibrin-selective thrombolytics.

The active substance of Fortelyzin® is Forteplase, which is a single-chain molecule consisting of 138 amino acids with a molecular weight of 15.5 kDa.

Forteplase® is an original recombinant protein obtained using genetically engineered *E. coli* and containing the amino acid sequence of modified (non-immunogenic) staphylokinase.

In contrast to the native staphylokinase, 3 amino acids in the immunodominant epitope were replaced in the Forteplase® molecule.

Forteplase® is not an enzyme and activates plasminogen forming a stoichiometric complex at a 1:1 ratio.

Lysine-11 of the N-terminal site of plasminogen and glutamine-46, lysine-50, glutamine-65, and asparagine-69 of the C-terminal site of the drug play a key role in the interaction of Forteplase® and plasminogen.

Along with this, the N-end of the substance acts as a "moving arm" that captures the plasmin molecule and, as a result, a ternary complex composing of plasminogen-Forteplase®-plasmin, is formed.

Depending on different conditions, plasminogen can take three different conformations: α, β, and γ.

Plasminogen was determined to have a closed α-conformation in the bloodstream; a semi-open β-conformation occurs when plasminogen is bound to intact fibrin and a fully open γ-conformation occurs when plasminogen is bound to partially degraded fibrin.

The conversion of plasminogen to plasmin by plasminogen activators requires an open γ-conformation. Forteplase® interacts only with plasminogen in the γ-

conformation and does not bind to plasminogen in the closed form, i.e., which is in the circulation.

The mechanism of Forteplase® fibrinolytic action involves its initial binding to plasmin generated on the fibrin clot with subsequent activation of γ-plasminogen. Fortelyzin®, compared to the classic plasminogen activator Actilyse®, is the only thrombolytic that forms a ternary complex: plasmin-Forteplase-plasminogen activator.

The second mechanism of Forteplase® fibrin selectivity results from the difference in the inhibition rate of the plasmin-Forteplase® complex in the bloodstream and on the surface of fibrin, i.e. on the clot. This complex is neutralized by α_2-antiplasmin in blood plasma 100 times faster than on the fibrin surface.

Fibrin selectivity of Fortelyzin® increases the therapeutic efficacy and prevents the activation of circulating plasminogen, and may also increase the safety of thrombolytic therapy by minimizing exposure to circulating blood fibrinogen.

The thrombolytic fibrin selectivity also causes its dose-dependent effect. The dose of the moderately selective thrombolytic - Actilyse® is 100 mg for AMI patients and 90 mg for patients with ischemic stroke, while the dose of the fibrin-selective thrombolytic- Fortelyzin® - is 15 mg for AMI and 10 mg for ischemic stroke.

Also, Fortelyzin® does not cause the formation of neutralizing anti-staphylokinase antibodies in the blood. In rare cases, the transient formation of anti-drug antibodies (in low titers) may be observed.

After intravenous administration of Fortelyzin®, there is a slight decrease in blood fibrinogen, less than 10 %, within 1 day after administration.

Fortelyzin® has a short half-life. The main organ for excretion of the drug (75%) is the liver.

Pharmacokinetics of a single bolus injection of Fortelyzin® at a dose of 15 mg for AMI patients showed a short half-life (t1/2 α) of 5.7 minutes and t1/2 β in the so-called terminal phase of 30 minutes.

Pharmaceutical form: Lyophilizate for preparation of a solution for intravenous administration.

One vial of Fortelyzin® contains:

Active substance: Forteplase® (a recombinant protein containing the amino acid sequence of staphylokinase), 5 mg (745,000 IU).

Excipients: L-arginine - 15,0 mg, L-histidine - 2,0 mg, glycine - 30,0 mg, povidone - 17:20,0 mg, polysorbate -20:0,4 mg.

Solvent: 5mL ampule of sodium chloride 0.9% solution for injection.

The results of Fortelyzin® preclinical studies

Preclinical study of toxicological properties was conducted in the Russian Cardiology Research Complex of the Ministry of Health of the Russian Federation (Director – Academician Chazov E.I.) in accordance with the requirements set out in the "Manual on Experimental (Preclinical) Study of New Pharmacological Substances" (Moscow, 2005) of the Federal Service for Surveillance in Healthcare and Social Development of the Russian Federation.

A single intraperitoneal administration of Fortelyzin® in the range of tested doses of 1250-28750 mg/kg to BALB/c mice did not cause any signs of intoxication and death of the animals. The highest dose tested on mice -28750 mg/kg - was more than 216,000 times higher than the highest therapeutic dose for humans (10 mg/person or 0.133 mg/kg).

The toxicity study of Fortelyzin® in subchronic experiments, when the drug was administered intravenously to rabbits for 2 weeks at doses of 2.66 mg/kg (substance) and 1.33 mg/kg (dosage form), did not show the damaging effect of the drug on the main organs and body systems of experimental animals. The doses tested were 20 and 10 times higher than the highest therapeutic dose recommended for humans. It was found that Fortelyzin® does not have mutagenic properties.

Also, a 2-week intravenous administration of Fortelyzin® to experimental animals did not establish any local irritant effect of the drug.

At the tested dose of 2.66 mg/kg, Fortelyzin® did not cause any deformities and embryo malformations and did not affect the reproductive function of the animals.

Fortelyzin®, at the doses and sensitization schemes tested, did not reveal any allergizing effect and had no effect on the humoral and cellular immunity, which indicates the absence of immunotoxicity of the drug.

According to the results of the preclinical study, Fortelyzin® was recommended for clinical trials as a fibrinolytic agent – plasminogen activator (Pharmacotherapeutic Group of the State Registration of Medicinal Products).

Fortelyzin® clinical trial according to the Protocol "An open-label, prospective study to evaluate the safety, tolerability, and efficacy of Fortelyzin® (a recombinant protein containing the amino acid sequence of staphylokinase) vs. Actilyse® in patients with acute myocardial infarction (phase I-II)", the authorization of the Federal Service for Supervision in Healthcare (hereinafter–“Roszdravnadzor”) No. 400 dated 01.10.2009, showed the following:

– a bolus and bolus+infusion i.v. injection of Fortelyzin® at a dose of 10 mg in comparison with Actilyse® in patients with acute myocardial infarction with ST-segment elevation (STEMI) showed comparable tolerability and safety of the study drug.

– a bolus and bolus+infusion i.v. injection of Fortelyzin® at a dose of 10 mg in comparison with Actilyse® in patients with acute myocardial infarction with ST-segment elevation (STEMI) showed the efficacy of the study drug comparable to that of Actilyse®;

– it is recommended to increase the dose of Fortelyzin® to 15 mg, administered according to the two schemes: the first scheme: 10 mg bolus + 5 mg bolus with an interval of 30 minutes; the second one: 10 mg bolus + 5 mg infusion for 30 minutes.

The further study assessed a bolus and bolus-infusion of Fortelyzin® at a dose of 15 mg vs. Actilyse® according to the Protocol "An open-label, prospective study of tolerability and efficacy of Fortelyzin® vs. Actilyse® in patients with acute myocardial infarction (phase III)", the authorization of Roszdravnadzor No. 196 dated 28.04.2010.

A total of 90 people were screened in 6 clinics, and 41 patients in the Fortelyzin® study group and 13 patients in the Actilyse® study group were included in the study.

In the Fortelyzin® group, a combination of bolus + infusion was administered to 21 patients, and 20 patients received a double bolus dose.

Assessment of the tolerability of the study drug revealed no serious adverse events (SAE) or hemorrhagic stroke in any of the groups observed. There was one adverse event in the form of "no-reflow" syndrome in the Fortelyzin® group and one bleeding in the Fortelyzin® group (considered as not related to the drug administration) and in the Actilyse® group (related to the drug administration).

There were no allergic reactions, nausea, and vomiting after administration of the drugs in both groups of patients. The maximum drop in blood fibrinogen on the first day after administration of Actilyse® was 38 %, and after administration of Fortelyzin® - only 7 %. The difference is statistically significant.

There was no decrease in blood pressure, increased pulse, and heart rate in both groups of patients.

Thus, the clinical study of bolus and bolus+infusion administration of Fortelyzin® showed their comparable tolerability with Actilyse® in STEMI patients.

Direct and indirect methods were used to assess the blood flow through the coronary arteries.

Coronary angiography was performed as a direct method (within 180 minutes after administration of Fortelyzin® or Actilyse®). Assessment of coronary blood flow was carried out according to the TIMI criteria. Evaluation of the TIMI frame count (TFC), the so-called aggregated frame-by-frame count, was carried out.

As an indirect method, the control of the QRST complex dynamics of the electrocardiogram was performed. When coronary blood flow was restored, there was a rapid decrease in the ST segment in the leads in which it was elevated, and the formation of negative ("coronary") T-waves were observed.

At the patient’s first visit, the following parameters were evaluated: a reduction of > 50% of the initial ST segment in the lead, where its elevation was maximal 3 hours after the administration of drugs; the rate of a reduction of the initial ST

segment in the lead, where its elevation was maximal after 90 and 180 minutes after drug administration in % (30%, 50% and 70% of the initial value); the blood flow through the coronary arteries according to the results of coronary angiography (TIMI and TFC scales).

Table 1. Clinical Efficacy Assessment of Fortelyzin® and Actilyse®

Parameters	Fortelyzin®			Actilyse®
	Double bolus (n = 20)	bolus + infusion (n = 21)	Total (n = 41)	Total (n = 13)
Clinical signs of reperfusion	17	19	36	11
ECG signs of reperfusion	16	19	35	11

Table 1 demonstrates that the clinical efficacy expressed in the absence of signs of angina was observed in 36 patients (88%) in the Fortelyzin® group, with 35 of them (85%) having electrocardiographic (ECG) signs of reperfusion. In the Actilyse® group, 11 out of 13 patients (85%) also had clinical and ECG signs of reperfusion.

Table 2. Angiographic assessment of the efficacy of Fortelyzin® and Actilyse®

Parameters	Fortelyzin®			Actilyse®
	Double bolus (n = 20)	bolus + infusion (n = 21)	Total (n = 41)	Total (n = 13)
TIMI 0	3	2	5	1
1	1	1	2	2
2	6 (30%)	6 (29%)	12 (29%)	6 (46%)
3	10 (50%)	12 (57%)	22 (54%)	4 (31%)
TIMI 2 + 3	16 (80%)	18 (86%)	34 (83%)	10 (77%)

Table 2 shows that after bolus administration of Fortelyzin®, a complete reperfusion of blood flow (TIMI 3) is observed in 10 patients (50%), incomplete reperfusion of blood flow (TIMI 2) in 6 patients (30%), in total – in 16 patients (80 %).

After bolus infusion of Fortelyzin®, the reperfusion of blood flow according to TIMI 3 criteria was observed in 12 patients (57%); according to TIMI 2 criteria – in 6 patients (29%); in total, in 18 patients (86%). The blood flow did not restore in

4 patients (20 %) after bolus injection of Fortelyzin® and in 3 patients (14%) after bolus infusion of the drug.

In total, in the Fortelyzin® group (n = 41), the blood flow was restored in 34 patients (83%), in the Actilyze® group – in 10 patients (77 %). It should be noted that according to TIMI criteria 3, a higher degree of blood flow reperfusion is observed in the Fortelyzin® group (54%) compared with the Actilyse® group (31%).

Thus, the results of the study show the comparable efficacy of the drugs both in bolus and bolus-infusion administration.

Fortelyzin® was recommended for medical use, including its bolus administration at the prehospital stage by emergency medical teams.

According to the results of preclinical and phase I-II and III clinical trials, Fortelyzin® is registered as a medicinal product for medical use: registration certificate LP-001941 as of December 18, 2012.

The project of creating an innovative domestic drug Fortelyzin® involved the participation of leading medical and scientific centers: Lomonosov Moscow State University (MSU), I. M. Sechenov First Moscow State Medical University, RUDN University (the Peoples' Friendship University of Russia), Tomsk Research Institute of Cardiology SB Russian Academy of Medical Sciences and others and took more than 12 years.

When developing the Fortelyzin® substance and pharmaceutical form, the requirements of the Russian, British, European, and American Pharmacopoeias were taken into account.

The production of Fortelyzin® is carried out in compliance with the current regulations and GMP rules in the Russian Federation (License No. 11035-LS-P of February 11, 2011).

Monitoring the efficacy and safety of Fortelyzin®. Continuous monitoring of the efficacy and safety of Fortelyzin® is carried out and covers 1,531 STEMI patients for the period from June 2013 to June 2015.

The average age of STEMI patients who received Fortelyzin® was 65 years, 322 people were older than 75 years old (of the total number of patients, 61% are men). According to the localization of myocardial infarction, the patients were distributed almost equally (Table 3).

Table 3. Main characteristics of patients treated with Fortelyzin®

Number of patients	Sex, m/f	Average age, Min-max (years old)	Number of patients > 75 years old	Localization of infarction		Average door-to-needle time Min-max (min)
				anterior	posterior	
1531	924 / 607	65 32-89	322	789	742	176 40-720

The time from the onset of symptoms of myocardial infarction to the administration of Fortelyzin® was 176 min., which is 10% less than in 40 regions of the Russian Federation implementing the Vascular Program, but 50% higher than in the studies carried out by Tomsk Research Institute of Cardiology and almost 2 times higher than in the STREAM trial.

ECG evidence of myocardial reperfusion was observed in 1,148 patients out of 1,531, which was 75%. Angiography confirmed coronary reperfusion in 141 out of 179 patients (79%), and TIMI 3 was observed in 104 out of 179 patients, which was 58%.

Hemorrhagic stroke occurred in 7 out of 1,531 patients (0.46%), with 2 patients older than 75 years. The 30-day mortality rate was 5.3%. Major non-cerebral bleeding was observed in 3.7%, of which: 1.6% required blood transfusion; minor bleeding from the puncture sites was noted in 10.7% of patients (Table 4).

Table 4. Evaluation of the efficacy and safety of Fortelyzin®

Signs of reperfusion (reduction in the ST elevation by 50 %) (N = 1531)	TIMI (N = 177)			intracranial hemorrhage	Death within 30 days	Major non-cerebral bleeding	Bleeding required blood transfusion	Minor bleeding
	3	2	3 + 2					
1148 74,9 %	104 58 %	37 21 %	141 79 %	7 0,46 % among them 2 > 75 years old	81 5,3 %	56 3,7 %	25 1,6 %	165 10,7 %

These data on the efficacy and safety of Fortelyzin®, based on the monitoring of 1,531 STEMI patients, indicate the results comparable to thrombolytics Actilyse® and Metalyse®.

Since 2015, the original drug Fortelyzin® (a recombinant protein containing the amino acid sequence of staphylokinase) has been included by the RF Government in the updated list of vital and essential medications for medical use.

Background for the dose of Fortelyzin® in patients with ischemic stroke

To find a safe and effective dose of Fortelyzin® for AIS patients, *in vitro* and *in vivo* experiments were performed.

At the first stage, the *in vitro* studies of molecular mechanisms of fibrinolysis of the fibrin-selective thrombolytics used in the treatment of AIS patients were carried out to compare Actilyse® and Purolase with Fortelyzin® used in the treatment of AMI.

The trial was performed in cooperation with the laboratory of fibrinolysis of the MSU Chemistry Department (the head of the laboratory prof. R. B. Aisina, the head of the Department of Chemical Enzymology, the member of the Russian Academy of Sciences (RAS) - S. D. Varfolomeev).

The study of the drug amidolytic activity revealed that Fortelyzin® is more fibrin-selective thrombolytic than Actilyse® and Purolase, which determined its dose:

10 times less than Actilyse® dose and 5 times less than Purolase. It was also found that Fortelyzin® does not bind to fibrinogen placed in a 0.9% NaCl solution *in vitro*, compared to Actilyse® and Purolase, which reduce fibrinogen in the NaCl solution by 50% of its initial value.

Thus, the experimental studies performed *in vitro* to compare Fortelyzin® with Actilyse® and Purolase used in IS patients confirmed the principal possibility for Fortelyzin® application both in AMI patients and in patients with AIS.

Thus, it was necessary to make a comparison of these three drugs on AIS models in animals, the choice of which was associated with their different species specificity. It is known that staphylokinase is fibrin-selective in humans, primates, pigs, and rabbits, neutral in rats, and fibrin-nonselective in dogs, which caused massive hemorrhages and animal death in experimental studies.

Therefore, it was decided to make an experimental AIS model in rabbits.

The creation of such model was a separate scientific task performed jointly with the Laboratory of Drug Toxicology of the Russian Cardiology Research Complex (RCRPC) of the RF Ministry of Health (the head of the laboratory, prof. E. V. Arzamassev, Director – Academician E. I. Chazov). The creation of a rabbit model of acute ischemic stroke is described in detail in the Investigator's Brochure.

Evaluation of thrombolytic efficacy involved the determination of the degree of lysis of the embolus inserted in the right middle-cerebral artery of the rabbit, the size of cerebral infarction before and after injection of thrombolytics, and neurological status of the rabbit according to the modified Bederson scale (MBS) (Bederson JB, et al. Rat middle cerebral artery occlusion: evolution of the model and development of a neurologic examination. Stroke. 1986;17:472-476.) before and after thrombolysis:

A grade of 0 indicates no neurological damage;

A grade of 1 indicates minor to moderate neurological damage;

A grade of 2 indicates severe neurological damage, including hemiparesis, persistent sopor/stupor (Persistent Vegetative State (PVS), and circadian behavior;

A grade of 3 indicates death.

Safety assessment of the thrombolytic use in AIS rabbits involved the determination of the number of deaths, hemorrhagic transformation, bleeding, and the concentration of blood fibrinogen.

Based on the results of the *in vitro* studies of Fortelyzin®, Actilyse® and Purolase, the following doses of the drugs were chosen for their administration to AIS rabbits: Fortelyzin® - 0.133 mg/kg, Actilyse® - 1.33 mg/kg, and 0,665 mg/kg of Purolase, which, based on the rabbit weight equal on average to 3 kg, is 0.4 mg of Fortelyzin®, 4 mg of Actilyse® and 2 mg of Purolase.

These doses correspond to 10 mg of Fortelyzin®, 100 mg of Actilyse®, and 8 mln/IU of Purolase in humans.

Fortelyzin® at a dose of 0.4 mg was injected intravenously as a bolus (5 seconds), and 4 mg of Actilyse® and 2 mg of Purolase were injected intravenously for 30 minutes.

Efficacy evaluation revealed a 50% reduction in the size of the embolus inserted into the right middle cerebral artery of rabbits when using each of the thrombolytics.

The pathomorphological study of the brain of AIS rabbits showed the greatest reduction in the size of brain infarction when using Fortelyzin® – 530 mm^3, Actilyse® – 450 mm^3, Purolase – 400 mm^3 from the initial 1250 mm^3 (the difference between the drugs was not statistically significant).

Injection of Fortelyzin® reduced the neurological damage by 0.91 points of MBS, Actilyse® – 0.65 points of MBS, and Purolase by 0.58 points of MBS from the initial 2.3 MBS scores in each group of the thrombolytics.

Application of Fortelyzin® revealed a high correlation between the reduction in the size of brain infarction and a degree of reduction in the neurological symptoms according to MBS ($r = 0.67$, $p < 0.001$).

Safety assessment of the doses used - Fortelyzin® 0.4 mg, Actilyze® – 4 mg, and Purolase® 2 mg revealed no deaths, hemorrhagic transformations and bleeding in AIS rabbits.

When applying 0.4 mg of Fortelyzin®, the reduction of fibrinogen was 15% from the baseline, when applying Actilyse® and Purolase® – 40% and 52%, respectively.

At the next stage, the efficacy and safety of the double doses of Fortelyzin® (0.8 mg), Actilyse® (8.0 mg), and Purolase® (4.0 mg) in thrombolysis in rabbits with ischemic stroke were evaluated. The double doses studied correspond to 20 mg of Fortelyzin®, 200 mg of Actilyse®, and 16 mln/IU of Purolase® in humans.

Evaluation of the efficacy of double doses of the studied drugs in AIS rabbits has shown that such doses do not increase the area of lysis of the embolus, decrease the volume of brain infarction and reduce the amount of neurological damage according to the modified Bederson scale (MBS).

However, the double doses of Actilyse® and Purolase® led to a catastrophic drop in blood fibrinogen of more than 90%, causing the massive cerebral hemorrhages and death of animals.

The data obtained correspond to the literature data on the application of immunogenic staphylokinase in rabbits with ischemic stroke in comparison with Actilyse® (S. Vanderschueren, et al. Intravenous Thrombolysis With Recombinant Staphylokinase Versus tissue-type Plasminogen Activator in a Rabbit Embolic Stroke Model. Stroke. 1997; 28:1783-1788).

Therefore, the study of the two doses of Fortelyzin® - 0.4 mg and 0.8 mg - in rabbits with ischemic stroke, corresponding to 10 mg and 20 mg of Fortelyzin® in humans, showed their equal effectiveness and greater safety of a dose of 0.4 mg.

In connection with the above, a 10 mg dose of Fortelyzin® was selected for the treatment of patients with ischemic stroke.

First, such choice is due to the fact that the dose of 10 mg equivalent to 0.4 mg of Fortelyzin® in AIS rabbits has shown its high safety and comparable effectiveness with the doses of Actilyse® (4 mg) and Purolase (2 mg) used in ischemic stroke.

Secondly, the 10 mg dose of Fortelyzin® has also shown high efficacy and safety in the clinical trials (phase I and II) (page 18 of this protocol) of Fortelyzin® in comparison with Actilyse® (100 mg) in AMI patients.

Indications

Ischemic stroke in men and women aged 18 and older

After 80 years with caution

The time from the onset of stroke symptoms is less than 4.5 hours.

Contraindications

Cerebral

1. Neuroimaging (CT, MRI) signs of intracranial hemorrhage, brain tumors
2. Hemorrhagic stroke or stroke of an unspecified nature in the anamnesis
3. Rapid improvement or mild symptoms by the time of the beginning of TLT
4. Signs of a severe stroke: clinical [score on the National Institutes of Health Stroke Scale (NIHSS) > 25), neuroimaging (based on CT of the brain and/or MRI of the brain in the diffusion-weighted imaging (DWI) mode, the focus of ischemia extends to the territory of more than 1/3 of the CMA pool)
5. Seizures in the onset of the disease, if there is no certainty that the seizure is a clinical manifestation of ischemic stroke with a postictal residual deficiency
6. Previous stroke or severe traumatic brain injury within 3 months
7. Stroke of any genesis in the history combined with diabetes mellitus
8. Suspected subarachnoid hemorrhage (SAH)
9. Surgical intervention on the brain or spinal cord in the anamnesis

Cerebral and somatic

10. Arterial aneurysms, defects in the development of arteries or veins
11. Tumors with a high risk of bleeding

Somatic

12. Hypersensitivity to any component of the drug
13. Hemorrhagic diathesis

14. Arterial hypertension greater than 185/105 mm Hg or the need for intensive reduction below these figures

15. Bacterial endocarditis, pericarditis

16. Gastrointestinal bleeding or bleeding from the genitourinary system in the last 3 weeks. Confirmed exacerbations of gastric ulcer and duodenal ulcer during the last 3 months.

17. Liver failure (cirrhosis, active hepatitis, portal hypertension)

18. Acute pancreatitis

19. Present bleeding or extensive bleeding in the last 6 months

20. Extensive surgery, trauma, delivery, puncture of non-compressible vessels, cardiopulmonary resuscitation within the last 10 days

21. Pregnancy. Before the study, women of childbearing age will be asked to have a urine pregnancy test (determination of chorionic gonadotropin (HCG) in the urine).

22. Data on bleeding or acute injury at the time of examination.

Laboratory

23. Administration of warfarin and INR > 1,3

24. Use of heparin for 48 hours with increased aPTT

25. Thrombocytopenia less than 100,000/μl

26. Glycemia less than 2.8 and more than 22.5 mmol/l

Posology and method of administration

For Intravenous Use Only!

Fortelyzin® is administered intravenously as early as possible from the onset of clinical symptoms, but no later than 4.5 hours from its onset. The content of each of the two vials of 5 mg (745,000 IU) is diluted in 5 ml of isotonic sodium chloride solution. Fortelyzin® administered intravenously as a bolus dose of 10 mg for 5-10 s. The solution should be prepared immediately before use and should not be stored.

According to clinical guidelines, when conducting thrombolytic therapy with Fortelyzin® in the intensive care unit, it is necessary to ensure that the following vital signs are monitored for at least 24 hours:

* blood pressure;
* heart rate;
* frequency of respiratory movements;
* body temperature;
* oxygen saturation.

During thrombolytic therapy and within 24 h after its completion, it is necessary to monitor the dynamics of neurological status.

Drug-Drug Interactions

Fertilyzin® should not be administered concurrently with other medications. If severe bleeding occurs (especially from non-compressible vessels), the administration of the thrombolytic should be stopped, and fresh frozen plasma should be administered.

Due to the possible development of arterial hypertension observed during thrombolysis with Actilyse®, according to clinical guidelines antihypertensive drugs can be used in dosage forms for intravenous administration:

* angiotensin-converting enzyme inhibitors -enalaprilate (Enap R); however, simultaneous use of angiotensin-converting enzyme inhibitors with alteplase increases the risk of angioedema;
* nitrates – isosorbite dinitrate (isoket), nitroglycerin; however, the frequent side effect of nitrates is headache, which can mask the development of intracranial hemorrhage;
* vasodilators – sodium nitroprusside;
* ganglioblockers-azamethonium bromide (Pentamine);
* alpha-blockers-urapidil (Ebrantil), proxodolol (Albetor);
* calcium channel blockers - nimodipine (Nimotop).

Side effect/Adverse events

Bleeding of varying severity is possible. Bleeding events associated with thrombolytic therapy can be divided into two large groups:

– external bleeding (usually occurred at the sites of blood vessel punctures);

– internal bleeding in any part or cavity of the body.

The possibility of allergic reactions is not excluded.

As a rule, therapeutic doses of Fortelyzin® do not lead to arterial hypotension, but the possibility of its occurrence cannot be excluded.

Experience of using Fortelyzin® in AMI patients with simultaneous use of anticoagulant and dual antithrombotic therapy showed a minimal percentage of hemorrhagic stroke (0.46 %) or major bleeding requiring blood transfusion (1.6%).

However, due to the short half-life of Fortelyzin® and its minimal effect on the blood coagulation system (drop in fibrinogen is less than 10 %), no clotting factor replacement is required.

In the case of bleeding, accompanied by a decrease in hemoglobin by more than 3 g/dl, the use of blood components transfusion is required.

Bleeding that occurs within the first 24 hours after Fortelyzin® administration can be regarded as associated with drug use.

Hemorrhagic transformation of the brain lesion is symptomatic if its development leads to an increase in the total NIHSS score by 4 points or more. In most cases of intracerebral hemorrhages, asymptomatic hemorrhagic transformation is registered after thrombolytic therapy. It is detected by CT or MRI and often accompanied by clinical improvement and is evidence of reperfusion.

If intracerebral hemorrhage is suspected, neuroimaging (brain CT or MRI) must be performed. If necessary, the patient should be consulted by a neurosurgeon.

Overdose (intoxication)

Adverse drug effects from overdose can result in hemorrhagic complications.

In the case of local bleeding – it can be stopped without stopping the drug administration.

If life-threatening bleeding occurs, Fortelyzin® administration should be stopped and fresh frozen plasma or whole blood should be prescribed. If necessary, to neutralize the effect of Fortelyzin®, an anti-fibrinolytic agent can be administered, for example, aminocaproic acid, or tranexamic acid.

3. POTENTIAL BENEFITS AND RISKS TO PARTICIPANTS

The expected benefit of using the drug is to restore normal blood circulation in the ischemic areas of the brain, which will normalize brain functions and avoid further disability of patients.

The possible undesirable effects of Fortelyzin® is the likelihood of bleeding. Local bleeding (injection sites, oral cavity), as a rule, does not require additional treatment.

Internal bleeding may be caused by latent forms of peptic ulcer, esophageal erosion, bleeding from hemorrhoidal veins, esophageal veins, etc. Thus, careful history taking with special attention to the above-mentioned diseases and a clear selection of patients taking into account all contraindications allows, in most cases, to minimize the risk of bleeding.

During thrombolytic therapy in patients with ischemic stroke, hemorrhagic transformation of the focus is possible. However, it is believed that the benefit of using drugs of this group in patients with thrombosis significantly exceeds the risk of bleeding.

During the study, all adverse events will be carefully controlled. If they appear, all available data will be evaluated and appropriate measures will be taken by the staff of the research centers.

All participants in this clinical study are insured. If a patient is harmed in the trial and this harm is a direct result of the action of the study drug and/or medical manipulations used in accordance with the Study Protocol, the Insurance Company undertakes to fully reimburse the participant all expenses for the necessary medical care.

4. REGULATORY FRAMEWORK AND ETHICAL ASPECTS OF THE STUDY

The clinical trial is conducted in accordance with the guidelines of the International Conference on Harmonization Good Clinical Practice (ICH GCP), the ethical principles set out in the World Medical Association Declaration of Helsinki "Recommendations guiding physicians in biomedical research involving human subjects" (1964-2008), the Directive 2001/20/EC of the European Parliament and the requirements of the legislation of the Russian Federation:

- Federal Law No. 61-FZ "On Circulation of Medicines" (as amended of October 11, 2010);
- Federal Law No. 323-FZ of November 21, 2011 “On the fundamentals of health protection in the Russian Federation” (as amended of July 2, 2013);
- ICH Harmonized Tripartite Guidelines for Good Clinical Practice, 1996
- Rules for quality clinical trials in the Russian Federation (OST 42-511-99);
- Rules of clinical practice in the Russian Federation (2003);
- National Standard of the Russian Federation GOST R 52379-2005 "Good Clinical Practice" (approved by the order of the Federal Technical Regulation and Metrology Agency (Rosstandart) No. 232-ST of September 27, 2005);
- RF Government Regulation of September 13, 2010, No. 714 “On the approval of model rules for Compulsory Insurance of Life and Health of a patient involved in clinical trials of a medicinal product” (as amended by the RF Government No. 393 of May 18, 2011);
- Order of the Ministry of Health and Social Development of the Russian Federation No. 774 of August 31, 2010 “On Ethics Board”;
- Guideline for quality clinical trials of medicinal products. Edited by A. N. Mironov, Moscow, 2012;
- RF Government Resolution No. 1012 of December 27, 2007, and No. 186 of March 2, 2009;

- Order of the Ministry of Health and Social Development of the Russian Federation No. 389n of July 6, 2009 “On Approval of Medical Care Procedure to patients with Acute disorders of Cerebral Circulation”;
- Order of the Ministry of Health of the Russian Federation No. 928n of November 15, 2012 “On Approval of Medical Care Procedure to patients with Acute disorders of Cerebral Circulation”;
- Russian clinical guidelines for thrombolytic therapy in ischemic stroke, developed by the Russian Society of Neurologists and the National Stroke Association (Principal Investigator – Academician of RAS E. I. Gusev and corresponding member of RAS V. I. Skvortsova), 2012;
- Methodological guide "Thrombolytic therapy in ischemic stroke", of the Ministry of Health and Social Development of the Russian Federation, edited by a corresponding member of RAS V. I. Skvortsova, Moscow, 2010;
- Recommendations for thrombolytic therapy in patients with ischemic stroke. Moscow, 2014;
- Guidelines for the Early Management of Patients with Acute Ischemic Stroke: A Guideline for Healthcare Professionals from the American Heart Association/American Stroke Association. Stroke, 2013; 44; 870-947.

The trial will be initiated in all research centers only after receiving the written permission to conduct the study, approval of the Ethics Committee, and receiving signatures on the clinical trial protocol of each of the parties involved in the study.

Researchers will be timely familiarized with the materials of the study before it begins. The qualification of researchers will meet the requirements necessary to conduct high-quality clinical trials.

The selection of prospective study participants is made on a volunteers basis.

4.1. Ethics Committee

The materials of the clinical trial (including the protocol, Information Sheet, and materials provided to the patient) will be approved by the Ethics Committee of the

Ministry of Health of the Russian Federation before the start of the study. All amendments to the Protocol and updated versions of the Patient Information Sheet will also be submitted to the Ethics Committee.

The clinical trial will be conducted in accordance with the Protocol approved by the Ethics Committee.

4.2. Patient Information Sheet

Informed consent will be obtained from all prospective study participants before any research procedures are initiated. Prior to this, the Investigator must fully inform a patient about all aspects of clinical research, including its purposes, study procedures, expected risks and benefits of participation, and voluntariness of participation.

The patient must be provided with written information about the study (Information Sheet), approved by the Ethics Committee. The patient's information sheet will contain all information about the planned clinical trial, as well as the terms of confidentiality and the use of the patient’s data. The rights, safety, and well-being of the participants will take priority and prevail over the interests of science and society. If necessary, information about the procedure for obtaining informed consent from patients/volunteers can be submitted to the Ethics Committee.

Prior to the patient's participation in the clinical trial, two (2) copies of the Informed Consent Form with the patient's name must be signed and personally dated by the patient or the patient's legal representative, or by a person who conducted the informed consent discussion. One copy of signed and dated written Informed Consent will be provided to the patient, and the other will be kept by the Investigator.

When the patient's condition does not allow him/her to express his/her will and there is no legal representative and the need for treatment is urgent, the question of medical intervention in the interest of the patient and his/her inclusion in the study is resolved by a council of physicians, and if it is not possible – by his/her attending (duty) physician with following notification of the Principal Investigator, officials of the medical institution and the legal representative.

The Patient Information Sheet and Informed Consent Form must be reviewed and approved by the Ethics Committee. The final version of the Study Protocol, Individual Patient Registration Form, information for the patient, and Informed Consent Form must be approved by the Russian Ministry of Health and the Local Ethics Committee (if any) at the Research Center.

All subsequent amendments to the above-mentioned documents will also be submitted for approval to these institutions. In addition, these same institutions will be informed about all adverse events associated or possibly related to the use of the study drug.

The information obtained during the study that may reveal the patient's identity will be kept secret and can only be disclosed within the limits established by law.

5. TRIAL AIM

Assessment of the efficacy and safety of single-bolus intravenous injection of Fortelyzin® in comparison with bolus infusion of Actilyse® in patients with ischemic stroke.

6. TRIAL OBJECTIVES

- To assess the efficacy of single-bolus intravenous injection of Fortelyzin® in comparison with bolus infusion of Actilyse® in patients with ischemic stroke.
- To assess the safety and possible adverse events of single-bolus intravenous injection of Fortelyzin® in comparison with bolus infusion of Actilyse® in patients with ischemic stroke.

7. TRIAL POPULATION

Description of the trial population

This trial is planned to involve adult patients of both sexes aged 18 years and older with a verified diagnosis of ischemic stroke.

7.1. Inclusion criteria

- Men and women aged 18-80 (inclusive) years old.
- Verified diagnosis of ischemic stroke (neuroimaging (CT/MRI) signs of ischemic stroke, from 5 to 25 points on the NIHSS scale).
- Duration of symptoms of ischemic stroke is not more than 4.5 hours before starting thrombolytic therapy.
- The patient's consent to use reliable contraceptive methods throughout the trial and within 3 weeks after:

– women who have a negative pregnancy test and use the following means of contraception: intrauterine devices, oral contraceptives, a contraceptive patch, long-acting injectable contraceptives, a double-barrier method of contraception. Women who are not at risk of pregnancy for health-related reasons (documented conditions: hysterectomy, tubal ligation, infertility, menopause for more than 1 year) may also be involved in the trial.);

– men who use barrier contraception. Men who unable to fertilize (documented conditions: vasectomy, infertility) may also be involved in the trial.

- A participant provided signed and dated informed consent of participation in the trial.

7.2. Exclusion criteria

- The time of the onset of the first symptoms is more than 4.5 hours from the onset of the disease or the time of the onset of the first symptoms of a stroke is not known (for example, the development of a stroke during sleep - the so-called "night stroke").

- Increased sensitivity to alteplase, gentamicin (residual traces from the production process).
- Systolic blood pressure greater than 185 mm Hg or diastolic blood pressure greater than 110 mm Hg or the need for intravenous administration of drugs to reduce the blood pressure to these boundaries.
- Neuroimaging (CT, MRI) signs of intracranial hemorrhage, brain tumors, arteriovenous malformation, brain abscess, aneurysm of cerebral vessels.
- Surgery on the brain or spinal cord.
- Suspicion of subarachnoid hemorrhage.
- Signs of severe stroke: clinical signs (stroke scale NIH> 25), neuroimaging (according to CT of the brain and/or MRI of the brain in the DWI, the ischemia focuses on the territory of more than 1/3 of the MA pool).
- Simultaneous administration of oral anticoagulants, for example, warfarin with INR> 1.3.
- The use of direct anticoagulants (heparin, heparinoids) in the 48 hours preceding the stroke with APTT values above the norm.
- Prior stroke or severe head injury within 3 months.
- Significant regression of neurological symptoms during the observation of the patient
- Light neurological symptoms (NIH <4 points)
- Hemorrhagic stroke or stroke, unspecified in history.
- Strokes of any genesis in the history of a patient with diabetes mellitus.
- Gastrointestinal bleeding or bleeding from the genitourinary system in the last 3 weeks. Confirmed exacerbations of gastric ulcer and duodenal ulcer during the last 3 months.
- Extensive bleeding, present or within the previous 6 months.

- Severe liver disease, including liver failure, cirrhosis, portal hypertension (with varicose veins of the esophagus), active hepatitis.
- Acute pancreatitis.
- Bacterial endocarditis, pericarditis.
- Aneurysms of arteries, malformations of arteries and veins. Suspicion of exfoliating aortic aneurysm.
- Neoplasms with an increased risk of bleeding.
- Large operations or severe injuries within the last 14 days, minor surgery or invasive manipulation in the last 10 days.
- Puncture of uncompensated arteries and veins during the last 7 days.
- Prolonged or traumatic cardiopulmonary resuscitation (more than 2 min).
- Pregnancy, obstetrics, 10 days after delivery.
- The number of platelets is less than 100,000/μL.
- Blood glucose less than 2.8 mmol/l or more than 22,5 mmol/l.
- Hemorrhagic diathesis, including renal and hepatic insufficiency.
- Data on bleeding or acute trauma at the time of examination.
- Seizures in the onset of the disease, if there is no certainty that the seizure is a clinical manifestation of ischemic stroke with a postictal residual deficiency.

7.3. Trial completion and post-trial therapy

The trial for a patient will be considered completed after the 6th visit on the 90th day after thrombolysis.

7.4. Criteria for early termination of trial participants

A patient should be withdrawn from the trial immediately after any of the following situations occur:

- a patient or his/her legal representative or attending physician sends a request to exclude the patient from the trial, based on the withdrawal of Informed Consent (the patient's unwillingness to continue participation in the trial);
- pregnancy;
- significant violation of the Protocol procedures;

The reason for each unscheduled termination should be noted in the Case Report Form (CRF) in the section "Trial Completion". Follow-up will be performed for all patients who have dropped out of the trial due to adverse events or other safety parameters.

Follow-up will also be performed for all patients who have dropped out of the trial for other reasons, but also experience adverse events or have other safety parameters that might lead to discontinuation of therapy. In the case of early termination, the treatment of the underlying disease continues in accordance with recommendations and health standards.

Follow-up should be continued until the patient's condition is restored; until the diagnosis of an adverse event or a change in the safety parameter is stabilized or established chronically; as long as there are clinical indications for follow-up, or the duration of follow-up is determined by an attending physician-investigator.

In the case of pregnancy, every effort should be made to fully monitor the course of pregnancy until the moment of delivery, and child growth.

Randomized patients who did not complete the trial for reasons including those related to the trial drug will be included in the analysis of efficacy and safety data. Replacement of the removed randomized patients with newly included ones is not provided.

7.5. Criteria for early termination of the trial

The trial may be completed prematurely:

• if the trial reveals serious adverse events associated with the use of the trial drug and makes its further use unacceptable from an ethical point of view;

• when new information is received that indicates a high risk for the trial participants;

• in the case of circumstances considered as force majeure events;

• at the request of the Federal Regulatory Authorities and at the decision of the Sponsor.

7.6. Exclusion of Research center

The trial conducted at a separate clinical center may be terminated if the Sponsor, Investigator, Regulatory Authorities, or the Ethics Committee of the clinical center deem it necessary for any reason.

8. DESIGN AND PLAN OF RESEARCH ACTIVITIES

The current trial is a multicenter, open-label, randomized, parallel-group, phase III b non-inferiority trial to compare the efficacy and safety of the trial drug.

The trial will include 336 patients with ischemic stroke. The patients will be monitored for 90 days: 1-2 days in the Neurological Intensive Care Unit and the Department of Neurology in the remaining days before discharge (on average 14 days), with an outpatient visit on the 90th day.

Start of research, initiation of centers: «_____» _____20___

Enrollment of patients: «_____» _____20___

Result processing and reporting: «_____» _____20___

The enrollment period may be extended to total patient enrollment if a research center fails to enroll the planned number of patients. The end date of the trial is considered to be the end date of the trial in all patients involved.

In each research center, the subject of the trial is assigned a serial number that corresponds to the sequence of patient inclusion in the trial. The serial number is reflected in the Case Report Form (CRF). The list of patient's identification is stored in the trial file. Patients are included in the trial only after signing the Informed Consent Form.

Before the trial, all patients will undergo the following procedures: collection of thorough medical history, measurement of vital signs, physical examination, assessment of neurological status, CT/MRI scans of the brain, duplex scanning, and angiography of the brain vessels (if necessary), ECG, blood and urine samples for laboratory tests:

***complete blood count**: hemoglobin, red blood cells, hematocrit, leukocyte formula: neutrophils, eosinophils, basophils, monocytes, lymphocytes, platelets, ESR;

***biochemical blood parameters:** total protein, creatinine, urea, AST, ALT, CK, total bilirubin, glucose, sodium, potassium, cholesterol, HDL-C, triglycerides;

***coagulogram:** fibrinogen, activated partial thromboplastin time (aPTT), INR, prothrombin;

***common urine analysis:** color, transparency, reaction, density, glucose, protein, bilirubin/urobilinogen, white blood cells, red blood cells.

After enrollment, patients will be randomized using the "envelope method" into two equal groups of 168 patients each (a total of 336 people, including 10% who may have dropped out) and assigned to receive either Fortelyzin® or Actilyse®. Fortelyzin® will be given as single i.v. bolus over 5 - 10 seconds. Actilyse® will be administered following the instruction for use.

To compare the efficacy and safety of Fortelyzin® vs Actilyse®, patients will be monitored for 90 days after administration of the drug. During this period, patients will have 6 visits (Table 5).

Table 5. Plan of the trial

	V 1	V 2	V 3	V 4	V 5	V 6
	0	1 day	2 days	7 days	14 days	90 days
Signing Informed consent	+	–	–	–	–	–
Demographic data	+	–	–	–	–	–
Previous treatment	+	–	–	–	–	–
CT/MRI SCAN	+	+	–	+	–	–
ASPECTS	+	+	–	+	–	–
Dynamics of neurological status	–	+	+	–	–	–
Changes by organs and systems	–	+	+	+	+	–
TOAST criteria	–	–	+	–	–	–
Medical history	+	–	–	–	–	–
Measurement of vital signs	+	+	+	+	+	+
Physical examination	+	–	–	–	–	–
Concomitant diseases	+	–	–	–	–	+
NIHSS	+	+	+	+	+	+
Bartel	–	–	–	+	+	+
Rankin	+	–	+	+	+	+
Meningeal symptoms	+	+	+	+	+	–
ECG	+	–	–	+	+	–
Duplex scanning of the major arteries of the head (if necessary)	+	–	–	+	–	–
Coagulogram	+	+	+	+	+	–
Complete blood count	+	–	+	+	+	–
Biochemical blood analysis	+	–	–	+	+	–
Blood sampling for **Pharmacokinetic** analysis	–	+	–	–	–	–
Common urine analysis	+	–	+	+	+	–
Inclusion/Exclusion criteria	+	–	–	–	–	–
Angiography of cerebral vessels (if necessary)	+	–	–	–	–	–
Administration of Fortelyzin® or Actilyse®	–	+	–	–	–	–
Change in concomitant therapy	–	+	+	+	+	+
Registration of AE	–	+	+	+	+	+
Bleeding assessment	–	+	+	+	+	–

9. TRIAL PROCEDURES

Visit 1 (Start of the trial)

Prior to administration of the trial drug, all patients will undergo the following procedures:

- determination of inclusion/non-inclusion criteria
- signing of Informed consent
- collection of demographic data (date of birth, sex)
- collection of medical history (duration of the underlying disease, previous therapy, allergological anamnesis, etc.)
- physical examination
- evaluation of changes by organs and systems
- assessment of neurological status using the NIHSS and modified Rankin scales (mRS)
- Duplex scanning of the major arteries of the head (if necessary)
- CT/MRI examination of the brain
- assessment of CT changes according to ASPECTS (Alberta Stroke Program Early CT Score)
- angiography of the cerebral vessels (if necessary)
- blood sampling for hematological and biochemical analyses
- coagulogram
- common urine analysis
- ECG
- assessment of the patient's compliance with the inclusion/exclusion criteria
- randomization

Assignment of individual patient identification code (IPIC)

The Investigator must ensure the anonymity of the trial subjects. The full names of all patients participated in the trial are strictly confidential.

After signing the Informed Consent Form, each patient will be assigned an Individual Patient Identification Code (IPIC) in accordance with the current legislation (Table 6).

Table 6. Individual Patient Identification Code

Permission of the Ministry of Health of the Russian Federation No.	Permission's date of issue, DD/MM/YYYY/	Serial number of the medical organization specified in the Permission	Patient's initials	Patient's date of birth, DD/MM/YYYY/	A unique number assigned to the patient by the Investigator, consisting of letters and numbers (the name of the protocol and the screening number under which the patient was included in the trial)

The individual patient identification code (IPIC) is established by the Insurant based on the patient data provided by the Investigator. The individual patient identification code is reported by the Insurant to the Investigator for inclusion in his medical documentation. The IPIC will be reflected in the primary documentation, Case Report Form, and Patient Information Sheet with the informed consent form. The IPIC will be transmitted by the Code Registry by the Insurant to the Insurer.

The individual identification code assigned to the patient is not subject to change.

The Investigator should maintain a separate Identification Registry containing the individual numbers, surnames, dates of birth, addresses, phone numbers, and medical records (if available). Only the initials, dates of birth, and unique patient numbers that are part of the Individual Patient Identification Code will be used in the report and trial documentation.

The randomization plan will be known by the Principal Investigator and Regulatory Authorities.

Visit 2.

- Administration of Fortelyzin® or Actilyse®.
- On Visit 2, the changes in neurological status will be recorded, as well as registration and evaluation of bleeding and adverse events based on patient complaints, data from neurological, physical, and laboratory-instrumental studies.

Table 7. Monitoring of parameters at the moment of thrombolysis

Trial parameter	**Frequency of examination**
BP	Every 15 minutes to 2 hours after the start of thrombolysis
Vital signs	Every 30 minutes for 6 hours
NIHSS	Every 15 minutes for 1 hour
Meningeal symptoms	Every 15 minutes for 1 hour

Table 8. Monitoring of parameters during the first day (24h) after thrombolysis

Trial parameter	**Frequency of examination**
BP	Every hour until the end of the day (within 24 hours)
Vital signs	Every 30 minutes to 6 hours, then every hour until the end of the day
Meningeal symptoms	Every hour for 24 hours
NIHSS	Every hour for 24 hours

- CT/MRI 24 hours after TLT or if the patient's condition worsens
- assessment of CT changes according to ASPECTS
- registration of adverse events (AE)
- assessment of bleeding

All adverse events identified should be recorded in the Case Report Form (CRF). The Investigator should report the adverse events to Roszdravnadzor within the next 24 hours. The assessment of bleeding is carried out according to the corresponding scales (see Safety assessment). Monitoring of coagulogram parameters (fibrinogen) will be carried out within 24 h from the time of Fortelyzin® or Actilyse®

administration. CT/MRI examination of the brain will be repeated 24 hours after the thrombolysis or if the patient's condition worsens.

During the visit, patients will receive treatment in accordance with the Russian Clinical Guidelines for Thrombolytic Therapy in Ischemic Stroke, 2015.

Visit 3.

- verification of the stroke subtype according to TOAST criteria
- measurement of vital signs
- physical examination
- assessment of neurological status scales scores
- coagulogram
- complete blood count
- common urine analysis

Visit 4.

- measurement of vital signs
- physical examination
- assessment of neurological status scales scores
- CT/MRI scans
- assessment of CT changes according to ASPECTS
- ECG
- ultrasound examination of brain vessels (if necessary)
- coagulogram
- complete blood count
- biochemical analysis of blood
- common urine analysis.

Visit 5.

- measurement of vital signs
- physical examination
- assessment of neurological status scales scores

- ECG
- coagulogram
- complete blood count
- biochemical analysis of blood
- common urine analysis.

During visits 2-5, information about adverse events and bleeding will be collected and recorded into the Case Report Form. The data on adverse events will be shared with the Sponsor. In addition, information about changes in concomitant therapy will also be recorded.

Visit 6.

- measurement of vital signs
- collection of information about SAE and AE, as well as recording the Bartel index scores, Rankin and NIHSS scale scores
- collection of information about concomitant diseases.

Unscheduled visits

Unscheduled visits may be made at the discretion of the Investigator or at the initiative of the patient. For all unscheduled visits, the corresponding CRF page should be filled out.

9.1. Description of some research procedures

9.1.1. Anamnesis

Collection of the anamnesis of the studied disease will be carried out during the screening, as well as information on significant past or current concomitant diseases (for example, allergic reactions, congenital and acquired heart and vascular diseases, other serious diseases, organ transplantation, mental disorders).

9.1.2. Collection of information about concomitant and previous therapy

All medications taken within 30 days prior to screening must be registered in the primary documentation. Separately, it will be clarified whether the patient took any prohibited drugs that prevent his/her inclusion in the trial.

9.1.3. Physical examination

Physical examination should be performed by the research physician. It includes (at a minimum) assessment of the patient's general condition and the following body systems: lymph nodes, mouth, and pharynx, lungs, cardiovascular system, abdominal cavity, extremities, musculoskeletal system, nervous system, and skin.

All changes considered clinically significant should be reported as an anamnesis before the study drug administration, and after the patient received the study drug, all changes should be reported as adverse events.

9.1.4. Vital signs

Vital signs include blood pressure (BP), heart rate (HR), and respiratory rate (RR), which must be measured and recorded by the staff of the research center.

Measurements of blood pressure and heart rate should be taken at the same body position throughout the trial.

9.1.5. Electrocardiography

Electrocardiography (ECG) is performed in accordance with normal clinical practice. A prerequisite is the registration of all 12 standard leads.

ECG interpretation is performed by an authorized researcher. It is necessary to indicate the date of ECG registration and provide a general conclusion. ECG records should be kept in the patient's medical records.

9.1.6. Complete and biochemical blood analysis

The tests are carried out in accordance with the methodology adopted in the clinical center laboratory. The following parameters will be measured:

– complete blood count (number of red blood cells, white blood cells, platelets, hemoglobin, hematocrit, leukocyte formula, ESR)

– biochemical blood parameters (glucose, ALT, AST, bilirubin, creatinine, total protein, sodium, potassium, CK, urea, cholesterol, HDL-C, triglycerides)

– coagulogram (fibrinogen, INR, APTT, prothrombin index)

In the case of clinically significant abnormalities, these phenomena are recorded as a concomitant disease (during the screening) or as an adverse event (during the trial).

9.1.7. Common urine analysis

To perform the analysis, approximately 50 ml of the first-morning portion of urine is taken, and the analysis is carried out according to local practice. The following parameters are measured:

– General characteristics (color, transparency, pH, density, protein, glucose, bilirubin/urobilinogen).

– Microscopy of urinary sediment (red blood cells, white blood cells, epithelium, cylinders, bacteria, salts).

In the case of clinically significant abnormalities, these phenomena are recorded as a concomitant disease (during the screening) or as an adverse event (during the trial).

10. THERAPY DURING THE TRIAL

Study drug: Fortelyzin®

Manufacturer: Supergene, LLC, Russia

Drug description

Pharmaceutical form: lyophilizate for preparation of a solution for intravenous administration**,** supplied with a solvent

Detailed description:

Lyophilizate: tablet-like freeze-dried mass of white or almost white color.
Solvent: colorless transparent liquid.
Reconstituted solution: colorless, transparent or slightly opalescent liquid.

Composition:

Active substance: Forteplase® (recombinant protein containing the amino acid sequence of staphylokinase) 5 mg (745,000 IU).
Excipients: L-arginine - 15,0 mg, L-histidine - 2,0 mg, glycine - 30,0 mg, povidone - 17:20,0 mg, polysorbate -20:0,4 mg.

Solvent: 5mL ampule of sodium chloride 0.9% solution for injection

Dosage regimen: Fortelyzin® will be assigned at a dose of 10 mg (1,490,000 IU).

Route of administration: intravenously in the form of a single bolus injection for 5-10 sec. The solution of Fortelyzin® is prepared immediately before administration: the content of a 5 mg vial (745,000 IU) is diluted in 5 ml of 0.9% sodium chloride solution. Non-storable!

Labeling: «Product for clinical trial only».

Active Comparator: Actilyse®

Manufacturer: Boehringer Ingelheim Pharma GmbH & Co. KG

Drug description

Pharmaceutical form: lyophilizate for preparation of a solution for infusion supplied with a solvent.

Detailed description:

Lyophilizate: freeze-dried mass of white or light yellow color.

Solvent: colorless transparent liquid

Composition:

Active substance: Alteplase 50,0 mg

Excipients: L-arginine: 1742.0 mg,

Phosphoric acid, 85 %: up to pH 7.2 ± 0.2,

Polysorbate 80: 3.5-5.0 mg.

Note

The excess is 3.5 % of the nominal amount of the active ingredient and excipients in the vial.

Residual traces: gentamicin (used in the production process).

Each vial of solvent contains:

Water for injection: 50 ml

1 ml of the solution after dilution contains 1 mg of alteplase.

Dosage regimen:

Actilyse ® is administered in accordance with the instructions for use in ischemic stroke at a dose of 0.9 mg/kg, maximum 90 mg, if a patient's weight exceeds 55 kg:

- 10% of the dose is administered intravenously via a syringe for one minute (bolus);
- the main dose of the drug (90% of the dose) is administered within 60 minutes (using a syringe dispenser / infusomat);

- during the infusion of the main dose, it is allowed to stop and continue the administration of Actilyse®, but the total duration of drug administration cannot exceed one hour after bolus (10% of the dose).
- Labeling: «Product for clinical trial only».

Previous treatment

Every effort should be made to find out what treatment the patient received within 30 days before administration of the study drug. The names of the drugs, the start and end dates of their use should be registered in the CRF.

Permitted concomitant therapy

In the first 24 hours after thrombolysis, the use of drugs to lower blood pressure is allowed: angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists. If blood pressure drops during thrombolysis, the use of vasopressors (dobutamine, dopamine, and norepinephrine) is allowed.

In the case of allergic reactions during thrombolysis, the use of glucocorticoids and antihistamines is allowed.

If severe bleeding (especially from non-compressible vessels) occurs, the thrombolytic should be stopped and administration of fresh frozen plasma or fresh blood should be performed.

After 24 hours after thrombolysis, the use of antiplatelet agents – ASA, clopidogrel, ticlopidine, ticagrelor, anticoagulants: unfractionated or low-molecular-weight heparin, warfarin, dabigatran, bivalirudin, apixaban, rivaroxaban, fondaparinux is allowed. These drugs are permitted after thrombolysis only after CT/MRI examination to exclude intracranial hemorrhages (symptomatic and asymptomatic) and hematomas (Clinical recommendations for thrombolytic therapy in ischemic stroke, 2015).

In the absence of hemorrhagic transformation, the use of ASA is allowed during the entire follow-up period (90 days) after thrombolysis.

Prohibited concomitant therapy

In this trial, the use of antiplatelet agents (ASA, clopidogrel, ticagrelor, ticlopidine), anticoagulants (NPH, NMG, fondaparinux), oral anticoagulants (phenylin, syncumar), vitamin K inhibitor (warfarin), direct inhibitors of thrombin and blood clotting Factor X (dabigatran, bivalirudin, apixaban, rivaroxaban), glycoprotein IIb/IIIa receptor blockers, drugs that affect the rheological properties of blood (agapurin, vinpocetine) is prohibited within 24 hours after thrombolysis.

11. EFFICACY ASSESMENT

This trial involves a comparison group. Evidence of Fortelyzin® efficacy will be compared with the results of the application of Actilyse®.

Criteria for the efficacy evaluation of thrombolytic therapy

- The primary efficacy endpoint is a good functional recovery on day 90 (modified Rankin scale (mRS), 0-1 score).
- Secondary combined efficacy endpoint on day 90 – mRS (0-1) + NIHSS (0-1 score) + Barthel Index (BI) - 95 points or higher.
- Additional efficacy criterion – NIHSS score after 24 hours and after 90 days.

12. SAFETY ASSESSMENT

- Safety endpoint - overall mortality on day 90.
- Safety endpoint - hemorrhagic transformation (all cases).
- Safety endpoint - symptomatic hemorrhagic transformation (as defined by ECASS III).
- The number of SAE and AE by organs and systems.

The assessment of safety is based on the number and severity of adverse events that may occur in the course of the trial. The drug is considered as well tolerated if no severe adverse events are registered during the trial (fatal outcome or life-threatening conditions of the patient), which, according to the Investigator, may be associated with the application of the drug studied.

Adverse events

Adverse event (AE) is any unfavorable medical occurrence in a patient or clinical trial subject administered a pharmaceutical product, which may not have a causal relationship with its use. Thus, an adverse event can be any adverse symptom (including deviation of the laboratory value from the norm), a complaint, or a disease, the time of occurrence of which does not exclude a causal relationship with the use of the medicinal product, regardless of the presence or absence of such communication.

Information about adverse events should be collected at each visit by interviewing the patient, as well as during physical examination and evaluation of laboratory and instrumental data. All adverse events that occur during the trial should be evaluated and documented.

When registering an adverse event, the following information should be reflected, if possible:

- the exact time of AE occurrence и разрешения
- the nature of the adverse event
- severity (mild, moderate, severe)
- causal relationship with the study drug
- whether the adverse event is serious
- actions taken (whether AE required any therapeutic measures)
- outcome

Adverse Event Grading:

- **Mild**: the presence of signs or symptoms that do not affect daily activities.
- **Moderate**: event has sufficient intensity to interfere with daily activities.
- **Severe**: inability to work or perform daily activities.

The connection between the development of an adverse event and the use of the study drug is determined by the WHO scale:

- ***Certain*:** Clinical manifestations of unforeseen adverse reaction (UAR), laboratory findings deviations that occur during the period of drug administration, can not be explained by the presence of existing diseases and the influence of other factors.

Manifestations of UAR regress after the drug discontinuation and occur again after repeated administration.

- ***Probable***. Clinical manifestations of UAR, laboratory findings deviations are associated with the time of drug administration, unlikely to be related to concomitant diseases or other factors, and which regress with drug withdrawal. Response to the drug re-challenge is unknown.
- ***Possible***. Clinical manifestations of UAR, laboratory findings deviations associated with the time of drug administration, but they can be explained by the presence of concomitant diseases or other drug administration and the influence of chemical compounds. Information on drug withdrawal response is unclear.
- ***Questionable***. Clinical manifestations of UAR, changes in laboratory findings occur in the absence of a clear temporal association with the product administration; other factors are present (medicinal products, diseases, chemicals), which may be the cause of their occurrence.
- ***Unlikely***. Clinical manifestations of UAR, laboratory findings deviations related to UAR, are difficult to assess. Additional data are needed for assessment, or the data are currently being analyzed.
- ***Unclassified***. Reports on suspected UAR cannot be assessed, due to insufficient information, or it is contradictory.

In the case of adverse events, the choice of measures (follow-up, non-drug or drug therapy, hospitalization, and/or prolongation of existing hospitalization) is made by the physician-investigator based on the patient's interests to create optimal conditions and provide the patient with needed medical care.

Patients are monitored in the center until the full completion of any adverse event that occurred during the trial, and in the case of the development of a chronic disease - until the condition is stabilized. Adverse events in the form of changes in laboratory findings are observed until the normalization of the values or until the diagnosis of the chronic disease that caused such changes.

Serious adverse event (SAE)

A serious adverse event (SAE) is defined as any adverse event (AE) that at any dose results in:

* death and/or disability;
* development of a life-threatening condition;
* prolongation of existing hospitalization;
* development of congenital anomalies or malignant tumors.

In addition, any severe bleedings will be considered as SAEs in this trial:

- "major bleeding" according to the TIMI classification
- "severe or life-threatening bleeding" according to the GUSTO classification
- symptomatic intracerebral hemorrhage (SICH) was defined as any hemorrhage with neurologic deterioration, as indicated by an NIHSS score for 4 points and more than its value at baseline or the lowest one at 7 days after thrombolysis or any hemorrhage leading to death and had been identified as a predominant cause of neurologic deterioration (definition ECASS III)

Adverse events that do not fall into these categories should not be regarded as serious.

Any SAE, regardless of the causal relationship with the drug under trial, must be reported to Roszdravnadzor no later than 24 hours after the Investigator became aware of the SAE.

All paragraphs in the appropriate SAE report form must be filled in. Information about the SAE should be included in the Case Report Form.

Bleedings

All bleeding events should be registered in the patient's CRF. Each bleeding is classified according to the GUSTO and TIMI scales. Bleeding that is classified according to TIMI as "major" or "severe or life-threatening " – according to the GUSTO scale are considered as SAE in this trial.

Safety assessment is carried out based on the number and severity of AEs and SAEs detected during the trial.

If a serious adverse event occurs, the Investigator must:

- provide (if necessary) appropriate qualified medical care, including laboratory and instrumental studies;
- inform Roszdravnadzor within 24 hours about the occurrence of SAE and send (fax, e-mail) a Report form on a serious adverse event;
- indicate all adverse events in the patient's CRF;
- continue to monitor the patient’s condition until the SAE is fully completed.

In addition, if there is major or moderate bleeding, its localization is indicated:

- gastrointestinal tract;
- urogenital tract;
- retroperitoneal space;
- central nervous system;
- parenchymal organs.

In minor bleeding occurs, the immediate cause of its occurrence and/or localization is indicated:

- puncture;
- damage to blood vessels;
- bleeding from the gums.

13. CASE REPORT FORM

Primary data means all the information contained in the original medical records and their certified copies, describing the results of clinical observations, examinations, and other activities, allowing to restage the trial and assess it. Primary data is included in the primary documentation (originals or certified copies thereof).

Data entered in the CRF will be confirmed by other primary documents.

For the tests performed in the central laboratory, it is necessary to attach a copy of the analysis results to the CRF.

All information obtained during the trial about the patient's condition, data from laboratory and instrumental studies, and adverse events are recorded in the CRF.

Erroneous records are crossed out with a single line so that it can be read. Correct records are made nearby. All records and corrections are dated and signed by the physician-investigator.

The original documents should be available for periodic review. The original CRFs are transferred to the Sponsor upon completion of the trial.

14. DRUG STORAGE AND ACCOUNTING

The study drug will be properly labelled. The label will indicate the name and dose of the drug, expiration date, storage conditions. The drug package will have an inscription: ”For clinical trial use only”. It is unacceptable to use the drug in patients not included in this trial.

The drug is delivered to a research center on demand and stored in a dry place at a temperature of 2-25 °C. Only Investigators have access to the drug. The packaging of the used drug must be preserved and returned to the Sponsor.

The Regulatory Authorities will require accounting for all the necessary drugs received by each research center. The following information should be entered into the register: the date of issue, date of administration, dose, and patient to whom the drug was administered. When using each dose of the drug, the corresponding record is made in the patient's medical history and in the patient's CRF (date and time, batch number of the drug, and the initials of the person who prepared the drug for administration).

The patient's number, date, and time of administration should also be indicated on the drug package.

Drug accounting will be controlled by the Monitor during visits to the research center.

At the end or termination of the trial, the form for accounting the trial product should be completed and verified; all discrepancies should be clarified, and their cause should be indicated in the documentation. According to the requirements of the Regulatory Authorities, all unused research materials must be returned to the Sponsor at the end of the trial. It is allowed to dispose of empty vials in the research center, but only after the use of the drug has been checked by the Monitor.

15. CONFIDENTIALITY

Confidentiality of a patient’s personal data is protected by the current laws and legislation. The names and surnames of all patients participating in the trial are strictly confidential. Participants are identified by their initials and the individual number assigned.

The surname and other personal information will not be included in the reports and publications related to this trial. Direct access to the medical records will be available to the trial Monitor, the audit, and representatives of the control bodies when verifying the authenticity of the information obtained during the trial.

16. PARTICIPATION IN THE TRIAL AND COMPENSATION

No payments and/or compensation are provided for participation in this trial. The trial drug, medical examinations, laboratory, and instrumental studies are free of charge for all participants. The patient has the right to information about the results of medical examinations.

In accordance with Article 44 of the Federal Law of April 12, 2010, No. 61-FZ "On the Circulation of Medicines", all patients involved in a clinical trial are subject to compulsory life and health insurance. In the case of harm to the patient's health as a

result of the clinical trial, the patient is obliged to provide the Insurer with an individual identification code for receiving the insurance premium.

17. CALCULATION OF SAMPLE SIZE

No multicenter randomized clinical trials of thrombolytic therapy in ischemic stroke have been conducted in the Russian Federation before. Available scientific literature is related to the pilot application of Actilyse® in a limited number of AIS patients (10-16 people) (V. I. Skvortsova et al., 2006, N. A. Shamalov, 2015). Thus, the data of the SITS-MOST international register and the results of randomized multicenter clinical trials of Actilyse® in comparison with placebo in patients with ischemic stroke were used as the basis for the sample size justification.

The calculation of the number required was carried out taking into account the capacity of 80 % and planning of a non-inferiority study in parallel groups [V. I. Sergienko, I.B. Bondareva "Mathematical statistics in clinical trials"; GEOTAR-Media, Moscow, 2006]. The null hypothesis (H_0) was that the thrombolytic therapy with Fortelyzin® is worse than the standard thrombolytic therapy with Actilyse®. This means that frequency of the primary efficacy criterion in the comparison group ($P_{Actilyse}$) is higher than in the group of the study drug ($P_{Fortelyzin}$), taking into account the “no worse” boundary (δ):

H_0: $P_{Fortelyzin} - P_{Actilyse} \leq -\delta$

The alternative hypothesis (HA) is that Fortelyzin® therapy is no less effective than the standard thrombolytic therapy with Actilyse:

H_A: $P_{Fortelyzin} - P_{Actilyse} > -\delta$

The calculation of the sample size for the “no worse" hypothesis, the primary criterion representing the proportion of respondents, and even 1:1 distribution in therapy groups is carried out according to the following formula [Chow S-Ch., Shao J., Wang H., "Sample Size Calculations in Clinical Research", 2003]:

$$n = \frac{(z_{1-\alpha} + z_{1-\beta})^2}{(\delta - \Delta)^2}[p_1(1-p_1) + p_2(1-p_2)]$$

where n – the number of patients in each of the groups compared;

p_1 – the proportion of **respondents** (reaching the primary efficacy criterion) in the group of the study drug;

p_2 – the proportion of **respondents** in the comparison group with Actilyse®;

$\Delta = p_1 - p_2$ – the expected difference between the proportions of respondents in the groups of the study drug and the comparison drug;

δ – "no worse" boundary

α and β – probabilities of type I and II errors, respectively;

$Z_{1-\alpha}$ and $Z_{1-\beta}$ – critical values of the standard normal distribution.

The limit of clinical significance was chosen to be 16.9% and was calculated as follows.

The frequency of reaching the primary efficacy criterion in the thrombolytic therapy of ischemic stroke using Actilyse®, namely, the percentage of good functional recovery 3 months after thrombolysis (MRS – 0-1 score) according to the International Register (SITS-MOST) is 54.8 % (total number of patients is 6 483) (Wahlgren N et al., SITS-MOST investigators. Multivariable analysis of outcome predictors and adjustment of main outcome results to baseline data profile in randomized controlled trials; Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS-MOST). Stroke 2008; 39: 3316-3322).

The frequency of reaching the primary efficacy criterion when using placebo – the percentage of good functional recovery (MRS-0-1 score) in patients with ischemic stroke 3 months after thrombolysis, is on average 37.9%. This value was obtained according to the ECASS II, Atlantis, and ECASS III studies, where the favorable effect when using placebo was 36.6%, 32 %, and 45.2%, respectively (Hacke W et al, for the Second European-Australasian Acute Stroke Study Investigators. Randomized double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II. Lancet 1998:352:1245-1251),

(Clark W.M., et al., for the Atlantis Study Investigators. for the Second European-Australasian Acute Stroke Study Investigators. Recombinant tissue-type plasminogen activator for ischemic stroke 3 to 5 hours after symptom onset. The Atlantis Study: A Randomized Controlled Trial. JAMA 1999;282:2019—26),

(Hacke W, et al. "Thrombolysis with Alteplase 3 to 4.5 Hours after Acute

Ischemic Stroke". ECASS III The New England Journal of Medicine. 2008. 359(13):1317-1329).

Thus, the difference between a good clinical recovery in patients with AIS with the use of Actilyse® and placebo is 54,8% – 37,9% = 16,9%. Thus, the “no worse " boundary was set as 16%.

Assuming equal proportions of reaching the primary efficacy criterion in the study drug group and the comparison drug group of 54.8% ($\Delta = 0$), with the established “no worse” boundary of 16% and demonstration at the bilateral significance level of 5%, the minimum number of patients included in the trial is estimated as 152 people in each therapeutic group to maintain 80% of the comparison power. Taking into account the possible 10% dropout, it is recommended to increase the sample size to 336 patients (168 patients in each group).

18. STATISTICAL ANALYSIS OF RESEARCH MATERIALS

For Statistical Analysis R 3.5.1 package will be used.

Normally distributed variables will be described as mean (SD), ordinal variables as median (IQR), and categorical variables as number and percentage per category. For calculation of significant differences between proportions, we shall use the χ^2 method and for medians the Mann-Whitney U test (with a two-sided 5% level of significance). Ninety-five percent confidence intervals will be calculated for quantitative data.

The primary outcome and other continuous variables will be analyzed with a linear regression model. Binary variables will be tested using logistic regression models adjusted for the same stratification variables, p-values for the difference between treatment groups will be extracted from these models.

19. MONITORING AND AUDIT

Monitoring and audit will be conducted by Roszdravnadzor in accordance with the requirements of the GCP. At the same time, Investigators should provide direct access to the center's documentation and medical documentation.

In order to ensure that all the requirements of the Protocol are met, monitoring visits will be carried out periodically during the trial. A research center may also be

subject to inspections by other Regulatory Authorities. The primary medical documentation will be checked to verify its compliance with the data entered in the CRF.

National Regulatory Authorities may conduct audits during and after the trial.

The audit may include but is not limited to, monitoring of the correct storage and delivery of the study drug, availability of necessary documentation, compliance with procedures of signing the Informed Consent, medical records management, compliance with the Protocol of the trial as a whole, and comparison of data reflected in the CRFs with the information included in the primary documentation.

Prior to the start of the trial, the Monitor should conduct an initiating visit to the research center to ensure that all materials (CRF, study drug, etc.) have been received by the center in proper condition, and to train the Investigator and the center's staff to comply with the procedures and requirements of the Protocol.

Monitoring of the research center can be carried out through visits and telephone contacts. The Monitor of clinical research (Alexey A. Nikonov, Ph.D., Professor of the Department of Neurology, Neurosurgery and Medical Genetics of the Medical Faculty of the Pirogov Russian National Research Medical University of the RF Ministry of Health, mob. phone: +7 916 141-95-48) will perform monitoring visits in accordance with a pre-determined schedule and with sufficient frequency to assess the rate of patient’s inclusion, verification of data entered in the CRF in comparison with the data of the primary documentation, monitor the compliance with the Trial Protocol and the study drug handling.

After the completion of participation of all patients, the Monitor will conduct a final visit.

20. REPORTING

Each research center compiles a report, which is signed by the Principal Investigator and approved by the head of the medical institution on the basis of which the center is located.

The statistical processing is carried out by an independent statistical expert on the basis of the CRF.

The deadline for report submission is up to 1 month after the completion of the trial.

The final report will contain clinical and statistical reports, as well as appendixes with individual data and statistical analysis.

The suggested structure of the report: the purpose of the trial, methods (including any deviations from the Trial Protocol), evaluation of the results, discussion of adverse events, and their interpretation.

21. PUBLICATIONS

After the end of the trial, the data will be summarized and prepared for publication.

Principal Investigator's Signature	Signature Date	