P R O T O C O L

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 massive pulmonary embolism

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
Version:	2.0, April, 16, 2021

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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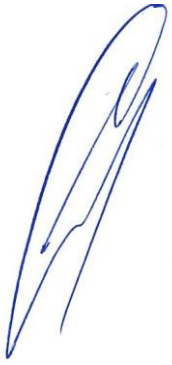
I read all the pages of the Clinical Trial Protocol «Multicenter open-label randomized comparative trial of the efficacy and safety of single bolus injection of Fortelyzin® («SuperGene», LLC) and bolus-infusion of Actilyse® (Boehringer Ingelheim Pharma GmbH) in patients with massive pulmonary embolism», 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:



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LIST OF ABBREVIATIONS

AE	– adverse event
ALT	– alanine aminotransferase
APPT	– activated partial thromboplastin time
AST	– aspartate aminotransferase
BARC	– Bleeding Academic Research Consortium
BP	– blood pressure
CAG	– coronary angiography
CI	– confidence interval
CPK	– creatine phosphokinase
CRF	– case report form
CTPA	– computed tomography pulmonary angiogram
CVD	– cardiovascular desease
EC	– ethics committee
ECASS	– European Cooperative Acute Stroke Study
ECG	– electrocardiography
EchoCG	– echocardiography
EDD	– end-diastolic diameter
GCP	– good clinical practice
Hb	– hemoglobin
HDL-C	– high-density lipoprotein cholesterol
Ht	– hematocrit
ICH	– International Conference On Harmonisation
INR	– international normalized ratio
IPIC	– individual patient identification code
K^+	– blood serum potassium
LV	– left ventricle
MRI	– magnetic resonance imaging
mRS	– modified Rankin scale

Na^+	– sodium in blood
NIHSS	– National Institutes of Health Stroke Scale
NOACs	– new oral anticoagulants
NT-proBNP	– N-terminal prohormone of brain natriuretic peptide
PASP	– pulmonary artery systolic pressure
PE	– pulmonary embolism
PESI	– Pulmonary Embolism Severity Index
RV	– right ventricle
RVD	– right ventricle dysfunction
SAE	– serious adverse event
STEMI	– ST elevation myocardial infarction
TIMI	– thrombolysis in myocardial infarction
WHO	– World Health Organization

PROTOCOL SUMMARY

Official Title:	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 massive pulmonary embolism (PE)
Short title:	FORPE
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 massive PE
Trial objectives:	• To assess the efficacy of single-bolus intravenous injection of Fortelyzin® in comparison with bolus infusion of Actilyse® in patients with massive PE • 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 massive PE
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 155 patients each (a total of 310 people, including 10 % of those who may have dropped out) and assigned to receive either Fortelyzin® or Actilyse®. The drugs will be administered after signing the informed consent. Fortelyzin® in a dose of 15 mg

	will be given as single i.v. bolus over 10-15 seconds. Actilyse® will be administered in accordance with the instruction for use. Patients will be monitored for 30 days: 1-2 days in the Intensive Care Unit and the Department of cardiology in the remaining days before discharge (on average 14 days), with an outpatient visit on the 30th day. Enrollment of patients into the trial will be conducted on a competitive basis.
Trial duration:	The trial completion date is the 30th day after the final trial patient was included in the trial. All patients will be examined for 30 days
Trial population:	Men and women aged 18 years and older with a diagnosis of massive PE
Sample size:	The total number of patients included in the trial – 310 people, including 10 % of possible dropouts
Study drug:	**Fortelyzin®**, registration number – LP001941 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 and older • Massive PE (confirmed by CTPA) • Signs of RV overload/dysfunction (at least one)[1] associated with persistent hypotension or shock[2] • Patient consent to use reliable contraceptive methods throughout the study and for 3 weeks after: - women who have a negative pregnancy test and use the following contraceptives: intrauterine devices, oral contraceptives, contraceptive patch, long-acting injectable contraceptives, double barrier method of contraception. Women who are not capable of bearing children (documented conditions: hysterectomy, tubal ligation, infertility, menopause for more than 1 year) can also take part in the study; - men using barrier contraceptives. The study may also include men who are not capable of bearing children (documented conditions: vasectomy, infertility) • Signed and dated informed consent from the patient to participate in the trial
Exclusion criteria:	• Increased risk of bleeding: - extensive bleeding at present or within the previous 6 months, hemorrhagic diathesis;

1
– RV end-diastolic diameter more than 30 mm (parasternal long axis);
– RV/LV end-diastolic diameters ratio more than 0.9;
– hypokinesis of RV-free wall (Mc Connel sign);
– tricuspid regurgitant jet velocity in systole more than 2.6 m/s [1, 2].

2
– cardiac arrest;
– obstructive shock (systolic BP <90 mmHg or vasopressors required to achieve a BP≥90 mmHg despite an adequate filling status, in combination with end-organ hypoperfusion);
– persistent hypotension (systolic BP <90 mmHg or a systolic BP drop ≥40 mmHg for >15 min, not caused by new-onset arrhythmia, hypovolaemia, or sepsis) [1, 2].

	- intracranial (including subarachnoid) hemorrhage at present or in history, suspicion of a hemorrhagic stroke; - hemorrhagic stroke or unknown etiology stroke in anamnesis; - ischemic stroke or transient ischemic attack within the last 6 months, with the exception of the current acute ischemic stroke within 4.5 hours; - central nervous system diseases in anamnesis (including neoplasms, aneurysm, surgery on the brain or spinal cord); - major surgery or major trauma within the previous 3 months, recent traumatic brain injury; - prolonged or traumatic cardiopulmonary resuscitation (> 2 min), labor within the previous 10 days, recent puncture of an uncompressible blood vessel (eg, subclavian or jugular vein); - severe liver disease, including liver failure, cirrhosis, portal hypertension (including esophageal varices) and active hepatitis; - confirmed peptic ulcer of the stomach or duodenum within the last three months; - a neoplasm with an increased risk of bleeding; - simultaneous treatment with oral anticoagulants, for example, warfarin with INR> 1.3; - arterial aneurysms, defects in the development of arteries / veins; - severe uncontrolled arterial hypertension; - acute pancreatitis; - bacterial endocarditis, pericarditis; - suspicion of a dissecting aortic aneurysm; - any other conditions, in the opinion of the doctor, associated with a high risk of bleeding.

	• Lactation, pregnancy • Known hypersensitivity to Actilyse®, Fortelyzin®.
Trial procedures:	• collection of anamnesis, physical examination, measurement of vital signs (blood pressure, respiratory rate, heart rate, SpO_2) • PESI stratification • CTRA for massive PE confirmation • ECG • EchoCG • ultrasound diagnostic system of the inferior vena cava system • venous blood sampling for complete blood count (with platelet count) and biochemical blood analysis (with NT-proBNP level determination), coagulogram (INR, APTT, fibrinogen, D-dimer) • general urine analysis
Efficacy criteria:	• Primary efficacy outcome – death from any causes within 7 days • Secondary efficacy outcome – PASP (V_1, V_2, V_4, V_5) • Secondary efficacy outcome – hemodynamic collapse within 7 days • Secondary efficacy outcome – recurrent PE within 7 days • Secondary efficacy outcome – death from PE within 7 days • Secondary efficacy outcome – death from any causes within 30 days • Secondary efficacy outcome – clinical composite of hemodynamic collapse within 7 days + recurrent PE within 7 days + death from any causes within 30 days
Safety criteria:	• Safety outcomes – ischemic and hemorrhagic strokes within 7 days • Safety outcomes – bleedings BARC type 3 and 5 • number and severity of SAEs and AEs in organs and systems

Conclusion:	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.

1. BACKGRAUND FOR THE TRIAL

Pulmonary embolism (PE) is a complex clinical problem. The prevalence of PE is high and reaches 30-40 people per 100 000 populations in Russia [1]. PE is one of the leading causes of death, morbidity and hospitalization in Europe [2]. It has been shown that in six EU countries (population about 450 million people), the mortality rate from PE is higher than from AIDS, breast cancer, prostate cancer and road traffic accidents combined, and is 11% of the entire population surveyed [2].

In the Russian Federation, mortality from PE is very high and ranges from 10-30%, depending on modern diagnostics and the use of adequate methods of treatment, primarily thrombolytic therapy.

The indication for thrombolytic therapy is massive PE, defined by size scales and risk stratification. Massive PE is defined in the presence of signs of shock or hypotension, which corresponds to III-V class PESI [1].

In accordance with European [1] and Russian [2] Guidelines for massive PE treatment, the following thrombolytic drugs are used:

1) streptokinase according to the standard scheme of administration for 12-24 hours or accelerated scheme for 2 hours;

2) urokinase according to the standard (12-24 hours) or accelerated schemes for 2 hours;

3) alteplase according to the standard scheme of 100 mg for 2 hours.

The recently completed PEITHO study used the «gold standard» thrombolytic therapy for acute ST-segment elevation myocardial infarction (STEMI) tenecteplase in patients with intermediate-risk PE with signs of right ventricular (RV) disease according to a single bolus administration at a dose of 30-50 mg, depending on body weight. Tenecteplase was shown to be highly effective, combined with a large number (6.3%) of hemorrhagic stroke, which did not allow tenecteplase to be included in the list of recommended thrombolytics for PE [3].

FRIDOM 1 study showed equal efficacy and safety of Fortelyzin®, 15 mg as a single bolus, regardless of body weight, compared with Metalyse® (tenecteplase), administered as a single bolus of 30-50 mg depending on body weight, in patients with STEMI. This study demonstrated the high efficacy of Fortelyzin® and its safety, the absence of major bleeding and hemorrhagic stroke in patients with STEMI complicated by cardiogenic shock [5, 6].

Fortelyzin®, given as a single bolus of 10 mg regardless of body weight, was non-inferior to Actilyse® (alteplase) bolus infusion at a dose of 0.9 mg/kg (maximum 90 mg) for patients with acute ischemic stroke within 4.5 hours of symptom onset in a recently completed FRIDA trial. Mortality, symptomatic intracranial hemorrhage, and serious adverse events did not differ significantly between groups [7].

These results were decisive for resolving the issue of the possibility of using Fortelyzin® in patients with massive PE accompanied by shock and hypotension, where thrombolytic therapy is the method of choice.

Taking together, the purpose of this trial is to evaluate the efficacy and safety of the Fortelyzin® with its single bolus administration in comparison with the bolus-infusion administration of the Actilyse® in patients with massive PE.

Rationale for the Fortelyzin® dose in patients with massive PE

The FRIDOM1 study Fortelyzin® was used as a single bolus injection of 15 mg in patients with STEMI, including patients with cardiogenic shock. Thrombolytic therapy with Fortelyzin® at a dose of 15 mg in these patients showed high efficacy and safety.

The proposed comparison drug Actilyse® is the «gold standard» in the massive PE treatment. It used at a dose of 100 mg, the same as for STEMI.

In this regard, the dose of Fortelyzin® should be identical to that used for STEMI, 15 mg administered as a single bolus.

Thus, Fortelyzin® in patients with massive PE can be considered justified in comparison with Actilyse® as the “gold standard” for PE thrombolytic therapy.

2. GENERAL INFORMATION ABOUT THE STUDY DRUG

Brand name: Fortelyzin®, valid registration number – LP001941 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 STEMI patients and 90 mg for patients with ischemic stroke, while the dose of the fibrin-selective thrombolytic – Fortelyzin® – is 15 mg for STEMI 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 STEMI patients showed a short half-life ($t_{1/2\alpha}$) of 5.7 minutes and $t_{1/2\beta}$ 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 E.I. Chazov Russian Cardiology Research Complex of the Ministry of Health of the Russian Federation 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 trials

Phase I-II

According to the Protocol «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, were 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 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 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 scheme: 10 mg bolus + 5 mg infusion for 30 minutes.

Phase III

The further study assessed a bolus and bolus-infusion of Fortelyzin® at a dose of 15 mg vs. Actilyse® according to the Protocol «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.

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

FRIDOM 1

The relevance of thrombolysis in the prehospital or early hospital stages in Russia was one of the reasons for conducting a post-registration multicenter

randomized comparative study FRIDOM1 in order to evaluate Fortelyzin® efficacy and safety with its single bolus administration in patients with STEMI in comparison with Metalyse® single bolus administration in patients with STEMI in the first 12 hours from the onset of the disease.

The study included 382 patients, 191 patients each in the Fortelyzin® and Metalyse® groups. Randomization was carried out using the «envelope» method.

All 382 randomized patients had a classic anginal attack that began no more than 12 hours before thrombolytics administration. In 381 patients (190 in the Fortelyzin® group and 191 in the Metalyse® group), ST segment elevation of more than 1 mm in two or more consecutive limb leads and/or more than 2 mm in the precordial leads was noted; in 1 patient myocardial infarction was not accompanied ST segment elevation (this patient was excluded from further efficacy analysis).

All enrolled patients received prehospital and hospital treatment, which included acetylsalicylic acid, clopidogrel, unfractionated heparin or low molecular weight heparin in standard dosages, Fortelyzin® or Metalyse®. Rescue PCI was carried out in case of ineffective thrombolysis and planned PCI – 3-24 hours after thrombolysis, accompanied by a positive ECG dynamics. If indicated, PCI with stenting of the infarct-related coronary artery was performed.

Fortelyzin® was administered in a dose of 15 mg, regardless of body weight, as a bolus over 10-15 seconds, Metalyse® was administered as a bolus at a dose of 30-50 mg, depending on body weight, according to the instructions for medical use.

The FRIDOM1 study was designed as a non-inferiority study. Primary efficacy outcome was conducted in ITT population.

Restoration of coronary blood flow according to TIMI 2 + TIMI 3 criteria in the Fortelyzin® group was observed in 133 patients (70%); in the Metalyse® group – in 131 patients (71%).

Rescue PCI was performed in 40 patients (21%) in the Fortelyzin® group and in 38 patients (21%) in the Metalyse® group. In 2 patients in the Fortelyzin® group with positive ECG dynamics, pain persisted, and therefore they underwent rescue PCI.

Planned PCI was performed in 150 of 190 patients (79%) in the Fortelyzin® group and in 147 of 185 (80%) in the Metalyse® group.

Coronary blood flow TIMI 2 + TIMI 3 after thrombolysis + PCI was restored in 184 of 190 patients (96%) in the Fortelyzin® group and in 179 of 185 patients (97%) in the Metalyse® group.

According to coronary angiography (CAG), 5 out of 8 patients with cardiogenic shock in Fortelyzin® group experienced restoration of blood flow according to TIMI 2 + TIMI 3 criteria. In 3 patients, rescue PCI was performed.

In the Metalyse® group, according to CAG data, restoration of blood flow according to TIMI 2 + TIMI 3 criteria was observed in 5 out of 7 patients with cardiogenic shock. In 2 patients with cardiogenic shock in the Metalyse® group, rescue PCI was performed.

Efficacy data in Fortelyzin® group, to whom the drug was administered after 6 hours from the onset of the disease, showed that blood flow restoration according to CAG was observed in 69% of cases, which corresponds to the CAG results obtained in patients to whom Fortelyzin® was administered before 6 hours from the onset of the disease – 70%.

The safety criteria were (Table 1):

1. Frequency of major bleeding;
2. Intracranial hemorrhage;
3. Blood transfusion;
4. Frequency of minor bleeding.

Table 1. FRIDOM1 trial. Safety criteria

criteria	**Fortelyzin® (n = 191) n (%)**	**Metalyse® (n = 191) n (%)**	**p**
1. Frequency of major bleeding	1 (0,5)	1 (0,5)	>0,99
2. Intracranial hemorrhage	0	0	>0,99
3. Blood transfusion	7 (3,7)	20 (10,5)	0,02
4. Frequency of minor bleeding	1 (0,5)	1 (0,5)	>0,99

There were no intracranial bleedings in both groups. Ischemic stroke was diagnosed in one patient in the Metalyse® group.

No patients with cardiogenic shock in the Fortelyzin® and Metalyse® groups experienced major bleeding or hemorrhagic stroke.

In the FRIDOM1 study, CVD death within 30 days of randomization was 3,8% in both the Fortelyzin® and Metalyse® groups, and was consistent with the 3,3% mortality rate in the STREAM study.

In the FRIDOM1 study, cardiogenic shock within 30 days of randomization was observed in 4,7% of patients in the Fortelyzin® group and 5,3% in the Metalyse® group, for comparison – in the Metalyse® group in the STREAM study this indicator was 4,4%. According to CAG usage of Fortelyzin® in patients with Killip III and Killip IV cardiogenic shock results in blood flow restoration in 60% of cases and is not accompanied by the development of hemorrhagic stroke and major bleeding.

The primary combined endpoint in the FRIDOM1 study defined as deaths from any causes + recurrent STEMI + cardiogenic shock was the same in the Fortelyzin® and Metalyse® groups – 12,63% and 12,56%, respectively, comparable to a similar endpoint in the study STREAM – 12,4%.

The results of the FRIDOM1 study showed that the timing of PCI after thrombolysis does not affect the thrombolysis safety.

FRIDOM1 trial confirmed the «non-inferiority» hypothesis. The results of the efficacy and safety endpoints showed that Fortelyzin® administered as a single bolus in a dose of 15 mg regardless of body weight is non-inferiority to Metalyse® administered as a bolus at a dose of 30-50 mg depending on body weight in patients with STEMI no later than 12 hours from the onset of the disease. This trial has demonstrated a similar safety profile.

The Ministry of Health of the Russian Federation conducted an examination of FRIDOM1 trial results and approved updated Fortelyzin® instructions, allowing its

single bolus administration at a dose of 15 mg in patients with STEMI no more than 12 h symptoms onset, including in patients with cardiogenic shock.

Fortelyzin® efficacy and safety monitoring

Continuous monitoring of the Fortelyzin® efficacy and safety is carried out and covers 4123 STEMI patients for the period from June 2013 to March 2015.

The average age of STEMI patients who received Fortelyzin® was 64 years, 610 people were older than 75 years old (72% of the total number of patients were male). According to the localization of myocardial infarction, the patients were distributed almost equally (Table 2).

Table 2. 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	
4123	2981/ 1142	64 32–93	610 (15%)	2300	1823	206 40–720

The time from the STEMI symptoms onset to Fortelyzin® administration was 206 minutes. Signs of myocardial reperfusion, according to ECG data, by the 90th minute with a non-invasive strategy were observed in 2542 of 3139 patients (81%), with a pharmacoinvasive strategy – in 826 of 984 patients (83%).

With a pharmacoinvasive strategy, according to coronary angiography, coronary blood flow restoration according to TIMI 3 criteria was observed in 403 of 984 patients (41%), according to TIMI 2 criterion – in 305 patients (31%), in total, according to the sum of TIMI 2-3 – in 708 of 984 patients (72%).

Hemorrhagic stroke occurred in 9 (0,3%) patients with the non-invasive strategy and in 3 patients (0,3%) with the pharmacoinvasive strategy.

Mortality during hospitalization was slightly higher in the non-invasive group compared to the pharmacoinvasive group – 4,5 and 3,2%, respectively.

Number of bleedings requiring blood transfusion and minor bleedings within 7 days was the same in both groups – 0,7 and 2,5%, respectively (Table 3, 4).

Table 3. Fortelyzin® efficacy and safety monitoring with a pharmacoinvasive strategy (thrombolysis + PCI) (n = 984)

Signs of reperfusion (reduction in the ST elevation by 50 %)	TIMI (N = 177)			Intracranial hemorrhage	Death within 30 days	Bleeding required blood transfusion	Minor bleeding
	3	2	3 + 2				
826 (83%)	403 (41%)	305 (31%)	708 (72%)	3 (0,3%)	31 (3,2%)	7 (0,7%)	24 (2,5%)

Table 4. Fortelyzin® efficacy and safety monitoring with a non-pharmacoinvasive strategy (n = 3139)

Signs of reperfusion (reduction in the ST elevation by 50 %)	Intracranial hemorrhage	Death within 30 days	Bleeding required blood transfusion	Minor bleeding
2542 (81%)	9 (0,3%)	141 (4,5%)	22 (0,7%)	72 (2,3%)

Since 2015, Fortelyzin® has been included in the list of vital and essential drugs. Since 2013, clinical use has increased rapidly, spread throughout Russia and has over 20000 patients treated.

During the period of Fortelyzin® administration from 2013 to the present, the Ministry of Health of the Russian Federation has registered only 3 undesirable expected reactions to the drug Fortelyzin®, which were not reliably related to the use of the drug, which is extremely small for such a disease as STEMI.

3. POTENTIAL BENEFITS AND RISKS TO PARTICIPANTS

The expected benefit of using the drug is to restore normal blood circulation in the pulmonary artery, which will normalize pulmonary perfusion and avoid patient's death.

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 hemorrhoid 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 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 On Dugs Circulation of Medicines;
- Federal Law No. 323 On the fundamentals of health protection in the Russian Federation;
- ICH Harmonized Tripartite Guidelines for Good Clinical Practice, 1996
- Rules for quality clinical trials in the Russian Federation (OST 42-511-99);
- National Standard of the Russian Federation GOST R 52379-2005 Good Clinical Practice (2005);
- Order of the Ministry of Health and Social Development of the Russian Federation No. 774 On Ethics Board;
- Guideline for quality clinical trials of medicinal products. Edited by A. N. Mironov, Moscow, 2012;
- Order of the Ministry of Health and Social Development of the Russian Federation No. 389 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. 928 On Approval of Medical Care Procedure to patients with Acute disorders of Cerebral Circulation;
- Guidelines on the diagnosis and management of acute pulmonary embolism. The Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC) / Eur. Heart J. – 2008. – №29. –P. 2276-2315.

- 2014 ESC Guidelines on the diagnosis and management of acute pulmonary embolism / Eur. Heart J. – 2014. – №35. – P.3033-3080.
- Management of Massive and Submassive Pulmonary Embolism, Iliofemoral Deep Vein Thrombosis, and Chronic Thromboembolic Pulmonary Hypertension / Circulation. – 2011. – №123. – P.1788-1830.

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, Informed consent form, 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 Informed consent form 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. Informed consent form

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 (Informed consent form), approved by the Ethics Committee. The patient's informed consent form contains 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 science and society interests. 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 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 form 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 clinical site and the legal representative.

The Informed consent form must be reviewed and approved by the Ethics Committee. The final version of the Study Protocol and Informed consent form must be approved by the Russian Ministry of Health and the Local Ethics Committee (if any) at the clinical site.

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 massive PE.

6. TRIAL OBJECTIVES

- To assess the efficacy of single-bolus intravenous injection of Fortelyzin® in comparison with bolus infusion of Actilyse® in patients with massive PE.
- 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 massive PE.

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 massive PE.

7.1. Inclusion criteria

- Men and women aged 18 and older.
- Massive PE (confirmed by CTPA).
- Signs of RV overload/dysfunction (at least one)[3] associated with persistent hypotension or shock[4].

[3]
– RV end-diastolic diameter more than 30 mm (parasternal long axis);
– RV/LV end-diastolic diameters ratio more than 0.9;
– hypokinesis of RV-free wall (Mc Connel sign);
– tricuspid regurgitant jet velocity in systole more than 2.6 m/s [1, 2].

[4]
– cardiac arrest;
– obstructive shock (systolic BP <90 mmHg or vasopressors required to achieve a BP ≥90 mmHg despite an adequate filling status, in combination with end-organ hypoperfusion);
– persistent hypotension (systolic BP <90 mmHg or a systolic BP drop ≥40 mmHg for >15 min, not caused by new-onset arrhythmia, hypovolaemia, or sepsis) [1, 2].

- Patient consent to use reliable contraceptive methods throughout the study and for 3 weeks after:
  - women who have a negative pregnancy test and use the following contraceptives: intrauterine devices, oral contraceptives, contraceptive patch, long-acting injectable contraceptives, double barrier method of contraception. Women who are not capable of bearing children (documented conditions: hysterectomy, tubal ligation, infertility, menopause for more than 1 year) can also take part in the study;
  - men using barrier contraceptives. The study may also include men who are not capable of bearing children (documented conditions: vasectomy, infertility).
- Signed and dated informed consent from the patient to participate in the trial.

7.2. Exclusion criteria

- Increased risk of bleeding:
  - extensive bleeding at present or within the previous 6 months, hemorrhagic diathesis;
  - intracranial (including subarachnoid) hemorrhage at present or in history, suspicion of a hemorrhagic stroke;
  - hemorrhagic stroke or unknown etiology stroke in anamnesis;
  - ischemic stroke or transient ischemic attack within the last 6 months, with the exception of the current acute ischemic stroke within 4.5 hours;
  - central nervous system diseases in anamnesis (including neoplasms, aneurysm, surgery on the brain or spinal cord);
  - major surgery or major trauma within the previous 3 months, recent traumatic brain injury;
  - prolonged or traumatic cardiopulmonary resuscitation (> 2 min), labor within the previous 10 days, recent puncture of an uncompressible blood vessel (eg, subclavian or jugular vein);
  - severe liver disease, including liver failure, cirrhosis, portal hypertension (including esophageal varices) and active hepatitis;

- confirmed peptic ulcer of the stomach or duodenum within the last three months;
- a neoplasm with an increased risk of bleeding;
- simultaneous treatment with oral anticoagulants, for example, warfarin with INR> 1.3;
- arterial aneurysms, defects in the development of arteries / veins;
- severe uncontrolled arterial hypertension;
- acute pancreatitis;
- bacterial endocarditis, pericarditis;
- suspicion of a dissecting aortic aneurysm;
- any other conditions, in the opinion of the doctor, associated with a high risk of bleeding.

- Lactation, pregnancy
- Known hypersensitivity to Actilyse®, Fortelyzin®.

7.3. Trial completion and post-trial therapy

The trial for a patient will be considered completed after the 6th visit on the 30th day after thrombolysis.

Therapy after the study will be carried out in accordance with current guidelines.

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 form (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 guidelines 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 allowed.

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 clinical site

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

8. DESIGN AND PLAN OF RESEARCH ACTIVITIES

This 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 310 patients with massive PE. The patients will be monitored for 30 days: 1-2 days in the Intensive Care Unit and the Department of cardiology in the remaining days before discharge (on average 14 days), with an outpatient visit on the 30th 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 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, signs of hemodynamic collapse, risk stratification to assess the prognosis according to the PESI classification, a physical examination, CTPA, ECG, EchoCG with the definition of PASP, ultrasound diagnostics of the inferior vena cava will be performed; blood and urine samples will be taken 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, NT-proBNP, total bilirubin, glucose, sodium, potassium, total cholesterol, HDL-cholesterol, triglycerides;

- **coagulogram:** fibrinogen, aPTT, INR, prothrombin, D-dimer.
- **general urine analysis:** red blood cells.

After enrollment, patients will be randomized using the «envelope method» into two equal groups of 155 patients each (a total of 310 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 10-15 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 3 days after administration of the drug. During this period, patients will have 6 visits (Table 5).

Table 5. FORPE trial plan

Procedure / Visit number and its period	V_1 0	V_2 Day 1	V_3 Day 2	V_4 Day 7	V_5 At discharge	V_6 Day 30 (outpatient)
Inclusion and exclusion criteria	+	–	–	–	–	–
Signing informed consent form	+	–	–	–	–	–
Demographics	+	–	–	–	–	–
Anamnesis	+	–	–	–	–	–
Previous treatment	+	–	–	–	–	–
PESI risk stratification	+	–	–	–	–	–
CTPA	+	+	–	–	–	–
EchoCG with PASP definition	+	+	–	+	+	–
Ultrasound diagnostics of the inferior vena cava	+	+	–	+	–	–
Assessment of vital signs	+	+	+	+	+	+
Determining the presence of hemodynamic collapse	+	+	+	+	–	–
Physical examination	+	–	–	–	–	–
Accompanying illnesses	+	–	–	–	–	+
Changes in organs and systems	–	+	+	+	+	–
ECG	+	+	–	+	+	–
Coagulogram	+	+	–	–	–	–
General blood test	+	+	–	+	+	–
Biochemical blood test	+	–	–	–	+	–
General urine test	+	+	–	–	+	–
Fortelyzin® or Actilyse® administration	+	–	–	–	–	–
Concomitant therapy changes	–	+	+	+	+	+
Registration of AE and SAE	–	+	+	+	+	+
Assessment of bleedings	–	+	+	+	+	–

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 form
- collection of demographic data (date of birth, sex, body weight)
- collection of medical history (duration of the underlying disease, previous therapy, allergological anamnesis, etc.)
- physical examination
- evaluation of changes by organs and systems
- PESI mortality stratification
- CTPA
- EchoCG
- ultrasound diagnostics of the inferior vena cava
- determining the presence of hemodynamic collapse
- ECG
- blood sampling for hematological and biochemical analyses
- coagulogram
- general urine analysis
- randomization
- Fortelyzin® or Actilyse® administration

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)

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, CRF, and in the informed consent form. The IPIC will be transmitted by the Code Registry by the Insurant to the Insurer.

IPIC 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 IPIC part will be used in the report and trial documentation.

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

Visit 2. (The first day after thrombolysis)

- assessment of vital signs – every hour
- determining the presence of hemodynamic collapse
- changes in organs and systems
- CTPA
- EchoCG
- ultrasound diagnostics of the inferior vena cava
- ECG
- blood sampling for hematological analyse
- coagulogram
- general urine analysis
- registration of AE and SAE
- assessment of bleedings
- concomitant therapy changes

Visit 3. (The second day after thrombolysis)

- Assessment of vital signs (twice per day)
- determining the presence of hemodynamic collapse
- changes in organs and systems
- registration of AE and SAE
- assessment of bleedings
- concomitant therapy changes

Visit 4. (The 7th day after thrombolysis)

- assessment of vital signs (twice per day)
- determining the presence of hemodynamic collapse
- changes in organs and systems
- EchoCG
- ultrasound diagnostics of the inferior vena cava
- ECG

- blood sampling for hematological analyse
- registration of AE and SAE
- assessment of bleedings
- concomitant therapy changes

Visit 5. (Discharge)

- assessment of vital signs
- changes in organs and systems
- EchoCG
- ECG
- blood sampling for hematological and biochemical analyses
- general urine analysis
- registration of AE and SAE
- assessment of bleedings
- concomitant therapy changes

Visit 6. (Day 30 after thrombolysis)

- assessment of vital signs
- registration of AE and SAE
- assessment of bleedings
- concomitant therapy changes

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 investigator. 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, heart rate, and respiratory rate, 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 CRF.

9.1.6. CTPA

The most common method for PE diagnosing is computed tomography of the pulmonary arteries (CTPA, level of evidence 1A). It has broad capabilities for

visualizing the lumen of the pulmonary arteries, the nature of damage to the vascular bed, identifying pulmonary infarctions, and, while simultaneously performing a native study of the lungs, to make a differential diagnosis.

The presence of PE is undoubted when thrombi are detected in the segmental (distal) and more proximal branches of the pulmonary arteries.

Currently, CTPA is the standard for PE non-invasive diagnosis due to its high sensitivity and specificity.

The embolic lesion system is assessed using the Qanadli index [15]. The Qanadli index in the absence of lesion is 0, in case of partial lesion – 1 and in case of complete lesion – 2.

In the Qanadli system, each lung is divided into 10 segments (20 in total), i.e. The maximum Qanadli index with complete damage to both lungs will be 40. Qanadli index was calculated by dividing the patient score by the maximal total score (40) and by multiplying the result by 100 at baseline and 24 h after drug administration.

Interpretation of the CTPA results will be performed by certified specialists who are unaware of the goals and objectives of this clinical trial and the therapy performed.

9.1.7. EchoCG

EchoCG is an informative and accessible non-invasive method for examining patients with PE. According to EchoCG, PE presence can be predicted or denied with high accuracy. EchoCG examination has many fairly specific symptoms for PE diagnostics. PE is supported by: enlargement of the right chambers of the heart, bulging of the interventricular septum towards the left parts, paradoxical movement of the interventricular septum in diastole, direct location of the thrombus in the pulmonary artery, severe regurgitation on the tricuspid valve, 60/60 sign, McConnell sign.

Positive echocardiography results may be the basis for PE diagnosis.

The criterion for right ventricular dysfunction (RVD) is an increase in pulmonary artery systolic pressure (PASP) of more than 30 mm Hg in combination with RV enlargement more than 30 mm. The following parameters are also assessed: right atrium diameter, RV/LV end-diastolic (EDD) ratio, RV free wall hypokinesis, thrombosis of the right chambers heart, paradoxical movement and/or flattening of the interventricular septum.

9.1.8. Utrasound diagnostics of the inferior vena cava

The study is carried out to identify PE source (thrombosis) and the likelihood of recurrent PE (the presence of floating blood clots). The deep veins of the leg, popliteal veins, superficial femoral veins, common and deep femoral veins, external and common iliac veins, the inferior vena cava, as well as the great and small saphenous veins are examined on both limbs. If there is no thrombosis in these veins, it is necessary to examine the internal iliac, gonadal (testicular), renal and hepatic veins. A reliable sign of thrombosis should be considered the absence of closure of the vein walls during compression by a sensor installed across the vein.

9.1.9. General and biochemical blood analysis

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

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

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

– coagulogram (fibrinogen, INR, APTT, prothrombin, D-dimer)

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).

9.1.10. General 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 quantity of red blood cells will be measured.

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 15 mg (2 235 000 IU).

Route of administration: intravenously in the form of a single bolus injection for 10-15 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 mg,

Phosphoric acid, 85 %: up to pH 7,2 ± 0,2,

Polysorbate 80: 3,5-5,0 mg.

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 100 mg.

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.

Patients treatment is carried out in accordance with current Guidelines [1, 2].

After thrombolysis, anticoagulant therapy with unfractionated heparin must be started within 48 hours:

– intravenously with a bolus of 80 units/kg (or 5000 units) and infusion at an initial rate of 18 units/kg/h (or 1250-1300 units/h), then dose selection according to aPTT values. The goal is to maintain an aPTT of 1,5 to 2,5 times the laboratory-specific upper limit of normal (or anti-Xa activity values of 0,6 to 1,0 units/mL using the amidolytic method).

If anticoagulant therapy with unfractionated heparin is initiated at the prehospital stage, an intravenous bolus is not administered, but its infusion is continued.

After 48 hours, a switch is made to low molecular weight heparin, which is administered for 7 days:

– Enoxaparin sodium – subcutaneously 100 units (1 mg/kg) 2 times a day followed by indirect anticoagulants:

– Vitamin K antagonists – orally, dose selection in order to maintain the INR in the range from 2 to 3;

or NOACs:

– Dabigatran etexilate –150 mg orally 2 times daily;

– Rivaroxaban – 15 mg orally 2 times a day for 21 days, then 20 mg once daily;

– Apixaban – 5 mg orally 2 times daily.

Anticoagulants are taken for at least 6 months.

In case of an allergic reaction during thrombolysis, the use of glucocorticoids and antihistamines is permitted.

In case of severe bleeding (especially from non-compressible vessels), the administration of thrombolytics should be discontinued. The administration of fresh frozen plasma or fresh blood is indicated.

Prohibited concomitant therapy

In patients with massive PE, massive fluid resuscitation is not recommended because aggressive volume loading may worsen right ventricular dysfunction.

11. EFFICACY ENDPOINTS

- Primary efficacy outcome – death from any causes within 7 days;
- Secondary efficacy outcome – PASP (V1, V2, V4, V5);
- Secondary efficacy outcome – hemodynamic collapse within 7 days;
- Secondary efficacy outcome – recurrent PE within 7 days;
- Secondary efficacy outcome – death from PE within 7 days;
- Secondary efficacy outcome – death from any causes within 30 days;
- Secondary efficacy outcome – clinical composite of hemodynamic collapse within 7 days + recurrent PE within 7 days + death from any causes within 30 days.

12. SAFETY ENDPOINTS

- Safety outcomes – ischemic and hemorrhagic strokes within 7 days;
- Safety outcomes – bleedings BARC type 3 and 5;
- number and severity of SAEs and AEs in organs and systems.

Adverse events

Adverse event (AE) is an unfavorable medical occurrence in a patient administered a pharmaceutical product, which may not have a causal relationship with its use. Thus, an AE 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 AEs should be collected at each visit by interviewing the patient, as well as during physical examination and evaluation of laboratory and instrumental data. All AEs 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 AE
- severity (mild, moderate, severe)
- causal relationship with the study drug
- whether the AE is serious
- actions taken (whether AE required any therapeutic measures)
- outcome

AEs Grading:

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

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

- ***definitely related*:** Clinical manifestations of AE, laboratory findings deviations that occur during the period of drug administration, cannot be explained by the presence of existing diseases and the influence of other factors. Manifestations of AE regress after the drug discontinuation and occur again after repeated administration.
- ***Probable***. Clinical manifestations of AE, 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 AE, 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 AE, 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 AE, 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 AE cannot be assessed, due to insufficient information, or it is contradictory.

In the case of AEs, 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 AE that occurred during the trial, and in the case of the development of a chronic disease – until the condition is stabilized. AEs 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 (type 3 and 5) according to BARC classification;
- hemodynamic collapse within 7 days.

AEs 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 the Ministry of Health of the Russian Federation 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 CRF.

Bleedings

All bleedings should be registered in the patient's CRF. Each bleeding is classified according to BARC. Bleeding that is classified according to BARC as type 3 or 5 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 to the Sponsor within 24 hours about the occurrence of SAE and send (fax, e-mail) a Report form on a SAE;
- indicate all adverse events in the patient's CRF;
- continue to monitor the patient’s condition until the SAE is fully completed.

In connection with SAE occurrence, the sponsor is obliged to:

• notification of administrative control authorities;

• suspend or cancel the clinical trial.

Contact information for security specialists is listed below:

Company name – Sponsor	The Russian Academy of Sciences
Legal address	14 Leninsky Prospekt, Moscow, 119991
Actual address	14 Leninsky Prospekt, Moscow, 119991
Phone fax	+7 (495) 698-56-50
e-mail	info@ras.ru
Authorized representative of the Sponsor	Alexander I. Kirienko +7 (916) 128-40-62

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.

It is necessary to attach CTPA data on CD media, ECG, EchoCG with description, and an ultrasound protocol of the inferior vena cava system to 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 No. 61 "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. SAMPLE SIZE CALCULATION

In the Russian Federation, multicenter randomized clinical trials of PE thrombolytic therapy have not previously been conducted. The largest clinical material of PE thrombolysis – 236 patients over 15 years – was collected in the Faculty of Surgery Clinic of N.I. Pirogov Russian National Research Medical University [1].

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 [10]. The null hypothesis (H_0) was that the thrombolytic therapy with Fortelyzin® is inferior to 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 ($P_{Fortelyzin}$) (δ):

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

The alternative hypothesis (HA) is that Fortelyzin® therapy is non-inferior to the standard thrombolytic therapy with Actilyse:

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

The calculation of the sample size for the “non-inferiority" 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 [11]:

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

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 – “non-inferiority” 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 10% and was calculated as follows.

According to published results, hospital mortality from massive PE without treatment is 30% [12]. Since placebo control is unethical and given the characteristics of the disease (the insignificant role of the placebo effect is obvious), the absence of treatment can be considered equivalent to the placebo effect.

In-hospital mortality after thrombolysis with bolus and bolus-infusion alteplase ranges from 8% to 13,4% with an average of 10% [13, 14].

The expected mortality in both treatment groups is 10%. The clinically acceptable difference limit was chosen to ensure that at least 50% of the effect of the comparator drug relative to placebo/no treatment is retained. Thus, the difference between mortality is (30% – 10%) / 2 = 10%. With a planned study power of 80% and a one-sided confidence interval of 2,5%, the minimum number of patients enrolled in the study is estimated to be 141 patients per treatment group to maintain 80% power for the comparison. Taking into account a possible 10% dropout rate, the sample size was increased to 310 patients to 155 patients in each group.

18. STATISTICAL ANALYSIS OF TRIAL RESULTS

All indicators entered into the clinical database will be listed for each patient and sorted by group (Fortelyzin® or Actilyse®), patient number and visit.

Statistical analysis was carried out using R Foundation for Statistical Computing и Graph Pad Prism 7, Graph Pad Software Inc (version 4.2). Continuous variables will be presented as mean (SD) or median (IQR). Categorical variables will be presented as n (%) and corresponding 95% Clopper-Pearson confidence intervals (CI).

Mann-Whitney U test will be used to compare continuous variables and the two-sided Fisher's exact test will be used to compare categorical variables.

Odds ratios (ORs) will be estimated and presented with 95% CIs. Outcomes will be considered as statistically significant if the p value will less than 0,05.

19. MONITORING AND AUDIT

Monitoring and audit will be conducted by the Ministry of Health of the Russian Federation in accordance with the requirements of the GCP. At the same time, Investigators should provide direct access to the clinical sites' 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 clinical site 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 form, CRFs 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 clinical site 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 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.

Efficacy and safety results will be analyzed by an Independent Data Monitoring Committee (Steering committee) when 25%, 50% and 75% patient enrollment will be reached.

20. REPORTING

Each clinical site 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 one month after the completion of the trial.

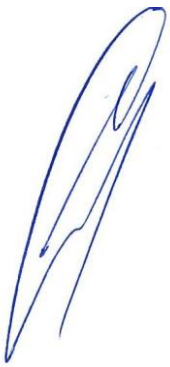
The final report will contain clinical and statistical reports, as well as appendixes with individual data and statistical analysis.

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.

Alexander I. Kirienko
Principal Investigator's Signature



April, 02, 2021
Date

22. REFERENCES

1. Bokeriya LA, Zatevakhin II, Kirienko AI, et al. Russian guidelines for the diagnosis, danger and prevention of venous thromboembolic complications. *Flebologiya*. 2015; 9(4,2): 3-52. (In Russ.).

2. Konstantinides SV, Torbicki A, Agnelli G, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *European Heart Journal.* 2014; 35(43): 3033-69, 3069a-3069k.

3. Meyer G, Vicaut E, Danays T, et al. Fibrinolysis for patients with intermediate-risk pulmonary embolism. *The New England Journal of Medicine.* 2014;370(15): 1402-1141.

4. Vanderschueren S, Dens J, Kerdsinchai P, et al. Randomized coronary patency trial of double-bolus recombinant staphylokinase versus front-loaded alteplase in acute myocardial infarction. Am Heart J 1997; 134: 213-219.

5. Markin SS, Semenov AM, Markov VA, Nizov AA, Ponomarev EA, Lebedev PA. Clinical trial of fibrinselective thrombolytic pharmaceutical agent FORTELYZIN (III Phase). *Rudn Journal of Medicine.* 2012; 1: 105-110. (In Russ.).

6. Markov VA, Duplyakov DV, Konstantinov SL, et al. Fortelyzin® in comparison with Metalyse® for ST-elevated myocardial infarction: one-year results and clinical outcomes of a multicenter randomized study FRIDOM1. *Russian Journal of Cardiology.* 2018; 11: 110-116. (In Russ.).

7. Gusev EI, Martynov MYu, Nikonov AA, et al. Non-immunogenic recombinant staphylokinase versus alteplase for patients with acute ischaemic stroke 4·5 h after symptom onset in Russia (FRIDA): a randomised, open label, multicentre, parallel-group, non-inferiority trial. *Lancet Neurol.* 2021; 20(9): 721-728.

8. Torbicki A, Perrier A, Konstantinides S, et al. ESC Committee for Practice Guidelines (CPG). Guidelines on the diagnosis and management of acute pulmonary embolism: the Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC). *European Heart Journal.* 2008;29(18):2276-315.

9. Yavelov IS. Venous thrombosis and pulmonary embolism: prevention, diagnosis and treatment (part 2). *Atherothrombos.* 2010; 1 (4): 55-86. (In Russ.).

10. Sergienko VI, Bondareva IB. *«Matematicheskaya statistika v klinicheskih issledovaniyah»*. M.: GEOTAR-Media; 2006. (In Russ.).

11. Chow S-Ch, Shao J, Wang H, Shao J. Sample Size Calculations in Clinical Research, 2nd Edition. New York, Chapman and Hall/CRC; 2007.

12. Olin JW. Pulmonary embolism. *Reviews in Cardiovascular Medicine.* 2002; 3(2): 68-75.

13. De Bonis S, Rendina D, Vargas G, Di Minno D, Piedimonte V, Gallotta G, Postiglione A. Predictors of in-hospital and long-term clinical outcome in elderly patients with massive pulmonary embolism receiving thrombolytic therapy. *Journal of the American Geriatrics Society.* 2008; 56(12): 2273-2277.

14. Capstick T, Henry MT. Efficacy of thrombolytic agents in the treatment of pulmonary embolism. *European Respiratory Journal.* 2005; 26(5): 864-874.

15. Qanadli SD, El Hajjam M, Vieillard-Baron A, Joseph T, Mesurolle B, Oliva VL, Barré O, Bruckert F, Dubourg O, Lacombe P. New CT index to quantify arterial obstruction in pulmonary embolism: comparison with angiographic index and echocardiography. *American Journal of Roentgenology.* 2001; 176(6): 1415-1420.