1 Sosnovaya St., Pokrov village, 142143, Podolsk District, Moscow Region, Russia

CLINICAL STUDY PROTOCOL

A Multicenter, Double-Blind, Randomized, Comparative Study of Safety, Reactogenicity, Immunogenicity, and Efficacy of Quadrivalent Inactivated Subunit Influenza Vaccine Grippol® Quadri (NPO Petrovax Pharm, LLC, Russia) and Trivalent Inactivated Polymer-Subunit Vaccine Grippol® Plus (NPO Petrovax Pharm, LLC, Russia) in Parallel Groups, in Volunteers of 18 to 60 Years Old.

Study ID: GriQv-III-16

Version of study protocol: **3.0 dated November 18, 2016**

Study phase: **Phase II-III**

Study Drug:

Grippol® Quadri, a quadrivalent inactivated subunit influenza vaccine

INN or generic name: Influenza vaccine [inactivated] + Azoximer bromide

Dosage form: suspension for intramuscular and subcutaneous injection.

Dosage: 0.5 mL (1 dose), *active ingredients:* type A (H1N1) influenza virus antigen (5 µg), type A (H3N2) influenza virus antigen (5 µg), type B (Yamagata lineage) influenza virus antigen (5 µg), type B (Victoria lineage) influenza virus antigen (5 µg), and 500 µg of Polioxidonium® (Azoximer bromide)

Manufacturer: NPO Petrovax Pharm, LLC

Study sponsor: NPO Petrovax Pharm, LLC (1 Sosnovaya St., village of Pokrov, Podolsk district, Moscow region, Russia, 142143) Tel/fax: +7 (495) 926-21-07

Confidentiality

1 Sosnovaya St., Pokrov village, 142143, Podolsk District, Moscow Region, Russia

SIGNATURE / APPROVAL PAGE (PRINCIPAL INVESTIGATOR, SPONSOR)

INVESTIGATOR INFORMED CONSENT

I, _____

investigator, have reviewed the following protocol provided by NPO Petrovax Pharm, LLC designed for the clinical study: Code GriQv-III-16 version dated November 18, 2016 "Multicenter, Double-Blind, Randomized, Comparative Study of Safety, Reactogenicity, Immunogenicity, and Efficacy of Quadrivalent Inactivated Subunit Influenza Vaccine Grippol® Quadri (NPO Petrovax Pharm, LLC, Russia) and Trivalent Inactivated Polymer-Subunit Vaccine Grippol® Plus (NPO Petrovax Pharm, LLC, Russia) in Parallel Groups, in Volunteers of 18 to 60 Years Old," phase II-III. I affirm that this protocol contains all the information necessary to conduct this study.

I understand that this protocol contains confidential information, which is the property of NPO Petrovaks Farm, LLC. I have discussed the objectives of this clinical trial and the content of this protocol in full with the representative(s) of the Sponsor.

I agree to conduct this clinical study in accordance with this protocol, to comply with all the requirements of the protocol and all ethical standards and safety matters regarding the drug.

I understand that the sponsor may decide to prematurely terminate or suspend the study at any time and for any reason, and I will be notified about such a decision in writing. If I decide to terminate my participation in this clinical study, I will immediately notify the sponsor in writing.

PRINCIPAL INVESTIGATOR

FULL NAME_____

Date: _____ __, 20__ Signature_____

SPONSOR'S REPRESENTATIVE

FULL NAME_____

Date: _____ __, 20__ Signature_____

RESPONSIBLE STUDY PERSONNEL

Sponsor:	**NPO Petrovax Pharm, LLC** 1, Sosnovaya St., Pokrov village, Podolsky District, Moscow Oblast, 142143 President: Arkhangleskaya E. V.
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Trial sites, principal investigator, laboratories:	The list of clinical centers participating in this clinical study (indicating the address and telephone numbers of clinical centers, the name and position of investigators responsible for conducting the study), detailed information about the principal investigator, as well as information about central and local laboratories are presented in a separate document.
In case of serious adverse events, please notify:	**Anna Viktorovna Tsymbal** Tel.: **8-800-234-44-80** Tel.: (495) 730-75-45 (+114) *e-mail*: TSymbalAV@petrovax.ru 22, Krasnaya Presnya Str., 123022, Moscow, Russia **Ethics Board of the MH RF** Tel.: (495) 625-44-21 3, Rakhmanovsky per., 127994, Moscow, Russia

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ABBREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
VAS	Visual Analog Scale
ULN	Upper Limit of Normal
WHO	World Health Organization
HA	Hemagglutinin
DBP	Diastolic Blood Pressure
BMI	Body Mass Index
CRF	Case report form
CRO	Contract Research Organization
LDH	Lactate Dehydrogenase
LEC	Local Ethics Committee
INN	International Non-Proprietary Name
MoH	Ministry of Health
NSAID	Non-Steroid Anti-Inflammatory Drug
ADR	Adverse Drug Reaction
AE	Adverse Event
POx	Polyoxidonium®
HAIR	Hemagglutination Inhibition Assay
SBP	Systolic Blood Pressure
GMAT	Geometric Mean Antibody Titer
SUADR	Serious Unexpected Adverse Drug Reaction
SAE	Serious Adverse Event
ESR	Erythrocyte Sedimentation Rate
CNS	Central nervous system
HD	Human Dose
AP	Alkaline phosphatase
ECG	Electrocardiography
CPMP	Committee for Proprietary Medicinal Products
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IWRS	Interactive web response system
Ig	Immunoglobulin

SYNOPSIS

Study Title	A Multicenter, Double-Blind, Randomized, Comparative Study of Safety, Reactogenicity, Immunogenicity and Efficacy of Quadrivalent Inactivated Subunit Influenza Vaccine Grippol® Quadri (NPO Petrovax Pharm, LLC, Russia) and Trivalent Inactivated Polymer-Subunit Vaccine Grippol® Plus (NPO Petrovax Pharm, LLC, Russia) in Parallel Groups, in Volunteers of 18 to 60 Years Old.
Study Design	A multicenter, double-blind, randomized, comparative study in parallel groups.
Clinical study phase	Phase II-III
Number of investigation sites	The list of clinical centers participating in this clinical study (with addresses and telephone numbers of clinical centers, names and positions of investigators responsible for conducting the study) is presented in a separate document. Up to 6 study centers in the Russian Federation are to participate in the study.
Study Drug	*Name:* Grippol® Quadri, a quadrivalent inactivated subunit influenza vaccine *Dosage form:* suspension for intramuscular and subcutaneous injection. Dosage: 0.5 ml (1 dose) *Active ingredients:* - type A (H1N1) influenza virus antigen 5 µg; - type A (H3N2) influenza virus antigen 5 µg; - type B (Yamagata lineage) influenza virus antigen 5 µg; - type B (Victoria lineage) influenza virus antigen 5 µg; - immunoadjuvant Polyoxidonium® (azoximer bromide) 500 µg. *Administration route:* intramuscular or deep subcutaneous injection *Sponsor:* NPO Petrovax Pharm, LLC *Manufacturer:* NPO Petrovax Pharm, LLC
Comparators	*Name:* Grippol® Plus, trivalent inactivated polymer-subunit influenza vaccine containing Yamagata lineage type B influenza virus antigen. *Dosage form:* suspension for intramuscular and subcutaneous injection. Dosage: 0.5 ml (1 dose) *Active ingredients:* - type A (H1N1) influenza virus antigen 5 µg; - type A (H3N2) influenza virus antigen 5 µg; - type B (Yamagata lineage) influenza virus antigen 5 µg; - immunoadjuvant Polyoxidonium® (azoximer bromide) 500 µg. *Administration route:* intramuscular or deep subcutaneous injection *Sponsor:* NPO Petrovax Pharm, LLC

	Manufacturer: NPO Petrovax Pharm, LLC
	Name: Grippol® Plus, trivalent inactivated polymer-subunit influenza vaccine containing Victoria lineage type B influenza virus antigen. *Dosage form:* suspension for intramuscular and subcutaneous injection. Dosage: 0.5 ml (1 dose) *Active ingredients:* - type A (H1N1) influenza virus antigen 5 µg; - type A (H3N2) influenza virus antigen 5 µg; - type B (Victoria lineage) influenza virus antigen 5 µg; - immunoadjuvant Polyoxidonium® (azoximer bromide) 500 µg. *Administration route:* intramuscular or deep subcutaneous injection *Sponsor:* NPO Petrovax Pharm, LLC *Manufacturer:* NPO Petrovax Pharm, LLC
Number of subjects	The total number of volunteers who will receive the study vaccines is 612 (taking into account the possible 5 % of volunteers whose data cannot be evaluated). It is planned to obtain data on the primary efficacy endpoint that would be measurable in at least 579 volunteers (193 volunteers in each treatment group). Accounting for the possible dropout of volunteers at the screening stage (about 25 %), it is planned to obtain a permission from the Ministry of Health of the Russian Federation to conduct a clinical study with a total of up to **765 volunteers.**
Study Objective	The objective of this study was assessment of safety, reactogenicity, immunogenicity, and efficacy of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri (NPO Petrovax Pharm, LLC, Russia) versus trivalent inactivated polymer-subunit vaccine Grippol® Plus (NPO Petrovax Pharm, LLC, Russia) in subjects from 18 to 60 years old.
Purposes of the Study	1) Study and comparative assessment of immunogenicity of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri versus trivalent inactivated polymer-subunit vaccine Grippol ® Plus in subjects from 18 to 60 years old. 2) Safety and reactogenicity assessment of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri versus trivalent inactivated polymer-subunit vaccine Grippol® Plus in subjects from 18 to 60 years old. 3) Study and comparative assessment of immunogenicity of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri versus trivalent inactivated polymer-subunit vaccine Grippol® Plus in subjects from 18 to 60 years old.

Treatment groups	Group A: quadrivalent inactivated subunit influenza vaccine Grippol® Quadri, suspension for intramuscular and subcutaneous injections. Group B: Grippol® Plus, trivalent inactivated polymer-subunit influenza vaccine, based on type B influenza virus of Yamagata lineage, suspension for intramuscular and subcutaneous injections. Group C: Grippol® Plus, trivalent inactivated polymer-subunit influenza vaccine, based on type B influenza virus of Victoria lineage, suspension for intramuscular and subcutaneous injections. ***Dosing:*** All the vaccines used in the study are administered as a single intramuscular injection of 0.5 ml (1 dose) into the upper third of the outer surface of the shoulder (the deltoid muscle).
Study duration	Proposed study duration — 12 months: • Proposed enrollment period — 3 months. • Duration of vaccination period — once (1 day). • Duration of observation period – 180±5 days.
Visits	Visit 0 (Days [-3] to [0], inclusion of volunteer into the study, assessment of baseline characteristics) Visit 1 (Day 0 — vaccination) Visit 2–7 (Days 1–22±1 — follow-up) Visit 8–11 (Days 43±5–180±5 — telephone calls)
Methodology	***Study diagram and the order of examinations are presented on Figure 1 and in Table 2.*** Within 1–3 days after signing the informed consent, the volunteers will undergo screening laboratory investigations, and baseline data will be assessed during physical examination. Upon the screening period and the final confirmation of compliance with all eligibility criteria, volunteers will be randomized into study groups of intramuscular injection of 0.5 ml of one of the three study vaccines. After the vaccination, the volunteers remained under the observation of investigating physician for 2 hours. The frequency of measurements of vital functions, body temperature, local and general reactions after vaccination are described in the procedure table. The volunteers were given diaries for daily records of the body temperature, concomitant therapy, and adverse events. Follow up of volunteers includes 7 outpatient visits (up to Day 22±1 post immunization) and 4 telephone calls (up to Day 180±5 post immunization). ***Randomization:*** The immunization group was determined randomly. Randomization will be based on a computer-generated randomization algorithm (randomization list), where each volunteer will be assigned a randomization number. That number will define the immunization group (A, B, or C) according to the randomization plan. That number will be assigned to the study subject for the entire period of the study and will be documented in volunteer's CRF.

	The volunteers will be randomized into 3 groups (at a ratio of 1:1:1). A block randomization method is used. An interactive web-response system (IWRS) with technical support service will be used for randomization. Investigators will be trained on working with IWRS system, and IWRS user guide will be handed out to all of them. An identification name (ID) and a password will be used to access the system. These identifiers will be given to the authorized staff member of the investigation site prior to study initiation. Upon the volunteer randomization, the investigator receives information through the IWRS system on volunteer assignment to one of the vaccination groups that will be given to the volunteer during the study. Volunteer stratification (by gender and age) will be made in the IWRS system for uniform assignment to groups.
Inclusion criteria	1. Signed and dated volunteer's informed consent for participation in the study. 2. Men and women from 18 to 60 years old. 3. Healthy volunteers without signs of acute or chronic disorders, without history of chronic respiratory, cardiovascular, nervous system disorders, hepatic or renal disorders. 4. Previously not immunized, or previous influenza immunization occurring ≥ 12 months before this study. 5. Subjects without history of influenza within ≥ 12 months before this study. 6. Consent of volunteers (men and women) to use adequate methods of contraception (cervical caps with spermicide, diaphragms with spermicide, condoms with spermicide, intrauterine devices, oral contraceptives) or full abstinence for the whole period of the study.
Non-Inclusion criteria	*Specific:* 1. Contraindications listed in the protocol and prescribing information for inactivated influenza vaccines: - acute infections and non-communicable disorders, including the period of reconvalescence of at least one month from the time of clinical and laboratory evidence of recovery; - hepatitis or meningococcal infection occurred less than 6 months after recovery; - exacerbations of chronic disorder or decompensated disorders that may impact the study (organic central nervous system disorders, decompensated cardiovascular disorder, acute renal or hepatic failure); - malignant neoplasms (including hematological disorders); - primary immunodeficiency (laboratory-confirmed); - HIV infection or HIV-associated disorders; - systemic disorders of connective tissue;

	- haemophilia (and other blood coagulation disorders); - severe neurological disorders; - Guillain–Barré syndrome (post infection demyelinating polyradiculoneuropathy of autoimmune nature with peripheral limb muscle palsy related to inflammation and destruction of myelin sheath of peripheral nerves; may acquire an ascending nature, involving muscles of face, pharynx, larynx); - history of severe vaccine-associated reactions (body temperature exceeding 38.5 °C) or local reactions (hyperemia and/or oedema at the site of injection of over 5 cm in diameter); - history of severe allergic disorders (angioedema, polymorphic exudative erythema, serum disease, etc.); - hypersensitivity to chicken protein or vaccine components; - blood and components transfusion within the last 6 months. 2. Indications for immunomodulating therapy. 3. Body temperature over 37.0 °C at screening or before injection. 4. Potential evidence of a chronic infection (periodic episodes of fever within the last 6 months), or antiviral (and/or antibacterial) treatment indicated. 5. History of disorders or conditions, which, according to investigator's judgment may impact the thermal regulation (chronic infections, neuroendocrine disorders [thyrotoxicosis, pheochromocytoma, etc.], climacteric syndrome, malignant hyperthermia, CNS disorders, malignant neoplasm, connective tissue disorders, systemic vasculitis, and information on excessive physical stress or work-rest regimen deviations [within the last 2 months: night shifts, significant change of time zones, overheating]). 6. Use of antipyretics (including non-steroidal anti-inflammatory drugs and anilides) within 24 hours before randomization. 7. Surgical interventions within less than 90 days before the screening visit. 8. Systolic blood pressure of over 130 mm Hg or less than 100 mm Hg and/or diastolic blood pressure of over 90 mm Hg or less than 60 mm Hg. 9. Any other disorder, which, in the opinion of the investigator, may prevent inclusion of the volunteer due to safety reasons or may impact the study results. *General:* 10. Pregnant and nursing women. 11. Lack of ability to visit daytime inpatient facility according to the study schedule, unavailability for adequate follow-up of the volunteer. 12. Body mass index of less than 18.5 or over 30.0 kg/m2 based on the weight-height Quetelet's index. 13. Participation in another clinical study of medicinal drugs within 3 months before the start of this study. 14. Mental, physical, or other reasons which prevent adequate assessment of own behavior and prevent from meeting the study protocol conditions. 15. History of narcotic and/or drug abuse, and/or inhalant addiction, current signs of alcoholic intoxication.

	16. Intake of at least 5 alcohol units per week or history of alcohol, drug, or medicinal product abuse. One alcohol unit corresponds to 360 ml of beer, 120 ml of wine, or 30 ml of a strong alcoholic beverage. 17. Suspected lack of compliance with treatment or inability to undergo treatment and observe the limitations according to the study protocol. 18. Volunteers acknowledged by the court to be disabled or under guardianship. 12. Any other conditions that make the volunteer ineligible for the study according to a justified opinion of the study doctor or Sponsor.
Criteria of volunteer drop out from the study	Early withdrawal of the volunteer from the study is possible at any time. The study doctor shall withdraw subjects from the study continuation in the following cases: • Informed consent recall. • Occurrence of a severe AEs or serious adverse events. • The volunteer is found to meet any of the non-inclusion criteria related to the safety of the volunteer participation in the study. • If a female-volunteer becomes pregnant. • The volunteer takes medicines not allowed in this study. • The volunteer is lost to follow-up. • In a situation which, in investigator's opinion, may adversely impact the volunteer if he/she continues participation in the study. • For administrative reasons (study termination by the Sponsor or regulatory authorities) or in case of major protocol violations which may significantly impact the study results.
Not allowed concomitant therapy	During the study, volunteers must not receive: • <u>only at screening and on the day of immunization</u>: - antipyretics (including non-steroidal antiinflammatory drugs and anilides); - any drugs or food supplements which, according to the investigator's judgment, may affect the body temperature of the volunteer (including caffeine); - physiotherapeutic procedures.

	• For the whole period of the study: - medicinal products suppressing central nervous system functions (barbiturates, opiates, sedative drugs), tranquilizers, neuroleptics, tricyclic antidepressants etc.; - cytotoxic drugs; - anticoagulants (including warfarin), antiagregants (including clopidogrel, acetylsalicylic acid). Hormonal contraceptives or hormone replacement therapy is only allowed if the female volunteer has been on a stable dosing regimen maintained for at least 6 months before the screening visit and remains on a stable dosing regimen for the whole study period.
Immunogenicity and efficacy endpoints	**Primary:** • The share of subjects achieving seroconversion (the number of volunteers with antibody titer [of at least 1:40] increased more than 4-fold versus baseline [assessed by HAIR]) for antigens: influenza virus type A — H1N1, influenza virus type A — H3N2, influenza virus type B — Yamagata and Victoria lineage, based on assessment at Day 21 after immunization (Visit 7). **Secondary:** • Coefficient of increase of mean geometric antibody titer (antigens H1N1, H3N2, Yamagata and Victoria lineages) based on assessment data after 21 days following vaccination (visit 7). • Geometric mean of serum antibodies to antigens: influenza virus type A — H1N1, influenza virus type A — H3N2, influenza virus type B — Yamagata and Victoria lineage, based on assessment at Day 21 after immunization (Visit 7). • Seroprotection: share of subjects achieving protective antibody titer (1:40 and more) to antigens H1N1, H3N2, Yamagata and Victoria lineage, based on assessment at Day 21 after immunization (Visit 7). • Incidence of influenza and ARI based on assessment 180±5 days after immunization (Visit 11). • Severity and duration of reported cases of influenza and ARI, complications.
Safety and reactogenicity parameters	• Quantitative assessment and nature (severity and duration) of local and systemic reactions to vaccine administration. • AEs and SAEs associated with the study vaccines. • Immediate type AEs, emerging within one hour after immunization. • Incidence and nature of intercurrent disorders • Vital function assessment results. • Results of physical examination. • Neurological examination results. • Results of blood hematology and biochemistry tests.

	• Urinalysis results. • Immunological parameters evaluation results: serum immunoglobulin content (ELISA: IgE).
Statistical analysis	See Section 8 of the protocol.
Determining the sample size	For details see Section 8 of the protocol. Calculation of the sample size was performed based on the primary endpoint, the share of subjects achieving seroconversion according to the evaluation at Day 21 after immunization (Visit 7), and testing of the statistical hypothesis of non-inferiority of the test vaccine (Grippol® Quadri) versus the reference vaccine (Grippol® Plus). The following non-inferiority margin was used for comparative evaluation of immunogenic efficacy in the non-inferiority model: - upper limit of the two-sided 95 % confidence interval of the difference between parameters (R-T) of subjects achieving seroconversion in the study quadrivalent vaccine and reference trivalent vaccine groups shall not exceed 15 %. This value is selected based on the minimum threshold for influenza vaccines > 40 % of individuals with seroconversion, established harmonization requirements for influenza vaccine of the Committee for Proprietary Medicinal Products (CPMR), as well as the expected level of seroconversion in comparison groups over 60 %. For testing of non-inferiority null hypothesis for Grippol® Quadri and Grippol® Plus vaccines, taking into account the following parameters: • Power: 80 % • One-sided significance level: 2.5 % • Non-inferiority margin: 15 % • Expected share of subjects achieving seroconversion in Grippol® Plus group: 60 % • Expected actual difference for the parameter in groups: 1 % according to the calculation using software package PASS 12, each treatment group shall have at least 193 volunteers (579 volunteers in total). Taking into account the dropout during the study (5 %), the required number of volunteers has to be increased to 204 per group (612 in total). Taking into account the possible dropout of 25 % of volunteers, up to 765 volunteers are planned to be screened during the screening.
Intermediate data analysis	Not applicable.

Ethical and legal aspects	The study will be conducted in accordance with the Constitution of the Russian Federation; the current version of the Federal Law of the Russian Federation of April 12, 2010 No. 61-FZ “On Circulation of Medicines”; the current version of the Federal Law of the Russian Federation of November 21, 2011 No. 323-FZ “Basics of Health Protection of the Citizens in the Russian Federation” Guidelines for the Expertise of Medicines (Volume 1.- M .: Grif and K, 2013, p. 328); GOST R52379-2005 "Good Clinical Practice"; the ethical principles of the Helsinki Declaration of the World Medical Association, last revision; Government Decree of the Russian Federation of September 13, 2010 No. 714 “On approval of the Typical rules for compulsory insurance of the life and health of a Patient involved in Clinical Trials of a medicinal product” (as amended on September 4, 2012); Government Decree of the Russian Federation of May 18, 2011 No. 393 “On Amendments to the Model Rules for Mandatory Life Insurance of a Patient Participating in Clinical Studies of a medicinal product”; Order of the Ministry of Healthcare and Social Development of Russia dated August 23, 2010 No. 703n “On approval of the reporting form on completion, suspension or termination of a clinical trial of a medicinal product for medical use”; By order of the Ministry of Health of the Russian Federation dated February 8, 2013 No. 58n "On approval of the Regulation on the Ethics Council in the field of circulation of medical devices."

1. STUDY RATIONALE

1.1. Name and description of study drugs

Seasonal epidemics of influenza are still a significant public health issue associated with significant morbidity and mortality rates in different parts of the globe every year. The illness in human is caused by influenza viruses types A/H1N1, A/H3N2, and B. International surveillance over influenza demonstrates that seasonal viruses can actively migrate between hemispheres and continents. Droplet transmission and high variability of influenza virus poses the whole world population at threat as a new variant of the virus emerges in any part of the globe: as it can be seen from the practical experience, the virus rapidly spreads within just a few months [1, 2].

Influenza A and B viruses belong to orthomixoviruses; however, A strains can infect both humans and animals, and birds, while type B is only virulent for humans. Concurrent circulation of type A and B influenza viruses in the community has been documented since the 1970s, which formed the basis for recommendation of composition of seasonal influenza vaccines based on three influenza strains — two A and one B. It has long been conjectured that B influenza virus is significantly less virulent and does not cause serious illness or large epidemics, unlike type A viruses. Since 1985, experts isolated two phylogenetic lineages of type B virus strains with different antigens — Victoria and Yamagata lineages — circulating in different seasons or simultaneously within one epidemic period [3]. According to requirements to influenza vaccines, the composition of seasonal vaccines still included only one strain of type B virus, which did not always match the seasonal circulating strain or both viruses circulated within one epidemiological season. It led to suboptimal effectiveness of vaccination in the epidemiological seasons when the vaccine strain B did not match the actually circulating strain: the vaccine strain B does not provide full protection against the wild strain of another lineage [4, 5, 6, 7].

Post-hoc analysis of data on circulation of B viruses of both lineages and of associated infections in different populations within different epidemic seasons has shown that the incidence of influenza B significantly varies by different years and by continents and may reach high numbers if the vaccination strain does not match the wild-type virus. According to the US Centers for Disease Control in 2001/2002 to 2010/2011 seasons (including the pandemic season of 2009/2010), in USA cases of influenza caused by the type B virus in all populations, predominantly in pediatric population, reached 44 % [8]. European data show similar trends: In the Netherlands, the number of cases of influenza caused by the type B virus in the period from 1992/1993 to 2006/2007 in various seasons reached 82 % [9]; in Finland, the number of confirmed cases of type B influenza reached 20 % within the period from 1980 to 1999, with most patients affected in the pediatric population of < 17 years [10]. The analysis of data on influenza virus circulation form 1999 to 2012 by Finnish investigators confirmed the epidemic significance for community of mismatch between the vaccine and wild type B influenza virus, particularly in children and adolescents [11].

Impossibility to accurately predict the specific type B strain to be prevalent in the next season at a specific region and data of monitoring of seasonal epidemics formed the basis for development of quadrivalent influenza vaccines, in addition to two type A and one type B virus strains containing the second type B virus, the so-called quadrivalent vaccines.

The first quadrivalent vaccine was launched in Europe in 2012; later, quadrivalent influenza vaccines were approved in the US, Canada, Australia, China. Experts expect significant cost effectiveness of immunization. European researchers using a mathematic model calculated that using quadrivalent influenza vaccine in the UK will help decrease the incidence of seasonal influenza by 1,393,720 cases; reduce the number of visits by 439,852; complications, by 167,357 cases; complication-related hospitalizations, by 26,424; influenza mortality, by 16,471 versus trivalent vaccines. The quality of life adjusted incremental cost-effectiveness ratio (ICER) would be £ 5,299 [12].

Currently, only trivalent influenza vaccines have been approved in Russia. No quadrivalent influenza vaccines are approved in the Russian pharmaceutical market. There are no local developments available either. Development of the first Russian quadrivalent vaccine will help improve the effectiveness of vaccination and significantly improve the cost-effectiveness of preventive immunization.

Petrovax Pharm has a rich legacy in the field of development, creation, and manufacture of effective subunit influenza vaccines based on highly purified antigens and immunoadjuvant Polyoxidonium® (PO). Petrovax Pharm has all the required resources: state-of-art pharmaceutical manufacture, competent and qualified personnel, as well as quality assurance service according to Good Manufacturing Practice (GMP) standards.

Many years of positive experience of using local vaccines Grippol® and Grippol® Plus with reduced antigenic load and addition of 500 μg of POx further highlights the significance of such vaccines and introduction into the practical healthcare in terms of decreased incidence and improved cost effectiveness.

In view of the foregoing, the new development of NPO Petrovax Pharm, LLC — a Russian inactivated subunit quadrivalent influenza vaccine containing highly purified antigens of four influenza strains and POx as immunoadjuvant — is of significant value.

The complex of own nonclinical studies of the quadrivalent inactivated influenza subunit vaccine Grippol® Quadri confirms the high specific activity and high safety profile of the product [13-19].

Animal studies on specific activity have shown that Grippol® Quadri vaccine has a pronounced immunogenicity comparable with trivalent vaccine Grippol®Plus. The presence of the antigen to the fourth B strain of the influenza virus, of Victoria lineage in Grippol® Quadri does not reduce the immunogenicity of the other antigens included in the vaccine.

Non-clinical studies have shown that induction of the specific immune response to the vaccination does not prevent adequate immune responses to the antigens heterologous to those included into the vaccine.

Phase I safety and tolerability study of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri in healthy volunteers was completed [20]. The study included 3 groups of 30 subjects, overall 90 subjects. Subjects from group 1 (30 volunteers) received a single 0.5 ml dose of Grippol® Quadri; Group 2 (30 volunteers) received a single 0.5 ml dose of the reference vaccine Grippol Plus; Group 3 (30 volunteers) received a single 0.5 ml dose of placebo (saline solution). Overall 53 AEs were reported in the study, including 26 AEs in 11 subjects (36.7 %) in the Grippol® Quadri Group, 13 AEs in 9 subjects (30.0 %) in the Grippol® Plus group, and 14 AEs in 6 subjects (20.0 %) in the Placebo Group. No serious adverse events (SAE) or AEs leading to withdrawal from the study were reported. All the AEs were of a mild to moderate severity. Reactogenicity assessment showed mild or indefinite severity local reactions with no significant differences between the groups. All systemic reactions were considered to be mild without significant differences between the groups.

In conclusion, the evidence of non-clinical studies and a phase I clinical study supports safety of Grippol® Quadri vaccine (NPO Petrovax Pharm, LLC, Russia) and helps to recommend it for further investigation in healthy volunteers to evaluate its immunogenicity, efficiency, safety, and reactogenicity.

The objective of this study was assessment of safety, reactogenicity, immunogenicity, and efficacy of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri (NPO Petrovax Pharm, LLC, Russia) versus trivalent inactivated polymer-subunit vaccine Grippol® Plus (NPO Petrovax Pharm, LLC, Russia) in subjects from 18 to 60 years old. Due to the identity of antigens used in the production of tetravalent and trivalent vaccines, the difference in composition of the developed vaccine and the comparator vaccine only in terms of valence, the absence of need to select the optimal dose and the possibility
to adequately assess protective potentials of vaccines on immunological parameters, the proposed study belongs to phase II-III and is registrational.

1.2. The results of nonclinical and clinical studies relevant to this study.

A detailed description of the results of preclinical and clinical trials of influenza tetravalent inactivated subunit vaccine Grippol® Quadri (NPO Petrovaks Farm, Russia) is presented in Investigator's Brochure.

Nonclinical studies

Antigenic activity (immunogenicity) of Grippol® Quadri [13].

Antigen activity of Grippol® Quadri was assessed by the level of specific antibodies to hemagglutinin in serums of immunized mice. The level of antibodies was determined by hemagglutination inhibition test (HAIR) and by enzyme-linked immunosorbent assay (ELISA). The conducted studies have shown that antibodies to hemagglutinin of each strain of the vaccine are produced in mice in response to administration of Grippol® Quadri, as was shown in all the performed tests. The geometric mean antibody titers (GMAT) determined in animals after administration of Grippol® Quadri did not significantly differ from those for the trivalent vaccine Grippol®Plus. In one test, the level of antibodies after administration of quadrivalent vaccine was even higher than that for a similar dose of trivalent vaccine. GMAT obtained by HAIR for Grippol® Quadri were 1:113, 1:242, 1:86, 1:92 for strains A/H1N1/California/07/09, A/H3N2 /Switzerland/9715293/13, B/Phuket/3073/13 and B/Brisbane/60/08 respectively. Equivalent values for Grippol Plus: 1:75, 1:80, 1:65. The B/Brisbane/60/08 strain was not included in the trivalent vaccine, so the level of antibodies to it was not measured. Thus, it can be concluded that the inclusion of additional five micrograms of hemagglutinin strain B/Brisbane/60/08 in the vaccine does not reduce the intensity of the immune response to other strains. In addition, the studied vaccines provided 100 % seroprotection of animals, i.e. GMAT to hemagglutinin of each strain was ≥ 1:40.

It was shown that POx acts as an adjuvant in the composition of tetravalent vaccine, in particular, it enhances the production of antibodies to hemagglutinin. GMAT for polyoxide-free antigens was 1:87, 1: 113, 1:80 for strains A/H1N1/California/07/09, A/H3N2/Switzerland/9715293/13, B/Phuket/3073/13 respectively. Equivalent values for Grippol® Plus: 1:123, 1:247, 1:104.

ELISA results support the data obtained by HAIR. Immunization of animals led to the production of antibodies to hemagglutinin of both IgG1 and IgG2a subclasses. It is known that an increase in the level of IgG1 antibodies reflects activation of humoral, and an increase in IgG2a — cellular immunity.

In the second test, the amount of cytokine IL-4 in supernatants of mouse splenocyte pre-stimulated with a mixture of hemagglutinins was determined. According to the obtained results, it can be concluded that the humoral immunity is activated in mice that received Grippol® Quadri. Index of Il-4 stimulation was 3.9. Same parameter for trivalent vaccine was 4.3

During the nonclinical efficacy studies of the Grippol® Quadri influenza vaccine containing hemagglutinin and neuraminidase of four influenza virus strains, the antigenic activity of the novel product was investigated. In the three studies with laboratory mice it was shown that the Grippol® Quadri vaccine represented high antigenic activity comparable or even exceeding that of the trivalent vaccine. The presence of the 4th strain B/Brisbane/60/08 Victorian line influenza virus antigens in Grippol® Quadri did not reduce immune activity of other vaccine antigens.

It should be noted that even considering the 4th strain, the total amount of hemagglutinin in one dose of Grippol® Quadri was 20 µg/dose, which is almost twice less than that usually included in the trivalent inactivated influenza vaccines without adjuvant (45 µg/dose). Grippol® Quadri vaccine includes POx making the antigen dose of 20 µg sufficient and effective. The amount of POx in Grippol® Quadri is 500 µg. This amount of POx in the trivalent polymer-subunit vaccines Grippol® plus and Grippol® with a total HA content of 15–21 µg has proven its efficacy and safety. The studies showed that the "antigen-adjuvant"-related antibody response for each product significantly exceeded the antigen-mediated immune response without adjuvant.

Assessment of administration safety

Acute toxicity of Grippol® Quadri vaccine [14]

The acute toxicity of the Grippol® Quadri vaccine was evaluated in 40 male and female white mice (10 mice per group) when administered subcutaneously at a single dose of 1,600 and 3,200 µg HA/kg that, respectively, 2,000 and 4,000 times exceeds the maximum permitted dose in human (based on a child weighing 10 kg, i.e. about 0.8 µg/kg). In the control groups, the animals were administered normal saline solution and vehicle (vaccine solvent) — phosphate saline buffer solution.

The criteria for assessment of toxicity were: indicators of body weight change, signs of impaired health in animals during a clinical examination, indicators of changes in peripheral blood, a macro- and microscopic picture of cavities and internal organs.

During the study, no animal death, as well as clinical signs of toxicity following a single-dose subcutaneous administration of the Grippol® Quadri vaccine to the male and female mice at the doses of 1,600 µg HA/kg and 3,200 µg HA/kg were observed. The necropsy carried out on animals 7 days after the administration show no macroscopic changes in the organs or tissues, their location relative to each other, size, shape, color, or consistency; no pathological changes in the structure of the walls, mucous membranes, intracranial, abdominal, or pleural cavities. As a result of the analysis of histological specimens, a moderate reaction of the organs of the immune system (thymus, spleen, regional lymph node) was revealed. Thymocyte emigration, delymphatization of thymus-dependent areas in the white pulp of the spleen and a regional lymph node, an increase in the number of plasma cells in the lymph node medulla were observed. These structural transformations of the lymphoid organs are the hallmark of the immune reactions occurring in response to vaccination with significant doses. In other organs and tissues, as well as at the administration site no changes were detected.

Repeat dose toxicity [15]

Study products were administered to male and female Wistar rats intramuscularly once a week for 6 weeks at doses of 20 and 100 HA µg/kg. Animals in control group received in a similar way an equivalent amount of saline and the vehicle (vaccine diluent) — phosphate saline buffer.

During the observation period, test animals did not show decreased body weight gain, clinically significant abnormalities in food and water intake or functional parameters of central nervous system and cardiovascular system, as well as changes in the peripheral blood cell counts and serum biochemical values.

Urine biochemical analysis in female rats following 6 intramuscular injections of the Grippol® Quadri vaccine at a maximum dose of 100 HA µg/kg revealed a significant decrease of 24-hour excretion of chlorides and urea along with a decline in the daily urine output. The blood serum urea and chlorides levels were within the normal ranges; therefore, there were no reasons to suggest any serious tubular reabsorption impairment.

Histological findings in the liver and kidneys demonstrated moderate vascular reactions and changes in convoluted tubules epithelium; in other studied internal organs and tissues, when compared to the control, any significant pathological abnormalities have not been revealed.

Therefroe, the studies showed that there were no significant deviations in the health status of study animals following the immunization with Grippol® Quadri vaccine, manufactured by NPO Petrovax Pharm, LLC, containing locally produced influenza virus antigens at a dose of 20 µg per 0.5 ml, which indicates the safety of the study drug.

Assessment of local effect.

The assessment of local effect was included in the acute and chronic toxicity studies in mice and rats based on the clinical examination data and the results of the histological examination of the injection site (muscle and surrounding tissues).

Macroscopic examination of the injection site after a single subcutaneous injection of Grippol® Quadri to mice at doses of 1,600 and 3,200 HA µg/kg and after a course of intramuscular administration to the Wistar rats showed no significant differences in the skin, subcutaneous tissue, and adjacent tissues condition, following the administration of the investigational product and physiological saline solution as a control.

Histological examination of the injection site after 6 intramuscular administrations of the vaccine to male and female rats at doses of 20 and 100 HA µg/kg has shown the presence of small haematomas in isolated cases in the control and test groups of males and females. This non-specific reaction is a response to the mechanical impact, it is not the result of the local irritant effect.

Assessment of immunologic safety [16].

Immunological safety assessment of the Grippol® Quadri vaccine (in total, 20 µg of HAs of the three strains per dose plus 500 µg of Polyoxidonium) consisted of nonclinical investigation of the immunoreactivity changes in the experimental animals (mice, guinea pigs) associated with the specific immune response to the immunization. The vaccines containing similar hemagglutinin and POx levels were used as comparators: commercially available vaccine Grippol® Plus (a total of 15 µg HA of the 3 strains per dose and 500 µg of POx) and vaccine Grippol® (a total of 21 µg HA of the 3 strains per dose and 500 µg of POx).

The ability of vaccines to influence the humoral, cellular, and macrophage parts of the immune response at different times after vaccination was evaluated in mice. Immunological reactivity of mice after the study vaccine administration was estimated according to the accumulation of antibody-producing cells in mice spleen after their immunization with sheep red blood cells (a model of humoral immune response), the development of delayed-type hypersensitivity during the plantar test (a model of cell-mediated immune response). Activity of phagocytes was estimated according to the respiratory burst process in the spontaneous and induced chemical luminescence setting. Investigators also studied the possibility of the polyclonal effect of the study vaccines by the ability to nonspecific stimulation of antibody-forming cells to various test antigens.

The findings of the animal studies performed have demonstrated that the specific immune response to the vaccine does not preclude both acute and long-term post-immunization normal immune reaction to the antigens heterologous to those presented in vaccines. The stimulating effect of the Grippol® Quadri vaccine on the humoral immune response to the heterologous test antigen (the ram erythrocytes) has been proven. It was shown that immunoadjuvant action is not associated with polyclonal properties, which is the immune system safety indicator. The investigation of the study vaccine effect on the multi-local phagocytic activity and the cellular immune response did not reveal effects specific for the possible immunotoxic effect of immunization.

The Grippol® Quadri vaccine allergenic properties [17] were assessed in animals (guinea pigs and mice) using the immediate (ITH) and delayed type (DTH) response testing with multiple administrations of the product.

Like the Grippol® vaccine, the Grippol® Quadri vaccine was not found to have any local irritative effect (at a dose of 1/10 BH) and did not cause the delayed-type allergic reaction following multiple administration of the maximum possible testing amount (1/5 and 1/10 of BH) to mice.

During evaluation of the possibility to sensitize guinea pigs (the most sensitive animal species) in the immediate-type hypersensitivity reaction, there were no signs of sensitization revealed with repeated Grippol® Quadri injections, suggesting an extremely low probability of allergic reactions associated with the vaccine administration to humans.

Summarizing the results of the conducted research, we can conclude that the domestic tetravalent vaccine for the prevention of influenza Grippol® Plus containing 2 strains of influenza virus types A and B in nonclinical studies has shown high efficiency and safety. It can be expected that the use of Grippol® Quadri vaccine will provide better protection to the population against influenza.

Clinical studies [20]

Phase I safety and tolerability study of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri was conducted in healthy volunteers [20].

The study included 3 groups of 30 subjects, overall 90 subjects. Subjects from group 1 (30 volunteers) received a single 0.5 ml dose of Grippol® Quadri; Group 2 (30 volunteers) received a single 0.5 ml dose of the reference vaccine Grippol Plus; Group 3 (30 volunteers) received a single 0.5 ml dose of placebo (saline solution).

Over the study period, there was a minimum of 1 AE in the Grippol® Quadri group, Grippol® Plus group, and Placebo group in 36.7 %, 30.0 %, and 20.0 % of the subjects respectively. The majority of all registered AEs were classified as post-immunization reactions (reactogenicity symptoms — local and system reactions) and were considered by the Investigator as related to the study product (strength of relation: "definite", "probable", and "possible").

During the evaluation of the reactogenicity in the Grippol® Quadri group, the local reactions were detected in 33.3 % of the subjects, in the group Grippol® Plus, there were 23.3 % of the subjects, and in the Placebo group, 13.3 % of the subjects. Among the local reactions, there were observed pain at the injection site, erythema, swelling and induration, and itching at the injection site. Pain at the injection site was the most common local reaction in all three study groups. No additional medical intervention was needed for the local reactions in the subjects. Local reactions were mild to non-identified by severity. During the comparative evaluation, there was no evidence of the statistically significant difference between the proportion of mild, moderate, and severe local reactions in the Grippol® Quadri group, Grippol® Plus group, and Placebo group ($p = 1.00$ and $p = 1.00$ respectively).

Only the subjects without any intercurrent conditions within the first five days after immunization were included in the system reactions analysis: in total 29 of the 30 subjects from the group Grippol® Quadri, 28 of 30 subjects from the group Grippol® Plus, and 29 subjects from the Placebo group. During the evaluation of the reactogenicity, the general reactions were detected in the group Grippol® Quadri in 20.7 % of the subjects, in the group Grippol® Plus in 3.6 % of the subjects, and in the Placebo group in 10.3 % of the subjects. Among the general reactions recorded during the study, there were the following: rise in body temperature above 37 °C, malaise, headache, nausea, myalgia / arthralgia. The most common general reaction in the group Grippol® Quadri was the development of headache — in 13.8 % of the subjects, whereas in the group of Grippol® Plus and in the Placebo group headache was observed only in 3.6 % and 3.4 % of the subjects respectively. The rise in body temperature above 37 °C was recorded in 6.9 % of subjects in the group Grippol® Quadri (within subfebrile with a maximum registered value of 37.2 °C), and also in 6.9 % of subjects in the Placebo group (within subfebrile with a maximum registered value of 37.5 °C). All the occurred systemic reactions were regarded as mild. During the comparative evaluation, there was no evidence of the statistically significant difference between the proportion of mild, moderate, and severe local reactions in the Grippol® Quadri group, Grippol® Plus group, and Placebo group ($p = 0.102$ and $p = 0.470$ respectively).

The results of the laboratory tests and neurological status assessment did not reveal any significant changes over the study period. In addition, the results of the analysis of all vital signs excluding body temperature (clinically significant subfebrile increase of body temperature in 2 subjects during the vital signs assessment) did not reveal any considerable changes over the study period. Clinically significant body temperature abnormalities were recorded within the frames of the registered AEs. The results of the physical examination evaluations, except for the data on the condition of the respiratory system and mucous membranes (in 1 subject diagnosed with respiratory tract infection, nasopharyngitis), were within the normal range. Clinically significant abnormalities were registered as AEs.

1.3. Known and potential risks and benefits for study subjects

Known and potential risks of the study vaccine and comparison vaccine are described below. When determining the causal relationship of the adverse events that occur during this study, the investigator must take into account all the information described below, as well as information from the current version of the draft of the instruction for medical use and information from the latest version of the investigator's brochure on the study drug.

Potential risks of using the study drug:

Inactivated influenza vaccines are highly purified drugs and are well tolerated. Based on the published literature, the results of non-clinical studies and the phase I clinical study, it is expected that the safety profile of quadrivalent vaccine under investigation will be the same as the profile of trivalent comparison vaccines. Adverse events and their frequency are listed below.

Common (> 1/100, < 1/10). Local reactions in the form of sore, redness, swelling, and itching in the administration site. Systemic reactions: malaise, fatigue, subfebrile temperature.

Infrequent (> 1/1,000, < 1/100). Systemic reactions such as mild runny nose, sore throat, headache, temperature above subfebrile.

Usually the above mentioned reactions pass off in 1–2 days.

Rare (> 1/10,000, < 1/1,000). Allergic reactions including the immediate ones.

Very rare (< 1/10,000).

- nervous system disorders: neuralgia, paresthesia, neurological disorders;
- locomotor system disorders: myalgia.

53 adverse events in 26 volunteers have been recorded during phase I clinical study. All the AEs were of a mild to moderate severity. None of the adverse events was a reason for the subject's withdrawal from the study. Among the AEs, there were no serious adverse events (SAE). Characteristics of adverse events (system organ classes and association to the study drug) are presented in Table 1.

Table 1. Number of Volunteers with Adverse Events Associated With the Study Drug (Degree of Association: "definite", "probable", and "possible") distributed according to the System organ class and the preferred term (MedDRA)

System Organ Class: preferred Term, n (%)*	*Degree of Association With the Study Drug*	*Group Grippol® Quadry N = 30*	*Group Grippol® Plus N = 30*	*Group Placebo N = 30*
Total number of subjects with adverse events (any AEs)	**Definite**	**1 (3.3 %)**	**1 (3.3 %)**	**0 (0.0 %)**
	Likely	**9 (30.0 %)**	**5 (16.7 %)**	**4 (13.3 %)**
	Possible	**1 (3.3 %)**	**1 (3.3 %)**	**1 (3.3 %)**
General disorders and administration site conditions:	**Definite**	**1 (3.3 %)**	**1 (3.3 %)**	**0 (0.0 %)**
	Likely	**9 (30.0 %)**	**5 (16.7 %)**	**4 (13.3 %)**
	Possible	**0 (0.0 %)**	**1 (3.3 %)**	**0 (0.0 %)**
Erythema at the injection site	Likely	1 (3.3 %)	1 (3.3 %)	1 (3.3 %)
Pain at the injection site	Definite	1 (3.3 %)	1 (3.3 %)	0
	Likely	9 (30.0 %)	4 (13.3 %)	4 (13.3 %)
	Possible	0	1 (3.3 %)	0
Itching at the injection site	Likely	1 (3.3 %)	1 (3.3 %)	0
Swelling at the injection site Malaise	Likely	0	0	1 (3.3 %)
	Possible	2 (6.7 %)	0	1 (3.3 %)
Laboratory and instrumental data:	**Possible**	**3 (10.0 %)**	**0**	**2 (6.7 %)**
Increased body temperature	Possible	3 (10.0 %)	0	2 (6.7 %)
Skeletal muscle and connective tissue disorders:	**Possible**	**0**	**0**	**1 (3.3 %)**
Arthralgia	Possible	0	0	1 (3.3 %)
Myalgia	Possible	0	0	1 (3.3 %)
Nervous system disorders:	**Likely**	**2 (6.7 %)**	**0**	**0**
	Possible	**3 (10.0 %)**	**1 (3.3 %)**	**1 (3.3 %)**
Headache	Likely	2 (6.7 %)	0	0
	Possible	3 (10.0 %)	1 (3.3 %)	1 (3.3 %)
Drowsiness	Likely	1 (3.3 %)	0	0

* — number of subjects with adverse events

The expected benefits from the use of the study drug and the comparator drug:

For a long time, trivalent influenza vaccines have been successfully used on the territory of the Russian Federation and around the world as an efficient and safe measure for prevention of influenza. The development of a quadrivalent vaccine containing strains of two lines of influenza B viruses will provide for a more reliable protection of the population, thus, increasing the immunization efficiency. Several trivalent vaccines (Fluarix Tetra, FluQuadri, Afluria, etc.) produced in Europe and the USA have already been successfully used in the world. In the Russian Federation, such drugs are currently not available.

Quadrivalent vaccine induces high-level specific anti-influenza immunity formation. Post-vaccination protective effect usually comes in 8–14 days, and the immunity retains up to 12 months, including elderly people. Protective anti-influenza antibody titers are detectable among 75–95 % of the vaccinated people of different age.

Development of the first russian quadrivalent vaccine will help improve the effectiveness of vaccination and significantly improve the cost-effectiveness of preventive immunization.

1.4. Description and justification of the mode of administration, dosage, dosage regimen, and course of treatment

As there are no quadrivalent inactivated influenza vaccines approved on the territory of the Russian Federation and also due to similarity of antigens used for manufacture of quadrivalent and trivalent vaccines, the use of trivalent inactivated polymer-subunit influenza vaccine Grippol® Plus as a reference product is considered to be justified.

Therefore, to get comparative data for all the four antigens of the quadrivalent vaccine, the study will use two trivalent reference vaccines with different type B influenza virus antigens.

The study will be conducted in three groups (A, B, C):

Group A: Grippol® Quadri, quadrivalent inactivated subunit influenza vaccine, suspension for intramuscular and subcutaneous administration.

Group B: Grippol® Plus, trivalent inactivated polymer-subunit influenza vaccine, based on type B influenza virus of Yamagata lineage, suspension for intramuscular and subcutaneous injections.

Group C: Grippol® Plus, trivalent inactivated polymer-subunit influenza vaccine, based on type B influenza virus of Victoria lineage, suspension for intramuscular and subcutaneous injections.

Dosing: All the vaccines used in the study are administered as a single intramuscular injection of 0.5 ml (1 dose) into the upper third of the outer surface of the shoulder (the deltoid muscle).

The recommended dose and mode of administration of vaccines correspond to those described in the instructions for medical use of comparator vaccines. The duration of the observation of volunteers to identify adverse events after a single vaccination will be 6 months. A detailed description of the vaccination procedure by study vaccines is presented in Section 5.1.

1.5. Indication that this clinical trial will be conducted in accordance with the protocol, these rules, and regulatory requirements.

This clinical study will be conducted in accordance with this protocol, current principles of good clinical practice (ICH GCP), ethical principles of conducting medical research involving human subjects of the Helsinki Declaration of the World Medical Association, as well as all the requirements of regulatory authorities of the Russian Federation.

1.6. Description of study population

Healthy eligible volunteers will participate in this study.

1.7. References to literary sources and data relevant to the study that constitute the rationale for this study.

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2. OBJECTIVES AND PURPOSES OF THE STUDY

The objective of this study was assessment of safety, reactogenicity, immunogenicity, and efficacy of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri (NPO Petrovax Pharm, LLC, Russia) versus trivalent inactivated polymer-subunit vaccine Grippol® Plus (NPO Petrovax Pharm, LLC, Russia) in subjects from 18 to 60 years old.

Study goals:

- Study and comparative assessment of immunogenicity of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri versus trivalent inactivated polymer-subunit vaccine Grippol ® Plus in subjects from 18 to 60 years old.
- Assessment of safety and reactogenicity of the quadrivalent inactivated subunit influenza vaccine Grippol® Quadri compared to the influenza vaccine trivalent inactivated polymer subunit vaccine Grippol® Plus in volunteers ages 18–60 years old.

- Study and comparative assessment of immunogenicity of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri versus trivalent inactivated polymer-subunit vaccine Grippol® Plus in subjects from 18 to 60 years old.

3. STUDY DESIGN

3.1. Primary and additional study parameters that will be evaluated during the study

Primary immunological efficacy endpoint:

- The share of subjects achieving seroconversion (the number of volunteers with antibody titer [of at least 1:40] increased more than 4-fold versus baseline [assessed by HAIR]) for antigens: influenza virus type A — H1N1, influenza virus type A — H3N2, influenza virus type B — Yamagata and Victoria lineage, based on assessment at Day 21 after immunization (Visit 7).

Secondary efficacy endpoints:

- Coefficient of increase of mean geometric antibody titer (antigens H1N1, H3N2, Yamagata and Victoria lineage), based on assessment at Day 21 after immunization (Visit 7).
- Geometric mean of serum antibodies to antigens: influenza virus type A — H1N1, influenza virus type A — H3N2, influenza virus type B — Yamagata and Victoria lineage, based on assessment at Day 21 after immunization (Visit 7).
- Seroprotection: share of subjects achieving protective antibody titer (1:40 and more) to antigens H1N1, H3N2, Yamagata and Victoria lineage, based on assessment at Day 21 after immunization (Visit 7).
- Incidence of influenza and ARI based on assessment 180±5 days after immunization (Visit 12).
- Severity and duration of reported cases of influenza and ARI, complications.

Safety parameters:

The safety parameters of this study are the following:

- Quantitative assessment and nature (severity and duration) of local and systemic reactions to vaccine administration.
- AEs and SAEs associated with the study vaccines.
- Immediate type AEs emerging within 60 minutes after immunization.
- Incidence and nature of intercurrent disorders
- Vital function assessment results.
- Results of physical examination.
- Neurological examination results.
- Results of blood hematology and biochemistry tests.
- Urinalysis results.
- Immunological parameters evaluation results: serum immunoglobulin content (ELISA: IgE).

3.2. Description of the type / design of the study and a schematic representation of its design, procedures, and stages

This was a phase II-III, 6-month, multicenter, double-blind, randomized comparative study of safety, reactogenicity, immunogenicity and efficacy of quadrivalent inactivated subunit influenza vaccine Grippol® Quadri versus trivalent inactivated polymer-subunit influenza vaccine Grippol® Plus in parallel groups in volunteers of 18 to 60 years. Study design flow and schedule of procedures are shown in Figure 1 and in Table 2.

Figure 1. Schematic design of the study

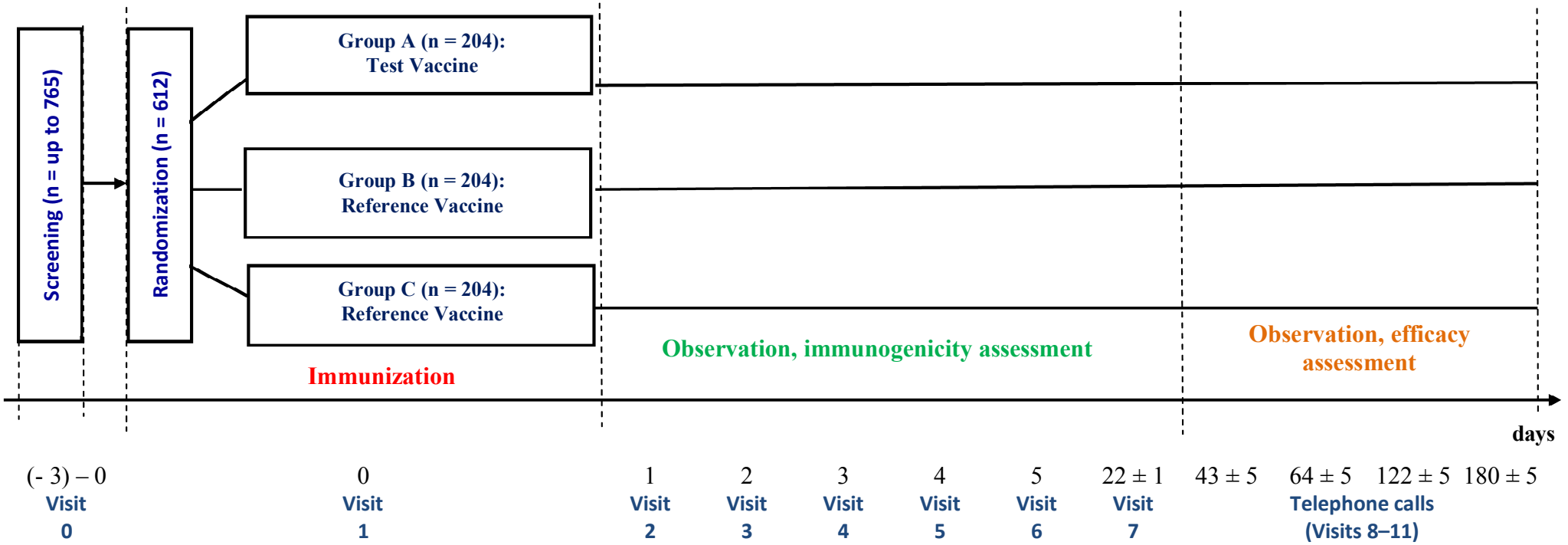

Table 2. Schematic design of study procedures and stages

	Visit 0	Visit 1		Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11
	Screening	Immunization		Outpatient follow-up						Telephone call			
Day after immunization	Day (-3) – 0	Day 0		Day 1	Day 2	Day 3	Day 4	Day 5	Day 22±1	Day 43±5	Day 64±5	Day 122±5	Day 180±5
		Before	In 2 hours										
Signing of informed consent	X												
Collection of demographic data and history	X												
Measurement of body weight, height, calculation of BMI	X												
Physical examination	X	X	X	X	X	X	X	X	X				
Vital signs assessment	X	X	X		X			X	X				
Body temperature measurement	X	X	X	X	X	X	X	X	X				
Complete blood count[1]	X				X				X				
Blood biochemistry test[2]	X				X				X				
Urinalysis[3]	X				X				X				
Tests for syphilis, HBV, HCV, HIV	X												
Urine test for pregnancy	X								X				
Serological test (serum antibody titer test)	X								X				
Immunoglobulins (IgE)		X							X				
Neurological examination		X			X								
Assessment of volunteer's compliance with inclusion and non-inclusion criteria	X	X											
Assessment for exclusion criteria	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization		X											
Immunization		X											
Distribution of volunteer's diaries and instructions[4]			X										
Review of volunteer's diary				X	X	X	X	X	X				
Return of volunteer's diaries									X				
Interviewing on ARI symptoms for the period									X	X	X	X	X
Recording of concomitant therapy	X	X	X	X	X	X	X	X	X	X	X	X	X
Recording of local and systemic reactions, adverse events, and intercurrent disorders		X	X	X	X	X	X	X	X	X	X	X	X

[1] - Hemoglobin, RBC, hematocrit, PLT, WBC and WBC differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), ESR.
[2] - Glucose, urea, creatinine, total bilirubin, total protein, ALT, AST, AP.
[3] - Relative density, protein, sugar, RBC, WBC.
[4] - Self-measurement of the body temperature by the volunteer, with the information logged into the diary.

Screening (Visit 0 (-3), – 0 days):

Volunteers will be invited to participate in the study and undergo the corresponding screening procedures:

- Signing of the informed consent form for participation in the study.
- Collection of demographic data (age, gender, race).
- Collection of medical history, history of infections and previous immunizations.
- Physical examination, measurement of height, body weight (calculation of BMI, kg/m2).
- Measurements of vital functions (pulse, SBP, DBP).
- Body temperature measurement (t°)
- Laboratory investigations:
  - Blood hematology (hemoglobin, RBCs, hematocrit, platelets, WBC and WBC differential [neutrophils, lymphocytes, monocytes, eosinophils, basophils], ESR).
  - Blood biochemistry (glucose, urea, creatinine, total bilirubin, total protein, ALT, AST, AP).
  - Urine test with sediment microscopy (specific density, protein, sugar, RBC, WBC).
  - Tests for syphilis, HBV, HCV, HIV infection.
  - Urine test for pregnancy (only for female subjects).
  - Blood collection for serology (baseline serum antibody titer).
- Preliminary assessment of volunteer's compliance with inclusion and non-inclusion criteria.
- Assessment for exclusion criteria.
- Recording of concomitant therapy within 3 months prior to screening, including collection of information on vaccinations within the last year.
- Oral instructions (in addition to volunteer's information leaflet) on not allowed medicinal drugs during screening and for the whole study period.

Within the study period, volunteers will not be allowed to take medicinal drugs listed in section 5.2.1.

The period of Screening could last up to 3 days. The duration has to be sufficient to obtain all the parameters required to assess the selection criteria. In the volunteer's best interests, procedures of Visit 0 and Visit 1 can be pooled on condition of collection of all the required parameters within one visit.

Visit 1 (Immunization day):

Final assessment of volunteer's eligibility, including measurement of the body temperature, is performed at Visit 1 before the procedure of randomization and before immunization.

If the volunteer meets all the inclusion criteria and none of the non-inclusion criteria, and if the volunteer did not take any of the unallowed drugs prior to the Screening visit, the volunteer is eligible for immunization.

The volunteer remains under the study doctor's observation within the first 2 hours after immunization.

Therefore, the following procedures are performed at Visit 1:

- Physical examination before immunization and 2 hours after immunization.
- Measurement of the body temperature (t°) before immunization and 2 hours after immunization.
- Measurement of vital signs (pulse, SBP, DBP) before immunization and 2 hours after immunization.
- Neurological examination before immunization.
- Laboratory investigations:
  - Blood collection prior to immunization to determine the baseline IgE level.
- Final conclusion on volunteer's compliance with inclusion and non-inclusion criteria.
- Randomization.
- Immunization.
- Provision of a volunteer's diary and instructions on keeping the diary (2 hours after immunization).
- Assessment for exclusion criteria (prior to immunization and 2 hours after immunization).
- Recording of concomitant therapy.
- Recording local and systemic reactions, adverse events, and intercurrent disorders.

After immunization and conclusion of follow-up (2 hours) the volunteer is instructed on how to keep a diary. The volunteer has to log the first records (body temperature in the morning) into the diary at the trial site to ensure that records in the diary are made correctly.

Visit 2 (Day 1 after immunization):

The following procedures are performed at Visit 2:

- Physical examination.
- Body temperature measurement (t°).
- Review of volunteer's diary.
- Assessment for exclusion criteria.
- Recording of concomitant therapy.
- Recording local and systemic reactions, adverse events, and intercurrent disorders.

Visit 3 (Day 2 after immunization):

The following procedures are performed at Visit 3:

- Physical examination.
- Body temperature measurement (t°).

- Measurements of vital functions (pulse, SBP, DBP).
- Neurological examination.
- Laboratory investigations:
  - Blood hematology (hemoglobin, RBCs, hematocrit, platelets, WBC and WBC differential [neutrophils, lymphocytes, monocytes, eosinophils, basophils], ESR).
  - Blood biochemistry (glucose, urea, creatinine, total bilirubin, total protein, ALT, AST, AP).
  - Urine test with sediment microscopy (specific density, protein, sugar, RBC, WBC).
- Review of volunteer's diary.
- Assessment for exclusion criteria.
- Recording of concomitant therapy.
- Recording local and systemic reactions, adverse events, and intercurrent disorders.

Visit 4 (Day 3 after immunization) and Visit 5 (Day 4 after immunization):

The following procedures are performed at Visits 4 and 5:

- Physical examination.
- Body temperature measurement (t°).
- Review of volunteer's diary.
- Assessment for exclusion criteria.
- Recording of concomitant therapy.
- Recording local and systemic reactions, adverse events, and intercurrent disorders.

Visit 6 (Day 5 after immunization):

The following procedures are performed at Visit 6:

- Physical examination.
- Body temperature measurement (t°).
- Measurements of vital functions (pulse, SBP, DBP).
- Review of volunteer's diary.
- Assessment for exclusion criteria. Recording of concomitant therapy.
- Recording local and systemic reactions, adverse events, and intercurrent disorders.

Visit 7 (Day 22±1 after immunization):

The following procedures are performed at Visit 7:

- Physical examination.
- Body temperature measurement (t°).

- Measurements of vital functions (pulse, SBP, DBP).
- Laboratory investigations:
  - Blood hematology (hemoglobin, RBCs, hematocrit, platelets, WBC and WBC differential [neutrophils, lymphocytes, monocytes, eosinophils, basophils], ESR).
  - Blood biochemistry (glucose, urea, creatinine, total bilirubin, total protein, ALT, AST, AP).
  - Urine test with sediment microscopy (specific density, protein, sugar, RBC, WBC).
  - Blood collection for IgE test.
  - Blood collection for serology (serum antibody titer).
  - Urine test for pregnancy (only for female subjects).
- Interviewing on ARI symptoms within the period.
- Review and return of the volunteer's diary.
- Assessment for exclusion criteria.
- Recording of concomitant therapy.
- Recording adverse events and intercurrent disorders.

Telephone calls (Visits 8–11):

Telephone calls are made on Day 43±5 (Visit 8), Day 64±5 (Visit 9), Day 122±5 (Visit 10), Day 180±5 (Visit 11) and included the following procedures:

- Interviewing on ARI symptoms within the period.
- Assessment for exclusion criteria.
- Recording of concomitant therapy.
- Recording adverse events and intercurrent disorders.

3.3. Measures for minimization / elimination of subjectivity

Subjectivity in the study is minimized / eliminated due to randomization and the double-blinded design of the study.

3.3.1. Randomization

The immunization group was determined randomly. Randomization will be based on a computer-generated randomization algorithm (randomization list), where each volunteer will be assigned a randomization number. That number will define the immunization group (A, B, or C) according to the randomization plan. That number will be assigned to the study subject for the entire period of the study and will be documented in volunteer's CRF.

The volunteers will be randomized into 3 groups (at a ratio of 1:1:1). A block randomization method is used. An interactive web-response system (IWRS) with technical support service will be used for randomization.

Investigators will be trained on working with IWRS system, and IWRS user guide will be handed out to all of them.

An identification name (ID) and a password will be used to access the system. These identifiers will be given to the authorized staff member of the investigation site prior to study initiation. Upon the volunteer randomization, the investigator receives information through the IWRS system on volunteer assignment to one of the vaccination groups that will be given to the volunteer during the study.

Volunteer stratification will be performed in the IWRS system according to the age and gender.

In an emergency medical case, the investigator can obtain information about a vaccine received by a volunteer through the IWRS system. Information about storage and unblinding of randomization codes is provided in Section 3.8.

3.3.2. Blinding

This study is double-blind, neither the doctor nor the volunteer will know what kind of vaccine the study participant will receive.

3.4. The treatment used in the study, dosages, regimens, dosage form, packaging, labeling, and storage of study drugs

All the vaccines related to this study will be stored away from other vaccines and drugs in a safe place under relevant storage conditions with temperature control. *All vaccines related to this study will be checked for the expiry date before use. Study subjects cannot be immunized using vaccines with expired date.*

Treatment (immunization), dosages, dosing regimen:

This study includes 3 study groups:

- Group A: Grippol® Quadri, quadrivalent inactivated subunit influenza vaccine, suspension for intramuscular and subcutaneous administration.
- Group B: Grippol® Plus, trivalent inactivated polymer-subunit influenza vaccine, based on type B influenza virus of Yamagata lineage, suspension for intramuscular and subcutaneous injections.
- Group C: Grippol® Plus, trivalent inactivated polymer-subunit influenza vaccine, based on type B influenza virus of Victoria lineage, suspension for intramuscular and subcutaneous injections.

Dosing: All the vaccines used in the study are administered as a single intramuscular injection of 0.5 ml (1 dose) into the upper third of the outer surface of the shoulder (the deltoid muscle).

Description of study vaccines:

Grippol® Quadri, a quadrivalent inactivated subunit influenza vaccine:	
INN or generic name:	Influenza vaccine [inactivated] + Azoximer bromide
Dosage form:	suspension for intramuscular and subcutaneous injection.
Composition:	One immunizing dose (0.5 ml) contains: Active ingredients: • Influenza type A virus (H_1N_1) antigen with hemagglutinin - 5 µg. • Influenza type A virus (H_3N_2) antigen with hemagglutinin - 5 µg. • Influenza type B virus (Yamagata lineage) antigen with hemagglutinin, 5 µg. • Influenza type B virus (Victoria lineage) antigen with hemagglutinin, 5 µg. • Polyoxidonium®, lyophilizate for the preparation of dosage forms and vaccines (Azoximer bromide), supplied by NPO Petrovax Pharm, LLC, Russia. Russia, 500 µg. Excipients: • Phosphate saline buffer solution up to 0.5 ml.
Description:	Colorless or yellowish, slightly opalescent liquid.
Presentation:	0.5 ml (1 dose) in ampules or vials, hermetically sealed with rubber stoppers and crimped with aluminum caps. 5 ampoules or vials are placed into a blister packaging of polyvinyl chloride film. 1 or 2 blisters together with Instruction on use per carton pack. Or 5 or 10 ampoules or vials without blister packaging are placed into a carton pack with a package insert.
Storage conditions:	Store away from light at the temperature of 2 to 8 °C. Keep out of reach of children. **Do not freeze!** Do not use the product which has been frozen. Shipping by all the types of roofed transport in lightproof containers temperature of 2–8 °C in conditions excluding freezing. Transportation is acceptable at a temperature of ≤ 25 °C for 6 hours.
Shelf life:	1 year. Do not use the product after the expiry date.
Manufacturer:	NPO Petrovax Pharm, LLC, Russia

Grippol® Plus is a trivalent inactivated polymer-subunit influenza vaccine:	
INN or generic name:	Influenza vaccine [inactivated] + Azoximer bromide
Dosage form:	suspension for intramuscular and subcutaneous injection.
Composition:	One immunizing dose (0.5 ml) contains: Active ingredients: • Influenza type A virus (H_1N_1) antigen with hemagglutinin - 5 µg. • Influenza type A virus (H_3N_2) antigen with hemagglutinin - 5 µg. • Influenza virus subtype B antigen with hemagglutinin, 5 µg. • Polyoxidonium®, lyophilizate for the preparation of dosage forms and vaccines (Azoximer bromide), supplied by NPO Petrovax Pharm, LLC, Russia. Russia, 500 µg. Excipients: • Phosphate saline buffer solution up to 0.5 ml.
Description:	Colorless or yellowish, slightly opalescent liquid.
Presentation:	0.5 ml (1 dose) in ampules or vials, hermetically sealed with rubber stoppers and crimped with aluminum caps. 5 ampoules or vials are placed into a blister packaging of polyvinyl chloride film. 1 or 2 blisters together with Instruction on use per carton pack. Or 5 or 10 ampoules or vials without blister packaging are placed into a carton pack with a package insert.
Storage conditions:	Store away from light at the temperature of 2 to 8 °C. Keep out of reach of children. **Do not freeze!** Do not use the product which has been frozen. Shipping by all the types of roofed transport in lightproof containers temperature of 2–8 °C in conditions excluding freezing. Transportation is acceptable at a temperature of ≤ 25 °C for 6 hours.
Shelf life:	1 year. Do not use the product after the expiry date.
Manufacturer:	NPO Petrovax Pharm, LLC, Russia

Packaging and labeling

Upon arrival to the trial site, the primary and secondary packaging of the study drugs will be labeled in accordance with the requirements set forth in Federal Law No. 61 of the Russian Federation "On the Circulation of Medicines" and be marked "For clinical studies".

Study vaccines in 0.5 ml ampoules will be supplied by NPO Petrovaks Farm, LLC in a package in accordance with the specification. Packages with the study drug were labeled as follows:

Protocol: GriQv-III-16

Study Drug: inactivated influenza vaccine

Suspension for intramuscular and subcutaneous injection.

1 ampule = 0.5 ml

Shake before use

Code of the study drug: 100, 200, or 300

Volunteer ID (No.)

Sponsor: NPO Petrovax Pharm, LLC

1, Sosnovaya St., Pokrov village, Podolsk district, Moscow region, 142143, Russia

Tel.: +7 (495) 730-75-45

Trial site:

Investigator:

Batch:

Date of issue:

Expiry date:

Store away from light at the temperature of 2 to 8 °C.

Labeling: "For clinical studies only"

Storage of study products

Vaccines supplied for clinical studies should be kept in a restricted access area (i.e. in a locked cabinet) and in strict compliance with the conditions recommended by the manufacturer. The storage temperature should be checked and recorded daily. Dispensing and return of the unused product is reflected in the reports which were then transferred to NPO Petrovax Pharm, LLC. Vaccines and placebo should be stored in a place protected from light and inaccessible to children at a temperature of 2 to 8 °C (in the refrigerator).

Do not use the product that has been frozen.

Transportation is acceptable at a temperature of ≤ 25 °C for 6 hours.

Responsibility for storage of the drug

All vaccine samples for the clinical study are transferred to the trial sites and stored strictly in accordance with the documentation. Unused vaccine samples must be returned to NPO Petrovax Pharm, LLC. Principal investigator is in charge of the proper product storage at the trial site.

The investigator should make accurate records of deliveries and release of the drug in a drug log. The accurate record containing the date and amount of drug administered to each volunteer should be available for inspection at any time.

All delivered drugs should be used only in accordance with this protocol, use for any other purpose is prohibited.

3.5. The duration of participation of subjects in the study, a description of the sequence and duration of all study periods

The duration of participation of subjects in the study is 183±5 days. The study includes Screening (days (-3)– 0), Vaccination and Post-Vaccination Observation and Efficacy / Safety Evaluation (Days 1–180 ± 5).

3.6. "Rules of discontinuation" and "Exclusion criteria" for individual subjects, parts of a study or study in general

Since the present study does not provide for interim analysis of efficacy or safety, the "rules of discontinuation" of the entire study are not defined for these parameters.

If a volunteer withdraws participation from the study early, the investigator must document the reason for this withdrawal. Regardless of the reasons for why the volunteers drop out of the study early, they are not replaced since the sample size is calculated taking into account the early withdrawal of volunteers at a 5 % level.

In order for the Sponsor to evaluate the benefit and risk ratio for study subjects, an investigator is obligated to immediately notify the project manager of the CRO or the Sponsor about all the health risks that have arisen for each specific study subject who dropped out of the study. The study subject should also be notified about any new information on the study drug.

Early termination of the entire study:

Early termination criteria of the study include, but are not limited to:

- Sponsor's decision for medical or administrative reasons. The current review of medical data and information about the safety of study treatment together with the investigator is a standard procedure. As a result of this review, it may be decided to discontinue the study before all volunteers have completed it.
- Inability to recruit the needed number of volunteers.

In the event of early termination of the study, regardless of the reason for such decision, the sponsoring company will notify the investigator and the regulatory authorities in writing about study termination or suspension, indicating the reasons for its termination or suspension. LEC should be immediately notified about termination or suspension of the study with indication of the reasons for such decision. The investigator must immediately notify all volunteers and provide them with appropriate follow-up and, if necessary, further treatment.

Early termination of the study in a trial site:

Criteria for early termination of the study in a trial site include, but are not limited to:

- The investigator is not complying with protocol requirements, the Helsinki Declaration, standards of the good clinical practice (ICH GCP) and the relevant requirements of regulatory authorities.
- The researcher provides false or incomplete information about the study to the Sponsor, CRO, LEC or regulatory authorities (including repeated omission of data entry in the CRF).
- The investigator cannot recruit the required number of volunteers within a specific period of time.
- The investigator withdraws his/her consent for study participation.

If the study is terminated prematurely or suspended for any reason, the investigator or the trial site should immediately notify the study subjects, verify the adequacy of their treatment and follow-ups and, if required by law, inform the regulatory authorities.

Early termination for separate volunteers:

Volunteer's informed consent for study participation states that the volunteer has a right to terminate his/her participation in the study at any time and for any reason, and that the volunteer may terminate his/her participation in the study at the discretion of the investigator or the Sponsor, which should not affect the quality of medical care provided to him/her in the future.
The investigating physician should exclude a volunteer from the study (criteria for early termination of study participation) in a case of:

- Informed consent recall.
- Occurrence of a severe AE or serious adverse events.
- The volunteer is found to meet any of the non-inclusion criteria related to the safety of the volunteer participation in the study.
- If a female-volunteer becomes pregnant.
- The volunteer takes medicines not allowed in this study.
- The volunteer is lost to follow-up.
- In a situation, which, to the investigator's judgment, may adversely impact the volunteer if he/she continues participating in the study.
- For administrative reasons (study termination by the Sponsor or regulatory authorities) or in case of major protocol violations which may significantly impact the study results.

If any of these events occur, the investigator, whenever possible, immediately conducts an early study termination visit for the volunteer to evaluate safety and efficacy parameters.

The date of termination of study participation of a volunteer and the reason for early termination must be recorded in source documents and the CRF.

If a female volunteer drops out due to pregnancy, the investigator provides the sponsor with the information about the outcome of pregnancy (see Section 7.3.5).

Volunteers with unresolved adverse events are observed until the adverse event is resolved or until the condition is stabilized.

3.7. Procedure for study drug accountability

The investigator is responsible for drug accountability. The pharmacist or another authorized employee of the trial site must receive the study drugs, check the inventory, and fill out the drug receipt confirmation form. A copy of this form remains at the trial site, and the original must be sent to the sponsoring company or an organization authorized by it. In the course of the study, the pharmacist or another authorized employee of the trial site keeps records of the release of study drugs in a special log on drug release. The following information is recorded in this log for each volunteer:

- Volunteer's initials.
- Volunteer No.
- Study drug code (assigned to a volunteer during randomization).
- Time and date when the study drug was dispensed.
- Batch numbers of dispensed drugs.
- Expiry date of the dispensed drugs.
- Time and date when the study drug packages were returned.

The drug release log is maintained and stored in such a manner that none of unauthorized persons have access to the information contained in this log.

All primary and secondary packaging of study drugs, both used and unused, should be stored at the trial site for accounting. After the end of the study, the investigator must return all used or unused packages to the study Sponsor or its authorized organization.

3.8. Storage of randomization codes and unblinding procedures

The randomization scheme (randomization list in the IWRS system) will only be known to the Sponsor, the investigators will not be able to influence the group selection, to which a specific volunteer is assigned during randomization.

If any of the members of the research team learn about accidental / intentional disclosure of the information as to which group a volunteer was randomized into, he/she should immediately notify the sponsor company.

Procedure for unblinding of randomization codes

In a case of a medical emergency (including pregnancy of a female volunteer), for which knowledge of the nature of study investigational drug is critical to further treatment, the investigating physician may send a request to the sponsor for disclosure of the code for this volunteer. Unblinding can be performed only if it is fully justified. In other cases, especially when an emergency situation is not clearly associated with the study drug, the problem should be solved based on the assumption that a volunteer is receiving an unauthorized study drug.

At the request of the Principal Investigator, the sponsor company will inform the investigating physician on which vaccine a volunteer is receiving — the emergency unblinding procedure for the assigned vaccine through electronic randomization system (IWRS). Instructions for emergency unblinding procedure will be provided to the trial site along with guidance for use of IWRS.

If it is not possible to contact IWRS for emergency unblinding, the Principal Investigator shall contact the Sponsor's representative.

Emergency unblinding of the study therapy should be described and justified in volunteer's primary documentation and recorded in the appropriate section of the CRF.

3.9. The list of all data recorded directly in the CRF and considered as source data

All medical data about volunteers must first be recorded in the source documents on a paper or electronic medium and only then entered into the CRF.

Baseline data is described in detail in Section 12.1.

4. SELECTION AND EXCLUSION OF STUDY SUBJECTS

4.1. Subject inclusion criteria

1. Signed and dated volunteer's informed consent for participation in the study.
2. Men and women from 18 to 60 years old.
3. Healthy volunteers without signs of acute or chronic disorders, without history of chronic respiratory, cardiovascular, nervous system disorders, hepatic or renal disorders.
4. Previously not immunized, or previous influenza immunization occurring ≥ 12 months before this study.
5. Subjects without history of influenza within ≥ 12 months before this study.

6. Consent of volunteers (men and women) to use adequate methods of contraception (cervical caps with spermicide, diaphragms with spermicide, condoms with spermicide, intrauterine devices, oral contraceptives) or full abstinence for the whole period of the study.

4.2. Subject non-inclusion criteria

Specific:

1. Contraindications listed in the protocol and prescribing information for inactivated influenza vaccines:

- acute infections and non-communicable disorders, including the period of reconvalescence of at least one month from the time of clinical and laboratory evidence of recovery;
- hepatitis or meningococcal infection occurred less than 6 months after recovery;
- exacerbations of chronic disorder or decompensated disorders that may impact the study (organic central nervous system disorders, decompensated cardiovascular disorder, acute renal or hepatic failure);
- malignant neoplasms (including hematological disorders);
- primary immunodeficiency (laboratory-confirmed);
- HIV infection or HIV-associated disorders;
- systemic disorders of connective tissue;
- haemophilia (and other blood coagulation disorders);
- severe neurological disorders;
- Guillain–Barré syndrome (post infection demyelinating polyradiculoneuropathy of autoimmune nature with peripheral limb muscle palsy related to inflammation and destruction of myelin sheath of peripheral nerves; may acquire an ascending nature, involving muscles of face, pharynx, larynx);
- history of severe vaccine-associated reactions (body temperature exceeding 38.5 °C) or local reactions (hyperemia and/or oedema at the site of injection of over 5 cm in diameter);
- history of severe allergic disorders (angioedema, polymorphic exudative erythema, serum disease, etc.);
- hypersensitivity to chicken protein or vaccine components;
- blood and components transfusion within the last 6 months.

2. Indications for immunomodulating therapy.
3. Body temperature over 37.0 °C at screening or before injection.
4. Potential evidence of a chronic infection (periodic episodes of fever within the last 6 months), or antiviral (and/or antibacterial) treatment indicated.
5. History of disorders or conditions, which, according to investigator's judgment may impact the thermal regulation (chronic infections, neuroendocrine disorders [thyrotoxicosis, pheochromocytoma, etc.], climacteric syndrome, malignant hyperthermia, CNS disorders,

malignant neoplasm, connective tissue disorders, systemic vasculitis, and information on excessive physical stress or work-rest regimen deviations [within the last 2 months: night shifts, significant change of time zones, overheating]).

6. Use of antipyretics (including non-steroidal anti-inflammatory drugs and anilides) within 24 hours before randomization.
7. Surgical interventions within less than 90 days before the screening visit.
8. Systolic blood pressure of over 130 mm Hg or less than 100 mm Hg and/or diastolic blood pressure of over 90 mm Hg or less than 60 mm Hg.
9. Any other disorder, which, in the opinion of the investigator, may prevent inclusion of the volunteer due to safety reasons or may impact the study results.

General:

10. Pregnant and nursing women.
11. Lack of ability to visit daytime inpatient facility according to the study schedule, unavailability for adequate follow-up of the volunteer.
12. Body mass index of less than 18.5 or over 30.0 kg/m2 based on the weight-height Quetelet's index.
13. Participation in another clinical study of medicinal drugs within 3 months before the start of this study.
14. Mental, physical, or other reasons which prevent adequate assessment of own behavior and prevent from meeting the study protocol conditions.
15. History of narcotic and/or drug abuse, and/or inhalant addiction, current signs of alcoholic intoxication.
16. Intake of at least 5 alcohol units per week or history of alcohol, drug, or medicinal product abuse. One alcohol unit corresponds to 360 ml of beer, 120 ml of wine, or 30 ml of a strong alcoholic beverage.
17. Suspected lack of compliance with treatment or inability to undergo treatment and observe the limitations according to the study protocol.
18. Volunteers acknowledged by the court to be disabled or under guardianship.
19. Any other conditions that make the volunteer ineligible for the study according to a justified opinion of the study doctor or Sponsor.

4.3. Subject exclusion criteria

Volunteer exclusion criteria are provided in Section 3.6.

5. TREATMENT (VACCINATION) OF SUBJECTS

5.1. Treatment (vaccination) with study drugs

Name, description, and dosages of vaccines are provided in Section 3.4.

The investigator or an authorized person will be responsible for controlling the administration of the vaccine to enrolled study participants in accordance with the procedures defined in this study protocol. All vaccines will be administered only by staff members that have the right to perform this function in accordance with applicable local laws and trial site rules.

Detailed instructions for preparing and administering the vaccine will be provided to investigators in additional documentation prior to study initiation.

The vaccine cannot be used in the following cases:

- when the ampoule is damaged;
- when foreign matter is present;
- in case of a missing label or missing information from it;
- in case of violation of storage conditions.

Dosing: All the vaccines used in the study are administered as a single intramuscular injection of 0.5 ml (1 dose) into the upper third of the outer surface of the shoulder (the deltoid muscle). The exact anatomical site of each injection must be carefully recorded in the medical record and transferred to the CRF.

Standardized immunization practices should be strictly followed, and the vaccine should be administered intramuscularly. **DO NOT INJECT intravenously.** Before vaccine administration, the vaccination site should be disinfected with an appropriate skin disinfectant (70 % ethyl alcohol). Wait until the skin is dry. After vaccine injection, the vaccination site shall be wiped again with 70 % ethyl alcohol.

As with all injectable vaccines, trained medical personnel with proper medical treatment should be available if anaphylactic reactions should occur after vaccine administration. For example, a 1:1,000 solution of epinephrine, diphenhydramine, and/or other drugs for the treatment of anaphylaxis should be available.

In case any foreign particles are detected, and/or any changes in the appearance of the vaccine are observed, the vaccine should not be administered to the participant, and the issue should be reported to the manufacturer. The vaccine should not be discarded without sponsor's permission. Any unused medication or waste must be disposed of in accordance with the local regulations.

Administration error and overdose:

This study is designed for a one-time intramuscular injection of vaccine. The erroneous administration of vaccine is defined as the administration of a the vaccine under study in a dosage that does not comply with the instructions or inappropriate mode of administration. An overdose of study vaccine (accidental or deliberate) is defined as the administration of a dosage exceeding a

single recommended dose of study vaccines.

Any erroneous administration or an overdose of study vaccine must be registered as an adverse event, and if the erroneous administration of the vaccine or an overdose cause a severe response, the Sponsor should be informed within 24 hours.

5.2. Drugs / treatments that are permitted or not permitted before and / or during the study

5.2.1. Contraindicated drugs and treatments

The use of the following drugs (regardless of the mode of administration) and treatment methods is prohibited during the study:

- only at screening and on the day of immunization:
- antipyretics (including non-steroidal antiinflammatory drugs and anilides);
- any drugs or food supplements which, according to the investigator's judgment, may affect the body temperature of the volunteer (including caffeine);
- physiotherapeutic procedures.
- For the whole period of the study:
- medicinal products suppressing central nervous system functions (barbiturates, opiates, sedative drugs), tranquilizers, neuroleptics, tricyclic antidepressants etc.;
- cytotoxic drugs;
- anticoagulants (including warfarin), antiagregants (including clopidogrel, acetylsalicylic acid).

Hormonal contraceptives or hormone replacement therapy is only allowed if the female volunteer has been on a stable dosing regimen maintained for at least 6 months before the screening visit and remains on a stable dosing regimen for the whole study period.

5.2.2. Permitted drugs and treatments

All unprohibited products and treatments are allowed.

5.3. Methods of control of compliance with procedures ans subjects

For the whole post-immunization follow-up period, the study subjects had to log in their volunteer's diaries information on the body temperature, any adverse events, and treatment with any medicinal drugs. At Visits 2–8, the investigator reviews the volunteer's diary and assesses the incidence and severity of adverse reactions, as well as the volunteer's compliance with the rules of concomitant therapy.

Additionally, the investigator assesses the regularity of the subject's visits to the trial site. The investigator can withdraw the subject from the study in case of subject's non-compliance with the requirements to concomitant therapy or if the study subject was not able to comply with the medical recommendations, in the investigator's opinion.

Study protocol does not provide for self-administration of the study products, the single dose immunization is performed at the trial site. The trial site personnel has to ensure that all the procedures related to the study product accountability are complied with (see Section 3.7).

As some of the study procedures (e.g., recording of AEs, blood sample collection, urine sample collection, vital signs measurement, physical examination, neurological examination, etc.) are performed by the investigator or another authorized personnel of the trial site at the study Visits, no methods of compliance control for such procedures are required.

6. EFFICACY EVALUATION

6.1. List of efficacy parameters

Parameters of immunogenic activity and efficacy are listed in Section 3.1.

6.2. Methods and terms of evaluation, recording and analysis of efficacy parameters

Evaluation of efficacy parameters includes the following procedures:

- serum antibody to antigens titer test: influenza virus type A — H1N1, influenza virus type A — H3N2, influenza virus type B — Yamagata and Victoria lineages prior to and at Day 21 after immunization;
- influenza and ARI incidence recording within 6 months after immunization;
- evaluation of severity and duration of reported cases of influenza and ARI, complications.

Serologic testing (serum antibody titer test):

Immunological efficacy (antigenic activity) of the vaccine is evaluated by its ability to induce the specific immune response, which can be identified under laboratory conditions by serum antibody titer to each antigen.

Antibody titer is determined using the hemagglutination inhibition reaction (HAIR), the method recommended by the WHO for influenza vaccine efficacy evaluation. The study is conducted at the central laboratory. As an additional method for 150 volunteers (50 volunteers in each group), a microneutralization assay is planned (the data will be used to evaluate seroprotection). The detailed description of the study methods is provided in the laboratory guide.

Blood samples for antibody titer test and calculation of mean geometric antibody titer will be collected for determination of background antibody titer after the immunization (Visit 0, Screening) and evaluation of functional immune reaction three weeks after immunization (Visit 7, Day 22±1).

Study parameters:

- The share of subjects achieving seroconversion (the number of volunteers with antibody titer [of at least 1:40] increased more than 4-fold versus baseline [assessed by HAIR]) for antigens: influenza virus type A — H1N1, influenza virus type A — H3N2, influenza virus type B — Yamagata and Victoria lineages.

- Coefficient of increase of mean geometric antibody titer (antigens H1N1, H3N2, Yamagata and Victoria lineages) (assessed by HAIR).
- Geometric mean of serum antibody to antigens titer (assessed by HAIR): influenza virus type A — H1N1, influenza virus type A — H3N2, influenza virus type B — Yamagata and Victoria lineages.
- Seroprotection: share of subjects achieving protective antibody titer (1:40 and more, assessed by HAIR and microneutralization test) to antigens H1N1, H3N2, Yamagata and Victoria lineages.

The detailed instruction on blood and serum sampling, labeling of tubes, storage and transportation of samples is presented in a separate laboratory guide provided by the central laboratory.

Influenza and ARI incidence recording:

Influenza and ARI incidence recording is performed during 6 months after immunization by means of follow-up and telephone calls to the study subjects.

A case of influenza and ARI is considered as a case of flu-like symptoms (according to: ECDC TECHNICAL DOCUMENT Protocol for case-control studies to measure pandemic and seasonal influenza vaccine effectiveness in the European Union and European Economic Area Member States / European center for Disease Prevention and Control, 2009):

if a subject seeks physician's advice regarding unexpected occurrence of

- at least one of four systemic symptoms: 1) chills or fever, 2) distress, 3) headache, 4) myalgia and

- - of at least one of three respiratory symptoms: 1) cough, 2) sore throat, 3) respiratory distress.

Evaluation of severity and duration of reported cases of influenza and ARI, complications:

When recording a case of influenza or ARI, a study doctor will perform an evaluation of its duration, making additional telephone calls, where necessary, and determine its severity. In this study, a case with at least one of the complications listed below will be considered severe:

- the necessity of hospitalization,
- influenza-associated pneumonia,
- influenza-associated cardiovascular disorder,
- death.

7. SAFETY EVALUATION

7.1. Review of the safety parameters

Safety parameters are listed in Section 3.1.

7.2. Methods and terms of evaluation, recording, and analysis of safety parameters

Evaluation of local and systemic reactions, as well as AEs, will be performed from the moment of immunization and within 6 months of follow-up. Recording of safety parameters will include the following procedures:

- clinical interview of a study subject;
- recording of thermometry results and complaints of a study subject related to his/her medical condition in a volunteer's diary;
- physical examination (including neurological status);
- vital signs;
- laboratory investigations
  - complete blood count and blood chemistry;
  - urinalysis;
  - evaluation of immunological parameters: serum immunoglobulin content (ELISA: IgE);
- quantitative assessment and nature (severity and duration) of local and systemic reactions to vaccine administration (reactogenicity);
- recording of AEs and SAEs associated with the study vaccines;
- recording of immediate type AEs, emerging within one hour after immunization;
- evaluation of incidence and nature of intercurrent disorders.

Clinical interview of a study subject:

During Visits 1–11 (post-immunization) the investigator shall perform a clinical interview of a study subject to collect information on the study subject's medical condition. At that, the investigator shall ask a study subject the following routine question at every visit:

"Have there been any changes in your condition since our last conversation / meeting?"

Clinically significant symptoms will be recorded by the investigator in the source medical documentation and CRF as AEs.

Recording of thermometry results and complaints of a study subject in a volunteer's self-observation diary:

A study subject will record axillary temperature in a self-observation diary every day, in the morning and in the evening, starting from Visit 1 after immunization and till Visit 7 (further measurements — depending on condition). He/she will also record concomitant therapy and complaints related to his/her medical condition during the day (if any); to do so, a subject will be offered to answer the

following question in the volunteer's diary in writing:

"Have there been any changes in your condition since yesterday?"

Clinically significant symptoms will be recorded by the investigator in the CRF as AEs.

Physical examination (including neurological status):

The investigator shall perform complete physical examination of a study subject during Visits 0, 1, 2, 3, 4, 5, 6, and 7. Apart from that, neurological examination (recording of neurological status) will be performed by a neurologist during Visits 1 and 3. Height and weight measurements of a study subject will only be taken during Screening visit. Clinically significant deviations identified during physical and neurological examination will be recorded by the investigator in the primary medical documentation and CRF as AEs.

Vital signs:

During the Screening visit and Visits 1, 3, 6, and 7, the investigator shall measure the following vital signs: SBP, DBP, heart rate. Clinically significant deviations of vital signs will be recorded by the investigator in the source medical documentation and CRF as AEs.

Laboratory investigations:

Blood and urine samples of a study subject will be collected during the Screening visit and Visits 3 and 7 for laboratory safety studies. Valid certificates of laboratories conducting laboratory tests and their norms are included in the study archive. The investigator is responsible for evaluation of all laboratory investigation results of the study subjects, as well as evaluation of clinical significance of any deviations of laboratory parameters. Clinically significant deviations of laboratory analyses results are recorded by the investigator in the primary medical documentation and CRF as AEs.

Laboratory investigation parameters for treatment safety evaluation are presented in Table.

Table 3. Laboratory investigation parameters for treatment safety evaluation.

Laboratory investigation	Visits	Parameters
Complete blood count	Screening, Visits 3 and 7	hemoglobin, RBC, hematocrit, PLT, WBC and WBC differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), ESR
Blood chemistry	Screening, Visits 3 and 7	glucose, urea, creatinine, total bilirubin, total protein, ALT, AST, AP
Urinalysis	Screening, Visits 3 and 7	relative density, protein, sugar, RBC, WBC

Table 3. Laboratory investigation parameters for treatment safety evaluation (continued).

Laboratory investigation	Visits	Parameters
Infection blood test	Screening	RW blood test, HBV, HCV, HIV infection tests
Determination of blood immunoglobulin levels	Visits 1 and 7	Immunoglobulin E (IgE)

Assessment of local and systemic reactions to vaccine administration (reactogenicity)

For assessment of local and systemic reactions to vaccine administration, the results of daily examinations are recorded in a Case Report Form and an Outpatient Medical Record for each subject. Particular attention is paid to symptoms corresponding to clinical manifestations of influenza, allergic and neurological reactions. A local reaction is evaluated by the diameter of a hyperaemic skin area at the injection site, skin tenderness and oedema, and enlargement of regional lymph nodes.

The assessment is performed before immunization, 2 hours after immunization, then once a day for 5 post-immunization days; thermometry is mandatory. Subjects who responded to the vaccination are monitored until these reactions pass off completely. In case of development of any unusual reactions the subject will be examined by an appropriate specialist. The following parameters are used for evaluation of tolerability of the study drugs.

Local reactions:

1. Pain at the injection site
2. Redness (mm)
3. Swelling, induration (mm)
4. Infiltrate (mm)
5. Itch
6. Enlargement of regional lymph nodes

Systemic reactions:

1. Increase of body temperature over 37 ˚C
2. Malaise
3. Headache
4. Sleep disturbance
5. Appetite disturbance
6. Nausea
7. Vomiting
8. Abdominal pains
9. Stuffy nose

10. Cough
11. Pharynx hyperaemic
12. Increased sweating
13. Tremor
14. Myalgia, arthralgia.

The nature and duration of immunization reactions in vaccinated subjects is compared upon interpretation of all study materials. An analysis of this data will make it possible to formulate a conclusion and resolve the issue of tolerability of study vaccines.

The course of the post-immunization period is assessed as smooth or complicated. Complicated course of the post-immunization period is considered as integration of intercurrent diseases after immunization. The absence of intercurrent diseases is considered as smooth post-immunization period. Fever responses were classified by temperature increase as follows: mild (low-grade fever up to 37.5 °C), moderate (temperature rise from 37.6 °C to 38.5 °C), and severe (temperature rise above 38.6 °C).

Local immunization reactions are classified as mild, moderate, and severe. Erythema without infiltration up to 50.0 mm in diameter at the injection site or infiltration up to 25.0 mm in diameter is estimated as a mild local reaction; erythema with a diameter more than 50.0 mm or an infiltrate with a diameter 26.0 to 50.0 mm was estimated as a moderate local reaction; infiltration more than 50.0 mm in diameter was estimated as a severe local reaction (Methodological Guidelines MU 3.3.2.1758-03 "Methods for determination of quality indicators of immunobiological products for prevention and diagnostics of influenza"). When the reaction is free of redness and/or infiltration, the reaction severity is accepted as indeterminate.

Local reactions will be assessed in all subjects, since local reactions were directly related to the administration of the vaccine.

Occurrence of general symptoms on the first 3 days after (and even on the day of immunization) may be associated with immunization (systemic reactions). However, it does not rule out their causal relationship with diseases, accidentally occurring within the first days of post-immunization period.

According to the accepted methods of interpretation of the vaccinial process course, assessment of general reactions will only be performed in volunteers with a smooth course of vaccinial process, because in a complicated course of post-immunization process, the observed symptoms may be associated with an intercurrent disease rather than with immunization. Thus, in case of development of an infectious intercurrent disease, including ARD/ARI in a volunteer within the first days after immunization, the general symptoms observed should be excluded from systemic reactions recorded within the first 5 days.

*List of references:

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Lokotkina E. A., Kharit S.M., Chernyaeva T. V., Brusov N.K. Post-vaccination complications (clinical presentation, diagnosis, treatment, prevention). Physician Guideline. Edited by Ivanova V. V. SPb, 2004, p. 79
Briko N.I. Ensuring the safety of immunization is one of the main criteria for the quality of vaccine prevention / Attending Physician. 2015. No. 7. P. 74.
Kharit S.M. Vaccine prevention: problems and prospects. *Infectology Journal*. 2009;1(1):61-65.

7.3. Reporting requirements, procedures for recording and reporting of adverse events and intercurrent diseases

An **adverse event (AE)** is any adverse change of a study subject's condition upon administration of the medicinal product, irrespective of the casual relationship with its administration. AEs include all adverse and unintended changes (e.g., deviation of a laboratory parameter), symptoms or diseases, where the time of occurrence does not rule out the casual relationship with administration of the medicinal product (manifestations absent in a subject prior to enrollment and developing during participation in a study, or manifestations observed in a volunteer prior to enrollment, the severity of which increases during the study upon administration of study products), irrespective of relationship with administration of the medicinal product.

An **adverse drug reaction (ADR),** with regard to pre-approval clinical use of a new medicinal product, includes all negative reactions associated with administration of any dose of the medicinal product. The term "associated with administration of the medicinal product" means at least a minimum possibility of presence of a causal relationship between the medicinal product and the adverse event, i.e. correlation cannot be excluded. For registered medicinal products, the term "adverse drug reactions" means all negative reactions associated with administration of the medicinal product in standard doses used for prevention, diagnostics, or treatment of diseases, as well as for modification of physiological functions.

7.3.1. Recording of adverse events

Procedures for recording the AEs are described in Section 7.2. AEs are recorded in the source medical documentation of a study subject, as well as in the respective sections of the CRF. Adverse events are recorded from the moment of administration of the first dose of the study product till the Study end visit.

The following information should be specified during recording of AEs:

- Nature of AEs (preferably with indication of the diagnosis, rather than the list of symptoms).
- Time of AE onset (*day, month, year, hour, minute,* where possible).
- Time of AE resolution (*day, month, year, hour, minute,* where possible).
- Severity of AE (*mild, moderate, severe*).

- Association with the study drug administration (*doubtful, possible, probable, definite*).
- Incidence of AEs (*single, intermittent*);
- Measures taken with regard to AE (*none, change of the study drugs dosing regimen, suspension of the study drugs administration, withdrawal of the study drugs, concomitant therapy*);
- Compliance of AE with SAE criteria (see Section 7.3.2; *yes, no*).
- Outcome of AE (*resolved without residual complications; resolved with residual complications; observed during the Study end visit; death of a study subject; lost to follow-up; stabilization of AE and its consequences*).

The investigator should try to establish the diagnosis based on the signs, symptoms, and other clinical information. If a diagnosis is determined, then the diagnosis itself should be recorded as an AE. If the diagnosis is unknown, then each complaint and symptom must be recorded as a separate AE.

7.3.1.1. Evaluation of severity of adverse event

The investigator should assess the severity of each AE using the following AE severity classification:

- mild: with or without symptoms, which are easily tolerated without treatment;
- moderate: minimum or local non-surgical treatment is indicated, may interfere with daily activities;
- severe: may interfere with daily activities, may require hospitalization or extension of existing hospitalization.

If severity of the AE changed in the course of its development, maximum severity should be reported.

7.3.1.2. Evaluation of casual relationship between the adverse event and administration of the study product

All available information which could help to reveal the causal relationship between the AE and the study product should be considered during evaluation of this relationship, namely:

- mechanism of action of the study product;
- information on known side effects according to the study protocol, investigator's brochure, and draft instruction for medical use of the product;
- characteristic of AE;
- temporal association of AE with the product administration;
- time of AE resolution;

- AE resolution upon withdrawal of the product or down titration;
- administration of other medicinal products or effect of chemical substances;
- natural course of the main disease or other conditions;
- study procedures;
- effect of other factors.

Based on analysis of the obtained information, the investigator will determine the causal relationship between the AE and the study product using one of the following categories:

- Doubtful: Clinical manifestations of AE occur in absence of a clear temporal association with the product administration. Other factors (medicinal products, diseases, chemical substances) which may cause their occurrence are observed.
- Possible: Clinical manifestations of AE have a temporal association with the product administration, but they can be attributed to concomitant diseases or administration of other medicinal products and to the effect of chemical substances. Information on a reaction to the product withdrawal is ambiguous.
- Probable: Clinical manifestations of AE have a temporal association with the product administration, can hardly be attributed to concomitant diseases or other factors and decrease after the product withdrawal. The response to re-challenge of the product is unknown.
- Definite: Clinical manifestations of AE occur during the product administration and cannot be attributed to existing diseases and the effect of other factors. Manifestations of ARs decrease after the product is discontinued and reoccur upon its re-initiation.

AEs with a doubtful casual relationship with the study product are considered as not associated with the study product administration. AEs with a possible, probable, or definite casual relationship with the study product are considered as associated with the study product administration.

7.3.2. Serious adverse events

A SAE is any AE corresponding to one or several criteria listed below:

- AE causing death of a volunteer.
- AE posing a threat to life of a volunteer.
- AE requiring hospitalization of a study subject or leading to extension of existing hospitalization, except for:
  - Optional treatment.
  - Monitoring of the study disease.

- For procedures as per the protocol, not associated with a deterioration in a volunteer's condition.
- Treatment of the existing disease, associated or not associated with the study disease, which has not worsened after administration of the first dose of the study product.
- Treatment received in an emergency unit due to an event which does not comply with the above mentioned criteria and which did not lead to hospitalization.

- AE leading to permanent or pronounced incapacity for work or disability.
- AE leading to congenital abnormalities / developmental diseases in children whose parents were exposed to the study product.

Apart from that, SAEs may include important medical manifestations in case when, based on the investigator's medical experience, such manifestations, if left untreated, may lead to one of the outcomes described above.

Death of a study subject is considered as an outcome of the AE. In case of death of a study subject, the investigator has to report medical condition of the study subject or the event causing the death, e.g., a disease, an accident, etc. as an SAE.

AE is considered as "posing a threat to life of a study subject" if it constitutes immediate danger to life of a study subject at the time of its development. This category does not include AEs, which could theoretically lead to subject's death if their severity was higher.

According to SAE criteria, hospitalization is considered as inpatient treatment for more than one calendar day, i.e. a hospital stay for at least one night.

7.3.3. The investigator's responsibilities for submission of reports on serious adverse events

Completion of SAE report form:

In case of development of SAE, the investigator or other authorized employee of the trial site has to complete the SAE report form with indication of information on this case. SAE report forms will be provided to investigators during the training on completion of SAE report form. The information stated in the SAE report has to correspond to the data provided in CRF sections "Adverse events", "Concomitant therapy", and "Results of laboratory investigations". SAE has to be included in source medical documents of a study subject.

Report on SAEs:

The investigators has to report in a prompt manner on all cases of SAE developing in study subjects from the moment of administration of the first dose of the study product until the Study end visit.

When reporting on the SAE, the investigator has to complete an SAE report form and send it to the sponsor's contact person by fax or send a scanned copy of the report by e-mail within 24 hours from recording of SAE.

All serious adverse events have to be reported on in a prompt manner by sending a completed SAE report form to the sponsor's contact person by fax and/or e-mail:

Anna Viktorovna Tsymbal

Tel.: 8-800-234-44-80

Tel.: (495) 730-75-45 (+114)

e-mail: TSymbalAV@petrovax.ru

22, Krasnaya Presnya Str., 123022, Moscow, Russia

Before sending a report about an SAE, the study monitor should be notified by phone about the submission of the fax.

The SAE report has to be prepared irrespective of relationship, detected by the investigator or an authorized employee, between the observed SAE and administration of the study product.

After submitting an urgent SAE report, the investigator must, within 48 hours, submit a detailed report with detailed information on SAE to the study sponsor, which will allow the latter to make an assessment about the need to review the benefit-risk ratio of the clinical study.

If an SAE associated with administration of the study product is recorded in a study subject, it has to be reported even if the study has been completed.

All SAEs and their complications have to be monitored up to their resolution or stabilization of a study subject's condition.

7.3.4. The sponsor's responsibilities for submission of SUADR reports and other safety reports

Reporting of SUADRs:

A serious unexpected adverse drug reaction (SUADR) is an SAE associated with the study product administration, i.e. a reaction the nature and severity of which does not correspond to the known information on the medicinal product. The following documents shall be used for determination of predictability of the SAE: investigator's brochure, draft instruction for medical use, and the study protocol.

Pharmacovigilance officer of the sponsor company has to send express reports on SUADR to LECs of trial sites and to regulatory authorities. Pharmacovigilance officer of the sponsor company also has to inform the investigators on all SUADRs recorded during the study.

Reports on SUADRs causing death or posing a threat to life of a study subject has to be sent to LECs of trial sites and to regulatory authorities within 7 calendar days from the date of receipt of the investigator's SAE report. If full information on a death or development of a life-threatening condition is not available to the sponsor as of the date of the urgent report, the sponsor has to take all measures to obtain full information provided in the form of a subsequent urgent report on identified SUADRs within 8 calendar days from the date of submission of initial report.

Reports on all other SUADRs have to be submitted by the study sponsor to LECs of trial sites and to regulatory authorities within 15 calendar days from the date of receipt of the investigator's SAE report.

If new significant information on identified SUADR becomes available to the sponsor, this information has to be provided in the form of a subsequent report within 15 calendar days from the date of its receipt.

Requirements to submission of information on SUADR applied to the study product, including the study quadrivalent vaccine.

Submission of other treatment safety reports:

Within 15 calendar days from the date of receipt of the information, the sponsor has to provide regulatory authorities and LECs of trial sites with other safety information which can affect evaluation of the risk-benefit balance associated with the treatment performed or can require significant modifications of the treatment performed during the study or the study methods. This safety information may include:

- Clinically significant increase of the expected rate and a change in the nature of the expected serious adverse reactions.
- SUADRs that occur in a study subject after completing the participation in a clinical study.
- New data related to the clinical study that may affect the safety of volunteers, such as:
  - Study-related SEAs, on the basis of which changes are required to study protocol.
  - The lack of effectiveness of the study drug used in a life-threatening pathology.
  - New important safety data from recently completed animal studies (identified carcinogenic effects and effects similar in severity and importance).

- Premature study termination or suspension of the study in another country (countries) due to a change in the safety profile of a similar study drug.
- Other safety data that alter the benefit-risk profile for study subjects.

- Recommendations of the independent evaluation committee of the results of a clinical study regarding the safety profile of a study drug.

Apart from submission of urgent reports, the sponsor company has to submit an annual periodic report on safety of the study product to regulatory authorities during the whole period of the clinical trial.

Information on the periodic report on safety of the study product is submitted to LECs of trial sites in the form of a brief description of the report basic content with an organized list of serious adverse drug reactions attached.

7.3.5. Pregnancy

If a female volunteer becomes pregnant while participating in the study, administration of the study drug must be discontinued immediately.

Pregnancy as such is not an AE except in cases where there is a reason to believe that the use of the study drug led to a decrease in the efficacy of contraceptives. Congenital abnormalities and birth defects in children of study subjects are considered SAEs. Medical abortions, as well as any serious complications during pregnancy (including spontaneous abortions) should be recorded as SAEs. Planned abortions without complications are not considered AEs.

All cases of pregnancy observed during the study (including cases of pregnancy of sexual partners of volunteers participating in the study) should be appropriately recorded. If a pregnancy is confirmed, the investigator must notify the sponsor's authorized representative on pharmacovigilance in the form of a pregnancy report. Then, the sponsor shall be provided with the information about the pregnancy outcome. Pregnancy of a study participant and sexual partners of male volunteers is recorded from the moment of the first injection of the study drug and until the Visit of study completion.

The outcome of each pregnancy (spontaneous abortion, elective abortion, birth of a normal child or a child with congenital anomalies or birth defects) must be recorded, even if the female volunteer has terminated her participation in the study.

7.4. The method and duration of observation of subjects after the occurrence of adverse events

If AEs or their complications are not resolved, the investigator has to conduct a follow-up of study of subject's condition. Follow-up has to be conducted until resolution or stabilization of the AE and its consequences in a condition considered as appropriate by the investigator or until the identification of the cause of the AE not associated with the study product administration.

8. STATISTICAL ASPECTS OF CLINICAL STUDY

8.1. Description of statistical methods

Descriptive statistics will be provided for all demographic and other baseline characteristics, immunogenicity, efficacy, and safety, as well as for changes (if applicable) in the course of the study for the time points of assessment and for therapeutic groups (groups A, B, C, and pooled B and C group). Descriptive statistics for quantitative variables will include mean, standard deviation (SD), median, first and third quartiles, minimum and maximum, and the number of valid observations. Qualitative parameters will be shown as frequencies and shares in per cent. 95 % confidence intervals around point estimate are also provided (where applicable). Descriptive statistics for immunogenicity and immunological efficacy parameters will be provided for antigens.

Efficacy analysis

The following immunogenicity parameters will be presented with the respective two-sided 95 % confidence intervals for antigens and therapeutic groups:

Before and after immunization:

- Geometric mean antibody titers (GMAT).
- The share of subjects achieving seroconversion.

After immunization:

- The share of subjects achieving seroconversion.
- Coefficient of increase of mean geometric antibody titer versus baseline before immunization (GMAT ratio before and after immunization).

Efficacy parameters will be evaluated by available data, without restoration of missing data.

Testing of statistical hypotheses will be performed for Group A (Grippol® Quadri vaccine) as compared with:

- pooled data of groups B and C (Grippol® Plus) for antigens H1N1 and H3N2, separately for each antigen;
- data of group B for Yamagata lineage antigen;
- data of group C for Victoria lineage antigen.

Primary variable

For comparison, the primary change in the proportion of persons with seroconversion (the ratio of the number of volunteers in whom the antibody titer [at least 1:40] increased by more than 4 times compared with the baseline [method of determination — hemagglutination-inhibition reaction]) according to an assessment 21 days after vaccination (Visit 7, Day 22 ± 1) the statistical hypothesis will be tested that the study vaccine is “not worse than” the comparator vaccines with an established 15 % limit of “not worse”.

H_0: $\pi_1-\pi_2 \geq 0.15$ H_A: $\pi_1-\pi_2 < 0.15$,

π_1 и π_2 – the share of subjects achieving seroconversion for the reference vaccine and the test vaccine respectively.

The numbers of subjects achieving seroconversion according to the evaluation at Day 21 after immunization are presented with the respective two-sided 95 % confidence intervals for each antigen, by therapeutic groups. Testing of the hypothesis will be performed by calculation of two-sided 95 % confidence interval for the difference of shares based on logistic regression with the vaccine as a fixed factor and the baseline antibody titer as a covariate. The clinical center, sex, and age will also be considered as potential factors of the model. The conclusion that the test vaccine is not inferior to the comparator for the primary endpoint, the percentage of subjects achieving seroconversion according to the assessment on Day 21 after immunization will be made if the upper limit of the calculated 95 % confidence interval does not exceed the established limit of 0.15 (15 %). The calculation of the exact two-sided 95 % confidence interval for the difference in proportions will be presented as a supporting analysis. In case of proven non-inferiority of the test vaccine versus the reference vaccines for Yamagata and Victoria lineages antigens, the same two-sided 95 % confidence interval will be used for testing of the hypothesis of predominant efficacy of the test vaccine for the primary endpoint, the share of subjects achieving seroconversion according to the evaluation on Day 21 after immunization. The conclusion on predominant efficacy of the test vaccine can be made if the upper limit of the calculated 95 % confidence interval does not exceed 0. Since a statistically significant conclusion when testing "non-inferiority" hypotheses should be obtained in relation to one primary variable, the adjustment of significance level due to the presence of one primary variable will not be performed. The successive transition from testing of the non-inferiority hypothesis to testing of the statistical advantage hypothesis does not require adjustment of the significance level as well.

Secondary study endpoint

- Geometric mean titers of serum antibodies to antigens according to assessment at Day 21 after immunization (Visit 7)

The comparison will be performed by calculation of two-sided 95 % confidence interval using ANCOVA covariance analysis upon log base 10 transformation. ANCOVA model will include the vaccine as a fixed factor and the baseline antibody titer log as a covariate. The clinical center, sex, and age will also be considered as potential factors of the model. The difference between mean values in the compared groups (comparator test) will be evaluated together with the respective 95 % confidence interval. Point estimate of mean differences and the respective confidence interval will be subjected to inverse transformation.

If the upper limit of the calculated 95 % confidence interval for GMAT1/GMAT2 ratios (GMAT1 and GMAT2 — geometric mean titers of serum antibodies to antigens for the reference vaccine and the test vaccine respectively) does not exceed the limit of 1.5, the test vaccine will be considered as not inferior to the comparator for the endpoint, the share of subjects achieving seroconversion according to the evaluation at Day 21 after immunization. In case of proven non-inferiority of the test vaccine versus the reference vaccines for Yamagata and Victoria lineages antigens the same two-sided 95 % confidence interval shall be used for testing of the hypothesis of predominant efficacy of the test vaccine for this endpoint. This conclusion will be made if the upper limit of the calculated 95 % confidence interval does not exceed 1.

- Coefficient of increase of mean geometric antibody titer (antigens H1N1, H3N2, Yamagata and Victoria lineages), based on evaluation at Day 21 after immunization versus baseline (Visit 7)

GMAT ratio before and after immunization will be evaluated with the respective two-sided 95 % confidence interval based on log transformation (log_{10}), point estimate of the difference and the confidence interval, and inverse transformation of the obtained values. The lower limit of the obtained confidence interval for GMAT ratio shall be positioned to the right of the defined limit of 2.5.

- Seroprotection: share of subjects achieving protective antibody titer (1:40 and more) to antigens H1N1, H3N2, Yamagata and Victoria lineage, based on assessment at Day 21 after immunization (Visit 7).

The shares of subjects achieving seroprotection will be presented with two-sided exact 95 % confidence intervals. Intergroup comparisons will be performed based on the exact Fisher test, as well as based on logistic regression with the vaccine as a fixed factor and the baseline antibody titer log as a covariate. The clinical center, sex, and age will also be considered as potential factors of the model.

- Incidence of influenza and ARI based on assessment 180±5 days after immunization (Visit 11).

Incidence of the disease will be presented with the respective two-sided 95 % confidence interval for therapeutic groups. The parameter will be analyzed using the Kaplan-Meier survival method (time to the first episode of the disease).

- Evaluation of severity and duration of reported cases of influenza and ARI, symptoms and complications will be presented descriptively for therapeutic groups.

Safety analysis

The safety analysis will be conducted by descriptive methods in the safety population. Adverse events will be coded using MedDRA. The number (percentage) of participants with AEs and the number of AEs will be presented in tables by system organ class and preferred term, as well as by the study therapy and severity.

With that, within a system organ class and preferred term, each subject will be considered once with regard to study therapy and maximum severity. The number (percentage) of volunteers with local and general reactions to vaccination will be presented taking into account severity and duration.

Laboratory findings and their changes after vaccination compared to the baseline and “shift tables” relative to normal values will be tabulated by therapeutic groups and by assessment points.

The results of the assessment of vital functions will be tabulated by therapeutic groups for each assessment point together with changes from the last available measurement prior to vaccination.

The results of physical examination will be presented descriptively by therapeutic groups and by assessment points.

Immunological parameters evaluation results: serum immunoglobulin content (ELISA: IgE) and their changes after vaccination compared to baseline values will be tabulated by therapeutic groups.

8.2. Planned number of subjects

Sample size was calculated based on the primary endpoint, the percentage of subjects achieving seroconversion according to the evaluation at Day 21 after immunization (Visit 7), and testing of the statistical hypothesis of non-inferiority of the test vaccine (Grippol® Quadri) versus the reference vaccine (Grippol® Plus).

The following non-inferiority margin was used for comparative evaluation of immunogenic efficacy in the non-inferiority model:

- upper limit of the two-sided 95 % confidence interval of the difference between parameters (R-T) of subjects achieving seroconversion in the study quadrivalent vaccine and reference trivalent vaccine groups shall not exceed 15 %.

Justification of the "non-inferiority margin" :

In placebo-controlled studies of inactivated influenza vaccines, the seroconversion parameter in placebo group for the study antigens amounts to 0–1 %. The placebo does not contain the respective antigens, so it demonstrates no immunogenic activity [1].

Taking into account the expected seroconversion level in reference groups > 60 % and the minimum limit for influenza vaccines > 40 % of subjects achieving seroconversion, established by the guidelines for harmonization of requirements to influenza vaccine of the Committee for Proprietary Medicinal Products (CPMP), the non-inferiority margin 15 % is significantly below the superiority of the comparator over the placebo, which is appropriate.

For testing of non-inferiority null hypothesis for Grippol® Quadri and Grippol® Plus vaccines, taking into account the following parameters:

- Power: 80 %
- One-sided significance level: 2.5 %
- Non-inferiority margin: 15 %
- Expected share of subjects achieving seroconversion in Grippol® Plus group: 60 %
- Expected actual difference for the parameter in groups: 1 %

1 % of the calculated sample size for non-inferiority hypothesis, primary dichotomized parameter and uniform assignment into groups (1:1) is based on the following formula [5,6]:

$$n = \frac{(z_\alpha + z_\beta)^2}{(\delta - \Delta)^2}[\pi_1(1-\pi_1) + \pi_2(1-\pi_2)]$$

where n — the number of patients in each group being compared;
π_1 — percentage of subjects achieving seroconversion in the reference vaccine group;
π_2 — percentage of subjects achieving seroconversion in the test vaccine group;
$\Delta = \pi_1 - \pi_2$ — the expected difference between percentages of subjects achieving seroconversion in the reference vaccine and test vaccine groups;
Z — critical values of standardized normal distribution;
δ — non-inferiority margin.

according to the calculation as per the given formula using software package PASS 12, there shall be at least 193 volunteers in each therapeutic group (579 volunteers in total).

Thus, the minimum required sample size should be: 193 volunteers in each of the three groups (579 volunteers in total).

Taking into account the dropout during the study (5 %), the required number of volunteers has to be increased to 204 per group (612 in total).

Accounting for the possible dropout of volunteers at the screening stage (about 25 %), it is planned to obtain a permission from the Ministry of Health of the Russian Federation to conduct a clinical study with a total of **up to 765 volunteers.**

Replacement of discontinued volunteers with backups is not provided for.

References:

1. Jackson LA, Gaglani MJ, Keyserling HL, Balser J, Bouveret N, Fries L, Treanor JJ. Safety, efficacy, and immunogenicity of an inactivated influenza vaccine in healthy adults: a randomized,placebo-controlled trial over two influenza seasons. BMC Infect Dis. 2010 Mar 17;10:71. doi: 10.1186/1471-2334- 10-71.

2. Clinical study report "Clinical trials of influenza trivalent inactivated polymer-subunit influenza vaccine Grippol plus (phase II)", GOU VPO St. Petersburg State Medical University named after Acad. I.P. Pavlov, 2008.

3. Committee for Proprietary Medicinal Products (CPMP). Note for guidance on harmonization of requirements for influenza vaccines. CPMP/BWP/214/96. The European Agency for the Evaluation of Medicinal Products (EMEA), March 1997.
4. FDA Guidance for Industry Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines, 2007.
5. Chow S-Ch., Shao J., Wang H., "Sample Size Calculations in Clinical Research", 2003.
6. Fleiss, J. L., Levin, B., Paik, M.C. 2003. Statistical Methods for Rates and Proportions. Third Edition. John Wiley & Sons. New York

8.3. Significance level

Level of statistical significance: bilateral 5 %.

8.4. Statistical criteria for suspension and discontinuation of study

Not provided by the protocol.

8.5. Selection of study subjects

Data analysis is performed in the following populations:

Safety analysis population. This population will include all volunteers who received a dose of the vaccine. The whole safety analysis will be provided in that population of volunteers.

Full Analysis Set (FAS). This analysis set will include all volunteers from the safety analysis set who had at least one measurement of serum antibody titer to antigens made. This analysis set was used for the analysis of efficacy and immunogenicity.

Per-protocol population. This population includes all the volunteers from the full analysis set meeting the inclusion / non-inclusion criteria, having received a dose of the vaccine according to the randomization, who provided data before and after vaccination to asses immunogenicity according to the study schedule, who did not receive any of the prohibited drugs, and who completed the study without any significant protocol deviations. This analysis set will be used for the analysis of immunogenicity parameters.

8.6. Procedures for recording missing, falsified data and data that cannot be analyzed.

The employee who maintains the database, after entering all the data checks the database for inconsistencies, erroneously entered and missing data. If questions arise, the investigator will be sent a form “Questions for investigators”, the request for which must be answered in writing within 7 business days after receipt. After receiving answers to the questions, the officer responsible for maintaining the study database checks for inconsistencies, erroneously entered data, and missing data. After the final completion of the collection and data entry of all centers, the database is closed, after which statistical processing can begin.

8.7. Procedures for communication on any deviations from the initial statistical plan

If the application of initially defined statistical methods is not possible, the statistical report and the final report on the study will provide a justification for making changes, with references to the calculations made, statistical indicators and a general analysis of the reasons for these changes. The decision to make extraordinary deviations (assumptions, modifying data) is the competence of the study Sponsor, and must be explained and justified, including in the text of the final report on the clinical study.

9. DIRECT ACCESS TO SOURCE DATA / DOCUMENTS

The investigator and the medical institution where the study is conducted are obligated to provide direct access to source documents (original records or certified copies) to study monitors, auditors, representatives of the medical unit of LEC, as well as to representatives of regulatory authorities.

Direct access to source documents is granted to specified persons for the purpose of verifying the data entered in the CRF, assessing the compliance of the study with the protocol and normative documents, as well as protecting the rights of study subjects. To accomplish these goals, these individuals may conduct study-related monitoring, audits, ethical examinations, and inspections.

10. QUALITY CONTROL AND ASSURANCE

The quality control of this study is provided through monitoring, verification of data against the baseline, audits by representatives of the sponsoring company, standardized data entry and processing. All the above procedures are carried out according to sponsor's standard operating procedures that have been composed in accordance with the requirements of ICH GCP.

Training of investigators

During the initial visit, the trial site monitor will conduct a training on the clinical trial protocol, as well as on all procedures and materials required for appropriate conduct of the study with the study team members.

Monitoring of the study

Study monitors must regularly contact the investigator and visit the trial site. Upon request, the investigator and the medical institution that conducts the study should provide them with direct access to all study documents (CRFs and other documents relevant to the study), subject to the requirements of confidentiality of the personal data of volunteers and conducting an audit in accordance with legal regulations.

The duties of the monitor of the trial site include verification of data entered in the CRF against the baseline data, monitoring of the compliance of the study center with the requirements of the study protocol, monitoring of the compliance with the requirements of the good clinical practice, and monitoring of the compliance with the applicable legal requirements.

The investigator and other staff members at the trial site participating in the clinical study must be prepared to allow time to discuss the data with the clinical study monitor. The investigator should assist the monitor and cooperate with him/her in order to resolve any problems identified during the visits to the trial site.

The frequency of monitor's visits to a trial site depends on a set of study subjects; at the beginning and end of the study, more frequent visits of the monitor to the trial site are possible as compared to the middle of the study.

Before initiation of the study, the monitor verifies that the trial site complies with all of the study requirements and discusses the clinical study protocol and the CRF with the investigator and the rest of the trial site staff responsible for the implementation of key study elements.

During the study, the monitor checks the compliance of the study with all the requirements specified above, including checking the informed consents, records and storage conditions of the study drugs, study documentation, baseline data, and reports on AEs.

When data inconsistencies are identified during the validation of study database, the description of these inconsistencies is addressed to the investigator in a form of data correction forms.

Monitors will be provided with separate guidelines for monitoring the study, containing additional instructions on monitoring procedures.

Audits:

The purpose of the audit by the study sponsor is an assessment of the conduct of study and its compliance with the requirements of the protocol, standard operating procedures, standards for good clinical practice, and applicable legal requirements independent of normal monitoring and quality control procedures. An audit can be conducted in all trial sites and orgaizations participating in the study.

11. ETHICAL ASPECTS

11.1. Helsinki Declaration of the World Medical Association

The investigator is obligated to ensure that the study is conducted in strict accordance with the Helsinki Declaration of the World Medical Association, adopted in 1964, in a 2013 edition.

11.2. Approval by the regulatory authorities

This study will be conducted in compliance with the requirements of the regulatory authorities of the Russian Federation. In addition to the approval of LEC trial sites before initiation of the study, a study permission must be obtained from relevant regulatory authorities, including the Ethics Council. In addition, all amendments to study documents must be submitted to the regulatory authorities for approval, as well as reports on SUADR, annual reports on the safety of study treatment and notification of the completion of the study.

11.3. Approval by the LEC of a medical institution

Before study drugs are sent to the trial site, and the site begins recruiting study subjects, the LEC of the medical institution must receive a documented approval to conduct the said study, as well as approval of the study protocol, amendments to it (if any), volunteer information sheet and the informed consent form for participation in the study, any other documents provided to the volunteers for promotional purposes, as well as information on payments and compensation to volunteers in the study.

Amendments to study documents, which must be approved by the LEC, take effect only upon receipt by the sponsoring company of a copy of the written approval of the LEC amendment.

If an amendment to the study protocol is necessary in order to eliminate the obvious and immediate risk to volunteers, it can be enforced before the approval of the LEC of the medical institution. However, in this case the approval has to be obtained within the shortest possible period of time after introduction of the amendment.

11.4. Informed consent for study participation

The investigator is responsible for obtaining the written consent from study subject to participate in the study. Consent from the study subject must be obtained before the start of any study procedures and after the study subject has been provided with adequate information about the objectives and methods of the study, the expected benefits and possible risks associated with study participation. The study subject must be issued one copy of the information leaflet and the informed consent form for the volunteer to participate in the study in his/her native language. The signed and dated informed consent form should be kept in the archives of the trial site and may be requested by the study monitor, employees of the sponsoring company or representatives of regulatory authorities during monitoring, audits, and inspections.

12. DATA PROCESSING AND RECORD KEEPING

12.1. Baseline data and comparison with baseline data

Source documents and source records with the results of all assessments and surveys conducted as part of this study should be kept in the study archive. The source medical records in the study archive are the source of baseline data of the study subject. The investigator is responsible for complete and accurate filling out of CRF. All data registered in the CRF must also be reflected in the source medical documentation of the study subject in the form of records made by the investigator or other authorized person according to the form on assignment of responsibilities. The data in the CRF must correspond to the source documentation from which they are transferred; discrepancies should be explained.

12.2. Study archive in the trial site

The investigator must keep an archive of the study, where all documentation is stored. The investigator shall store the documents related to the study until the sponsor company notifies him that further storage of these documents is no longer required. All primary medical documentation, drug registration logs, correspondence, confirmation of receipt of participation consent, and other documents related to the conduct of the study should be kept in the medical institution for as long as possible.

Essential study documents, including informed consent forms and primary medical records, must be kept for at least 15 years after the end of the study.

It is the sponsor's responsibility to inform the investigator / medical institution about the end of the period of documentation storage.

Documents must be stored in such a way as to be accessed later and easily identified in case of an inspection. Special attention should be paid to document storage conditions.

The destruction of study documents is possible only after the written approval by the sponsor and investigator. If the investigator wishes to delegate the duties for document archiving to another person or change the archive storage location, he/she must obtain written permission from the sponsoring company.

12.3. Confidentiality of study subject's data

The investigator shall ensure subject's confidentiality. Only subject's ID number is used in CRF and other documents submitted to the sponsoring company, to a laboratory and to safety and efficiency assessment commissions. Documents that are not forwarded to the study sponsor (for example, signed informed consent forms for participation in the study) must be kept by the investigator in compliance with confidentiality requirements

When a volunteer is included in the study, the investigator is assigned a unique number (screening number) consisting of numbers and letters, having the following structure: eight consecutive ranks placed from left to right

G	R	I					

Ranks from 1 to 3 are defined for this study with the following sequence: GRI (where GRI is the first letter of the protocol number) and the same for all volunteers, the next two digits correspond to the number of the trial site (for example, 01, 02, etc.), and the last three digits are the volunteer's number at the screening stage (numerical values of 001, 002, 003, etc.).

Based on the unique screening number, the investigator generates an individual volunteer identification code to record it in "Volunteer Information Sheet and the Informed Consent for Participation in a Clinical Study" and its medical documentation.

Individual identification code of the volunteer looks as follows:

																														G	R	I					
Number of permission for study participation			Permission issue date MM.DD.YYYY								Serial number of the medical organization specified in the permit			Volunteer's initials			Volunteer's date of birth MM.DD.YYYY								Unique volunteer number assigned at screening							

The investigator must maintain a separate Identification Log containing individual numbers, surnames, addresses, telephone numbers and medical record numbers (if available). The investigator must keep documents that are not intended to be submitted to the Sponsor in strict confidentiality, in particular, the signed Informed Consent Forms and the Identification Log.

The investigator is obligated to provide access to CRFs and primary medical documentation of study subjects to the authorized representatives of the sponsoring company, regulatory authorities and LEC of the medical institution for the purpose of their inspection during monitoring visits, audits, and inspections.

12.4. Disclosure

Information about the study, patent applications, procedures, as well as data of a scientific nature, and other information relevant to the study are the property of the sponsoring company. The investigator must understand that the sponsoring company may use the information obtained during this study to further develop the study drug, and therefore this information may be passed on to other researchers participating in clinical trials, as well as to regulatory authorities.

In order to make it possible to use the information obtained during this study, the investigator obliges to provide the sponsor with the results of all tests conducted during the study, as well as any other information obtained during the study. Discussion of the study results in oral or written form until study completion and registration of all data is possible only with the written permission of the sponsoring company.

13. FINANCING AND INSURANCE

13.1. Financing

Financing terms are described in a separate contract.

13.2. Insurance

The sponsoring company provides the necessary insurance for the study subjects required in accordance with the national legislation on clinical studies. Insurance conditions for study subjects are described in a separate contract.

14. PUBLISHING OF CLINICAL STUDY RESULTS

Study results are planned to be published in specialized scientific publications after the completion of final report on the clinical study.

The investigator has no right to publish the study results without prior consent of the sponsoring company. The investigator obliges to submit a manuscript or draft report containing the results of this study to the sponsor in order to obtain consent from the sponsor for publication or public presentation of these results. In this case, the investigator shall submit to the sponsor a manuscript or draft report no less than 30 days prior to the submission of the manuscript for publication.