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Safety, reactogenicity, and immunogenicity of a viral vector vaccine against influenza A: A Phase I open clinical trial

Maxim M. Shmarov¹✉, Svetlana V. Alekseeva¹, Nina A. Dovzhenko¹,
Alina S. Bandelyuk¹, Ilias B. Esmagambetov¹, Dmitrii N. Shcherbinin¹,
Lyudmila V. Verkhovskaya¹, Svetlana V. Volchihina², Yana V. Simakova¹,
Vladimir F. Babira², Denis Y. Logunov¹, Alexander L. Gintsburg¹

¹ National Research Center for Epidemiology and Microbiology named after the honorary
academician N.F. Gamaleya, 18 Gamaleya St., Moscow 123098, Russian Federation

² Branch No. 7, Main Military Clinical Hospital named after academician N.N. Burdenko, 4 Novaya St.,
Sergiev Posad-6, Moscow Region 141306, Russian Federation

✉ Maxim M. Shmarov; mshmarov@gamaleya.org

ABSTRACT

INTRODUCTION. The development of universal influenza vaccines based on conserved influenza virus antigens is a promising strategy for preventing pandemic influenza. Recombinant vaccines based on adenoviral vectors have high antiviral potential and have proven their effectiveness during the COVID-19 pandemic. In this regard, the development and clinical evaluation of a viral vector vaccine against influenza A seem relevant.

AIM. The aim was to evaluate the safety, reactogenicity, and immunogenicity of a broad-spectrum viral vector vaccine against influenza A after a single intranasal administration in healthy volunteers.

MATERIALS AND METHODS. This clinical trial studied the GamFluVac recombinant human adenovirus serotype 5 (rAd5)-based vaccine against influenza A (National Research Center for Epidemiology and Microbiology named after the honorary academician N.F. Gamaleya). The clinical trial enrolled 36 volunteers. The follow-up period was 28 days. The safety assessment of the viral vector vaccine was based on the incidence, nature, and severity of adverse events (AEs) after a single intranasal administration at doses of 2.5×10^{10} (Group 1), 1.0×10^{11} (Group 2), and 2.5×10^{11} (Group 3) viral particles. Immunogenicity was evaluated by measuring serum IgG antibodies against influenza A (H5N2) by enzyme immunoassay on Day 0 and Day 28.

RESULTS. No serious or severe AEs were reported during the clinical trial. Most AEs associated with the vaccine manifested as respiratory disorders, abnormal blood findings, and general disorders (elevated body temperature, headache, chills, malaise, arthralgia, and myalgia). Statistically significant differences ($p=0.0285$) were identified in the incidence of general disorders and administration site conditions in Group 1 (0%), Group 2 (16.7%), and Group 3 (33.3%). Group 3 demonstrated the highest increase in the geometric mean titers of specific IgG antibodies (2.8 times the baseline) on Day 28. In this group, 100% of volunteers had a ≥ 2 -fold seroconversion rate, and 41.7% of volunteers had a ≥ 4 -fold seroconversion rate.

CONCLUSIONS. This phase I clinical trial of the GamFluVac viral vector vaccine against influenza A demonstrated the immunogenicity and favourable safety profile of the vaccine after a single intranasal administration.

REGISTRATION. This clinical trial was registered at ClinicalTrials.gov (NCT03651544) on August 29, 2018.

Keywords:

influenza; universal influenza vaccine; viral vector vaccine against influenza A; intranasal vaccine; clinical trial; safety; reactogenicity; immunogenicity; adverse events; adverse drug reactions

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Disclosure. There is a potential conflict of interest in connection with the affiliation of the authors of this paper with the National Research Center for Epidemiology and Microbiology named after the honorary academician N.F. Gamaleya of the Ministry of Health of the Russian Federation. However, when writing this paper, the authors were guided by considerations of the scientific value of the material obtained and declare impartiality in the assessment of the data obtained. A.L. Gintsburg and D.Y. Logunov have been members of the Editorial Board of *Biological Products. Prevention, Diagnosis, Treatment* since 2021 and 2024, respectively.

Безопасность, реактогенность и иммуногенность векторной вакцины против гриппа А: открытое клиническое исследование I фазы

М.М. Шмаров^{1,✉}, С.В. Алексеева¹, Н.А. Довженко¹, А.С. Банделюк¹, И.Б. Есмагамбетов¹,
Д.Н. Щербинин¹, Л.В. Верховская¹, С.В. Волчихина², Я.В. Симакова¹, В.Ф. Бабира²,
Д.Ю. Логунов¹, А.Л. Гинцбург¹

¹ Федеральное государственное бюджетное учреждение «Национальный исследовательский центр эпидемиологии и микробиологии имени почетного академика Н.Ф. Гамалеи» Министерства здравоохранения Российской Федерации, ул. Гамалеи, д. 18, Москва, 123098, Российская Федерация

² Филиал № 7 федерального государственного бюджетного учреждения «Главный военный клинический госпиталь имени академика Н.Н. Бурденко» Министерства обороны Российской Федерации, ул. Новая, д. 4, г. Сергиев Посад-6, Московская обл., 141306, Российская Федерация

✉ Шмаров Максим Михайлович; mshmarov@gamaleya.org

РЕЗЮМЕ

ВВЕДЕНИЕ. Разработка универсальных противогриппозных вакцин на основе консервативных антигенов вируса гриппа представляет собой перспективную стратегию для предотвращения пандемий гриппа. Рекомбинантные вакцины на основе аденовирусных векторов обладают высоким противовирусным потенциалом и доказали свою эффективность в период пандемии COVID-19. В связи с этим представляется актуальной разработка и клиническая оценка векторной вакцины против гриппа А.

ЦЕЛЬ. Изучение безопасности, реактогенности и иммуногенности векторной вакцины широкого спектра действия против гриппа А при однократном интраназальном введении у здоровых добровольцев.

МАТЕРИАЛЫ И МЕТОДЫ. В исследовании использовали векторную вакцину против гриппа А на основе рекомбинантных аденовирусов человека 5 серотипа ГамФлюВак (ФГБУ «НИЦЭМ им. Н.Ф. Гамалеи» Минздрава России). В клиническом исследовании принимали участие 36 добровольцев. Период наблюдения составлял 28 сут. Оценку безопасности проводили на основании данных о частоте, характере и степени тяжести нежелательных явлений (НЯ) при однократном интраназальном введении векторной вакцины в дозах (по количеству вирусных частиц): $2,5 \times 10^{10}$ (группа 1), $1,0 \times 10^{11}$ (группа 2) и $2,5 \times 10^{11}$ (группа 3). Для изучения иммуногенности определяли уровень IgG антител к вирусу гриппа А (H5N2) в сыворотке крови методом иммуноферментного анализа на 0 и 28 сут исследования.

РЕЗУЛЬТАТЫ. В ходе проведения исследования серьезных нежелательных явлений и НЯ тяжелой степени не было зарегистрировано. Наиболее часто НЯ, связанные с исследуемым препаратом, проявлялись со стороны дыхательной системы, крови, а также в виде общих расстройств (повышение температуры тела, головная боль, озноб, недомогание, артралгия,

миалгия). Оценка НЯ категории общих расстройств и нарушений в месте введения вакцины показала, что различия в частотах возникновения НЯ при сравнении групп 1 (0%), 2 (16,7%) и 3 (33,3%) были статистически значимыми ($p=0,0285$). В группе 3 через 28 сут выявлено максимальное увеличение среднего геометрического значения титров специфических IgG антител — в 2,8 раза. Показатель частоты сероконверсии в 2 и более раза составил 100%, в 4 и более раза — 41,7%.

ВЫВОДЫ. В клиническом исследовании I фазы векторной вакцины против гриппа А ГамФлюВак при однократном интраназальном введении продемонстрирована иммуногенность и благоприятный профиль безопасности вакцины.

РЕГИСТРАЦИЯ: Идентификатор клинического исследования ClinicalTrials.gov; NCT03651544. Зарегистрировано 29.08.2018.

Ключевые слова: грипп; универсальная противогриппозная вакцина; векторная вакцина против гриппа А; интраназальная вакцина; клиническое исследование; безопасность; реактогенность; иммуногенность, нежелательные явления; нежелательные реакции

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Финансирование. Государственное задание «Разработка вакцины широкого спектра действия против вирусов гриппа А на основе рекомбинантных вирусных векторов», номер госрегистрации АААА-А17-117030110102-3.

Потенциальный конфликт интересов. Существует потенциальный конфликт интересов в связи с аффилиацией авторов данной научной работы с ФГБУ «НИЦЭМ им. Н.Ф. Гамалеи» Минздрава России. Однако при написании рукописи они руководствовались соображениями научной ценности полученного материала и заявляют о беспристрастности оценки полученных данных. А.Л. Гинцбург и Д.Ю. Логунов являются членами редакционной коллегии журнала «БИОпрепараты. Профилактика, диагностика, лечение» с 2021 и 2024 г. соответственно.

INTRODUCTION

Previous influenza pandemics were caused by influenza A viruses, including the subtypes H1N1 (1918, 1977, and 2009), H2N2 (1957), and H3N2 (1968) [1, 2]. The threat of a new pandemic remains due to the high mutational variability of the influenza A virus genome [3]. Spontaneous genetic reassortment between zoonotic and anthroponotic virus variants in a reservoir host could lead to the emergence of a potentially pandemic influenza virus with new properties capable of transmission from animals to humans. New viral variants can cause a pandemic if they acquire the ability to rapidly spread among the population in the absence of pre-existing immunity and cause clinically manifested forms of symptomatic infection [1, 4, 5]. If an influenza pandemic occurs, developing a vaccine based on a new strain will take several months, making access to the vaccine difficult in the early

stages of a pandemic due to limited production capacity and the need for mass vaccination¹.

The World Health Organization (WHO) coordinates an influenza surveillance system that serves as a global alert mechanism for the emergence of influenza viruses with pandemic potential². Following a risk assessment, candidate viruses are selected, based on which pre-pandemic (zoonotic) vaccines are developed during the interpandemic period. Recommendations for candidate viruses are updated annually on the WHO website³. This approach is important for creating a vaccine stockpile and could reduce the lead time for vaccine availability in the event of an influenza pandemic. However, pre-developed vaccines based on zoonotic viruses with pandemic potential may demonstrate low cross-protection against a new pandemic influenza virus due to significant antigenic differences [6]. At the same time,

¹ <https://www.who.int/publications/i/item/WHO-CDS-EPR-GIP-2006-1>

² Report by the Open-Ended Working Group of Member States on Pandemic Influenza Preparedness: sharing of influenza viruses and access to vaccines and other benefits. Pandemic influenza preparedness: sharing of influenza viruses and access to vaccines and other benefits; 2011. https://apps.who.int/gb/ebwha/pdf_files/WHA64/A64_8-en.pdf

³ <https://www.who.int/teams/global-influenza-programme/vaccines/who-recommendations/zoonotic-influenza-viruses-and-candidate-vaccine-viruses>

the number of influenza viruses recommended by the WHO as vaccine candidates has increased. The number of influenza virus subtypes for the Northern Hemisphere has increased from one subtype, A(H5N1), for the 2010–2011 season to eight subtypes: A(H5N1), A(H5), non-A(H5N1), A(H7), A(H7N9), A(H9N2), A(H3N2), A(H1), and A(H3N8) for the 2023–2024 season⁴. Maintaining stockpiles of pre-pandemic vaccines requires significant financial expenditures, which would prove ineffective if the antigenic characteristics of the pandemic virus strain do not match those used in the candidate vaccine [7].

A promising approach to solving this problem is the development of universal, broad-spectrum vaccines that protect against various variants of the influenza A virus, regardless of the pandemic strain. According to the WHO strategy, the preferred approach is to develop universal influenza A vaccines by 2027, the key characteristic of which is the ability to protect against influenza A for at least five years⁵ [8]. The mechanism of action of such vaccines is based on the induction of a humoral immune response to conserved epitopes of the influenza virus and/or stimulation of a specific cellular immune response. Candidates for universal influenza vaccines are currently being developed and are at various stages of research⁶.

Vaccines based on adenovirus vectors have proven effective, demonstrating a favorable safety profile and high epidemiological efficacy, during the COVID-19 pandemic [9]. Adenoviral vectors also hold promise for the creation of influenza vaccines, as they can express various influenza virus antigens, including conserved ones, inducing humoral and T-cell immune responses in immunized individuals [10]. Important advantages of these vectors are the possibility of administration through the upper respiratory tract, the affinity for tissues and organs of the respiratory system, and the ability to generate a mucosal immune response directly at the portal of entry [11, 12]. The intranasal route of administration is convenient for

patients, especially in the case of a pandemic. Several vaccines designed for intranasal use are in various stages of clinical trials [13, 14].

The GamFluVac viral vector vaccine against influenza A is being developed at the National Research Center for Epidemiology and Microbiology named after the honorary academician N.F. Gamaleya. The vaccine is based on recombinant replication-defective human adenovirus serotype 5 (Ad5). The consensus genes for the NP nucleoprotein and the M2 ion channel protein of the influenza A virus were integrated into a single vector, as they are the closest sequences to all those available for the human influenza A virus in the Influenza Virus Resource database⁷. The second vector contains the influenza A virus hybrid hemagglutinin protein gene, artificially obtained from known hemagglutinin sequences⁸ of human influenza A virus strains of the H1, H2, and H5 (HA125) subtypes, containing the largest number of epitopes for T- and B-cells. Previously, in preclinical studies, the authors demonstrated the safety of the GamFluVac vaccine and its ability to provide 90–100% protection against avian, swine, and human influenza A viruses of the H7N2, H1N1, H5N2, H3N2, H2N3, and H9N2 subtypes, demonstrating its potential for the creation of a broad-spectrum vaccine against influenza A [15, 16].

The aim of the study was to evaluate the safety, reactogenicity, and immunogenicity of a broad-spectrum viral vector vaccine against influenza A after a single intranasal administration in healthy volunteers.

MATERIALS AND METHODS

Study design

This was an open-label study of the safety, reactogenicity and immunogenicity of the GamFluVac viral vector vaccine against influenza A in healthy volunteers in three groups with a dose escalation (Clinical Study Protocol No. 03-GamFluVac-2018). This protocol was approved by the Ministry of Health of the Russian Federation (the approval No. 435 dated

⁴ Ibid.

⁵ WHO preferred product characteristics for next generation influenza vaccines. WHO; 2017. <https://www.who.int/publications/item/9789241512466>

Global influenza strategy 2019–2030. Prevent. Control. Prepare. WHO; 2019. <https://www.who.int/publications/item/9789241515320>

⁶ <https://ivr.cidrap.umn.edu/universal-influenza-vaccine-technology-landscape>

⁷ <https://www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-select.cgi?go=database>

⁸ Ibid.

August 24, 2018)⁹, by the Ethics Council of the Ministry of Health of the Russian Federation (extract from the minutes of the Ethics Council meeting No. 175 dated August 21, 2018) and by the independent local ethics committee of the clinical site (Branch No. 7, Main Military Clinical Hospital named after academician N.N. Burdenko). The trial was registered at ClinicalTrials.gov¹⁰: NCT03651544.

The study was conducted in compliance with the requirements of Federal Law No. 61-FZ¹¹, the ethical principles of the World Medical Association's Declaration of Helsinki¹², the ICH Guideline for Good Clinical Practice¹³, GOST R 52379-2005 National Good Clinical Practice guidelines and the Order No. 200n of the Ministry of Health of Russia¹⁴. Written informed consent was obtained from the volunteers to participate in the study and for publication of the study data. The administration of the vaccine and observation of volunteers was carried out between October and December 2018.

The study consisted of 3 periods: the screening (up to 7 days), the period of administration of the study medicinal product (hospitalization for 3 days), and the follow-up period (28 days from the date of administration). Based on the screening results, participants were sequentially recruited into three groups with different vaccine doses. The total number of volunteers who received the GamFluVac vaccine was 36.

Volunteers

Men and women aged 18 to 55 years took part in the clinical trial. Women of childbearing potential were warned of the potential risks during pregnancy. The body weight was within the normal range for the age. Volunteers' eligibility for participation in the study was confirmed using standard laboratory tests, medical history, and physical examination. Volunteers included in the study met generally accepted inclusion and exclusion criteria¹⁵ used for studies of vaccines being administered to humans for

the first time, as well as exclusion criteria specific to nasal vaccines: recent frequent nosebleeds (>5 in the past year); chronic rhinitis, nasal septal defects, nasal polyps, or other significant abnormalities; history of nasal surgery or trauma within 6 months. Volunteers were also required not to have received an influenza vaccination within 6 months prior to the start of the study (including participation in other clinical trials).

Study medicinal product

The GamFluVac viral vector vaccine against influenza A, nasal drops, 0.5 mL/dose, manufactured by the N.F. Gamaleya National Research Center for Epidemiology and Microbiology (Moscow, Russia), was supplied in 3 doses of recombinant adenoviruses: dose 1, 2.5×10^{10} viral particles (v.p.); dose 2, 1.0×10^{11} v.p.; and dose 3, 2.5×10^{11} v.p.

Method of administration and dosage of the study medicinal product

The vaccine was administered intranasally to volunteers in a single-dose schedule by instillation of 0.25 mL into each nostril using a sterile medical pipette. Volunteers received the GamFluVac vaccine depending on the study group: in Group 1, at dose 1 (2.5×10^{10} v.p.); in Group 2, at dose 2 (1.0×10^{11} v.p.); in Group 3, at dose 3 (2.5×10^{11} v.p.).

Safety and reactogenicity analyses

The safety analysis was based on the registration of adverse events (AEs) during the whole study.

Primary outcome measures were the assessment of the frequency, nature, and severity of adverse events (AEs) after the use of the study medicinal product.

Secondary outcome measures were changes in instrumental (ECG) and laboratory parameters (clinical and biochemical blood tests, urinalysis), changes in vital signs (blood pressure, heart rate, body temperature). Laboratory tests included

⁹ The State Register of Medicines. The Register of Approved Clinical Trials. Number and date of permission of the RF Ministry of Health: No. 435 dated August 24, 2018. <https://grls.rosminzdrav.ru/CIPermissionMini.aspx?CIStatementGUID=7a60ea73-0051-48fa-a218-9d469cd252e7&CIPermGUID=df9bd9e4-096a-4247-a3f2-acbadf562874>

¹⁰ <https://www.clinicaltrials.gov/>

¹¹ Federal Law of the Russian Federation of 12.04.2010 No. 61-FZ "On the Circulation of Medicines".

¹² WMA Declaration of Helsinki – ethical principles for medical research involving human subjects. <https://www.wma.net/policies-post/wma-declaration-of-helsinki/>

¹³ Integrated addendum to ICH E6(R1): Guideline for good clinical practice E6(R2). https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf

¹⁴ The Order of the Ministry of Health of Russia dated 01.04.2016 No. 200n "On approval of the rules of good clinical practice".

¹⁵ Guidelines for Conducting Clinical Trials of Medicinal Products. Part One. Moscow: Grif and K; 2012.

a clinical blood test, a biochemical blood test, a coagulation profile, C-reactive protein determination, and urinalysis. Blood and urine samples were collected during the screening phase, before vaccination, and on Days 3, 7, 14, and 28 post-vaccination. Female volunteers were given a pregnancy test at the screening stage and on the day of vaccination.

The immunological safety of the medicinal product was determined based on an assessment of immune status indicators. Volunteers' immunograms were analyzed using data on the number and relative content of T- and B-lymphocyte subpopulations (CD3, CD4, CD8, CD16, CD19), the CD4/CD8 ratio, the phagocytic index, the serum levels of immunoglobulins of the main classes (A, M, G, E), and circulating immunocomplexes during the study.

The reactogenic properties of the vaccine were assessed based on local reactions (at the administration site) and general reactions (the body's systemic reactions to the administration). These were recorded by a physician based on an examination and interview of the volunteers, conducted in accordance with the study visit schedule and procedures. While the volunteers were in the hospital, various organs and systems were examined daily (for 3 days) and during outpatient visits (up to 28 days), in particular the organs of vision, ENT organs, organs of the cardiovascular system, organs of the respiratory system, organs of the gastrointestinal tract, the skin, the musculoskeletal system, the nervous system, lymph nodes; including examination of visible mucous membranes, and abdominal palpation. In addition, the volunteers were examined by an otolaryngologist.

AEs were assessed based on the following parameters: the maximum severity of the AE; the AE outcome; actions related to the study medicinal product; the relationship of the AE with the study medicinal product; and necessary medical correction of the AE. AEs related to the study medicinal product were classified as adverse drug reactions (ADRs).

To assess the severity of AEs, the study doctor used tables compiled based on the guidelines of the US National Institutes of Health (NIH)¹⁶. The degree of certainty of causality was determined according to the Guidelines for the Examination of Medicinal Products¹⁷. The frequency of AEs was assessed taking into account the classification adopted by the WHO¹⁸.

Immunogenicity analysis

The level of specific IgG antibodies to the influenza A virus subtype H5N2 in the blood serum of volunteers was assessed using an enzyme-linked immunosorbent assay (ELISA) before the first vaccine administration (baseline values) and on Day 28 of the study.

ELISA

The level of specific IgG antibodies in blood serum samples was determined using an indirect ELISA. Whole serum was used for the determination. The sorption antigen was sucrose gradient-purified influenza virus strain A/Mallard Duck/Pennsylvania/10218/84(H5N2), adapted to mice [17], at a concentration of 5 µg/mL. Non-specific binding was blocked with a 5% ovalbumin solution. Blood serum samples were titrated using two-fold dilutions starting at 1:400 and added to the wells of the plate. Incubation was carried out for 1 hour at 37 °C. The bound antigen-antibody complex was detected using a peroxidase-conjugated antibody (Goat anti-human IgG-HRP, cat. no. A0170, Sigma) at a dilution of 1:10,000. Incubation was carried out as described above. A TMB indicator mixture (cat. no. T0404, Sigma-Aldrich) was used to detect the reaction. Incubation time was 15 minutes at room temperature in the dark. The optical density of the stained product was measured using an iEMS Reader MF (LabSystems, Finland) at a wavelength of 450 nm. The antibody titer was determined as the reciprocal of the serum dilution.

The method for determining the level of specific IgG antibodies in blood serum using indirect

¹⁶ Common terminology criteria for adverse events (CTCAE), version 5.0, November 27, 2017. U.S. Department of Health and Human Services. National Institutes of Health. National Cancer Institute. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. Guidance for industry. U.S. Department of Health and Human Services. Food and Drug Administration. Center for Biologics Evaluation and Research. September 2007.

¹⁷ Guidelines for the examination of medicinal products. Vol. III. Moscow: Polygraph-plus; 2014 (In Russ.).

¹⁸ Guidelines for preparing core clinical-safety information on drugs. Report of CIOMS Working Group III. Chapter 5. Good safety information practices. Geneva: WHO; 1995.

ELISA was validated for sensitivity, reproducibility, and specificity.

Statistical analysis

Statistical analysis of safety data was performed using Microsoft Excel 2016 and STATISTICA 12.0 (StatSoft). One-way analysis of variance (ANOVA) was used to compare age groups. When comparing the incidence of AEs/ADRs in different groups, the χ^2 test for linear trend was used to assess the effect of dose on the incidence of AEs/ADRs in study subjects. The data were presented using the arithmetic mean (*M*) and standard deviation (*SD*). Statistical calculations for immunogenicity and graphical presentation of results were performed using GraphPad Prism 8.0 (GraphPad Software, USA).

The validity of the results was assessed using the Wilcoxon signed-rank test for dependent groups and the Mann–Whitney test for independent groups. A *p* value of <0.05 was considered statistically significant.

RESULTS

During the clinical trial of the GamFluVac vaccine, 60 individuals were screened to determine eligibility/non-eligibility. Forty-one individuals were enrolled in the clinical trial, of which 36 volunteers received the vaccine. Five persons were replacement subjects, but their participation in the study was not required. The full analysis set (FAS), safety population, and immunogenicity population included 36 volunteers. Since the medicinal product was being studied for the first time in humans, 5 volunteers from Group 1 were initially hospitalized and received the study medicinal product (0.5 mL) at a dose of 2.5×10^{10} v.p. After confirming the medicinal product's safety based on observation results 7 days after vaccination, the study was continued with the participation of 7 more volunteers in this group.

Thereafter, following a similar scheme (each time after an interim safety assessment on Day 7), volunteers from Group 2, who received the medicinal product (0.5 mL) at a dose of 1.0×10^{11} v.p., and Group 3, who received 2.5×10^{11} v.p. (Fig. 1) were included in the study. The decision on the possibility of dose esca-

tion after an interim analysis of safety data was made by the Research Center's Ethics Committee.

The age of participants ranged from 18 to 55 years. No statistically significant age differences were found between the compared groups (*p*=0.25). Volunteers included in the study in all groups were generally comparable in weight and height. The mean age, demographic and anthropometric parameters of the volunteers who participated in the study are presented in Table S1 (available on the journal website¹⁹).

The majority of volunteers had a normosthenic build: 11 people in Group 1 (91.7%), 10 people each in Groups 2 and 3 (83.3%). One participant in Group 1 (8.3%) had an asthenic build, and two participants each in Groups 2 and 3 (16.7%).

Based on the data from the conducted objective examination, general and biochemical blood tests, urine analysis, and coagulation profile, it was shown that there were no significant differences between the groups of volunteers included in the study at the time of screening, confirming the comparability of the study participant groups.

Safety analysis of the viral vector vaccine against influenza A

During dynamic observation of volunteers, no effect of the vaccine on vital organs or systems was detected. Within 28 days after administration of the vaccine, no cases of serious AEs or unexpected AEs were registered. There were also no cases of early discontinuation of the study due to the development of adverse events. No clinical manifestations of allergic reactions to the vaccine administration—local (e.g., urticaria, rash) or general (anaphylactic reactions)—were recorded.

In all three groups AEs of various categories according to system organ class (SOC) classification according to the MedDRA (Medical Dictionary for Regulatory Activities²⁰) were reported: 13 AEs²¹ were related to somatic status; 25 AEs were local reactions associated with various respiratory symptoms; 461 AEs were associated with abnormal laboratory tests²².

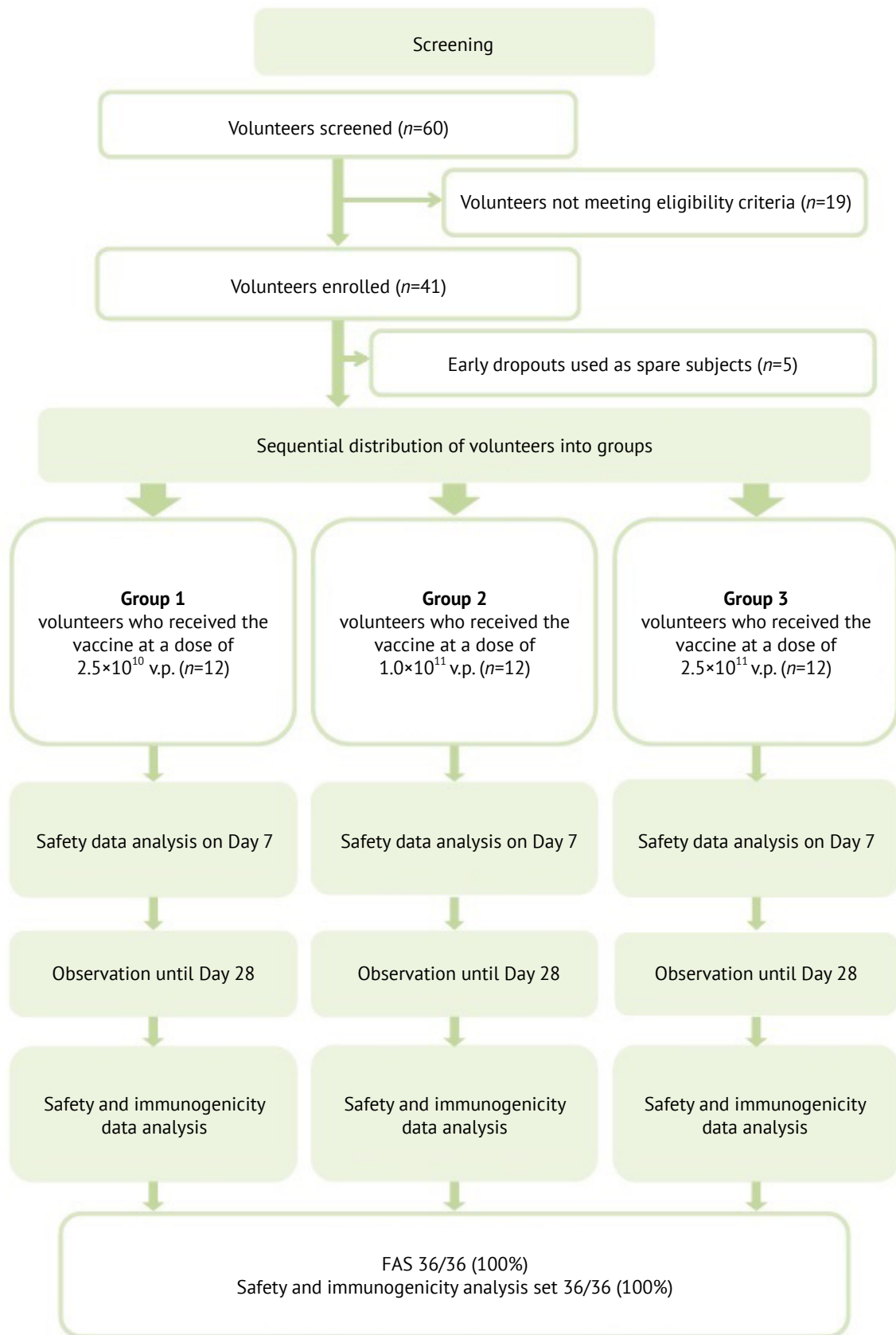
The adverse events recorded during the study were mostly of mild severity and typically developed 1–7 days after vaccination. All

¹⁹ <https://doi.org/10.30895/2221-996X-2025-25-1-7-21-table-s1>

²⁰ <https://www.meddra.org/>

²¹ The same adverse event observed multiple times in the same volunteer was counted as one adverse event.

²² Not all cases of increase/decrease in laboratory parameters were assessed by the researchers as adverse events.



The figure was prepared by the authors using their own data

Fig. 1. Study design and volunteer allocation for the clinical trial of the viral vector vaccine against influenza A. FAS, full analysis set; v.p., viral particles.

Table 1. Adverse drug reactions (ADRs) reported in volunteers after a single intranasal administration of the viral vector vaccine against influenza A (according to the MedDRA classification)

System Organ Class	Group 1 (n=12)		Group 2 (n=12)		Group 3 (n=12)	
	Number of volunteers with ADRs (group incidence rate, %)	Number of ADRs	Number of volunteers with ADRs (group incidence rate, %)	Number of ADRs	Number of volunteers with ADRs (group incidence rate, %)	Number of ADRs
Respiratory, thoracic and mediastinal disorders	6 (50.0)	8	3 (25.0)	3	7 (58.3)	8
Blood and lymphatic system disorders	11 (91.7)	25	10 (83.3)	14	8 (66.7)	28
General disorders and administration site conditions	0 (0)	0	2 (16.7)	4	4 (33.3)	7
Renal and urinary disorders	2 (16.7)	3	0 (0)	0	0 (0)	0

The table was prepared by the authors using their own data

Note. MedDRA, Medical Dictionary for Regulatory Activities²³.

AEs resolved within 1–2 days, typically without symptomatic treatment.

In Group 1 there were AEs assessed as moderate AEs: 1 case of leukocytosis, 9 cases of changes in immunogram data (5 cases of increased CD16, 1 case each of decreased CD16, CD19, and CD4, and 1 case of increased immunoglobulin E to 1629 IU/mL). The remaining general and local AEs, as well as changes in laboratory parameters in Group 1, were assessed as mild AEs. In Group 2, general and local AEs of moderate severity (increased CD16, increased immunoglobulin E (up to 614 IU/mL), headache) were recorded in 3 cases; the remaining AEs were mild. In Group 3, all AEs were of mild severity.

The causal relationship of AEs with the vaccine was established by the study doctor and was assessed as “definite” or “possible/probable” for local AEs (swelling, hyperemia, burning), and also for symptoms such as increased body temperature and various symptoms of intoxication (headache, chills, malaise, myalgia, and arthralgia). The causal relationship of AEs for laboratory data (a general clinical analysis (lymphocytosis, monocytosis, fluctuations in neutrophil and leukocyte levels, an increase in the erythrocyte sedimentation rate) and a biochemical analysis (increased levels of alanine and aspartate aminotransferases, C-reactive protein) was assessed

as “possible/probable”. The causal relationship in the case of similar manifestations in the late stages of outpatient observation was often classified as “possibly not” or “probably not,” due to the significant influence of factors not related to the medicinal product, but characteristic of the volunteer’s usual lifestyle and any conditions during the outpatient period. It can also be noted that when assessing the immunogram, changes were of a multidirectional nature: both an increase and a decrease in indicators were noted within the groups.

The adverse drug reactions reported during the clinical trials were categorized according to the MedDRA classification (Table 1). When comparing groups 1, 2, and 3, the differences in the incidence of adverse drug reactions were statistically insignificant in most categories ($p>0.05$): respiratory, thoracic and mediastinal disorders ($p=0.6812$), blood and lymphatic system disorders ($p=0.8132$), and renal and urinary tract disorders ($p=0.0747$). The exception was one category (general disorders and administration site conditions), in which the differences were statistically significant ($p=0.0285$).

Thus, the adverse events identified during the study and considered medicinal product-related are typical of most vaccines. No serious or severe adverse events were reported, indicating

²³ <https://www.meddra.org/>

that the study medicinal product does not have a negative impact on vital organs or systems, nor does it significantly affect laboratory parameters.

Immunogenicity analysis of the viral vector vaccine against influenza A

The vaccine's immunogenicity was not fully assessed during the phase I clinical trial due to the small sample size of volunteers in each group and the lack of a placebo group. Therefore, a preliminary immunogenicity assessment was conducted to refine the doses for the Phase II clinical trial. The efficacy (immunogenicity) population coincided with the safety population.

Venous blood was collected from volunteers before vaccination (on the day of vaccine administration, Day 0) and after vaccination (Day 28) to obtain serum samples and determine the IgG antibody titer to the influenza A (H5N2) virus using the ELISA method (Fig. 2).

The administration of the vaccine to volunteers in Group 1 at the lowest dose (2.5×10^{10} v.p.) did not result in an increase in the level of specific IgG antibodies in the blood serum on Day 28 compared to baseline values before vaccination (Fig. S1 A, available on the journal website²⁴). The geometric mean titer (GMT) of specific IgG antibodies was 1131 on both Day 0 and Day 28. No increase in individual IgG antibody titers by 2 or more times was detected in the blood serum of immunized individuals.

The administration of the viral vector vaccine against influenza A to Group 2 volunteers at a median dose (1.0×10^{11} v.p.) resulted in a significant increase in serum specific IgG antibody levels (Fig. S1 B²⁴). The serum specific IgG antibody titer before vaccination (Day 0) was 1425.44, and, an increase in serum specific IgG antibody titers to 2397.3 on Day 28 after vaccination ($p=0.0039$) was observed. In addition, an increase in individual IgG antibody titers by 2 or more times was observed in 9 of the 12 immunized individuals.

In Group 3 volunteers were immunized with the highest dose of the viral vector vaccine (2.5×10^{11} v.p.), serum samples revealed the development of a high-level immune response (Fig. S1 C²⁴). The GMT value of antibodies to influenza A virus before immunization was 5382, and, it significantly increased to 15222

($p=0.0005$) on Day 28. Moreover, an increase in individual IgG antibody titers by 2 or more times was recorded in 12 out of 12 volunteers, and by 4 or more times in 5 out of 12 volunteers.

A comparative analysis of the results obtained in three groups of immunized volunteers is presented in Table 2.

An immunogenicity assessment of the viral vector vaccine against influenza A in volunteers revealed a dose-dependent effect (Table 2). The maximum increase of the GMT of specific IgG antibodies on Day 28 after vaccination (2.8-fold) was observed in volunteers in Group 3, immunized with the highest dose of the vaccine (2.5×10^{11} v.p.). All individuals in this group showed a 2-fold or more increase in the titer of post-vaccination serum antibodies on Day 28 after immunization, thus, the seroconversion rate was 100%. The administration of the vaccine to volunteers in Group 2 at the medium dose (1.0×10^{11} v.p.) also resulted in the induction of a humoral immune response with a significant increase in the GMT of specific IgG antibodies (a 2-fold increase in titers), however, the seroconversion rate was 75%, lower than in Group 3. When volunteers in Group 1 were administered the lowest dose of the vaccine (2.5×10^{10} v.p.), the induction of a specific immune response was not determined.

It should be noted that a 4-fold or greater increase in antibody titers was observed only in Group 3, with a seroconversion rate of 41.7%. Thus, the results of a preliminary assessment of the immunogenicity of the GamFluVac vaccine in healthy volunteers in three groups with increasing doses of the medicinal product showed that the vaccine dose containing 2.5×10^{11} recombinant adenovirus particles was most effective in inducing a humoral immune response.

DISCUSSION

A phase I clinical trial evaluating the safety and immunogenicity of the GamFluVac viral vector vaccine administered intranasally to healthy volunteers examined the potential use of serotype 5 adenovirus vectors carrying the genes for conserved influenza A virus proteins—M2, NP, and the HA125 hybrid hemagglutinin gene—as an influenza vaccine.

The selection of three vaccine doses (2.5×10^{10} , 1.0×10^{11} , 2.5×10^{11} v.p.) was guided by literature data and the results of our own animal stu-

²⁴ <https://doi.org/10.30895/2221-996X-2025-25-1-7-21-fig-s1>

Table 2. Results of assessing the seroconversion levels on Day 28 after a single intranasal administration of the viral vector vaccine against influenza A in healthy volunteers ($n=36$)

Group	Geometric mean titer of antibodies (95% confidence interval)		Fold rise in antibody titers on Day 28	Number of volunteers with seroconversion on Day 28	
	Before vaccination (Day 0)	Day 28		≥2-fold IgG antibody titer rise, n (%)	≥4-fold IgG antibody titer rise, n (%)
Group 1, $n=12$	1131 (759.6–1685)	1131 (759.6–1685)	1	0 (0)	0 (0)
Group 2, $n=12$	1425.44 (986.9–2059)	2397.29* (1911–3008)	1,7	9 (75)	0 (0)
Group 3, $n=12$	5382* (3861–7501)	15222** (10922–21215)	2,8	12 (100)	5 (41.7)

The table was prepared by the authors using their own data

Note. # $p<0.0001$ (level of significance of differences in antibody titers between Group 2 and Group 3 on Day 0); * $p<0.001$ and ** $p<0.0001$ (levels of significance of differences in antibody titers in groups between Day 0 and Day 28).

dies of specific activity and toxicity [18–20]. The lowest dose (2.5×10^{10} v.p.) was determined by applying a safety factor of 10 to the dose established in animal studies. The highest dose in the study was 2.5×10^{11} v.p. The use of higher doses was not carried out due to the lack of published data on their safety.

Safety is one of the key characteristics of new vaccines in clinical trials²⁵. Adenoviral vector-based vaccines have demonstrated their safety in a number of clinical trials, including those administered intranasally. Thus, the data obtained in this study regarding the nature and severity of AEs, the absence of significant effects on vital systems and organs, and a significant negative impact on the quality of life of volunteers are consistent with the results obtained during the NasoVAX influenza vaccine study involving 80 volunteers [21]. This vaccine is based on human adenovirus serotype 5, which expresses the full-length hemagglutinin gene of the influenza A/California/04/2009(H1N1) virus. The use of the vaccine in doses of 1×10^9 , 1×10^{10} , and 1×10^{11} v.p. demonstrated safety and good tolerability. The most common AEs were local reactions such as nasal congestion, sneezing, irritated nose, and sore throat, and systemic reactions such as headache and fatigue. All AEs were of mild or moderate severity, with the exception of headache and fatigue in one participant in the 1×10^9 v.p. dose group and one in the 1×10^{11} v.p. dose group. The duration of AEs ranged from 1 to 14 days [21]. Similar results were obtained during a safety study of intranasal administration

of the Salnavac vaccine (dose 10^{11} v.p.), containing recombinant vectors based on human adenoviruses of 26 and 5 serotypes expressing the S-protein gene of the SARS-CoV-2 virus [22]. Thus, vaccines based on adenovirus vectors, when administered intranasally, most often cause adverse drug reactions involving respiratory, thoracic and mediastinal disorders, and also general disorders and administration site conditions.

The most common adverse drug reactions reported after administration of the GamFluVac vaccine were headache, also respiratory, thoracic, and mediastinum-related disorders including various nasal symptoms and mucosal reactions (nasal mucus flow, swelling of the nasal passages, scratchy throat, hyperemia of the fauces and nasal passages, stuffiness in the nose et al.). These adverse drug reactions were expected and typical for viral vaccines administered intranasally.

Current standard methods for assessing the immunogenicity of influenza vaccines (hemagglutination inhibition reaction, microneutralization assay) are not always relevant for vaccines containing conserved influenza virus antigens. Therefore, research is being conducted to find new immunological markers, other than hemagglutinating and neutralizing antibodies, including those determined using ELISA, with the goal of establishing correlates of protection capable of predicting the effectiveness of universal influenza vaccines [23, 24].

In this study, the inactivated influenza virus A/Mallard Duck/ Pennsylvania/10218/84

²⁵ Guidelines for Conducting Clinical Trials of Medicinal Products (Immunobiological Medicinal Products). Part 2. Moscow: Grif and K; 2012 (In Russ.).

was used as an antigen to determine the level of IgG antibodies in blood serum samples using the ELISA method. The use of whole-virion antigen in ELISA to detect specific antibodies is a classic approach, since the virus contains all proteins in their native conformation, including hemagglutinin, nucleoprotein and the influenza virus ion channel protein M2. This allows us to evaluate the humoral immune response to all antigens present in the adenoviral vectors included in the vaccine.

It is important to note that in our study, all baseline serum samples collected before vaccination yielded IgG antibodies to the influenza A (H5N2) virus at titers of 1:400, indicating 100% seropositivity among participants at the initial stage of the study. The detection of antibodies to the avian influenza A (H5N2) virus, despite its absence of circulation among humans, is likely due to the presence of an immune response to conserved proteins of the influenza virus (M1, M2, NP, etc.).

Furthermore, cross-reactions with conserved epitopes of the influenza virus hemagglutinin are possible, as previously described elsewhere [25].

Thus, during a clinical trial of an influenza vaccine based on a replication-competent adenovirus type 4 expressing the hemagglutinin of the influenza A/Vietnam/1194/04 (H5N1) virus (Ad4-H5-Vtn), high baseline levels of specific mucosal IgG and IgA antibodies to the hemagglutinin of the influenza A (H5) virus were detected. According to the authors, the detection of antibodies in samples obtained before vaccination is associated with cross-reactivity to the hemagglutinin stem region of antibodies induced by previous exposure to other influenza virus strains [25].

Russian authors have also examined the mechanisms of induction of cross-reactive IgA antibodies to avian influenza viruses types A (H5N1) and (H5N2), due to the circulation of other influenza virus subtypes, in particular A (H1N1)²⁶. It should be noted that in our study, the baseline values GMT of IgG antibodies to influenza A (H5N2) differed across all groups and were 1131 (Group 1), 1425.44 (Group 2), and 5382 (Group 3), with a statistically significant difference only between Groups 2 and 3 ($p < 0.0001$).

It is important to note that enrolment criteria of volunteer included the absence of influenza vaccination in the past 6 months, but information about previous vaccinations was not available. Furthermore, study participants had not had acute respiratory infections in the 7 days prior to vaccination and had not had respiratory symptoms in the past 3 days.

The clinical trial was conducted in the middle of the 2018–2019 influenza epidemic season, which may have influenced the detection of elevated levels of specific IgG antibodies due to increased circulation of seasonal influenza viruses during the epidemic surge, as well as the prolonged sequential recruitment of volunteers into the groups. Asymptomatic forms of infection may have contributed to this process [26]. The potential influence of these factors (study limitations) on the results of the vaccine immunogenicity assessment should be taken into account when planning further studies, and a control group (placebo) should be included for comparative analysis of the dynamics of specific antibody titers.

An evaluation of the immunogenicity study results showed that a single intranasal administration of the GamFluVac vaccine containing 2.5×10^{10} v.p. (Group 1) did not result in an increase in the GMT value of IgG antibodies to the influenza A (H5N2) virus. The administration of a 1.0×10^{11} v.p. vaccine dose (Group 2) induced a humoral immune response with a 1.7-fold increase in the GMT value of specific IgG antibodies and a 75% seroconversion rate (a 2-fold increase). The highest immune response was induced after administration of a 2.5×10^{11} v.p. (Group 3): the increase in the GMT values of specific IgG antibodies was 2.8 times compared to the baseline value; the seroconversion rate was 100% (with a two-fold increase) and 41.7% (with a four-fold increase).

It is worth noting that a direct comparison of the immunogenicity of the GamFluVac vaccine with similar vaccines was not possible due to the lack of candidate vector vaccines based on conserved influenza virus antigens that had completed clinical trials. An indirect comparison is only possible with the intranasal influenza vaccine NasoVAX, which differs in that the adenoviral vectors carry influenza virus hemagglutinin

²⁶ Losev IV. Features of the development of the adaptive immune response to influenza viruses A (H5N1), A (H5N2) and A (H2N2): diss. ... Cand. Biol. Sci. St. Petersburg; 2017.

as a transgene rather than conserved antigens. An immunogenicity analysis of the NasoVAX vaccine showed that the increase in the GMT of hemagglutinating antibodies after administration of a dose of 1.0×10^{11} v.p. on Day 29 was 4.3, and the seroconversion rate was 33.3%. The proportion of individuals with a 4-fold or more increase in virus-neutralizing antibodies was 53.3% [21].

In our study, when compared with a similar dose level of 1.0×10^{11} v.p. (Group 2), the increase in the GMT of specific binding IgG antibodies was 1.7 times, while there were no volunteers with a 4-fold increase in individual IgG antibody titers.

This indicates a lower immunogenicity of the conserved antigens used in the development of the GamFluVac vaccine, compared to the immunodominant antigen—influenza virus hemagglutinin. It is important to note that the NasoVAX vaccine stimulated the production of antigen-specific IgA in the nasopharyngeal mucosa—an increase of approximately 2-fold compared to the baseline GMT value. This parameter was not assessed in our study.

Future clinical trials plan to study the ability of the GamFluVac vaccine to induce the production of antigen-specific IgA antibodies in the mucous membrane. Additionally, it is planned

to study the cellular immune response and the immune system's reaction to the adenoviral vector, including assessing its impact on immunogenicity during intranasal immunization. This will allow for a more complete characterization of the vaccine's immunogenic properties.

CONCLUSIONS

1. A Phase I clinical trial of the GamFluVac viral vector vaccine against influenza A, administered intranasally as a single dose, demonstrated a good safety profile for all three doses (2.5×10^{10} ; 1.0×10^{11} ; 2.5×10^{11} viral particles). All adverse drug reactions identified were expected and typical for viral vaccines administered intranasally.

2. In the group of volunteers who received the vaccine at a dose of 2.5×10^{11} v.p., a maximum increase in the geometric mean titer of specific IgG antibodies was observed after 28 days—a 2.8-fold increase, while the seroconversion rate for 2 or more times was 100%, and for 4 or more times, 41.7%.

3. Based on the results of the immunogenicity assessment of the viral vector vaccine against influenza A, a dose containing 2.5×10^{11} recombinant adenovirus particles for intranasal administration was selected for further clinical trials.

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vaccine immunogenicity evaluation. **I.B. Esmagambetov** designed the vaccine vector constructs. **D.N. Shcherbinin** developed the concept, critically revised and edited the manuscript. **L.V. Verkhovskaya** performed enzyme-linked immunosorbent assays to determine antibody titers in serum samples of volunteers. **S.V. Volchihina** analyzed the effect of the medicinal product on laboratory findings. **Ya.V. Simakova** systematized the results of safety assessment. **V.F. Babira** analyzed the effect of the medicinal product on vital body systems and organs. **D.Y. Logunov** analyzed and interpreted the results of the clinical trial. **A.L. Gintsburg** designed the clinical trial and approved the final version of the manuscript for publication.

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Authors / Об авторах

Maxim M. Shmarov, Dr. Sci. (Biol.) / **Шмаров Максим Михайлович**, д-р биол. наук

ORCID: <https://orcid.org/0000-0002-5268-1296>

Svetlana V. Alekseeva, Cand. Sci. (Biol.) / **Алексеева Светлана Викторовна**, канд. биол. наук

ORCID: <https://orcid.org/0000-0003-3313-1894>

Nina A. Dovzhenko, Cand. Sci. (Biol.) / **Довженко Нина Александровна**, канд. биол. наук

ORCID: <https://orcid.org/0000-0002-1382-1428>

Alina S. Bandelyuk / **Банделюк Алина Сергеевна**

ORCID: <https://orcid.org/0000-0001-8453-797X>

Ilias B. Esmagambetov, Cand. Sci. (Biol.) / **Есмагамбетов Ильяс Булатович**, канд. биол. наук

ORCID: <https://orcid.org/0000-0002-2063-2449>

Dmitrii N. Shcherbinin, Cand. Sci. (Biol.) / **Щербинин Дмитрий Николаевич**, канд. биол. наук

ORCID: <https://orcid.org/0000-0002-8518-1669>

Lyudmila V. Verkhovskaya, Cand. Sci. (Biol.) / **Верховская Людмила Викторовна**, канд. биол. наук

ORCID: <https://orcid.org/0000-0002-4731-1629>

Svetlana V. Volchihina / **Волчихина Светлана Владимировна**

Yana V. Simakova / **Симакова Яна Владимировна**

ORCID: <https://orcid.org/0000-0002-5033-6931>

Vladimir F. Babira / **Бабира Владимир Федорович**

Denis Y. Logunov, Dr. Sci. (Biol.), Acad. RAS / **Логунов Денис Юрьевич**, д-р биол. наук, академик РАН

ORCID: <https://orcid.org/0000-0003-4035-6581>

Alexander L. Gintsburg, Dr. Sci. (Biol.), Prof., Acad. RAS / **Гинцбург Александр Леонидович**, д-р биол. наук, проф., академик РАН

ORCID: <https://orcid.org/0000-0003-1769-5059>

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