

## ORIGINAL RESEARCH



Original Study Article

<https://doi.org/10.36233/0372-9311-661>



# A study of the safety and immunogenicity of a new vaccine for the prevention of COVID-19 based on virus-like particles in phase I clinical trials

Tatiana V. Grebennikova<sup>✉</sup>, Olga N. Zaykova, Alexey A. Plotnikov, Lyudmila V. Kostina, Yana Yu. Chernoryzh, Olesia V. Eliseeva, Oleg E. Latyshev, Viktor F. Larichev, Irina T. Fedyakina, Milana A. Losich, Ilya M. Kirillov, Ilya E. Filatov, Marina V. Balandina, Valery V. Tsibezov, Kirill I. Yurlov, Ekaterina I. Lesnova, Valeria M. Kondratieva, Alina A. Kozlova, Marina S. Baranets, Aleksandr L. Gintsburg

National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya, Moscow, Russia

### Abstract

**Introduction.** One of the more promising developments in preventing the spread of infections, including COVID-19, is the production of vaccines based on virus-like particles (VLP). Currently, in the National Research Center for Epidemiology and Microbiology named after N.F. Gamaleya of the Ministry of Health of the Russia has developed a VLP-based vaccine against COVID-19.

**The aim** of this study is to evaluate the tolerability, safety and immunogenicity of a new vaccine for the prevention of COVID-19 based on VLP compared with placebo for 21 days after two intramuscular injections in phase I clinical trials.

**Materials and methods.** A double-blind, placebo-controlled study of the tolerability, safety and immunogenicity of a vaccine for the prevention of COVID-19 based on VLP was conducted with a dose of the drug containing 40 and 80 micrograms of antigen, the placebo being 0.9% NaCl. The presence or absence of adverse events (AEs) after vaccination was noted in 180 volunteers aged 18 to 55 years; clinical and biochemical blood parameters, the intensity of humoral and cellular immunity before and after vaccination were assessed using enzyme immunoassay, neutralization reactions, lymphocyte blast transformation reactions and flow cytometry.

**Results.** An analysis of the tolerability and safety of the new COVID-19 VLP-vaccine showed that most adverse events were registered within the first 10 days after vaccination, mainly after the first vaccination. In the period from 11 to 21 days after vaccination, AEs were observed in isolated cases. No deaths, serious or other AEs have been reported. The administration of the studied vaccine to the volunteers had no negative effect on the basic vital signs. A comparative analysis of immunogenicity indicators in volunteers showed that the administration of a vaccine with both an antigen content of 40 µg and an antigen content of 80 µg leads to a pronounced and significant increase in the level of specific immunoglobulins, virus neutralizing antibodies and activation of a cell-mediated immune response. As part of the phase I clinical trials, a dose of 80 µg was selected as optimal.

**Conclusion.** It has been shown that a new vaccine for the prevention of COVID-19 based on VLP with an antigen content of 40 and 80 µg when administered intramuscularly to volunteers does not cause serious adverse events and induces a tense humoral and cellular immune response.

**Keywords:** COVID-19, SARS-CoV-2, virus-like particles, VLP

**Ethics approval.** The study was conducted with the voluntary informed consent of the patients. The study protocol was approved at a meeting of the Ethics Council of the Department of Regulation of the Circulation of Medicines and Medical Devices of the Ministry of Health of the Russian Federation (No. 310, dated 05.31.2022).

**Acknowledgement.** The authors of the article express their gratitude for the cooperation of RIC-Pharma, Elektrostal City Hospital, N.P. Bechtereva Institute of the Human Brain of the Russian Academy of Sciences

**Funding source.** State assignment of the Ministry of Health of the Russian Federation 121092400113-8 «Clinical trials of a VLP-based vaccine for the prevention of COVID-19 (phases 1-2)».

**Conflict of interests.** The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

**For citation:** Grebennikova T.V., Zaykova O.N., Plotnikov A.A., Kostina L.V., Chernoryzh Ya.Yu., Eliseeva O.V., Latyshev O.E., Larichev V.F., Fedyakina I.T., Losich M.A., Kirillov I.M., Filatov I.E., Balandina M.V., Tsibezov V.V., Yurlov K.I., Lesnova E.I., Kondratieva V.M., Kozlova A.A., Baranets M.S., Gintsburg A.L. A study of the safety and

immunogenicity of a new vaccine for the prevention of COVID-19 based on virus-like particles in phase I clinical trials. *Journal of microbiology, epidemiology and immunobiology*. 2025;102(2):135–149.  
DOI: <https://doi.org/10.36233/0372-9311-661>  
EDN: <https://www.elibrary.ru/LKOJHI>

Оригинальное исследование  
<https://doi.org/10.36233/0372-9311-661>

## Исследование безопасности и иммуногенности вакцины для профилактики COVID-19 на основе вирусоподобных частиц в рамках I фазы клинических испытаний

Гребенникова Т.В.<sup>✉</sup>, Зайкова О.Н., Плотников А.А., Костина Л.В., Чернорыж Я.Ю.,  
Елисеева О.В., Латышев О.Е., Ларичев В.Ф., Федякина И.Т., Лосич М.А.,  
Кириллов И.М., Филатов И.Е., Баландина М.В., Цибезов В.В., Юрлов К.И.,  
Леснова Е.И., Кондратьева В.М., Козлова А.А., Баранец М.С., Гинцбург А.Л.

Национальный исследовательский центр эпидемиологии и микробиологии имени почетного академика Н.Ф. Гамалеи, Москва, Россия

### Аннотация

**Введение.** Одним из перспективных направлений в предупреждении распространения инфекций, в том числе COVID-19, является получение вакцин на основе вирусоподобных частиц (virus like particles, VLP). В НИЦЭМ им. Н.Ф. Гамалеи разработана вакцина на основе VLP против COVID-19.

**Цель работы** — оценить переносимость, безопасность и иммуногенность новой вакцины для профилактики COVID-19 на основе VLP в сравнении с плацебо на протяжении 21 сут после двукратного внутримышечного введения в рамках I фазы клинических испытаний.

**Материалы и методы.** Двойное слепое плацебо-контролируемое исследование переносимости, безопасности, и иммуногенности вакцины для профилактики COVID-19 на основе VLP проводили с дозой введения препарата, содержащего 40 и 80 мкг антигена, плацебо — 0,9% NaCl. У 180 добровольцев в возрасте 18–55 лет отмечали наличие или отсутствие нежелательных явлений (НЯ) после вакцинации, оценивали показатели крови, напряжённость гуморального и клеточного иммунитета до и после вакцинации с помощью иммуноферментного анализа, реакции нейтрализации, реакции бласттрансформации лимфоцитов и проточной цитометрии.

**Результаты.** Анализ переносимости и безопасности новой вакцины против COVID-19 на основе VLP показал, что большинство НЯ регистрировались в течение первых 10 сут после вакцинации, преимущественно после 1-й вакцинации. В период с 11-х по 21-е сутки после вакцинации НЯ отмечались в единичных случаях. Летальных исходов, серьёзных и иных НЯ не зарегистрировано. Введение исследуемой вакцины добровольцам не оказало негативного влияния на основные жизненные показатели. Сравнительная характеристика показателей иммуногенности у добровольцев показала, что введение вакцины с содержанием антигена как 40, так и 80 мкг приводит к выраженному и достоверному росту уровня специфических иммуноглобулинов, вируснейтрализующих антител и активации клеточно-опосредованного иммунного ответа. В рамках I этапа клинических исследований доза 80 мкг была выбрана как оптимальная.

**Заключение.** Новая вакцина для профилактики COVID-19 на основе VLP с содержанием антигена 40 и 80 мкг при введении добровольцам внутримышечно не вызывает серьёзных НЯ и индуцирует напряжённый гуморальный и клеточный иммунный ответ.

**Ключевые слова:** COVID-19, SARS-CoV-2, вирусоподобные частицы, вирусоподобные частицы

**Этическое утверждение.** Исследование проводилось при добровольном информированном согласии пациентов. Протокол исследования одобрен на заседании Совета по этике Департамента регулирования обращения лекарственных средств и медицинских изделий Министерства здравоохранения Российской Федерации (№ 310 от 31.05.2022).

**Благодарность.** Авторы статьи выражают благодарность за сотрудничество ООО «РИК-Фарма», ГБУЗ МО «Электростальская центральная городская больница», ФГБУН «Институт мозга человека им. Н.П. Бехтеревой» РАН.

**Источник финансирования.** Государственное задание Министерства здравоохранения Российской Федерации 121092400113-8 «Клинические испытания вакцины на основе VLP для профилактики COVID-19 (1–2 фазы)».

**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

**Для цитирования:** Гребенникова Т.В., Зайкова О.Н., Плотников А.А., Костина Л.В., Чернорыж Я.Ю., Елисеева О.В., Латышев О.Е., Ларичев В.Ф., Федякина И.Т., Лосич М.А., Кириллов И.М., Филатов И.Е., Баландина М.В., Цибезов В.В., Юрлов К.И., Леснова Е.И., Кондратьева В.М., Козлова А.А., Баранец М.С., Гинцбург А.Л. Исследование безопасности и иммуногенности вакцины для профилактики COVID-19 на основе вирусоподобных частиц в рамках I фазы клинических испытаний. *Журнал микробиологии, эпидемиологии и иммунобиологии*. 2025;102(2):135–149.

DOI: <https://doi.org/10.36233/0372-9311-661>

EDN: <https://www.elibrary.ru/LKOJHI>

## Introduction

The pandemic of a new coronavirus infection has posed serious challenges to public health services worldwide in the prevention, treatment and diagnosis of this disease. COVID-19, a severe acute respiratory infection caused by the SARS-CoV-2 virus (*Coronaviridae*, *Orthocoronavirinae*, *Betacoronavirus*, *Sarbecovirus*), is characterized by a high mortality rate, which, according to different studies, ranges from 0.5 to 15% [1–3].

The first cases of disease caused by SARS-CoV-2 virus were registered in December 2019 in China. The virus spread quite rapidly to all continents, and according to the World Health Organization (WHO), as of December 2021, there were more than 260 million confirmed cases of COVID-19 worldwide, including 5.2 million deaths<sup>1</sup>.

The most severe manifestations of coronavirus infection are interstitial pneumonia with impaired respiratory function and multi-organ failure, which were often fatal [4–6]. The duration of post-infection immunity is not fully understood [7].

According to Rospotrebnadzor, as of May 12, 2024, about 785 million cases have been detected worldwide, the most unfavorable region being the Western Pacific. In Russia from 02.03.2020 to 05.05.2024 about 24 million cases were registered in 85 subjects<sup>2</sup>. Despite WHO statements that COVID-19 has moved into the category of seasonal infections and periodically causes outbreaks of the disease along with influenza and acute respiratory viral diseases, SARS-CoV-2 virus continues to infect people and claim their lives<sup>3</sup>.

The variability of SARS-CoV-2, namely mutations in the receptor binding domain (RBD) of the

S-protein, has led to the emergence of a variety of virus variants worldwide, of which Alpha (lineage B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529) are the most epidemically significant and of current concern to WHO [8]. The emergence of new variants of the virus, including those with reduced sensitivity to virus-neutralizing antibodies and vaccination with already available vaccines, requires regular molecular genetic monitoring of SARS-CoV-2 and the development of new highly effective vaccines that promote the formation of intense and long-lasting immunity against topical strains of the causative agent of coronavirus infection [8].

An effective vaccine against COVID-19 should be safe, non-reactogenic, and induce the formation of virus-neutralizing antibodies in titers sufficient to prevent the development of the infectious process. Furthermore, the vaccine should promote an effective immune response with the least amount of antigen used. This reduces the cost of the vaccine and makes it affordable [9].

A vaccine based on virus like particles (VLP) for COVID-19 prophylaxis has been developed in the Gamaleya Research Center for Ecological Medicine. VLPs are formed from 4 recombinant structural proteins (S, M, E, N) and are similar in structure to SARS-CoV-2 virion, but without viral RNA. The surface S-protein (S-spike) of SARS-CoV-2 is responsible for binding to specific receptors on the surface of susceptible cells. Particles containing S-protein with consensus mutations of clades 19A, Delta and Omicron are used in the vaccine formulation. Thus, it is expected that antibodies to strains of these clades will be synthesized in the body after immunization.

The structural spike and membrane proteins undergo significant mutational changes, whereas the envelope and nucleocapsid proteins are highly conserved, indicating differential selection pressure to which SARS-CoV-2 has been subjected during evolution. At the same time, the contribution of viral M, E, and N proteins to the formation of B- and T-cell immunity is equally important, which is confirmed by the development of vaccines based on these proteins [10, 11]. It was shown that the vaccine under development stimulates the T-cell immune response in golden hamsters after the 1<sup>st</sup> immunization, which also provides protection against various SARS-CoV-2 strains [12].

Data on preclinical trials of the drug were submitted to the Ministry of Health of the Russian Federation.

<sup>1</sup> WHO Director-General's opening remarks at the media briefing on COVID-19. 11 March 2020. URL: <https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020>  
WHO. COVID-19 Epidemiological Update. 06.11.2024. URL: [https://www.who.int/docs/default-source/coronaviruse/situation-reports/covid-19\\_epi\\_update\\_173.pdf?sfvrsn=457952e6\\_4&download=true](https://www.who.int/docs/default-source/coronaviruse/situation-reports/covid-19_epi_update_173.pdf?sfvrsn=457952e6_4&download=true)

<sup>2</sup> The epidemiological situation and the spread of COVID-19 in the world as of 8 Moscow time on 05/12/2024 /FCUN ROSNIPCHI "Microbe". Federal Service for Consumer Rights Protection and Human Welfare. (In Russ.) URL: <https://www.rospotrebnadzor.ru/12.05.2024%20г.%20Информация%20о%20случаях%20заболевания.docx>

<sup>3</sup> COVID-19 Cases, World / WHO Coronavirus (COVID-19) dashboard. URL: <https://data.who.int/dashboards/covid19/cases>

Permission to conduct clinical trials was obtained from 16.02.2022 No. 115.

**The aim** of this study was to evaluate the tolerability, safety and immunogenicity of a new VLP-based vaccine for COVID-19 prophylaxis compared to placebo for 21 days after double intramuscular administration within the framework of phase I clinical trials.

**Study objectives:**

1. To consistently evaluate the tolerability and safety of vaccine containing 40 and 80 µg antigen doses administered intramuscularly on the 10<sup>th</sup> day after single vaccination.

2. To evaluate the tolerability and safety of vaccine containing 40 and 80 µg antigen doses administered intramuscularly on the 21<sup>st</sup> day after double vaccination.

3. To evaluate the immunogenicity of the vaccine containing 40 and 80 µg antigen doses administered intramuscularly on the 21<sup>st</sup> day after double vaccination.

4. To determine the optimal vaccine dosage based on the immunogenicity and safety parameters obtained for the phase II clinical trial.

## Materials and methods

Double-blind randomized placebo-controlled multicenter prospective study of Phase I clinical trials with dose-ranging to assess tolerability, safety and immunogenicity of the vaccine for COVID-19 prophylaxis based on VLP (containing particles similar to SARS-CoV-2) when administered intramuscularly to volunteers aged 18–55 years was conducted from February to December 2022 on the basis of two research centers: Elektrostal Central City Hospital and N.P. Bekhtereva Institute of the Human Brain of RAS in accordance with the principles of the Declaration of Helsinki (2013), the ICH guidelines on overdose, ICH Guidelines for Good Clinical Practice (version E6, approved by SRMR/135/95), Federal Law No. 61-FL “On Circulation of Medicines” dated 12.04.2010; Order of the Ministry of Health of the Russian Federation No. 200n “On Approval of the Rules of Good Clinical Practice” dated 01.04.2016; National Standard of the Russian Federation GOST R 52379-2005 “Good Clinical Practice” approved by Order of the Federal Agency for Technical Regulation and Metrology No. 497-st. from 04.06.2014, the Rules of Good Clinical Practice of the Eurasian Economic Union” approved by the Decision of the Council of the Eurasian Economic Commission from 03.11.2016 № 79 and other applicable requirements of national legislation.

Prior to inclusion in the study, volunteers were familiarized with information about the study and signed an informed consent form. Researchers recruited to participate in the clinical trial provided signed and dated resumes describing their research activities and certificates confirming their qualifications before the start of the study.

A total of 180 male and female volunteers aged 18–55 years were included in the study. All volunteers were healthy, met the inclusion criteria (**Appendix 1** on the journal's website: <https://www.microbiol.crie.ru>) and were divided into 3 groups:

- Group 1 — 60 volunteers who were immunized with vaccine containing 40 µg antigen twice at an interval of 21 days intramuscularly;
- Group 2 — 60 volunteers who were vaccinated with 80 µg antigen vaccine twice at an interval of 21 days intramuscularly;
- Group 3 — 60 volunteers who received placebo vaccine twice at an interval of 21 days intramuscularly.

The vaccine was purified recombinant SARS-CoV-2 virus-like particles synthesized in a baculovirus expression system. The S surface protein within the virus-like particles was represented by variants 19A, Alpha, Delta, and Omicron. The vaccine composition included a squalene-based adjuvant. The volume ratio of adjuvant to antigen was 1 : 1. Vaccine with 40 and 80 µg antigen content was investigated. A 0.9% NaCl solution was used as placebo.

Volunteers were examined at visits:

- visit 1 (hospitalization, randomization, the 1<sup>st</sup> vaccination);
- visits 2, 3 (2<sup>nd</sup>–3<sup>rd</sup> days after the 1<sup>st</sup> vaccination);
- visit 4 (10<sup>th</sup> day after the 1<sup>st</sup> vaccination);
- visit 5 (21<sup>st</sup> day after the 1<sup>st</sup> vaccination, hospitalization, 2<sup>nd</sup> vaccination);
- visits 6, 7 (2<sup>nd</sup>–3<sup>rd</sup> days after the 2<sup>nd</sup> vaccination);
- visit 8 (10<sup>th</sup> day after the 2<sup>nd</sup> vaccination);
- visit 9 (21<sup>st</sup> day after the 2<sup>nd</sup> vaccination).

We analyzed data from the electronic self-monitoring diary, performed physical examination, assessed vital signs, collected data on concomitant therapy, identified and recorded adverse events (AEs) and serious AEs, and evaluated inclusion/non-inclusion criteria. Blood was drawn to assess cellular and humoral immunity. Biochemical, clinical tests, determination of total IgE, coagulogram, and urinalysis were performed. During visits 4, 5, 8, 9, nasopharyngeal swabs were taken to determine the absence of SARS-CoV-2 virus RNA by polymerase chain reaction (PCR) method.

*Lymphocyte Blast Transformation Reaction (LBTR).* Blood was collected on the 10<sup>th</sup> day after the 1<sup>st</sup> and 2<sup>nd</sup> vaccinations (visits 4 and 8). Mononuclear cell fractions from peripheral blood were isolated by centrifugation on a one-step density gradient of Ficoll-Pak (PanEco), the isolated mononuclear cells were washed twice in pure RPMI-1640 medium and seeded in 96-well microculture plates at a concentration of 10<sup>5</sup> cells/well and stimulants were added in 100 µl to final concentrations. We used the medium separately (spontaneous proliferation) as a negative control; concanavalin A mitogen (5 µg/mL, PanEco) as a non-specific positive control; SARS-CoV-2 virus: PMVL-12, depo-



sition number EPI\_ISL\_572398 in the GISAID database as a specific stimulant; Congo-Crimean hemorrhagic fever antigen as a nonspecific positive control; and Seppic adjuvant as a non-specific stimulant. Cells were cultured in RPMI-1640 medium containing 20% fetal calf serum, 2 mM glutamine, 4.5 g/L glucose, 50 µg/mL gentamicin, and 0.2 units/mL insulin at 37°C in a 5% CO<sub>2</sub> atmosphere. All of the mentioned processes were performed under sterile conditions.

Splenocyte proliferation was assessed by LBTR after 4 days using an inverted microscope (× 400). The results were expressed as proliferation stimulation index (PSI), calculated as the ratio of the mean number of lymphoblasts observed in the presence and absence of specific stimulants. A result was considered positive if the PSI > 2.

The obtained data were processed using the Prism Graphpad v. 8.4.3 program (GraphPad Software). Statistical analysis was performed using the Statistica v. 12.6 program (StatSoft Inc.). Reliability of differences was evaluated by Student's t-criterion. A value of  $p < 0.05$  was considered statistically significant.

**Cell staining for flow cytometry.** 1 million lymphocytes in a volume of 50 µl were transferred into centrifuge tubes and 5 µl each of anti-CD3, anti-CD4 and anti-CD8 monoclonal antibodies (Sorbent) were added and incubated for 45 min at 4°C. Washed twice in Hanks' solution (5 min at 200g). The supernatant was removed and cells were suspended in 200 µl of Hanks' solution and analyzed on a BD FACS Accuri C6+ flow cytometer. The obtained data were processed using the Cytotflex and Prism Graphpad 8.0 programs. The results were analyzed using the FlowJo program (Three Star).

**Humoral immunity** was assessed by enzyme-linked immunosorbent assay (ELISA) and neutralization reaction (NR). Blood was collected on the 10th day after the 1<sup>st</sup> and 2<sup>nd</sup> vaccination (visits 5 and 9). ELISA was performed using a reagent kit for immunoenzymatic detection of IgG to RBD of the S surface glycoprotein S of SARS-CoV-2 coronavirus (SARS-CoV-2-RBD-ELA-Gamalei, RU No. RZN 2020/10393).

The levels of neutralizing antibodies were determined by titrating sera from 1 : 10 to 1 : 1280 against 100 TCID<sub>50</sub> of three SARS-CoV-2 strains from the Molecular Diagnostics Laboratory collection: Wuhan, Delta (line B.1.617.2) and Omicron (variant XBB 1.5). Neutralization reactions were performed by micro-method in 96-well plates on Vero E6 green monkey kidney cell culture. Serum dilutions were incubated with viruses for 1 h at 37°C in an atmosphere of 5% CO<sub>2</sub> and transferred to a plate with a cell monolayer. After 72 h, the reaction was counted by the presence of cytopathic action of the virus. The serum titer (the last neutralizing dilution) was considered to be the dilution at which 100% cell protection (no cytopathic effect) was ensured.

**Cytokine concentrations in sera** were measured by ELISA using commercial test systems (Vector-Best): Interleukin-2-ELISA-BEST “Reagent kit for immunoenzymatic determination of interleukin-2 concentration in serum”; Gamma-Interferon-ELISA-BEST “Reagent kit for immunoenzymatic determination of gamma-interferon concentration in serum”; Alpha-TNF-ELISA-BEST “Reagent kit for immunoenzymatic determination of tumor necrosis factor-alpha concentration in serum”.

**Statistical processing of data** was performed using Microsoft Office Excel 2007-2016 and online statistical calculators (<https://math.semestr.ru>, <https://medstatistic.ru>). Mean, standard deviations, quartiles, minimum and maximum values, as well as frequencies, depending on the nature of the data, were used as descriptive characteristics of demographic and other baseline data parameters, as well as safety parameters and immunogenicity parameters.

To analyze quantitative indicators over time in each group, repeated-measures analysis of variance or Friedman analysis was used, depending on the nature of the data distribution. For a posteriori comparisons of values at screening and follow-up visits, Dunnett's criterion was used in the case of analysis of variance and Dunn's criterion with Bonferroni correction in the case of Friedman analysis.

Comparisons of groups with each other on qualitative features were performed using the  $\chi^2$  criterion or Fisher's exact test. Geometric mean titers (GMT) for each study group with 95% confidence intervals (CI) were estimated for each time point. Logarithmic transformation was applied to the raw data to analyze titers. For comparisons of the study groups with each other, analysis of variance (Bonferroni or Gates–Howell posterior comparisons if the variances were not equal) or Kruskal–Wallis analysis (Dunn posterior comparisons with Bonferroni correction) was applied to the logarithmic data, depending on the nature of the distribution. To assess the type of distribution, the Kolmogorov–Smirnov criterion was used, as well as the asymmetry and kurtosis indices.

Levene's criterion was used to test the homogeneity of dispersions, and Spearman's correlation was used to assess the relationship between neutralizing and specific antibody titers. 95% CI was calculated for the correlation coefficient. The frequencies with 95% CI calculated by the Clopper–Pearson method are given as descriptive characteristics for the efficacy parameters.

## Results

In a safety and immunogenicity study of the novel VLP-based COVID-19 prophylaxis vaccine in a phase I clinical trial, screened volunteers ( $n = 180$ ; 107 males and 73 females) were categorized into 3 groups, with 84% completing the study ( $n = 151$ : 52 in group 1,

53 in group 2, and 46 in group 3). Demographic data are summarized in **Table 1**.

### *Evaluation of vaccine tolerability and safety*

In the **Appendix 2** on the journal's website: <https://www.microbiol.crie.ru>, all AEs by groups are presented. During the entire period of post-vaccination follow-up within 21 days after twice-daily vaccination, 572 AEs were detected in 138 (76.7%) volunteers, including 216 AEs in 47 (78.3%) volunteers in Group 1, 226 AEs in 49 (81.7%) volunteers in Group 2, and 130 AEs in 42 (70%) volunteers in Group 3 (**Fig. 1**). The same volunteer could experience several different reactions simultaneously.

In 50.5% of cases, the most frequently reported general abnormalities and reactions at the injection site (in 37.7% — pain at the vaccination site and in 22.5% — fatigue), which in 95% of cases were observed in the first 10 days. Also, in 20.5% of cases, clinically significant abnormalities in laboratory tests were observed equally on both the 10<sup>th</sup> and 21<sup>st</sup> day after administration of the study drug, irrespective of vaccination. In 64.5% of cases, the registered AEs had a mild degree of severity, in 31.1% — moderate, in 4.4% — severe. In the majority of cases, the relationship between the development of AEs and vaccination was considered probable. The outcome in 95% of all cases was “recovery without consequences”, while in 5% of cases the outcome was “not yet recovered”. There were no fatalities, serious AEs, or other significant AEs that were considered to be of special interest due to their clinical significance.

The results of laboratory studies confirmed that administration of the investigated vaccine to volunteers aged 18–55 years had no negative effect on the main indicators of clinical and biochemical blood tests, IgE level and indicators of general urine analysis. Deviations of laboratory parameters from the norm were registered in all groups. The majority of deviations were regarded as clinically insignificant. The greatest number of clinically significant deviations was observed in the level of creatine phosphokinase and IgE. The results of electrocardiographic study before the beginning and after the completion of the administration of the preparations allow us to conclude that there was no effect of the administration of the studied vaccines on the work of the heart muscle, all results were within normal limits. Also, no development of vaccine-related neuro-

logic disorders was detected in vaccinated volunteers. Physical examination of the volunteers before vaccination and at each visit did not reveal any abnormalities in the health status of the vaccinated volunteers in any of the groups, except for cases associated with the manifestations of AEs.

### *Study of humoral immune response in the neutralization reaction*

At the screening in the research centers, all volunteers were negative in the rapid test for COVID-19, but antibodies were observed in ELISA and neutralization reactions. In the placebo group, GMT values fluctuated insignificantly, and an increase in GMT was observed in groups 1 and 2 against Wuhan, Delta and Omicron strains (**Fig. 2**).

Volunteers with low GMT ( $\leq 1/80$ ) before vaccination showed a significant increase in antibodies in the NR after vaccination (**Fig. 3**). In the presence of high titers ( $> 1/80$ ), lower rates of immunogenicity were observed in NR. In group 3, there was virtually no increase in antibody titers.

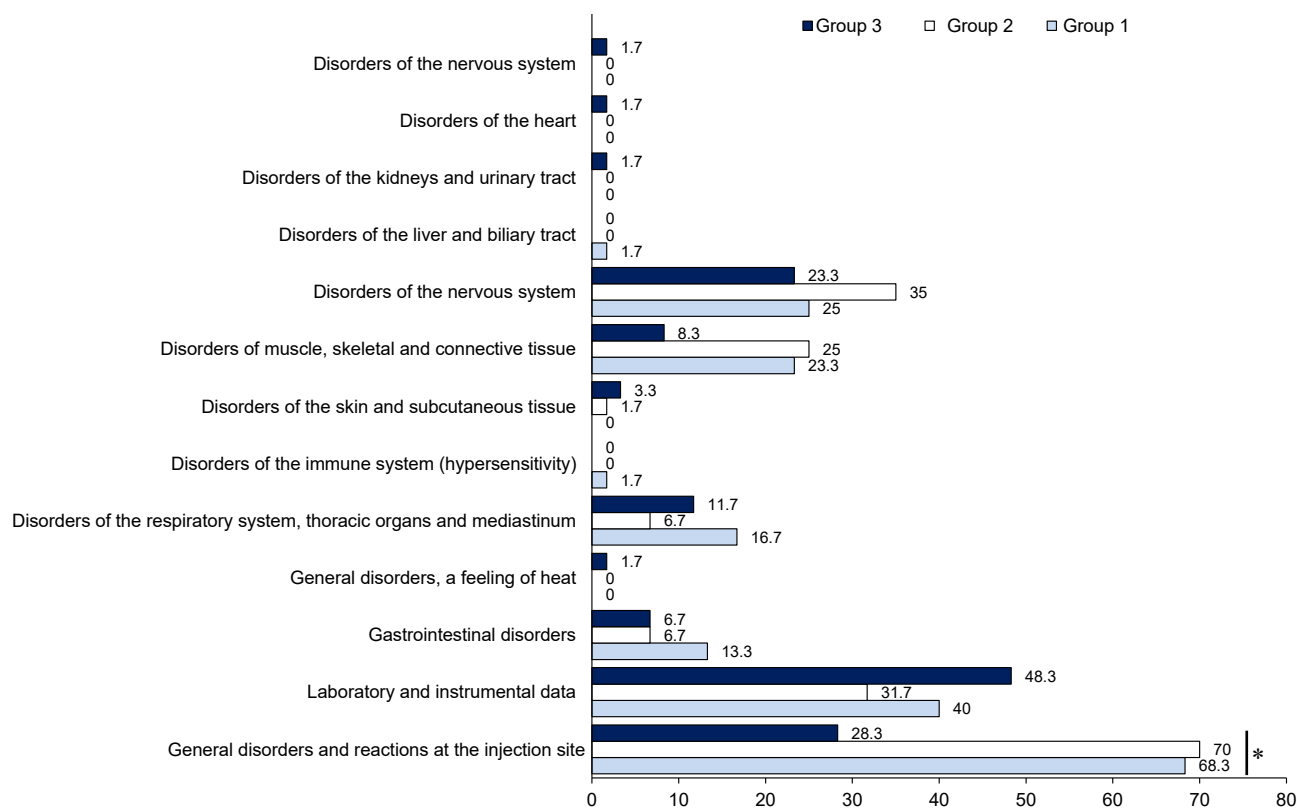
### *Study of humoral immune response by ELISA method*

In the study of humoral immune response in dynamics by ELISA method it was shown that in group 1 the increase of IgG titers to S-protein SARS-CoV-2 on the 21<sup>st</sup> day after a single injection was observed in 100% of volunteers, after a 4-fold injection — in 70%, GMT amounted to 1600, and on the 21<sup>st</sup> day after a double injection GMT already amounted to 2177.26, seroconversion level — 77.8% (**Fig. 4**). In group 2, on the 21<sup>st</sup> day after a single vaccine administration, IgG titers increased in 100% of volunteers, and after a 4-fold vaccine administration — in 86%, the GMT was 4306.88. After the 2<sup>nd</sup> administration of the vaccine with 80  $\mu$ g antigen content, the immunogenicity indices remained practically unchanged. In group 3, no increase in IgG titers was observed, and the GMT remained at the same level. The average indices of total IgA, IgM, IgG of volunteers during the dynamic observation underwent insignificant changes in all groups.

On the 21<sup>st</sup> day after the 1<sup>st</sup> vaccination, specific IgG to the S-protein of SARS-CoV-2 was detected in ELISA in all volunteers of group 1, with antibody titers of 1 : 400–1 : 12,800 and an GMT of 4201.57. The percentage of volunteers who showed an increase in antibody titer was 62.5%, of which those with

**Table 1.** Patient demographics,  $M \pm SD$

Groups	<i>n</i>	Age, years	Height, cm	Body mass, kg	Body mass index, kg/m <sup>2</sup>
All study subjects	180	29.91 $\pm$ 10.36	171.82 $\pm$ 8.21	68.02 $\pm$ 10.58	22.94 $\pm$ 2.50
Group 1	60	31.62 $\pm$ 12.00	172.18 $\pm$ 8.50	67.94 $\pm$ 9.76	22.84 $\pm$ 2.22
Group 2	60	26.58 $\pm$ 7.74	172.05 $\pm$ 8.82	66.97 $\pm$ 11.33	22.49 $\pm$ 2.55
Group 3	60	31.53 $\pm$ 10.25	171.22 $\pm$ 7.32	69.16 $\pm$ 10.66	23.49 $\pm$ 2.65



**Fig. 1.** Proportion of volunteers with AEs by organ system class.

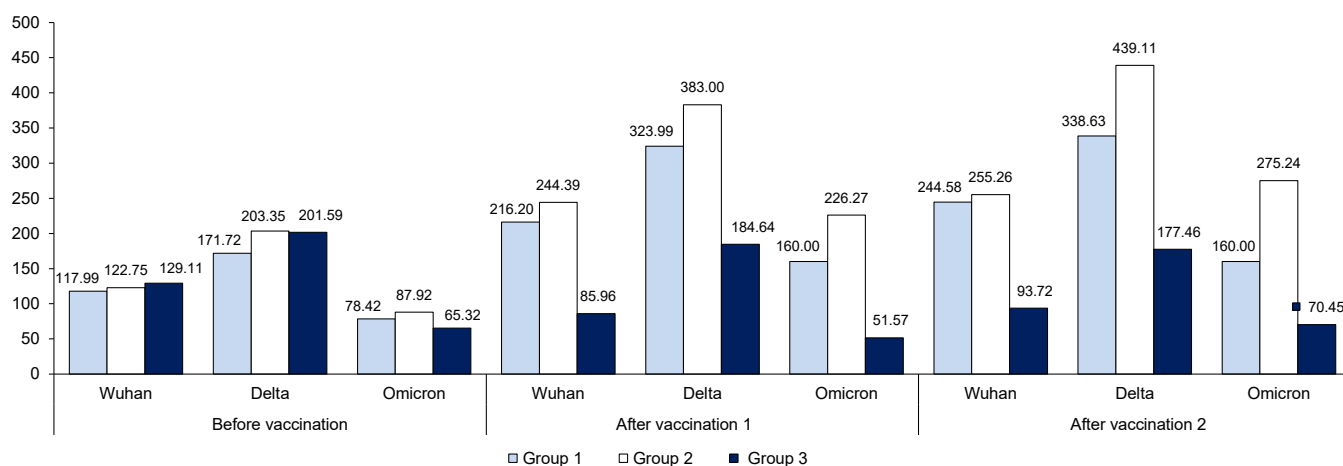
\* — statistically significant differences.

4-fold seroconversion were 19.6%. On the 21<sup>st</sup> day after the 2<sup>nd</sup> vaccination, the GMT was 4306.88 and the seroconversion rate was 20.4%. The multiplicity of increase in GMT relative to screening on the 21<sup>st</sup> day after the 1<sup>st</sup> vaccination was 2.1 and after the 2<sup>nd</sup> vaccination was 2.2.

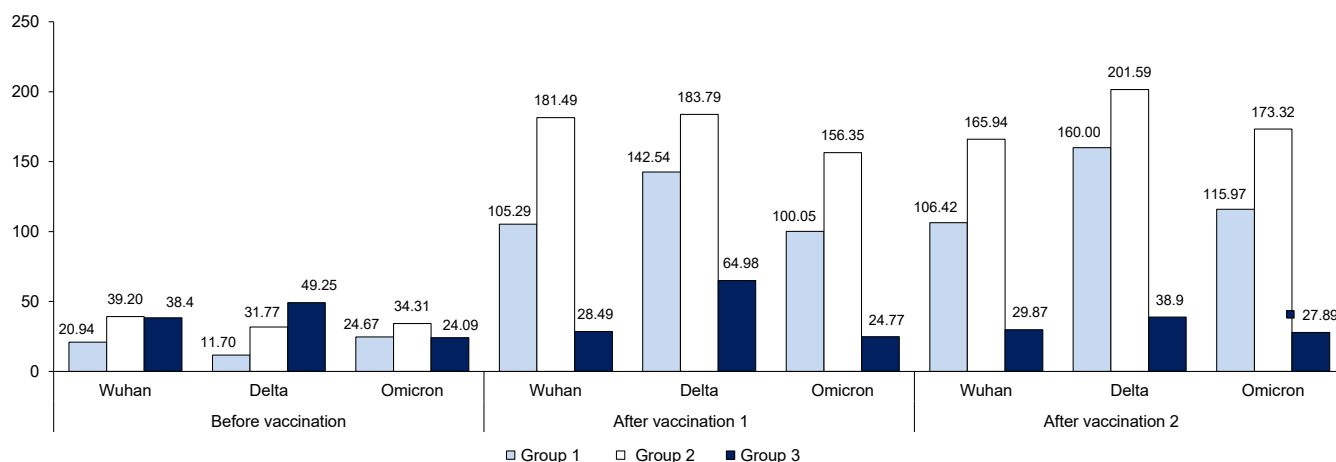
In group 2, on the 21<sup>st</sup> day after the 1<sup>st</sup> vaccination, specific IgG to S-protein SARS-CoV-2 was detected in ELISA in 100% of volunteers, with antibody titers of 1 : 1600–1 : 12 800, and the GMT was 4950.94. The proportion of volunteers with an increase in antibody

titer was 57.4%, including 14.8% with 4-fold seroconversion. On the 21<sup>st</sup> day after the 2<sup>nd</sup> vaccination, the GMT was 5261.46 and the seroconversion rate was 19.6%. The multiplicity of GMT increase relative to screening on the 21<sup>st</sup> day after the 1<sup>st</sup> vaccination was 1.7, after the 2<sup>nd</sup> vaccination — 1.8.

In group 3, specific IgG in ELISA on the 21<sup>st</sup> day after the 1<sup>st</sup> administration of placebo was detected in 91.7% of volunteers, with antibody titers of 0–1 : 12,800 and an GMT of 1241.36. The proportion of volunteers who showed an increase in antibody was 18.8%, with



**Fig. 2.** GMT of neutralizing antibodies in all volunteers.



**Fig. 3.** GMT of neutralizing antibodies in volunteers with baseline low antibody titers  $\leq 1/80$ .

4-fold seroconversion in 10.4%. On the 21<sup>st</sup> day after the 2<sup>nd</sup> administration, the GMT was 2311.60, with a seroconversion rate of 15.9%. At the same time, no COVID-19 cases were detected by PCR method.

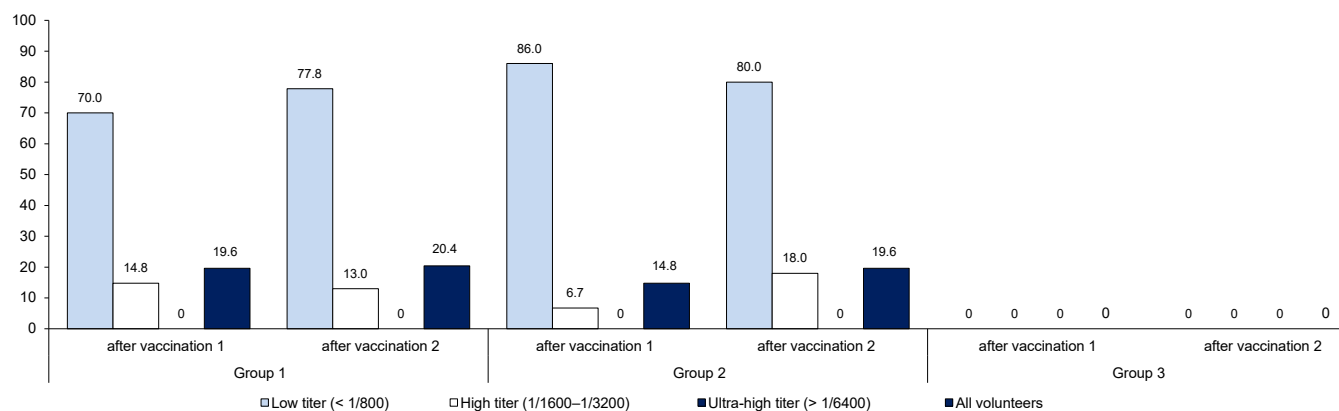
#### Assessment of cellular immunity

The studies were carried out initially (screening V), as well as on the 10<sup>th</sup> day after the 1<sup>st</sup> and 2<sup>nd</sup> vaccination. When assessing the dynamics of PSI as a result of immunization with VLP-vaccine, a significant ( $p < 0.05$ ) increase in the index in the studied groups compared to the screening was observed. As the number of vaccinations increased, a gradual increase in cellular response was noted ( $p < 0.05$ ). The mean PSI level reached a maximum value on the 10<sup>th</sup> day after the 2<sup>nd</sup> immunization with a vaccine containing 40  $\mu$ g antigen, per dose (2.17 [1.87; 3.05]) and with a vaccine containing 80  $\mu$ g antigen, per dose (2.57 [1.98; 3.17]). In LBTR, the number of stimulated lymphocytes was examined, and if they are stimulated by a specific stimulant, it may indicate a prior encounter with antigen, which is possible either post-vaccination or post-infection. In group 3, there were volunteers with very high PSI values, which may suggest post-infection stimulation,

as there were no such high PSI levels post-vaccination at any of the tested doses of antigen in the vaccine. Cellular and humoral immune response are not necessarily correlated. Both the presence of antibodies without the formation of cellular reactions and the absence of antibodies but with the formation of cellular immunity are frequently observed in COVID-19 convalescents. Therefore, despite the absence of seroconversion in the group of volunteers immunized with placebo, we cannot unequivocally state the absence of asymptomatic COVID-19 infection in volunteers with high levels of PSI with cellular response. It should be noted that such a high proliferative response to a specific antigen in the placebo group is only possible after an infection has occurred.

Examination of the CD4<sup>+</sup>/CD8<sup>+</sup>-lymphocyte ratio showed no abnormalities after vaccination with the VLP vaccine containing both 40  $\mu$ g and 80  $\mu$ g of antigen per dose, either toward helper or cytotoxic lymphocytes. This additional information about the absence of potential immunotoxicity can be observed after vaccination (according to ICH S8) (Table 2).

To study the immune status of volunteers, the dynamics of cytokine production during double immuni-



**Fig. 4.** Seroconversion rate at different study dates.

Y axis – proportion of volunteers with seroconversion (increase in specific antibody titer  $\geq 4$  times), %.

zation was also investigated. Sera from patients were taken before immunization and on the 10<sup>th</sup> day after the 1<sup>st</sup> and 2<sup>nd</sup> immunization (**Fig. 5**).

Analysis of statistical differences showed that in group 1, for the tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) concentration, there was a statistically significant difference between the screening visit (Me = 4.6) and visit 4 (Me = 7.81). No significant differences were found for the rest of the data.

The results show that the developed VLP vaccine, containing both 40 and 80  $\mu$ g of antigen per dose, can be an inducer of cell-mediated immune response, in which the CD4<sup>+</sup>/CD8<sup>+</sup>-lymphocyte ratio is not changed either toward helper or cytotoxic lymphocytes. And post-vaccination changes in cytokine levels are not sufficient for the development of immunopathologic conditions associated with excessive production of the proinflammatory cytokines under study.

## Discussion

Inactivated and live vaccines are the most widely used for the prevention of infectious diseases. However, the emergence and spread of new infections, the variability of their causative agents and the recent pandemic that affected everyone show the need to develop and improve means of specific therapy and prophylaxis. In parallel with the development of mRNA-based, viral vector-based, and subunit vaccine production technology, the technology of VLP production is gaining popularity, which represents an alternative platform for vaccine development. The advantage of such a platform is the possibility of creating multivalent vaccines that are capable of inducing humoral and cellular immune response with the production of virus-neutralizing antibodies of a broad spectrum. This is important in the development of vaccines for the prevention of infections

caused by viruses with high genetic variability, such as SARS-CoV-2. At the same time, the absence of genetic material in VLP-vaccines can guarantee an increased level of safety, which is confirmed by preclinical studies [13–15].

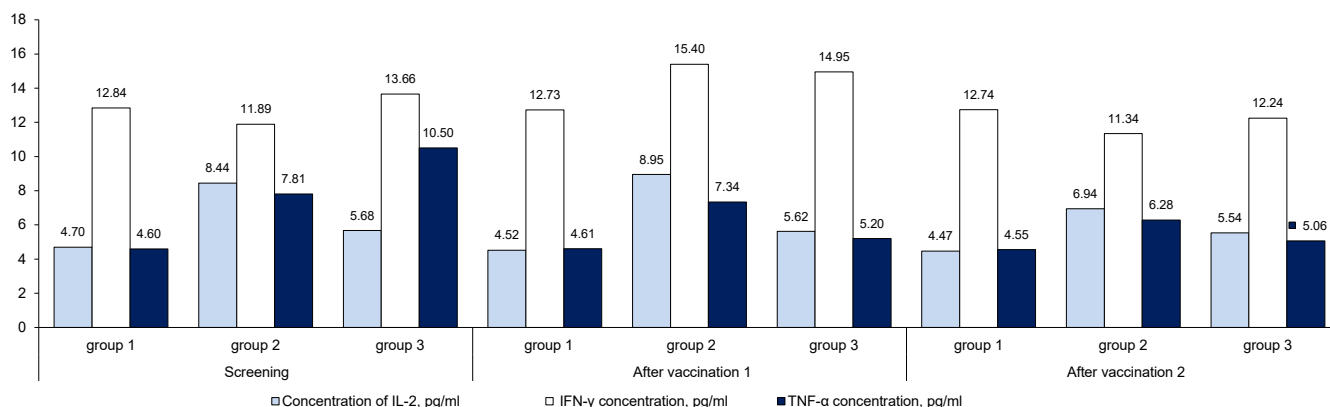
In the present study, the tolerability, safety and immunogenicity of a new vaccine against coronavirus infection caused by SARS-CoV-2 were investigated in phase I clinical trials in healthy volunteers. The drug is purified recombinant SARS-CoV-2 VLPs that are synthesized in a baculovirus expression system. The surface S-protein within the VLP is represented by variants 19A, Alpha, Delta and Omicron. The vaccine contains a squalene-based adjuvant.

The main objectives were to assess the tolerability, safety and immunogenicity of the vaccine compared to placebo for 21 days after double intramuscular administration, as well as to determine the optimal dose of antigen for further study of the safety and efficacy of the drug in phase II clinical trials.

When developing vaccines, including those for COVID-19 prophylaxis, special attention is paid to studies of safety, non-reactogenicity and tolerability of new vaccines, as these indicators directly affect the possibility of widespread use of these drugs and the level of public confidence in vaccination. In particular, studies of injectable forms of a number of mRNA vaccines from COVID-19 have revealed both local reactions, such as pain at the injection site, and serious adverse reactions, and some publications on vaccine safety have been withdrawn by journal editors despite the authors' objections. VLP-based vaccines have proven to be highly effective and safe, as demonstrated by vaccines against human papillomavirus (Gardasil, Gardasil9, Cervarix), hepatitis E (Hecolin) hepatitis B (Sci-B-Vac) and malaria (Mosquirix) [16–18].

**Table 2.** PSI data and CD4<sup>+</sup>/CD8<sup>+</sup> ratios in the studied groups, Me [Q<sub>1</sub>; Q<sub>3</sub>]

No.	Immunization	Indicator	Group 1	Group 2	Group 3
		<i>n</i>	60	60	60
1	Before vaccination	PSI	1.4 [1.15; 1.69]	1.43 [1.07; 1.83]	1.25 [1.11; 1.59]
		CD4 <sup>+</sup> /CD8 <sup>+</sup>	2.15 [1.71; 2.81]	2.44 [1.77; 2.97]	2.7 [1.97; 3.25]
		<i>n</i>	54	52	49
2	10 <sup>th</sup> day after the 1 <sup>st</sup> vaccination	PSI	1.75 [1.31; 2.21]	2.06 [1.51; 2.3]	1.59 [1.22; 2.15]
		CD4 <sup>+</sup> /CD8 <sup>+</sup>	2.08 [1.65; 3.38]	2.23 [1.61; 3.38]	2.86 [2.06; 4.23]
		<i>n</i>	47	49	45
3	10 <sup>th</sup> day after the 2 <sup>nd</sup> vaccination	PSI	2.17 [1.87; 3.05]	2.57 [1.98; 3.17]	2.17 [1.45; 2.79]
		CD4 <sup>+</sup> /CD8 <sup>+</sup>	3.3 [2.2; 4.37]	3.18 [2.28; 4.42]	4.15 [2.51; 5.09]
Post-hoc analysis of PSI adjusted for multiple comparisons			<i>p</i> <sub>1-2</sub> = 0.020	<i>p</i> <sub>1-2</sub> = 0.318	<i>p</i> <sub>1-2</sub> = 0.195
			<i>p</i> <sub>1-3</sub> < 0.0001	<i>p</i> <sub>1-3</sub> < 0.0001	<i>p</i> <sub>1-3</sub> < 0.0001
			<i>p</i> <sub>2-3</sub> = 0.020	<i>p</i> <sub>2-3</sub> = 0.004	<i>p</i> <sub>2-3</sub> = 0.006



**Fig. 5.** Dynamics of cytokine levels in blood serum of volunteers.

According to the results of the presented study, AEs were observed both in volunteers vaccinated with the vaccine with 40 and 80 µg of antigen per dose and in the group immunized with placebo. At the same time, the majority of AEs were registered during the first 10 days after vaccination, mainly after the 1<sup>st</sup> vaccination. In the period from the 11<sup>th</sup> to the 21<sup>st</sup> day after vaccination, there were only a few cases of AEs.

The majority of the reported AEs with a “probable” and “possible” relationship were related to expected adverse events after immunization to the effect of the vaccine preparation. AEs of particular interest associated with COVID-19 vaccination (Guillain–Barré syndrome, generalized seizures, anaphylaxis, thrombocytopenia, coagulopathy, etc.) were not reported in any volunteers.

The detected fluctuations in the mean values of clinical blood and urine indices before vaccination and at different periods after vaccination do not allow us to speak about the influence of vaccination on these indices and can be explained by random factors, restructuring of the immune system of the body of the vaccinated in response to the introduction of antigen.

The average values of vital function indices in the study group were within the normal range, and the changes in these parameters according to the results of measurements after the beginning of the drug administration, compared to the initial values, were insignificant and within the reference values.

Significant changes in individual laboratory parameters relative to screening were observed in all study groups. Importantly, these abnormalities occurred both in volunteers who received the study vaccine at both doses and in the group of volunteers who received placebo. The absence of serious vaccine-related AEs or deaths during the study suggests good tolerability and safety of the vaccine with both 40 mcg and 80 mcg of antigen per dose.

Researchers at Radboud University Medical Center in Nijmegen, the Netherlands, conducted a single-center clinical trial with dose-matched adjuvant ABNCov2

vaccine based on VLP or capsid-like particles (cVLP). The RBD of the spike glycoprotein SARS-CoV-2 glycoprotein was covalently attached to the cVLP carrier. Forty-five healthy volunteers, aged 18–55 years, who were immunized intramuscularly, twice, were studied. Participants had a total of 249 possibly vaccine-related NIs within a week of vaccination (185 grade 1; 63 grade 2; 1 grade 3). Two serious events occurred; one was classified as a possible adverse reaction [19].

VLPs of the developed vaccine are capable of exerting a strong immunostimulatory effect on the organism, activating T- and B-lymphocytes, as they contain the main immunogenic proteins SARS-CoV-2 in native conformation. They easily penetrate into lymph nodes and are taken up by antigen-presenting cells, in particular dendritic cells, with subsequent antigen presentation by molecules of the major histocompatibility complex class II [19, 20].

Studies of immunogenicity of the developed vaccine against SARS-CoV-2 in the framework of phase I clinical trials showed that administration of the vaccine containing 40 and 80 µg of antigen to volunteers induced a significant increase in GMT compared to placebo. At the same time, the immune response was stronger in volunteers who initially had low antibody titers (NR — titer ≤ 1/80, ELISA — titer ≤ 1/800). When volunteers had high antibody titers (1/1600–1/3200 and ≥ 1/6400 in ELISA) at screening, these immunogenicity rates were lower and no seroconversion was observed. Of note, virally neutralizing antibodies were produced to various SARS-CoV-2 strains, including clades 19A, Delta, and Omicron. In group 3, an increase in antibody titers was noted in a number of volunteers, but no 4-fold increase was observed. It should be noted that COVID-19 was not detected in those included in the study.

In group 2, the immunogenicity indices in ELISA were insignificantly but superior to both the values in the general population and in volunteers with initially low and high antibody titers. Indicators of immune response intensity, antibody titers increase, including 4-fold, in NR were also higher in group 2, especially



for the current and predominant Omicron strain. Thus, in this group the GMT was 275.24, a multiple of 3.1, while in group 1 the GMT was 160, a multiple of 2.0.

High indices of immune response in group 3 most likely indicate a COVID-19 disease in a latent form, which did not manifest itself clinically and was not confirmed by PCR-test. It is important to note that this study was conducted at the height of the epidemic, and the main infectious agent at that time was the Omicron strain.

The insufficient sensitivity of some commercial ELISA test systems for detecting antibodies to SARS-CoV-2 should also be noted [21]. For example, in influenza, antibodies appear in 50–80% of unvaccinated adults without signs of disease [22]. A number of researchers note that up to 80% of COVID-19 infections may be asymptomatic [23, 24].

Researchers from the Netherlands studied the immune response of 45 volunteers who were immunized with ABNCoV2 vaccine based on cVLP with different amounts of antigen: 6, 12, 25, 50 or 70 µg [19]. A dose-dependent antibody formation was observed after the 2<sup>nd</sup> vaccination when immunized with vaccines containing 25–70 µg of antigen. Antibodies neutralized the major SARS-CoV-2 variants, but viral neutralizing activity was lower with the Omicron variant (BA.1), specific interferon-γ (IFN-γ)<sup>+</sup>CD4<sup>+</sup> T cells were activated. The researchers' overall conclusion: immunization with the vaccine was well-tolerated, safe and resulted in a functional immune response.

SARS-CoV-2 infection induces immune responses that may have important implications for the development of vaccination strategies. T-cell immunity plays a central role in the control of SARS-CoV-2 infection. Antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells and neutralizing antibodies play a protective role against SARS-CoV-2, whereas impairment of the adaptive immune response, namely a lack of naive T cells, can lead to adverse disease outcomes.

In the study of the immune response, it is important to assess the degree of cytokine imbalance and immune cell activation. Antigen mimicry between viral and human proteins can lead to the development of immune-mediated hemolysis, decreased leukocyte counts, cytokine storm, procoagulant state, and macrophage activation [25]. The synthesis of cytokines IFN-γ, interleukin-2, and TNF-α accounts for the Th1-type immune response [26]. IFN-γ and interleukin-2 activate macro-

phages, natural killer cells, and cytotoxic lymphocytes, which are crucial for virus elimination. IFN-γ is the most potent factor in macrophage activation. Full activation of macrophages can be achieved by low levels of IFN-γ. In vaccine development, it is important to avoid toxicity associated with its over-activation.

Abnormal stimulation of T cells and antigen-presenting cells (dendritic cells, macrophages and B cells) can lead to the development of a cytokine storm generated by suppressive release of cytokines, particularly TNF-α, which promotes migration of neutrophils from vessels and activation of clotting pathways. Hyperinflammatory reactions correlate with increased levels of serum interleukin-2, -6 and -7 [27, 28].

The developed VLP-vaccine can be an inducer of cell-mediated immune response, in which the CD4<sup>+</sup>/CD8<sup>+</sup>-lymphocyte ratio does not change either toward helper or cytotoxic lymphocytes. At the same time, the post-vaccination change in cytokine levels is not sufficient for the development of immunopathologic conditions associated with excessive production of the proinflammatory cytokines under study.

When the developed vaccine was used, antibody formation and cellular response increased markedly as the number of immunizations increased. However, the immunogenicity parameters obtained after immunization with the vaccine containing 80 µg antigen per dose were superior to those obtained after immunization with the vaccine containing 40 µg antigen per dose (especially for the Omicron strain); therefore, this dose was selected as the optimal dose for phase I clinical trials.

## Conclusion

The tolerability, safety and immunogenicity of a new vaccine for COVID-19 prophylaxis based on VLP within the framework of phase I clinical trials on 180 volunteers were evaluated. It has been shown that vaccination with preparations containing 40 and 80 µg of antigen in the dose intramuscularly twice with an interval of 21 days does not cause serious AEs and induces humoral and cellular immune response. The CD4<sup>+</sup>/CD8<sup>+</sup>-lymphocyte ratio does not change either toward helper or cytotoxic lymphocytes. Post-vaccination changes in cytokine levels are not sufficient for the development of immunopathologic conditions associated with excessive production of the proinflammatory cytokines under study.



## REFERENCES

- Chen N., Zhou M., Dong X., et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395(10223):507–13. DOI: [https://doi.org/10.1016/s0140-6736\(20\)30211-7](https://doi.org/10.1016/s0140-6736(20)30211-7)
- Uskov A.N., Lobzin Yu.V., Rychkova S.V., et al. Course of a new coronavirus infection in children: some aspects of monitoring and analysis of mortality. *Journal Infectology*. 2020;12(3):12–20. DOI: <https://doi.org/10.22625/2072-6732-2020-12-3-12-20> EDN: <https://elibrary.ru/jyuxsy>
- Garafutdinov R.R., Mavzyutov A.R., Nikonov Yu.M., et al. Betacoronavirus SARS-CoV-2, its genome, variety of genotypes and molecular-biological approaches to combat it. *Biom-ics*. 2020;12(2):242–71. DOI: <https://doi.org/10.31301/2221-6197.bmcs.2020-15> EDN: <https://elibrary.ru/dhderx>
- Gorenkov D.V., Khantimirova L.M., Shevtsov V.A., et al. An outbreak of a new infectious disease COVID-19:  $\beta$ -coronaviruses as a threat to global healthcare. *Biological Products. Prevention, Diagnosis, Treatment*. 2020;20(1):6–20. DOI: <https://doi.org/10.30895/2221-996X-2020-20-1-6-20> EDN: <https://elibrary.ru/euulmy>
- Romanov B.K. Coronavirus disease COVID-19. *Safety and Risk of Pharmacotherapy*. 2020;8(1):3–8. DOI: <https://doi.org/10.30895/2312-7821-2020-8-1-3-8> EDN: <https://elibrary.ru/vzvbrk>
- Nikiforov V.V., Suranova T.G., Chernobrovkina T.Ya., et al. New coronavirus infection (COVID-19): Clinical and epidemiological aspects. *Archive of Internal Medicine*. 2020;10(2):87–93. DOI: <https://doi.org/10.20514/2226-6704-2020-10-2-87-93> EDN: <https://elibrary.ru/melbop>
- Tregoning J.S., Brown E.S., Cheeseman H.M., et al. Vaccines for COVID-19. *Clin. Exp. Immunol.* 2020;202(2):162–92. DOI: <https://doi.org/10.1111/cei.13517>
- Ozhmegova E.N., Savochkina T.E., Prilipov A.G., et al. Molecular epidemiological analysis of SARS-CoV-2 genovariants in Moscow and Moscow region. *Problems of Virology*. 2022;67(6):496–505. DOI: <https://doi.org/10.36233/0507-4088-146> EDN: <https://elibrary.ru/ergiwk>
- Ghafouri F., Cohan R.A., Noorbakhsh F., et al. An in-silico approach to develop of a multi-epitope vaccine candidate against SARS-CoV-2 envelope (E) protein. *Res. Sq.* 2020. Preprint. DOI: <https://doi.org/10.21203/rs.3.rs-30374/v1>
- Ayyagari V.S., Venkateswarulu T.C., Abraham Peele K., Sri-rama K. Design of a multi-epitope-based vaccine targeting M-protein of SARS-CoV-2: an immunoinformatics approach. *J. Biomol. Struct. Dyn.* 2022;40(7):2963–77. DOI: <https://doi.org/10.1080/07391102.2020.1850357>
- Gupta T., Gupta S.K. Potential adjuvants for the development of a SARS-CoV-2 vaccine based on experimental results from similar coronaviruses. *Int. Immunopharmacol.* 2020;86:106717. DOI: <https://doi.org/10.1016/j.intimp.2020.106717>
- Latyshev O.E., Zaykova O.N., Eliseeva O.V., et al. Development, production and characterization of SARS-CoV-2 virus-like particles (Coronaviridae: Orthocoronavirinae: Betacoronavirus: Sarbecovirus). *Problems of Virology*. 2024;69(2):175–86. DOI: <https://doi.org/10.36233/0507-4088-226> EDN: <https://elibrary.ru/gkxfed>
- Banihashemi S.R., Es-Haghi A., Fallah Mehrabadi M.H., et al. Safety and efficacy of combined intramuscular/intranasal RAZI-COV PARS vaccine candidate against SARS-CoV-2: a preclinical study in several animal models. *Front. Immunol.* 2022;13:836745. DOI: <https://doi.org/10.3389/fimmu.2022.836745>
- Vakhrusheva A.V., Kudriavtsev A.V., Kryuchkov N.A., et al. SARS-CoV-2 subunit virus-like vaccine demonstrates high safety profile and protective efficacy: Preclinical study. *Vaccines (Basel)*. 2022;10(8):1290. DOI: <https://doi.org/10.3390/vaccines10081290>
- Chernoryzh Ya.Y., Kondratieva V.M., Malkova A.P., et al. Pre-clinical safety studies of intranasal virus-like particles based vaccine for prevention of COVID-19. *Problems of Virology*. 2025;70(1):35–46. DOI: <https://doi.org/10.36233/0507-4088-278> EDN: <https://elibrary.ru/fzgyxe>
- Yang X., Chen M., Cao L., Zhao M. Bibliometric analysis of scientific papers on adverse reactions to COVID-19 vaccines published between 2019 and 2023. *Hum. Vaccin. Immunother.* 2023;19(3):2270194. DOI: <https://doi.org/10.1080/21645515.2023.2270194>
- Kombe Kombe A.J., Li B., Zahid A., et al. Epidemiology and burden of human papillomavirus and related diseases, molecular pathogenesis, and vaccine evaluation. *Front. Public Health*. 2021;8:552028. DOI: <https://doi.org/10.3389/fpubh.2020.552028>
- Nooraei S., Bahrulolum H., Hoseini Z.S., et al. Virus-like particles: preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers. *J. Nanobiotechnology*. 2021;19(1):59. DOI: <https://doi.org/10.1186/s12951-021-00806-7>
- Smit M.J., Sander A.F., Ariaans M.B.P.A., et al. First-in-human use of a modular capsid virus-like vaccine platform: an open-label, non-randomised, phase 1 clinical trial of the SARS-CoV-2 vaccine ABNCoV2. *Lancet Microbe*. 2023;4(3):e140–8. DOI: [https://doi.org/10.1016/s2666-5247\(22\)00337-8](https://doi.org/10.1016/s2666-5247(22)00337-8)
- Tariq H., Batool S., Asif S., et al. Virus-like particles: revolutionary platforms for developing vaccines against emerging infectious diseases. *Front. Microbiol.* 2022;12:790121. DOI: <https://doi.org/10.3389/fmicb.2021.790121>
- Okba N.M.A., Müller M.A., Li W., et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients. *Emerg. Infect. Dis.* 2020;26(7):1478–88. DOI: <https://doi.org/10.3201/eid2607.200841>
- Medunitsyn N.V., Olefir Yu.V., Merkulov V.A., Bondarev V.P. Vaccination contribute to the development of personal and herd immunity. *Biological Products. Prevention, Diagnosis, Treatment*. 2016;16(4):195–207. EDN: <https://elibrary.ru/xehmax>
- Krotkova E.N., Kuznetsov O.E., Gorchakova O.V. Assessment of population immunity to the SARS-CoV-2 virus among the population of Grodno. *Journal of the Grodno State Medical University*. 2021;19(5):489–95. DOI: <https://doi.org/10.25298/2221-8785-2021-19-5-489-495>
- Lai C.C., Liu Y.H., Wang C.Y., et al. Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Facts and myths. *J. Microbiol. Immunol. Infect.* 2020;53(3):404–12. DOI: <https://doi.org/10.1016/j.jmii.2020.02.012>
- Liu Y., Sawalha A.H., Lu Q. COVID-19 and autoimmune diseases. *Curr. Opin. Rheumatol.* 2021;33(2):155–62. DOI: <https://doi.org/10.1097/bor.0000000000000776>
- Mesentseva M.V., Antoshina I.F., Morozova O.V. The cytokines synthesis *in vitro* in the tick-borne encephalitis virus infected cells and in the presence of inactivated vaccine. *Russian Journal of Infection and Immunity*. 2014;4(1):37–42. EDN: <https://elibrary.ru/rzjgcz>
- Landete P., Quezada Loaiza C.A., Aldave-Orzaiz B., et al. Clinical features and radiological manifestations of COVID-19 disease. *World. J. Radiol.* 2020;12(11):247–60. DOI: <https://doi.org/10.4329/wjr.v12.i11.247>
- Luo X.H., Zhu Y., Mao J., Du R.C. T cell immunobiology and cytokine storm of COVID-19. *Scand. J. Immunol.* 2021;93(3):e12989. DOI: <https://doi.org/10.1111/sji.12989>

### Information about the authors

**Tatiana V. Grebennikova** — Dr. Sci. (Biol.), Professor, Corresponding Member of RAS, Head, Laboratory of molecular diagnostics, Head, Deputy Director for scientific work, D.I. Ivanovsky Institute of Virology, Head, Testing Center, NRCEM named after N.F. Gamaleya, Moscow, Russia, [t\\_grebennikova@mail.ru](mailto:t_grebennikova@mail.ru), <https://orcid.org/0000-0002-6141-9361>

**Olga N. Zaykova** — Cand. Sci. (Biol.), senior researcher, Laboratory of molecular diagnostics, researcher, Diagnostic and Prevention Research institute for Human and Animal Diseases, NRCEM named after N.F. Gamaleya, Moscow, Russia, [zaykova\\_o\\_n@mail.ru](mailto:zaykova_o_n@mail.ru), <https://orcid.org/0000-0003-4708-2069>

**Alexey A. Plotnikov** — postgraduate student, junior researcher, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [alesp@ya.ru](mailto:alesp@ya.ru), <https://orcid.org/0009-0009-1253-1152>

**Lyudmila V. Kostina** — Cand. Sci. (Biol.), senior researcher, Laboratory of molecular diagnostics, researcher, Diagnostic and Prevention Research institute for Human and Animal Diseases, NRCEM named after N.F. Gamaleya, Moscow, Russia, [lvkostina@mail.ru](mailto:lvkostina@mail.ru), <https://orcid.org/0000-0002-9556-1454>

**Yana Yu. Chernoryzh** — Cand. Sci. (Med.), researcher, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [revengeful\\_w@mail.ru](mailto:revengeful_w@mail.ru), <https://orcid.org/0000-0001-9848-8515>

**Olesia V. Eliseeva** — Cand. Sci. (Biol.), senior researcher, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [olesenka80@mail.ru](mailto:olesenka80@mail.ru), <https://orcid.org/0000-0002-0723-9749>

**Oleg E. Latyshev** — Cand. Sci. (Biol.), senior researcher, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [oleglat80@mail.ru](mailto:oleglat80@mail.ru), <https://orcid.org/0000-0002-5757-3809>

**Viktor F. Larichev** — Dr. Sci. (Med.), leading researcher, Laboratory of biology and indication of arboviruses, NRCEM named after N.F. Gamaleya, Moscow, Russia, [vlaritchev@mail.ru](mailto:vlaritchev@mail.ru), <https://orcid.org/0000-0001-8262-5650>

**Irina T. Fedyakina** — Cand. Sci. (Biol.), Head, Laboratory of virus ecology, leading researcher, Department of virus ecology, NRCEM named after N.F. Gamaleya, Moscow, Russia, [irfed2@mail.ru](mailto:irfed2@mail.ru), <https://orcid.org/0000-0001-6421-9632>

**Milana A. Losich** — Cand. Sci. (Biol.), senior researcher, Laboratory of molecular diagnostics, Group of comparative virology, NRCEM named after N.F. Gamaleya, Moscow, Russia, [mkohnovich@rambler.ru](mailto:mkohnovich@rambler.ru), <https://orcid.org/0000-0002-5618-1918>

**Ilya M. Kirillov** — senior researcher, Laboratory of virus ecology, NRCEM named after N.F. Gamaleya, Moscow, Russia, [iliyakirillov@yandex.ru](mailto:iliyakirillov@yandex.ru), <https://orcid.org/0000-0002-4933-850X>

**Ilya E. Filatov** — junior researcher, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [filat69rus@yandex.ru](mailto:filat69rus@yandex.ru), <https://orcid.org/0000-0001-5274-224X>

**Marina V. Balandina** — Cand. Sci. (Biol.), senior researcher, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [mbalandina77@mail.ru](mailto:mbalandina77@mail.ru), <https://orcid.org/0009-0002-8179-1379>

**Valery V. Tsibezov** — Cand. Sci. (Biol.), leading researcher, Laboratory of specific means of prevention of viral diseases, NRCEM named after N.F. Gamaleya, Moscow, Russia, [tsibezov@yandex.ru](mailto:tsibezov@yandex.ru), <https://orcid.org/0000-0003-2150-5764>

**Kirill I. Yurlov** — researcher, Laboratory of cellular engineering, NRCEM named after N.F. Gamaleya, Moscow, Russia, [kir34292@yandex.ru](mailto:kir34292@yandex.ru), <https://orcid.org/0000-0002-4694-2445>

**Ekaterina I. Lesnova** — researcher, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [wolf252006@yandex.ru](mailto:wolf252006@yandex.ru), <https://orcid.org/0000-0002-2801-6843>

**Valeria M. Kondratieva** — postgraduate student, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [1999valeriak@mail.ru](mailto:1999valeriak@mail.ru), <https://orcid.org/0000-0001-9163-4516>

**Alina A. Kozlova** — Cand. Sci. (Biol.), researcher, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [malinkakozlova88@gmail.com](mailto:malinkakozlova88@gmail.com), <https://orcid.org/0000-0003-2749-3258>

**Marina S. Baranets** — Cand. Sci. (Med.), researcher, Laboratory of molecular diagnostics, NRCEM named after N.F. Gamaleya, Moscow, Russia, [shizotorex@mail.ru](mailto:shizotorex@mail.ru), <https://orcid.org/0000-0002-3466-3588>

**Aleksandr L. Gintsburg** — Dr. Sci. (Biol.), Professor, Academician RAS, Director, NRCEM named after N.F. Gamaleya, Moscow, Russia, [gintsburg@gamaleya.org](mailto:gintsburg@gamaleya.org), <https://orcid.org/0000-0003-1769-5059>

**Информация об авторах**

**Гребенникова Татьяна Владимировна**<sup>✉</sup> — д-р биол. наук, профессор, чл.-корр. РАН, зав. лаб. молекулярной диагностики, зам. директора по научной работе Института вирусологии им. Д.И. Ивановского, руководитель Испытательного центра НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [t\\_grebennikova@mail.ru](mailto:t_grebennikova@mail.ru), <https://orcid.org/0000-0002-6141-9361>

**Зайкова Ольга Николаевна** — канд. биол. наук, с. н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [zaykova\\_o\\_n@mail.ru](mailto:zaykova_o_n@mail.ru), <https://orcid.org/0000-0003-4708-2069>

**Плотников Алексей Андреевич** — аспирант, м. н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [alesp@ya.ru](mailto:alesp@ya.ru), <https://orcid.org/0009-0009-1253-1152>

**Костина Людмила Владимировна** — канд. биол. наук, с. н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [lvkostina@mail.ru](mailto:lvkostina@mail.ru), <https://orcid.org/0000-0002-9556-1454>

**Чернорыж Яна Юрьевна** — канд. мед. наук, н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [revengeful\\_w@mail.ru](mailto:revengeful_w@mail.ru), <https://orcid.org/0000-0001-9848-8515>

**Елисеева Олеся Васильевна** — канд. биол. наук, с. н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [olesenka80@mail.ru](mailto:olesenka80@mail.ru), <https://orcid.org/0000-0002-0723-9749>

**Латышев Олег Евгеньевич** — канд. биол. наук, с. н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [oleglat80@mail.ru](mailto:oleglat80@mail.ru), <https://orcid.org/0000-0002-5757-3809>

**Ларищев Виктор Филиппович** — д-р мед. наук, в. н. с. лаб. биологии и индикации арбовирусов НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [vlaritchev@mail.ru](mailto:vlaritchev@mail.ru), <https://orcid.org/0000-0001-8262-5650>

**Федякина Ирина Тимофеевна** — канд. биол. наук, зав. лаб. экологии вирусов, в. н. с. отдела экологии вирусов НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [irfed2@mail.ru](mailto:irfed2@mail.ru), <https://orcid.org/0000-0001-6421-9632>

**Лосич Милана Анатольевна** — канд. биол. наук, с. н. с. лаб. молекулярной диагностики группы сравнительной вирусологии НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [mkohnovich@rambler.ru](mailto:mkohnovich@rambler.ru), <https://orcid.org/0000-0002-5618-1918>

**Кириллов Илья Михайлович** — с. н. с. лаб. экологии вирусов НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [iliyakirillov@yandex.ru](mailto:iliyakirillov@yandex.ru), <https://orcid.org/0000-0002-4933-850X>

**Филатов Илья Евгеньевич** — м. н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [filat69rus@yandex.ru](mailto:filat69rus@yandex.ru), <https://orcid.org/0000-0001-5274-224X>

**Баландина Марина Владимировна** — канд. биол. наук, с. н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [mbalandina77@mail.ru](mailto:mbalandina77@mail.ru), <https://orcid.org/0009-0002-8179-1379>

**Цибезов Валерий Владимирович** — канд. биол. наук, в. н. с. лаб. средств специфической профилактики вирусных болезней НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [tsibezov@yandex.ru](mailto:tsibezov@yandex.ru), <https://orcid.org/0000-0003-2150-5764>

**Юрлов Кирилл Иванович** — н. с. лаб. клеточной инженерии НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [kir34292@yandex.ru](mailto:kir34292@yandex.ru), <https://orcid.org/0000-0002-4694-2445>

**Леснова Екатерина Ивановна** — н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [wolf252006@yandex.ru](mailto:wolf252006@yandex.ru), <https://orcid.org/0000-0002-2801-6843>

**Кондратьева Валерия Михайловна** — аспирант лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [1999valeriak@mail.ru](mailto:1999valeriak@mail.ru), <https://orcid.org/0000-0001-9163-4516>

**Козлова Алина Александровна** — канд. биол. наук, н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [malinkakozlova88@gmail.com](mailto:malinkakozlova88@gmail.com), <https://orcid.org/0000-0003-2749-3258>

**Баранец Марина Сергеевна** — канд. мед. наук, н. с. лаб. молекулярной диагностики НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [shizotorex@mail.ru](mailto:shizotorex@mail.ru), <https://orcid.org/0000-0002-3466-3588>

**Гинцбург Александр Леонидович** — д-р биол. наук, профессор, академик РАН, директор НИЦЭМ им. Н.Ф. Гамалеи, Москва, Россия, [gintsburg@gamaleya.org](mailto:gintsburg@gamaleya.org), <https://orcid.org/0000-0003-1769-5059>

**Authors' contribution:** *Grebennikova T.V.* — study concept and design, data analysis and interpretation, text editing, preparation of the final version of the article for publication; *Zaykova O.N.* — cell culture processing, article writing, results presentation; *Plotnikov A.A.* — clinical trial monitoring, results presentation; *Kostina L.V.* — cell culture processing, drug production and characterization; *Chernoryzh Ya.Yu.* — cellular immunity study, statistical analysis; *Eli-seeva O.V.* — quality control of the obtained drug; *Latyshev O.E.* — drug production and characterization; *Larichev V.F., Fedyakina I.T.* — immunogenicity study in the neutralization reaction; *Losich M.A.* — cell culture processing, drug production and characterization; *Kirillov I.M.* — vaccine antigen purification; *Filatov I.E.* — immunogenicity study in the ELISA reaction (specific IgG); *Balandina M.V.* — cytokine production dynamics study; *Tsibezov V.V.* — study of immunogenicity in ELISA reaction, data interpretation; *Yurlov K.I.* — study of cellular immunity; *Lesnova E.I., Kondratieva V.M.* — study of cellular immunity; *Kozlova A.A., Baranets M.S.* — study of immunogenicity in neutralization reaction; *Ginzburg A.L.* — organizational aspect of clinical trials. All authors confirm that they meet the International Committee of Medical Journal Editors criteria for authorship, made a substantial contribution to the conception of the article, acquisition, analysis, interpretation of data for the article, drafting and revising the article, final approval of the version to be published.

The article was submitted 20.02.2025;  
accepted for publication 21.04.2025;  
published 28.04.2025

**Участие авторов:** *Гребенникова Т.В.* — концепция и дизайн исследования, анализ и интерпретация данных, редактирование текста, формирование окончательного варианта статьи для публикации; *Зайкова О.Н.* — работа с культурой клеток, написание текста статьи, оформление результатов; *Плотников А.А.* — мониторинг клинического исследования, оформление результатов; *Костина Л.В.* — работа с культурой клеток, получение и характеристика препарата; *Чернорыж Я.Ю.* — исследование клеточного иммунитета, статистический анализ; *Елисеева О.В.* — контроль качества полученного препарата; *Латышев О.Е.* — получение и характеристика препарата; *Ларичев В.Ф., Федякина И.Т.* — исследование иммуногенности в реакции нейтрализации; *Лосич М.А.* — работа с культурой клеток, получение и характеристика препарата; *Кириллов И.М.* — очистка антигена вакцины; *Филатов И.Е.* — исследование иммуногенности в реакции ИФА (специфические IgG); *Баландина М.В.* — исследование динамики продукции цитокинов; *Цибезов В.В.* — исследование иммуногенности в реакции ИФА, интерпретация данных; *Юрлов К.И.* — исследование клеточного иммунитета; *Леснова Е.И., Кондратьева В.М.* — исследование клеточного иммунитета; *Козлова А.А., Баранец М.С.* — исследование иммуногенности в реакции нейтрализации; *Гинцбург А.Л.* — организационная сторона клинических исследований. Все авторы подтверждают соответствие своего авторства критериям Международного комитета редакторов медицинских журналов, внесли существенный вклад в проведение поисково-аналитической работы и подготовку статьи, прочли и одобрили финальную версию до публикации.

Статья поступила в редакцию 20.02.2025;  
принята к публикации 21.04.2025;  
опубликована 28.04.2025