ANNOTATION

of dissertation work on the topic «Production and Evaluation of a Recombinant Protein Based on Baculovirus Technology for the Development of a COVID-19 Vaccine», submitted for the degree of Doctor of Philosophy (PhD) in the specialty 8D07201 – «Technology of Pharmaceutical Production ».

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Relevance of the research topic. In December 2019, the COVID-19 pandemic caused by the novel coronavirus SARS-CoV-2 acquired a global character, which necessitated the development of safe and effective vaccines. Since the pandemic was declared, various technological approaches to vaccine manufacturing have been developed, including recombinant viral vectors and vaccines based on recombinant proteins. In particular, the use of the baculovirus system allows efficient cloning of vectors and production of recombinant proteins, which makes it well suited for developing vaccines against a broad range of pathogens, including SARS-CoV-2.

The baculovirus system is distinguished by its ability to grow to high density in insect cell cultures and by its inability to replicate in mammalian cells, which ensures a high level of biological safety. Compared with other manufacturing approaches, it offers considerable advantages – low production cost and high scalability – which is particularly important for reducing the economic burden associated with global vaccination programmes. These aspects underline not only the medical but also the technological significance of baculovirus systems in the context of pharmaceutical manufacturing.

Given the emergence of new viral strains capable of evading the immune response induced by existing vaccines, the development of vaccines adapted to multiple strains has become critically important. The use of baculovirus technology to create a vaccine capable of inducing both humoral and cellular immune responses represents a promising direction in pharmaceutical development. Proceeding from the above, recombinant S and RBD proteins of SARS-CoV-2 obtained by baculovirus technology were used to develop the experimental COVID-19 vaccine samples.

To ensure a comprehensive approach to infection prevention, the present work adopted a technological strategy of developing two different dosage forms: for intramuscular and for intranasal administration. The adjuvanted intramuscular form is aimed at inducing durable systemic immunity and preventing severe disease. However, SARS-CoV-2 primarily infects the respiratory epithelium, and parenteral administration is unable to induce secretory IgA antibodies at the mucosal surface to block infection at the stage of entry. This determined the need to develop an intranasal form.

The use of simple physicochemical liquid forms (for example, conventional solutions or suspensions) for intranasal administration of protein antigens is of low efficiency because of their rapid enzymatic degradation, short mucosal residence time (mucociliary clearance) and poor uptake by antigen-presenting cells. A technological decision was therefore taken to encapsulate the antigen in polymeric chitosan nanoparticles. Unlike other delivery systems, such nanoparticles exhibit pronounced mucoadhesion, provide controlled antigen release owing to the hydrophilic biopolymer matrix and, through covalent conjugation with D-mannose, enable active targeted endocytosis by macrophages and dendritic cells, substantially enhancing the immune response.

Thus, the present work describes the technological aspects of preparing two experimental vaccines – from obtaining the active pharmaceutical ingredient to the finished dosage form, including finishing operations and packaging. Two dosage forms were developed: an adjuvanted form for intramuscular administration and a lyophilisate based on chitosan nanoparticles for the preparation of an intranasal solution.

The dissertation was carried out in line with the priority directions of science development of the Republic of Kazakhstan, in particular in the field of national biological security and the development of the domestic biotechnological and pharmaceutical industries. The development of proprietary high-technology recombinant subunit vaccines is a strategically important step in

implementing the state import-substitution programme in the pharmaceutical sector. This will make it possible to meet the need of the healthcare system of the Republic of Kazakhstan for safe immunobiological preparations, reduce dependence on foreign manufacturers and create export potential for international markets.

The research objectives were implemented under grant funding from the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (projects No. AP09259609 and No. AP09259192), carried out at the International Center for Vaccinology of KazNARU. Substantial support was also provided by KazNARU from its own funds, including payment for the preclinical GLP studies of the NARUVAX-C19/Nano vaccine.

Preclinical safety studies of the developed experimental samples were conducted at the Testing Center of the National Center for Expertise of Medicines and Medical Devices of the Ministry of Health of the Republic of Kazakhstan. The development and physicochemical characterisation of the nanosomal dosage form, including scanning electron microscopy, particle size and zeta potential determination, as well as lyophilisation, were performed at The Ohio State University (USA). The results of the NARUVAX-C19 vaccine development were obtained jointly with Vaxine Pty Ltd (Adelaide, Australia), whose representative serves as the foreign scientific consultant of the present work.

The purpose of scientific research. Development of the technology for preparing two experimental COVID-19 vaccines based on recombinant Spike/RBD proteins obtained using baculovirus technology – from the production of the active pharmaceutical ingredient to the finished dosage forms – and evaluation of their safety and efficacy.

Objectives of study:

1. To analyse SARS-CoV-2 genomic sequences from the GISAID database, including isolates circulating in the Republic of Kazakhstan, and to construct plasmids carrying inserts of the immunodominant antigens (S-ECD, RBD) of SARS-CoV-2;
2. To construct a baculovirus vector and to perform expression, purification and concentration, as well as quality control of the Spike/RBD proteins (specificity, purity, concentration, sterility and bacterial endotoxins, and reactivity in immunoblotting);
3. To develop the technology for preparing two dosage forms of experimental SARS-CoV-2 vaccines (formulation rationale, manufacturing flow chart, finishing operations and packaging): a subunit vaccine for intramuscular administration based on recombinant spike protein (Spike protein) with the extracellular domain (ECD) and a squalene-based nanoemulsion oil adjuvant (SWE), as well as a nanoparticle-based form in the shape of a lyophilisate for the preparation of a solution for intranasal use containing the receptor-binding domain (RBD) encapsulated in chitosan-mannose nanoparticles;
4. To evaluate the quality attributes (physicochemical and technological characteristics) of the developed experimental samples of the intramuscular and intranasal vaccines, and to study their stability during storage;
5. To evaluate the safety, immunogenicity and protective efficacy of the obtained recombinant proteins within the two developed dosage forms (the experimental adjuvanted subunit intramuscular vaccine and the intranasal nanovaccine prototype) in laboratory animal models, and to analyse protective efficacy based on the published results of collaborative studies.

Research Methods. Bioinformatic, molecular, statistical, physicochemical, chemical, pharmacopoeial, technological, microbiological and pharmacological methods were used in accordance with the requirements of regulatory documents recognised and in force in the Republic of Kazakhstan.

Design of the target antigens was performed by bioinformatic modelling (in silico) and genetic engineering; biosynthesis of the recombinant proteins was carried out in a suspension culture of ExpiSf9™ insect cells using the baculovirus expression system. Isolation and purification were performed by immobilised metal affinity chromatography on a Ni-NTA matrix and gel filtration; quality control was carried out by sodium dodecyl sulfate polyacrylamide gel electrophoresis, western blotting, enzyme-linked immunosorbent assay, determination of bacterial

endotoxins and sterility testing. Chitosan-mannose nanoparticles were obtained by ionic gelation and characterised by dynamic light scattering, zeta potential measurement and scanning electron microscopy. Safety, immunogenicity and protective efficacy were studied in non-clinical in vivo and in vitro studies in laboratory animal models, including non-human primates.

A detailed description of the methods with reference to the corresponding general pharmacopoeial monographs is given in subsection 2.2 of the dissertation; statistical processing was performed according to GPM 2.1.10.2 of the Pharmacopoeia of the Eurasian Economic Union at a critical significance level of $p < 0.05$.

Objects of study: the manufacturing process for subunit COVID-19 vaccine preparations based on recombinant SARS-CoV-2 proteins synthesised in the baculovirus expression system – from biosynthesis of the active pharmaceutical ingredient in a suspension culture of insect cells to the finished dosage forms for intramuscular and intranasal administration. The material carriers are the recombinant Spike protein with the extracellular domain (S-ECD) and the receptor-binding domain (RBD) of SARS-CoV-2, as well as the experimental preparations obtained from them: the NARUVAX-C19 subunit vaccine for intramuscular administration, containing the Spike protein and the SWE nanoemulsion oil adjuvant, and the NARUVAX-C19/Nano nanovaccine for intranasal administration, in which the receptor-binding domain is enclosed in a chitosan nanoparticle conjugated with mannose.

Subject of the study: the method of obtaining recombinant Spike/RBD proteins based on baculovirus technology; the manufacturing technology and quality attributes of the experimental subunit SARS-CoV-2 vaccine NARUVAX-C19 for intramuscular administration, based on recombinant Spike protein (Spike protein) with the extracellular domain (ECD) and a nanoemulsion oil adjuvant (SWE); the manufacturing technology and quality attributes of the experimental SARS-CoV-2 nanovaccine NARUVAX-C19/Nano for intranasal administration, containing the receptor-binding domain (RBD) enclosed in a chitosan nanoparticle conjugated with mannose; the safety, immunogenicity, protective efficacy and stability of the obtained recombinant proteins within the subunit adjuvanted intramuscular and the subunit intranasal nanovaccine against COVID-19 in laboratory animal models, including non-human primates.

The main provisions for defense:

1. The established baculovirus expression model based on the ExpiSf9™ suspension cell line provides highly efficient biosynthesis of conformationally stable and functionally active recombinant SARS-CoV-2 antigens (Spike S-ECD and RBD). The physicochemical and biological properties of the obtained target proteins meet the pharmacopoeial requirements for active pharmaceutical ingredients used to construct safe immunogenic intramuscular and intranasal vaccines.

2. The scientifically substantiated technological parameters for cultivating the ExpiSf9™ cell line (multiplicity of infection MOI = 5, cell density at infection $6.5–7.0 \times 10^6$ cells/mL, temperature 27 °C, expression time 72 h), combined with a two-stage purification scheme (immobilised metal affinity chromatography on a Ni-NTA matrix and gel filtration), provide the highest yield of the target recombinant SARS-CoV-2 antigens (1.5–2.0 mg/L for S-ECD and 2.0–2.4 mg/L for RBD) while achieving a high degree of purity (over 95 %) and biological safety – bacterial endotoxin content of no more than 1.0 EU/μg of protein, with actual values of 0.2 EU/μg for S-ECD and 0.15 EU/μg for RBD – and preserving biological activity.

3. The safety and reactogenicity profile of the experimental vaccines NARUVAX-C19 and NARUVAX-C19/Nano established in the preclinical studies performed. Comprehensive preclinical studies under GLP conditions confirm that the developed preparations belong to the class of relatively harmless substances. The vaccines show no acute or subchronic toxicity when administered at doses ten times the therapeutic dose and have no local irritant or sensitising effect.

4. The established biotechnological platform based on ExpiSf9™ suspension culture provides a consistently high yield (1.5–2.0 mg/L) and chromatographic purity (over 95 %) of the conformationally stable SARS-CoV-2 S-ECD antigen. The rational design of the injectable dosage form NARUVAX-C19, based on the use of the Sepivac SWE nanoemulsion adjuvant in a 50:50

volume ratio with the antigen, ensures the absence of phase separation during storage of the oil-in-water emulsion and the formation of a pronounced systemic Th1/Th2 cellular and humoral immune response.

5. The technology of targeted mucosal antigen delivery implemented in the NARUVAX-C19/Nano nanovaccine through ionic gelation of chitosan-mannose nanoparticles. This polymeric nanosystem is characterised by optimal physicochemical parameters (hydrodynamic size ~290 nm, ζ-potential ~+30 mV, RBD encapsulation efficiency 66.8 %). The use of this platform provides mucoadhesion, protection of the antigen against enzymatic degradation and its targeted uptake by antigen-presenting cells of the respiratory mucosa, inducing a pronounced secretory IgA response.

6. The scientific and technological rationale for the standardisation and scalability of the manufacture of the developed vaccine preparations. The pre-validation performed on three pilot batches of the RBD substance confirms the reproducibility of critical quality attributes (QbD); pre-validation batches of the S-ECD substance were not produced within the scope of this work. The established indicators of physicochemical and biological stability of the lyophilised and injectable forms during storage form an evidence base for approval of the regulatory documentation and provide grounds for considering the transition of the vaccine candidates NARUVAX-C19 and NARUVAX-C19/Nano to phase I clinical trials.

Scientific novelty of the work. The technological basis of the work – the baculovirus expression system, immobilised metal affinity chromatography, gel filtration and the ionic gelation method for producing chitosan nanoparticles – belongs to the state of the art and is not claimed as novel. The novelty of the present work lies in the specific combination and numerical values of the parameters at which this basis has for the first time been brought to a complete technological solution for two dosage forms under the conditions of the Republic of Kazakhstan:

1. For the ExpiSf9™ expression system, the operating range of critical parameters for the biosynthesis of conformationally stable S-ECD and RBD antigens of SARS-CoV-2 has been established and quantitatively substantiated: at a multiplicity of infection MOI = 5, cell density at infection of 6.5–7.0 × 10^6 cells/mL, a temperature of 27 °C and harvest after 72 h, a yield of 1.5–2.0 mg/L for S-ECD and 2.0–2.4 mg/L for RBD is achieved at a purity of over 95 %. Reducing the MOI to 3 lowers the yield to 0.8–1.2 mg/L, while increasing it to 7 lowers the yield to 1.0–1.4 mg/L with purity falling to 90–93 %.

2. A scalable two-stage isolation and purification (Downstream) scheme has been developed and pre-validated, comprising immobilised metal affinity chromatography on a Ni-NTA matrix and gel filtration. This technological approach made it possible to achieve a high degree of purity of the active pharmaceutical ingredient (over 95 %) and biological safety (bacterial endotoxin content of no more than 1.0 EU/μg of protein), meeting pharmacopoeial requirements.

3. An experimental subunit COVID-19 vaccine (NARUVAX-C19), in which the Sepivac SWE nanoemulsion oil adjuvant of the oil-in-water emulsion type is used as the immunostimulating component, has been proposed and studied in laboratory models of rodents and rhesus macaques.

4. The safety and immunogenicity of the experimental NARUVAX-C19 vaccine have been demonstrated in rhesus macaques, in which the administration of two intramuscular doses two weeks apart resulted in a sustained broadly neutralising humoral and T-cell IFN-γ response.

5. For the first time, a prototype nanovaccine against COVID-19 with an intranasal route of administration has been developed on the basis of chitosan-mannose nanoparticles.

6. It has been established that the experimental vaccine based on recombinant monomeric RBD protein loaded into nanoparticles of chitosan conjugated with mannose (NARUVAX-C19/Nano) induces antigen-specific IgA antibodies and a Th1 cellular immune response in mice after two intranasal immunisations 21 days apart, as well as protection against infection with the ancestral SARS-CoV-2 strain (D614G lineage) in the Syrian hamster model. In this study it was established for the first time that the efficacy of the experimental NARUVAX-C19/Nano form was achieved in the absence of detectable serum anti-RBD IgG (including the IgG1 and IgG2a subclasses), as well as of antibodies blocking the RBD–ACE2 interaction and of virus-neutralising antibodies.

7. Preclinical safety studies of the developed experimental vaccine samples were conducted at the Testing Center of the National Center for Expertise of Medicines and Medical Devices of the Ministry of Health of the Republic of Kazakhstan (NCEMMD). It was shown that both experimental vaccines have no toxic effect on the organism, are well tolerated and show no sensitising effect in tests on laboratory animals, including pregnant females. On the basis of the successful results of the preclinical safety studies (conducted in compliance with GLP standards), the experimental samples received the status of the vaccine candidates NARUVAX-C19 and NARUVAX-C19/Nano; according to the conclusion of the NCEMMD, the preparations were recognised as meeting the safety requirements for transition to the next stage of development.

Patent searches carried out in accordance with GOST R 15.011-96 in the international Google Patents database, with a search depth from 2014 (IPC classes A61K 39/215 and C12N 15/866), showed that the combination of features characterising the intranasal dosage form based on the SARS-CoV-2 receptor-binding domain encapsulated in chitosan nanoparticles conjugated with mannose was not found in the claimed combination in the patent documents analysed.

Practical significance of the obtained results Two new dosage forms of experimental COVID-19 vaccines have been developed, as well as a platform approach potentially applicable to other viral infections: an adjuvanted form for intramuscular administration and a nanoparticle-based form in the shape of a lyophilisate for the preparation of an intranasal solution.

A technology for producing the recombinant Spike protein based on baculovirus technology has been developed and implemented for the creation of NARUVAX-C19 – an experimental subunit COVID-19 vaccine, solution for intramuscular administration, 0.5 mL/dose, based on recombinant Spike protein (Spike protein) with the extracellular domain (ECD) and a nanoemulsion oil adjuvant (SWE). A laboratory protocol has been developed and implemented.

A technology for producing the recombinant RBD protein based on baculovirus technology has been developed and implemented for NARUVAX-C19/Nano – an experimental COVID-19 nanovaccine, lyophilisate for the preparation of a solution for intranasal administration, 0.5 mL/dose, containing the receptor-binding domain (RBD) enclosed in a chitosan nanoparticle conjugated with mannose. A laboratory protocol has been developed and implemented.

Drafts of regulatory documentation have been prepared and standardisation has been carried out for NARUVAX-C19 – an experimental subunit COVID-19 vaccine, solution for intramuscular administration, 0.5 mL/dose, and for NARUVAX-C19/Nano – an experimental COVID-19 nanovaccine, lyophilisate for the preparation of a solution for intranasal administration, 0.5 mL/dose; storage conditions and shelf lives have been determined for both dosage forms.

Pilot batches of both experimental vaccines have been manufactured at the International Center for Vaccinology of KazNARU (Republic of Kazakhstan, Almaty).

Personal contribution of a doctoral student

The candidate constructed the recombinant vector for introducing the genes of the SARS-CoV-2 S and RBD proteins into the baculovirus system. This stage included selection of the promoter, use of the melittin signal peptide to direct the target polypeptide into the secretory pathway, and integration of regulatory sequences optimising protein folding in the producer cells.

The candidate carried out protein expression in the baculovirus system. The work included obtaining the recombinant bacmid in competent E. coli cells, determining the titre of the viral stock, transfecting the producer cells and comprehensively selecting the fermentation parameters.

The candidate's contribution consists in developing the design of the plasmid constructs and performing the complete manufacturing cycle of cell biomass production and purification of the recombinant proteins. The applicant personally carried out the studies to determine the stability parameters of the obtained preparations during storage, and developed drafts of the regulatory documents and laboratory protocols for the production of the two dosage forms of the experimental vaccines. Alongside the technological part, the candidate took direct part in analysing the data of the preclinical studies, which confirmed the safety and specific biological activity of the developed dosage forms.

Certain stages of the work were performed with the participation of partner organisations. The physicochemical characterisation of the chitosan-mannose nanoparticles, scanning electron microscopy and development of the lyophilisation regime were performed at The Ohio State University (USA); the candidate took part in formulating the task and interpreting the data obtained. Preclinical safety studies in rodents were conducted by the Testing Center of the NCEMMD of the Ministry of Health of the Republic of Kazakhstan under GLP conditions; the candidate took part in drawing up the study programmes and analysing the raw data of the reports. The preclinical study of the safety and immunogenicity of the NARUVAX-C19 vaccine in rhesus macaques was performed by the Research Institute of Medical Primatology; the candidate provided production and quality control of the antigen, took part in analysing the raw data of the report and is the first author of the publication prepared on the results of this study. Immunogenicity and protection studies in mouse and Syrian hamster models were performed at the International Center for Vaccinology of KazNARU jointly with Vaxine Pty Ltd (Australia); the candidate provided production and quality control of the antigens.

Conclusions:

1. Using bioinformatic modelling (in silico) and genetic engineering, recombinant SARS-CoV-2 proteins were designed and produced in a suspension culture of ExpiSf9™ insect cells: for the injectable form, the full-length extracellular domain of the S protein (S-ECD) with the furin cleavage site removed; for the intranasal form, the receptor-binding domain (RBD) carrying the mutations of the Omicron BA.5 variant. The developed biotechnological process (Upstream and Downstream), at a multiplicity of infection MOI = 5, an isothermal cultivation regime of 27 °C and harvest of the culture fluid 72 hours after infection, made it possible to achieve yields of 1.5–2.0 mg/L of culture fluid for S-ECD and 2.0–2.4 mg/L for RBD at a chromatographic purity of over 95 % according to sodium dodecyl sulfate polyacrylamide gel electrophoresis. The physicochemical and biological properties of the obtained proteins, including a bacterial endotoxin content of 0.2 EU/μg of protein for S-ECD and 0.15 EU/μg of protein for RBD against a limit of no more than 1.0 EU/μg, meet the requirements for active pharmaceutical ingredients.

2. The reproducibility of the manufacturing process was confirmed on three consecutive pilot batches of the RBD substance; pre-validation batches of the S-ECD substance were not produced within the scope of this work, and the conclusion on reproducibility extends to it only in respect of the shared process steps and is subject to experimental confirmation at the scale-up stage. Laboratory protocols establishing the critical process parameters (MOI = 5, temperature profile, chromatographic purification parameters) have been approved. For correct transfer of the technology to pilot suspension bioreactors, the calculated transition parameters were determined: an oxygen mass transfer coefficient (kLa) in the range of 5–10 h^{-1}, a dissolved oxygen level of not less than 40 % of air saturation, limitation of the specific mixing power (P/V) and the addition of poloxamers to the culture medium as cell membrane protectants. The manufacturing process was developed in accordance with the principles of Quality by Design and the requirements of Good Manufacturing Practice.

3. On the basis of the obtained substances, formulations and manufacturing technologies for two dosage forms were developed. For intramuscular administration, the NARUVAX-C19 vaccine was created – a kinetically stable composition of the recombinant protein with the Sepivac SWE nanoemulsion oil adjuvant, which retained physical stability under the storage conditions studied. For intranasal (mucosal) use, a prototype of the NARUVAX-C19/Nano nanovaccine was developed, the technology of which is based on the ionic gelation method: encapsulation of the antigen in cationic chitosan nanoparticles modified with D-mannose made it possible to obtain a colloidal system with a mean hydrodynamic size of 290 nm, a high positive zeta potential (+30 mV) and a protein entrapment efficiency of about 66.8 %, which, according to published data, contributes to protection of the antigen against degradation and potentially provides its targeted delivery to antigen-presenting cells through the interaction of mannose residues with mannose receptors.

4. A set of preclinical safety studies was conducted in an accredited GLP laboratory (NCEMMD of the Ministry of Health of the Republic of Kazakhstan), which showed that both dosage forms belong to the class of relatively harmless substances (toxicity class VI). No signs of acute or subchronic toxicity were detected when the preparations were administered to rodents at doses ten times the therapeutic dose; the vaccines have no local irritant or sensitising effect. When administered to pregnant females, no fetal malformations were recorded; the isolated microfoci of circulatory disturbance and necrosis detected in embryonic tissues on histological examination were observed for both dosage forms, including the one not containing the squalene adjuvant, and their causal relationship with the action of the vaccine preparations or accompanying procedures could not be established with the design applied. Assessment of reproductive toxicity to the extent of segment II with appropriate comparison groups has been assigned to a separate stage of preclinical development.

5. The specific immunogenicity and protective efficacy of the intramuscular NARUVAX-C19 vaccine were established. Studies in mouse (BALB/c), Syrian hamster and rhesus macaque models showed that two-dose immunisation induces a systemic humoral response with the formation of anti-S IgG and virus-neutralising antibodies against strains of the Wuhan and Delta lineages, as well as a balanced Th1/Th2 cellular response. In non-human primates, the severity of pathological changes in the lungs upon challenge with live pathogen was reduced compared with the placebo group. The cross-neutralising effect against genetically distant strains indicates the ability of the vaccine to induce cross-neutralising antibodies.

6. For the first time, a prototype intranasal nanovaccine against COVID-19 based on recombinant RBD protein encapsulated in chitosan-mannose nanoparticles was developed and studied. It was established that two intranasal administrations of the preparation to mice and Syrian hamsters lead to the induction of antigen-specific secretory IgA in the respiratory tract and to the formation of a cellular immune response with Th1 polarisation (synthesis of IFN-γ, IL-2 and TNF-α). It was shown that delivery of the RBD protein within chitosan-mannose nanoparticles protects immunised animals against the development of lung pathology upon challenge with a SARS-CoV-2 strain, the protective effect being achieved in the absence of detectable serum anti-RBD IgG. At the same time, it was established that the intranasal form does not prevent transmission of the virus to contact animals; sterilising immunity was not achieved in the experiments performed when the mucosal route of administration was used in isolation.

7. Pharmaceutical development and standardisation of the two dosage forms were carried out: drafts of regulatory documents and quality control specifications were developed for the intermediate and finished products of the injectable (NARUVAX-C19) and intranasal (NARUVAX-C19/Nano) vaccines, and the conditions for stabilising the lyophilised nanoform and the parameters for producing the adjuvanted form were substantiated. Based on the stability studies, storage conditions and shelf lives were established: for NARUVAX-C19 – at a temperature of 2 to 8 °C without freezing, with a provisional shelf life of 12 months subject to confirmation in a stability study on three batches in accordance with ICH Q1A(R2) and ICH Q5C; for the NARUVAX-C19/Nano lyophilisate, according to preliminary data – not less than 12 months at a temperature of minus 17 to minus 30 °C and not less than 6 months at a temperature of 2 to 8 °C. Pilot batches of the two vaccine candidates for preclinical testing were manufactured at the International Center for Vaccinology of KazNARU (Republic of Kazakhstan, Almaty).

Thus, the objective set for the dissertation has been achieved. The data obtained on the safety, immunogenicity and protective efficacy of both dosage forms, established under the conditions of preclinical studies in laboratory animals, provide grounds for considering the transition of the vaccine candidates NARUVAX-C19 and NARUVAX-C19/Nano to phase I clinical trials.

Approbation of the results of the dissertation

The main provisions, results and conclusions of the dissertation were presented, reported and positively received at the following international scientific forums and conferences:

– 16th Vaccine Congress (September 12–14, 2022, Riva del Garda, Italy) – a poster presentation of the results of the development of the NARUVAX-C19 vaccine together with international partners (Vaxine Pty Ltd, Australia; The Ohio State University, USA);

– International Scientific and Practical Conference "Biotechnology and Biological Safety: Achievements and Development Prospects", dedicated to the 65th anniversary of the Research Institute for Biological Safety Problems (RIBSP) of the Ministry of Health of the Republic of Kazakhstan (September 6–8, 2023, Almaty, Republic of Kazakhstan) – with publication of proceedings on the topic "Evaluation of the immunogenicity and protective efficacy of an intranasal subunit nanovaccine against COVID-19 in laboratory models".

Publications. Based on the results of the research, 5 scientific works have been published, including 3 articles in international peer-reviewed scientific journals indexed in the Web of Science and Scopus databases and 2 conference abstracts in the proceedings of international scientific and practical conferences:

– 1 article in the journal Human Vaccines & Immunotherapeutics (WoS-Q1, IF-4.8, Scopus-68 %);

– 1 article in the journal Scientific Reports (WoS-Q2, IF-4.6, Scopus-92 %);

– 1 article in the journal Microorganisms (WoS-Q2, IF-4.9, Scopus-65 %).

Contribution of the doctoral student to each publication. In the article in Human Vaccines & Immunotherapeutics (Solomadin M. et al., 2023, Vol. 19, No. 2, 2258571) the doctoral student is the first author – he carried out the design of the plasmid constructs, the complete manufacturing cycle of cell biomass production and purification of the recombinant S-ECD protein, participation in the analysis of the preclinical study data and preparation of the manuscript text. In the article in Scientific Reports (Tabynov K., Solomadin M., Turebekov N. et al., 2023, Vol. 13, No. 1, 12115) the doctoral student is the second author – he carried out the production and quality control of the recombinant RBD protein and participated in the analysis of the results and preparation of the manuscript. In the article in Microorganisms the doctoral student carried out the analysis of the results and preparation of the manuscript. The 2 conference abstracts were prepared by the doctoral student with respect to the technological results of recombinant protein production and dosage form development.

Scope and structure of the dissertation

The dissertation is presented on 196 pages of typewritten text and contains 54 tables, 52 figures, 98 domestic and foreign literature sources and 6 appendices. The structure includes: normative references and a list of abbreviations, an introduction, a literature review, materials and methods of research, four sections of the experimental part (chapters 3, 4, 5 and 6), conclusions to the sections and a final conclusion.

Appendices A and B contain the drafts of the regulatory documents for the experimental vaccines NARUVAX-C19 and NARUVAX-C19/Nano; appendices C and D contain the laboratory protocols for the production and evaluation of the recombinant Spike and RBD proteins; appendix E contains the research report; and appendix F contains a copy of the poster presentation.