GENETIC DIAGNOSTICS AND THERAPY 21 LTD

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Basic information about the project

"DNA-vaccine against SARS-CoV-2"

Background information.

According to the World Health Organization (WHO), infectious diseases account for 25% of all deaths on the planet annually. Expectations in the prevention and treatment of infectious, as well as many other (oncological, autoimmune) diseases, are placed on advanced developments in genetic prophylaxis using DNA vaccines. Genetic prophylaxis, as well as gene therapy, is an essential part of modern molecular medicine.

Vaccines are divided into the following types, depending on the production methods thereof:

- live attenuated vaccines (LAV) (poliomyelitis, measles, rubella, influenza, mumps, varicella, tuberculosis, rotavirus infection),
- inactivated vaccines (IV) (influenza, typhoid fever, tick-borne encephalitis, rabies, hepatitis A, meningococcal disease, etc.),
- vaccines containing purified components of microorganisms (anatoxins, toxoids, e.g. pertussis, diphtheria, tetanus),
- recombinant vaccines containing components of microorganisms obtained via genetic engineering techniques, including DNA vaccines (recombinant hepatitis B vaccine).

Recombinant DNA-derived vaccine (also known as DNA vaccine, gene vaccine, nucleic acid vaccine) is a genetically engineered construct based, inter alia, on a double-stranded plasmid (plasmid vector, DNA vector, gene therapy DNA vector) carrying the DNA sequence encoding the immunologically relevant protein of the pathogenic microorganism (or tumour

antigen DNA) and regulatory genetic elements that ensure the expression of the protein encoded in the cell. Plasmids are produced using the cloning in the bacterial cell culture (E. coli), purified from impurities and other bacterial DNA (Anderson R. J.; Schneider J. Plasmid DNA and viral vector-based vaccines for the treatment of cancer (Eng) // Vaccine (Eng)Ru : journal. – Elsevier, 2007. – Vol. 25. – P. 24–34. – doi:10.1016/j.vaccine.2007.05.030). Following the injection of the purified construct into the cell, the DNA encoded protein (or tumour antigen) is expressed and elicits immune response against pathogen or cancer cell carrying the antigen. At the same time, plasmids containing the corresponding gene do not integrate into the DNA of human chromosomes, i.e. they do not have a mutagenic effect. One of the advantages of the approach is that genetic immunisation can provide a specific cytotoxic response (cell-mediated immunity), as well as antibody production (humoral immunity) (Ferraro B.; Matthew P. Morrow, Natalie A. Hutnick; Et al. Clinical Applications of DNA Vaccines: Current Progress (Eng) // Clin Infect Dis. (Eng) Ru : journal. – 2011. – Vol. 53, no. 3. – P. 296–302. – doi:10.1093/cid/cir334), which was previously achievable through live vaccines only. The cytotoxic T cell activation without the living pathogen injection is a distinctive feature of DNA vaccines.

The decisive advantage of DNA vaccines is the expression of viral agents in their native form. During immunisation with viral proteins, in the process of their production and purification, a three-dimensional protein structure may be changed (misfolding), which will reduce the effectiveness of immunization.

DNA vaccines are significantly superior to live attenuated vaccines or certain recombinant vaccines based on live virus vectors for safety, since the body only produces a single specific protein that featured antigenic properties but is unable to cause disease on its own.

Other advantages of DNA vaccines include the following: the expressed antigen can be selectively targeted to HLA-I or HLA-II pathway;

long-term antigen expression; ease of production and low production cost; modest storage requirements; can be used both for prevention and treatment; potentially effective against a wide range of diseases, including bacterial, viral, autoimmune, and oncological diseases.

There is much concern about the usage of DNA vaccines for the prevention of viral diseases that trigger epidemics, or even pandemics that affect a large number of people. A striking example of the pathogen of such disease is coronavirus SARS-CoV-2 (GenBank MN908947.3) discovered in China in late 2019 and triggered a pandemic. Vaccines for the prevention of the coronavirus, including the one that triggered the epidemic in 2003 (SARS) do not exist to this day.

When developing a DNA vaccine against the SARS-CoV-2 virus, it should be noted that the most likely candidates for antigens are immunogenic epitopes of S, M, N, and E proteins of SARS-CoV-2 virus (Zhang J, Zeng H, Gu J, Li H, Zheng L, Zou Q. Progress and Prospects on Vaccine Development against SARS-CoV-2. Vaccines (Basel). 2020 Mar 29;8(2). pii: E153. doi: 10.3390/vaccines8020153).

S protein (spike protein; protein that forms "spikes") is the most promising in terms of development of vaccines, including DNA vaccines. First, this protein is exposed on the virus surface and is directly recognised by the immune system. Second, it binds to the ACE2 receptor (human angiotensin converting enzyme 2) on the cell surface for subsequent penetration (Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.;Wang, Q.; Zhang, L.; et al. Crystal structure of the 2019-nCoV spike receptor-binding domain bound with the ACE2 receptor. BioRxiv 2020). It was demonstrated that S protein fragments feature immunogenic properties (Zhu, X.; Liu, Q.; Du, L.; Lu, L.; Jiang, S. Receptor-binding domain as a target for developing SARS vaccines. J. Thorac. Dis. 2013, 5 (Suppl. 2), S142–S148).

M protein (membrane protein, intrinsic protein) is the glycoprotein responsible for the virus assembly is the most represented protein on the surface of the viral particle (Neuman, B.W.; Kiss, G.; Kunding, A.H.; Bhella, D.; Baksh, M.F.; Connelly, S.; Droese, B.; Klaus, J.P.; Makino, S.; Sawicki, S.G.; et al. A structural analysis of M protein in coronavirus assembly and morphology. J. Struct. Biol. 2011, 174, 11–22).

N protein (nucleocapsid protein) is a highly antigen protein associated with genomic RNA, plays a role in the processes of vRNA replication and transcription (McBride, R.; van Zyl, M.; Fielding, B.C. The coronavirus nucleocapsid is a multifunctional protein. Viruses 2014, 6, 2991–3018).

E protein (envelope protein, coat protein) is the key factor of virulence of the SARS-CoV-2 and plays an important role in the morphogenesis and assembly of viral particles (Nieto-Torres, J.L.; DeDiego, M.L.; Verdia-Baguena, C.; Jimenez-Guardeno, J.M.; Regla-Nava, J.A.; Fernandez-Delgado, R.; Castano-Rodriguez, C.; Alcaraz, A.; Torres, J.; Aguilella, V.M.; et al. Severe acute respiratory syndrome coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis. PLoS Pathog. 2014, 10, e1004077.).

However, when developing formulation from gene therapy DNA vectors included in the DNA vaccine, it is preferable to use no more than three DNA vectors encoding the immunogenic epitopes of the virus proteins, because the development of formulation from more DNA vectors can be limited by the dose of the vaccine injected, which results in insufficient expression level of each antigen to generate a complete immune response.

Thus, the background of the invention indicates that DNA vaccines have the potential for large-scale vaccination of the population, in particular against SARS-CoV-2. This is why a DNA vaccine as a composition of gene therapy DNA vectors GDTT1.8NAS12-S, GDTT1.8NAS12-M, and GDTT1.8NAS12-N based on gene therapy DNA

vector GDTT1.8NAS12 encoding immunogenic epitopes of S, M, and N proteins of SARS-CoV-2 virus was developed within this project.

Analysis of approaches to develop DNA vaccines implies the practicability of use of different gene therapy vectors.

Gene therapy vectors are divided into viral, cell, and DNA vectors (Guideline on the quality, non-clinical, and clinical aspects of gene therapy medicinal Products EMA/CAT/80183/2014). Recently, gene therapy has paid increasingly more attention to the development of non-viral gene delivery systems with plasmid vectors topping the list. Plasmid vectors are free of limitations inherent in cell and viral vectors. In the target cell, they exist as an episome without being integrated into the genome, while producing them is quite cheap, and there is no immune response or side effects caused by the administration of plasmid vectors, which makes them a convenient tool for gene therapy and prevention of the genetic diseases (DNA vaccination) (Li L, Petrovsky N. // Expert Rev Vaccines. 2016;15(3):313-29).

However, limitations of plasmid vectors use in gene therapy and DNA vaccination are: 1) presence of antibiotic resistance genes for the production of constructs in bacterial strains; 2) the presence of various regulatory elements represented by sequences of viral genomes; 3) length of therapeutic plasmid vector that determines the efficiency of vector delivery to the target cell.

It is known that the European Medicines Agency deems it necessary to refrain from adding antibiotic resistance marker genes to newly engineered plasmid vectors for gene therapy (Reflection paper on design modifications of gene therapy medicinal products during development / 14 December 2011 EMA/CAT/GTWP/44236/2009 Committee for advanced therapies). This recommendation is primarily related to the potential danger of the DNA vector penetration or horizontal antibiotic resistance gene transfer into the cells of bacteria found in the

body as part of normal or opportunistic microflora. Furthermore, the presence of antibiotic resistance genes significantly increases the length of DNA vector, which reduces the efficiency of its penetration into eukaryotic cells.

It is important to note that antibiotic resistance genes also make a fundamental contribution to the method of production of DNA vectors. If antibiotic resistance genes are present, strains for the production of DNA vectors are usually cultured in medium containing a selective antibiotic, which poses risk of antibiotic traces in insufficiently purified DNA vector preparations. Thus, production of DNA vectors for gene therapy without antibiotic resistance genes is associated with the production of strains with such distinctive feature as the ability for stable amplification of therapeutic DNA vectors in the antibiotic-free medium.

In addition, the European Medicines Agency recommends avoiding the presence of regulatory elements in therapeutic plasmid vectors to increase the expression level of therapeutic genes (promoters, enhancers, post-translational regulatory elements) that constitute nucleotide sequences of genomes of various viruses (Draft Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products,http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2015/05/WC500187020.pdf). Although these sequences can increase the expression level of the therapeutic transgene, however, they pose risk of recombination with the genetic material of wild-type viruses and integration into the eukaryotic genome. Moreover, the relevance of overexpression of the particular gene for therapy or vaccination remains an unresolved issue.

The size of the therapy vector is also essential. It is known that modern plasmid vectors often have unnecessary, non-functional sites that increase their length substantially (Mairhofer J, Grabherr R. // Mol Biotechnol. 2008.39(2):97–104). For example, ampicillin resistance gene

in pBR322 series vectors, as a rule, consists of at least 1000 bp, which is more than 20% of the length of the vector itself. A reverse relationship between the vector length and its ability to penetrate into eukaryotic cells is observed; DNA vectors with a small length effectively penetrate into human and animal cells. For example, in a series of experiments on transfection of HELA cells with 383–4548 bp DNA vectors it was shown that the difference in penetration efficiency can be up to two orders of magnitude (100 times different) (Hornstein BD et al. // PLoS ONE. 2016;11(12): e0167537.).

Thus, when selecting a DNA vector, for reasons of safety and maximum effectiveness, preference should be given to those constructs that do not contain antibiotic resistance genes, the sequences of viral origin and length of which allows for the effective penetration into eukaryotic cells. A strain for production of such DNA vector in quantities sufficient for the purposes of gene therapy should ensure the possibility of stable DNA vector amplification using antibiotic-free nutrient media.

Project tasks.

The task of the project is a construction, study and launch onto the market of a DNA vaccine for SARS-CoV-2 virus as a composition of gene therapy DNA vectors based on gene therapy DNA vector GDTT1.8NAS12, combining the following properties:

I) Efficiency of gene therapy DNA vectors in order to increase the expression level of therapeutic genes in eukaryotic cells included in the DNA vaccine and the DNA vaccine.

II) Possibility of safe use in human gene therapy and vaccination due to the absence of regulatory elements representing the nucleotide sequences of viral genomes in the gene therapy DNA vectors.

III) Possibility of safe use in human gene therapy and vaccination due to the absence of antibiotic resistance genes in the gene therapy DNA vector.

IV) Producibility and constructability DNA vaccine on an industrial scale.

The effectiveness of DNA vaccine under spec I is ensured by including in its composition the optimal number of gene therapy DNA vectors based on the gene therapy DNA vector GDTT1.8NAS12 carrying sequences that encode the most immunogenic epitopes of SARS-CoV-2 proteins. This approach is desirable due to the fact that when constructing a single gene therapy DNA vector to which several sequences encoding immunogenic epitopes of SARS-CoV-2 proteins have been cloned, the expression level of therapeutic genes encoding viral antigens can be reduced due to an increase of the gene therapy vector length, which reduces the efficiency of DNA vector penetration into the cells.

Specs II and III are provided for herein in line with the recommendations of the state regulators for gene therapy medicines and, specifically, the requirement of the European Medicines Agency to refrain from adding antibiotic resistance marker genes to newly engineered plasmid vectors for gene therapy (Reflection paper on design modifications of gene therapy medicinal products during development / 14 December 2011 EMA/CAT/GTWP/44236/2009 Committee for advanced therapies) and refrain from adding viral genomes to newly engineered plasmid vectors for gene therapy (Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products / 23 March 2015, EMA/CAT/80183/2014, Committee for Advanced Therapies).

The other task of the project is a construction of strains carrying gene therapy DNA vectors for the development and production of these gene therapy DNA vectors and DNA vaccines on an industrial scale.

Current outcomes of the project implementation:

1. A DNA vaccine has been constructed in the form of a composition of gene therapy DNA vectors based on the gene therapy DNA vector GDTT1.8NAS12 encoding the three most immunogenic epitopes of the SARS-CoV-2 virus proteins - S, M, and N.

A distinctive feature of the DNA vaccine is that each of the constructed gene therapy DNA vectors included in the DNA vaccine, due to the limited size of GDTT1.8NAS12 vector part, not exceeding 2600 bp, has the ability to efficiently penetrate into human and animal cells and express the target proteins of the SARS-CoV-2 virus cloned into it.

Another difference of the DNA vaccine is that each of the constructed gene therapy DNA vectors included in the DNA vaccine has nucleotide sequences as structural elements, which are not antibiotic resistance genes or regulatory elements of viral genomes, which ensures its safe use for gene therapy and human vaccination.

2. Producer strains on the basis of Escherichia coli JM110-NAS have been obtained, carrying gene therapy DNA vectors for their development with a possibility of selection without the use of antibiotics when obtaining a gene therapy DNA vector for their inclusion in the DNA vaccine for vaccination of humans against SARS-CoV virus.
3. A method of production of the DNA vaccine on a commercial scale has been developed in the form of a composition of gene therapy DNA vectors on the base of gene therapy vector GDTT1.8NAS12, encoding the three most immunogenic epitopes of the SARS-CoV-2 virus proteins

4. Samples of the DNA vaccine in the volume necessary for preclinical studies have been produced.

5. A bank of producer strains has been created for the production of DNA vectors included in the composition of the DNA vaccine on a commercial scale.

6. Patent for base vector GDTT1.8NAS12 № 2712838 was issued on 31.01.2020 (priority date 04.09.2018).

7. The intellectual property of the SARS-CoV-2 DNA vaccine is protected. A package of international patent procedures was completed, priority date - 20 April, 2020.

8. Initial in vitro studies, confirming the DNA vaccine effectiveness, have been performed.

9. Technological documentation for industrial production, ensuring acceptable price parameters, has been prepared.

10. Currently:

- preparation for the launch of preclinical studies on rats to assess a specific pharmacological activity of the DNA vaccine is under way.
- preparation for the launch of preclinical studies to assess the acute and chronical toxicity is under way.