

## APPLICATION OF GENE ENGINEERING IN THE TREATMENT OF PATIENTS WITH COVID-19

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**Background.** Modern therapeutic options for the treatment of COVID-19 combine the use of drugs that affect both the virus itself and the components of the body's immune response. Despite the fact that the pathogenetic mechanisms of the infectious disease have been partially investigated, treatment methods still do not live up to expectations, which is largely due to the development of adverse drug reactions and conflicting treatment results. This situation necessitates the analysis of modern scientific sources regarding the prospects, advantages and disadvantages of the use of virus-neutralizing monoclonal antibodies, natural killers, mesenchymal stem cells and monoclonal antibodies to interleukin-6.

**Aim:** to examine the present studies on monoclonal antibodies used in treatment of severe cases of coronavirus disease caused by SARS-CoV-2 and to mark its benefits.

**Materials and methods.** The article uses the bibliographic method and is a review of existing works on PubMed and Google Scholar.

**Results.** Data on the presence of neutralizing antibodies in the blood plasma of sick patients gave an impetus to obtaining humanized or fully human monoclonal antibodies, potentially able to become the basis for the development of drugs for the targeted therapy of SARS-CoV-2. When using biological therapy, the maximum selectivity of the impact on the immune system is created, which makes it possible to eliminate one of the links of the pathogenetic chain without significantly affecting the cells of other organs and systems. Also, the possibility of requalification of existing drugs for cell therapy is being considered.

**Conclusions.** Thus, strategies involving the use of virus-neutralizing monoclonal antibodies, natural killers, mesenchymal stem cells, and monoclonal antibodies to interleukin-6 are promising in the treatment of patients with COVID-19.

**Key words:** COVID-19, monoclonal antibodies, mesenchymal stem cells.

**Background.** One of the most relevant topics at the moment is the viral infection caused by the SARS-CoV-2 coronavirus, which has acquired not only medical, but also, of course, social significance. The World Health Organization declared the COVID-19 pandemic on March 11, 2020. Modern therapeutic options for the treatment of COVID-19 combine the use of drugs that affect both the virus itself and the components of the

body's immune response. Despite the fact that the pathogenetic mechanisms of the infectious disease have been partially investigated, treatment methods still do not live up to expectations, which is largely due to the development of adverse drug reactions and conflicting treatment results. Currently, the number of experimental genetic engineering molecules proposed for the treatment of SARS-CoV-2 is constantly growing, which neces-

sitates the analysis of modern scientific sources regarding the prospects, advantages and disadvantages of the use of virus-neutralizing monoclonal antibodies, natural killers, mesenchymal stem cells and monoclonal antibodies to interleukin-6.

**Aim:** to examine the present studies on monoclonal antibodies used in treatment of severe cases of coronavirus disease caused by SARS-CoV-2 and to mark its benefits.

## MATERIALS AND METHODS

The article uses the bibliographic method and is a review of existing works on PubMed and Google Scholar.

## RESULTS AND DISCUSSION

Infection with the highly pathogenic SARS-CoV-2 coronavirus sometimes leads to the development of a severe viral disease (COVID-19), sometimes with a fatal outcome [1]. The immunopathogenesis of COVID-19 is associated with the development of an unbalanced immune response, accompanied by insufficient synthesis of interferon at the beginning of the disease, with subsequent hyperproduction of pro-inflammatory cytokines, which leads to the development of an inadequate inflammatory response, which is the basis of acute lung damage. Monoclonal antibodies to viral surface proteins are currently in use. Their therapeutic effectiveness against a number of viruses and the possibility of use in immunotherapy of patients with COVID-19 have been proven [2].

Data on the presence of neutralizing antibodies in the blood plasma of sick patients gave an impetus to obtaining humanized or fully human monoclonal antibodies, potentially able to become the basis for the development of drugs for the targeted therapy of SARS-CoV-2. When using biological therapy, the maximum selectivity of the impact on the immune system is created, which makes it possible to eliminate one of the links of the pathogenetic chain without significantly affecting the cells of other organs and systems.

Treatment strategies with virus-neutralizing monoclonal antibodies are largely based on data from a few small completed studies or a few interim

analyses. Neutralizing antibodies prevent the spread of the virus in the body, blocking the proteins necessary for its penetration into human cells and stimulating the immune response against the pathogen. In general, the principle of their work is the same as that of convalescent plasma, which also contains antibodies to the virus. Similar methods belong to the so-called "passive immunization". The half-life of antibodies is about three weeks, therefore, unlike a vaccine, they are not able to provide long-term protection, but can only interrupt the current active infectious process [2].

Many global pharmaceutical companies are currently engaged in the creation of antibodies that neutralize SARS-CoV-2 - for example, British-Swedish Astra Zeneca, Korean Celltrion, American Regeneron and Japanese Takeda. Basically, they can be aimed at the S-protein of the virus, which prevents the virus from entering human cells, in particular, at the RBD-domain (receptor-binding domain) of the S-protein [3].

Monoclonal antibody 47D11, obtained by scientists from the Netherlands and Germany, affects the S-protein, with the help of which the causative agent of the new coronavirus SARS-CoV-2 penetrates into cells - the antibody does not allow it to penetrate into human cells. The study indicated that this antibody is capable of neutralizing not only SARS-CoV-2 (the causative agent of COVID-19), but also the related SARS-CoV virus, which causes acute respiratory distress syndrome. Genetically modified mice were used for the synthesis of 47D11. The antibody was shown to exhibit virus-neutralizing activity, after which the antibody was humanized to create a human version [4].

In addition to 47D11, monoclonal antibodies S309 [5] are tested in the experiment; VHH-72 [6]; ADI55689/ADI56046 [7]. All of them, with the exception of VHH-72, are fully human IgG molecules.

Studies conducted on laboratory animals have shown the high neutralizing capacity and therapeutic potential of monoclonal antibody BD-368-2 in the treatment of COVID-19. BD-368-2 neutralizes SARS-CoV-2 by completely blocking ACE2 recognition because it occupies all three receptor binding domains (RBDs) simultaneous-

ly, regardless of their "ascending" or "descending" conformation. BD-368-2 showed efficacy in the treatment of infected animals using low doses by various routes of administration, in contrast to the placebo control group, which developed severe interstitial pneumonia [8].

Importantly, the use of single monoclonal antibodies can activate selective pressure, potentially increasing the possibility of mutational escape of the target antigen. This risk can be reduced by the combination of several monoclonal antibodies targeting non-overlapping epitopes. In an animal model, the protective effect of a "cocktail" of two fully humanized monoclonal antibodies that bind to different regions of the SARS-CoV2 spike protein is shown.

REGN-COV2 is a combination of two monoclonal antibodies (REGN10933 and REGN10987) and was developed specifically for SARS-CoV-2 therapy [9]. Baum A. et al. (2020) evaluated the efficacy of an in vivo REGN-COV2 antibody "cocktail" in both macaques, which modeled mild disease, and golden hamsters, which modeled more severe disease. Macaques and golden hamsters treated with REGN-COV2 had significantly lower levels of subgenomic viral mRNA at both prophylactic and therapeutic doses. In macaques, a decrease in mRNA was observed in swabs from the oral cavity and nasopharynx, as well as in bronchoalveolar lavage [10].

Regeneron Pharmaceuticals researchers tested an experimental serum with REGN-COV2 antibodies, and stated that this drug reduces the concentration of coronavirus disease in the blood and has a positive effect on the symptoms of non-hospitalized patients.

In the bioRxiv preprint database, the results of testing the effectiveness of combinations of neutralizing monoclonal antibodies REGN-COV2 against SARS-CoV-2 on laboratory animals have appeared. REGN-COV2 includes the REGN10987 and REGN10933 antibodies developed by Regeneron Pharmaceuticals. Research results show that REGN-COV2 has pronounced prophylactic and therapeutic properties: in animals that received antibodies, the viral load was lower, and the manifestations of infection were less pronounced compared to placebo groups. It was previously shown

that the D614G amino acid substitution in the SARS-CoV-2 S-protein does not affect the neutralizing capacity of REGN10987 and REGN10933 antibodies.

Clinical studies of the REGN10987+REGN10933 combination (NCT04426695, NCT04425629 and NCT04452318) are currently underway [11].

The Omeros company is investigating the experimental drug narsoplimab for the treatment of patients with acute respiratory distress syndrome against the background of the coronavirus disease. Narsoplimab is a high-affinity fully human IgG4 monoclonal antibody that binds lectin-associated serine protease-2 (MASP-2) and blocks the lectin pathway of complement system activation. The lectin pathway is one of the major complement pathways and is primarily activated by tissue injury and microbial infection. MASP-2 inhibition does not affect the classical pathway of complement activation as a critical component of the adaptive immune response to infectious agents. Because MASP-2 also acts directly on the coagulation cascade and contact system by cleaving prothrombin to thrombin and forming fibrin clots, its inhibition with narsoplimab prevents thrombus formation associated with microvascular damage and MASP-2-mediated activation [12].

Monoclonal antibodies have been successfully used for a long time in the therapy of autoimmune diseases, in oncology and oncohematology. Currently, the use of monoclonal antibodies to interleukin-6 (IL-6) in the treatment of COVID-19 is at the stage of clinical research. IL-6 is a key mediator of the systemic inflammatory response in the state of hypercytokinemia, which is found in patients with severe acute respiratory distress syndrome due to SARS-CoV2. Its impact allows you to prevent the development of a pro-inflammatory cascade, including avoiding the activation of antigen-presenting cells, T- and B-lymphocytes, monocytes and macrophages, endothelial cells and fibroblasts, which, in turn, prevents excessive synthesis of connective tissue components [13].

Several mechanisms of action of drugs of this group are distinguished:

- inhibition of cytokines: sirukumab, olokizumab;
- IL-6 receptor inhibition: tocilizumab, sarilumab, levilimab;

- inhibition of signaling pathways (janus kinase): tofacitinib, baricitinib, upadacitinib [14].
- Regarding certain drugs that are at the stage of clinical research.

**Tocilizumab.** The manufacturer of tocilizumab is the American biotechnology company Genentech Roche Group. The Food and Drug Administration, an agency of the US Department of Health and Human Services, has now approved a double-blind, randomized, phase III clinical trial of tocilizumab for use in standard-of-care regimens in adults with severe SARS-CoV-2 pneumonia.

**Sarilumab.** Human monoclonal antibody (IgG) to IL-6 receptor. It specifically blocks both soluble and membrane receptors and inhibits IL-6-mediated signaling involving ubiquitous signaling glycoprotein 130 and STAT-3 proteins. In functional studies on human cells, it was proven that the drug works only in the presence of IL-6 [15, 16].

Currently, the possibility of requalification of existing drugs for cell therapy is being considered. In particular, natural killer (NK) drugs, which normally cause the lysis of cells affected by the virus, are at the stage of clinical trials, which can be used in SARS-CoV-2 therapy. Such drugs include Tanira-leucel (CYNK-001). Production of CYNK-001 from placental stem cells is carried out by Sorrento Therapeutics and Celularity, the range of application of the drug includes oncohematological pathology, in particular, acute myeloid leukemia and multiple myeloma. Currently, the possibility of using CYNK-001 for the treatment of COVID-19 is being investigated [17]. Clinical studies on the use of genetically engineered NK cells obtained from the blood of immunized persons are registered in the "Register of candidate drugs for the treatment and prevention of COVID-19".

Another option of cell therapy is the use of mesenchymal stem cells (MSCs), which have anti-inflammatory and immunomodulatory effects. In the "Register of candidate drugs for the treatment and prevention of COVID-19" 16 clinical studies on the use of MSCs are registered. Their use is suggested for acute and chronic lung damage and acute respiratory distress syndrome. Given the pathogenesis and high lethality of SARS-CoV-2 lung damage, such therapy can be considered justified [18, 19]. The introduction of MSCAGEII had

a positive effect on the treatment of patients with pneumonia caused by COVID-19, especially in the case of patients whose condition was critical, while a decrease in the level of pro-inflammatory and an increase in the level of anti-inflammatory cytokines were established [20].

Modern therapeutic options for the treatment of COVID-19 combine a diverse arsenal of drugs that target both the virus and the immune response. Although the pathogenic mechanisms of the new infection have been partially deciphered, current treatment methods have not yet lived up to initial expectations, due to the presence of side effects and generally conflicting research results.

The number of experimental molecules proposed against SARS-CoV-2 is constantly increasing in an attempt to offer short-term solutions, especially for patients with severe disease. As a result of numerous clinical studies, these options have been proven to be effective in the treatment of SARS-CoV-2, although they may require further analysis and adaptation regarding the complex pathogenetic mechanism of the development of this disease. [21, 22]. Molecules that are effective at one stage of the disease may be destructive at another. Therefore, the best treatment option is likely to be a molecule that has the ability to prevent the penetration of the virus and the development of an unpredictable immune response. That is why the main direction of work in the long term has become the creation of vaccines [23, 24]. While the development of monoclonal antibodies is the best treatment option in the short term, it requires further in-depth studies of cellular invasion and immune dysregulation [25].

## CONCLUSION

Thus, strategies involving the use of virus-neutralizing monoclonal antibodies, natural killers, mesenchymal stem cells, and monoclonal antibodies to interleukin-6 are promising in the treatment of patients with COVID-19.

Monoclonal antibodies were developed on the basis of antibodies from the blood plasma of convalescents, which block the penetration of SARS-CoV-2 into target cells and stop the progression of the disease.

Monoclonal antibodies are used in patients with COVID-19 with a mild or moderate degree of the disease in the first days of the disease, with a high risk of progression to a severe disease (diabetes, chronic diseases of the kidneys, cardiovascular system, pregnant women, etc.).

Treatment with monoclonal antibodies is carried out in health care facilities that provide inpatient medical care to patients with COVID-19.

**Conflict of interests.** The authors of this manuscript claim that there is no conflict of interest during the research and writing of the manuscript.

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## ЗАСТОСУВАННЯ ГЕННОЇ ІНЖЕНЕРІЇ В ЛІКУВАННІ ХВОРИХ НА COVID-19

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**Актуальність.** Сучасні терапевтичні варіанти лікування COVID-19 поєднують використання препаратів, які впливають як на сам вірус, так і на компоненти імунної відповіді організму. Незважаючи на те, що патогенетичні механізми інфекційного захворювання вивчені частково, методи лікування все ще не виправдовують очікувань, що значною мірою пов'язано з розвитком побічних реакцій на препарати та суперечливими результатами лікування. Така ситуація зумовлює необхідність аналізу сучасних наукових джерел щодо перспектив, переваг і недоліків використання віруснейтралізуючих моноклональних антитіл, природних кілерів, мезенхімальних стовбурових клітин і моноклональних антитіл до інтерлейкіну-6.

**Ціль:** вивчити поточні дослідження моноклональних антитіл, які використовуються для лікування важких випадків коронавірусної хвороби, викликані SARS-CoV-2, і відзначити їх переваги.

**Матеріали та методи:** у статті використано бібліографічний метод і є оглядом наявних праць на PubMed та Google Scholar.

**Результати та обговорення.** Дані про наявність нейтралізуючих антитіл у плазмі крові хворих пацієнтів дали поштовх для отримання гуманізованих або повністю людських моноклональних антитіл, потенційно здатних стати основою для розробки препаратів для таргетної терапії SARS-CoV-2. При застосуванні біологічної терапії створюється максимальна вибірковість впливу на імунну систему, що дає можливість усунути одну з ланок патогенетичного ланцюга без істотного ураження клітин інших органів і систем. Також розглядається можливість перекваліфікації існуючих препаратів для клітинної терапії.

**Висновки.** Таким чином, стратегії, що передбачають використання віруснейтралізуючих моноклональних антитіл, природних кілерів, мезенхімальних стовбурових клітин і моноклональних антитіл до інтерлейкіну-6, є перспективними в лікуванні пацієнтів з COVID-19.

**Ключові слова:** COVID-19, моноклональні антитіла, мезенхімальні стовбурові клітини.