

Brief information about the project

Title	IRN AP26102743 «Nanobodies (single-domain antibodies) as next-generation tools for tuberculosis therapy»
Relevance	Tuberculosis (TB) is one of the most common infectious diseases, which is the main cause of widespread mortality, especially among people living with HIV. The disease is caused by a type of bacteria called <i>Mycobacterium tuberculosis</i>. According to data from the World Health Organization, 10.6 million people were infected with <i>mycobacterium tuberculosis</i> (MT) and 1.3 million people died from TB in 2022 (including 167 000 people with HIV). The spread of antibiotic resistance is one of the most pressing public health problems, threatening to turn tuberculosis into an incurable disease with a high mortality rate, especially in resource-limited settings. According to projections, if no action is taken, tuberculosis, along with malaria and <i>Escherichia coli</i>, will become one of the three main factors causing economic damage due to antimicrobial resistance, which accounts for approximately 2.5-3.0% of the global gross domestic product (GDP) and will reach US\$ 100 trillion by 2050. In 2023, 41.6 billion tenge was allocated from the republican budget of Kazakhstan for the treatment of tuberculosis. Every year in Kazakhstan, about 7 thousand new cases of tuberculosis are registered at the outpatient and polyclinic service level, of which 2 thousand people fall ill with multidrug-resistant tuberculosis. Seven-eight percent of them die. The development of more effective treatments for tuberculosis (TB) is critical, especially given the rise of drug-resistant strains. The progress in TB drug development has been slow and none of the new drugs tested so far have allowed standard treatment regimen shortening. Host-directed therapy using immunomodulators is a promising approach which must be explored for better control of TB. One such approach has been the development of monoclonal antibodies (mAbs) to prevent infections or treat infections in combination with antibiotics. Although mAbs can neutralize pathogens, their instability, large size (150 kDa), high cost and labor-intensive production, and low efficacy due to low tissue penetration pose many challenges. The discovery of heavy chain antibodies (HCAbs) found naturally only in camelids (<i>Camelidae</i>) has ushered in a new era in the engineering of highly specific antibodies. Therapy with nanobodies is now considered a very promising alternative for the treatment of dangerous bacterial infections. Nanobodies are the smallest functional fragments (15kDa) of heavy chain-only antibodies naturally occurring in <i>Camelidae</i> and represent the antigen-binding variable domain. Nanobodies have several advantages, including cost-effectiveness and ease of production in large quantities in bacteria, good solubility, resistance to significant temperature and pH fluctuations. Due to their small size, nanobodies can penetrate tissues

	and cells more effectively than conventional antibodies. This property is especially useful for targeting intracellular pathogens such as MT, which reside inside host cells. In addition, nanobodies can be formulated as inhalation aerosols, allowing direct delivery to the lungs, where MT primarily infects host cells. Inhalation nanobodies can improve drug delivery efficiency and minimize systemic side effects. Based on these features, nanobodies become a more promising tool for diagnosing and treating various diseases, including tuberculosis, compared to conventional antibodies.
Goal	Development and evaluating therapeutic potential of nanobodies targeting to a key protein in the pathogenicity and cell wall synthesis of <i>Mycobacterium tuberculosis</i> .
Tasks	1. Expression of <i>Mycobacterium tuberculosis</i> Ag85A and Ag85B proteins as correctly folded recombinant protein and evaluation of antigenicity of the expression products; 2. Construction of a phage-displayed cDNA-VHH-library from camels immunized with Ag85A and Ag85B proteins and isolation of antigen specific nanobodies; 3. Generation anti-Ag85A and Ag85B nanoantibodies fused with the Fc fragment of human IgG (VHH-Fc); 4. Characterization <i>in vitro</i> and <i>in vivo</i> therapeutic potential of nanobodies (VHHs and VHH-Fc).
Expected and Achieved Results	Tuberculosis is one of the top infectious disease killers worldwide. Developing more effective diagnostics and treatments would contribute to the global fight against TB, aligning with international goals such as the WHO's End TB Strategy, which aims to reduce TB deaths by 90% by 2030. Nanobody-based therapies, which are cheaper to produce than traditional monoclonal antibodies, can reduce TB treatment costs. This has the potential to make treatment more accessible, especially in resource-limited settings. With the rise of multi-drug-resistant TB (MDR-TB), new therapies like nanobodies offer novel mechanisms of action, potentially addressing the challenge of drug-resistant TB strains. Results from this project could lead to the development of new diagnostic tools and treatments, creating opportunities for pharmaceutical companies to commercialize these innovations. This could increase exports, drive revenue growth, and strengthen the economy. As a result of the project implementation (in the relevant field of science), at least 1 (one) Doctor of Philosophy (PhD) will be prepared. In this case, the dissertation defense is possible no later than 2 (two) years after the completion of the project. According to the competition documentation for grant funding for scientific and (or) scientific and technical

	projects for 2025-2027, according to the results of the project for the entire implementation period, it is planned to publish: - at least 3 (three) articles and (or) reviews in peer-reviewed scientific publications indexed in the Science Citation Index Expanded of the Web of Science database and (or) having a CiteScore percentile in the Scopus database of at least 50 (fifty); at least 1 patent for an invention (including a positive decision on it); at least 2 (two) articles or reviews in a peer-reviewed foreign or domestic publication recommended by the CQAFSHE; - or at least 2 (two) article or review in a peer-reviewed scientific publication included in the 1st (first) or 2nd (second) quartile of the impact factor in the Web of Science database and (or) having a CiteScore percentile in the Scopus database of at least 65 (sixty-five); at least 2 (two) articles or reviews in a peer-reviewed foreign or domestic publication recommended by the CQAFSHE; - or at least 1 (one) article or review in a peer-reviewed scientific publication that is included in the 1st (first) quartile by impact factor in the Web of Science database and (or) has a CiteScore percentile in the Scopus database of at least 80 (eighty); at least 1 patent for an invention (including a positive decision on it); at least 1 (one) articles or reviews in a peer-reviewed foreign or domestic publication recommended by the CQAFSHE. Based on the results of 2025 and 2026, the following was published: 1) Izat Smekenov, Nazym Tleumbetova, Sagit Bektassov, Amangeldy Bissenbaev. Revisiting the Antigen 85 Complex as a Target for Functional Antibody-Based Strategies Against Mycobacterium tuberculosis // MDPI Microorganisms. At the review stage.
Names and Surnames of Research Group Members with Their Identifiers (Scopus Author ID, Researcher ID, ORCID, if available) and Links to Corresponding Profiles	1. Bisenbaev Amangeldy Kuanbaevich, Doctor of Biological Sciences, Professor, Academician, NAS RK; http://orcid.org/0000-0001-7837-8685; Scopus Author ID – 24343057700 2. Smekenov Izat Temirgalievich, PhD, http://orcid.org/0000-0002-7739-7777; Scopus Author ID - 56688607600 3. Alybaev Sanzhar Dosanovich, https://orcid.org/0000-0002-7909-1835; Scopus Author ID - 57203727066 4. Kuanbai Aigerim Kurmanbekkyzy, PhD, https://orcid.org/0000-0001-6509-4085; Scopus Author ID - 57222715698 5. Baltakhozha Nurayim Bolatkyzy, https://orcid.org/0000-0003-2827-644X; Scopus Autor ID-59404177200 6. Tleumbetova Nazim Zholdasovna, https://orcid.org/0000-0001-9928-8021; Scopus Autor ID-57204759445 7. Marat Zhanar Zhanatkyzy 8. Ibragimova Leila Adikovna

Usage	Nanobodies represent a promising tool for the diagnosis and treatment of tuberculosis, caused by the bacterium <i>Mycobacterium tuberculosis</i>. Due to their small size, high stability, and ability to penetrate host tissues and cells, nanobodies can effectively bind to mycobacterial antigens and neutralize the pathogen. For therapeutic purposes, nanobodies can be used as standalone antimicrobial agents or in combination with traditional anti-tuberculosis drugs to enhance treatment efficacy, especially in drug-resistant forms of the infection. Their ability to penetrate infected macrophages makes them promising for targeting intracellular bacteria. Furthermore, nanobodies can be used to develop highly sensitive diagnostic tests for the rapid detection of mycobacterial antigens. They can also be formulated as inhaled drugs, enabling direct delivery to the lungs—the primary organ affected by tuberculosis. Thus, nanobodies can be used in three main areas: therapy, combination therapy with antibiotics, and diagnostics of tuberculosis, making them an important tool in the fight against infection caused by <i>Mycobacterium tuberculosis</i>.
Publications list with links to them	1. Zutterling C., Mursalimov A., Talhaoui I., Koshenov Z., Akishev Z., Bissenbaev A.K., Mazon G., Geacintov N.E., Gasparutto D., Groisman R., Zharkov D.O., Matkarimov B.T., Saparbaev M. Aberrant repair initiated by the adenine-DNA glycosylase does not play a role in UV-induced mutagenesis in <i>Escherichia coli</i>// PeerJ. –2018. –Vol.2018, №12. - Article number e6029. DOI: 10.7717/peerj.6029. Citation –0,73; Q1; Procentile -83%. 2. Kuanbay A., Taipakova S., Smekenov I., Saparbaev M., Ishchenko A., Bissenbaev A. <i>Arabidopsis thaliana</i> Poly(ADP-ribose) polymerases ADP-ribosylate DNA oligonucleotides// Journal of biotechnology. –2018. - Vol.280. - P.S87. DOI: 10.1016/j.jbiotec.2018.06.285. Citation -0,85; Q2; Procentile -71%. 3. Bazlekowa-Karaban M., Prorok P., Baconnais S., Taipakova S., Akishev Z., Zembrzuska D., Popov A.V., Endutkin A.V., Groisman R., Ishchenko A.A., Matkarimov B.T., Bissenbaev A., Le Cam E., Zharkov, D.O., Tudek B., Saparbaev M. Mechanism of stimulation of DNA binding of the transcription factors by human apurinic/aprimidinic endonuclease 1, APE1// DNA repair. –2019. –Vol.82. - Article number 102698. DOI: 10.1016/j.dnarep.2019.102698. Citation -0.82; Q2; Procentile -74%. 4. Smekenov I., Bakhtambayeva M., Saparbayev M., Taipakova S., Bissenbaev A.K. Heterologous secretory expression of β-glucosidase from <i>Thermoascus aurantiacus</i> in industrial <i>Saccharomyces cerevisiae</i> strains// The Brazilian Journal of Microbiology –2020. –Vol.51. – P.107–123. DOI: 10.1007/s42770-019-00192-1. Citation -0.681; Q3; Procentile -41%. 5. Smailov B., Alybayev S., Smekenov I., Mursalimov A., Saparbaev M., Sarbassov D., Bissenbaev A. Wheat germination is dependent on plant target of rapamycin signaling// Frontiers in Cell and Developmental

Biology – 2020. – Vol. 8. – Article №606685. DOI: [10.3389/fcell.2020.606685](https://doi.org/10.3389/fcell.2020.606685). Citation - 0.986; Q1; Procentile -84%.

6. Taipakova S., **Kuanbay A.**, Saint-Pierre C., Gasparutto D., Baiken Y., Groisman R., Ishchenko A, Saparbaev M., **Bissenbaev A.** The *Arabidopsis thaliana* Poly(ADP-Ribose) Polymerases 1 and 2 Modify DNA by ADP-ribosylating terminal phosphate residues // Frontiers in Cell and Developmental Biology – 2020. – Vol. 8. – Article № 606596. DOI: [10.3389/fcell.2020.606596](https://doi.org/10.3389/fcell.2020.606596) Citation - 0.87; Q1; Procentile -69%.

7. Smekenov I., **Alybavev S.**, Ayupov T., Rakhmatullaeva G., **Bissenbaev A.** A polyclonal antibody against a recombinantly expressed *Triticum aestivum* RHT-D1A protein // J Genet Eng Biotechnol. – 2020. - Vol 18, №52. DOI: [10.1186/s43141-020-00072-4](https://doi.org/10.1186/s43141-020-00072-4). Citation -1,038; Q2; Procentile -71%.

8. Akbay B., Germini D., **Bissenbaev A.K.**, Musinova Y., Sheval E., Vassetzky Y., Dokudovskaya S. Hiv-1 tat activates akt/mTORC1 pathway and aicda expression by downregulating its transcriptional inhibitors in B cells // International Journal of Molecular Science – 2020. - Vol 22, №52. DOI: [10.3390/ijms22041588](https://doi.org/10.3390/ijms22041588). Citation -0.71; Q1; Procentile -87%.

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Patent information	Absent

