GENERAL INFORMATION

1. NAME OF THE CENTER AND LOCATION

Department of Immunology
Institute of Microbiology "Stephan Angelov"
Bulgarian Academy of Sciences – Centre of Competence - *Fundamental, Translational and Clinical Investigations of Infections and Infectious Immunology*
1113, Sofia
Bulgaria

2. TYPE OF THE RESEARCH INFRASTRUCTURE AND/OR SCIENTIFIC EXPERTISE

Identify the type of the RI, equipment/facilities/ specific research, and in particular linked to COVID-19:	Scientific equipment for biomedical and pharmaceutical research & development: ❖ Cell culture rooms with HEPA clean air filter system and laminar flow cabinets, CO2 incubators; ❖ Barrier-type SPF animal facility with temperature control and HEPA system for breeding of laboratory mice; ❖ Bank of cell hybridomas and lines. ❖ BD™LSR II FACS with FCS Express™ Diva software allowing analysis of 18-different cell parameters; ❖ SACURA™ microtome, Leica Cryostat CM1950, Leica TP1020 Automatic Tissue Processor, and Leica Embedding Arcadia equipment for histology and immunohistochemistry; ❖ Nikon™ light and Leica™ DM2000, Leica™ DMi8 invert and light microscopes with digital cameras for picture capturing; ❖ Leica DM6 B fully automated upright fluorescent microscope; ❖ High performance Microplate Reader with advanced LVF MonochromatorsTM for Fluorescence and Luminescence ❖ Confocal laser scanning microscopy (CLSM) Nikon Eclipse Ti-U CLSM ❖ ELISpot ImmunoSpot® Series 6 Ultimate UV fluorescent Image Analyzer; ❖ Bio-Tek™ ELISA plate reader with Gen 5.0™ software; ❖ PCR - C1000 Touch™ Thermal Cycler with Dual 48/48 Fast Reaction Module ❖ Eppendorf™ micro UV/VIS spectrophotometer; ❖ Eppendorf BioSpectrometer;

- ❖ Arrayit InnoScan 1100 Microarray Scanner;
- ❖ MACS® cell sorting equipment and products;
- ❖ BioRad™ and SciePlas™ systems for electrophoresis and immunoblot;
- ❖ Countess™ II FL Automated Cell Counter;
- ❖ BioRad™ ChemiDoc™ MP Imaging System;
- ❖ Basic laboratory instruments and BioRad™ NGC Quest™ 10 Plus Chromatography System;

The main research interests of the Laboratory of Experimental Immunology are directed to animal models, vaccines and immunomodulators. The work is focus on the development of new approaches for generation of immune response through genetic engineering and protein molecules. A topic of interest is the development of humanized mouse models of human diseases and for vaccines tests. Widely covered in the activity of the team are studies on immunomodulatory activity of black sea and garden snail proteins as adjuvants for bacterial and viral vaccines.

KEY WORDS:
Vaccine development; Multi-epitope vaccines, nanoparticle delivery, Humanized animal models;

3. TYPE OF THE RESEARCH

Provide information on the research carried on or planned in regard with COVID-19 and other viruses	We hypothesize that it may be possible to generate protective anti-SARS-CoV-2 immune response in humanized NSG transfer mouse model. This will be achieved by administering to them of lipid-based nanoparticles carrying peptide molecules, comprising dominant immunogenic B and T cell epitopes from coronavirus SARS-CoV-2, predicted by EpiDOCK server for prediction of peptide binding to HLA class I/II proteins. The aim of this proposal is the knowledge-based development of next-generation coronavirus vaccine. The project starts with *in silico* analysis of public coronavirus sequence to identify immunogenic coronavirus B and T cell epitopes using sophisticated bio-informatics. Then, the synthesized peptides comprising T- and B-cell immune-dominant coronavirus epitopes will be included within delivery lipid-based nanoparticles. The *in vitro* data will be validated with the animal data and patient´s data by which we expect the reduction of animal experiments for dose

	findings. The NSG/RAG2 mice lack mature B- and T- and NK-cells, which allows the transfer of foreign cells of human origin. Therefore, NSG mice are perfect recipients of normal human lymphoid cells, and the humanized animals are a suitable model to investigate the efficiency of therapeutic interventions. The major expectations from the novel adjuvanted multi-epitope coronavirus vaccine formulation are to (i) achieve prolonged duration of protective immune responses, based on neutralizing antibodies and memory CD8+ T cells; (ii) exhibit a good safety profile.
4. WEBSITE	
Provide the internet address:	www.labexpimm.eu www.microbio.bas.bg
5. BACKGROUND, PUBLICATIONS AND OPEN DATA REPOSITORY	
leading research team AND Scientific publications of the research group on the topics of related to coronaviruses research results**;** **link to open data repository**	We used already experimental humanized NOD/SCID mouse model to test a new anti-flu vaccine. The eukaryotic expression vector system pTriEx-3 Neo, encoding a scFv fragment from mouse anti-human FcγRI monoclonal antibody, coupled to a sequence encoding a T- and B-cell epitope-containing influenza A virus hemagglutinin intersubunit peptide was used as a naked DNA vaccine and introduced directly to experimental humanized NOD/SCID mice with or without boosting with the expressed fusion protein. Immunization with the generated DNA chimeric molecules, and prime-boost with the expressed recombinant proteins induced significant serum levels of anti-influenza IgG antibodies and strong CTL activity against influenza virus-infected cells in humanized animals. **Research team:** **Head:** Assoc. Professor Andrey Tchorbanov, PhD Head, Department of Immunology Institute of Microbiology Stephan Angelov Bulgarian Academy of Sciences 26, Georgi Bonchev str; 1113, Sofia, Bulgaria ☎: +359 2 979 6357; ✉: tchorban@microbio.bas.bg

Members:

Assist. Professor Nikolina Mihaylova, PhD, mihaylova_n@microbio.bas.bg

Assist. Professor Kalina Nikolova-Ganeva, PhD, nikolova_k@microbio.bas.bg

Assist. Professor Petya Ganova, PhD, pganova@gmail.com

Assist. Professor Nikola Kerekov, PhD, nikolakerekov@gmail.com

Assist. Professor Iliyan Manoylov, PhD, iliyanmanoylov@gmail.com

Silviya Bradyanova, PhD student, silvy.bradyanova@gmail.com

Gabriela Boneva, PhD student, gabriela.v.boneva@gmail.com

Nikola Ralchev, MSc, niki_951101@abv.bg

Emiliya Stoyanova, MSc, emi24_bg@abv.bg

Blagovesta Boneva, MSc, blagi_52@abv.bg

1. Ivanova I., Mihaylova N., Manoylov, I., Makatsori, D., Lolov, S., Nikolova M., Mamalaki, A., Prechl J., Tchorbanov A. Targeting of Influenza viral epitopes to antigen presenting cells by Genetically Engineered Chimeric Molecules in Humanized NSG transfer model. Human Gene Therapy 2018, 29(9), 1056-1070.

2. Moussa, M, Arrode-Brusés, G, Manoylov, I, Malogolovkin, A, Mompelat, A, Ishimwe, H, Smaoune, A, Ouzrout, B, Gagnon, J, Chebloune, Y. A novel non-integrative single-cycle chimeric HIV lentivector DNA vaccine. Vaccine, 2273-2282, 2015.

3. Gesheva V., Chausheva S., Stefanova N., Mihaylova N., Doumanova L., Idakieva K., Tchorbanov A. *Helix pomatia* hemocyanin - a novel bio-adjuvant for viral and bacterial antigens. Int Immunopharmacol 2015, 26(1):162-168. DOI: 10.1016/j.intimp.2015.03.01;

4. Kerekov N., Ivanova I., Mihaylova N., Nikolova M., Prechl J., Tchorbanov A. Built-in adjuvanticity of Genetically and Protein Engineered Chimeric Molecules for Targeting of Influenza A peptide epitopes. Immunol Research 2014, 60(1), 23-34;

5. Gesheva V., Idakieva K., Kerekov N., Nikolova K., Mihaylova N., Doumanova L., Tchorbanov A. Marine gastropod hemocyanins as adjuvants of non-conjugated bacterial and viral proteins. Fish and Shellfish Immunol 2011, 30 (1), 135-142.

6. Ivanovska N., Tchorbanov A., Prechl J., Maximova V., Voynova E., Vassilev T. Immunization with a DNA chimeric molecule encoding a hemaglutinin peptide and a scFv CD21-specific antibody fragment induces long-lasting IgM and CTL responses to influenza virus. Vaccine 2006, 24, 1830 – 1837.

https://scholar.google.com/citations?user=11xS71IAAAAJ&hl=en
https://www.scopus.com/authid/detail.uri?authorId=6603347197

6. COORDINATOR

Full name of the coordinator organization;
Institute of Microbiology Stephan Angelov
Bulgarian Academy of Sciences – Centre of Competence - *Fundamental, Translational and Clinical Investigations of Infections and Infectious Immunology*
1113, Sofia
Bulgaria

Contact person;
Assoc. Professor Andrey Tchorbanov, PhD
Head, Department of Immunology
Institute of Microbiology Stephan Angelov
Bulgarian Academy of Sciences
26, Georgi Bonchev str; 1113, Sofia, Bulgaria
☎: +359 2 979 6357;
✉: tchorban@microbio.bas.bg

7. POSIBLE PARTNERS

Indicate the partner organizations

Full name of the partner
Sofia University “St Kliment Ohridski”
Sofia
Bulgaria
www.uni-sofia.bg

Contact person;
Assoc. Professor Christo Tzachev, PhD
Head of Laboratory of Pharmaceutical Technology, Faculty of Chemistry and Pharmacy,

	Sofia University “St Kliment Ohridski” ✉: christo.tzachev@gmail.com
	Full name of the partner Medical University of Sofia, Faculty of Pharmacy 1000 Sofia Bulgaria www.mu-sofia.bg *Contact person;* Professor Irini Doytchinova, DSc Head, Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, ✉: idoytchinova@pharmfac.mu-sofia.bg
	Full name of the partner National Center of Infectious and Parasitic Diseases National Reference Laboratory of Immunology 26, Yanko Sakazov Boul. 1504 Sofia, Bulgaria www.ncipd.org *Contact person;* Professor Maria Nikolova, MD, Dsc Head, Reference laboratory of Immunology Head, Immunology and Allergy Department National Center of Infectious and Parasitic Diseases ✉: mstoimenova@ncipd.org
	Full name of the partner University Eötvös Lorand Department of Immunology; Budapest, Hungary; www.elte.hu/en *Contact person;* Assoc. Professor Jozsef Prechl, MD, PhD

	Department of Immunology; Immunology Research Group, Hungarian Academy of Sciences ✉: jprechl@gmail.com
	Full name of the partner University Tor Vergata Rome, Italy www.web.uniroma2.it *Full name of the partner* Professor Maurizio Mattei, PhD Director of the Station for Animal Technology - University of Rome "Tor Vergata" ✉: mattei@uniroma2.it

8. IMPLEMENTED AND RUNNING PROJECTS

Projects related to virology, vaccines, infection diseases …	**1.** Institute Pasteur International Network Grant - IP Corona Task Force 2 "*VacciNanoCor - Development of novel nano-based multi-epitope vaccine against coronavirus SARS-CoV-2 - Generation of Humanized NSG transfer mouse model for vaccine testing*" 2020-2021; Project leader A. Tchorbanov **2.** Science and Education for Smart growth Operational Programme grant - Creation and Development of Centres of Competence - „Fundamental, Translational and Clinical Investigations of Infections and Infectious Immunology"; BG05M2OP001-1.002-0001; 2018–2023. Project coordinator for IM-BAS A. Tchorbanov **3.** H17/40 NFSI – Bulgaria "*Multiplex Detection of Bacterial Toxins Using a Magnetic nanoparticle-Immunoassay Technology*" 2017–2021; Project leader T. Godjevurgova; project leader for IM-BAS A. Tchorbanov; Copernicus Contract CIPA CT 94-0152 *„Genetically Engineered Vaccines"* 1995-1998; Project leader P. Capel **4.** ACIP - ACTIONS CONCERTEES INTERPASTEURIENNES. „*Viral Antigen Targeting by Genetically Engineered Chimeric Molecules"* 2013-2015; Project leader A. Tchorbanov

5. Bulgarian-Hungarian international join project "*Genetically engineered viral DNA vaccines"* 2010-2013; Project leader A. Tchorbanov

6. NFSI – Bulgaria DTK 02/19 "*DNA vaccines: Viral Antigen Targeting by Genetically Engineered Chimeric Molecules*" 2010-2016; Project leader A. Tchorbanov