

## EXPERT OPINION

**By:** Assoc. Prof. Dr. Ivanka Georgieva Tsacheva, Department of Biochemistry, Sofia University "St. Kliment Ohridski", member of the scientific jury appointed by order No I-39 / 23.04.2021 of the Director of Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences, Prof. Penka Petrova, Dr.

**Re:** The materials submitted for participation in a competition for the academic position „Associate Professor" at the Department of Immunology, Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences in Higher Education area 4. *Natural sciences, mathematics and informatics*; Professional area 4.3. *Biological sciences*, scientific specialty "Immunology".

The competition for the academic position "Associate Professor" in Higher Education area 4. *Natural sciences, mathematics and informatics*; Professional area 4.3. *Biological sciences*, scientific specialty "Immunology" has been launched for the needs of the Department of Immunology, Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences in SG no. 20/09.03.2021. Assist. Prof. Dr. NIKOLINA MIHAYLOVA MIHAYLOVA is the only applicant who has submitted documents for this competition within the deadline regulated by the law. She currently works on a permanent position at the same department.

### **General presentation of the procedure and the applicant**

The presented materials are in compliance with the requirements of the Act for the Development of the Academic Staff in Republic of Bulgaria, the Regulations for its implementation, and the Regulations for the conditions and the order for acquiring scientific degrees and holding academic positions in the Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences. They also meet the recommended criteria for holding the academic position of "Associate Professor" in Professional area 4.3. *Biological sciences*. The documentation for the competition is structured in a way that fully reflects the educational, scientific and applied research activities of the applicant in both qualitative and quantitative aspects.

Assist. Prof. Dr. Nikolina Mihaylova graduated from Sofia University "St. Kl. Ohridski", Faculty of Biology in 2005 with a Master degree in Cell Biology and Pathology. She defended her PhD thesis "Immunomodulating activity of new experimental IgM and IgG preparations" in 2008 at the Department of Immunology, Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences. She began her academic career at the same department in 2004 as a research associate. In 2007 she conducted a medium-term specialization at INSERM Institute, France in the laboratory headed by Dr. Srinivasa Kaveri.

Assist. Prof. Dr. Nikolina Mihaylova has authored 36 articles in peer-reviewed and indexed journals including 26 scientific articles with a total IF of **93,672**, 8 articles in journals without IF and 1 chapter in a book published by a foreign publishing house. According to Scopus/Web of science research databases, the presented papers are cited 256 times. Twenty-three publications are presented in the competition for the academic position "Associate Professor". Among them, 19 research papers in international peer-reviewed and indexed journals with a total IF of **61,011**, distributed in quartiles as follows: Q1 - 6 papers, Q2 - 11 papers, Q3 - 2 papers, Q4 - 1 paper and

1 book chapter. The reference in Scopus/Web of science and other databases shows that these articles were cited 102 times. Her H-index is 9. The results of the research activity were reported at 69 international and national scientific forums. The intensive scientific and applied research, and educational activity of Assist. Prof. Dr. Nikolina Mihaylova and her professional skills in the field are reflected in her participation in 16 projects.

## **Assessment of academic achievements of the applicant**

### *Scientific papers*

The reference for compliance with the minimal state requirements in accordance with Art. 2b of the Act for the Development of the Academic Staff in the Republic of Bulgaria for Higher Education area 4. *Natural sciences, mathematics and informatics*; Professional area 4.3. *Biological sciences*, scientific specialty "*Immunology*", indicates that the applicant research achievements fully fit the stipulated criteria, as follows:

✓ Indicators of group A: PhD thesis	<b>50 p.</b> (min 50)
✓ Indicators of group B: monograph	<b>185 p.</b> (min 100)
✓ Indicators of group C: research articles	<b>262 p.</b> (min 200)
✓ Indicators of group D: citations	<b>204 p.</b> (min 50)
✓ Indicators of group E: projects	<b>213 p.</b> ( <i>not required</i> )

Clearly, the applicant's academic achievements exceed the minimal state requirements. Assist. Prof. Dr. Nikolina Mihaylova also exceeds the additional requirements set by the Institute of Microbiology "Stefan Angelov", Bulgarian Academy of Sciences for a H-index of 5 and participation in 3 projects.

### *Scientific and applied research contribution*

The applicant's research contribution is in the field of experimental immunology. These contributions can be grouped as achievements of scientific and applied research.

#### Major scientific contribution:

✓ *Selective inhibition of pathological autoreactive cells by protein-engineered antibodies and by monoclonal antibodies in mouse and human models of autoimmunity.*

The publications included in this competition present the search of specific approaches to the therapy in autoimmune diseases, in particular **Systemic lupus erythematosus (SLE) and autoimmune type 1 diabetes**. The developed therapeutic approaches include an innovative selective suppression of autoreactive B lymphocytes. Protein-engineered chimeric molecules comprising an antibody specific for an inhibitory B-cell receptor and conjugated antigenic peptides have been constructed. This selective approach was tested in three experimental model systems - in lupus-prone animals, in pristane-induced lupus and in humanized mice. The selectivity was achieved by:

- Hybrid molecules constructed by binding of the DNA mimotope (DWEYSVWLSN peptide) and the CD22-binding STN epitope with free terminal sialic acid to a murine monoclonal IgG antibody. The engineered chimera specifically inhibits the *in vivo* production of anti-DNA IgM and IgG antibodies and slows the development of glomerulonephritis in lupus-prone animals by inhibiting B- and T-cell proliferation, and limiting the number of anti-DNA-producing plasma cells.
- a chimeric molecule composed of monoclonal antibody against a human inhibitory receptor for complement type 1 associated with the DNA mimotope.
- suppression of autoreactive B and T cells is alternatively tested by a monoclonal antibody against ANX A1 in a pristane-induced mouse model of lupus. Administration of this monoclonal antibody results in inhibition of T cell activation and proliferation, inhibition of IgG anti-dsDNA secreting plasma cells and urinary protein deposition, reduced disease activity and prolonged animal survival compared to controls.
- suppressing anti-GAD65 IgG-producing B lymphocytes in a murine C57BL / 6J model of STZ-induced type 1 autoimmune diabetes by bispecific chimeric molecules containing 2.4G2 monoclonal antibody conjugated to epitope peptides from the GAD65 molecule.

✓ Natural biological molecules with anti-tumor and adjuvant properties.

- A mouse model of colon cancer is developed in which the anti-tumor and anti-proliferative properties *in vivo* of hemocyanins isolated from *Rapana thomasiana* (RtH) and *Helix pomatia* (HpH) was tested. In animals immunized with RtH and HpH, suppression of solid tumor formation, splenomegaly and lung metastases was observed and the survival of treated animals was extended. An increase in the humoral anti-tumor response was also observed.
- Hemocyanins isolated from the land snail *Helix pomatia* (HpH) and *Rapana thomasiana* (RtH) were characterized as bio-adjuvants in combination with standard antigens. Immunization with HpH-TT (purified HpH in combination with a peptide from the viral hemagglutinin (IP) or with tetanus toxoid (TT)) resulted in increased levels of anti-TT IgG-producing plasma cells and induction of B- and T-cell proliferation. Immunization of experimental animals with the combination HrH-IP caused the generation of a strong cytotoxic anti-influenza response. RtH in combination with a viral peptide, part of the hemagglutinin molecule, resulted in the generation of highly immunogenic molecule. These results demonstrate for the first time that RtH and/or its subunits can be used in various immunization protocols as adjuvants or as carrier proteins.

✓ Immunomodulatory activity of immunoglobulin molecules.

- Modulation of the immunoreactivity of pooled human IgG *in vivo* under the influence of the aggressive inflammatory environment.

