



## REVIEW

by Assoc. Prof. Lyudmila Vladimirova Kabaivanova, PhD, The "Stephan Angeloff" Institute of microbiology- BAS, on the materials submitted for participation in a competition for the academic position "Associate Professor" in the professional field 4.3. Biological sciences, scientific specialty "Virology", code 01.06.13.

Candidate: Assist. Prof. Ani Krasimirova Georgieva, PhD

### Common part

The competition for the academic position of "Associate Professor" in the professional field 4.3. "Biological Sciences", scientific specialty "Virology", code 01.06.13, was published in the State Newspaper, issue 57 of 16. 07. 2021, for the needs of the section "Pathology" in IEMPAM - BAS. The only candidate is Assistant Professor Dr. Ani Krasimirova Georgieva from the same institute. The review of the documents shows that the procedure for opening and announcing the competition has been followed. The presented set of materials is also prepared in accordance with the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria (RASRB) and the Regulations for its application and the Regulations for Development of the Academic Staff of IEMPAM - BAS, meeting the criteria for acquiring the academic position "Associate Professor".

### Brief biographical data

Ani Krasimirova Georgieva received her master's degree from Sofia University "St. Kliment Ohridski" - Faculty of Biology, speciality in Molecular Biology and specialization: Animal and Human Physiology. The candidate has been working at IEMPAM-BAS since 2002, successively passing through the positions of laboratory assistant, specialist - molecular biologist, assistant and chief assistant - 2015. She is a full-time doctoral student and acquired the ESD "Doctor" in Virology at the same institute in 2014, after successfully defending a dissertation on "*In vitro*" and "*in ovo*" models of chemical and retrovirus-induced carcinogenesis. For more than 16 years the candidate has been gaining professional experience in the field of cell biology, virology and experimental pathology.

### General presentation of the scientific papers

Dr. Georgieva has published a total of 63 scientific papers. Five of the publications are on the list for obtaining the educational and scientific degree "Doctor". She participated in the competition with 35 publications. Of these, 23 are publications that are referenced and



indexed in world-famous databases of scientific information (IF = 34.123) and **12** have been published in other scientific journals. Her works have been cited **116** times, and the participating in the competition - **93** times, with h-index - **6**. The results of her research work have been presented at more than **70** national and international scientific forums.

According to the submitted report for fulfillment of the minimum requirements for the academic position "Associate Professor", it can be seen that the candidate fully meets all requirements - she has submitted materials corresponding to **617 points** out of the required 430. In the groups of indicators **A - 50 points; C - 100 points; D - 281** of the required 220 and **E - 186** of the required 60.

Dr. Georgieva's scientific activity can be highly valued based on her high scientometric indicators.

### **Project and educational activity**

Ani Georgieva has participated in the implementation of 14 research projects, 10 of which are funded by the NSF of the Ministry of Education and Science, and two are contracts with higher education institutions in the country and two - under European programs. She also takes part in the training of graduates from higher education institutions in the country and abroad.

### **Scientific contributions**

The main contributions of Dr. Georgieva's research are in the following areas: virology, oncovirology and experimental oncology.

The research and the conducted experimental work are aimed at developing new approaches and strategies for prevention and treatment of oncological diseases and elucidation of the factors and mechanisms responsible for the initiation and development of neoplastic processes. It is shown that by applying a complex approach in studying the biological activity and safety of various chemical compounds *in vitro*, *in ovo* and model systems, the effectiveness of some natural products, newly synthesized chemical compounds and new nanostructured materials, their potential application in the treatment of neoplastic and virus-induced diseases was proved.

The topics she develops are undeniably relevant due to the fact that the number of cancer victims is constantly increasing, which gives reason to consider it a leading disease worldwide, and the right actions for prevention and treatment would lead to longer life expectancy and improving people's quality of life.

In her research work, Ani Georgieva applies a wide range of contemporary molecular biological, cellular biological, virological, histopathological and morphological methods to



develop new experimental model systems for research on carcinogenicity and antitumor effects of natural and synthetic substances.

In the field of virology, her research is aimed at taxonomic identification of isolated in Bulgaria avian oncogenic retroviruses and determination of their phylogenetic relationship with other representatives of the group of avian leukemia and sarcoma viruses, model systems for studying the oncogenic properties of viruses and the mechanisms of virus - induced neoplastic processes.

Participates in the introduction of an innovative molecular biological approach to study the genetic and species diversity of viruses in honey bees in Bulgaria and to clarify their phylogenetic origin and possible paths of their geographical distribution.

In particular, contributions may be distributed as follows:

Scientific and applied contributions in the field of **virology**

1. Molecular biological tests for detection and identification of six of the most common viruses in honey bees *Apis Mellifera* have been conducted and the most common viruses in bees in Bulgaria have been identified, and their origin and distribution have been clarified.
2. It has been established that the wing deforming virus (DWV) is the most widespread in all studied regions of the country. The sequence analysis showed that the virus belonged to the most pathogenic strain type A.
3. The vesicular rot virus (SBV) ranks second among the viruses studied in terms of the frequency of infection. The greatest homology of the Bulgarian strains with those from the Pacific region has been established, which reveals a new, hitherto unclear path of SBV spread in Europe.
4. The acute paralysis virus (ABPV) is detected with a lower frequency, only in samples from bee families in Northern Bulgaria, as a significant contribution is to clarify the geographical distribution of ABPV in Southeast Europe. The black queen virus (BQCV) shows the lowest prevalence.

Scientific and applied contributions in the field of **oncovirology**

5. A permanent Grafi virus-induced myeloid tumor cell line was derived from a hamster, allowing the screening of a large number of substances for antitumor activity under *in vitro* conditions and the selection of the most active ones for further studies in experimental animals, as well as and to determine optimal doses to be administered in conducting *in vivo* experiments. This new *in vitro* model system contributes to the reduction of experimental animals in the experiments.

Scientific and applied contributions in the field of **experimental oncology - cytotoxicity, genotoxicity and carcinogenicity**



6. The fungicide mancozeb exhibits a concentration-dependent cytotoxic effect on primary cultures of Syrian Golden Hamster embryonic cells and on cell cultures of the BALB / c 3T3 constant cell line, but is not able to induce morphological transformation of cells in *in vitro* tests for cancer and causes no statistically significant increase in the number of erythrocytes with micronuclei in an *in ovo* genotoxicity test.
7. The cytotoxic effect of the mycotoxin fumonisin B1 in cell cultures of embryonic cells from birds and mammals has been demonstrated, with mycotoxin causing a concentration-dependent decrease in cell viability and the effect being more pronounced in avian cells. Cytomorphological studies of cells cultured in the presence of fumonisin B1 show pronounced apoptotic and necrotic changes, the mechanism of action showing that fumonisin B1 crosses the cell and nuclear membrane and is localized mainly in the perinuclear space and in the cell nucleus.
8. Newly synthesized anthracene-containing  $\alpha$ -aminophosphonates and their derivatives have been shown to have low cytotoxicity, no genotoxic and carcinogenic effects in *in ovo* model systems, and a weak clastogenic effect in the genotoxicity study in mice which are suitable candidates for medical use due to their structural similarity to  $\alpha$ -amino acids, their lipophilicity, allowing easy penetration through the cell and nuclear membrane and are promising in the design of new antiviral and antitumor drugs.
9. Strong cytotoxic and antiproliferative activity of the newly synthesized anthracene-containing  $\alpha$ -aminophosphonates and their derivatives was found in a panel of human tumor cell lines (HeLa - cervical adenocarcinoma; HT-29 - colorectal adenocarcinoma; MCF-7 and - MDA-MB-32 low invasive and highly invasive adenocarcinoma of the mammary gland; HepG2 - hepatocellular carcinoma; 647-V - bladder carcinoma) and in the Ehrlich carcinoma cell line in mice, which allows their use in the treatment of cancer.
10. In *in vitro* and *in vivo* model systems, the antitumor activity of alkylphosphocholine erufosine has been established - a new and promising class of antitumor agents, which by chemical nature are synthetic analogues of cellular phospholipids, by inhibiting the proliferation of Graft tumor cells, inducing cytoskeletal elements and apoptosis. The mechanism of action differs in that the main target is not the genetic apparatus of tumor cells, but in this case is due to disruption of lipid metabolism of tumor cells and modulation of lipid-dependent signal transduction involved in the control of cell proliferation, differentiation and death.
11. The protective antitumor effect of erufosine, administered alone or in combination with the conventional cytostatic doxorubicin in hamsters with experimental Grafi's experimental myeloid tumor, has been demonstrated *in vivo* as the applied therapy leads to decrease in metastatic activity and prolongation of median survival time. These effects are most pronounced in experimental animals treated with the combination of the two anticancer agents and show it as an antitumor agent useful in complex chemotherapy with increased efficacy and reduction of adverse side effects of the cytostatics used.
12. The antitumor properties of nanostructured polymeric materials with different design were shown, obtained by electrofibration and loaded with biologically active substances of natural origin (curcumin, ferulic acid, caffeic acid), by inducing apoptosis in tumor cells, such as low solubility in water, chemical instability, rapid metabolism and elimination from the human body, low bioavailability and limited therapeutic applications, which is overcome by including natural biologically active compounds in a suitable polymer matrix. The results show a higher cytotoxic and antiproliferative effect against HeLa cervical carcinoma cells of the tested materials compared to that of the free biologically active



substance and predetermine the possibility of future use in the local treatment of cervical tumors.

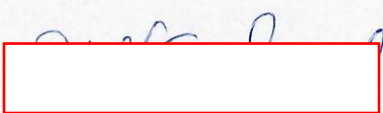
13. Different degrees of antitumor / antiproliferative activity of non-steroidal anti-inflammatory drugs against cells of 3 human tumor lines - cervical adenocarcinoma (HeLa), adenocarcinoma of the colon (HT-29) and breast cancer (MCF-7) have been demonstrated. An inhibitory effect of NSAIDs on the processes of adhesion and migration of cancer cells, which are key to the spread and metastasis of tumors, and thus to the discovery of new characteristics - antitumor properties of established and widely used in practice nonsteroidal anti-inflammatory drugs, their mechanisms of action on human cancer cells in *in vitro* experiments was shown.
14. The *in vitro* antitumor activity of hemocyanins isolated from sea snail *Rapana venosa*, garden snails *Helix lucorum* and *Helix aspersa*, as well as mucus from *Helix aspersa* in human colorectal carcinoma HT-29 cell line was studied, and the obtained experimental data show that hemocyanins and mucus from *Helix aspersa* show significant antineoplastic activity and the potential to develop new therapeutic agents for the treatment of colorectal cancer.

#### **Conclusion:**

The documents and materials submitted by Ani Georgieva meet all the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria (RASRB), the Regulations for its implementation and the Regulations for the Development of the Academic Staff of IEMPAM, BAS, exceeding the minimum national requirements for the acquisition of the academic position of "Associate Professor".

The presented materials give me a reason to express convincingly my positive opinion regarding the submitted candidacy for the academic position of "Associate Professor". It can be seen that Ani Georgieva is an established specialist with authority in the scientific community. As a member of the Scientific Jury for the announced competition, I give a positive assessment and recommend to the members of the respected Scientific Council of IEMPAM at BAS to elect Assistant Professor Dr. Ani Georgieva to the academic position of "Associate Professor" in a professional field 4.3. Biological sciences; scientific specialty Virology, code 01.06.13.

15.11.2021 г.

  
/Assoc. Prof. Lyudmila Kabaiyanova/