To Prof. Sarafian, MD, PhD, DSc
Chairman of the scientific jury,
Designated by a written order No. R - 2300/ 03.07.2024
of the Vice Rector of Science and Research of
the Medical University - Plovdiv

Regarding your protocol №1/ 16. 07 2024

Reviews

Concerning a competition to fill up the position of "Professor"
In the scientific specialty "Medical Biology" 4.3. Biological Sciences
announced the needs of MU-Plovdiv, Department "Medical Biology"
in state newspaper issue, No. 35/ 19.04.2024

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Reviews

By Prof. Spaska Angelova Stanilova, PhD, DSc
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Member of the Scientific Jury, confirmed by order No. R - 2300/ 03.07.2024 of the Vice Rector of Science and Research of MU-Plovdiv

Regarding: Competition for the academic position of " Professor" in the field of higher education 4. Natural sciences and mathematics, professional direction 4.3. Biological sciences and scientific speciality "Medical Biology", announced for the needs of MU-Plovdiv, Department of "Medical Biology" in the SG, no. 35/ 19.04.2024

Only one candidate submitted documents for participation in the competition - Assoc. Prof. Maria Kazakova, PhD from the Department of Medical Biology of MU-Plovdiv. After consideration of the submitted documents, the candidate is allowed to participate in the competition.

I. Biographical data and career profile of the candidate.

Assoc. Prof. Maria Hristova Kazakova completed her higher education at the Faculty of Biology of Plovdiv University "Paisiy Hilendarski" with a bachelor's degree in molecular biology and a master's degree in cell biology in 2005 and 2006, respectively. In 2005, he started working as a specialist biologist in the Department of "Developmental Biology" at the Faculty of Biology of Plovdiv University "Paisiy Hilendarski".

In 2007, after successfully passing a competition, Maria Kazakova was elected as an assistant in Medical Biology at the Department of Medical Biology at the Medical University - Plovdiv. As an Assistant Professor in the same Department, he took a specialist in Medical Biology on 01.01.2013. The same year, she successfully defended her PhD thesis on "Immunobiological studies on YKL-40 in some inflammatory joint and tumor processes" on 24.10.2013, for which she was awarded the degree of "Doctor of Immunology" by the Scientific Jury. Since 2014, Maria Kazakova has been elected as the head assistant in the same Department.

Head assistant Maria Kazakova obtained a master's degree in applied research management from the PU "P. Hilendarski" in 2015.

In 2016 after successfully winning a competition, she was elected by the Faculty of Medicine and appointed to the academic position of Associate Professor of Medical Biology at the Department of Medical Biology at the Medical University of Plovdiv, where she is currently working.

As an assistant, head assistant and associate professor, she conducts practical classes in Parasitology, Molecular Biology, and Cellular and Molecular Immunology in Bulgarian and English for first-year students of Medicine, Dental Medicine and Pharmacy, as well as lectures, mainly in Medical Parasitology in the Faculty of Medicine " Biology of Parasites".

Throughout her career, Assoc. Prof. Maria Kazakova has conducted a large number of courses and specializations in Bulgaria and abroad in Molecular and Cell Biology, including training in flow cytometry, cell culture, RT2 - PCR, new strategies in vaccine development, as well as several specialized courses in Immunology, personalized medicine, etc., detailed in the CV. This additional qualification helps her career development. As a result of her overall activity, Associate Professor Maria Kazakova has established herself as a well-accepted researcher and teacher with many years of experience in the scientific field of Medical Biology and its applications in Medical Practice. The candidate is proficient in English, for which she presents a certificate and teaches English students in the disciplines taught at the Department.

In addition, she also speaks Italian, which allows her to spend an effective two-month stay in a laboratory at the University of Catania, as well as to conduct two Erasmus mobilities at the University Campus Bio-Medico of Rome, Rome, Italy - 2018 and 2022.

In 2023-2024, Associate Professor Maria Kazakova completed an online training course on Leadership: Creating Public Value from Harvard University.

II. General description of the submitted materials for the competition.

The set of documents submitted by the candidate in an electronic variant is by the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation and the relevant Regulations of the MU-Plovdiv dated 28.01.2021. The documents are prepared by the LIST OF DOCUMENTS for occupying the academic position of "Professor" in the field of Natural Sciences of the MU-Plovdiv. Evidence materials for all indicators and the required declarations are presented.

III. Evaluation of the candidate's scientific works for his overall academic development.

Assoc. Prof. Maria Kazakova participated in the competition for the academic position "Professor" with 22 scientific articles published after taking up the position of associate professor, 16 of which were published in scientific journals, referenced and indexed in Scopus and/or Web of Science. They are distributed by quartiles as follows: Q1-6; Q2-5; Q3- 3, Q4-1 and 1 in a non-Q journal. Also, 14 of them are in Clarivate Analytics impact factor journals and 2 of them are in SJR journals but no impact factor. This issue does not include 5 publications used to replace a monographic work. Apart from them, 6 of the 22 scientific articles were published in non-refereed Bulgarian peer-reviewed journals. A peer-reviewed book chapter is also presented. It is also worth noting the fact that for the entire scientific and teaching career to date, she presents a list of 58 publications, 30 of them in journals with an impact factor. The scientific results have been presented at a total of 98 scientific congresses and conferences, of which 33 were international.

Associate Professor Kazakova summarizes her scientific activity in two main directions, defining her specific scientific profile. One of them is related to the determination and characterization of new Biomarkers and molecular signatures in inflammation and tumorigenesis, which would provide clinically significant information about the specific condition of the patient, the dynamics of the disease development and the results of concomitant therapy. One of these biomarkers is the extracellular matrix glycoprotein - YKL-40, also known as chitinase-3-like protein-1 (CHI3L1), involved in apoptosis and angiogenesis. The other two proteins are lysosome-associated membrane glycoproteins LAMP-1 and LAMP-2, which are a major feature of the lysosomal membrane and active participants in autophagy.

The long-term work of the team with the leader role of Assoc. Prof. Kazakova on YKL-40 includes publications in which for the first time have been presented results for the serum levels of YKL-40 in healthy, asymptomatic persons from the Bulgarian population, which allows the tracking of changes in this protein in autoimmune and inflammatory diseases. The team demonstrates that significantly high values of YKL-40 in the synovial fluid of patients with rheumatoid and psoriatic arthritis are an indication of an ongoing inflammatory process, which defines the role of YKL-40 in monitoring these diseases. In a group of patients with the autoimmune disease systemic sclerosis (SS), elevated levels of YKL-40 protein were found to correlate with decreased levels of miRNA-214 in plasma by *in silico* and *in vitro* experiments with blood samples. The ROC analysis

performed shows that serum levels of YKL-40 and plasma levels of miRNA-214 can be used as biological markers to distinguish patients with systemic sclerosis, as well as those with diffuse and local skin manifestations. Based on these data, a mechanism for the regulation of YKL-40 by miRNA-214 in patients with systemic sclerosis has been proposed (2). A positive correlation has also been established between YKL-40 and the pro-inflammatory cytokines IL-1β and TNF-α in rheumatoid arthritis, as well as with IL-6 in patients with systemic sclerosis and traumatic brain injuries (1;3).

Part of the study includes the epigenetic mechanisms of regulation in the synthesis of YKL-40, to elucidate the post-transcriptional mechanisms of control of its gene expression in patients with SS. Through the performed molecular biological analyses (RT-PCR by TagMan of 7 miRNAs and three dsRNAs), followed by bioinformatics, the presence of a dsRNA/miRNA-30e/CHI3L1 regulatory axis involving sequential interactions between long dsRNAs, miRNAs and YKL-40 was established. mRNA that controls glycoprotein synthesis in SS (7).

In terms of new markers for tumorigenesis, assoc. Prof. Kazakova's main contribution is related to the evaluation of YKL-40 in the development of metastatic colorectal carcinoma and its clinical application, a study initiated by her PhD thesis. New information was obtained about the role of this glycoprotein in metastatic colorectal carcinoma and its potential use as a diagnostic marker in predicting response to therapy. Part of the new results are the first data on the clinical significance of tissue expression of YKL-40 as a prognostic marker in metastatic colorectal carcinoma. The most recent data in this study show that two colorectal carcinoma cell lines, one wildtype Caco2 (KRAS-WT/p53mut) and the other KRAS-mutated HCT116 (KRASmut/p53-WT) are characterized by high YKL-40 expression, increased proliferation, mobility and invasiveness, and increased epithelial-mesenchymal transformation. Increased expression of YKL-40 in tumor tissue has also been found to enhance sensitivity to targeted therapy in patients (8). Strong expression of YKL-40 at the tumor front in CRC may serve as a prognostic indicator (Kazakova et al. 2024; 12). The other two proteins subject to long-term research in the department, LAMP-1 and LAMP-2, were studied in high-grade gliomas, where increased gene and protein expression of LAMP-1 was found to be associated with tumor progression in Glioblastoma multiforme.

The second main direction includes the development of more effective drugs and monitoring of the therapy carried out at the cellular level, such as changes in cellular metabolism related to mitochondrial function.

For the first time, the biological action of new fluorenylspirohydantoins, organic compounds used in medicine as aldose reductase inhibitors, having antiepileptic, antiarrhythmic and anticonvulsant effects, was investigated. Isolation of single crystals and tests of biological activity of substituted fluorenylspirohydantoins, as well as their cytotoxic effect on the human tumor cell line A2058 by WST-1 test and antimicrobial activity against bacteria and yeast, were carried out (4, 5).

In this direction, Prof. Kazakova also includes the research of bioenergetic and metabolic pathways in the cell by measuring mitochondrial activity in living cells in several groups of patients. Mitochondrial activity was investigated in Parkinson's patients treated with different therapeutic regimens, simultaneously with quantitative expression and protein analysis of YKL-40, LAMP-1 and LAMP-2. New data revealing changes in mitochondrial activity and YKL-40 levels in patients with Parkinson's disease are reported. Mitochondrial activity improves after treatment (7;12;16).

Research on the metabolic status and key parameters of mitochondrial function in patients with rheumatoid arthritis (PA) treated with both therapeutic regimens (Methotrexate and JAK inhibitors) allowed the authors to establish that increased ATP values in the group of patients treated with JAK -inhibitors positively correlated with improvement in patient's clinical and laboratory tests, as well as with ultrasonographic DAS28 and GUS7 scores after therapy. Mitochondrial dysfunction has also been demonstrated in isolated PBMCs from newly diagnosed rheumatoid arthritis patients (11;14).

Associate Professor Kazakova is the head of 2 international scientific and educational projects and 4 national scientific and educational projects. She is also a participant in 2 other international and 5 national scientific and educational projects, as well as in 11 intra-university scientific projects.

IV. Evaluation of the monography or equivalent publications submitted for participation in the competition by the candidate.

Assoc. Prof. Kazakova instead of a monograph, presents 5 articles published in scientific journals, referenced and indexed in Scopus and Web of Science, two of which have Q1, and the remaining three - Q2, presented in the list in item 4 of group B in the table for the mandatory minimum science indicators and in full text. Two of the articles are review papers.

These articles are in a well-defined field and present new results on the clinical relevance of the expression of the CHI3L1 gene, encoding the polypeptide sequence of the YKL-40 protein. Gene expression at the mRNA and protein level has been studied about inflammation in the central

nervous system and neurodegeneration processes associated with ageing and diseases such as Alzheimer's and Parkinson's. The expression of *CHI3L1* in five brain regions: cerebellum, dorsolateral prefrontal cortex, prefrontal cortex, hippocampus, and visual cortex was investigated in age-matched healthy controls and Alzheimer patients.

The authors found differences in CHI3L1 expression levels depending on age and sex. Females showed higher expression of CHI3L1 than males in brain tissue, with differences most evident in older healthy subjects. Analysis of CHI3L1 expression in different brain regions in Alzheimer's patients also showed gender differences, with female patients showing greater expression in the cerebellum than male patients. Sex-related differences in CHI3L1 expression were not observed in the hippocampus of patients. The authors conclude that CHI3L1 expression in the brains of cognitively intact subjects and Alzheimer's patients is closely related to age and sex, most evident in the cerebellum. This pattern of YKL-40 expression is explained by the apparent involvement of glial cells in pathological processes accompanying any neurodegenerative disease. At the same time, reduced NSE levels are probably associated with low metabolic activity and increased neuronal death. YKL-40 is a more reliable biomarker in neurological diseases than neuron-specific enolase (NSE).

In summary of the experimental results and biological pathways, the clinical relevance and relationship between YKL-40 and NSE as biomarkers in the monitoring and prognosis of neurological diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and multiple sclerosis are evaluated. The role of autophagy in the pathology of these diseases is also discussed.

V. Reflection (citation) of the candidate's publications in the national and foreign literature (publication image).

Assoc. Prof. Kazakova presents 119 citations after 2017 year, all in scientific journals, referenced and indexed in Scopus and Web of Science. It also includes 7 reviews. The applicant's overall Hirsch index is h-index (from Scopus) = 11 at the time of review.

VI. Comprehensive, qualitative evaluation of the teaching-methodical and teaching activity, incl. scientific guidance for students, doctoral students, and specialists.

Consecutively, as an assistant and associate professor at the Department of Medical Biology, Maria Kazakova participates in illustrating and conducting practical exercises in medical biology and parasitology and also develops and presents in English a part of a lecture course on medical parasitology for English-language medical students. He is a member of the council for educational and scientific research activities of MU-Plovdiv.

Assoc. Prof. Kazakova presents many contributions to manuals for students in Bulgarian and English (total number -20, of which 10 after associate professor) in medical biology and parasitology, as well as in a textbook on Parasitology in English for students of Medicine. All mentioned study textbooks, including the parasitology textbook in English, are currently used in the teaching process at MU-Plovdiv. She has also co-authored 3 biology textbook study manuals for candidate students.

Assoc. Prof. Kazakova is the supervisor of one successfully defended doctoral PhD student and one still in the doctoral program. She is also a scientific consultant to a graduate student and has provided scientific guidance to outstanding students in the preparation and defence of scientific reviews, as well as to two medical biology specialists. She participated as a member of scientific juries for the defence of the PhD and the acquisition of academic positions.

He has long-term experience (13 years) as an examiner of candidate-student competitive works in biology, since 2 years she has been the Chairman of the Biology committee for candidate students in Bulgarian and of the Biology committee for English-language education (1 year).

VII. The general assessment of the applicant's compliance with the minimum national requirements with the Law of Development of Academic Development in the Republic of Bulgaria, respectively, to the mandatory qualitative and quantitative criteria and indicators by academic development at the Medical University - Plovdiv.

The overall comparative assessment of the candidate is presented in the Comparative Table.

Comparative assessment of the compliance with the minimum requirement for holding the academic position "Professor"

Group of indicators	Indicators	Value required for "**Professor**"	**Assoc. Prof. Maria Kazakova**
А	Dissertational thesis for PhD	50	50
В	Habilitation thesis - monography or 10 publications in Scopus and Wos	100	110
Г	Indicators from 5 to 10 Number of publications	250 **20 articles, of which 15 in journals referenced in Scopus and/or Web of Science, including 8 articles with IF**	377 **22 articles, of which 16 in journals referenced in Scopus and/or Web of Science, including 14 articles with IF**
Д	Indicator 11 Number of citations (Scopus)	100	**252**
Е	Indicators from 12 to 20	50	**394**
Ж	Teaching workload Academic position	360 200	**786** **700**
Sum		**1210**	**2 669**

A proposal for the position

The candidate who submitted the documents for the competition - Associate Professor Maria Kazakova, not only meets but also exceeds all the mandatory and specific conditions and science criteria for the academic position "Professor" in the Ministry of Education of the MU-Plovdiv. The submitted documents certify the significant experience and skills of the candidate as a university professor and scientific researcher with extensive experience

in project activity, with significant scientific output in the field of cellular and molecular biology, such as articles and citations.

In conclusion, I recommend the members of the Scientific Jury and the Faculty Council to vote positively for the election of Associate Professor Maria Hristova Kazakova to the academic position of "Professor" in Medical Biology in the Department of Medical Biology of MF at the Medical University of Plovdiv.

27.08.2024

Prepared by:
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