

To

the Chairman of the Scientific Jury,

according to Vice-Rector's Order, MU-Plovdiv, No R -1306/25.02.2026

15a V. Aprilov Blvd., 4002 Plovdiv

Medical University – Plovdiv

I hereby submit:

OPINION

on

Dissertation for the award of the Scientific Degree "Doctor of Sciences" in the scientific specialty "Medical Biology", field of Higher Education 7. Health and Sports, Professional Field: 7.1. Medicine, Doctoral program "Rheumatology"

in connection with the procedure for public defense of a dissertation for the Scientific Degree "Doctor of Sciences" in the scientific specialty "Medical Biology" of **Prof. Maria Hristova Kazakova-Velinova, PhD, from the Department of Medical Biology, Faculty of Medicine, Medical University of Plovdiv**

Prepared by: Prof. Dr. Dimitrina Kirilova Dimitrova-Dikanarova, MD, PhD

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OPINION

on

Dissertation for the award of **the Scientific Degree "Doctor of Science" in the scientific specialty "Medical Biology"**, field of Higher Education 7. Health and Sports, Professional field: 7.1. Medicine, Doctoral program "Rheumatology", in connection with the procedure for the public defense of the dissertation work

The dissertation work was developed by Prof. Maria Hristova Kazakova-Velinova, PhD, from the Department of Medical Biology, Faculty of Medicine, Medical University of Plovdiv and is entitled "Molecular biological and immunological studies on chitinase-like proteins *CHI3L1* and *CHI3L2* in inflammatory processes, tumorigenesis and neurodegeneration".

The OPINION was developed by Prof. Dr. Dimitrina Kirilova Dimitrova - Dikanarova, MD, PhD, Professor of Biology at the Department of Biology, Faculty of Medicine, Medical University of Sofia, participating in the composition of the scientific jury, according to Vice-Rector's Order, MU-Plovdiv, No R -1306/25.02.2026 after preliminary discussion by the reinforced Department Council (pr. No. 2/13.02.2026) and acceptance by the Faculty Council (pr. No. 2/23.02.2026) of the Ministry of Finance of the developed dissertation.

The opinion was developed in accordance with the requirements of the Law on the Development of Academic Staff in the Medical University of Plovdiv and the Regulations on the Implementation of the Law on the Development of Academic Staff in the Medical University of Plovdiv (2021), containing: the requirements for acquiring the academic degree "Doctor of Sciences", Rules/sample structure of a review/opinion on a competition for acquiring the **Scientific Degrees** in the Medical University of Plovdiv and Appendix 1 of the Regulations on scientometric indicators for the Medical University of Plovdiv, as well as in accordance with the texts set out in Art. 10 and Art. 11 of the Law on the Development of Academic Staff and Art. 40 and Art. 41 of the Regulations on the Implementation of the Law on the Development of Academic Staff in the Medical University of Plovdiv, in connection with the other texts of the law and the regulations on its implementation, concerning the scientific degree "Doctor of Sciences".

General presentation of the procedure and the candidate

The candidate - Prof. Maria Hristova Kazakova - Velinova, PhD is a Doctor of

Immunology, a specialist in medical biology, a professor at the Department of "Medical Biology" at the Medical University - Plovdiv, as well as the Head of the Department.

She has submitted all the necessary documents in accordance with the requirements of the Act on the Development of the Academic Staff and the Regulations on the Structure and Activities of the Medical University - Plovdiv. The documents are arranged precisely and comprehensively according to the Recommended Requirements for the Competition for the Acquisition of the Scientific Degree "Doctor of Sciences".

Brief data on the professional development of the dissertation candidate

Maria Hristova Kazakova graduated from the Plovdiv University "Paisiy Hilendarski" as a bachelor with a degree in "Molecular Biology" in 2005. In 2006, she acquired the educational and qualification degree "Master" in the specialty "Cell Biology". Since 2015, she has also held the educational and qualification degree of Master in the specialty "Management of Innovation and Research Activities". After graduating as a Master, during the period: May 2006 - February 2007, Maria Kazakova worked as a biologist, junior expert at the University of Plovdiv "Paisiy Hilendarski", after which, after successfully passing a competitive exam, she successively held the academic positions: assistant professor (November 2007 - May 2014), and chief assistant professor (from 29.05.2014) in the Department of Medical Biology at the MF, Medical University - Plovdiv. In January 2013, she obtained a specialty in the field of healthcare "Medical Biology", and in October 2013 - she also obtained the educational and scientific degree "Doctor" in Immunology by defending a dissertation on the topic: "Immunobiological studies on YKL-40 in some inflammatory joint and tumor processes" under the scientific supervision of Prof. Dr. Victoria St. Sarafyan, MD, PhD, DMSc. Her postgraduate qualification during the period 2009 - 2024 includes a large number (13) courses and specializations of varying duration on basic problems and techniques of molecular biology and immunology, as well as on: medical-biological and social problems, issues of personalized medicine, reproductive medicine, etc. She has also participated in training courses for project management from the Priority "Health" under European programs. Prof. Kazakova's activities have been evaluated through prestigious national awards from various scientific events.

Since 2016, Maria Kazakova has held the Academic Position "Associate Professor" in the Department of Medical Biology after a successful competition, since September 2024 she has held the AD "Professor" in Medical Biology in the same Department, and since April 2024 she has been elected as the Head of the Department. Maria Kazakova has been elected as a member of the Council for Educational Activities of MU-Plovdiv (2018, 2022),

and as a Member of the Council for Scientific and Research Activities of MU-Plovdiv (2024). ***As of the date of the public defense of the Scientific Degree "Doctor of Sciences" Prof. Maria Hristova Kazakova, PhD has continuous, consistent and in-depth academic development in the field of medical biology, having received very high attestation grades and distinctions for her overall activity at MU-Plovdiv.***

The structure of the dissertation

The discussed dissertation work is developed on 198 pages, **is structured with the necessary ratio of its components and includes the following main sections, which are in accordance with the requirements of the Regulations for the Application of the Act on the Development of the Academic Staff at MU - Plovdiv: List of abbreviations used (1 page); Introduction (2 pages), Literature review (with structure, conclusion), (38 pages); Purpose of the study and two main tasks with three subtasks (1 page); Materials and methods (26 pages); Results (77 pages); Discussion (22 pages); Conclusions (2 pages); Contributions (1 page); Literature, as well as Financing, and List of published scientific works in connection with the dissertation work. Annexes are included as separate documents to the dissertation work.**

The bibliographical reference is based on literary sources directly related to the discussed problem. The bibliography includes 213 literary sources, of which 211 are in Latin and 2 in Cyrillic. A large part of the cited articles were published in recent years (2018-2026), with 37 of them being printed in the period 2021-2026.

The results included in the dissertation work have been published in 16 articles, of which 14 have an Impact Factor.

THE DISSERTATION includes illustrative material of 41 figures and 31 tables of very high informative quality and complex nature, of which: 4 figures and 1 table included in the literature review; 4 tables and 1 figure included in "Materials and Methods"; 26 tables and 36 figures included in "Results".

The presentation in the dissertation work is very clear, consistent, logical and well documented with complex figures, tables and schemes. The following are shown: the causal relationship between the analyzed facts and the own data, as well as the in-depth medical-biological and clinical knowledge of the dissertation candidate.

Relevance and significance of the dissertation

The dissertation submitted to me for opinion is a current, in-depth and original experimental study with fundamental medico-biological and applied clinical value. The

dissertation is aimed at research on the pathogenesis of autoimmune, neurodegenerative, malignant diseases and the importance of: changes in mitochondrial function, inflammation, autophagy, oxidative stress and other processes, in connection with improving the diagnosis and therapy of patients suffering from such diseases. In this regard, some of the members of the conservative family of Chitinase-like proteins (CLPs) and the best studied of them (*CHI3L1/YKL-40 and CHI3L2/YKL-39*), at high concentrations in biological fluids and increased expression of the genes encoding them, are associated with some autoimmune, malignant and neurodegenerative diseases. These proteins are important for influencing signaling pathways in the immune system, inflammation, tumorigenesis, regeneration and fibrosis. The two proteins have different profiles and expression. They have been studied and characterized in the dissertation as markers of various pathological processes and provide information about the activity of different cell populations in these processes. They could be used as new diagnostic biomarkers in clinical practice and scientific research, which determines the relevance and significance of the dissertation.

Knowledge of the problem and creative assessment of the literature on the problem

The literature review is comprehensive in accordance with the topic of the dissertation work and contains a critical review of the cited data, but also shows the awareness of the Candidate, as well as her excellent academic preparation. The review characterizes important biomarkers with the aim of validating and introducing new ones with an emphasis on the **"potential/ideal biomarker"**. Among the biomarkers used, which are of importance in a number of immunobiological processes, the glycosyl hydrolase family 18 (GH18) with representatives of chitinase-like proteins (CLPs) stands out. In the literature review, they are discussed in depth, with competence, and a specific medical-biological focus, and their representatives are analyzed, mainly the ***proteins YKL-40, YKL - 39***, as well as the genes encoding them. The following are analyzed: the primary structure of these proteins, their spatial organization, potential ligands, the cells secreting the corresponding proteins, the expression of their mRNA, the function of these proteins as biomarkers (in biological fluids and in the course of the disease) of the proinflammatory response in various diseases. Data on chitinase-like proteins and their relationship with mitochondrial activity and cellular metabolism in patients with neurodegenerative pathology, autoimmune diseases and tumorigenesis are also presented. The mechanisms of tolerance to self-antigens, the importance of T and B-cell populations in the

mechanisms of autoimmune processes, and the **importance of CHI3L1/YKL-40 and CHI3L2/YKL-39** in: **rheumatoid arthritis, systemic sclerosis, neurodegenerative processes associated with inflammation, malignant diseases (colorectal carcinoma, glioblastoma)** are considered. *However, at this stage, there are no generally accepted data in the literature on: the reference values of the two molecules in healthy individuals, as well as on the correlation between the levels of the two proteins in their local expression in colorectal carcinoma, and their levels in autoimmune and neurodegenerative diseases. Against this background, the goal and main tasks of the dissertation work have been derived, which are related to the study of the complex expression and biological role of the **chitinase-like proteins CHI3L1 and CHI3L2** in the processes of inflammation, tumorigenesis and neurodegeneration.*

Research methodology

The materials and methods used were selected very appropriately. They are also a prerequisite for reliable qualitative and quantitative analysis and achieving the set goal. All studies were conducted in strict compliance with the ethical rules for working with patients. 270 patients were studied, divided into three groups according to the diseases they suffer from: I. autoimmune diseases and inflammatory processes; II. neurodegenerative diseases and inflammatory processes, and III. malignant diseases. For each group of patients, the corresponding control group was also included according to strict criteria. In the indicated patient groups, the following were studied: the concentrations of the **CHI3L1 and CHI3L2 proteins** by ELISA; mRNA from leukocytes and the gene expression of **CHI3L1 and CHI3L2** by qRT-PCR. *Molecular biological and pathohistological studies, analysis of mitochondrial activity, cell cultivation, and a wide range of statistical methods were applied. The studies were conducted with a precise algorithm of steps and accurate justification for the three groups of diseases.*

Results and their analysis. Characteristics and assessment of the dissertation work and its contributions

The description of the own results is presented in-depth and logically. It shows the methodological preparation of the Candidate and her awareness in the work. The presentation logically follows the structure of the literature review and the main tasks. **The presented dissertation work is a very large-scale study with an interdisciplinary approach, with the aim of establishing the molecular and cellular mechanisms associated with the response during the course of therapy.** In autoimmune diseases with inflammation and joint degeneration, increased serum levels of proinflammatory cytokines and **CHI3L1** and

correlation with the activity of the disease are established, **proving the importance of *CHI3L1* as a biomarker for the activity and progression of rheumatoid arthritis (and also as a potential autoantigen).** The decrease in *CHI3L1* in patients treated, according to a certain regimen and with certain medications: TOFA - tofacitinib and MTX - methotrexate, suggests direct modulation of inflammation. From the results obtained, Professor Kazakova concludes that the two drugs used affect, through different mechanisms, important pro-inflammatory cytokines that are important for the pathogenesis of rheumatoid arthritis. Professor Kazakova emphasizes that the increased gene expression of *CHI3L1* is most likely associated with a more preserved mitochondrial function at the metabolic level. The dissertation emphasizes the importance of *CHI3L1* as a growth factor for connective tissue (in patients with systemic sclerosis). The expression of the *CHI3L2* genes, associated with the immune environment in patients, as well as with tissue remodeling and fibrosis, was also analyzed.

Professor Kazakova and her collaborators assume that *CHI3L1* is associated with inflammatory mediators and enzymes in the ***pathogenesis of osteoarthritis, and CHI3L2 is detected in increased concentration in the disease and is a marker for its progression.*** *CHI3L1* is expressed in inflammatory reactions in patients with CNS pathology, and the Candidate found that the concentration of *CHI3L1* in the cerebrospinal fluid correlates with the severity and prognosis of encephalitis. *CHI3L1* is elevated in various neuroinflammatory and neurodegenerative diseases. The author also demonstrates an increased concentration of *CHI3L2* in the cerebrospinal fluid in patients with CNS infections, a correlation with specific paraclinical markers (CRP) and disease activity (higher values were found in viral etiology of the infection, i.e. *CHI3L1* could be a discriminative marker of the infection). Professor Kazakova and colleagues found increased plasma levels of *CHI3L2* in the pre-thrombolytic phase of ischemic stroke, suggesting the protein's involvement in tissue remodeling and in the response to occlusion. The Candidate believes that during the acute phase of ischemia, mitochondria as a result of severe stress have a limited functional reserve and adaptive capacity. The Candidate found a statistically significant negative correlation between *CHI3L1* and the indicator of the part of oxygen consumption used directly for ATP synthesis (in terms of losses through proton transfer). These data point to the idea that ***CHI3L1 is a marker for mitochondrial dysfunction.***

The Candidate's research was also conducted in conditions with autism spectrum disorders (with and without regression) on mitochondrial dysfunction and the presumed

importance of both proteins in the pathophysiology of the diseases. Changed dynamics in mitochondrial parameters between subgroups and controls were found.

Prof. Kazakova found increased gene expression of **CHI3L1** in tumor tissues from patients with glioblastoma compared to healthy controls. In patients with colorectal carcinoma and metastatic carcinoma, it was found that *increased expression of **CHI3L1** stimulates the metastatic phenotype and is correlated with poor prognosis and therapeutic response.* In a study of a group of Bulgarian patients for immunohistochemical expression of the two proteins, Prof. Kazakova found a similar localization with high intensity of the reaction in the area of tumor budding. The two proteins have structural similarities, but exhibit different biological functions in colorectal carcinoma and glioblastoma.

CHI3L1 (chitinase-3-like protein-1) has characteristics that are important as a cellular biomarker of inflammation, fibrosis, tumor progression, an indicator of active progression of group I diseases, and the degree of joint damage. The increased concentration of **CHI3L1** in the cerebrospinal fluid is characteristic of neurodegenerative diseases. The concentration of **CHI3L1** increases with poor prognosis and increased risk of metastasis in oncological diseases.

CHI3L2 (chitinase-3-like protein-2) is important in oncological diseases and neurodegenerative processes and is a marker of inflammation, proliferation, angiogenesis, and participates in the immune response in autoimmune conditions.

*The dynamics of **CHI3L1/ CHI3L2** expression has an important diagnostic and prognostic value in diseases with complex etiopathogenesis.*

The main formulated contributions of the dissertation work are of original scientific and applied nature and exactly correspond to the obtained results. They are directly related to diagnostics and therapy in diseases associated with inflammation, tissue remodeling and neurodegeneration, and increased gene and protein expression of **CHI3L1 and CHI3L2** in these diseases; better antioxidant protection, and therapy of some autoimmune diseases; mechanisms of tumor cell proliferation, invasion and metastasis; modulation of inflammation and the tumor microenvironment.

Assessment of the publications and personal contribution of the Candidate

The Candidate-Professor Kazakova has a publication activity that meets and exceeds the requirements of the Regulations for the Development of the Academic Staff and the normative documents of MU - Plovdiv. The results included in the dissertation work have been published in 16 articles, of which 14 are with Impact Factor, 7 of which are in journals with Impact Factor and Q1, 6 articles in journals with Impact Factor and Q2, 1

article - in a journal with IF and Q4, 2 articles - in refereed and reviewed authoritative publications. Prof. Kazakova is the first author of 6 of these publications. The results have been reported and are included in the published materials of 30 forums, 15 of which are international. Prof. Kazakova has also included data on 12 scientific projects related to the dissertation work, of which: 3 are international; 2 are national and 7 - intra-university. **The total number of points from the published articles of Prof. Kazakova in group G is 133** (The total number of required points under Group G indicator, according to Appendix 1, is 100 points, as the Candidate exceeds the requirements for published articles in journals with an impact factor and for first authorship).

Reflection (citation) of the candidate's publications in the national and foreign literature:

The citations related to the topic of the dissertation work in the last two years are 166, which corresponds to 2490 points in group D with 200 points required according to the quantitative criteria of MU-Plovdiv, Table 1.)

The total number of points from the candidate's groups of indicators A, B, B, G and D is 2773 points, with 450 points required.

The Summary of the dissertation is made according to the requirements and accurately reflects the main results obtained by the dissertation candidate.

CONCLUSION:

The dissertation work submitted for opinion, entitled "***Molecular biological and immunological studies on chitinase-like proteins CHI3L1 and CHI3L2 in inflammatory processes, tumorogenesis and neurodegeneration***" is a current, in-depth and original experimental study with fundamental medical-biological and applied clinical value. The dissertation is aimed at research on the pathogenesis of autoimmune, neurodegenerative, malignant diseases and the importance of: changes in mitochondrial function, inflammation, autophagy, oxidative stress and other processes, in connection with improving the diagnosis and therapy of patients suffering from such diseases. A thorough experimental characterization of important biomarkers has been made in order to validate and introduce new ones with an emphasis on the "**potential/ideal biomarker**". The scientific research work included in the analyzed dissertation is very large in volume. The results are competently discussed and have an original and important scientific-theoretical, clinically-applicable, as well as methodological contribution in the field of

molecular biology, immunology and diagnostics and monitoring of therapy in medicine. The dissertation work, as well as the publication and scientific research activity of the Candidate - Prof. Maria Hristova Kazakova-Velinova, PhD, fully meet the requirements of the ZRASRB, the Regulations for its application and the Regulations for the acquisition of scientific degrees and holding academic positions at MU - Plovdiv, with a view to awarding the scientific degree "Doctor of Sciences". **The qualities of the dissertation work**, namely: the very thorough and competent review of the literature data, the relevance and significance of the analyzed problems, the application of modern experimental methods with high sensitivity and specificity, the originality and the scientific-applied and clinical-diagnostic significance of the contributions in the fundamental medical-biological field and clinical practice, **give me reason to positively assess: the dissertation work, the summary, the achieved results and contributions. I confidently declare to the Honorable members of the Scientific Jury that Professor Maria Hristova Kazakova-Velinova, PhD, fully meets the requirements for acquiring the scientific degree "Doctor of Sciences". I propose to the Honorable Scientific Jury to award the scientific degree "Doctor of Sciences" in the scientific specialty "Medical Biology", field of Higher Education 7. Health and Sports, Professional Direction: 7.1. Medicine to Prof. Maria Hristova Kazakova-Velinova.**

Signature:



15.04 . 2026 г.

Sofia

(Prof. Dr. D.Dimitrova-Dikanarova, MD, PhD)