

TO
The Chairman
Of the Scientific Jury
MF at MU-Plovdiv

REGARDING
Procedure for the defense of a dissertation
for the acquisition of the scientific degree
Doctor of Medical Sciences

OPINION

by Associate Professor, Dr. Velislava Ilieva Terzieva, MD, PhD

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on the defense of the dissertation of Prof. Maria Hristova Kazakova, DB for the award of the
scientific degree “Doctor of Sciences” in the field of higher education 7. Health and Sports,
professional field 7.1. Medicine, scientific specialty “Medical Biology”

By order No. R-1306/25.02.2026 of the Deputy Rector of the Research Institute (based on
Order No. R-1300/25.02.2026) Assoc. Prof. Dr. V. Kondeva-Glavinkova, MD, I am elected as an
external member of the Scientific Jury, and on the basis of protocol No. 1 of 06.03.2026 I am
assigned to prepare a statement in Bulgarian and English on the procedure for awarding the
scientific degree "Doctor of Sciences" in the field of higher education 7. Health and Sports,
professional field 7.1. Medicine, scientific specialty "Medical Biology" to Professor Maria
Kazakova, PhD from the Department of "Medical Biology", MF, MU-Plovdiv.

**Topic of the dissertation: “Molecular biological and immunological studies on the
chitinase-like proteins CHI3L1 and CHI3L2 in processes of inflammation, tumorigenesis and
neurodegeneration”**

General presentation of the procedure

Professor Maria Kazakova is the head of the Department of “Medical Biology” at the Faculty
of Medicine of MU-Plovdiv. The scientific work was discussed by an extended Department Council
with protocol No. 2/13.02.2026. . It was adopted by the Faculty Council with protocol No.
2/23.02.2026.

The documents presented to me are in accordance with the requirements of the ZRASRB and
the Regulations for Academic Development of MU-Plovdiv.

Biographical data and career development

Professor Maria Kazakova holds a Bachelor's degree in Molecular Biology, a Master's degree in Cell Biology and a Doctorate in Immunology from the Plovdiv University "Paisiy Hilendarski" and the Medical University-Plovdiv, respectively. She holds a Master's degree in Applied Research Management from the Plovdiv University "Paisiy Hilendarski" and has completed training in Leadership: Creating Public Value by Harvard University. Since 2024, she has been a professor in the Department of Medical Biology at MU-Plovdiv, and for the period from 2019 to the present, she has been or is an active member of the Academic Council of the Faculty of Medicine of MU-Plovdiv, of the Academic Council of MU-Plovdiv, of the Research Council of MU-Plovdiv. She has completed numerous specializations in the areas of current and research activities and two mobilities under the Erasmus program at the University Campus Bio-Medico of Rome, Rome, Italy. Professor Kazakova has been the Head of the Department of Medical Biology - MU Plovdiv since 2024.

Relevance of the topic of the dissertation

The relevance of the topic of the dissertation stems from the need to find new biomarkers that, against the backdrop of rapidly developing biomedical science and the desire to introduce minimally invasive diagnostic tests, can provide sufficiently reliable and clinically significant information.

Structure of the dissertation

The dissertation is written on 184 pages. The relevant evidences are presented in 41 figures and 31 tables. At the end of the dissertation, 4 appendices are systematized.

The Introduction indicates the main reasons for the choice of CHI3L1/YKL-40 and CHI3L2/YKL-39 as potential biomarkers related to the degree of inflammation, joint damage and oncological prognosis.

The literature review begins with a characterization of the concept of “biomarker”, clearly presenting the qualities and advantages necessary for an ideal biomarker. The essence and function of chitinases and chitinase-like proteins are presented in detail. The two chitinase-like proteins YKL-40 and YKL-39 are described in detail, with the emphasis on immune function and the grounds for discussing them as potential biomarkers in the bloodstream and in the cerebrospinal fluid.

Attention is also paid to other chitinase-like proteins: Ym1/Ym2 (Chi3l3/Chi3l4), Oviductin (mucin 9, oviduct-specific glycoprotein), SI-CLP. From the comparative organization of the domains of the GH18 family in Figure 3, and the summarized information about the family is given in Table 1, the differences between the individual members of the family are visible not only in structure, but also in function. The difference between the two chitinase-like proteins is clearly marked, despite the significant genetic similarity.

I was impressed by the reference to the dissertation's own studies on serum levels of CHI3L1 in sera of healthy individuals, which is presented on page 17 of the review. I accept these data as evidence of experience in the direction of the dissertation, and the dissertation itself as a consistent upgrade of the previous results. The need for in-depth research on CHI3L2 is also well argued (pp. 17-18).

The review chapter provides an overview of the participation of the studied indicators in the immunopathogenesis of autoimmune, degenerative processes, incl. inflammatory diseases of the CNS, ischemic strokes, autism spectrum disorder, neoplastic diseases of high social significance. The literature sources cover a period of more than 20 years, which I accept as evidence of the excellent awareness of Prof. Kazakova in the research area.

Based on the review of the literature, Prof. Kazakova has made three conclusions: aimed at: a) the potential of the two chitinase-like proteins to be prognostic/diagnostic markers for the activity of inflammatory, degenerative and tumor processes, and b) the connection with mitochondrial activity and cellular metabolism.

The purpose of the study is a logical consequence of these conclusions. Two groups of tasks have been derived for its implementation. The first has three subtasks, corresponding to three main pathophysiological processes - inflammation, neurodegeneration and tumorigenesis.

The **Materials and Methods** section is impressive for the large number of subjects studied - a total of 270 patients distributed across three areas - autoimmune, neurodegenerative and malignant diseases. Appropriate control groups are also included. Each group contains a sufficient number of subjects for reliable statistical processing of the results. The study was approved by an ethics committee, and the participants filled out an informed consent form. To achieve the set goal, Professor Kazakova applies a combination of clinical and laboratory approaches. Laboratory studies were performed on various biological materials - peripheral blood, tissues, cerebrospinal fluid, which I accept as an expression of broad methodological knowledge and a basis for a complete analysis of the chitinase-like proteins CHI3L1 and CHIL2. They include a variety of methods that allow for a comprehensive assessment of the studied indicators - from nucleic acids to protein, including its cellular and tissue localization. The reagents used are also presented in detail and methodically justified. I positively evaluate the summary (Figure 5, page 61) of the included pathologies, biological materials, and methods, including the assessment of mitochondrial activity.

The results of the study are summarized in 25 tables and 35 figures in a volume from p. 65 to p. 139. The diagnostically related presentation of the data, including autoimmune, neurological and tumor diseases, is striking. I highly appreciate the chosen approach for displaying the found trends in tables after each nosological unit, including the dependencies between the individual parameters. This makes the results accessible, useful and understandable.

It is interesting whether there is a relationship between the levels of CHI3L1 in synovial fluid and the severity of clinical symptoms and/or with the duration of the disease, according to the last paragraph on p. 85 and the beginning of p. 86?

An interesting finding is the difference in the concentration of the two CLPs in plasma in Colorectal carcinoma. Apart from being a factor that stimulates angiogenesis, can it be associated with the stage of the process and if so - at which stage? Does it matter whether the tumor is endophytic or exophytic? How can it be explained?

The relationship between the two chitinase-like proteins and CNS infections is also very interesting. Although the studies were conducted on a limited number of cases, the results deserve serious attention in order to continue research on age-related changes in immune reactivity.

Of interest is the fact that the two markers are related differently to proinflammatory factors in different diseases. This suggests that there are serious opportunities for upgrading and continuing the research and justifies the need to measure both indicators simultaneously as prognostic markers.

A parallel study was also conducted in patients with ischemic stroke. In fact, studies in this condition are very limited. Prof. Kazakova found significant differences in the plasma levels of the two inhibitors, but not in the levels of gene expression.

How could this finding be explained?

Another important direction in the dissertation work is the study in patients with autism spectrum disorder. On this problem, Professor Kazakova was the head of a successfully defended scientific project funded by the National Science Foundation. Although no change in the expression of the two studied genes was found, and the plasma protein levels were extremely low, a significant correlation was demonstrated between the levels of CHI3L1 in plasma and the clinical ADOS scale. I accept the presented results as highly specific for the specific pathology and extremely promising in the direction of the dissertation work.

The dissertation also presents the results obtained from patients with glioblastoma and colorectal carcinoma. From the results summarized in Table 28, as well as from the previous figures and histological documentation, it is seen that the plasma levels of CHI3L1 and CHI3L2 have a specific expression that distinguishes them from healthy controls, and the local protein expression in the tumor tissue has tumor-specific characteristics.

The dissertation presents for the first time data on the bioenergetic profile in patients with rheumatoid arthritis, ischemic stroke and autism spectrum disorder by assessing mitochondrial activity in a Bulgarian cohort. In patients with RA, the results of the analyses show impaired overall mitochondrial function, but also a significant improvement in proton leakage and maximum reserve capacity after therapy.

The same initial bioenergetic state of the cells was found in children with autism and in control samples, an expression of the high reliability of the measurements performed. Evidence is presented for increased values of maximum respiration (41%) in RAS patients compared to normally developing children. These data demonstrate the importance of mitochondrial function indicators as potential markers for the assessment and stratification of patients with RAS. Professor Kazakova proves that the predictive model, based on the indicators of maximum respiration and respiratory reserve capacity, is statistically reliable and allows the stratification of the studied patients into those with and without regression.

At the end of the "Results" section, an analysis of gene interactions of CHI3L1 and CHI3L2 is presented in Figure 41 and shows their relationship with 24 genes, including ETS proto-oncogene, progesterone receptor, and others, as listed in Table 31.

The discussion covers 20 pages and 213 literature sources for the period from 1988 to 2026. The emphasis is placed on the place and importance of the two chitinase-like proteins in the mechanisms of the inflammatory response. The positive result of the therapy is interpreted based on the molecular mechanisms of the respective drug and the participation of the studied indicators in them. The discussion of the results in the different nosological units is based on the role of CLPs in the pathophysiology of the diseases and the activity of the processes. Professor Kazakova also shows interesting directions for future research on chitinase-like proteins as an important element of the mechanisms of the inflammatory response. Special attention is paid to the studies of mitochondrial respiration and mitochondrial function. It is hypothesized that the observed metabolic switch in RAS patients is more likely to be a result than a cause of inflammation. In one of the most common tumors - colorectal carcinoma, a relationship between the expression of CHI3L1 and tumor invasiveness has been found.

The conclusion of the dissertation summarizes the results obtained and indicates prospects for further studies on the two proteins, not only as biomarkers, but also as potential targets for therapy.

Seven conclusions have been made that briefly and clearly correspond to the set goals and the results obtained.

Three main contributions have been made that confirm the meaning of the future application of the two molecules.

In connection with the dissertation work, 18 publications have been presented, distributed as follows: Q1-7, Q2-7, two materials are under review, one is in an international journal and one - in a Bulgarian scientific journal. The results have been presented at 30 national and international scientific forums. Professor Kazakova has participated in 12 scientific projects. It is evident from the attached documents that Professor Kazakova's indicators exceed the minimum requirements for scientific and teaching activities of candidates for obtaining a scientific degree.

The abstract is written on 84 pages. It corresponds to the structure of the dissertation work and presents the goal and objectives, materials and methods, results, conclusions and contributions clearly and briefly enough.

I have no critical notes. I asked the questions in the course of the presentation - page 4, paragraphs 1, 2 and 5.

I assess this dissertation work as extremely promising and useful in the fields of biomedical sciences and medical practice.

Conclusion

The dissertation work of Prof. Maria Kazakova represents a comprehensive study on the role of chitinase-like proteins CHI3L1/YKL-40 and CHI3L2/YKL-39 in the processes of inflammation, tumorigenesis and neurodegeneration. Using highly informative and state-of-the-art methods, a thorough comparative analysis of the two molecules in different groups of diseases has been made - all of which have a pronounced medical and social significance. The study is innovative, since the studied indicators are discussed relatively rarely in the world scientific literature, and for Bulgaria these are the first such data. I believe that the dissertation fully meets the requirements of the Law on the State of the Republic of Bulgaria, the Regulations for the Implementation of the Law on the State of the Republic of Bulgaria and the Regulations for Academic Development of MU-Plovdiv for the acquisition of the degree of Doctor of Sciences.

All of the above gives me reason to support and confidently recommend to the esteemed scientific jury to vote positively for the award of the scientific degree of Doctor of Sciences to Professor Maria Hristova Kazakova, PhD, in the field of higher education 7. Health and Sports, professional field 7.1. Medicine, scientific specialty "Medical Biology".

Sofia City

April 17, 2026

Prepared the opinion:

(Assoc. Prof. Dr. Velislava Ilieva Terzieva, MD, PhD)