

Review
of Prof.Dr. Krassimir T.Methodiev, MD, PhD, DScmed

Ref. Order N.P-1306/25.02.2026

According to #30, al.3, of the Regulations for Academic affiliations in Bulgaria, as well as the procedure for public acknowledgement of the dissertation for obtaining the scientific degree Doctor of Science, in the specialty “Medical Biology”, given to Prof.Dr. Maria Hristova Kazakova-Velinova, PhD, from Dept. Medical Biology of the Medical Faculty, Medical University Plovdiv, and after a preliminary decision of the Department Council (#N.2/13.02.2026) and approved by the Faculty Council (N.2/23.02.2026) of the Medical Faculty, the dissertation work, titled “Molecular-Biologic and Immunologic Study of Chitinase-like Proteins CHI3L1 and CHI3L2 in the Processes of Inflammation, Tumorigenesis and Neurodegeneration” ,

I, the undersigned Prof.Dr. Krassimir T.Methodiev, have been approved as a member of the Scientific Jury, by the Order of the Deputy-Rector of SIA of Plovdiv Medical University.

To comply with the aforementioned Order and after the analysis of the applied documents by the author and candidate for the Scientific Degree Doctor of Sciences Prof. Maria Hristova Kazakova-Velinova, PhD, from Dept. Medical Biology of the Medical Faculty, Medical University Plovdiv, hereby, I apply the following review:

The dissertation of the colleague Prof. M.Kazakova is presented in 182 standard pages, with the additional applications.

The literature, used by the author, includes 213 titles, in Cyrillic and Latin, 2 Cyrillic and 211 Latin (English language).

The scientific publications, associated to the dissertation are 18, in 6 of them Prof. M.Kazakova is the first author, and in the rest she is co-author with other colleagues. All of her publications are included in respected international scientific journals, among them 14 have impact factor.

Participations in scientific meetings: the results of the dissertation have been reported at 30 scientific meetings, 15 of them national and the other 15 at international congresses and conferences.

Research projects: the author presents a total of 12 projects, 2 national, 3 international and 7 interuniversity.

Resulting from the completed dissertation, the conclusions are altogether 7, as indicated here:

- The gene and protein expression of CHI3L1 and CHI3L2 is significantly increased in patients with RA before the therapy start, and significantly decreased, according to the type of the applied treatment, thus representing the inflammation reduction.
- The increased plasma level of CHI3L1 in the patients with SSc, their link to clinical scales and anti-inflammatory cytokines, illustrate the ongoing chronic autoimmune inflammation, and determine CHI3L1 as a marker of patients' stratification.
- The expression of CHI3L1 with CNS infections is able to help the early distinguishing between viral and bacterial infections.
- CHI3L1 and CHI3L2 indicate different models of expression, associated to the aggression of KKK and GBM.
- The tissue expression of CHI3L1 in KKK, in combination with the tumor budding, are a trustful indicator for evaluation of metastatic tumor potential.
- CHI3L1 can be determined as a marker of mitochondrial dysfunction, which correlates with the clinical scales of RAS.
- New aspects for the link between CHI3L1, CHI3L2 and the mitochondrial function/dysfunction, are presented, thus giving more options for evaluation of the inflammation, clinical course and applied therapy with a number of diseases.

The author of the dissertation reports the following three contributions to the results of her scientific-practical investigation:

- New data of gene and protein expression of CHI3L1 and CHI3L2 in diseases, associated with inflammation, tissue remodeling and neurodegeneration are presented.
- Early identification of mitochondrial dysfunction in processes, associated with neurodegeneration and inflammation would allow better antioxidant defense, selection and monitoring of the therapy in cases with RAS and RA.
- CHI3L1 stimulates the proliferation of the tumor cells, invasion and metastatic progress, thus modulating the inflammation and tumor micro area.

The content of the dissertation is well depicted and logical, in association with the overall work on the proposed topic:

Introduction

I.Literature

II. Conclusions of the literature

III.Aim and tasks

IV.Material and methods

V.Results

VI.Discussion

VII.Conclusions

VIII.Original achievements

IX.Scientific papers, associated with the dissertation

X.Literature

The dissertation has been realized with the financial support of projects of Med.Uni. Plovdiv (HO 01/2022; HO 1/2024; HO15/2025), European Union – NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0007-C01; project BG-RRP-2.004-0007-C03, together with the Bulgarian Association of Muscular-Skeleton ultrasound (BAMSU), thus predicting and proving the importance and value of the dissertation.

A total of 40 abbreviations of the used terms, indexes and signs to the text have been used by the author, thus providing a well-expressed view on the included in the dissertation parameters.

In the **Introduction** of her dissertation Prof. Maria Kazakova correctly determines that the Chitinaze-linked proteins (CLPs) are a group of proteins, which structurally are similar to the enzyme chitinases, but do not have the option to disintegrate chitin. These proteins are important fo the activation of the signal paths in the immune system, inflammation and tumorigenesis. They also are included in the restoration of tissues and fibrosis, and definitely the CLPs could be valuable biomarkers, depicting the degree of inflammation, joint damage and oncologic prognosis.

The **Literature review** of the dissertation of Prof. M.Kazakova is presented in 31 standard pages, and in this part of her thesis the author of the dissertation correctly and trustfully gives a very balanced basis of her research project, accenting on the role of CHI3L2 for the GBM development, even because it had been presented in just a few publications. This fact indeed had been used for planning of the study, assuming that CHI3L2 is a new prognostic biomarker, associated with the immune infiltration in GBM. Based exactly on the insufficiently clear functions, it is expected in the near future the expression and secretion of CHI3L2, relatively compared, to be included in the author’s dissertation study, under current investigation.

As a conclusion of the detailed literature review the author accents that based on the importance of the cell metabolism in the development of a number of pathologic processes, the investigations in this aim are basic to clear the pathogenic role of both chitinases.

And logically accents that the combination of these tests and facts tends to the necessity of realization of the aims, included in the dissertation, an aim which undoubtedly had been planned with very precise evaluation.

The **aim** of the study in the dissertation is to examine the complex expression and biologic role of chitinasesimilar proteins (CHI3L1 and CHI3L2) in processes of inflammation, tumorigenesis and neurodegeneration.

The **tasks** for completion of the aim, as depicted by the author, include complex study of the chitinasesimilar proteins in processes associated with inflammation, neurodegeneration, and tumor genesis, in correlation with proved clinicolaboratory parameters, as well as determination of the link between chitinasesimilar proteins, cell metabolism and mitochondrial function in various pathologies.

In the basic part of her study **Materials and Methods**, Prof. M.Kazakova includes as a contingent in her research a total of 270 patients, divided into three basic groups:
-autoimmune diseases and inflammatory processes, n=129 (PA, SSc, OA)
-neurodegenerative diseases and inflammatory processes, n=88 (iCNS, II, RAS)
-malignant diseases, n= 53 (CRK и GBM).

The contingent of the study includes patients from several basic hospitals in Plovdiv, with control groups added to each pathology.

The author uses the following clinical methods: PA, SSc and OA.

To investigate the patients with PA, SSc and OA, a number of instrumental and clinical methods is applied (echo diagnostic, double-size ultrasonography), which corresponds completely to the modern diagnostic approach, especially when it concerns a dissertation study.

A similar approach is used by Prof. Maria Kazakova in her author's study of patients with iCNS, II, RAS, GBM, CRK, thus maximally broadening the inclusion of patients, as basic part of her dissertation.

Important approach is the use of inclusion and delete criteria, an issue throwing additional light on the author's dissertation.

Prof. M.Kazakova applies in her study laboratory methods with specific direction: isolation of biologic materials, based on the literature analysis, and from the included in the study patients the following samples are taken: plasma, serum, liquor, cells or tissues, following strictly the standard clinical requirements.

Plasma/serum for immunoenzyme analysis, liquor, isolation of WBCs for RNA analysis, isolation of iRNA from WBCs and tissues, ELISA, synthesis of cDNA using RT-PCR, quantitative polymerase chain reaction, (qRT-PCR), pathologic tissues, routine histology, paraffin slices, antigen demasking, biotin-streptavidin-peroxidase method, analysis of mitochondrial activity – all these methodologic

approaches to work on the dissertation project add certain bonus to the author of the study.

The used reagents, plus the statistical methods comply completely to the evaluation of the achieved results.

The **Results** of the performed investigation are verified and realistic, especially that CHI3L1 is under intensive investigation, whereas the data for CGI3L2 are scarce in the international literature.

The author of the dissertation for the first time presents, according to the available literature, complex results for parallel expression (gene and protein level) and secretion of both CLPs in inflammatory, degenerative and tumor processes, which in general, adds a peculiar bonus to the dissertation study.

The correlation analysis of the links between the changes of the target indexes, determined after the applied therapy, has a remarkable value for the author's study, and the evaluation of the data is a result of the difference between the starting level and after the applied therapy, which is a compliment for the author.

Very well impression from the presented dissertation to me for evaluation and review of the entire study have the included **tables, graphs, photos and figures**, which provide completely realistic picture and impression for the author's plan, realization and analyses of her work on the topic.

One of the figures in the application (fig. 41), presented by Prof. M.Kazakova, shows the analysis of the gene interactions of CLPs, by using web-based instrument for prognosis, an approach which is absolutely innovative and most probably is used for the first time in Bulgaria, a fact adding compliments to the author's investigation.

The chapter **Discussion**, which determines the results of the author's study, is another compliment for Prof. M.Kazakova, and we find her multiplastic and interdisciplinary approach to the analysis of the pathogenesis of a number of diseases, with a peculiar focus on the role of inflammation, neurodegeneration and mitochondrial function in patients with autoimmune, tumor and neurodegenerative processes.

The author confirmatively depicts how the achieved results throw light on the important molecular and cellular mechanisms, which are at the start of the therapeutic answer, and correctly opens proofs to support the personalized approach in the treatment and monitoring of certain diseases, what can be seen as major part of her dissertation.

The value of the author's study is even more expressed, especially based on the multi approach, selected material, used method and inclusion of specially designed groups of patients, and when looking at the available literature, I could not find other published papers for the gene or protein expression of CHI3L1 in patients with iCNS, in terms of the etiology agent.

Thus, the dissertation of Prof. M.Kazakova is a pioneer study, not only in our country, but internationally.

Another positive impression from the author's study is the fact that Prof.M.Kazakova had taken over a very important research project, especially at the background of completely insufficient investigations on the link between the CLPs' expression and the tumor budding with CRK.

Her results indicate a link between the tumor budding and lymph invasion, thus supporting the potential role of these glycoproteins in the invasion of CRK, whereas the lack of a parallel between the increased tissue expression of CHI3L2 and low plasma concentrations in CRK is considerable, a fact which throws an additional light on the mechanisms of the pathology under our study.

The chapter **Conclusions** is used by the author to prove that CHI3L1 and CHI3L2 are conservative proteins playing role as biomarkers, and presenting as functional participants in the pathogenesis of diseases, associated with inflammation, tissue remodeling and neuro degeneration, despite their structural resemblance. They often indicate different, sometime even opposite functions, which in complex issues would be able to broaden the pathologies, linked to autoimmunity, neurodegeneration and cancer genesis, requiring detailed study of their posttranscript modification of both proteins. Thus, the importance of the environmental micro area in terms of their complex immunobiologic functions could be clear.

The chapter **Conclusions** is used by Prof.M.Kazakova for a detailed, analytical, proved and established existence protocol of her dissertation, by showing in 7 conclusions the worked out by her new aspects of the link between CHI3L1 and CHI3L2, with the mitochondrial function/dysfunction, thus presenting a new approach and possibility to evaluate the inflammation, clinical course and applied therapy in cases with a number of pathologic diseases.

The chapter **Achievements**, which I wish to add according to the author's original, is a depiction of her own evaluation of the results of the project, where the achievements and proofs are original, convincing and credible:

1.New data for gene and protein expression of CHI3L1 and CHI3L2 in diseases, associated with inflammation, tissue remodeling and neurodegeneration are presented.

2.Early identification of mitochondrial dysfunction in processes associated with neurodegeneration and inflammation would allow better antioxidant defense, choice and monitoring of the therapy of RAC and RA.

3.CHI3L1 stimulates the proliferation of tumor cells, invasion and metastatic process, modulating the inflammation and tumor microenvironment

In conclusion of my review on the dissertation of Prof. Maria Kazakova to obtain the scientific degree Doctor of Sciences in the specialty Medical Biology, titled “Molecular-Biologic and Immunologic Study of Chitinase-like Proteins CHI3L1 and CHI3L2 in the Processes of Inflammation, Tumorigenesis and Neurodegeneration”,

I, the undersigned Prof.Dr. Krassimir T. Metodiev, confirmatively and logically, based on my profound analysis of all provided materials, give my **absolutely positive and deserved evaluation, voting “YES”**, in my position of reviewer for the procedure of the scientific degree Doctor or Sciences, in Medical Biology, according to the Order № P-1306/25.02.2026, to complete the obligation according to #30, al.3 of the Rules of Plovdiv Medical University.

I hope that my colleagues from the Juri Committee shall follow my vote as well.

Varna, 21 April 2026

Prof.Dr. Krassimir T. Metodiev, MD, PhD,DScmed

Заличено на основание
Чл.5 §1, б. “В” Регламент (ЕС)2016/679