

PEER REVIEW

From Associate Professor Dr. Jeliazko Iliev Arabadjiev, MD, Ph.D.

Head of the Clinic of Medical Oncology, Acibadem City Clinic University Hospital Tokuda
EAD, Sofia

According to order № P-109-110 / 11.03.2022 of the Rector of the Medical University - Varna and Protocol №1 / 25.03.2022. , I have been chosen to prepare a peer review of the dissertation of Dr. Margarita Krasenova Maneva.

Regarding the dissertation thesis entitled:

”PREDICTIVE AND PROGNOSTIC VALUE OF A MARKER FOR NECROPTOSIS - RIPK3 IN PATIENTS WITH COLON CANCER IN METASTATIC STAGE”

for awarding the educational and scientific degree "Doctor" in the scientific specialty "Oncology", in the field of higher education "Health and Sports", professional field 7.1. "Medicine".

Author: Dr. Margarita Krasenova Maneva

The review is prepared according to the requirements of:

- The Law for development of the academic staff of the Republic of Bulgaria
- Regulations for application of the Law on the Protection of Human Rights and Fundamental Freedoms
- Regulations for the development of the academic staff of MU-Varna and the specific requirements for obtaining the scientific degree "Doctor".

Biographical information of the candidate:

Dr. Margarita Krasenova Maneva graduated in Medicine at the Medical University - Varna with a degree in Medicine in 2015. In the period January 2016 until November 2016 works as a resident doctor in SBALOZ "Dr. Marko A. Markov" - Varna, Department of Medical Oncology and Palliative Care. From November 2016 started working in the Clinic of Medical Oncology at the University Hospital "St. Marina" - Varna, and in March 2017. was selected as a full-time assistant at the Department of Propaedeutics of Internal Medicine at MU - Varna, English language training. From 2018 is enrolled as a doctoral student full-time study at the same department. Since 2020, she has been reassigned to the newly formed Department of Oncology. He speaks English and German. He is a member of the European Society of Medical Oncology (ESMO). The doctoral student has attended numerous scientific congresses and conferences at home and abroad.

Characteristics of the presented dissertation:

The dissertation has a classical structure. The dissertation contains 102 standard pages and contains the following sections: literature review 57 pages, purpose and tasks 1 page, patient population and research methods 10 pages, own results 11 pages, discussion 4 pages, conclusion 1 page, conclusions 1 page, contributions 1 page and bibliography. The work is illustrated with 15 tables and 20 figures of high quality and informativeness. The reference includes 227 literary sources in Latin. The structure of the dissertation is well balanced, the proportions between the separate sections are observed and it meets the requirements of the Law for the development of the scientific staff in the Republic of Bulgaria.

The dissertation has selected for his dissertation an important topic in the field of oncology, which he has studied in detail and presented in depth in his work. Colorectal cancer is one of the most common malignancies worldwide, and is one of the leading causes of death in cancer patients. Despite significant advances in the development of medicine in recent years, especially in the field of diagnosis and treatment, the 5-year survival in patients with metastatic stage remains low. In her **literature review**, Dr. Maneva has presented in depth the main points of the etiology, epidemiology, pathogenesis, staging, diagnosis and treatment of CRC. The wide information provided by the dissertation, the accurate use of scientific terminology and correct citation of the authors of scientific publications in the field make a strong impression. Current data on the epidemiology of the CRC in Bulgaria and the world are presented. This was followed by an expert presentation by Dr. Maneva of detailed information on the nature and

molecular characteristics of the necroptosis process, a recently identified form of programmed cell death that, unlike apoptosis, is caspase-independent and mediated by receptor kinase activity. -interacting proteins such as RIPK1 and RIPK3. It is the expression levels of the necroptosis marker - RIPK3 in the primary tumor of patients with mRCC that are the subject of an in-depth study by Dr. Maneva. The dissertation demonstrates extremely in-depth knowledge in the field of necroptosis, presenting its signaling pathways, structural characteristics of its mediators, skillfully comparing necroptosis with other forms of cell death such as apoptosis and necrosis. The good visualization of the information synthesized in a large number of tables is impressive. The dissertation presents in detail the key mediators of necroptosis and their influence not only in the development of neoplastic processes, but also in a number of other inflammatory, neurological and vascular diseases, which defines Dr. Maneva as a well-trained researcher.

The **main goal** of the dissertation is to study the relationship between the expression levels of the essential marker for necroptosis - RIPK3 in patients with CRC in the metastatic stage and some clinical and pathological characteristics such as gender, age, degree of tumor differentiation, KRAS mutation status, the relationship to the biological behavior of the tumor, as well as progression-free survival and overall survival. The aim of the dissertation is a logical continuation of the literary review.

The realization of this **goal** is related to the implementation of the main tasks set by the dissertation, namely:

- Selection of patients with colon cancer in the metastatic stage.
- Comparative study of the levels of immunohistochemical expression of RIPK3 in the primary tumor of patients with colon cancer.
- To analyze the correlations between the immunohistochemical levels of RIPK3 expression with the clinical and pathological characteristics of patients with colon cancer.
- To analyze the predictive ability of RIPK3 expression in the primary tumor to respond to 5-FU based first-line chemotherapy.
- To analyze the prognostic potential of RIPK3 expression in the primary tumor in terms of progression-free survival and overall survival.

The set main tasks are formulated clearly and precisely, they are specific and correspond to the set goal, which shows the good theoretical preparation and mastery of the problem by the dissertation.

The **materials and research methods** are well selected and thoroughly clarified. Different methods for medical and statistical data processing were used to analyze the collected data.

The dissertation is based on a retrospective analysis of 74 patients with unresectable, metastatic colon cancer who underwent at least three courses of first-line 5-FU-based chemotherapy in January 2012. until December 2015 The dissertation student has precisely summarized and illustrated with tables and figures the distribution of patients by groups according to gender, age, performance status, RAS mutation status, location of the primary tumor, degree of differentiation.

The **results** are presented in detail and systematically on 11 pages, following the course of the set tasks and are illustrated in detail with statistically processed digital tables and graphs. Expert analysis of the results shows that there is no relationship between the expression of RIPK3 in the primary tumor and gender, the degree of differentiation of carcinoma and KRAS mutation status. According to the dissertation results presented by the dissertation, patients with moderately differentiated G2 tumors showed a significantly longer overall survival compared to the group of patients with low G3 differentiation. A significant association between RIPK3 expression and the degree of differentiation has not been demonstrated. a significant difference in mean progression-free survival was found for the low-expression group compared to the high-expression group of RIPK3. A significant difference was also reported in terms of overall survival in the two groups, with significantly longer reported in patients with high expression compared to the group with low expression of RIPK3.

In the **discussion**, Dr. Maneva skillfully synthesized the analyzed results and compared them with similar ones worldwide, showing the innovativeness of the study.

The **contributions** synthesized by Dr. Maneva show the exceptional importance of the dissertation due to the fact that for the first time in Bulgaria the relationship between the level of expression of the marker for necroptosis RIPK3 in patients with mRCC is studied. His contribution worldwide is also impressive, as for the first time in the world literature the relationship between the necroptosis marker RIPK3 and patients' response to treatment with 5-FU based first-line HT in patients with mRCC has been studied and reported.

The data summary of 44 pages corresponds to the content of the dissertation and provides brief information on the main points clarified in the development - materials and methods, goals and objectives, results, discussion, contributions and conclusions of the dissertation. A list of Dr. Maneva's publications related to the topic of the dissertation is also provided.

Conclusion:

The dissertation of Dr. Margarita Krasenova Maneva "Predictive and prognostic value of a marker for necroptosis - RIPK3 in patients with colon cancer in metastatic stage" is an in-depth and accurate scientific development of an extremely important and useful topic in the field of oncology.

The dissertation has set clear, well-formulated goals and objectives, which are realized expertly and precisely. The theoretical conclusions made are comprehensive, correct and well-founded. The contributions have theoretical and practical value and are the basis for future research. The dissertation covers all indicators for obtaining the educational and scientific degree "DOCTOR" and fully meets the requirements of Law for development of the academic staff of the Republic of Bulgaria

Having in mind the above, I give a positive assessment of the dissertation of Dr. Margarita Krasenova Maneva and recommend to the esteemed Scientific Jury to award her the scientific and educational degree "DOCTOR".

Assoc. Prof. Dr. Jeliazgo Iliev Arabadjiev, Ph.D.