

# PEER REVIEW

**FROM PROF. DR. KRASIMIR IVANOV, MD, PhD, DSc**

**CHAIRMAN OF THE SCIENTIFIC JURY**

**SELECTED BY ORDER № P-109-110 / 11.03.2022**

**OF THE RECTOR OF THE MEDICAL UNIVERSITY - VARNA**

**SUBJECT:** the dissertation thesis of Dr. Margarita Krasenova Maneva, for awarding the educational and scientific degree "Doctor" at the Medical University "Prof. Dr. Paraskev Stoyanov "- Varna, specialty - Oncology, entitled " Predictive and prognostic value of a marker for necroptosis - RIPK3 in patients with colon cancer in metastatic stage" with scientific supervisor Assoc. Prof. Dr. Eleonora Georgieva Dimitrova - Gospodinova, PhD

## **Biographical information of the candidate**

Dr. Margarita Krasenova Maneva graduated from the Nikola Y. Vaptsarov Language High School in Shumen with German and English. In 2015 graduated from the Medical University - Varna with a degree in Medicine. In January 2016 started working as a doctor in SBALOZ "Dr. Marko A. Markov" - Varna, Department of Medical Oncology and Palliative Care. From November 2016 works in the Clinic of Medical Oncology of MHAT "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. Since the same month she has been enrolled as a specialist in Medical Oncology at the University Hospital "St. Marina" - Varna. From 2018 is a doctoral student full-time form of study at the same department. Since 2020, it has been reassigned to the newly formed Department of Oncology. She speaks English and German. She is a member of the European Society of Medical Oncology (ESMO)

Globally, colorectal cancer (CRC) ranks third in incidence among cancers, with the number of global new cases projected to increase. Despite advances in the diagnosis and treatment of CRC in recent years, the 5-year survival of advanced patients remains low. 5-fluorouracil (5-FU) is the most commonly used chemotherapeutic drug for advanced CRC and in combination with other agents, and its clinical applications are severely limited due to drug resistance.

Necroptosis is a newly discovered pathway of regulated cell death mediated by receptor-interacting protein kinases 1 and 3 (RIPK1 and RIPK3) as well as the mixed linear kinase domain (MLKL). Necroptosis has been shown to be a key process in the pathogenesis of cancer, and therefore a deeper understanding of its mechanisms is essential for developing new approaches to its management. The discovery of new predictive and prognostic markers aims to improve outcomes from treatment in patients with metastatic CRC.

The dissertation of Dr. Maneva sought a relationship between the expression levels of the essential marker RIPK3 for necroptosis in the primary tumor in patients with metastatic colon cancer and some clinical and pathological characteristics - sex, age, degree of tumor differentiation, KRAS mutation status, its relationship to the biological behavior of the tumor, as well as progression-free survival and overall survival.

### **Characteristics of the dissertation submitted for review**

The dissertation is presented in a volume of 102 pages and is illustrated with 15 tables and 20 figures. The scientific work has the following structure: "Introduction" - 2 pages, "Literary Review" - 57 pages, "Purpose and objectives of the study" - 1 page, "Patient population and research methods" - 10 pages, "Results" - 11 pages, "Discussion" - 4 pages, "Conclusion" - 1 page, "Conclusions" - 1 page, "Contributions to scientific work" - 1 page, "Scientific publications and communications related to the dissertation" - 2 pages (Presented 7 scientific publications, 2 of which have been published in international publications with an impact factor). The literature contains 227 Latin titles.

The main focus of the **literature review** is an in-depth analysis of the etiology, treatment, signaling pathways involved in the pathogenesis of CRC, as well as related prognostic and predictive factors. Up-to-date epidemiological data are presented, both for Bulgaria and for other countries in the European Union and the world. A detailed and systematic acquaintance was made with the key process of necroptosis for oncological diseases. The doctoral student presents in detail its mediators and their influence not only in the development of neoplastic processes, but also in a number of other disease states. Particular attention is paid to the acquaintance with a large number of mediators of necroptosis, expression of necroptotic factors in some cell lines, necroptosis inhibitors, the role of the process in overcoming therapeutic resistance, all well illustrated with appropriate figures and tables.

The dissertation is based on a clearly stated **goal** - to assess the relationship between the expression levels of the key necroptosis marker RIPK3 in patients with metastatic colon cancer and some clinical and pathological characteristics such as gender, age, degree of tumor differentiation,

KRAS mutation status, its relationship to the biological behavior of the tumor, as well as progression-free survival and overall survival. To achieve the set goal, the author outlines the following **tasks**:

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

## **Results:**

The dissertation presents the results of a retrospective single-center clinical trial in which the cases of 74 patients with unresectable, metastatic colon cancer were studied. The study included patients treated with first-line 5-FU-based chemotherapy in January 2012. until December 2015 at our University Hospital "St. Marina". All patients underwent at least 3 courses of I line HT. The dissertation has well synthesized and illustrated with tables and figures the distribution of patients in different groups according to gender, age, ECOG / performance status, RAS mutation status, location of the primary tumor.

In a detailed analysis of the test results, no correlation was found between the expression of RIPK3 in the primary tumor and gender, the degree of differentiation of the carcinoma and the KRAS status. The study found a significant difference in mean progression-free survival 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. According to the systematized results of the study, patients with moderately differentiated tumors showed significantly longer overall survival compared to the group of patients with G3 differentiation. A significant association between RIPK3 expression and the degree of differentiation has not been demonstrated.

The discussion on the obtained results emphasizes their importance, comparing them with other tests worldwide and provides guidelines for future developments.

Five clearly formulated **conclusions** have been synthesized, which briefly and accurately give summarized information about the work and its results, fully meet the set goals and objectives. The dissertation clearly pointed out the **contributions** of the dissertation not only in Bulgaria but also worldwide.

The **data summary** provides concise information on the main points marked in the dissertation - materials and methods, goals and objectives, results, discussion, contributions and conclusions of the dissertation.

**Critical remarks and recommendations:**

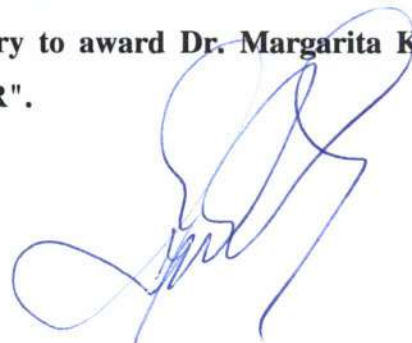
In his retrospective study, the dissertation included a relatively small group of patients (74 patients), which necessitates additional studies. However, the results obtained most likely suggest that the level of RIPK3 expression in primary tumors is a new independent potential predictive biomarker for progression-free survival in patients with metastatic CRC. Despite the great workload, functions and responsibilities of the dissertation, I believe that he will have the opportunity to supplement and expand his knowledge and contributions in the field of the topic in the coming years.

**Conclusion**

The dissertation of the dissertation of Dr. Margarita Krasenova Maneva on "Predictive and prognostic value of a marker for necroptosis - RIPK3 in patients with colon cancer in metastatic stage" presents results and conclusions with original contributions to science and meets all the requirements of the Law for the development of the academic staff of the Republic of Bulgaria and the Regulations of MU-Varna.

The dissertation shows that the dissertation of Dr. Margarita Krasenova Maneva has acquired in-depth theoretical knowledge and demonstrates qualities and skills for independent research. Due to the above, I confidently give my positive assessment of the dissertation.

**I propose to the highly respected Scientific Jury to award Dr. Margarita Krasenova Maneva an educational and scientific degree "DOCTOR".**



Date: 04.05.2022

Varna

**Prof. Dr. Krasimir Ivanov, MD, PhD, DSc**