

To the Chairman of the Scientific Jury,
determined by Order No. P-109-110 / 11.03.2022

on

Rector of the Medical University – Varna

STANDPOINT

by Prof. Dr. Zhasmina Mihailova Milanova, Ph.D.

Head of the Clinic of Medical Oncology at

Department of Hematology, Oncology, Pathology and Radiobiology,

Military Medical Academy, Sofia

Subject: Dissertation on "*Predictive and prognostic value of a marker for necroptosis - RIPK3 in patients with metastatic colon cancer*" of full-time doctoral student Dr. Margarita Krasenova Maneva, for awarding the educational and scientific degree "DOCTOR" in professional field 7.1. Medicine, in the field of higher education 7.3 Health and sports, in the scientific specialty "Oncology".

Scientific Supervisor:

Assoc. Prof. Eleonora Georgieva Dimitrova-Gospodinova, Ph.D.

I. Administrative evaluation

Dr. Margarita Krasenova Maneva is enrolled as a doctoral student in full-time education at the Department of Propaedeutics of Internal Medicine, MU-Varna as of 16.07.2018 for a period of three years with the topic "Predictive and prognostic value of a marker for necroptosis - RIPK3 in patients with metastatic colon cancer " with supervisor Assoc. Prof. Dr. Eleonora Georgieva Dimitrova-Gospodinova, MD, with order № R-109- / 16.07.2018 of the Rector of the Medical University - Varna.

According to a report by the head of the Department of Oncology, the dissertation of Dr. Margarita Krasenova Maneva on "Predictive and prognostic value of a marker for necroptosis - RIPK3 in patients with metastatic colon cancer" was approved and proposed for work completion with the right to defense. By order № P 109-110 of 11.03.2022 of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna, full-time doctoral student Dr. Margarita Krasenova Maneva was proposed for work completion with the right to defense. According to Order No. R-109-110 / 11.03.2022 of the Rector of the Medical University and on the basis of Protocol No. 1 of 25.03.2022, a Scientific Jury consisting of five habilitated persons was elected.

II. Brief biographical data

Dr. Margarita Krasenova Maneva graduated in medicine at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna in 2015, and in 2016 began specialization in the Clinic of Medical Oncology at the University Hospital St. Marina Varna. Dr. Maneva is an assistant at the Department of Propaedeutics of Internal Medicine, later renamed the Department of Oncology, and since July 2018 she is a doctoral student in full-time education in the specialty "Oncology".

Dr. Maneva is a member of the Bulgarian Medical Association and the European Society of Medical Oncology (ESMO).

III. Evaluation of the dissertation

1. Relevance of the topic

Necroptosis is a major and best-studied form of regulated necrosis. RIPK3 (Receptor-interacting protein kinase 3) is a key component of the RIPK1-RIPK3-MLKL (mixed lineage kinase domain-like) complex called necrosom. Necroptosis is a regulated caspase-independent necrotic cell death and serves as an alternative to programmed cell death. RIPK1 and RIPK3 are involved in the process of necroptosis and are considered to be its main regulators. In the most widely studied model, TNF-R1 induces programmed necrosis by activating RIPK1 and RIPK3. Subsequently, programmed necrosis is achieved through mitochondrial fragmentation. More and more research in recent years has shown that changes and disorders in the process of necroptosis are associated with

tumorigenesis in cancer. On the other hand, therapeutic resistance through antiapoptotic progression is due to dominant selective advantages against the apoptosis of certain cancer cell populations. In connection with the relevance of the topic of the doctoral student evidence is the following data. A search of the Pubmed database of the keywords „necroptosis and cancer“ yielded 1496 publications as of 03.05.2022 and for „necroptosis and colorectal cancer“ only 89 publications. There is a great interest in the prognostic and predictive role of markers of necroptosis in malignant diseases and the presence of still few studies of these markers in patients with colorectal cancer, which makes the dissertation timely performed.

2. General characteristics of work

The dissertation is designed in accordance with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, namely - developed on 102 standard pages - of which: "Introduction" - 2 pages, "Literary Review" - 44 pages, "Objective and research tasks" - 1 page, "Materials and methods" - 11 pages, "Results" - 12 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.

The dissertation is illustrated with 20 figures and 15 tables. The bibliography contains 227 sources, all of which are English-language literature.

The scientific work of the doctoral student Dr. Maneva is properly structured as a sequence and volume of sections. There is a good illustration with figures and tables.

The abstract contains 43 pages and fully corresponds to the text of the dissertation.

3. An aim and tasks

The aim of the dissertation is clearly stated. The set 5 tasks are logically related to the goal and give grounds to assume that by mastering and applying the methodology of scientific research, they will be successfully solved. In this sense, the tasks are related to the search for a link between the immunohistochemical expression of RIPK3 in the primary tumor and the prognosis of patients, as well as the definition of RIPK3 as a predictive marker for the response to 5Fu-based therapy.

4. Research methodology

A study consists of 74 patients treated in the Clinics of Medical Oncology of the University Hospital St. Marina - Varna. Inclusion and exclusion criteria are well described, as well as specific methods of immunohistochemical examination, as well as statistical methods of processing the results.

5. Results

The results obtained are original and are the result of the research work of the doctoral student. The results are presented in five subsections, namely

- Clinical and pathological characteristics of patients
- Statistical analysis and data processing
- Relationship between RIPK3 expression and clinical and pathological characteristics of patients
- Prognostic value of the degree of differentiation
- Effect of RIPK3 expression on overall survival and progression-free survival

6. Evaluation of the dissertation and contributions

The doctoral student Dr. Margarita Krasenova Maneva presented the conclusions and contributions in two sections, formulating 5 conclusions. More significant conclusions are:

- The better overall survival was found in patients with high RIPK3 expression, making it a potential prognostic marker.
- The better response to 5-FU based first-line chemotherapy has been found in patients with higher RIPK3 expression.

The most significant contributions are:

- For the first time in Bulgaria, the potential of the level of RIPK3 expression to be used as a prognostic factor in terms of overall survival in patients with colon cancer has been reported.

- For the first time in the world literature, the relationship between the necroptosis marker RIPK3 and patient response to treatment with 5-FU based first-line therapy in patients with mCRC has been studied and reported.

IV. Scientific production

The scientific production of the candidate related to the topic of the dissertation includes 7 publications, all of which are full-text publications, two publications are in a referenced journal with Impact Factor - Biomedicines and Clinical and Investigative medicine

V. Conclusion

The dissertation of Dr. Margarita Krasenova Maneva is a valuable study on "Predictive and prognostic value of a marker for necroptosis - RIPK3 in patients with metastatic colon cancer."

As a member of the Scientific Jury, I believe that the presented research is well done and with contributions to oncology science. Currently, there are relatively few studies available on the relationship between necroptosis, its markers and mRCC (89 published in Pubmed), so more research is needed to elucidate the exact molecular mechanisms of interaction in this research area. Thus, Dr. Maneva's study fulfills this task by establishing a prognostic for overall survival and a probable predictive role for the response to first-line 5-Fu based therapy of RIPK3 expression assessed by IHC.

The content of the dissertation, as well as the obtained results show that the doctoral student has mastered the methodology of scientific research and is able to apply it. All this gives me reason to convincingly suggest to the members of the scientific jury to vote positively for obtaining the scientific and educational degree "DOCTOR" in the scientific specialty "Oncology" of Dr. Margarita Krasenova Maneva.

With respect:

/ prof. Dr. Zhasmina Mikhailova, MD /