

STATEMENT

BY PROF. DR. PETRANKA TROYANOVA, DM,

Professor of the scientific specialty "Oncology", Professional field 7.1. Medicine, Sphere of higher education 7. Health and sports,
at the Department of Nuclear Medicine, Radiation Therapy and Medical Oncology, Faculty of Medicine, Medical University - Sofia,
member of the scientific jury according to order № P-109-110 / 11.03.2022 of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna

About: Public defense of the dissertation of Dr. Margarita Krasenova Maneva for the award of educational and scientific degree "Doctor" in the Sphere of higher education 7. Health and Sports, Professional field 7.1. Medicine, in the scientific specialty "Oncology", with the topic of the dissertation "PREDICTIVE AND PROGNOSTIC VALUE OF A MARKER FOR NECROPTOSIS - RIPK3 IN PATIENTS WITH COLORECTAL CANCER IN METASTATIC STAGE"

I. Brief data on the candidate's career development

Dr. Margarita Maneva was born on August 20, 1990 in the town of Shumen. In 2015 he graduated from the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

Since 2016 she is a specializing physician at the University Hospital "St. Marina" - Varna in the clinical specialty "Medical Oncology".

By order of the Rector of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna is enrolled as a PhD student in full-time education in 2018 at the Department of Propaedeutics of Internal Medicine at the Faculty of Medicine, MU-Varna with supervisor Assoc. Prof. Dr. Eleonora Dimitrova, Ph.D. m.

Dr. Maneva is an assistant at the Department of Propaedeutics of Internal Medicine at the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

Dr. Maneva has a strong affinity for research. He has participated in national and international scientific events. In connection with the developed dissertation, Dr. Margarita Krasenova Bogdanova-Maneva has presented 8 scientific publications, 2 of which in refereed publications with impact factor.

Dr. Maneva's documents meet the legal requirements for the development of the academic staff of Law on the development of the academic staff in the Republic of Bulgaria and the Rules for the development of the academic staff in the Medical University "Prof. Dr. P. Stoyanov" - Varna. They are well arranged and contain sufficient evidence.

II. Relevance of the dissertation

Colorectal cancer (CRC) is the third most common solid tumor worldwide and one of the leading causes of cancer death in both sexes. The main therapeutic approach in patients with CRC in the early stage (I-II) is surgical treatment, but in metastatic or unresectable disease (stage IV) drug therapy is leading. In the latter case, the discovery and application of new prognostic and predictive markers that help better understand the biological behavior of the tumor is essential for a more accurate diagnosis, to predict the effect of therapy, and to improve survival.

Necroptosis is a newly discovered pathway of regulated apoptosis, the action of which requires the involvement of receptor-interacting protein kinases 1 and 3 (RIPK1 and RIPK3), as well as the mixed linear kinase domain (MLKL). As necroptosis is increasingly considered an important process in the pathogenesis of cancer, a deeper understanding of its mechanisms is essential for the development of new approaches to its regulation in neoplastic processes. Expression in the primary tumor of RIPK3 involved in the mechanisms of necroptosis may

have prognostic potential in terms of progression-free survival and overall survival and predictive significance for the effect of 5-FU-based first-line chemotherapy.

Therefore, the study of the possible correlation between the expression of RIPK3 in the primary tumor in patients with metastatic colon cancer and some clinical and pathological characteristics - sex, age, degree of tumor differentiation, KRAS mutational status is particularly important and makes the dissertation extremely interesting and up-to-date, contributing to a better understanding of the prognostic and predictive significance of the necroptosis marker - RIPK3 in patients with metastatic colon cancer.

III. Characteristics and evaluation of the dissertation

The dissertation contains 102 standard pages and is illustrated with 15 tables and 20 figures. The literature reference includes 227 literary sources, of which 227 are in Latin.

The aim of the study is correctly defined and gives the main directions of the research work - to look for a correlation between the expression of RIPK3 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 tumor biological behavior, as well as progression-free survival and overall survival.

To achieve the goal, 5 tasks are formulated precisely and clearly:

- To select patients with colon cancer in the metastatic stage;
- To compare 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.

In the dissertation work a detailed and analytical literature review on the topic is made, in which the most modern developments are presented. To date, the available studies examining the relationship between necroptosis, its markers and mCRC are relatively few and limited, so new studies are needed to elucidate the exact molecular mechanisms of interaction between different types of cell death, their role in neoplastic processes and the potential for - good therapeutic strategies for the treatment of patients with malignant diseases and in particular those with CRC. This proves the relevance of the topic of the dissertation and the usefulness of its development.

In the developed dissertation work are applied modern clinical, diagnostic, epidemiological and statistical methods that are sufficient to solve the tasks to achieve the scientific goal. The study was approved by the Commission on Ethics of Research (KENI) of the Medical University "Prof. Dr. Paraskev Stoyanov" - Varna.

A retrospective non-interventional single-center clinical trial was conducted, which included 74 patients with unresectable, metastatic colorectal cancer treated with first-line 5-FU-based chemotherapy in the period from January 2012 until December 2015 at the University Hospital "St. Marina" -Varna. They were all staged with CT or PET / CT before starting treatment. Performance / ECOG status of patients was assessed at <2.

Medical records, routine clinical trials and biological markers, as well as applied treatment were analyzed. Specific methods of examination of the biopsy materials of these patients have been applied. Immunohistochemical expression of RIPK3 was assessed using H-score (histo-score) on tissue samples. The RAS mutation status of the studied tissue samples was studied using an allele-specific device for quantitative PCR based analysis in real time (Real-Time qPCR System). All patients studied received at least 3 months of treatment

with 5-FU based first-line chemotherapy. The most commonly used regimen is FOLFOX4 / CAPEOX +/- Bevacizumab / Panitumumab (55.4% of cases). FOLFIRI +/- Bevacizumab / Panitumumab was administered to 29.7% of patients and De Gramont to the remaining 14.9%. Patients with low levels of RIPK3 expression show significantly lower levels of response to treatment, which is why high expression of RIPK3 is a positive prognostic marker for the response rate.

Imaging in all patients was performed before the start of first-line treatment and every three months regularly (4-6 treatment cycles) during systemic treatment until disease progression according to RECIST 1.1 criteria. The imaging methods used are CT of the chest, abdomen and pelvis or by PET / CT. In the course of systemic treatment, the parameters PFS (progression-free survival) and OS (overall survival) were monitored.

The statistical data are processed in detail with the help of statistical software package SPSS version 23. The obtained results are described in detail and accurately and are illustrated with tables and figures, following the set tasks.

Based on the obtained reliable and representative results, an in-depth discussion was developed and the relevant conclusions were formulated:

- There is no correlation between the level of RIPK3 expression in primary tumors and the clinical and pathological characteristics of patients with CRC in the metastatic stage;
- Better overall survival was found in patients with high RIPK3 expression, making RIPK3 expression a potential prognostic marker;
- There is a better response to 5-FU based first-line chemotherapy in patients with higher RIPK3 expression;
- Potential prognostic value of RIPK3 as a predictive factor for 5-FU based HT has been established;
- There was no statistically significant relationship between the level of RIPK3 expression and progression-free survival in patients with CRC.

The presented conclusion is logical and justifies the conclusions of the dissertation. In the present study, the significance of RIPK3 necroptosis marker expression levels was first investigated in patients with mRCC who underwent I-line 5-FU-based HT in terms of certain patient characteristics, OS, PFS, and response to treatment. It is clear that the marker for necrosis of RIPK3 can serve as an independent prognostic marker for overall survival in patients with advanced mRNA, which is supported by the latest literature data.

Its value for first-line treatment with 5-FU-based HT in these patients has also been proven, making it a potential predictor.

The contributions of the dissertation are significant, with theoretical and applied significance:

1. For the first time in Bulgaria the connection between the level of expression of the marker for necroptosis RIPK3 in patients with metastatic CRC is studied.
2. For the first time in Bulgaria, the potential for 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.
3. For the first time in Bulgaria, the potential of the level of RIPK3 expression to be used as a predictive factor for 5-FU-based first-line chemotherapy in patients with mCRC has been reported.
4. For the first time in the world literature, the relationship between the necroptosis marker RIPK3 and the response of patients to treatment with 5-FU based first-line HT in patients with m CRC has been studied and reported.

IV. CONCLUSION

The dissertation work developed by Dr. Margarita Maneva "PREDICTIVE AND PROGNOSTIC VALUE OF A MARKER FOR NECROPTOSIS - RIPK3 IN PATIENTS

WITH COLORECTAL CANCER IN METASTATIC STAGE” covers all scientometric requirements of Law on the development of the academic staff in the Republic of Bulgariain and the Rules for the development of the academic staff in the Medical University "Prof. Dr. P. Stoyanov "- Varna for the acquisition of educational and scientific degree "Doctor ". This, as well as the presented scientific production, are grounds for convincingly giving my positive assessment and recommending to the members of the esteemed scientific jury to award the educational and scientific degree "Doctor" to Dr. Margarita Krasenova Maneva.

May 4, 2022
Varna

Prepared the statement:
PROF. DR. P. TROYANOVA, DM,

A handwritten signature in blue ink, consisting of several overlapping loops and a long vertical stroke extending downwards.