STATEMENT

From Associate Professor Dr. Jeliazko Iliev Arabadjiev, MD, Ph.D.
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According to order № P-109-11 / 11.03.2022 of the Rector of the Medical University - Varna and Protocol №1 / 25.03.2022. , I have been chosen to prepare an opinion on the dissertation of Dr. Rostislav Radoslavov Manev.

Regarding the dissertation thesis entitled:

"SINGLE NUCLEOTIDE POLYMORPHISMS IN THE GENES FOR NON-CODING RNA AS DIAGNOSTIC AND PROGNOSTIC MARKERS IN PATIENTS WITH METASTATIC COLORECTAL CANCER"

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. Rostislav Radoslavov Manev

1. Significance of the problem and formulation of the goal and tasks:

Colorectal cancer (CRC) is the third most common malignancy, leading to death in cancer patients worldwide. In recent decades, the number of registered cases of CRC has increased dramatically, but the development of diagnostic methods, surgical treatment and the application of individual algorithms for anticancer treatment have significantly improved the quality of life and overall survival in patients with CRC. However, the 5-year survival in patients with stage IV CRC is below 14%. Lack of screening in people over the age of 50 increases the likelihood of developing invasive CRC. For this reason, stable and reliable predictive and prognostic markers are sought in patients with stage IV colorectal cancer as an attempt to optimize their treatment.

Single nucleotide polymorphisms (SNPs) are considered as a biomarker for predicting the risk of solid tumors with different primary sites, including CRC. Microribonucleic acids (miRNAs) are RNA molecules that play an important role in various biological processes, such as embryonic development, cell differentiation, proliferation, apoptosis, insulin secretion and oncogenesis, and others. Microribonucleic acids do not encode proteins, but have an important function in regulating gene expression. Single nucleotide polymorphisms in miRNA genes - miR-SNPs are able to modulate siRNA expression and thus affect the risk of cancer development, treatment efficacy and patient prognosis. There are few studies in this direction in the world, mainly in Asian populations, and such have not been conducted in the Bulgarian population so far.

For these reasons, the dissertation of Dr. Manev is of current importance, both from a theoretical and practical point of view and has contributed to the strategy in the diagnosis and treatment of CRC.

2. Structure of the dissertation:

The dissertation has a classical structure. The dissertation contains 146 standard pages and contains the following sections: literature review 58 pages, purpose and tasks 1 page, researched groups and methods 15 pages, own results 30 pages, discussion 2 pages, conclusion 1 page, conclusions 1 page, contributions 1 page and bibliography. The work is illustrated with 40 tables and 35 figures of high quality and informativeness. The literature includes 257 literature sources, of which 3 are in Cyrillic and 254 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.

3. Literary awareness of the dissertation:

The literature review is written with expert and thorough. The epidemiology, etiology, genetic predisposition, diagnosis, staging and treatment of CRC at the current level are discussed. The main place is given to microrobinucleic acids (miRNAs) and their polymorphisms as diagnostic and prognostic markers in CRC. The scientific publications on the topic are critical and the main problems and unresolved issues in this direction are presented.

The wide information that the dissertation handles, the correct use of the scientific terminology and the full and correct citation of the authors of the scientific publications in the field are impressive. It should be noted the precise focus and pragmatized comprehensiveness of the review of the problem, which defines Dr. Manev as a versatile and well-trained researcher. The bibliography includes 257 literature sources. The topicality of the used literature makes a very good impression, as more than 60% of the sources are from the last 10 years.

The aim of the dissertation is a logical continuation of the literature review, namely: To identify new diagnostic and prognostic molecular biological biomarkers in Bulgarian patients diagnosed with CRC in metastatic stage by examining the presence of five selected single nucleotide polymorphisms (SNPs) in genes encoding micro-RNAs and the possibility of their specific application in practice.

There are seven main tasks, which are formulated clearly and precisely, they are specific, well-planned and consistent with the goal, which shows the good theoretical preparation and mastery of the problem by the dissertation.

4. Methodological level and design of research:

In the section Research groups and methods are presented the patients included in the scientific work with correctly defined inclusion criteria and description of the used methods. The dissertation is based on a prospective analysis of 101 patients with metastatic CRC who received first-line fluoropyrimidine-based chemotherapy alone or in combination with anti-VEGF or anti-EGFR targeted therapy. The analysis was conducted in comparison with a group of 90 healthy volunteers, similar in demographic indicators to the patients. All participants in the study provided written informed consent, and the study itself was approved by a protocol of the Commission on Research Ethics at MU-Varna.

5. Correspondence between the goal, the results and the conclusions

There is a logical correspondence between the set goal, the obtained results, the discussion and the conclusions made. The own results are presented on 30 pages, following the course of the set tasks and are presented in detail, illustrated with statistically processed digital tables and graphs.

The correlations of the specific genotype / allele of the five SNPs studied in the siRNA genes are detailed and consistent in terms of the prediction of the risk of developing CRC, overall survival, and in terms of miRNA and plasma levels - in terms of overall patient survival.

The results are the subject of analytical discussion in relation to literary references, creative hypotheses are formulated.

6. Analysis of the conclusions and contributions:

Based on the results obtained, the author synthesizes 8 conclusions, which are written specifically and clearly and are relevant to the goal and the tasks developed.

The conclusions from the analysis of the samples of patients with mRCC and healthy volunteers substantiate the diagnostic and prognostic correlations of the five studied single nucleotide polymorphisms in mi-PKNs: (1) similarity was found in the distribution of studied polymorphisms in healthy individuals and other European cohorts; (2) Heterozygous individuals with rs2910164 - miRNA-146a are characterized by a low risk of developing the disease (3) the polymorphism rs2682818 - miR-618 also appears to be protective against CRC (4); Carriers of the dominant A allele in the homozygous state with rs353293 - miRNA-143/145 are characterized by a high risk of developing CRC; (5) carriers of the TT genotype with rs7372209 have significantly longer average overall survival (OS); (6) patients homozygous for the A allele (AA) of rs353293 - miRNA-143/145 also had a longer mean overall survival (OS); (7) The TT rs7372209 genotype was assessed as a risk factor for right colon tumor development; (8) Plasma levels of miRNA-26a-1, miRNA-146a, miRNA-618 and miRNA-181b are of diagnostic importance.

I accept the presented contributions of the dissertation, which can be defined as theoretical and scientific-applied.

For the first time in national clinical oncology and genetics, data on the allelic frequency and genotypic distribution of polymorphisms in the genes for non-coding miRNAs among healthy individuals are presented. There are also original interpretations of the relationship between the presence of definite nucleotide polymorphisms in the genes for non-coding RNAs and their role as potential biomarkers that could predict the risk of disease development and the prognosis of CRC. The presented data confirm the meaning and the need for genetic profiling for the presence of certain genetic alterations - single polymorphisms and genotypic signatures - for early diagnosis and correct follow-up therapeutic strategies in metastatic CRC.

In connection with the dissertation Dr. Manev presents 9 real publications in refereed international and national publications with impact factor.

The dissertation is written clearly and concisely, in good literary Bulgarian. The abstract is written concisely and adequately reflects the content of the dissertation.

7. Conclusion:

The dissertation of Dr. Rostislav Radoslavov Manev is an in-depth and precisely conducted research.

The dissertation is a topical work of scientific and applied value. The goal and tasks are precisely set, the results obtained are convincing, the conclusions are clearly formulated and comprehensive. The contributions have theoretical and practical value and are the basis for future research. The dissertation covers the quantitative indicators for obtaining the educational and scientific degree "Doctor" and fully meets the requirements of the Law for the development of the scientific staff in the Republic of Bulgaria.

Having in mind the above, I give a positive assessment of the dissertation of Dr. Rostislav Radoslavov Manev and recommend to the esteemed Scientific Jury to award him the scientific and educational degree "DOCTOR".

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