PEER REVIEW

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

CHAIRMAN OF THE SCIENTIFIC JURY

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OF THE RECTOR OF THE MEDICAL UNIVERSITY - VARNA

SUBJECT: the dissertation of Dr. Rostislav Radoslavov Manev, for awarding the educational and scientific degree "Doctor" at the Medical University "Prof. Dr. Paraskev Stoyanov "- Varna, specialty - Oncology, entitled " Single nucleotide polymorphisms in the genes for non-coding RNA as diagnostic and prognostic markers in patients with metastatic colorectal cancer. " with scientific supervisors Assoc. Prof. Dr. Nikolay Vladimirov Conev, Ph.D. and Assoc. Prof. Maria Atanasova Radanova, Ph.D.

Biographical information of the candidate

Dr. Rostislav Radoslavov Manev graduated in Medicine at the Medical University - Varna in 2016. In 2016 he began working as a doctor in the Clinic of Medical Oncology at the University Hospital "St. Marina" - Varna and as a part-time assistant in the Department of Medicine. Propaedeutics of Internal Medicine at the Medical University - Varna. In 2017 he was appointed as a specialist doctor in the Clinic of Medical Oncology at the University Hospital "St. Marina" - Varna and a regular assistant in the Department of Propaedeutics of Internal Medicine at the Medical University - Varna. From 2018 is a doctoral student full-time form of study at the same department. In 2020 he was reassigned to the newly formed Department of Oncology. Participates annually in international training courses, including those organized by ESMO. Dr. Rostislav Manev actively participates in conducting clinical trials on the territory of the Clinic of Medical Oncology. Fluent in English.

He is a member of the European Society of Medical Oncology (ESMO).

Colorectal cancer (CRC) is one of the leading malignancies worldwide, leading to death in cancer patients. Despite the development of medical treatment algorithms over the last decade, the 5-year survival rate in patients with stage IV clinical events does not exceed 10%.

Single nucleotide polymorphisms (SNPs) are genetic variations that are involved in various functional processes affecting individual susceptibility to certain cancers. 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. In recent years, a large number of SNPs have been studied worldwide through the use of specific genome-associated studies as directly related to the risk of developing CRC. This is one of the reasons why SNPs are considered as potential biomarkers in patients with CRC.

To date, research on the potential diagnostic, predictive and prognostic role of miR-SNPs has been limited in number, mainly in Asian colorectal cohorts. Studies on the presence of miR-SNPs and their role as diagnostic, prognostic and predictive factors have not been conducted among the Bulgarian cohort of patients.

The identified facts determine the need to introduce new diagnostic, predictive and prognostic biomarkers in patients with CRC in order to improve existing treatment algorithms and quality of life in patients with advanced colon cancer.

Characteristics of the dissertation submitted for review

The dissertation is presented in a volume of 146 pages and is illustrated with 40 tables and 35 figures. The scientific work has a classical structure: "Introduction" - 2 pages, "Literary Review" - 58 pages, "Purpose and objectives of the study" - 2 pages, "Specific research methods" - 6 pages, "Results" - 46 pages, " Discussion "- 7 pages," Conclusion "- 1 page," Conclusions "- 1 page," Contributions to scientific work "- 1 page," Scientific publications and communications related to the dissertation "- 2 pages (9 scientific publications are presented, 7 of which have been published

in international publications with an impact factor). The literature contains 257 titles, of which 3 in Cyrillic and 254 in Latin.

In the **literature review,** the dissertation initially considers separately general information about CRC covering the epidemiology, etiology, pathogenesis and drug treatment of the respective cancer. The diagnostic, prognostic and predictive biomarkers currently used in real clinical practice are described in detail. Information on the association of microribonucleic acids (siRNAs) and single nucleotide polymorphisms in the corresponding genes for non-coding siRNAs with CRC is presented sequentially and systematically. The author makes an in-depth literature review of the available data from global studies examining the relationship between single nucleotide polymorphisms and CRC covering different ethnicities and races, which are illustrated in 16 tables. 13 different single genetic polymorphisms in 14 siRNAs were examined in detail. The dissertation pays special attention to five selected single nucleotide polymorphisms (SNPs) in the genes encoding microRNAs - rs7372209 in the microRNA gene-26a-1, rs2910164 in the microRNA gene-146a, rs2682818 in the microRNA gene. -RNA-618, rs353293 in the promoter region of the gene cluster for microRNA-143 and microRNA145 and rs322931 in the gene for microRNA-181b, which frames the need for research in the field.

The dissertation sets a clearly stated **goal**, namely: to identify new diagnostic and prognostic molecular biological biomarkers in Bulgarian patients diagnosed with CRC in the metastatic stage by examining the presence of five selected single nucleotide polymorphisms (SNPs) in genes encoding microRNAs. and - rs7372209 in the micro-RNA-26a-1 gene, rs2910164 in the micro-RNA-146a gene, rs2682818 in the micro-RNA-618 gene, rs353293 in the promoter region of the micro-RNA-143 gene cluster and micro- RNA145 and rs322931 in the microRNA-181b gene. To achieve the set goal, the author outlines the following **tasks**:

To construct and characterize in detail a Bulgarian cohort for testing for the presence of five selected SNPs. Selection and stratification of patients with CRC in metastatic stage, conducted first-line treatment, by demographic and clinicopathological indicators. Follow-up of response to therapy. Selection and characterization of a control group of healthy individuals, similar in demographic indicators to patients. Study of the allelic distribution and genotypic frequency of the five selected

SNPs in the selected cohort of patients with metastatic colorectal cancer. Comparison of the allelic and genotypic frequencies of the selected five SNPs in the Bulgarian group of healthy individuals with the available data for other cohorts. Search for an association between the carrier of a certain genotype / allele of the five SNPs studied in the micro-RNA genes and the ability to predict the risk of developing CRC. Search for an association between the carrier of a certain genotype / allele of the five studied SNPs in the micro-RNA genes and the overall survival in patients. Comparison of plasma levels of micro-RNAs whose genes contain the tested SNPs, in patients with mRNA and in the healthy control group. Study of the association between plasma expression levels of micro-RNAs whose genes contain the SNPs studied and overall survival in patients.

Dr. Rostislav Manev examined retrospectively 101 patients who passed through the Clinic of Medical Oncology at the University Hospital "St. Marina" EAD - Varna and the Clinic of Medical Oncology - Military Medical Academy (MMA) - Sofia. Inclusion and exclusion criteria are clearly defined to select suitable patients for the study. The **methodology**, including descriptive information about patients, the specific methods of studying the five selected polymorphisms in the microRNA genes and the statistical design of the research are duly presented by the author.

Results

The study analyzed data from 101 patients with metastatic CRC. A descriptive analysis of the patient population was performed, and the main demographic and clinicopathological characteristics are well illustrated in tabular form and with the help of figures. The cohort is well balanced in terms of gender, performance status, RAS mutation status, primary tumor location, and secondary lesion location. The dissertation presents and illustrates the results well with the help of tables and figures with an accurate description of the type of statistical analysis and the degree of statistical significance.

Some highlights of the results

When comparing the allelic and genotypic frequencies of the selected five SNPs in the Bulgarian group of healthy individuals with the available data for other cohorts, a similar frequency distribution of the studied polymorphisms in Bulgarian individuals and European cohorts was found.

An association was found between the carrier of a certain genotype / allele in three (rs2910164 - miRNA-146a, rs2682818 - miR-618 and rs353293 in the promoter region of the gene cluster for miRNA-143/145) of the five SNPs studied in the microRNA genes. and the ability to predict the risk of developing CRC.

For two of the studied polymorphisms (rs7372209 and rs363293) a statistically significant association was found with the overall survival of the patients. Multivariate regression Cox analysis showed a tendency to associate the TT rs7372209 genotype with a better prognosis of metastatic CRC. Multivariate regression Cox analysis also showed that carriers of the rs363293 AA genotype may be associated with a better prognosis.

A comparison of the plasma levels of the microRNAs whose genes contained the tested SNPs (in patients with metastatic CRC and in the healthy control group) showed that four of them showed different expression in healthy and sick (miRNA-26a- 1, miR-146a, miRNA-618 and miRNA-181b), i. with relatively high sensitivity and specificity can distinguish patients with metastatic CRC from healthy controls.

The **discussion** is a critical analysis of the results obtained in the context of the literature. The discussion is closely related to the set goals and objectives and the formulated goals and objectives. The dissertation is self-critical, emphasizing some of the limitations of the dissertation.

Conclusions: 10 clear conclusions have been formulated, which represent a strictly differentiated synthesis of the overall development and meet the set goals and objectives. The results and conclusions of the scientific work lead to logical contributions, which are indicated as contributions related to communications for the first time worldwide and contributions related to communications for the first time in Bulgaria.

The **data summary** is presented in a volume of 84 pages and contains the main chapters of the dissertation. The purpose and tasks of the research, the materials and methods, the results, the discussion with the conclusion, the conclusions and the contributions of the dissertation are presented. The scientific publications and announcements related to the dissertation are also attached.

Critical remarks and recommendations:

In the results of the research the doctoral student compared the allelic and genotypic frequencies of the selected five SNPs in the Bulgarian group of healthy individuals and European cohorts of individuals. The frequency of distribution of the selected five SNPs in the Bulgarian group of healthy individuals should be compared with other cohorts other than the European race.

Another shortcoming of the scientific work is that an association has been sought only between the plasma expression levels of micro-RNAs whose genes include the SNPs studied and the overall survival in patients. It is appropriate to look for an association between plasma expression levels of microRNAs whose genes include SNPs and other clinical and pathological features such as progression-free survival (PFS), RAS mutation status and first-line antitumor therapy. treatment. Given the self-criticism shown by the doctoral student regarding the listed problems, I believe that this scientific work will be expanded and optimized in the near future.

The scientific production of the candidate, related to the topic of the dissertation, includes 9 publications, 7 of which are in international publications with impact factor.

Conclusion

The dissertation of Dr. Rostislav Manev on "Single nucleotide polymorphisms in genes for non-coding RNAs as diagnostic and prognostic markers in patients with colorectal cancer in metastatic stage" is a completed, accurately structured dissertation. It contains results with original contribution to science and meets all the requirements of the Law on the Development of the

Academic Staff of the Republic of Bulgaria and for implementation of the Regulations of MU-Varna.

The dissertation shows that the doctoral student Dr. Rostislav Radoslavov Manev has 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. Rostislav Radoslavov Manev an educational and scientific degree "DOCTOR".

Date: 04.05.2022
Varna

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