To the Chairman of the Scientific Jury,

determined by Order No. P-109-111 / 11.03.2022

on

Rector of the Medical University – Varna

REVIEW

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

Head of the Clinic of Medical Oncology at

Department of Hematology, Oncology, Pathology and Radiobiology,

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Subject: Dissertation on the topic: ***"Single nucleotide polymorphisms in the genes for non-coding RNAs as diagnostic and prognostic markers in patients with metastatic colorectal cancer "*** of the full doctoral student Dr. Rostislav Radoslavov Manev for awarding the educational and scientific degree "DOCTOR" in a professional field 7.1. Medicine, in the field of higher education 7.3 Health and sports, in the scientific specialty "Oncology".

Scientific Supervisors:

Assoc. Prof. Dr. Nikolay Tsonev, Ph.D.

Assoc. Prof. Maria Radanova, Ph.D.

I. Administrative evaluation

Dr. Rostislav Radoslavov Manev is enrolled as a doctoral student in full-time education at the Department of Propaedeutics of Internal Medicine, MU-Varna as of July 16, 2018 for a period of three years with the topic "Expertise of some non-coding RNA as predictive and prognostic markers in patients with metastatic colorectal cancer ” with supervisor Assoc. Prof. Dr. Nikolay Tsonev, MD, with order № R-109-434 / 16.07.2018 of the Rector of the Medical University - Varna.

Based on the proposal of the Head of the Department of Oncology and on protocol 3 from 21.02.2022, the dissertation of Dr. Rostislav Radoslavov Manev ***on "Single nucleotide polymorphisms in genes for non-coding RNAs as diagnostic and prognostic markers in patients with metastatic colorectal cancer"*** was approved and proposed for work completion with the right to defense.

According to Order No. R-109-111 / 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 was elected consisting of five habilitated persons.

II. Кратки биографични данни

Dr. Rostislav Radoslavov Manev graduated from the National School of Natural Sciences and Mathematics "Academic Lyubomir Chakalov" in Sofia in 2010.

Dr. Manev graduated in medicine at the Medical University "Prof. Dr. Paraskev Stoyanov "- Varna in September 2016, and from 06.11.2016 begins specialization in the Clinic of Medical Oncology at the University Hospital St. Marina Varna. Since February 28, 2017 he has been an assistant at the Department of Propaedeutics of Internal Medicine, later renamed the Department of Oncology, and since July 2018 he has been a doctoral student in full-time education in the specialty "Oncology".

Dr. Rostislav Radoslavov Manev is the author of 5 full-text publications in connection with the dissertation, one of which is in a refereed journal with Impact Factor - International Journal of Molecular Sciences. Dr. Manev is the author of 6 publications in abstract form, in connection with the dissertation, all published in Impact Factor journals Annals of Oncology and Journal of Clinical Oncology.

Dr. Manev 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

Single nucleotide polymorphisms ("single nucleotide polymorphisms", SNPs) are the simplest form of genetic polymorphism, as they consist of a change in a single nucleotide in the context of a genetic sequence. They are heterogeneously distributed throughout the genome and are found in both coding (exons) and non-coding (intron and promoter regions) regions of genes, as well as in regions of the genome that do not contain known genes (sometimes called "Junk DNA "). Single nucleotide polymorphisms can affect different functional processes in malignancies, which makes them biomarkers for the risk of developing malignancies with different primary sites. In recent years, many single nucleotide polymorphisms have been studied in direct connection with the risk of developing colorectal cancer. On the other hand, micro-ribonucleic acids (miRNAs) are RNA molecules that play an important role in various biological processes, as they do not encode proteins, but have an important function to regulate gene expression (namely, regulate the expression of proto-oncogenes and tumor suppressor genes). Single nucleotide polymorphisms in miRNA genes - miR-SNPs - can modulate siRNA expression and thus determine the risk of developing malignancy, and can have diagnostic, predictive and prognostic functions. Worldwide research on the various functions of miR-SNPs in malignancies is small in number, mainly in Asian cohorts. Studies on the importance of miR-SNPs in Bulgarian patients with colorectal cancer have not been performed to date. In this sense, the topic of the doctoral student is extremely relevant.

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 146 standard pages - of which: "Introduction" - 2 pages, "Literary Review" - 44 pages, "Objective and research tasks "- 2 pages," Materials and methods "- 15 pages," Results " - 31 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.

The dissertation is illustrated with 35 figures and 40 tables. The bibliography contains 257 sources, all of which are English-language literature.

The scientific work of the doctoral student Dr. Manev is properly structured as a sequence and volume of sections. There is an excellent and modern visualization with appropriately selected graphic means - figures and tables.

The abstract contains 84 pages and fully corresponds to the text of the dissertation.

3. An aim and tasks

The aim of the dissertation is clearly formulated, specific and feasible - „To identify new diagnostic and prognostic molecular biological biomarkers in Bulgarian patients diagnosed with metastatic colorectal cancer (CRC) by studying five selected single nucleotide polymorphisms in the genes encoding microRNAs - rs7372209 in gene microRNA-26a-1, rs2910164 in the microRNA gene-146a, rs2682818 in the microRNA gene-618, rs353293 in the promoter region of the gene cluster of microRNA-143 and microRNA-145 and rs322931 in the microRNA gene -RNA-181b "

The set 7 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 to study the allelic distribution and genotypic frequency of the five selected SNPs in patients with mCRC and in healthy controls, and also to look for a relationship between the carrier of a particular allele or genotype of the five SNPs in micro RNAs genes, and the possibility of predicting the risk of developing CRC.

4.Research methodology

A study of 101 patients treated in the Clinics of Medical Oncology of the University Hospital St. Marina and the Military Medical Academy - Sofia. The tests were performed at the Department of Biochemistry, Molecular Medicine and Nutrigenomics of MU - Varna.

The specific methods of research are described in detail, such as - DNA extraction, SNP genotyping, isolation of RNA from plasma for research of micro-RNAs, research of the levels of expression of circulating siRNAs in the blood.

Also described in detail are statistical methods such as statistical grouping method, statistical estimation method, method for detecting non-random associations between two category variables, chance ratio calculation method, nonparametric analyzes, ROC analysis, survival analysis, and Cox proportional hazards models. Genetic inheritance patterns were assessed by analyzing the association of SNPs with the advent of mCRC.

5. Results

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

- Clinical and pathological stratification of patients
- Allelic distribution and genotypic frequency of selected SNPs in the selected cohort of patients with metastatic CRC
- 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.
- 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
- 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 metastatic CRC and in the healthy control group
- Association between plasma expression levels of microRNAs whose genes include the SNPs studied and overall survival in patients

6. Evaluation of the dissertation and contributions

The doctoral student Dr. Rostislav Radoslavov Manev presented the conclusions and contributions in two sections, formulating 10 clear conclusions. More significant conclusions are

- Carriers of the dominant A allele in homozygous state with rs353293 - miRNA-143/145 are characterized by a high risk of CRC.

- TT rs7372209 genotype was assessed as a risk factor for right colon tumor development.
- The polymorphism studied for the first time in patients with CRC rs322931 – miR-181b is not associated with disease risk and prognosis
- Heterozygous individuals with rs2910164 - miRNA-146a are characterized by a low risk of developing the disease in the over-dominant genetic model.
- The polymorphism rs2682818 - miR-618 also appears to be protective against CRC in both heterozygous genetic models - codominant and superdominant.

The most significant contributions are:

- For the first time in Bulgaria, data were obtained on the role of rs2910164 – miR-146a, rs2682818 – miR-618 and rs353293 – miR-143 / miR-145 as potential diagnostic biomarkers for CRC patients.
- For the first time in Bulgaria, data on the allelic frequency and genotypic distribution of polymorphisms in the genes for non-coding miRNAs among healthy individuals have been obtained.
- rs322931 – miR-181b was tested for the first time in patients with CRC.
- For the first time, an association of the TT rs7372209 genotype with a longer mean OS has been reported in patients with metastatic CRC
- For the first time, an association of the AA rs353293 genotype with a longer mean OS has been reported in patients with metastatic CRC.
- The expression levels of miR-26a-1, miR-618, miR-181b were examined for the first time in connection with the presence of a certain polymorphism (rs7372209, rs2682818 and rs322931, respectively).

IV. Scientific production

The scientific production of the candidate related to the topic of the dissertation includes 11 publications. Dr. Rostislav Radoslavov Manev is the author of 5 full-text publications in connection with the dissertation, one of which is in a refereed journal with Impact Factor - International Journal of Molecular Sciences. Dr. Manev is the author of 6 publications in

abstract form, in connection with the dissertation, all published in Impact Factor journals Annals of Oncology and Journal of Clinical Oncology.

V. Conclusion

The dissertation of Dr. Rostislav Radoslavov Manev is a significant study on "Single nucleotide polymorphisms in genes for non-coding RNAs as diagnostic and prognostic markers in patients with metastatic colorectal cancer" The design of the study is scientifically sound. Scientific methods are appropriate and well explained. As a member of the Scientific Jury, I believe that the presented scientific work is up-to-date, accurately made and with significant contributions to oncology science.

The analysis of results showed a similar frequency distribution of the studied polymorphisms (rs7372209, rs2910164, rs2682818, rs353293 and rs322931) in the genes for miRNAs in Bulgarian individuals with that in European cohorts. An association with the risk of CRC development was established for three of the studied polymorphisms in the Bulgarian cohort of patients with metastatic CRC (rs2910164 in the miRNA-146a gene, rs2682818 in the miR-618 gene and rs353293 in the promoter region of the miRNA-143 gene cluster and miRNA-145). For two of the studied polymorphisms, a statistically significant association was found with overall patient survival (TT genotype for rs7372209-miR-26a-1 polymorphism and AA genotype for rs353293 in the promoter region of the miRNA-143 and miRNA-145 gene cluster). This study is the first in Bulgaria to study and prove the association of single nucleotide polymorphisms in the genes for non-coding RNAs as potential biomarkers that could predict the risk of developing the disease and have a potential relationship with the prognosis of CRC.

The content of the dissertation, as well as the obtained results show that the doctoral student proves that he has mastered the methodology of scientific research and that he 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. Rostislav Radoslavov Manev.

With respect:

/ Prof. Dr. Zhasmina Mihaylova, MD /