SCIENTIFIC OPINION

by **Prof. Dr. Radka Stefanova Tincheva- Yordanova MD, PhD**
of a dissertation for awarding the educational and scientific degree 'doctor'

Professional field- Medicine
Doctoral program- Medical Genetics
Author: Dr Nelly Nedyalkova Miteva-Marcheva
Form of doctoral studies: Full-time
Department: Pediatrics and Medical Genetics
Topic: "Pharmacogenetic Markers Associated With Drug Metabolism in Cancer Patients"
Scientific supervisor: Prof. Dr. Vili K. Stoyanova, MD

The presented set of materials on paper is in accordance with Art. 70 (1) of I. Section. Acquisition of educational and scientific degree "DOCTOR" and scientific degree "DOCTOR OF SCIENCES" at MU-Plovdiv; Regulations of MU-Plovdiv dated 28.01.2021.

Dr. Neli Nedyalkova Miteva-Marcheva graduated from the Medical University in the city of Plovdiv in 2015. From 2015 to 2019, she specializes in pediatrics, and from 2020 - in Medical Genetics at the Medical University - Plovdiv, Department of Pediatrics and medical genetics. She obtained a specialty in pediatrics in 2019. From the same year until now, he has been an assistant at the Department of Pediatrics and Medical Genetics at the Medical University of Plovdiv. Dr. Miteva she participated in a course on pediatric cardiology in Salzburg in 2017. She speaks English, Russian and Spanish. Participated in three projects-"Targeted genomic profiling for diagnosis and behavior in colorectal carcinoma"- 2019, "Liquid biopsy – an innovative method for non-invasive monitoring of therapeutic response and development of resistance in non-small cell lung carcinoma (NSCLC)"- 2019, "Program for strategic research and innovations for the development of MU-Plovdiv (SNIIR-MUP)", Creation of a network of research higher schools, National plan for recovery and sustainability, financed by the European Union - Next Generation EU 2023.

The idea that genetic factors might be responsible for how a drug works in some patients developed in the late 1950s. The term "pharmacogenetics" was coined in 1959 to describe a new scientific discipline that deals with inherited differences in drug response. It has been suggested that the selection of drug therapy based on a patient's genetic profile may lead not only to improved therapeutic response, but also has clinically important implications for reducing adverse drug reactions. Pharmacogenetics relies on genetic information to determine the likelihood of a response to a drug or the likelihood of a significant side effect to it. It has a key role in optimizing the treatment of malignant diseases, since many of the drugs have a small therapeutic window, i.e. at low doses they are ineffective, and at higher doses they can be toxic. Pharmacogenetics studies the genetic basis of variation in drug response, studying inherited genetic differences in drug metabolic pathways that may influence individual responses to drugs both in terms of therapeutic effect and adverse effects. With pharmacogenetics, individual genes that have a large phenotypic effect and determine increased, decreased or absent therapeutic effect of drugs. In pharmacogenomics, genome-wide studies are performed to detect small-effect variants involved in complex biological pathways that cause adverse drug effects. Pharmacogenetics is still a developing discipline, a very active area of research, promising to revolutionize therapy through "personalization of medicine" or, more precisely, "individualized therapy". In principle, individualized drug

prescribing based on genotype should be more effective in improving response rates and reducing the burden of adverse drug reactions.

The aim of the present work is to study the germline pharmacogenetic variants related to drug metabolism in patients with oncological diseases.
Tasks:
1. To isolate genomic/cell-free DNA from patients with non-small cell lung carcinoma (NSCLC), colorectal carcinoma (CRC), breast cancer (BRC) and 40 healthy individuals.
2. To carry out targeted sequencing of a panel of genes of the isolated samples.
3. To determine the germline pharmacogenetic variants that are associated with the metabolism of chemotherapeutics used in the course of treatment, by using the "PharmGKB" database.
4. To characterize the frequency and clinical significance of the identified genetic polymorphisms.
5. Evaluation of the frequency of genetic polymorphisms in the Bulgarian population compared to the human population.

The dissertation contains 147 pages and is structured as follows: 6 main sections, conclusions, contributions and a bibliography containing 197 sources in English. The relevance of the problem and the subject of the doctoral work are clearly defined. The literature review shows the doctoral student's good knowledge of the state of the problem. Pharmacogenetics and its relevance to the clinic, personalized medicine, genetic biomarkers, methods for their discovery and the importance of genetic polymorphism for response to a given therapy are presented in detail.
The review presents the incidence of malignant diseases worldwide, the principles of their treatment, the clinical pharmacology of anticancer drugs and the variability in drug response in individual patients. The most frequently used groups of chemotherapeutics are described in detail.
The chosen research methodology allows to achieve the set goal and to obtain an adequate answer to the tasks solved in the dissertation work. The criteria for inclusion and exclusion in the study are precisely formulated when selecting the samples.
A total of 90 participants were included in the study, of which 50 were cancer patients (26 were diagnosed with colorectal carcinoma, 13 with non-small cell lung carcinoma, 11 with breast cancer) and 40 were healthy individuals. The selection of patients was made on the basis of the high prevalence of carcinomas both in Bulgaria and worldwide, the frequency of adverse drug reactions among patients with these 3 groups of diseases, as well as a certain coincidence regarding the chemotherapeutic agents used in the treatment regimens .
The following methods were used in the doctoral thesis - isolation of genomic DNA (50 samples, of which 10 from patients and 40 from healthy individuals), isolation of free cell DNA (40 patients) and next-generation sequencing (NGS).
The obtained results are presented on 28 pages, illustrated with tables and diagrams and their statistical significance.
In the discussion, the PhD candidate analyzed common (in more than one patient) single nucleotide polymorphisms in specific genes associated with drug metabolism of chemotherapy used in the studied patient group, namely: MTHFR, DPYD, CYP1B1, XPC, ABCG2, SLC22A2, SOD2, EGFR, ABCB1, ABCC2, GSTP1, ATM, SLCO1B3, TP53, XRCC1, ERCC2. The function of these genes, the variation in clinical therapeutic response, compared with the worldwide database to which the established polymorphism can lead, are presented in detail.

The frequency of the genetic variants found in the studied patients was compared with that of the 40 healthy individuals, as well as with the global frequency of the non-cancer group in GnomAD, using Fisher's exact test and Z-score at a confidence interval for p-value 0.05. The frequency of established germline pharmacogenetic variants in the group of studied patients and healthy individuals was also compared with the frequency of the same variants in the global database (GnomAD).

In her doctoral work, Dr. Miteva identified and analyzed germline pharmacogenetic variants associated with the occurrence of drug toxicity in patients undergoing chemotherapy for the treatment of colorectal carcinoma, non-small cell lung cancer, and breast cancer. The choice of appropriate medical treatment based on the individual genetic profile of the patient is leading in the direction of reducing adverse drug reactions and improving the therapeutic effect. The detected germline pharmacogenetic variants with an increased allelic frequency among the Bulgarian population can be included in a genetic panel provided to patients in advance, before the start of medication therapy. The PhD student believes that the study confirms the need to conduct genetic testing for polymorphisms associated with the pharmacological metabolism of chemotherapeutics for all cases of colorectal carcinoma, non-small cell lung cancer and breast cancer undergoing platinum-containing drugs, 5-FU /Capecitabine, Leucovorin, taxanes, EGFR inhibitors, anthracyclines and Cyclophosphamide.

The PhD student draws the following conclusions based on the results obtained:

1. In a study of patients with colorectal carcinoma, non-small cell lung carcinoma and breast cancer, the following 23 germline pharmacogenetic variants in 16 genes associated with the metabolism of chemotherapeutic agents used in the studied patients, leading to ADRs, were found: MTHFR c.1286A>C, MTHFR c.665C>T, DPYD c.2194G>A, DPYD c.1627A>G, DPYD c.496A>G, DPYD c.85T>C, CYP1B1 c.1294G>C, XPC c.2815C>A, XPC c.1496C>T, ABCG2 c.421C>A, SLC22A2 c.808T>G, SOD2 c.47T>C, EGFR c.1562G>A, ABCB1 c.2677T>G, ABCC2 c. .1249G>A, GSTP1 c.313A>G, ATM c.5557G>A, SLCO1B3 c.334T>G, SLCO1B3 c.699G>A, TP53 c.215C>G, XRCC1 c.1196A>G, ERCC2 c. 2251A>C, ERCC2 c.934G>A.
2. Three germline pharmacogenetic variants in 3 genes (SLC22A2 c.808T>G, DPYD c.2194G>A, XRCC1 c.1196A>G) were detected with a higher frequency among the patients compared to the group of 40 healthy subjects examined, and compared to the group healthy in GnomAD, 4 germline pharmacogenetic variants were found in 4 genes. (SLC22A2 c.808T>G, DPYD c.2194G>A, XRCC1 c.1196A>G, EGFR c.1562G>A).
3. Two germline pharmacogenetic variants in one gene (XPC c.2815C>A, XPC c.1496C>T) were detected with a lower frequency among the patients compared to the group of 40 healthy subjects studied, and compared to the healthy group in GnomAD, only one germline pharmacogenetic variant in one gene (ERCC2 c.934G>A).
4. The following 6 germline pharmacogenetic variants in 6 genes are found with a higher frequency among the Bulgarian population compared to the world population: MTHFR c.665C>T, DPYD c.2194G>A, XPC c.2815C>A, EGFR c.1562G >A, XRCC1 c.1196A>G, ERCC2 c.2251A>C.
5. The following 4 germline pharmacogenetic variants in 4 genes are found with a lower frequency among the Bulgarian population compared to the world population: MTHFR c.1286A>C, DPYD c.85T>C, ABCG2 c.421C>A, ERCC2 c.934G >A.

Dr. Miteva's work also has certain scientific and practical contributions.

Contributions of a scientific nature:

1. For the first time, the frequency of germline pharmacogenetic variants associated with the metabolism of chemotherapy regimens among the Bulgarian population was investigated.

2. Liquid biopsy is a highly sensitive method for the detection of germline pharmacogenetic variants.
3. Found with a lower frequency in the Bulgarian population compared to the world population are 4 germline pharmacogenetic variants, determining toxic effects during chemotherapy, in 4 genes, and with a higher frequency – 6 germline pharmacogenetic variants in 6 genes.
Contributions of an applied nature:
1. Germline pharmacogenetic variants found with increased allelic frequency among the Bulgarian population can be included in a genetic panel provided to patients with upcoming chemotherapy to select the right medication in an appropriate dose and minimal risk of ADR.
2. Cell-free DNA sequencing is a sensitive method for the study of germline pharmacogenetic variants determining chemotherapy toxicity.
3. The DNA bank of patients with non-small cell lung carcinoma, colorectal carcinoma and mammary gland cancer at the Department of Pediatrics and Medical Genetics, Medical University - Plovdiv has been enriched, providing an opportunity for larger-scale future studies.

The PhD student presents three publications:
1. Miteva-Marcheva NN, Ivanov HY, Dimitrov DK, Stoyanova VK. Application of pharmacogenetics in oncology. Biomark Res. 2020 Aug 17;8:32. doi: 10.1186/s40364-020-00213-4. PMID: 32821392; PMCID: PMC7429778. IF 8.633; Q1.
2. Nelly Miteva-Marcheva, Hristo Ivanov, Veselin Popov, Gabriela Raycheva, Zhanet Grudeva-Popova & Vili Stoyanova (2023) Liquid biopsy: an innovative and reliable method for detecting not only somatic, but also germline mutations in patients with colorectal and non-small cell lung carcinoma, Biotechnology & Biotechnological Equipment, 37:1, DOI: 10.1080/13102818.2023.2249560. IF 1,4; Q4.
3. Nelly Miteva-Marcheva, Gabriela Raycheva, Dimitar Dimitrov, Momchil Topalov, Hristo Ivanov. Liquid biopsy – a sensitive tool for detecting genetic variants in solid tumors, Science and Youth Conference 2022, Scientific Reports, ISSN 2683-0922

Participation in scientific forums:
1. Nelly Miteva-Marcheva, Hristo Ivanov, Gabriela Raycheva, Janet Grudeva-Popova, Dimitar Dimitrov, Ivan Zheliazkov, Aleksandar Linev, Momchil Topalov, Peter Shopov, Vili Stoyanova, "DPYD*6 as a risk factor for drug toxicity in patients treated with 5-fluorouracil – preliminary results", European Human Genetics Conference 2022
2. Dimitar Dimitrov, Hristo Ivanov, Nelly Miteva-Marcheva, Gabriela Raycheva, Janet Grudeva-Popova, Aleksandar Linev, Ivan Zheliazkov, Peter Shopov, Momchil Topalov, Vili Stoyanova, "Significance of ARID1A mutations in colorectal cancer", European Human Genetics Conference 2022
3. Hristo Ivanov, Gabriela Raycheva, Janet Grudeva-Popova, Peter Shopov, Nelly MitevaMarcheva, Ivan Zheliazkov, Aleksandar Linev, Dimitar Dimitrov, Momchil Topalov, Vili Stoyanova, "Somatic variant PARP4 c.3509C>T identified in ctDNA in patients with colorectal cancer, preliminary results", European Human Genetics Conference 2022
4. Nelly Miteva-Marcheva, Hristo Ivanov, Momchil Topalov, Aleksandar Linev, Ivan Zheliazkov, Dimitar Dimitrov, Gabriela Raycheva, Veselin Popov, Janet Grudeva-Popova, Vili Stoyanova, "Association of AURKA c.169A>G and c.91T>A polymorphisms with higher cancer risk – preliminary results", European Human Genetics Conference 2023
5. Hristo Ivanov, Gabriela Raycheva, Veselin Popov, Ivan Zheliazkov, Aleksandar Linev, Nelly Miteva-Marcheva, Dimitar Dimitrov, Momchil Topalov, Vili Stoyanova, Zhanet Grudeva, "The clinical utility of droplet digital PCR for detecting PIK3CA mutations in

circulating tumor DNA in breast cancer patients – preliminary results", European Human Genetics Conference 2023
6. Dimitar Dimitrov, Hristo Ivanov, Nelly Miteva-Marcheva, Gabriela Raycheva, ZhanetGrudeva, Veselin Popov, Aleksandar Linev, Ivan Zheliazkov, Momchil Topalov, Vili Stoyanova, "Association of SLC22A2 c.808T>G (rs316019) in the occurrence of hematological toxicity of Oxaliplatin in patients with colorectal cancer", European Human Genetics Conference 2023

The abstract is made according to the requirements of the regulations and reflects the main results achieved in the dissertation. It contains 66 pages, it is structured according to the requirement in eight sections - introduction, aim and tasks, materials and methods, results, discussion, conclusion, conclusions and contributions. It is illustrated with tables and diagrams.

CONCLUSION

The dissertation contains scientific-applied and applied results, which represent an original contribution to science and meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the Regulations of the Ministry of Education - Plovdiv. The presented materials and dissertation results fully correspond to the specific requirements adopted in connection with the Regulations of the Ministry of Education - Plovdiv for the application of the ZRASRB.
The dissertation shows that the doctoral student Dr. Neli Nedyalkova Miteva-Marcheva possesses in-depth theoretical knowledge and professional skills in the scientific specialty of medical genetics, demonstrating qualities and skills for independent conduct of scientific research. Due to the above, I confidently give my positive assessment of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions and I propose to the honorable scientific jury to award the educational and scientific degree "doctor" to Dr. Neli Nedyalkova Miteva- Marcheva in a doctoral program in medical genetics.

12.01.2024 Prepared the opinion: