OPINION

by Assoc. Dr. Delyan Penev Delev, PhD - Head of the Department of Pharmacology and Clinical Pharmacology, Faculty of Medicine, MU-Plovdiv

of a dissertation for awarding the educational and scientific degree 'doctor'of a dissertation for awarding the educational and scientific degree **'doctor'**

professional direction *7. Health care and sports; 7.1. Medicine*
Doctoral Program *Medical genetics.*

Author : *Dr. Neli Nedyalkova Miteva-Marcheva*

Form of doctoral studies: independent preparation

Department: Pediatrics and Medical Genetics
Topic : Pharmacogenetic markers associated with drug metabolism in patients with oncological diseases

Research supervisor : *Prof. Dr. Vili Krasteva Stoyanova, d.m*
(academic title, first name, surname, last name, scientific organization)

1. General presentation of the procedure and the doctoral student

The presented set of materials on paper / electronic medium **is** 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 and includes the following documents:

- Application to the Rector of MU-Plovdiv for disclosure of the procedure for the defense of a dissertation work
- curriculum vitae in European format with the doctoral student's signature
- a notarized copy of a higher education diploma
- orders for enrollment in doctoral studies, interruption of studies (due to maternity) and for continuation of studies; for deduction with right of defense
- order for conducting an exam from the individual plan and corresponding protocol for a passed exam or doctoral minimum in the specialty
- minutes of the departmental council for the preliminary discussion of the pre-dissertation work and the decisions made for the disclosure of the procedure and for the composition of the scientific jury
- dissertation work
- abstract
- list of scientific publications on the topic of the dissertation
- copies of scientific publications
- list of participations in scientific forums
- list of noticed citations
- declaration of originality and authenticity of the attached documents
- other documents related to the course of the procedure

The PhD student has attached 3 publications .

Notes and comments on the documents - I don't have any

Presentation of the doctoral student with an emphasis on biographical data related to the procedure.

Dr. Neli Nedyalkova Miteva-Marcheva graduated with a Master's degree in "Medicine" at the Medical University of Plovdiv in 2015. In 2019, the specialty of Pediatrics was added. From 2020 to the present, she is a specialist in Medical Genetics. Since 2019, she is an assistant at the "Department of Pediatrics and Medical Genetics" of the Medical University of Plovdiv. She is enrolled as a doctoral student of independent study by order P-393/16.03.2022. She is a member of the following organizations: BMA, Bulgarian Society of Human Genetics and Genomics and Society of Cardiologists in Bulgaria. Participated in 3 scientific projects. Fluent in English (C1), Russian and Spanish (B1). She has completed the required number of doctoral school credits of 102 with a minimum of 86.

2. Relevance of the topic

Personalized medicine is a new concept for targeted therapy of a certain group of patients, which uses molecular biomarkers to select treatment and monitor the effect of therapy. Targeted therapy has the potential to improve medical outcomes, reduce adverse drug reactions in patients, and reduce the cost of ineffective or unnecessary treatment. Genomic analyzes offer enormous opportunities for personalized treatment and to achieve better therapeutic effects.

The dissertation work of Dr. Neli Miteva is dedicated to the study of pharmacogenetic pharmacogenetic variants related to the metabolism of chemotherapeutics used to treat patients with colorectal carcinoma, non-small cell lung carcinoma and breast cancer with the aim of more effective and safer therapy. Considering the social importance, the high population incidence of cancers and their high mortality, the search for new approaches in the treatment of patients suffering from malignant diseases is undoubtedly relevant.

3. Knowing the problem

The literature review is comprehensive, with specific data on the discussed topic. A detailed analysis was made of the contribution of pharmacogenetics and pharmacogenomics to the development of personalized medicine, the main concept of which is to make a precise diagnosis, monitor the disease process and determine effective treatment based on the patient's individual genome.

The significance of genetic biomarkers in malignant tumors, as well as molecular approaches for their identification, are presented in depth and informatively. The groups of drugs included in chemotherapy regimens for cancer treatment are discussed in detail.

In-depth knowledge of the subject has contributed to a precise formulation of the aim of the dissertation: to study the germinal pharmacogenetic variants related to drug metabolism in patients with oncological diseases. The tasks are logically and consistently set and correspond to the purpose of the study.

4. Research methodology

A total of 50 patients with oncological diseases and 40 healthy individuals were included in the study. Of the 50 patients, 26 were diagnosed with colorectal carcinoma, 13 with non-small cell lung carcinoma, and 11 with breast cancer.

The analyzes were carried out with appropriately selected and detailed modern molecular genetic methods - targeted and full exome DNA sequencing of the new generation (NGS) after isolation of genomic or cell-free DNA. A comprehensive solid tumor target panel including 484 genes associated with pharmacogenetics in cancer patients was used for DNA sequencing .

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 worldwide frequency of a non-malignant group in a worldwide database using appropriate statistical methods - Fisher's exactly test and Z- score at confidence interval for p- value 0.05.

5. Characterization and evaluation of the dissertation work and contributions

The dissertation contains clearly separated sections, presented in 149 standard pages, structured as follows: table of contents - 3 pages, list of abbreviations used - 7 pages, introduction - 2 pages, literature review - 44 pages, aim and objectives of the dissertation paper - 1 p., materials and methods - 9 p., results - 29 p., discussion - 21 p., conclusions - 1 p., contributions - 1 p., 2 appendices - 4 p. and cited literature - 26 p. The bibliography covers 197 sources.

The data from the performed genetic studies are appropriately systematized. In order to evaluate the clinical significance of genetic variants associated with the metabolism of chemotherapeutic agents based on similarity in regimens, patients were grouped into 7 groups. The first group included 35 patients treated with

platinum-containing chemotherapeutics , in which 11 pharmacogenetic germline variants in 7 genes – MTHFR 1286A>C, MTHFR c.665C>T, XPC c.2815C>A, XPC c.1496C>T, SLC22A2 c.808T>G, ABCC2 c.1249G>A, GSTP1 c.313A >G, SLCO1B3 c.334T>G, SLCO1B3 c.699G>A, ERCC2 c.2251A>C, ERCC2 c.934G>A. The second group included 26 patients treated with pyrimidine analogues (5-Fluorouracil/ Capecitabine), in which 14 variants were found in 10 genes associated with the metabolism of chemotherapeutics - MTHFR 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, ABCG2 c.421C>A, ABCB1 c.2677T>G, ABCC2 c.1249G >A, GSTP1 c.313A>G, TP53 c.215C>G, XRCC1 c.1196A>G, ERCC2 c.2251A>C. The third group included 21 patients treated with folinic acid (Leucovorin), in which 3 variants were found in 2 genes – MTHFR 1286A>C, MTHFR c.665C>T, ERCC2 c.2251A>C. In the fourth group, there were 11 patients treated with EGFR inhibitors, in which only one variant in the EGFR gene related to the metabolism of these chemotherapeutics was found – c.1562G>A. The fifth group includes 15 patients who underwent chemotherapy with taxanes , with 5 variants with pharmacogenetic significance in 4 genes - SOD2 c.47T>C, ABCB1 2677T>G, SLCO1B3 c.334T>G, SLCO1B3 c.699G>A, ERCC2 c.2251A>C. The last two groups included the same 8 patients who underwent therapy with Cyclophosphamide and Farmorubucin , in which 7 pharmacogenetic variants were found in 7 genes associated with the metabolism of chemotherapeutic agents - CYP1B1 c.1294G>C, ABCG2 c.421C>A, ABCC2 c .1249G>A, GSTP1 c.313A>G, ATM c.5557G>A, TP53 c.215C>G, XRCC1 1196A>G. The degree of toxicity was evaluated according to the latest criteria in the field of oncology - CTCAE.

In each group, the frequency of germline variants in the studied patients with malignant diseases was evaluated and compared with the group of 40 healthy individuals, as well as with that of the healthy group (without cancer) in the GnomAD global database . A comparative assessment of the frequency of genetic variants was made between the examined patients+healthy from the Bulgarian population (90 in total) and the world database (again including oncological patients and healthy). The pharmacogenetic variants with a higher and lower frequency among oncology patients compared to the examined healthy individuals from the Bulgarian population and compared to the healthy individuals from the world database, as well as the Bulgarian compared to the world population, were determined . 3 germinals were found pharmacogenetic variants in 3 genes with increased frequency among patients compared to healthy Bulgarians – SLC22A2 c.808T>G, DPYD c.2194G>A, XRCC1 1196A>G, and 4 variants in 4 genes compared to healthy people in the world database – SLC22A2 c.808T >G, DPYD c.2194G>A, XRCC1 1196A>G, EGFR c.1562G>A. 2 variants in 1 gene – XPC c.2815C>A, XPC c.1496C>T – were found with a lower frequency in patients compared to healthy patients, and 1 variant in 1 gene – ERCC2 c.934G>A compared to non-oncological patients in GnomAD . The detected variants with a higher frequency among the Bulgarian population compared to the world population are 6 in 6 genes - MTHFR c.665C>T, DPYD c.2194G>A, XPC c.2815C>A, EGFR c.1562G>A, XRCC1 1196A>G , ERCC2 c.2251A>C , and with a lower one – 4 variants in 4 genes – MTHFR 1286A>C, DPYD c.85T>C, ABCG2 c.421C>A, ERCC2 c.934G>A. With the study done, liquid biopsy proves to be a sensitive and suitable method for detecting pharmacogenetic variants.

The results are discussed and compared with the literature data in the last section "Discussion". The creative maturity of the doctoral student was demonstrated, presenting herself not only as a researcher with an excellent command of modern innovative technologies and methods, but also as a person with in-depth knowledge of the problem and the ability to analyze scientific results.

Based on the obtained results, 6 conclusions were drawn, which correctly reflect the content of the dissertation work. They correspond to the set tasks and are a consequence of the stated goal of the present study.

The dissertation was developed at a very high level, and the obtained results can be used to solve problems of practical importance: the germinal pharmacogenetic variants found with an increased allelic frequency in the Bulgarian population can be included in a genetic panel provided to patients with upcoming chemotherapy for the selection of an accurate drug in an appropriate dose and minimal risk of ADRs. In connection with the dissertation, 3 articles were published, in which Dr. Neli Miteva is the lead author. The results were also reported at 6 international congresses. On the basis of the dissertation, the author forms 6 contributions,

namely:

Contributions of a scientific nature

germinal was investigated pharmacogenetic variants associated with the metabolism of chemotherapy regimens in the Bulgarian population.

2. Liquid biopsy is a highly sensitive method for detecting germinal cells pharmacogenetic variants.

3. Found with a lower frequency in the Bulgarian population compared to the world population are 4 germinal pharmacogenetic variants, determining toxic effect during chemotherapy, in 4 genes, and with a higher frequency – 6 germline pharmacogenetic variants in 6 genes.

Contributions of an applied nature

1. Germinatives pharmacogenetic variants found with an increased allelic frequency among the Bulgarian population can be included in a genetic panel provided to patients with upcoming chemotherapy to choose the right medication in an appropriate dose and minimal risk of ADR.

2. Sequencing of cell-free DNA is a sensitive method for investigating germ pharmacogenetic variants determining toxic effects in chemotherapy.

3. The DNA bank of patients with non-small cell lung carcinoma, colorectal carcinoma and breast cancer in the Department of Pediatrics and Medical Genetics, Medical University - Plovdiv, was enriched, providing the opportunity for larger-scale future studies.

6. Evaluation of the publications and personal contribution of the doctoral student

3 publications are presented, of which 2 with an impact factor and 1 in a peer-reviewed collection. Dr. Miteva-Marcheva is the first author of all three, which proves her personal participation in the conducted dissertation research, as well as that the formulated contributions and obtained results are her personal merit. The doctoral student also presents 6 participations in scientific forums. I have no significant criticisms.

7. Abstract

The abstract (66 pages) is made according to the requirements and reflects the main results achieved in the dissertation.

CONCLUSION

The dissertation ***contains scientific, scientific-applied and applied results, which represent an original contribution to science*** and **meet all the** requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the law and the Regulations of the MU-Plovdiv. The presented materials and dissertation results **fully** correspond to the specific requirements adopted in connection with the Regulations of the MU-Plovdiv for the application of the law.

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 Pediatrics and 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 ***offer to the honorable scientific jury "Yes" awarded the educational and scientific degree "doctor"*** to Dr. Neli Nedyalkova Miteva-Marcheva in doctoral program in Pediatrics and Medical Genetics.

18.12. 2023.

Prepared the opinion : .....

(Assoc. Prof. Delyan Delev, PhD)