REVIEW

regarding PhD study:

„Analysis of molecular-genetic markers in patients with acute myelogenous leukemia"

for award of educational and scientific degree "Doctor"

in the area of higher education: 4. Natural sciences, mathematics and informatics:

professional direction: 4.3. Biological science;

scientific specialty: "Genetics"

Author:	Dinnar Ali Yahya
Research supervisors:	Prof. Dr. Ilina Dimitrova Micheva, MD, PhD Assoc. Prof. Dr. Trifon Georgiev Chervenkov, MD, PhD
Reviewer:	Prof. Savina Petrova Hadjidekova, MD, PhD Head of the Department of Medical Genetics, Medical University of Sofia

1. General presentation of the procedure and the PhD student

The review was prepared according to Order RK109-82/21.03.2024 of the Rector of the Medical University of Varna and Protocol 1/22.03.2024 from the I-st session of the Scientific Jury.

The presented set of materials are in accordance with the Procedure for the acquisition of the educational and scientific degree "Doctor" in MU of Varna.

The documents presented by the applicant are in accordance with the requirements of the regulation for the acquisition of the educational and scientific degree "Doctor" and the Rules of the Medical University of Varna.

2. Brief data from the professional biography of the candidate

The PhD student Dinnar Ali Yahya graduated as "Master" in Medicine at the Medical Faculty of Medical University "Prof. Dr. Paraskev Stoyanov" Varna in 2017. In 2023 - obtained a

degree in medical genetics at the same university. Since 2020 she is enrolled as a full-time PhD student in the scientific field of Genetics.

From 2019 to the present moment she holds the position of Assistant Professor at the Department of Medical Genetics, Medical University "Prof. Dr. Paraskev Stoyanov" Varna.

The dissertant is a member of three international scientific organizations - European Society of Human Genetics (ESHG), European Cytogeneticists Association (ECA), International Society for Prenatal Diagnosis (ISPD), as well as two Bulgarian ones - Bulgarian Society of Human Genetics and Genomics and National Society of Hematology and Hemotransfusion.

The candidate is author and co-author of 6 full-text scientific publications and has 22 published abstracts of scientific events in foreign and Bulgarian journals. He is involved in 1 scientific project; he is a mentor in a scientific exchange project (SCORE) of two foreign students organized by IFMSA (International Federation of Medical Students' Associations). She has participated in 18 specializations and courses in Bulgaria and abroad.

She is fluent in written and spoken English, Turkish, Russian and has basic level in Italian.

3. Relevance and significance of the topic

Dr. Dinnar Ali Yahya's dissertation, entitled "Investigation of Molecular Genetic Markers in Patients with Acute Myelogenous Leukemia", is an analysis of the role of molecular genetic markers in the context of acute myelogenous leukemia (AML). The study is prospective in nature, covers the period February 2022 - May 2023 and was conducted at the Medical Genetics Laboratory of St. Marina University Hospital, Varna.

The study of genetic markers in patients with acute myelogenous leukemia (AML) is key to understanding disease heterogeneity, prognosis and therapeutic response.

AML is characterized by a variety of genetic alterations, including chromosomal abnormalities, gene mutations, and altered gene expression. These alterations contribute to the pathogenesis and progression of CML and affect patient outcomes. Conventional cytogenetic analysis remains the cornerstone of risk stratification in CML. Common chromosomal abnormalities include translocations involving genes such as *RUNX1-RUNX1T1* and *CBFB-MYH11*, as well as deletions in chromosomes 5, 7 and 17. These abnormalities are associated with various clinical and prognostic implications. Next-generation sequencing (NGS) technologies have facilitated the identification of recurrent gene mutations in CML. Mutations in genes such as FLT3, NPM1, DNMT3A and IDH1/2 are frequently observed and have prognostic significance. For example, *FLT3*-ITD mutations are associated with a poor prognosis, whereas NPM1

mutations confer a favorable outcome in the absence of *FLT3*-ITD. Gene expression profiling reveals different molecular subtypes of AML with different biological characteristics and clinical outcomes. Gene expression signatures associated with specific mutations or cytogenetic abnormalities provide insight into disease mechanisms and can guide personalized treatment strategies.

Incorporation of genetic markers into risk stratification algorithms is critical for determining prognosis and selecting optimal treatment for CML. Targeted therapies directed against specific genetic aberrations, such as *FLT3* inhibitors and IDH inhibitors, have shown promising results in clinical trials. In addition, surveillance of minimal residual disease based on genetic markers allows early detection of disease relapse and informs therapy after remission.

This dissertation highlights the importance of integrating molecular genetic profiling into routine diagnostic and prognostic evaluations for patients with CML. As genetic markers increasingly determine treatment choices and risk stratification algorithms, this dissertation represents a timely scientific work with relevance not only for academia, but also offers substantial benefits for patients as well as the broader oncology community.

4. A review on the structure and content of the dissertation

The dissertation is consistent with the usual appereance of work of this type, according to the standards. The dissertation comprises 130 pages including: Introduction - 3 pages, Literature review - 43 pages, Aim and objectives - 1 page, Material and methods - 7 pages, Results - 14 pages, Discussion - 22 pages, Conclusion - 3 pages, Conclusions - 1 page and Contributions - 2 pages, Literature cited - 19 pages, Bibliography comprises 201 references, of which 5 in Cyrillic and 196 in Latin. The sources cited in this dissertation are relevant and up-to-date, reflecting a comprehensive review of the literature. The author appropriately highlights the contributions of previous researchers in the field.

Publications and research communications included in the dissertation - 2 p. Acknowledgements - 1 p. Appendix - 5 p. The work is illustrated with 15 figures and 12 tables in the text.

Literature review

The structure, content and volume of the literature review show excellent knowledge of the problem and comprehensively reflect the studies of the literature data, with a logical structure. The classification of the birth defects, etiology of diseases, and epidemiology are successively reviewed. The approaches to genetic diagnosis are described - genealogical method, physical examination, laboratory-genetic methods, and a comparative analysis of the resolution of

genetic analyses is made. In addition, modern software and computer dysmorphology programs and sources used in the interpretation of clinical phenotypes and laboratory results are indicated. Special emphasis is placed on genetic counselling as a highly specialized activity in the diagnosis of genetic pathology.

The overview shows that the PhD student is very well versed in the subject she is working on. I give a high rating to the literature review not only because of the excellent knowledge and analysis of the literary data, but also because it is systematized and aimed at the specific tasks of the dissertation.

Aim and tasks

The aim of this dissertation is to evaluate the applicability of the MLPA method for the consideration of molecular genetic markers characteristic of CML in the routine clinical and diagnostic evaluation of patients with newly diagnosed CML.

To accomplish this goal, 5 objectives were set and clearly and precisely formulated:

1. To introduce a molecular genetic method to identify significant molecular genetic markers associated with CML.
2. To select patients with newly diagnosed CML who meet the inclusion criteria for the prospective study.
3. To perform molecular genetic analysis of DNA isolated from venous blood leukocytes from patients with newly diagnosed OML before treatment and from a control group of healthy subjects.
4. To compare the data with those from a parallel CCA, and to summarize and analyze the results of the molecular genetic study.
5. To summarize the role of the molecular genetic method used in the initial genetic screening and to derive guidelines for improving the genetic evaluation of the contingent of newly diagnosed patients with CML.

Materials

A total of 61 patients (29 (47.5%) women and 32 (52.5%) men) were included in the study. The age of the subjects ranged from 20 to 89 years.

Based on the initial diagnosis, the patients were grouped into 4 groups. Group II - patients with previously diagnosed MDS - 7 (11.5%); Group III - patients with previously diagnosed BCR::ABL1 (-) MPN - 4 (6.6%); Group IV - patients with previously diagnosed CML - 2 (3.3%).

The control group included 21 individuals - 10 women aged 20-79 (median 64.5 years) and 11 men aged 37-73 (median 62 years).

DNA was isolated from venous blood leukocytes for molecular genetic analysis.

Methods

The following research methods were used in the implementation of the above-mentioned objective:

The following research methods have been used in fulfilling the above objective:

- Clinical methods
- Genetic laboratory methods including cytogenetic method, molecular genetic (DNA) methods - DNA isolation by salt extraction, MLPA analysis,
- The following methods were used for statistical analysis: graphical analysis, quantitative processing, non-parametric analyses - Mann-Whitney test, Kruskal-Wallis test, as well as chisquare test, Fisher's exact test, survival estimation by Kaplan-Meier test.
- Software analysis was performed using GraphPad Prism v. 9.5.1 (GraphPad Software, USA) and Microsoft Excel 2016 (Microsoft, USA).

From the review of the experimental techniques, it can be concluded that the PhD student has established herself as a highly skilled medical geneticist in the process of her training. The methods applied were adequate to meet the set goals and objectives.From the review of the experimental techniques, it can be concluded that in the process of her studies, the PhD student developed herself as a highly qualified medical geneticist. The applied methods are adequate to fulfil the set goals and tasks.

Results and discussion

More than three quarters - 78.7% of patients with newly diagnosed CML were without previous hematological (including malignant) diseases.

Analysis of MLPA results revealed no genetic changes in 27 (44.3%) and various changes in 34 (55.7%). Monogenic markers (NPM1, IDH2, DNMT3A) were diagnosed in one third (34.4%) of all patients examined. Thirty-one monogenic somatic variants were detected in 22 patients (64.7%), in isolated form in 11 and in combination with another genetic finding in the other 11. The NPM1 variant (865insTCTG) was the most frequent and was detected in 35.3% of patients with pathology.

Of the 53 patients included, cytogenetic analysis was successful in 38 (71.7%), while the remaining 15 (28.3%) had no metaphase plates detected for analysis. In 21 (55.3%), various

pathological results were found. Different mutations were found in chromosomes 1, 4, 5, 6, 7, 11, 14, 17 and 21 in 18 (52.9%) of the same 34 patients (29.5% of all in the study).

A comparison of the two methods in terms of their overall success rates and informativeness was made. The MLPA method revealed findings missed by cytogenetic analysis in one-fifth (18%) of all subjects. In nearly one-third (27.9%) of patients, conventional cytogenetic analysis detected pathology without an analogous result from the molecular genetic method. When the two methods were combined, genetic changes were detected in 78.7% of patients.

This work highlights the importance of integrating molecular genetic profiling into routine diagnostic and prognostic evaluations for patients with CML, as genetic markers increasingly guide the choice of therapeutics and risk stratification algorithms. By analyzing the molecular profile of AML and elucidating the prognostic and therapeutic implications of specific genetic aberrations, this dissertation helps to improve our understanding of the pathogenesis of AML, as well as highlights the importance of precision medicine for genomic-informed therapy selection.

Conclusions and contributions

Six conclusions are formulated which reflect the results and fully meet the objectives. Contributions of an original, confirmatory and applied nature are also outlined. I agree with the author's assessment of the contributions of the thesis.

5. Publications on the topic of the dissertation

In connection with the dissertation, the PhD student presents three full-text publications of which he is the first author, two of which are indexed in Scopus or Web of Science databases. The candidate also submits 4 abstracts from scientific events related to the dissertation topic. The presented scientific papers fully cover the dissertation topic and contain results of the conducted research.

6. Evaluation of the thesis summary

The thesis summary is written on 52 pages, meets the main sections of the dissertation work and correctly reflects the regulatory requirements.

7. Conclusion

The thesis "Investigation of molecular genetic markers in patients with acute myelogenous leukemia" is an in-depth study on the role of genetic markers in risk stratification, prognosis and optimal therapy selection in patients with AML. Ongoing research efforts aimed

at elucidating the genetic landscape of AML and applying the results to clinical practice are essential to improve the prognosis of patients with this heterogeneous disease.

The dissertation meets the requirements of the Law for the Development of the Academic Staff in the Republic of Bulgaria and the Regulation for the Development of the Academic Staff at the MU of Varna for awarding the scientific and educational degree "Doctor".

The work establishes the author as a responsible and reliable researcher who can independently conduct scientific research and interpret the results obtained.

All this gives me a reason to give a positive evaluation for the research conducted, presented by the above reviewed dissertation, abstract, results and contributions and I propose to the Honorable Scientific Jury to vote for awarding the educational and scientific degree "Doctor" to Dr. Dinnar Ali Yahya.

07 May 2024 Reviewer: .....

Sofia Prof. Savina Hadjidekova, MD, PhD