ACADEMIC REFERENCE LETTER

written by Associate Prof. Eleonora Dimitrova, MD, PhD
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According to order № P-109-141/ 09.04.2024 of the Rector of the Medical University in Varna, I was elected a member of the Scientific jury, and on the basis of Protocol №1/ 12.04.2024, I was appointed to write an academic reference letter about a procedure for obtaining an educational and scientific degree "Doctor" / PhD in the field of higher education 7. Healthcare and Sports, professional area 7.1. Medicine, doctoral program "Hematology and blood transfusion" in relation to the dissertation with a title: *"Biomarkers for evaluation of bone disease in multiple myeloma"*.

Author: Dr Vladimir Todorov Gerov, a self-study PhD student

Scientific advisor: Prof. Ilina Dimitrova Micheva, MD, PhD

1.   Brief information about the candidate

Dr Vladimir Gerov was born on the 24th of February, 1961 in the town of Shumen. In 1980 he graduated from the 4th Language Medium school "Frederic Joliot Curie|" with intensive learning of French. He graduated with a Master's degree in Medicine from the Medical University in Varna in 1988. In 1995 he obtained a specialty in Internal diseases, in 1997 – in Clinical hematology. In 2006 he also did a Master's degree in Healthcare management at MU – Varna. In 2016 he participated in a highly specialized training course on stem cell transplantation.

He began his professional career as a doctor-therapist in Hospital Medical Care, Shumen and from 1989 to 1991 he worked as a family doctor in DCC Shumen, from 1992 to 2014 he worked at the Hematology Clinic and since 2014 up to now he has been working in the ward for stem cell transplantation, at the Hematology clinic, "St. Marina" University multiprofile hospital for active treatment, Varna. Since 2010 up to now he has been a part-time assistant at the Second department of Internal diseases, MU, Varna. Dr Vladimir Gerov has specialized in transplantation centers in France (2015), Croatia (2016) and in Germany (2017). He participated in scientific research projects on the topic: "Oxidative stress detection during and after an anti-tumor treatment of patients with acute myeloblastic leukemias in order to create opportunities for influencing it with antioxidants" and "New molecular biomarkers for evaluation of bone disease in multiple myeloma" (Science Fund, MU, Varna). Dr Vladimir Gerov has scientific and professional interests in the field of diagnostics and treatment of acute leukemias, multiple

myeloma, transplantations in oncohematological diseases, oxidative stress in oncohematological diseases, etc. He is fluent in both French and English. He is a member of the Bulgarian Medical Association, the Bulgarian Medical Society of Hematology and the European Society for Blood and Marrow Transplantation.

2. Significance of the problem, formulation of the aims, objectives and hypotheses

Multiple myeloma is one of the most widely spread hematological malignant diseases with an ever growing occurrence in the world. Myeloma bone disease (MBD) is the most significant clinical expression of the multiple myeloma. Despite the progress in the treatment of MM, the introduction of new medications and therapeutic regimen, the myeloma-induced bone disease remains the main cause of disability and high mortality in patients with MM. The progression of the disease is frequently related to deterioration of MBD manifestations such as severe bone pain, pathologic fractures, spinal cord compression and bone marrow suppression, hypercalcemia, etc. The study of the pathophysiological and pathogenetic mechanisms of MBD could lead to new opportunities for implementing in the clinical practice biomarkers for effective monitoring of the development and progression of bone disease, for monitoring the effect of the treatment applied. The clarification of the signaling pathways that are related to the impaired balance between the osteoclastogenesis and osteoblastogenesis could contribute to the development of new medications targeted at its recovery, and thus the prevention of MBD progression. The biomarkers which are being used at the moment in the routine clinical practice serve for stratification of the risk of patients with MM and evaluation of the tumor load. Imagining investigations are the standard for bone disease evaluation and assessment.

The search for and the introduction of serum biomarkers for evaluating bone metabolism could supplement the data received with the help of imaging methods. They would also facilitate the development of new target therapies for treating MBD and improvement in the survival of the patients with MM.

All this has resulted in the definition of the following aim: "To evaluate the role of bone biomarkers sclerostin, Dkk-1, sRANKL, osteopontin and periostin for the development of bone disease in newly diagnosed patients with multiple myeloma and to monitor their dynamics in the course of the treatment applied". Eight objectives have been clearly formulated which serve the aim and hypotheses of the research.

3. Dissertation structure

The dissertation consists of 184 pages, of which –"Introduction" – 2 pages, "Literature review"– 40 pages, "Aim and objectives" – 1 page, "Materials and Methods" – 10 pages, "Results" – 68 pages, "Discussion" – 30 pages, "Conclusion" – 2 pages, "Contributions" – 2 pages, "References"– 24 pages.

The dissertation is illustrated with 50 tables and 52 figures.

The bibliography includes 424 literature sources, of which 1 in Cyrillic, 421 in Latin and 2 website sources.

The dissertation structure is well balanced and ordered. The separate sections have a logical sequence and a complex content. The scientific work meets the requirements of the Act for the development of the academic staff in the Republic of Bulgaria.

4. Awareness of the existing scientific literature

The PhD student presents an in-depth analysis of a large volume of scientific information. Data have been shared about the epidemiology, risk factors for the development of MM, the molecular mechanisms of interaction of the myeloma cells with the bone marrow stromal cells. Signaling pathways have been displayed that are related to the MBD pathogenesis. Detailed data are shown on the structure, biological role, the importance for development of different diseases, for the regulation of the immune response and the relation to MBD of the biomarkers Dickkopf-1, sRANKL (the RANK/RANKL axis), sclerostin, osteopontin, periostin, as well as the results available up to this moment of studies on their dynamics in the course of the disease progression and the application of different therapeutic regimens.

5. Study design and methodology

Results have been presented of a single-centered and prospective study of 74 individuals – 41 newly diagnosed patients with MM and 33 healthy people, forming the control group, carried out at the Hematology Clinic at "St. Marina" University multiprofile hospital for active treatment, Varna in the period from 01.06.2021 to 31.12.2022. The patients were selected according to the inclusion and exclusion criteria, described in detail in the dissertation work. The study design comprises the following stages T0 (before the beginning of the treatment), T1 (after four courses of chemotherapy were completed), T2 (after completion of the additional four courses of chemotherapy), TA (three months after the carrying out of autologous stem cell treatment). The routine laboratory, clinical and imaging research performed have been depicted as well as the specific investigations in all groups of patients at each separate stage of the study design. The methods used for statistical processing of the obtained results have also been demonstrated.

6. Alignment between aim, results and conclusions

The goal set and the objectives thus formulated correspond to the obtained results summarized in the discussion and the conclusions. The data collected from the study are visualized with tables, figures and graphs. Correlation searches were performed between the levels of the investigated biomarkers and some demographic indicators as well with routine hematological, biochemical and morphological indicators, the stage of the disease, the bone disease and the achieved effect of the treatment carried out in the separate groups of patients.

7.   Analysis of the conclusions and contributions

Twelve conclusions have been formulated that synthesize the most important of the results achieved. The conclusions are clear and specific.

Among the major contributions with an original scientific value of the dissertation are the following:

- an original combination of new bone biomarkers has been studied for the first time ever for evaluation of simultaneously suppressed osteoblast activity, increased osteoclast activity and changed bone marrow environment that favors the proliferation of myeloma plasma cells;

- it is proved for the first time that the investigated original combination of new bone biomarkers reflects the changes in the bone marrow and the clinical characteristics of the disease which allows for a better evaluation of the state of the patients with MM both at the moment of diagnosis and in the course of their monitoring;

- for the first time the dynamics in the serum levels of the new bone biomarkers in the course of treatment is investigated and their dependency on the therapy response carried out is analysed.

Among the major contributions with practical scientific value are the following:

- the new bone biomarkers could be used as additional parameters for evaluation of the stage and progression of MM;

- the new bone biomarkers could be used as additional information for evaluation of the bone disease in MM;

- the new bone biomarkers could be used as additional parameters for evaluation of the response of the newly diagnosed patients to the treatment applied;

- the proven potential of the new biomarkers to adequately reflect the pathogenetic mechanisms of the bone disease allows for them to be validated among a greater number of patients with MM;

- the results of the dissertation could serve for the development of new targeted cancer drugs directed against the progression of bone disease in MM patients;

The PhD candidate presents four scientific publications related to this dissertation work.

The dissertation Abstract is 115 pages long and contains all major sections of the dissertation work.

8.   Conclusion

Dr Vladimir Todorov Gerov's dissertation is in-depth research dedicated to an important medico-social problem – Evaluation of bone disease, the main reason for the disability and mortality in patients with MM, one of the most frequent diseases in the nosology of hematological malignancies. The theme is topical, relevant and broad. The methods applied in the investigation correspond to the formulated aim and objectives. The results are well analysed, the conclusions are clearly stated. The contributions thus analysed possess an original scientific value both theoretical and with potential for practical implementation.

The dissertation with the title "Biomarkers for evaluation of bone disease in multiple myeloma" meets the prerequisites for obtaining the educational and scientific degree "Doctor". It meets the requirements of the Act for the development of the academic staff in the Republic of Bulgaria and of the Guidelines for its application.

On the basis of all the information presented above, I give a positive evaluation of the dissertation written by Dr Vladimir Todorov Gerov and propose to the members of the esteemed Scientific jury that he be awarded the educational and scientific degree "Doctor of Philosophy" / PhD.

Заличено на основание чл. 5, §1, б. „В" от Регламент (ЕС) 2016/679

17.05.2024

Signature:..........................
/Assoc. Prof. Eleonora Georgieva
Dimitrova-Gospodinova, MD, PhD/

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