Посветени на хуманността
МЕДИЦИНСКИ УНИВЕРСИТЕТ – ПЛОВДИВ
4002 Пловдив, бул. Васил Априлов № 15А

www.mu-plovdiv.bg

Dedicated to humanity
MEDICAL UNIVERSITY – PLOVDIV
15A, Vasil Aprilov Blvd., 4002 Plovdiv, Bulgaria

TO
ASS.PROF. ELEONORA DIMITROVA-GOSPODINOVA, MD, PHD
CHAIR OF THE SCIENTIFIC JURY
DETERMINED BY ORDER No. R-109-141/04/09/2024
OF THE RECTOR OF MU- VARNA

REVIEW

From: Prof. Dr. Veselina Goranova-Marinova, MD, PhD
Head of the "Hematology" section, MF, MU-Plovdiv
External member of scientific jury according to order No. R-109-141/09.04.2024
of the Rector of MU-Varna "Prof. Dr. Paraskev Stoyanov"

Subject: Procedure for obtaining the scientific and educational degree "Doctor"
Field of higher education: 7. "Health and sports"
Professional direction: 7.1. "Medicine"
Doctoral program: "Hematology and blood transfusion"
Author: Dr. Vladimir Todorov Gerov, Department of Hematology, Second Department of Internal Medicine, Faculty of Medicine, Varna University
Form of doctoral studies: Independent preparation
Theme: "Biomarkers for assessment of bone density in multiple myeloma"
Research mentor: Prof. Dr. Ilina D. Micheva, MD

1.General presentation of the procedure. The presented set of materials on paper and electronic media under the procedure for the acquisition of educational and scientific degree "Doctor" in Varna Medical University is in accordance with the Regulations for the organization and activities of Varna Medical University; art. 68 para. 1 of the Higher Education Law Art. 7, para. 1 from ZRASRB; Art. 5, para. 1 and Art. 6, para. 1 of the Regulations for its application, the Regulations for the development of the academic staff of MU-Varna. All the necessary documents are presented, namely the orders for enrolling and deducting the doctoral student, the protocols of extended departmental councils and the doctoral minimum exam, which reflect all stages of the development of the doctoral student,

including the Dissertation itself. Attached to the set of materials is a list and copies of the publications related to the dissertation work, as well as a table presenting the doctoral student's compliance with the minimum national criteria for obtaining Education and Scientific Degree Doctor. A total of 4 publications were presented, in which the dissertation student was the lead author. Two are in prestigious international journals - J Clin Med, IF4.1 and Cancers with IF 5.6; one of the publications is in "Hematology" - the official journal of the Bulgarian Medical Society of Hematology, referenced and indexed in the international database "Scopus". Five scientific reports are presented, of which 3 are from participation in international scientific forums. In 4 of the presented scientific reports, the dissertation student is the lead author. ***The doctoral procedure has been followed.***

2. Presentation of the doctoral student. Dr. Vladimir Todorov Gerov graduated from Medical University - Varna in 1988. There are two specialties - in internal medicine and clinical hematology and a master's degree in health management. Since 1992, he has been working in the hematology clinic of the "St. Marina" UMBAL, Varna. Since 2010, he has been a part-time assistant at the Department of Hematology, Second Department of Internal Medicine, MU-Varna. In 2016, he oriented his professional career to the transplantation of hematopoietic stem cells, for which he successively conducted highly specialized courses at the University of Varna, in the Transplantation Unit of the University Hospital in Zagreb, Croatia, and in the Transplantation Unitof the University Hospital in Hanover, Germany. In 2015, he worked for 6 months at the Hematology Clinic with a transplant unit in the city of Colmar, France. The scientific interests of Dr. Vl. Gerov are in the field of acute leukemia, multiple myeloma, hematopoietic stem cell transplantation and oxidative stress in malignant hematological diseases. He is part of the teams of major international clinical trials, including in the field of multiple myeloma, non-Hodgkin's lymphomas, acute and chronic myeloproliferative diseases. He is fluent in French and very good in English. He has more than 30 years of experience in the field of clinical hematology. ***The doctoral student has theoretical knowledge, practical experience, additional qualifications and focused scientific and practical interests in the field of doctoral studies.***

3. Relevance of the topic and knowledge of the problem. In his dissertation, Dr. Vladimir Gerov develops an important, contemporary and significant problem of clinical hematology. Myeloma-induced bone disease (MBD) is the most typical clinical manifestation and "identity card" of multiple myeloma (MM). Approximately 80% of patients with MM have at least one or more osteolytic bone lesions at the time of diagnosis, often accompanied by compression fractures, and their frequency further increases in the evolution of the disease. The

progression of the disease is naturally accompanied by a worsening of MBD and its various clinical manifestations, which impairs the quality of life of patients and reduces their survival. Elucidation of the pathophysiology and pathogenesis of MBD provides opportunities for research and implementation in clinical practice of new biomarkers, with the help of which the development and progression of the bone disease could be effectively monitored on the one hand and the effect of the anti-myeloma therapy on the other. Bone biomarkers reflect how well treatment of the underlying disease improves and restores the severely disturbed balance between osteoclastogenesis and osteoblastogenesis. In routine clinical practice, a variety of clinical and laboratory parameters are used to assess tumor burden or serve to stratify the risk of patients with MM. Currently, clinical hematology does not have such indicators that give an idea of the degree of bone damage. Imaging studies remain the gold standard for MBD assessment. An important advantage of serum biomarkers for bone remodeling is the fact that they are more dynamically changing indicators and could provide important additional information to imaging studies, which significantly more slowly reflect metabolic changes in bone. All this necessitates the need to search for suitable biomarkers to effectively monitor MBD. In the long term, this is a prerequisite for the development of new biological agents for targeted therapy and adequate treatment of bone disease, which would improve the quality of life of MM patients and prolong their survival. ***The topic of the dissertation work is important, current, with big clinical significance.***

4. Knowledge of the scientific problem. The presented scientific hypothesis suggests that the bone biomarkers sclerostin, Dkk-1, sRANKL, osteopontin and periostin have an important role in the progression of MBD and have an altered expression at the diagnosis, in the evolution of the disease, depend on the phase of the treatment and the degree of clinical manifestation of bone disease. The author analyzes the role of sclerostin, Dkk-1, sRANKL, osteopontin and periostin as independent predictive risk factors for the development of MBD and prognostic risk factors for patient survival. The author knows the scientific problem in depth, can formulate a scientific hypothesis and conduct an analysis, the results of which would lead to an improvement in the diagnosis and follow-up of patients with multiple myeloma and bone disease as a prerequisite for prolonging their survival and increasing their quality of life.

5.Research methodology. The performed scientific study is single-center and prospective, conducted at the Hematology Clinic, UMBAL "St. Marina" - Varna for the period 01.06.2021 - 31.12.2022. A total of 74 people were studied - 41 newly diagnosed patients with multiple myeloma and 33 healthy people who formed the control group. The analyzed cohort of

patients recruited over a period of two years is sufficient in number and allows for statistical analysis. Routine clinical and laboratory parameters were examined according to generally accepted methods, imaging was performed with low-dose whole-body CT, which is the standard for MBD assessment, and the specific bone biomarkers sclerostin, Dkk-1, sRANKL, osteopontin and periostin were examined with a validated methodology that allows reproducibility of the results as their measurement is consistent with creatinine clearance in patients with myeloma nephropathy. The design has a clear clinical rationale and includes follow-up of the studied patients during the course of anti-myeloma therapy.

6. Characteristics and evaluation of the dissertation work. The dissertation is developed on 184 standard pages. Contains 50 tables and 52 figures. 424 literary sources, selected purposefully, are cited. Several formulas are derived. The mandatory sections of the scientific work are professionally developed, with an acceptable ratio between them. Clear and precise, grammatically correct Bulgarian language is used.

6.1 Literature review. The literature review is presented in 40 standard pages, in-depth and focused on the scientific problem. The pathogenesis of MBD is presented in detail, emphasizing that the multiple cell populations, cytokines, growth factors, adhesion molecules, receptors and their ligands in MBD form a vicious circle, in which the more active the MM, the more severe the bone disease and conversely, the more pronounced the bone disease, the more advanced the MM. Many authors argue that MM is a disease of the bone marrow microenvironment. Dr. Gerov presents numerous reliable data obtained as a result of long-term scientific developments of research teams from all over the world, which prove that MM cells suppress the differentiation of osteoblasts and stimulate the proliferation, differentiation, maturation and functional activity of osteoclasts. The molecular interactions of myeloma cells with osteoblasts, osteocytes, osteoclasts, T-lymphocytes and NK cells are presented in detail. The signaling pathways responsible for bone remodeling in normal and pathological conditions and their alteration in MM are described. The literature review continues with a detailed presentation of the structure, function, biological role and role in MM of the bone biomarkers analyzed in the dissertation: sclerostin, Dkk-1, sRANKL, osteopontin and periostin. The literature review concludes with a rationale for the need for further research to elucidate the role of sclerostin, Dkk-1, sRANKL, osteopontin and periostin involved in the regulation of osteoclasts, osteoblasts and osteocytes and their potential to be used in clinical practice to monitor MBD and the effect of the treatment.

6.2 Purpose and tasks. The aim of the dissertation is to evaluate the role of the bone biomarkers sclerostin, Dkk-1, sRANKL, osteopontin and periostin for the development of bone disease in newly diagnosed patients with multiple myeloma and to follow their

dynamics during the course of treatment. It is precisely and clearly formulated and with logically set 8 main tasks.

6.3 Material and Methods Section. The Material and Methods section is presented in 9 standard pages. The patients and the control group were examined in four time periods – before the start of treatment after C4, after C8 and 3 months after autologous hematopoietic stem cell transplantation (auto-HSCT). Bone metabolism markers sclerostin, Dkk-1, sRANKL, osteopontin, and periostin were analyzed for sex and age, MM activity indicators, International Staging System (ISS) stage, low-dose whole-body CT scan data in each one of the four periods and were calculated against creatinine clearance. Bone biomarkers were examined in serum using ELISA (enzymelinked immunosorbent assay) methods with ready-made commercial kits of Shanghai Sunred Biological Technology Co., Ltd, China. They are based on a non-competitive "sandwich" principle. The measurements were carried out in the Department of Biochemistry, Molecular Medicine and Nutrigenomics at the Faculty of Pharmacy of the Medical University of Varna in strict compliance with the manufacturer's instructions. Results were reported on a Biotek Synergy 2 Multi-Mode Plate Reader, USA. The statistical data are correctly selected and allow obtaining reliable results. The statistical processing of the obtained results was done competently with properly selected analyzes – descriptive, variational, comparative, correlational, regression, ROC, as well as the Shapiro-Wilks tests to assess the normal distribution and χ 2 non-parametric agreement test. The data were processed and graphically presented using GraphPad Prism, version 8.0.2 for Windows, USA and IBM and SPSS Statistics v. 23. ***I fully accept the "Materials and Methods" section***

6.4. The Results section is presented on 68 standard pages. The exposition of the section is voluminous, but it is presented comprehensively in tables, figures, graphs and diagrams. The sequence of tasks was followed logically. Demographic data and clinical characteristics of MM patients are presented. Twelve of them had myeloma nephropathy. Nine patients (21.9%) had absent and mild bone involvement and 32 patients (78.1%) had severe bone involvement. The author found that the serum levels of all investigated bone markers - Dkk-1, sclerostin, sRANKL, osteopontin and periostin - were significantly higher in newly diagnosed MM patients compared to healthy controls and increased with advanced clinical stages. They discriminate ill from healthy individuals, which is proven by the ROC analysis, while the positive correlation of the examined biomarkers with bone marrow infiltration and B2M, as well as the negative correlation with Hb, probably reflect tumor growth and disease progression. Serum levels of Dkk-1 and sclerostin in NDMM patients reflect suppressed osteoblastic activity in the bone marrow, while increased levels of sRANKL, osteopontin, and periostin reflect increased OK activity. Serum levels of Dkk-1, sclerostin, sRANKL, osteopontin and periostin in NDMM correlate with the severity of myeloma bone disease as

Dkk-1, sclerostin, osteopontin and periostin distinguish patients with milder bone involvement from those with more severe. Results that reflect the dynamics of sclerostin, Dkk-1, sRANKL, osteopontin and periostin over the course of treatment are important. With accumulation of cycles of therapy, their serum levels decrease, and Bortezomib-based therapy has the effect of not only suppressing the tumor process, but has the potential to interfere with bone remodeling processes and improve the imbalance between osteoclastogenesis and osteoblastogenesis. Lowest serum levels of Dkk-1, sclerostin, sRANKL, osteopontin, and periostin were found after ASCT in both patients with VGPR and CR, confirming the role of high-dose chemotherapy not only in reducing tumor burden, but also its beneficial effect on of bone metabolism. ***I accept the obtained results without reservations. As a result of important clinical importance, I note the data that, of all the studied biomarkers, the most sensitive and significant for the progression of myeloma bone disease are sclerostin and periostin.***

6.5 Discussion Section. In 30 standard pages, the PhD student analyzes his own results, discusses their significance and compares them with those of other author groups. The established differences with the data from other scientific studies, the author explains with arguments and concrete facts. Such is the case with the influence of age and gender on the expression of bone remodeling indicators. Dr. Gerov found no statistically significant differences in the serum levels of the studied indicators depending on gender and age and concluded that the influence of the pathological process outweighs the influence of the biological factors age and gender and is decisive for the observed significantly higher levels of Dkk-1 and Scl in the diagnosis of MM. The results obtained by Dr. V. Gerov regarding the correlations of sclerostin, Dkk-1, sRANKL, osteopontin and periostin with the clinical stage, the severity of MBD and their reduction after successful treatment completely coincide with the data of the well-known studies of E.Terpos and M .Politou. Unlike Dr. Gerov, E.Terpos et al. found sRANKL values in transplant patients still different from values in their control group. When conducting consolidation therapy according to the VTD protocol (6 courses), the authors indicated a significant change only in CTX levels, reaching those of controls, but not for the rest of the investigated bone biomarkers, including sRANKL. The dissertation explains these differences with the fact that the groups that achieved a therapeutic response are inhomogeneous, as well as that patients achieve a therapeutic response at a different stage of their treatment. The dissertation states that no data can be found in the literature regarding the dependence of serum levels of OPN and PON on the response achieved in the treatment of MM. Therefore, the results of the ROC analyzes confirm the high diagnostic significance of the studied bone biomarkers, and the regression analysis singles out the two of them -

sclerostin and periostin, which with the greatest degree of probability could predict the bone disease. I accept the Discussion section without objection.

6.6 The conclusions are 11 in number and clearly follow the set goals and objectives, being logically formulated according to the results obtained. The most important from a clinical point of view is the conclusion that of all studied biomarkers, the most sensitive and significant for the progression of myeloma bone disease are sclerostin and periostin. I agree with the conclusions thus formulated.

6.7 The bibliography covers 424 literary sources, of which 3 are by Bulgarian authors. The analyzed scientific publications in the last 5 years are 20% of the total number. These data testify to the topicality of the problem and the great ongoing research interest in the subject. ***I have no critical comments.***

7. Evaluation of the contributions of the dissertation work. The dissertation ends with the presentation of contributions that have a scientific, as well as a marked scientific-applied character. Three of the contributions are of an original nature and five of a confirmatory nature. **I accept the presented contributions.**

8.Personal participation of the doctoral student. The PhD student has a personal involvement in the formulation of the scientific idea, the collection of the material and the design of the study. His participation in the statistical processing of the data and the analysis of the obtained results is personal. The conclusions and contributions were also drawn with the participation of Dr. Gerov. The dissertation student is the first author in all pre-sat publications related to the dissertation, as well as in most scientific communications, which confirms his personal involvement in the development of the scientific work. **The doctoral student has mainly personal involvement in the development of the dissertation.**

9.Autoreferat. The autoreferat contains 115 pages, gives an overall idea of the dissertation work, fully reflecting the individual sections. All figures and tables are included, with the exception of those from the literature review, and they present in sufficient volume the necessary data.

10 Conclusion. The dissertation work of Dr. Vladimir Todorov Gerov complies with the requirements of ZRASRB, the Regulations for its implementation and the Regulations of MU-Varna for awarding the scientific and educational degree "Doctor". Although the topic of the dissertation is being developed by a number of author groups, including in Bulgaria, the selected panel of markers of bone metabolism is original, and the problems of the MBD are

still the subject of discussion on an international scale. The contributions of the dissertation have a scientific and marked scientific-applied nature, which is a good basis for the implementation of sclerostin and periostin in the algorithm for monitoring the effect of the treatment of ICD. In the dissertation work of Dr. Vladimir Gerov, developed qualities for the analysis and synthesis of scientific information, the ability to formulate conclusions and build scientific hypotheses are evident. They demonstrate in-depth scientific knowledge and practical skills in the specialty "Hematology and Blood Transfusion".

I give my positive assessment for the dissertation work on the topic "Biomarkers for the assessment of bone disease in multiple myeloma" and propose to the respected scientific jury to vote positively the award of a scientific and educational degree "Doctor" to Dr. Vladimir Todorov Gerov.

REVIEW BY

/Prof. Vesselina Goranova-Marinova, MD, PhD/