Opinion

from Assoc. Prof. Dr. Ilko Georgiev Bakardzhiev, Ph.D.
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on the PhD thesis of Dr. Lilyana Nikolova Petkova, on the topic "Expression of Cyclin D1, BCL2, p53 and other melanocyte markers in malignant melanoma of the skin and melanocyte nevi - comparative analysis of immunohistochemical expression, morphological profiles, and their importance for the diagnosis" for awarding the scientific-educational degree "Doctor", in the scientific specialty of pathoanatomy and cytopathology.

By decision of the Faculty Council at the Faculty of Medicine at MU-Varna (protocol № 57 / 21.12.2021) and by order № R-109-594 / 31.12.2021 of the Rector of MU-Varna, I was elected an internal member of the scientific jury, and on the basis of protocol №1 / 07.01.2022 I was appointed to prepare an opinion on the procedure for obtaining the educational and scientific degree "Doctor" of Dr. Lilyana Nikolova Petkova, PhD student in full-time education, MU-Varna. The presented documents are in accordance with the requirements of the Law for the development of the academic staff in the Republic of Bulgaria, the Regulations for its application and the Regulations for the development of the academic staff at the Medical University of Varna.

The presented thesis is written on 148 standard typewritten pages distributed as follows: Content and Abbreviations 6 pages, Introduction 2 pages, Literary review - 41 pages, Conclusion (review) - 2 pages, Aim and tasks - 1 page, Material and methods - 8 pages, Results 57, Discussion - 14 pages, Conclusions - 2 pages, Contributions - 1 page. It is illustrated with 41 figures and 30 tables. The bibliography contains 126 literature sources, 10 in Cyrillic and 116 in Latin.

Malignant melanoma is a tumor of melanocyte origin, unfavorable prognosis, and its incidence worldwide is constantly increasing. In Bulgaria, the epidemiological characteristics of cutaneous melanoma are presented with low morbidity, unfavorable growth trend, rapid rate of increase in both sexes and the lowest survival of patients among the countries in Eastern Europe. The diagnosis of malignant melanoma, despite the progress of diagnostic methods, the large number of pigmented neoplasms and discrete morphological differences with atypical melanocyte lesions mimicking this malignant tumor, sometimes make it extremely

difficult. In order to accurately diagnose and determine the behavior of the tumor, new markers are increasingly being sought to clarify the biology of the tumor. In addition to clinical and histological criteria for accurate diagnosis of tumors, markers are increasingly used which are even more important for early diagnosis and success of treatment and prognosis of the disease. Given the poor prognosis, increased mortality and the small number of immunohistochemical studies of cutaneous melanoma and melanocyte nevi in Bulgaria, all studies using modern methods are relevant, with completely scientifically justified need for our country.

The aim of the dissertation is clearly and precisely defined. For its implementation, 5 correctly formulated tasks have been set.

Material and methods are methodologically correctly developed on a sufficient clinical contingent. The subject of the present study are 91 pigmented neoplasms, including 57 (fifty-seven) benign melanocyte nevi, 10 (ten) atypical nevi and 24 (twenty-four) malignant melanomas, for the period 2014-2019 with analysis of clinical and anamnestic data from the accompanying biopsy sheet. The research methods are selected according to the tasks and are very well and informatively described. Transversal consecutive topographic sections with a thickness of 2 to 3 mm were prepared from the entire biopsy material, including the lesion with its edges - peripheral and deep, for subsequent histological processing. The materials thus prepared were examined histologically and immunohistochemically. The microscopic evaluation of each lesion follows the parameters required for morphological diagnosis of pigmented lesions, examined morphologically, morphometrically and by semi-quantitative methods. The evaluation included: microscopic symmetry of the lesion, cellular atypia with determination of the degree of expression, localization and distribution in the tumor, thickness of the lesion on the four-point Breslow scale, mitotic activity with mitosis by hot spot, Clark microinvasion, ulceration assessment and regression in melanomas, and the presence of tumor-infiltrating lymphocytes. Anti S100 protein, Anti Melanosome, clone HMB 45 (Human Melanoma Black), Ki 67 antigen clone MIB - 1, Antibody p53 protein, Antibody BCL2 oncoprotein, Antibody Cyclin D1 were used to perform the immunohistochemical study. The evaluation of the expression of the various antibodies used was performed by light microscopy, based on semi-quantitative analysis, on the basic requirements for the evaluation of immunohistochemical reactions and personal experience. A detailed report on the intensity and distribution of the expression of the markers in eleven groups was performed. The following methods of statistical analysis were applied: descriptive,

nonparametric, correlation analysis, t-test analysis, variation analysis, Spearman correlation analysis (rho). Statistical analysis programs were also used - IBM, SPSS v. 23. The results are presented in tables using Microsoft Excel v. 2010.
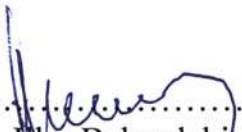
Results and discussion

The distribution of lesions by histological type showed: benign melanocyte lesions - 62.63% (57 cases), atypical nevi - 11% (10 cases), and malignant melanoma - 26.37% (24 cases). In all the material studied, the female sex dominates, almost 2:1 predominance of women with pigmented tumors compared to men, while in atypical nevi and malignant melanoma equality of both sexes is observed. The majority of cases of melanoma were diagnosed in patients over 40 years of age, and the difference compared to other age groups in which it is also detected (except for the group 11-20 years) is statistically significant. It follows that age in patients with melanoma can be considered as an independent prognostic factor, and with increasing age at the time of diagnosis, the prognosis for survival deteriorates. The results of the topographic distribution of the lesions in the target groups show that most benign nevi are in the head and neck, most atypical nevi are diagnosed in the skin of the abdomen, followed by those of the head and neck. Cases of malignant melanoma have been found, mainly in the skin of the lower extremities, back and chest, and according to literature data, the location of the tumor on the lower extremities has a better prognosis than the location on the trunk, head and neck. The study of the thickness after Breslow showed that the largest relative share was for Breslow 4 (58.33%), followed by Breslow 3 (33.33%). The thickness after Breslow is a specific independent prognostic factor of high importance, the main criterion for pT-staging of cutaneous melanoma. The analysis of other histological indicators is also an integral part of the correct diagnosis and prognosis, such as mitotic activity, level of invasion determined by Clark and tumor infiltrating lymphocytes. Special attention is paid to ulcerations, the presence of microsatellite lesions in the dermis and hypodermis and the regression of tumors. Priority for our country are the immunohistochemically studied expression of Cyclin D1, p53 protein, BCL2, S100 protein, Ki -67 Melanosime clone HMB45, important in the cell division cycle, tumor progression, melanocyte differentiation and tumor origin of pigment cells. Dr. Petkova found that immunohistochemical markers predicting malignant potential in pigmented tumors require their use in combination, with the best combination, according to the results, is a study of the three markers - Cyclin D1, p53 protein and HMB 45, which have the highest statistical reliability in the direction of malignancy. An 11-model scheme for

detailed recording of the intensity of expression and topographic distribution of marker-positive cells in the tumor has been introduced, which provides accuracy in the interpretation and determination of immunoreactivity of each marker, an important detail for increasing the certainty of morphological diagnosis and differential diagnosis in borderline lesions. The results are systematized, well presented, accurately illustrated and statistically confirmed. Discussion of the results is comprehensive. The obtained data are excellently compared with those used in the literary sources. Larger studies and the experience of other researchers are cited in detail, and their results are compared with the own results. 12 conclusions have been formulated, which give an idea of the performed scientific and research work in all stages of the study. The contributions are divided into original scientific-applied (2) and confirmatory (4). In principle, I accept the contributions indicated by the candidate. In connection with the dissertation, four publications in Bulgarian scientific journals have been presented. The abstract meets the requirements of the Law for the development of academic staff.

Conclusion:

The presented dissertation work of Dr. Lilyana Nikolova Petkova is original and relevant in its content and interpretation, methodologically correct, with a large number of studies. The conducted research is of scientific and practical orientation and give answers to the tasks. There are no elements of compilation and plagiarism. It fully meets the criteria of the Academic Staff Development Act in the Republic of Bulgaria, the regulations for its implementation and the regulations for the development of the academic staff at the Medical University of Varna, for obtaining the scientific-educational degree "Doctor" in pathology and cytopathology, code 01/03/03 The outstanding merits and contributions give me a reason to give my positive assessment and to confidently recommend it for a positive assessment of the esteemed members of the specialized scientific jury.

Varna
January 24, 2022.

Assoc. Prof. Dr. Ilko Bakardzhiev, Ph.D.