REVIEW OF DR. LILYANA PETKOVA'S DISSERTATION ON THE TOPIC:

Expression of Cyclin D1, BCL1, P53 and other melanocytic markers in malignant melanomas of the skin and melanocytic nevi - Comparative analysis of immunohistochemical expression, morphological profile and their significance for diagnosis and tumor progression

According to the order № P-109-594/ 31.12.2022 under the procedure for obtaining the PhD in the doctoral program "Pathology and Cytopathology", professional field 7.1.Medicine, field of higher education 7. Health and Sport.

By Ass. prof. Margarita Angelova Kamenova, M.D., PhD

Biographical data. Dr. Liliana Nikolova Petkova was born in 1961. Graduated from the Mathematical High School in Dobrich, 1979. She received her degree in Medicine at the Medical University "Prof. P. Stoyanov" , Varna in 1988 and immediately started working as a resident and later a part-time assistant at the Department of General and Clinical Pathology, Medical University, Varna - Dobrich affiliate until its closure in 1992. Dr. Petkova worked as a pathologist in Ruse /1994-1995/ and in Dobrich Hospital /1995-2014г/, where she was a head of the Pathology Department for the last 11 years. Since 2014 until now she is a resident doctor at the General and Clinical Pathology Department, University Hospital "St. Marina", Varna. She acquired her specialty "Pathology with cytopathology" in 1995 and professional qualification in cytopathology since 2000. Dr Petkova has a total work experience of 30 years in medicine. Maintains its classification with many courses in SDK, European Association of Pathology, European School of Oncology and others. She is a member of the BDP, SWB and ESP. Since 01.02.2019 she is enrolled as a full-time PhD student in the PhD program "Pathology and Cytopathology" at MU "Prof. P. Stoyanov", Varna.

Introduction. The diagnosis and prognosis of malignant melanoma (MM) is one of the biggest challenges for the pathologist, due to several factors: a) the diversity of morphological picture, which overlaps with multiple other malignant tumors, b) the difficult differential diagnosis of melanocytes nevi and c) the unpredictability of its evolution, which varies from dissemination at different times after its initial appearance to the possibility of spontaneous regression. There is no association between the histological type of melanoma and its biological behavior, which is why it is one of the few malignant tumors without definite degrees of differentiation. The presence of these difficulties does not change the requirement for an accurate diagnosis, which continues to maintain a significant frequency and causes high mortality.

The choice of the topic itself shows that the author has professional training, ambition and determination to explore one of the most difficult to interpret and diagnose malignant tumors.

Due to its wide range of histological phenotype, melanoma can be easily diagnosed by routine histological methods and simultaneously impossible to diagnose without additional immunohistochemical techniques. And even so, the difficulties in the differential diagnosis (DD) do not decrease, especially from benign and atypical melanocytik lesions. These assumptions justify the author to select a panel of appropriate biomarkers for

immunohistochemical (IHC) studies that have the potential for reliable demarcation between MM, benign melanocytic nevi and other malignant neoplasms.

The review is based on 126 sources with a strong predominance of up-to-date information without neglecting the founders of melanoma's study and the characteristic clinico-morphological features of pigmented lesions. An accent is put on the subtyping of MM with a characterization of clinical and morphological peculiarity of each subtype as well are striking on difficulties in their diagnosis and DD. Dysplastic nevi are described with special attention to the typical histological signs and additional Immunohistochemical (IHC) studies, necessary for objective discrimination between nevi and melanoma. Application of ICH biomarkers occupy important place in the review because of their role for determination of cytogenesis of the malignant neoplasms and for the clarification the cancer origin and establish of prognostic and predictive criteria. Used markers are divided in two groups – diagnostic factors for DD from other non-melanocytic malignant tumors and biomarkers - second generation, showing genetically injured proteins, giving information about cancer origin and prognosis of MM and its precursors. Comprehensive review permits to a dissertator to show up diagnostic and DD difficulty in the group of melanocytic lesions and determines the markers, which can support correct diagnosis as well these which are not enough studied or the object of discussion of their role as malignant or prognostic indicators.

Purpose and tasks. The purpose is clearly and precisely stated, as are the resulting objectives. It corresponds to the research topic and all parts of the dissertation are logically connected and subordinated to it. The purpose and tasks are focused on establishing significant differences between benign atypical melanocytic lesions and malignant melanoma which justify a correct diagnosis and predictability in their biological evolution.

Materials and methods: The applied routine and immunohistochemical methods derive from the set tasks and ensure the fulfillment of the aim and objectives of the dissertation. The appropriate statistical analysis creates conditions for additional and precise processing of the results and contributes to the reliability of the conclusions drawn. The routine methods are described following the strict algorithm of morphological diagnosis: precise localization, profile symmetry, cellular composition, presence of atypical cells and its distribution, Breslow thickness assessment, Clark invasion, mitotic activity, ulceration, stromal reaction, regression and satellite nodules.

The choice of IHC methods stems from some unresolved and controversial issues in the literature and is directly related to the aim and objectives. The used biomarkers are related both to evidence defining their cytogenesis, such as S-100 protein and HMB45, and to some nonresolution and discutabile diagnostic, differential diagnostic and prognostic problems of melanocytic skin lesions, such as Cyclin D1, p53 and others.

Studies of Cyclin D1 in pigmented lesions are relatively few, although it is a key factor in the growth cycle. There is also lack of consistency in publications, which has been attributed to methodological errors. Including of Cyclin D1 in present study is motivated from the observation for increased expression of connected to him different signal pathways during the transition of dysplastic nevi to MM. So, the activation of this gene is a warning sign.

Studies of **BCL-2** anti-apoptotic factor in the initiation phase of MM have not been conducted in contrast to the metastatic phase where there is evidence of its predictive function. Probably, this disproportion of the studies and not enough information for its role as diagnostic criteria has been tract an attention of the dissertator. Important fact is that the expression of BCL-2 in melanomatous metastases are associated with lower proliferative index and longer survival influenced from therapy with BCL-2 inhibitors. The mechanism of acting of BCL-family is not clear. It is proposed that melanocytes in phase of metastasis receive a stress and increase the expression of the pro-survival BCL-2 proteins. Probably the drugs inhibit anti-apoptotic proteins through a combination withBH3-only and engage BAK/BAN activators for good clinical results.

The role of **P53** gene in the process of malignant transformation of the dysplastic nevi is also unclear. By analogy with other malignant tumors in which its role in the early process of malignancy has been established, its involvement in the occurrence of MM and in aiding its diagnosis might be expected.

The examination of **S-100 protein** and melanosome class **HMB45** are involved in this study not only because of their close connection with cytogenesis but with a view to make more exact characteristic feature of their expression in types of melanocytic lesions.

Ki-67 is universal helper of a determination of proliferative index in neoplasms, but its values not exactly validated in melanocytic formations.

Dr. Petkova developped an original model to group IHC results in melanomas into 11 points according to the expression of applied biomarkers by *intensity*, defined in three grades and *distribution*, also in three subgroups: in the upper layer; mottled or diffuse covering the entire thickness of the lesion. Two of the groups represent no reaction and heterogeneous reaction. This method allows, after statistical processing, to accurately determine the diagnosis and DD parameters of the lesions. For all tested markers this model detailed immunoreactivity around one of the most common patterns in the group, called typical or characteristic. The other model is defined as atypical because of overlapping of the expression between types of lesion. As the assessment cannot be based on the expression of only one antibody in some cases, a combination of several markers has been used.

The material included 91 pigmented skin neoplasms, of which 57 were benign pigmented nevi, 10 atypical nevi and 24 malignant melanomas. The number of lesions studied is representative, and sufficient for the purpose of statistical analysis. The studies covered only one group of pigmented lesions located only in the skin, and MM was only in the promotion phase. It is a pure clinical model, allowing an accurate and specific interpretation of the set goal to refine the distinguishing features between melanocytic lesions without the influence of the phase of tumor progression with lymphatic and hematogenous dissemination, which is associated with other molecular-pathological changes.

Results: This is the fundamental part of the dissertation, describing the author's personal research, and its implementation is in accordance with the stated aim and methodology. The results are presented analytically as melanocytic lesions are evaluated by demographic and

topographic indices, morphological parameters and IHC results. Sex distribution showed a predominance of moles in the female sex and an equal distribution of atypical nevi and melanomas in both sexes. Statistically significant difference was found in the incidence of MM over 40 years, proving a positive correlation. In atypical nevi there is scatter of information, cases are few in number in different groups and not statistically significant. The topographic distribution shows that benign lesions are most frequent on the head and neck, followed by the back, chest and abdomen, upper limbs and least frequent on the lower limbs. MM is predominat in the lower extremities, followed by the back and chest. There was a difference in the localizations between benign lesions and MM, which strongly predominated in the lower extremities. Established differences of predilection localization were not connected with special clinical and prognostic significance. However, they are obligatory stage in the study of the tumors, need for their common clinical and morphological characteristic, turn to the diagnosis sometimes, as well by reason of the circumstance that some non-melanocytic tumors have localization closely correlated with prognosis.

The histologic characterization is highly informative, well written, with precise morphologic language, and contains all elements required for the study of target lesions. The types of benign nevi are presented according to their location in the skin, intradermal, compound and borderline, respectively, and according to the cell typing of groups A, B and C are reviewed. The histomorphology of rarely seen nevi such as Spitz nevus, Reed nevus, 2 congenital nevi, 1 blue nevus and 1 compound nevus are described. Atypical nevi are described according to their topographic appearance and expression of atypia. Dr. Petkova always compared the type of lesion and the expression of atypia with an effort to give them a quantitative score. Half of the atypical nevi were borderline, and the remaining were compound but with more pronounced atypia in the borderline component. This finding directs attention to the study of borderline nevi. The microscopic evaluation focuses on the uneven pigmentation of the cells (sometimes visible in the macroscopic picture) - architectural asymmetry with the formation of arches of horizontally oriented melanocytic nests, focal atypia including nucleolar, and extension of the border portion beyond the limits of the dermal component. All these details have practical meaning for setting the right diagnosis. Malignant melanomas were characterized according to their localization in the skin and already described special histologic feature. In the MM group there were predominantly nodular types (18 nodular), 3 are superficially advanced, 1 malignant blue nevus, 1 Spitz malignant melanoma and 1 childhood primary nodular dermal melanoma. The description of single special melanomas including pediatric one is contributory given the rarity among MM.

In addition to the critical evaluation of individual histologic features, Dr. Petkova emphasized the role of the combination of features in the final diagnosis of the lesion. This approach is also evident in the ICH examination and demonstrates her consistency and style in the diagnostic process.

The ICH studies were evaluated according to the described **eleven model grouping** of the findings, suitable for a comparative analysis and statistical data processing. Using this model Dr. Petkova defines the expression of applied biomarkers by intensity and distribution. The most common patterns in the group is called typical or characteristic; the following model

was determined as atypical because overlapping of the expression between types of lesion. In some case assessment cannot be based on the expression of one antibody, but on a combination of several. The model allows, after static processing, to identify convincing differences in favor of diagnosis and differential diagnosis of lesions. Reporting of the results is done with meticulous accuracy and precision. The results unify the parts of the study and provide a basis for discussion and conclusions that are logically linked. The results are illustrated with 41 figures and 30 tables with excellent performance.

Cyclin D1 revealed statistically significant differences in pigmented formations. The most common expression pattern in benign nevi was spotted positivity of moderate intensity and only in one case the reaction was diffuse but with low intensity. The highest immunopositivity was found in MM- diffuse or mottled throughout the all thickness of the tumor. There is no characteristic model in dysplastic nevi. Because of the significantly higher expression of Cyclin D1 in MM compared to that of benign nevi dr. Petkova accepte that Cyclin D1 is a suitable marker for revealing a malignant potential in atypical nevi and its strong positivity correlates with malignancy.

The results of **p53** expression reveal the statistically significant differences between the three groups - absence or very rarely immunopositivity in benign and small part of atypical nevi in contrast to MM, which typically express p53. These facts are interpreted as „a possibility this marker to be employed as a predictor of malignancy (sign of malignancy) in pigmented tumors ".

ICH and statistical analysis of **BCL-2** in the three groups reveal wide differences of frequency in three groups, dominated from high intensive diffuse reaction, observed in 100% in benign nevi, 66.66% in atypical nevi and 41. 67% in MM (10-th model of expression). Simultaneously it is found wide zone of overlapping the expression of this marker. These results lead to the conclusion, that BCL-2 does not correlate with malignancy and it is not an appropriate criterion for assessing their biological behave .

S-100 protein was expressed in all pigmented lesions, but the expression in benign nevi is diffuse and highly intensive in 100%, decrease in 83.33% in atypical nevi and in cases of MM the reaction was variable both in intensity and distribution throughout the thickness of the lesion.The difference in the intensity of expression in three groups, namely that it is consistently high in common nevi and moderate mainly in MM and some atypical nevi is characteristic feature useful for a diagnose.

The diversity of **HMB45** expression in the groups was striking. The lack of a gradient in MM and some atypical nevi is characteristic feature and should be considered as a predictor, (better sign) of malignancy. She found that absence of gradient in HMB45 expression in only a few proportion of atypical nevi requires a careful approach to determine their biological potential on the one marker and it should be based on a combined multifactorial analysis to avoid overdiagnosis of malignancy.

The study of proliferative activity by using of the **Ki-67** indicated a highest expression in MM, a lowest in benign nevi and an intermediate site in atypical nevi with statistically

significant differences. As isolated cases show moderate (6-10%), low (3-5%) or absence of Ki-67 positivity the dissertante considers that " the presence of MM with low proliferative activity does not permit using of this marker independently as a predictor of malignancy".

In present study along with high statistically significance for discrimination of different melanocytic lesions with aid of ICH methods there are the cases with overlapping results and blurring the boundaries between them. This motivated dr Pehkova to make statistical analysis for predictive (better diagnostic) assessment of combination of Cyclin D1, p53, HMB-45, markers which have been demonstrated the most essential differences in the expressions between groups, approved statistically. Specially about melanosome HMB45 she bases on statistically significant lacs of gradient of its expression in MM, reached to 100% sensitivity in contrast to pigmented nevi. Statistical analysis of markers' expression made with a few methods, show reliable differences between MM and dysplastic nevi and contributes for maximally accuracy of their diagnosis and demarcation.

Discussion: The discussion chapter is presented with a logical sequence and comparison of own results with the literature data. The demographic and localization parameters, routine histopathological data and the results of ICH studies are systematically and analytically interpreted in view of their relevance to the diagnosis and evaluation of the malignant potential of pigmented lesions. Consideration of clinical indicators are an important prerequisite to a correct diagnosis and discussion of their role demonstrates the aspiration of the dissertator to exhibit a comprehensive approach in making an integrative diagnosis. The authors' own results are supported by the literature data and point to the correct conclusion about their limited significance in the diagnostic process of cutaneous pigmented lesions and determination of prognosis. Attention has been paid to their morphological characteristics and to the parameters of high value for diagnosis according to modern requirements. Each of these parameters have been studied in depth for the three forms of melanocytic lesions and compared with published results for their prognostic role, as demonstrated by patient survival. It should be noted that the lack of health records of our patients, due to organizational problems in the health care system, does not allow Bulgarian researchers, in the case of Dr. Petkova, to conduct similar studies and draw conclusions about the prognosis of morphological indicators according to their own data.

The analysis of the authors' own results and the published data ends with a brief summary showing the role of the individual parameters for diagnosis. This leads to the conclusion that tumor thickness is an independent prognostic factor, related to survival, correlated with depth of invasion, mitotic activity and ulceration. The TIL score has significance as a positive prognostic factor - the results show concordance with previously published data on an existing correlation between stromal infiltration density and tumor thickness, with the latter decreasing with higher Breslow grade.

The need for further IHC analysis is justified due to the difficult and complex interpretation of malignancy and the predictability of atypical nevi and melanoma development based on routine histological methods alone. So, the introduction of an 11-model expression distribution scheme for intensity and topographic distribution was logical. In contrast to the

widely confirmed practice of high sensitivity of S -100 protein and HMB-45, used in DD, the found difference in their intensity and distribution with decreasing penetrance in MM is among the characteristic distinguishing features not so strongly accentuated in the literature. Concerning the role of HMB-45, the author emphasizes the great importance not only of the intensity but also of the gradient of its expression, which is highlighted only in moles, is absent in some atypical nevi and is a characteristic feature in MM, for which she finds confirmation in the literature. The antibody HMB- 45 shows at least focal deep reactivity in MM. Dr. Petkova shows a thorough interpretation of the expression of HMB-45 by intensity, nature of distribution (speckled or diffuse) or heterogeneous with varied intensity. Her ability to analyze small variations in immune reactivity and to infer them using statistical methods into rules to aid diagnosis should be emphasized. Based on these observations, the dissertation emphasizes and confirms an important fact in the diagnosis of MM, namely the use of HMB- 45 not only as a diagnostic marker determining the cytogenesis of MM, but also as an auxiliary marker for the detection of increased malignancy.

The role of **cyclin D1** as a carcinogenic factor in MM is discussed widely, but reports about the topography of its expression in different pigmented lesions were not reported frequently. Only some authors find expression localized in the upper parts of the nevi. The findings in this dissertation prove that moles also have focally distributed immunoreactivity throughout the lesion thickness with moderate intensity, not only in the upper part of the lesion and in nearly ¾ of them. Regarding the overexpression of the marker there is a consensus, which Dr. Petkova also observed - the expression covers the entire thickness of the MM; but she focuses her attention on the topography and intensity of the reaction and finds heterogeneity in intensity in part of the MM. In atypical nevi, there are also differences in the assessment, which is confirmed by the studies in the present work and conclusion is made that there is no characteristic pattern of intensity and distribution of the reaction product. Common conclusion from this research is that cyclin D1 overexpression is observed in atypical nevi and MM and correlates with the malignant phenotype of melanocytic lesions. These data coincide with recently documented activation of various related signaling pathways in the transition of dysplastic nevi to MM.

Comparative analysis of **p53** positivity in the three processes studies showed statistically confirmed results of its absence in the majority of nevi, limited expression in atypical nevi and highly dominant expression in MM. Detection of positive reaction in 1.75% of nevi restrain dissertator to confirm the role of p53 as an absolute criterion for determining malignant potential. Her statement finds confirmation in some literature data for higher value of expression than presented results in nevi - up to 15%.

The dissertation data on BCL-2 expression in the three studied groups showed positivity consistent with the immunophenotype of normal melanocytes confirming the general view of the published results. The author noted a decrease in intensity and partial positivity in atypical nevi, which were more prominent in nearly 60% of MM, also found in some reports. Substantiated is her opinion, that BCL-1 is not a suitable marker with diagnostic and prognostic value, confirmed also of the practice.

The Ki-67 assay is widely applicable in tumor pathology, but not always with validated percent for assessing tumor malignancy. Dr. Petkova's results showed a mean value for nevi of 0.9%, for dysplastic nevi of 2.75 , and for MM a mean proliferative activity of 14.96%. This is a statistically significant difference, close to the published results, but there are some details that deviate from the generally accepted ones. For nevi, 1-2% PI is usually quoted, and Dr. Petkova finds up to 10% in isolated cases, atypical ones reach up to 10%, bringing them closer to the MM range of 10 to 20%. The resulting deviations from the generally accepted as well the overlapping PI (Ki-67) in melanocytic lesions with different malignancy, limits according to the author, its significance as an absolute prognostic marker. In fact, Ki-67 is not used as an independent factor even in malignant tumors with a validated value, but is usually in combination with other indicators of malignancy. This is explainable given the irregular growth cycle in malignant tumors, influenced by many uncontrollable genetic impairments. Notwithstanding these considerations, the application of Ki-67 is an useful and common used marker to determine the malignancy of neoplasms in complex immunophenotypic study.

We should take in mind the fact that unlike highly specific markers associated with tumor cytogenesis, those characterizing the process of carcinogenesis and biological behavior exhibit different frequency and intensity of expression due to the pronounced heterogeneity of cellular composition, the complexity of molecular genetic relationships and genetic instability in malignant tumors. Complex genetic analysis is preferred in these cases – the same as in Dr. Petkova's choice.

Statistical analysis of expression of the combination of Cyclin D1, p53, and HMB-45, depending on the manifested most significant difference in the three studied pigmented lesions was made by several statistical methods. The combination of these markers confirm the presence of malignancy in MM and of malignant potential in dysplastic nevi. The demonstrated differences contribute to maximum accuracy of diagnosis and differentiation of malignant melanoma from dysplastic nevi. The significance of the multifactor analysis with introducing of 11 model scheme or noting the IHC intensity of the expression and distribution of the positive cells is the base of the many inferences and contributions in dissertation.

Inferences follow naturally and logically from the framework of the study, with the exception of the first one, which is very general and is postulated for the tumor nature and development of all types of tumors. Conclusion 4-th is similar and confirms the generally accepted importance of S-100 protein and HMB-45 as essential markers of diagnostic significance.

The second conclusion of particular importance. It concerns the importance of the precise morphological criteria with diagnostic value of the three pigmented lesions studied, expressed in melanoma in the determining principles of Breslow and Clark, lymphocyte infiltration, ulceration and mitotic activity.

The conclusion about the evaluation of the role of demographic indicators and localization, although less pronounced compared to morphological features, expresses the author's positive attitude towards the requirement for an integrative diagnosis, showing the role of other parameters of tumor growth besides morphology.

Of utmost importance are the confirmed peculiarities of HMB45 expression, concerning the lack of gradient in its expression distribution in malignant melanoma and its accumulation in benign nevi. Statistically significant differences in Cyclin D1 expression define it as a reliable prognostic and diagnostic marker for malignant melanoma.

A careful approach has been shown to the expression of p53 and Ki-67. The demonstration of p53 in a simple nevus is rarely observed in the literature, on this basis the thesis does not accept p53 as an absolute criterion of biological potential. This observation deserves pointed attention with practical utility - elevated p53 can also be found in nevi and should not mislead the pathologist into a diagnosis of malignancy. But, if there is p53 expression in lesion with a nevus phenotype, I would be recommended this fact should be noted and the patient actively followed.

Regarding the inference about the significance of PI /Ki-67/ I agree with the opinion that the range of expression varies widely and cannot serve as an independent prognostic criterion. Indeed, few human tumors have a validated PI or mitotic index, which are a decisive factor in determining malignancy. However, must be strike that in comparison with the other features of malignancy in melanocytic formations, the role of Ki-67 is not to be underestimated and coincides with the prevailing opinion in the literature of a low percentage (1-2%) in nevi, intermediate in atypical nevi and high in MM.

BCL2 testing in the target groups reasonably indicates that it is not an appropriate marker for predicting malignant potential. Few publications have demonstrated BCL-2 in common and atypical nevi but it is not included in the diagnostic results usually. This finding is a scientific fact confirming BCL-2 nonsignificant role in the diagnosis and prognosis of examined processes, in contrast to its application as a predictor of the therapy.

The conclusion about the importance of multifactorial analysis is justified by statistical results and is of a contributory nature. The last inference about the results of the introduction of the 11-module scheme for reporting the expression intensity and topographic distribution of positive cells deserves high praise due to the detail of the study and the immunoreactivity of the individual biomarker markers, which is the basis for most of the conclusions and contributions made in the thesis.

Contributions

The author's self-assessment of the contributions of the dissertation is correct, line with the actual justified results of the applied methodologies adequate to the topic and with the available literature on the problems addressed.

Original contribution is the study of the expression of essential markers for the diagnosis and determination of malignancy in melanocytic formations (Cyclin D1, p53 protein, BCL-2, S-100 protein, HMB45) using an 11-model scheme of assess the intensity and distribution of positive cells. The comparative analysis performed, supported by statistical processing, allows the consideration of the variability in the expression of individual markers to be used as a criterion for malignancy. The application of this model ensures that all feature of the

expression of each antibody are taken into account both in term of intensity, distribution and gradient.

Original is the proving of the significance of the proposed triple combination of markers - Cyclin D1, p53 protein, HMB45 as a diagnostic marker of malignancy in pigmented lesions.

Confirmatory contributions are as follows:

- The high value of S-100 protein and HMB-45 as markers of melanocytic origin was confirmed with their expression in all studied pigmented tumors of the 3 groups.
- The high intensity of S-100 protein was found in benign lesions, in the majority of atypical nevi and the peculiarities of HMB-45 expression were confirmed.
- BCL-2 is an inappropriate marker as a prognostic factor in pigmented lesions, as there are no statistically detectable differences in its expression in the different types.
- The variability in Ki-67 expression in the three groups was also found, which does not allow this marker to be accepted as an independent prognostic indicator. Its significance should be considered in the context of other malignancy criteria.
- Histological and immunohistochemical description of the rarely pigmented nevi and malignant melanomas is performed, which enriched this region of pathology.

Critical notes and recommendations. Prediction and predictive markers are sometimes used synonymously with prognosis or prognostic markers. The prediction is applicated here in the wide meaning of the word as foresight, but its meaning in the oncologic terminology is connected in all cases with influence after therapy. The prognosis and the prediction are terms with different semantic meanings - prognosis is the foreseeing of progression without drug administration, prediction is connected with the effect of administered drug on tumor evolution, i.e. it is showing what will be impact of some drugs on tumor evolution. In this sense, after my opinion, the use of the phrase "predictor for malignancy" as characteristic for some markers (Cyclin D1, p53) would be change with more exact "sign (mark) of malignancy". My recommendation to the author to keep to accepted terminology. These notes and recommendations support to the exactness of the text and do not change in essence the established and proved results of the research.

Conclusion

Dr. Petkova's dissertation is a thorough and contemporary study, the importance of which is determined by the medico-social significance of MM (high incidence and still high mortality) and the need to define its precise morphological diagnostic and prognostic criteria. The study was done according to the requirements for a dissertation with a clearly stated aim and objectives, selection of appropriate classical and molecular pathological methods, analytical analysis, including statistical processing of the results, compared with the published results in the discussion section. In their entirety, the facts presented lead to correct conclusions of

scientific and practical value. The assessment of the contributions of the present work are very much in line with the author's self-assessment and follow directly from the results described.

By its significance, correct structuring, justified conclusions and undoubted contributions to the diagnosis, DD and prognosis of the melanocytic lesions with emphasis on malignant melanoma, the presented dissertation deserves high evaluation and justification to award its author Dr. Petkova the scientific degree of Doctor of Medical Sciences. For the above reasons, I recommend the members of the esteemed scientific jury to give a positive vote for Dr. Liliana Nikolova Petkova to take „Doctor's Degree" of Medicine.

Ass. prof. Margarita Kamenova