REVIEW

by Assoc. Prof. EKATERINA BOYANOVA SOFTOVA-ZLATAROVA, MD, PhD

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SUBJECT: Defense of the dissertation of **Martina Georgieva Stoeva**, PhD student in full-time form in the doctoral program "Pathology and Cytopathology" at the Department of General and Clinical Pathology, Forensic Medicine and Deontology at the Medical University of Varna, for awarding the educational and doctoral degree in higher education 7. Health and Sports, Professional Field 7.1. Medicine, Scientific specialty "Pathology and Cytopathology", code 03.01.03

TOPIC: "IMMUNOHISTOCHEMICAL EXPRESSION OF THE NECROPTOSIS MARKER RIPK3 IN BREAST CANCER"

Scientific supervisor: Prof. Maria Angelova Tzaneva, MD, PhD

By decision of a meeting of the Faculty Council at the Faculty of Medicine at MU-Varna under Protocol № 58/14.01.2022 and by order № P-109-41/21.01.2022 of the Rector of MU-Varna, I was elected an external member of the Scientific Jury, and based on Protocol № 1/27.01.2022, I was appointed to prepare a review /in Bulgarian and English/ on the procedure for obtaining an educational and scientific degree "Doctor" with candidate Martina Georgieva Stoeva, MD at the Medical University - Varna.

As a doctoral student in full-time education in the doctoral program "Pathology and Cytopathology", professional field 7.1. Medicine, Dr. Martina Stoeva was enrolled by order № P-109-39/01.02.2019 by the Rector of MU-Varna, and with a report for deduction, ref. № 102-72 / 10.01.2022 by the Head of the Department Prof. Dr. Maria Tzaneva, and by Order of the Rector № R-109-41/21.01.2022, Dr. Stoeva was expelled with the right to defense.

The doctoral student has successfully passed the exams for the required doctoral minimum in a foreign language. For the competition, Dr. Stoeva presented on paper and electronic media a set of materials, including all necessary documents in accordance with the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations for the Development of the Academic Staff in MU-Varna.

Brief biographical data and professional development of the doctoral student

Dr. Martina Georgieva Stoeva was born on February 22, 1988, in Blagoevgrad. After graduating from Maths High School "Dr. Peter Beron" - Varna in 2006, she was accepted as a student at MU-Varna, where in 2012 she obtained a master's degree in Medicine, with professional qualification "doctor"/diploma for higher education series MUV, UI 001461, reg. 001897/1012/. From 01.05.2013 to 01.05.2017 Dr. Stoeva is enrolled as a resident in the Clinic of General and Clinical Pathology, and from 13.02.2015 to 01.10.2017 she is a part-time assistant in the Department of General and Clinical Pathology, Forensic medicine and Deontology" at MU - Varna. In October 2017, after a competition, Dr. Stoeva was appointed an assistant professor at the same department at MU-Varna. From 03.05.2017 without interruption, until now Dr. Stoeva works as a physician at the Clinic of Pathology at the University Hospital "St. Marina" - Varna. After

successfully passing the state exam in December 2017, she acquired a specialty in "General and Clinical Pathology" /certificate № 021479/20.02.2018 Series MUV № 3942/.

Dr. Stoeva has over 5 years of experience as a lecturer in "General and Clinical Pathology", actively participates in the educational activities of the Department by conducting practical classes in Bulgarian and English, lectures and exams for students of medicine, dental medicine, pharmacy and medical laboratory technicians. Dr. Stoeva is actively involved in the organization of courses for specialists in General and Clinical Pathology and FED of medical students. As a physician at the Clinic of General and Clinical Pathology at the University Hospital "St. Marina" - Varna Dr. Stoeva fully participates in the diagnostic biopsy and autopsy activities, applying in her daily work not only routine, but also many of the highly specialized, mostly immunohistochemical methods of research.

Dr. Stoeva has participated in 13 publications, incl. in 3 journals with IF. With scientific reports, posters and presentations she has participated in 5 scientific conferences and congresses with international participation in our country. She was involved in the development of 3 research projects under the Science Fund at MU-Varna. Dr. Stoeva is fluent in written and spoken English /C1/. She has excellent computer literacy and works well with Microsoft Office tools /Word, Excel and Power Point/. Her professional and scientific interests are diverse, with preferences for oncopathology and molecular pathology. Dr. Stoeva is a member of BMA and Bulgarian Society of Pathology.

Relevance and significance of the topic

Dr. Stoeva's dissertation, dedicated to breast cancer, is the result of in-depth and systematic morphological studies and research on a topical, increasingly social problem, creating therapeutic difficulties requiring strict clinical and morphological recognition in order to give diagnosis and administer treatment. It is known that breast cancer (BC) is the most common malignant tumor worldwide (11.7% of the total number of new cases). According to Globocan, in 2020, approximately 2.3 million new cases were diagnosed and 685,000 people lost their lives. As the leading cause of death from malignant tumors in women and men in the world, BC ranks 5th. Estimates of the International Association for the Analysis of Malignant Diseases show that by 2040 the incidence of BC will exceed 3 million new cases per year, and deaths will be over 1 million. Despite the success in elucidating some manifestations of BC, there are still some insufficiently studied issues and a number of difficulties caused by the high heterogeneity and combination of morphological findings in this tumor. Here, too, as a major challenge for the constellation of specialists in the field of medical oncology, there is a worldwide search for new mechanisms and molecules that, having the ability to modulate the interactions between the tumor and the elements of the tumor microenvironment, can to preserve or cause the death of tumor cells. Currently, the main goal of the various therapeutic methods used is the destruction of tumor cells by inducing apoptosis. However, it is known that sometimes in the course of treatment there are changes in signaling pathways that lead to resistance to therapy and the development of adverse side effects. In these cases, induction of non-apoptotic cell death, which includes necroptosis, could provide alternative pathways for the elimination of resistant tumor cells. Thus, the possible drug or other stimulation of necroptosis, causing or manipulating the necroptotic pathway, emerges as a new approach to overcoming the resistance of tumor cells to apoptosis. The marker for necroptosis RIPK3, which is located mainly in the cytoplasm of normal and tumor cells, has the ability to move in the nucleus, conducting signals for apoptosis, necroptosis, and activation of transcription factors. There are only isolated reports in the literature on the association of RIPK3 expression with clinical and morphological features, and its role in the development, prognosis and treatment of breast cancer. Research in this direction, enabling a new therapeutic strategy in the treatment of cancer, can be extremely valuable with scientific and practical application in the field of oncopathology. It is in the context of the above that Dr. Stoeva has focused her interests on scientific research related to the study of the expression of the marker for necroptosis RIPK3, in order to assess its role and importance for biological

behavior and development of breast cancer. The sufficient amount of material used, the skillfully selected morphological characteristics and the application of adequate IHC methods have enabled Dr. Stoeva to determine criteria and draw conclusions regarding the biological behavior of cancer and predict the likelihood of progression in its development. .

Structure and analysis of the dissertation. Dr. Stoeva's dissertation is properly structured, written with a very good, sound scientific style and terminology. The paper covers 133 standard pages and is illustrated with 42 figures and 50 tables. The distribution by sections is as follows: title page, content and abbreviations used - 5 pages; introduction 2 pages; literature review 42 pages; purpose and tasks 1 page; material and methods 8 pages; results and discussion - 55 pages; conclusions 1 page, contributions 1 page; list of publications and participation in scientific forums - 1 page; bibliography 17 pages, including a total of 205 titles, of which 4 in Cyrillic and 201 in Latin. Of these, 61% have been published in the last 10 years. The dissertation is accompanied by 2 full-text publications published in our journals and related to the topic of the dissertation, and one participation in the IMAB 2021 session with a scientific paper, also related to the topic of the dissertation.

Evaluation of the literature review

The literature review is structured in sections related to: 1. Epidemiology of breast cancer; 2. Etiology and risk factors for the development of cancer; 3. Classification of breast cancer; 4. Staging of the carcinoma; 5. Prognostic and predictive factors; 6. Treatment of cancer; 7. Types of cell death, 8. Conclusion.

The review of the literature shows the very good awareness of Dr. Stoeva regarding the achievements reflected in world science concerning breast cancer. This allows her to properly assess issues related to classification problems, diagnosis, morphology, IHC and molecular genetic profile of breast cancer. The data presented in the review highlight the author's ability to analyze literary sources, while expressing her own opinion on the existing controversial issues in some points. Dr. Stoeva pays deserved attention to the epidemiology of BC, emphasizing the continuing trend of increasing both diagnosed new cases and the number of women who lost their lives in the battle with the disease. The review presents in detail the etiological and risk factors favoring the development of cancer, divided by the author into 4 groups related to: 1. Family burden and individual features; 2. Reproductive factors, including pregnancy, lactation, administration of postmenopausal hormones 3. Nutrition, obesity and physical activity and 4. Influence of environmental factors. Examining the classifications of BC, Dr. Stoeva draws attention to the molecular classification, which includes the 4 main subtypes: Luminal A, Luminal B, HER2 positive and basaloid subtype. The characteristics of each of the subtypes are described and presented in a table with detailed interpretation. Dr. Stoeva examines in detail the triple-negative breast cancers, which from a molecular point of view partially overlap with the basaloid subtype, but the two terms are not synonymous. The author points out that not all basaloid carcinomas have a triple-negative phenotype, and not all triple-negative tumors are molecularly basaloid. The dissertation points out that despite the efforts made, there is still no established or clinically tested diagnostic approach in the classification of these aggressive cancers.

Of greatest practical importance for routine practice is the new histopathological classification of the WHO, published in late 2019. Following the approach of previous classifications and following the sequence of transition from benign to invasive carcinomas, Dr. Stoeva skillfully and accurately presents, with appropriate justification for their inclusion, the new units in each of the separate categories of epithelial neoplasms of the breast and their histopathological representatives, while drawing a parallel between the categories in the classification of 2012 and that of 2019.

The review examines in detail the principles for the staging of BC, performed on the basis of the 8th revision of the AJCC from 2018, with the included two staging systems - anatomical and prognostic stage. The pathological staging of BC based on the pTNM classification used is described in detail. The

tables present the individual categories for assessment of T-stage, the categories for pH and pM1 stage, and Dr. Stoeva emphasizes the fact that there are no categories pMo and pMx. The standard clinico-pathological prognostic factors are reflected in detail: age of the patient, stage, level of differentiation, histological type, condition of the resection margins and lymphovascular involvement. Hormonal receptors and HER2 status are considered as standard prognostic / predictive markers, and the important role of HER2-expression for the approach of the therapy is indicated. Ki67 expression is cited as an additional marker with the highest diagnostic and predictive / prognostic value, with the dissertation noting a complete understanding of the choice of therapy for low or high Ki67 expression. Last but not least, Dr. Stoeva pays attention to the applied methods of treatment, including surgery, neoadjuvant and adjuvant therapy, endocrine therapy, chemotherapy, HER2-targeted therapy and radiation therapy. It is correctly noted that the treatment of BC requires a multidisciplinary approach, involving surgeons, oncologists, pathologists and radiotherapists.

The part of the review devoted to the forms of cell death deserves special attention. Dr. Stoeva presents a simplified classification of different types of cell death, divided into two groups depending on the initiating factor, namely: programmed cell death / apoptotic and non-apoptotic / and unprogrammed cell death, including various species, incl. necroptosis. It is a relatively new form stimulated by classical cell death receptors identical to those of the external apoptosis pathway and mediated by the RIPK1 and RIPK3 receptor-interacting protein kinase families. There is evidence that necroptosis is involved in the regulation of various physiological processes, as well as in disorders arising from the regulation of signaling pathways underlying some viral and bacterial infections, neurodegenerative and especially malignant diseases in humans. Necroptosis is a complex process, and by understanding the mechanisms of its development, it was concluded that there is a possibility that it can be inhibited by chemical compounds, which in turn would help in the development of anticancer drugs. Because apoptosis and necroptosis are triggered by different molecular mechanisms, activation of necroptotic processes can be used as an alternative pathway for the destruction of apoptosis-resistant cells. However, the question of how necroptosis is controlled by oncogenic signals in neoplastic cells is still unclear. There are observations that suggest that neoplastic cells with antinecroptotic mechanisms are selected in the course of tumor progression, which ensures their survival but also allows them to manipulate necroptosis in order to inhibit tumor growth. Results from recent studies have shown that necroptosis supports natural or therapeutically induced antitumor immunity.

The presented literature review is a proof of Dr. Stoeva's ability not only to present the information reflected in the scientific literature related to a particular problem area, but also to interpret, discuss and compare the results of various scientific studies. In the conclusion to the review the dissertation rightly emphasizes the urgent need for further in-depth research in order to further clarify the interactions between markers of necroptosis and some clinical and morphological parameters in the development, tumor progression and treatment of patients with breast cancer.

Purpose and tasks. The aim of the dissertation is "To study the expression of the necroptotic marker RIPK3 in relation to clinical and morphological parameters, receptor status, proliferative marker Ki67 and survival of patients without progression in breast cancer." It is precisely and clearly formulated, specific and adequate to the dissertation topic. The 6 tasks arising from the set goal are correctly constructed and feasible and represent a logical continuation of the literature review.

Materials and methods

The basis for the realization of the dissertation are the Department of General and Clinical Pathology, Forensic Medicine and Deontology at MU-Varna, the Clinic of General and Clinical Pathology and the Clinic of Medical Oncology - University Hospital "St. Marina" -Varna. The present study included biopsies of a total of 98 patients - 79 diagnosed with BC and a control group of 19 patients without carcinoma, of which 10 cases with non-proliferative and 9 cases with proliferative type of fibrocystic breast

disease (FCBD). The target group included 16 cases of lobular and 63 cases of ductal carcinoma. The diagnosis and stage of 71 of the cases are determined according to the 5th edition of the WHO for 2019 for breast tumors. The patients underwent mastectomy with regional lymph dissection. The diagnosis of the remaining 8 cases of BC was made on a TRU-CUT biopsy with sufficient tumor tissue, and the stage was determined by imaging. In 58 of the cases, subsequent hormone, radiation and / or chemotherapy was performed at the University Hospital "St. Marina", and Disease free survival was determined. Cytoplasmic and nuclear expression of RIPK3 was reported in all patients.

In the study Dr. Stoeva has selected and included a wide range of adequate methods for the purposes of the study, namely: **1. Routine histological examinations:** In each case with BC were taken for histological examination 4-5 materials from the tumor and skin and mamilla when available; the lymph nodes sent to each case were examined; the materials were fixed in 10% neutral buffered formalin and treated by the standard paraffin method. 5 micron thick sections were stained with HE to assess histological changes in the tumor and for the presence of metastases in the sent lymph nodes. In all tumors, the histological type, degree of differentiation, TNM stage of disease were determined; and immunohistochemical status was assessed according to the expression of ER, PR, HER2 and Ki67. **2. Specific IHC - research methods.** The expression levels of the markers ER, PR, HER2 and Ki67 were assessed on pre-made preparations after the diagnosis of each tumor to determine its molecular profile. All preparations were re-examined and classified according to the criteria percentage of cells expressing the marker (area of PS expression), intensity of expression (IS) for ER and PR and final score-TS = sum of PS + IS. HER2-expression is determined depending on the degree of involvement of the tumor cell membrane in values from 0-negative reaction to 3 + - strongly positive reaction, when the expression is on the whole cell membrane in> 10% of tumor cells. The Ki67 marker was evaluated on the basis of the percentage of tumor cells with nuclear expression, with a value of 14% as the cut off value between low and high expression. The type of reagents used is indicated in Table 7. Indirect immunoperoxidase method for IHC analysis using the mini KIT high Ph DAKO K8024 was used to perform the IHC studies required for the dissertation, which assessed the expression of the Anti-RIPK3 antibody manufactured by ABCAM RabMab technology. In the process of carrying out ICH-reactions all elements of the methodology are observed, incl. technological discipline, description of localization of expression, positive and negative controls, etc., as the criteria for positivity are precisely formulated when reporting the results. The steps in the preparation of the biopsy materials for the IHC study, the IHC protocol, the origin of the antibodies used, and the imaging system are described in detail. The IHC expression of RIPK3 was assessed by H-score on tissue samples, and the intensity of cytoplasmic expression for each tumor and non-tumor tissue cell and the percentage of positive cells for each intensity were determined quantitatively in the range from 0 to 300 according to the attached scheme. Finally, the H-score is calculated by the appropriate formula. The H-score was also used to estimate the nuclear expression of RIPK3, when calculating the result by the same formula. The obtained data were processed and analyzed with the help of SPSS ver.23 software, using an extremely rich set of modern statistical methods for data analysis. The results reported as statistically significant at $p < 0.05$ are summarized in tables and illustrated with appropriate graphs.

Results and discussion

The results of the research obtained by Dr. Stoeva are presented in parallel with the discussion, observing the sequence of tasks. The analysis of the results is carried out at a very good methodological level, and the discussion is comprehensive, as own data are skillfully compared with data from the available literature. This is the first scientific study of its kind dedicated to breast cancer, with a complex clinical-morphological and IHC-characteristics of the tumor, using a large volume of indicators and statistical methods.

The biopsy materials included in the study from 79 patients diagnosed with breast cancer operated on at the St. Marina University Hospital in Varna for a period of 8 years / 2010-2018 / were divided into two groups - <65 years / 52 cases / and > 65 years / 27 cases /. The results show that the average age of women is 59.38 years, with a minimum age of 27 years, and max - 82 years. Histological type of tumor in 69 patients was defined as ductal carcinoma NOS, and in 16 patients - as lobular carcinoma. Of the 79 cases of carcinoma, 42 cases were in stage T2, in stage T1 - 33 patients, in stage T4 - three cases and one - in stage T3. In line with the literature, the highest number (33) of patients with moderate cell differentiation was found, followed by low-grade carcinoma in 29 cases and high-grade carcinoma in 17 cases. According to their prevalence, the results obtained in the study of Dr. Stoeva do not differ from those reported in the literature: most patients - 31 have a tumor located in the breast, the second group of 30 patients has - metastasis in regional lymph nodes, and the third group included 16 patients with distant metastases.

Analyzing the results of the values obtained by monitoring the receptor status and proliferative index Ki67 in tumor tissue in breast cancer, Dr. Stoeva found that the largest number of ER - / + / tumors - 32 cases, 30 of which are and PR - / + / positive; HER2 - / + / tumors are 26 and triple negative - 21 cases. In the distribution of patients according to the nuclear expression of Ki67, in 26 of them the marker was positive in <14%, and in 53 of the cases Ki67 was expressed in > 14% of the tumor nuclei. Among the 79 breast cancer patients studied, 58 had survival data. All patients underwent adjuvant hormone, radiation and / or chemotherapy. The observed mean progression-free survival in the presented sample of 58 patients showed that it was within 113.8 months.

The most important part of the dissertation is the IHC-study of RIPK3, a marker for necroptosis, and in all 79 patients its cytoplasmic and nuclear expression was monitored. The mean cytoplasmic expression determined by H-score was 119.6 with a minimum of 5 and a maximum of 230; the mean value of nuclear expression is 189.4- minimum 5, maximum-285. There were no significant differences from the mean values of cytoplasmic expression compared to non-mammary (in this case colorectal) carcinoma, while in nuclear expression the values are significantly lower. Dr. Stoeva notes that there are differences and to some extent contradictions in the data from the literature on this issue. When monitoring the normal distribution of nuclear and cytoplasmic expression, she found that the values obtained showed a significant difference in the distribution of cases, which means that the intensity of the RIPK3 response in the cytoplasm and nucleus of tumor cells in individual cases does not follow the normal distribution. Cytoplasmic and nuclear expression was found in the 19 controls in both types of FCBD, with no significant difference in cytoplasmic expression in the groups. As for nuclear expression, in FCBD it is lower in breast tissue compared to nuclear expression in non-tumor tissue of colorectal cancer. The obtained results give the dissertation the reason to conclude that it is very likely that nuclear and cytoplasmic expression in FCBD and non-tumor tissue from other organs are tissue-related.

Comparative analysis between the mean values of cytoplasmic and nuclear expression of RIPK3 in tumor and control tissue showed that there was a statistically significant difference in both types of expression, with the H-score showing higher cytoplasmic expression of RIPK3 in PCB tissues compared to that of tumor tissue. However, when monitoring nuclear expression, the data are opposite - in tumor tissue the expression is higher than in FCBD. In connection with the noted divergence of the results obtained in the data from various literature sources, Dr. Stoeva suggests that it is possible that in the course of progression the tumors have acquired mechanisms by which they can avoid necroptosis through reduced expression of key components of its signaling pathway.

The comparative analysis of cytoplasmic expression of RIPK3 in tumor tissue from breast cancer depending on the clinical and morphological characteristics of the tumor does not show dependence on the age of patients, T-stage, extent of tumor spread (metastases), HER2-status of the tumor, and is not associated with patient survival.

The comparative analysis of cytoplasmic expression of RIPK3 revealed a certain tendency for a relationship between the expression levels and the histological type of the tumor, but without a statistically significant difference. However, the additional Mann-Whitney test showed a statistically significant difference in the cytoplasmic expression of RIPK3, with 16 lobular carcinomas having value of 147.52, i.e. significantly higher than in 63 patients with ductal carcinoma in which the value is 112.6.

The comparative analysis of cytoplasmic expression depending on the level of differentiation is clear that highly differentiated tumors have higher average values of expression compared to tumors with a lower degree of differentiation (respectively 146.2 versus 98.3). The results obtained by Dr. Stoeva are in line with those of authors from the literature, uniting around the opinion that low cytoplasmic expression of RIPK3 occurs in tumors with low degree of differentiation, associating the expression with nuclear atypia.

Comparative analysis of cytoplasmic expression of RIPK3 by ER-expression shows that there is a weak, moderately positive, and a weak significant relationship between them. However, with regard to the cytoplasmic expression of the ER marker and IS, it can be seen that increased expression of RIPK3 in the nucleus increases cytoplasmic expression. The analysis between the indicators of cytoplasmic expression of RIPK3 and TS of ER shows the correspondence between the higher TS of ER in tumor cells and more intensive cytoplasmic expression of RIPK3.

In a comparative analysis of the cytoplasmic expression of RIPK3 depending on the area of PR expression, a strong / + / dependence was found, with significant difference between the indicators. Similar results were obtained when comparing the IS of PR and cytoplasmic expression-RIPK3, again with a significant relationship between the two indicators. Increased PR expression in tumor nuclei is accompanied by more intense cytoplasmic expression of RIPK3. There is also a very positive relationship between the cytoplasmic expression of RIPK3 and the Total score of PR.

The comparative analysis of cytoplasmic expression of RIPK3 in triple-negative carcinomas compared to all other carcinomas shows that the mean cytoplasmic expression of RIPK3 in all 21 cases of triple-negative BC is 80.5, and is significantly lower than the values of the other 58 patients whose tumors were ER + or HER2 + with 133.8 values, with a statistically significant difference in RIPK3 values between the two groups of tumors. The prevailing view is that in triple / - / carcinomas, low cytoplasmic expression of RIPK3 is significantly more common than high expression.

Statistically significant dependence was found in the comparative analysis of cytoplasmic expression of RIPK3 depending on the expression of the Ki67 marker - at low Ki67 expression is high cytoplasmic expression of RIPK3.

The comparative analysis of nuclear expression of RIPK3 according to clinical and morphological characteristics, molecular profile, Ki67 and patient survival did not show dependence on patient age, histological type of BC, T-stage and degree of tumor differentiation. There is no significant relationship between nuclear expression of RIPK3 and the area, intensity and overall score of ER and PR, HER2 status, Ki67 marker and triple-negative tumors relative to all other cancers. However, the analysis of nuclear expression relative to stage N and M-shows that there is a significant relationship between it and the presence of metastases in regional lymph nodes. Noting that there is no data in the literature on the relationship between nuclear expression of RIPK3 and the prevalence of cancer, Dr. Stoeva suggests that high nuclear expression may be perceived as a way to avoid tumor cells from necroptosis by retaining RIPK3 in the cell nucleus. Despite the still unclear mechanisms involved in this process, based on her own results and literature data, Dr. Stoeva concluded that low cytoplasmic and high nuclear expression increase the risk of regional lymph node metastases.

A first-time **comparative analysis of disease-free survival versus nuclear expression of RIPK3** found that in 31 cases, nuclear expression of RIPK3 was low and in 27 cases high, with a median survival of 126.0 months at low nuclear expression, and 99.9 months in cases of high expression. The difference in progression-free survival in patients with low and high nuclear expression was statistically significant. At high nuclear expression of RIPK3 in tumor cells, survival is lower than in patients with low expression levels. Based on literature data on the ability of RIPK3 to pass from the nucleus into the cytoplasm of cells and vice versa, Dr. Stoeva suggests that the option is to avoid the formation of necrosomes in the cytoplasm by retaining the necroptotic protein in the nuclei of tumor cells and to prevent cell death, which would lead to long-term survival of tumor cells.

Conclusions: From the conducted research 12 conclusions have been formed, which correspond to the set goals and objectives, are well formulated and are a logical in relation to the obtained results.

Contributions: Dr. Stoeva has formulated 5 contributions, divided into two groups, respectively two scientific contributions of original nature, and 3 scientific contributions of practical - applied nature:

The thesis summary is prepared in accordance with the accepted scientific requirements. It is printed on 76 pages, including all parts of the dissertation, tables and figures. Its content gives a sufficiently complete picture of the overall dissertation, reflecting the results achieved, conclusions and contributions. The publishing activity of Dr. Stoeva - according to resp. number of publications, content and value meet the requirements for presenting research results.

Conclusion:

Dr. Stoeva's dissertation is a complete, very well structured morphological study, with original scientific results, which has a definite contribution to solving still insufficiently studied clinical and morphological problems and possibilities for determining some predictive and prognostic markers related to diagnosis and treatment of patients with breast cancer. The work in which the dissertation stands out with a very good literary awareness and style in the interpretation of the results is entirely her personal work. The value of the paper is increased by the fact that for the first time in our country an analysis of disease-free survival in breast cancer patients was performed against the nuclear expression of the necroptosis marker RIPK3, and a comprehensive study was performed to determine its prognostic value. The results of the comparative analysis between cytoplasmic and nuclear expression of RIPK3 depending on the clinical and morphological characteristics, the molecular profile and the survival of the patients were statistically processed and presented the data from the obtained results. The purpose of the development and the resulting tasks have been achieved. The results are illustrated with tables, graphs and color figures of exceptionally good quality. The conclusions are clearly formulated, and the contributions have scientific and practical value and represent a basis for future research.

The obtained results and the contributions in the dissertation work of Dr. Stoeva meet all the requirements of the Law for the Development of Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations of MU-Varna. The dissertation shows that Dr. Stoeva not only has the necessary theoretical knowledge and skills in the scientific specialty "Pathological Anatomy", but also demonstrates qualities for independent research.

Given the above, I give my positive assessment of the proposed dissertation, and I will vote "FOR" /positive/ the awarding of Martina Georgieva Stoeva the degree of Doctor of Philosophy in the scientific specialty "Pathology and Cytopathology."

21.02.2022
Varna

Reviewer: .....
/Assoc. Prof. Ekaterina Boyanova Softova-Zlatarova, MD, PhD/