OPINION

ON THE COMPETITION FOR THE DOCTORAL DEGREE

AT MU-VARNA, DEPARTMENT OF GENERAL AND CLINICAL PATHOLOGY, FORENSIC MEDICINE AND DEONTOLOGY, FACULTY OF MEDICINE

By Assoc. Prof. Silvia Nikolaeva Genova, MD, PhD
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of a dissertation for the acquisition of an educational and scientific degree **"Doctor" protocol No. 46/29.09.2025 г.**

Professional field: Medicine, 7.1

PhD program: "Pathological Anatomy and Cytopathology," code 03.01.03

Author: **Dr. Plamen Petrov Vassilev**

Form of PhD program: regular

Department: "General and Clinical Pathology," MU-Plovdiv

Topic: **APOPTOSIS AND NECROPTOSIS IN BASAL CELL AND SPINOCELLULAR SKIN CANCER**

Research supervisor: **Prof. Maria Angelova Tsaneva, MD, PhD**

1. General presentation of the procedure and the doctoral student

The set of materials presented in paper and electronic form complies with Art. 70 (1) of the Procedure for Acquiring a Doctorate Degree at MU-Varna; the Regulations for the Development of Academic Staff at Medical University of Varna and includes all necessary documents.

Dr. Plamen Petrov Vassilev was born on October 13, 1974, in Burgas. He obtained a master's degree in medicine in 2000 from the Medical University of Sofia. He specialized in General and Clinical Pathology from 2013 to 2017 at MU "Prof. Dr. Paraskev Stoyanov" – Varna, St. Marina Universuty Hospital – Varna.

Since *November 2021 to October 2025*, he is enrolled in *the Doctoral Program in Pathological Anatomy and Cytopathology* at the Department of General and Clinical Pathology, Forensic Medicine and Deontology at Medical University "Prof. Dr. Paraskev Stoyanov"- Varna.

Dr. Vassilev obtained a specialty in General and Clinical Pathology in 2019, certificate of recognized specialty № 4091, Series МУВ № 022569, Medical University "Prof. Dr. P. Stoyanov"- Varna.

Dr. Vassilev's total professional experience in the department is 4 years.

Academic Career Development: Only training courses are presented.
Obtained a master's degree in Health Management in 2015. Reg. No. 2015009.
Speaks English at a good level.

2. Assessment of the doctoral student's personal contribution to the dissertation

The topic of the dissertation is relevant and does not repeat other studies in Bulgaria related to apoptosis and necroptosis in basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin.

The dissertation contains 141 standard pages and is illustrated with 66 tables and 35 figures. The bibliography includes 335 references, of which 6 are in Cyrillic and 329 are in Latin.

Skin carcinomas originating in the epidermis are of two main histological types: basal cell carcinoma (BCC) and spinocellular or squamous cell carcinoma (SCC), which together account for 99% of malignant epithelial tumors. Of these, BCC accounts for 2/3 of all neoplastic skin diseases.

The introduction presents the problems associated with melanoma and non-melanoma skin carcinomas, etiology, pathogenesis, and the main therapeutic methods, skillfully directing attention to unresolved scientific, diagnostic, and prognostic issues on the subject.

Dr. Vassilev demonstrates a thorough knowledge of the state of the problem and makes creative use of the literature, providing a 54-page literature review.

The doctoral student presents in detail the epidemiology of BCC and SCC, examining their prevalence and incidence in different countries, distribution by gender, age, location, and risk factors such as UV radiation, photosensitizing drugs, arsenic, and immunodeficiency conditions. The occurrence of BCC in areas less exposed to sunlight indicates the presence of genetic and molecular factors involved in the etiopathogenesis. The doctoral student examines the signaling pathways of Sonic Hedgehog, Hippo-YAP, heredity, rare genetic syndromes associated with BCC and Beta human papillomavirus, CDKN2A gene, NOTCH1 and NOTCH2 intercellular signaling pathways, p53, TP63, and other modified genes in SCC. The detailed study of molecular mechanisms as a factor in the development of skin carcinomas is impressive. The review is illustrated with 10 adapted figures representing the signaling pathways.

Morphological characteristics, staging, prognostic and predictive histological parameters in BCC and SCC are discussed in detail on 23 pages.

In the review, Dr. Vassilev examines the various forms of cell death with morphological signs of necrosis, which is regulated in a programmed manner through specific signaling pathways. He explains in detail the role of Caspase-3 in the tumor process and notes that Caspase-3 is one of the most important molecules in the apoptotic cascade, but the relationship between tissue expression and its prognostic significance in various neoplasms is quite controversial and unclear.

The literature review includes the epidemiology and social significance of BCC and SCC carcinomas, the forms of programmed necrotic cell death, necroptosis, which resembles apoptosis in mechanism and has morphological similarities to necrosis, and signaling pathways mediated mainly by Receptor Interacting Serine/Threonine Kinase 1 (RIPK1), Receptor Interacting Serine/Threonine Kinase 3 (RIPK3), and Mixed Lineage Kinase Domain Like Pseudokinase (MLKL). The passage of necrotic signaling components through the nucleus is a mechanism for regulating the formation of cytosolic necrosome and a form of regulation of necrotic cell death. Inhibition of cell death is a hallmark of malignant tumors. Necroptosis in various types of malignant diseases of breast, kidney, prostate, is discussed, showing an impressive continuity and development of studies in the Department of Pathological Anatomy at MU-Varna.

In conclusion, emphasis is placed on therapeutic behavior, which includes surgical intervention, targeted radiation, and chemotherapy, leading induction of apoptosis - a natural process of programmed cell death that eliminates tumor cells. The doctoral student emphasizes that if apoptosis cannot be induced due to congenital or acquired resistance, are necessary alternative pathways for cell death involving Caspase-3, RIPK3, and the mechanisms through which they induce apoptosis and necroptosis in malignant tumors. The studies will contribute to determining their predictive value and developing new therapeutic strategies.

GOAL AND OBJECTIVES:

The objective is clearly and precisely formulated, namely to study the immunohistochemical expression of the apoptosis marker Caspase-3 and the necroptosis marker Receptor-interacting kinase 3 (RIPK3) in patients with basal cell and squamous cell carcinomas of the skin and to determine their predictive value.

The first four tasks examine the clinical and morphological characteristics of patients with BCC and SCC and the immunohistochemical expression of Caspase-3, as well as the relationship with clinical and morphological indicators. Tasks from 5 to 7 examine the level of

immunohistochemical expression of RIPK3 in the tumor tissue of BCC and SCC and its relationship with clinical and morphological indicators.

A total of 91 patients were included in the study, 46 of whom had BCC of the skin and 45 had SCC of the skin. The material used in the study is sufficient to obtain statistically reliable results. All patients underwent radical surgical removal of the skin tumor, and the level of immunohistochemical expression of Caspase-3 and RIPK3 was determined.

The study was conducted at the Department of General and Clinical Pathology, Forensic Medicine and Deontology at the Medical University of Varna and the Diagnostic and Consultative Center "St. Marina" Varna. The research methods are modern, and their reporting, description, and commentary show that the dissertation author is skilled in their use.

The criteria for staging, degree of differentiation, and molecular classification of tumors are clearly and precisely defined. *TNM stage, tumor infiltrating lymphocytes, lymphovascular invasion (LVI), perineural invasion (PNI), and area of necrosis* are determined. The chosen research methodology allows the set goal to be achieved and an adequate response to the tasks addressed in the dissertation to be obtained. The statistical analysis of the data was performed using the specialized statistical analysis software package STATISTICA (StatSoft Inc, USA, STATISTICA Manual (Data analysis software system), Version 13.0, 2022). The graphs were constructed in Microsoft Excel for Windows. The statistical processing of the materials is very good, which gives credibility to the obtained results. The Results and Discussion chapter is presented on 40 standard pages. The results obtained from the studies are well explained, correctly described, logically arranged, and accompanied by well-structured tables, figures, and photographs. The quality of the photographic material is good.

The task set in the dissertation is to determine the cytoplasmic and nuclear expression of Caspase-3 and RIPK3 in the tumor tissue of BCC and SCC. A comparative analysis of the cytoplasmic expression of Caspase-3 and RIPK3 is made depending on the age of the patients, the histological type of the tumor, the T-stage of the tumors, localization, gender, tumor and non-tumor tissues, the area of necrosis, and TILs in BCC and SCC.

The correct choice of statistical methods used contributes to the accuracy of the conclusions drawn. Each result is accompanied by an explanation and statistical correlations.

The discussion of the results is structured according to the formulated tasks. A parallel is drawn between the data in the literature, global experience, and the results achieved. The discussion is of a very good standard, with clear and precise scientific and statistical language and style.

The results lead to 16 conclusions, 2 original contributions, and 8 scientific contributions of a practical and applied nature. The conclusions are based on the statistical correlation of the cases studied and the practical interpretation of the results according to the tasks set. With a decrease in the differentiation of SCC, the cytoplasmic and nuclear expression of the apoptosis marker Caspase-3 increases. The most significant of the results is that SCC suggests an active apoptotic form of cell death, which can be induced after chemotherapy and radiotherapy. The cytoplasmic and nuclear expression of RIPK3 in high-risk groups of BCC was assessed, and it was found that the cytoplasmic and nuclear expression of RIPK3 in BCC is higher in tumors with necrosis and pronounced infiltration of the tumor by TILs. The results support the presence of an active necroptotic cell death pathway that can be induced by the applied therapy.

Publications and personal contribution of the doctoral student. Dr. Vassilev has presented two full-text articles in a nationally refereed publication on the topic of the dissertation.

Based on the dissertation presented, I believe that Dr. Vassilev has independently developed the topic, and the results shown define him as a responsible and consistent colleague who is very skilled in handling scientific sources and facts, knows how to analyze, compare, and summarize the results obtained, and has analytical and practical thinking. It is evident that Dr. Vassilev demonstrates consistency and a desire to work in the field of science and to develop in his chosen professional direction.

Abstract

The abstract is 92 pages long and has been prepared in accordance with the requirements, including the main parts of the dissertation, the necessary tables, figures, and photographs. The content of the abstract provides an excellent overview of the entire dissertation and reflects the main results, conclusions, and contributions.

CONCLUSION

In conclusion, I would like to say that in the dissertation, researcher had investigated for the first time in our country necroptosis, nuclear and cytoplasmic expression of Caspase-3 and RIPK3 in BCC and SCC, and performs an extensive analysis of the expression of markers for necroptosis and apoptosis in tumor cells according to the histological type of tumor, T-stage of tumors, localization, gender, tumor and non- tumor tissues, area of necrosis, and TILs. The study ***contains scientific, scientific-applied, and applied results that represent an original contribution to science*** and **meet all** the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, and the Regulations of MU-Varna. The materials provided and dissertation results fully comply with the specific requirements adopted

in connection with the Regulations of MU-Varna for the application of the ADSRA.

Based on the above, I consider that Dr. Plamen Petrov Vassilev's dissertation is a fully completed scientific work and I give my **positive assessment by voting "For" the awarding of the an educational and scientific degree of "Doctor" to Dr. Plamen Petrov Vassilev.** I call on **the other members of the distinguished scientific jury to also vote positively.**

10. 11. 2025
Plovdiv

Opinion prepa

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

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