

OPINION

by

Assoc. Prof. Martina Georgieva Stoeva, MD, PhD

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Department of General and Clinical Pathology, Forensic Medicine and Deontology

Internal member and Chair of the Scientific Jury, appointed

by Order No. R-109-427/06.10.2025

for the procedure for acquisition of the educational and scientific degree “Doctor”

in field of higher education 7. Healthcare and Sports,

professional field 7.1. Medicine

scientific specialty: “Pathological Anatomy and Cytopathology”

on the dissertation thesis of Dr Plamen Petrov Vasilev

on the topic: “Apoptosis and necroptosis in basal cell and squamous cell carcinoma of the skin”

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

I. Data on the procedure

By Order No. R-109-427/06.10.2025 of the Rector of MU–Varna I was selected as a member of the scientific jury, and on the basis of Protocol No. 1/14.10.2025 I was appointed Chair of the Scientific Jury and tasked with preparing an opinion in the procedure for acquisition of the educational and scientific degree “Doctor” by Dr Plamen Petrov Vasilev, a full-time PhD student at the Department of “General and Clinical Pathology, Forensic Medicine and Deontology”, MU–Varna.

The set of documents submitted by the candidate in electronic format is in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and with the Regulations on the Conditions and Procedures for Acquisition of Scientific Degrees and Holding Academic Positions at MU–Varna.

II. Biographical data and career development

Dr Plamen Petrov Vasilev was born on 13.10.1974 in the city of Burgas. In 2000 he obtained a Master’s degree in Medicine at the Medical University – Sofia. In the period 2013–2017 he was a resident in General and Clinical Pathology at the Department of “General and Clinical Pathology, Forensic Medicine and Deontology” of MU–Varna. From 11.2020 to 10.2025 he has been a full-time PhD student in the Doctoral Program “Pathological Anatomy and Cytopathology”

at the Department of “General and Clinical Pathology, Forensic Medicine and Deontology”, Medical University “Prof. Dr Paraskev Stoyanov”, Varna.

Dr Vasilev obtained a specialty in General and Clinical Pathology in 2019, Certificate of Recognized Specialty No. 4091, Series MUV No. 022569, Medical University “Prof. Dr P. Stoyanov”, Varna. He has a good command of English.

III. Relevance of the topic of the dissertation

The topic of the dissertation is relevant and does not duplicate other studies in Bulgaria related to apoptosis and necroptosis in basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) of the skin. A detailed investigation of the expression of Caspase-3 and RIPK3 in patients with BCC and SCC may contribute to the identification of future prognostic and therapeutic approaches.

IV. Structure of the dissertation

The submitted dissertation is written on 139 pages and is structured according to the generally accepted scheme: List of abbreviations – 4 pages; Introduction – 2 pages; Literature review – 54 pages; Aim and tasks – 1 page; Materials and Methods – 5 pages; Results and Discussion – 43 pages; Conclusions – 1 page; Contributions – 1 page. It is illustrated with 66 tables and 35 figures. The reference list contains 335 literary sources, 6 in Cyrillic and 329 in Latin script.

In the **literature review** the epidemiology and risk factors for the development of BCC and SCC of the skin are described. Hereditary and genetic factors involved in tumorigenesis are examined in detail. The morphological characteristics, staging, prognostic and predictive histological parameters of BCC and SCC are presented. An overview is made of the different types of cell death – apoptosis, necroptosis, their molecular mechanisms and their role in the tumor process. The literature review is based on a large body of publications and reflects the PhD student’s detailed knowledge of the investigated problem.

The **aim** is clearly formulated, and the **tasks**, seven in total, correspond to the main aim.

The **materials and methods** are methodologically sound and correctly developed. The present study is retrospective and includes 91 patients, 46 with BCC and 45 with SCC of the skin for the period 2015–2021. The tumors were radically excised surgically and the level of immunohistochemical expression of Caspase-3 and RIPK3 (receptor-interacting serine/threonine-protein kinase 3) was determined. Clearly defined criteria were used to categorize each parameter – age, sex, localization, histological type of the tumor, TNM staging, tumor-infiltrating lymphocytes, vascular and perineural invasion, area of necrosis. As specific methods of investigation, an indirect immunoperoxidase method for immunohistochemical analysis using mini KIT high pH DAKO K8024 was applied.

For the **statistical analysis** of the data a specialized statistical software package STATISTICA (StatSoft Inc, USA, STATISTICA Manual (Data analysis software system), Version 13.0, 2022) was used. The graphs were constructed in Microsoft Excel for Windows. The following statistical methods were applied: descriptive statistical analysis, Student’s t-test for two

independent samples and analysis of variance (ANOVA). The methods of investigation are chosen in accordance with the set tasks and are clearly described and informative.

Results and Discussion. The obtained results are presented in two subsections and are well illustrated with a total of 61 tables and 24 figures. They provide a clear answer to the set tasks. The clinico-morphological characteristics of the patients with BCC and SCC are studied. The PhD student investigates the cytoplasmic and nuclear expression of RIPK3 in the tumor tissue of BCC and SCC and in the adjacent non-tumor tissue. A comparative analysis is carried out between the mean values of the expression of Caspase-3 and RIPK3 in the tumor tissue and the adjacent non-tumor tissue in patients with BCC and SCC.

He does not find a statistically significant difference in the cytoplasmic and nuclear intensity of Caspase-3 in the tumor and non-tumor tissue of BCC. The cytoplasmic expression of Caspase-3 in the tumor cells of SCC is higher compared to the non-tumor tissue, in contrast to the nuclear expression, for which no statistically significant difference is found. The increased level may indicate that the apoptotic pathway is active in SCC. The cytoplasmic and nuclear expression of Caspase-3 in the tumor tissue of BCC and SCC is not influenced by the sex of the patients, the area of tumor necrosis, the TILs in the tumor tissue or the localization of the tumor.

The obtained results show an inverse relationship between the cytoplasmic and nuclear expression of Caspase-3 and the differentiation of SCC. As the differentiation of the tumor decreases, the expression of Caspase-3 increases. Dr Vasilev establishes that the cytoplasmic expression of RIPK3 in the tumor tissue of BCC and SCC is higher compared to the adjacent non-tumor tissue, while the same dependence for nuclear expression is found only in SCC. The cytoplasmic and nuclear expression of RIPK3 in BCC is higher in tumors with necrosis compared to those without necrosis. In BCC and SCC the cytoplasmic and nuclear expression of RIPK3 are in a direct correlation with tumor-infiltrating lymphocytes (TILs). High expression of the antibody is associated with a higher intensity of TILs in the tumor tissue.

The obtained results support the concept of the immunogenic properties of necroptosis, because the marker of necroptosis RIPK3 is increased in both skin carcinomas when the intensity of TILs is highest. The data obtained are excellently compared with the findings known from the cited literature sources. Larger studies and the experience of other researchers are discussed in detail, and their results are compared and discussed with the author's own results. The discussion of the results is comprehensive.

From the obtained results, 16 **conclusions** have been drawn, which reflect the scientific and research work carried out at all stages of the conducted studies. A total of 10 **contributions** have been formulated, which I fully accept. They are divided into scientific contributions of original character (2) and scientific contributions of practical-applied character (8).

In connection with the dissertation, **two full-text publications** in a Bulgarian scientific journal have been presented. In one of the articles Dr Vasilev is the sole author, and in the other he is first author, which is evidence that the dissertation is his own work.

The **abstract** is written on 92 pages and reflects the structure and the main elements of the dissertation.

V. Conclusion

The submitted dissertation of Dr Plamen Petrov Vasilev is a comprehensive, in-depth and methodologically sound study, distinguished by its relevance and original contribution. The conducted investigations reveal new data regarding the expression and localization of Caspase-3 and RIPK3 in BCC and SCC of the skin, and for the first time in Bulgaria the role of necroptosis and its related markers is analyzed in such depth. The author presents a complex analysis, taking into account the histological characteristics of the tumors, their clinical stage, localization, the sex of the patients, as well as the features of the tumor and non-tumor tissue.

The obtained scientific and applied results fully meet the criteria of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the respective regulations and the requirements of the Medical University – Varna for awarding the educational and scientific degree “Doctor” in the specialty “Pathological Anatomy and Cytopathology”. The dissertation represents a completed, valuable and contributory scientific study.

On the basis of the above, I give my **positive assessment** and support the awarding of the **educational and scientific degree “Doctor” to Dr Plamen Petrov Vasilev**, and I recommend that the other members of the scientific jury also vote in favour.

19 November 2025
City of Varna

Prepared by
/Assoc. Prof. Martina Georgieva Stoeva, MD, PhD/

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