

OPINION

by Prof. Dr. Dimitar Kadiysky, Doctor of Medical Sciences,

Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM) at the Bulgarian Academy of Sciences, Sofia

Subject: dissertation for awarding the "doctor" degree in

Field of higher education: 4. Natural sciences, mathematics and informatics

Professional field: 4.3. Biological sciences

Scientific specialty: Morphology (code: 01.06.26)

Author: Dr. ROSSEN ALEXANDROV SPASOV

Topic: STUDY ON THE EXPRESSION OF PD-L1 IN UROTHELIAL CARCINOMA OF THE URINARY BLADDER AS A BASIS FOR THERAPEUTIC STRATEGY

Scientific supervisor: Prof. Radostina Ivaylova Aleksandrova, PhD

Form of doctoral studies: doctoral student of independent preparation at IEMPAM - BAS

By order NO-05-05-02 /21-01-2025 I am appointed as a member of the Scientific Jury for conducting a procedure for the defense of the dissertation of Dr. Rossen Alexandrov Spasov. The set of materials presented to me on an electronic medium by the author is in accordance with the articles of the Law on Academic Staff Development (LASD) in Bulgaria, concerning the defense of a dissertation for obtaining the educational and scientific degree "doctor" and the specific requirements in this regard of the Institute of Experimental Morphology, Pathology and Anthropology with a Museum (IEMPAM) at BAS. The set includes: dissertation, abstract and files with documents required according to the regulations for conducting a defense of a dissertation for obtaining the educational and scientific degree "doctor".

Dr. Rossen Alexandrov Spasov graduated from the Medical University - Sofia in 1996. with a degree of "Master of Medicine". He is currently a physician in the Department of Clinical Pathology of the Lozenets University Hospital. From 2012-2019, he has been a resident in the Department of Clinical Pathology of the Lozenets University Hospital for Oncology in Sofia. In parallel with this, since 2019, he has been performing the duties of Head of the Department of Clinical Pathology of the St. Mina University Hospital for Oncology in Blagoevgrad. In the period 1997-2008, he was a resident physician and, in parallel, Head of the Department of Clinical Pathology at the Dr. N. Vasiliev University Hospital in Kyustendil.

His research interest and career are directly related to the histomorphology and histopathology of oncological diseases, including due to his continuous biopsy, cytological and express intraoperative diagnostic activity, carried out during the years of his professional growth. During the period 2016 - 2019, Dr. Spasov was a full-time assistant professor at the University Hospital "Lozenets", where he successfully participated in the academic teaching activities of medical students.

The topic of his dissertation: "STUDY ON THE EXPRESSION OF PD-L1 IN UROTHELIAL CARCINOMA OF THE URINARY BLADDER AS A BASIS FOR THERAPEUTIC STRATEGY" is of particular relevance and scientific significance due to the fact that bladder carcinoma (BC) is among the leading causes of global mortality

caused by oncological diseases and represents a serious problem with significant medical and social burden. Finding reliable and specific diagnostic strategies and developing therapeutic approaches for this pathology requires *a priori* development and validation of biomarkers that will serve as a basis for refining the clinical behavior in patients with BC. The work on the doctoral student's dissertation was supervised and consulted by Prof. Radostina Alexandrova, PhD, who provided the guidelines and provided the laboratory conditions for the experiments.

The presented dissertation is written on 136 pages and contains 49 figures and 8 tables. The list of cited literature includes 224 titles.

The experiments were performed in the laboratories of the Institute of Experimental Morphology, Pathology and Anthropology with a Museum - BAS and in the Laboratory of General and Clinical Pathology and Forensic Medicine of the University Hospital "Lozenets". The dissertation was approved and directed for the defense of an extended assignment of the "Pathology" section, IEMPAM-BAS, conducted by order of the Director of IEMPAM-BAS No. RD-09-70 of 17.12.2024.

The realization of the set goal - study of the morphological and immunohistochemical characteristics of PD-L1 expression in urothelial carcinomas of the urinary bladder and establishment of their dependence on the degree of differentiation is associated with the implementation of seven specific research tasks.

The material for the study - tissue samples (tumor tissues from 110 diagnosed urothelial carcinoma of the bladder, 1 sarcomatoid variant of bladder carcinoma, 5 metastatic lymph nodes and 9 bone and soft tissue metastases), as well as tumor cell lines HT29, HeLa, LSR,-SF,-SR meet the set tasks, corresponding to the purpose of the development.

For the experimental study, classical and modern research methods such as routine histology, immunohistochemistry, indirect immunofluorescence, morphometry and statistical analysis were selected. These research approaches allowed the author, by performing tissue histological processing and tumor classification, to determine the status of the regional lymph nodes, as well as the stage of the disease by the degree of differentiation and to conduct an immunohistochemical study of the expression of PD-L1, CD8, CD68, Ki67. The results obtained are of sufficient number and quality, both for statistical evaluation and for formulating conclusions. They link the morphological characteristics of the studied pathology with the expression of PD-L1 - incl. the projection of this marker on the therapeutic behavior.

Another group of experiments implemented by culturing and storing permanent cell lines, assessing cell death - apoptosis/necrosis and isolating DNA from tumor tissues from urothelial carcinomas of the urinary bladder, create a basis for further exome and genomic DNA sequencing in future studies in this area. The conclusions and comparative analysis with existing data available in modern pathomorphology are the basis for interpreting the experimental results of Dr. Spasov. The author of the dissertation registered high levels of PD-L1 expression in poorly differentiated urothelial carcinomas, in metastatic lymph nodes and most clearly in osteolytic bone metastases. The importance of these results is evidenced by the fact that determining the PD-L1 status is essential before proceeding with immunotherapy. Nevertheless, the dissertation emphasizes the fact that the selection of patients who would benefit from the use of PD-L1 status for immunotherapy can only be done by taking into account other histomorphological characteristics - the presence of tumor-infiltrating lymphocytes (TILs), CD8+ T-lymphocytes, tumor-associated macrophages (TAMs), etc., as well as in the presence of metastatic foci.

In this regard, the literature data on the issue cited by the author neither confirm nor deny the role of PD-L1 values in the prognosis or therapy of certain types of cancer. Therefore, the author's experimental results are in agreement with the view that it remains controversial whether PD-L1 can serve as an accurate or additional biomarker in patients with urothelial carcinoma for the application of adjuvant chemotherapy. Regardless of the contradictory literature data, the anatomical, pathophysiological and immunochemical screening of the studied oncopathological objects, performed by the doctoral student in the development of the dissertation, demonstrates their importance in determining diagnostic approaches.

The conclusions made by the PhD student (7 in number) confirm the clinical-morphological results and their comparison with the PD-L1 status in the studied urothelial carcinomas of the urinary bladder. The registered prevalence of patients by gender (male) 70.23%, age - 67 years (7th decade) also has its medicobiological significance. In principle, establishing the dynamics of expression of key markers in different age groups helps to more precisely determine the individual benefit for each specific patient from the new immunotherapeutic and targeted strategies being introduced.

The presentation of the results through well-illustrated color immunohistochemical analysis, graphics and statistical processing, through discussion in a separate chapter (**V. Summary**) creates an understandable form of the dissertation work. I note the good quality of the illustrative material included in the dissertation, as well as the clarity of the formulated conclusions, emphasizing and specifying the contributions of the work.

CONCLUSION

The material presented to me for opinion, summarizing the research activity of Dr. Rossen Alexandrov Spasov, contains medicobiological scientific and applied results and specific pathomorphological conclusions, representing a contribution to science. The structure of the work is consistent with the classic view of a dissertation for obtaining the educational and scientific degree of "doctor", and its volume and well-presented illustrative material meet the standard requirements. The qualities of the dissertation work show that the doctoral student Dr. Rossen Alexandrov Spasov possesses in-depth theoretical knowledge and professional skills in the field of normal and pathological anatomy, histology, immunomorphology and experimental biomedicine. It is evident from the presented documentation that it meets the minimum points for the defense of a dissertation work according to the LASD in Bulgaria and the specific norms of IEMPAM - BAS. I remain fully convinced of his ability to independently conduct scientific research in parallel with his extensive diagnostic work carried out by him in several medical units.

In connection with the above, I confidently give my positive assessment of the research conducted, presented in the dissertation, the abstract and other materials related to the dissertation. I propose to the esteemed scientific jury to award the educational and scientific degree of 'doctor' to Dr. Rossen Alexandrov Spasov, a doctoral student in independent training at the Institute of Experimental Morphology, Pathology and Anthropology with a Museum at the Bulgarian Academy of Sciences.

14.03.2025.

Sofia

Prepared the opinion:


Prof. Dr. Dimitar Kadiysky, MD, DSci: