

OPINION PRESENTATION

by Professor Dr. Galina Kurteva, MD (Clinic of Medical Oncology, St. Catherine University Hospital, Sofia) - member of the Scientific Jury according to Order No. NO-05-05-2 dated 21.01.2025 of the Director of the Institute of Experimental Morphology, Pathology and Anthropology with Museum - Bulgarian Academy of Sciences (IEMPAM-BAS)

Regarding the dissertation on the topic: "Study on the expression of PD-L1 in urothelial carcinoma of the urinary bladder as a basis for a therapeutic strategy"

for awarding the educational and scientific degree "DOCTOR" in the scientific specialty "Morphology", code 01.06.26, professional direction "Biological Sciences" 4.3, field of higher education 4. "Natural Sciences, Mathematics and Informatics"

to Dr. Rossen Alexandrov Spasov, part-time doctoral student in the "Pathology" section of IEMPAM-BAS with scientific supervisor Prof. Radostina Ivaylova Alexandrova, Department of Pathology of IEMPAM-BAS.

1. Relevance and significance of the dissertation work

The relevance and significance of the presented dissertation work are determined by the persistently high levels of morbidity and mortality from oncological diseases and, in particular, the great medical and social burden of bladder carcinomas. Alarming facts are the decreasing age of patients, frequent relapses and multidrug-resistant tumors. Against this background, the possibility of applying modern antitumor immunotherapeutic strategies through therapeutic antibodies-inhibitors of immune checkpoints in recent years has revolutionized the treatment of bladder carcinoma. In order for therapy with immune checkpoint inhibitors to be successful, patients must be stratified by applying predictive biomarkers. Several clinical trials have reported promising durable responses in the presence of PD-L1 expression, but no clear correlation has been found between its level and therapeutic response, especially since patients with tumors without PD-L1 expression also show durable responses. Higher PD-L1 expression on tumor-infiltrating immune cells with a cutoff value of 5% is predictive of a better response to anti-PD-1/PD-L1 immunotherapy, but not that of tumor cells. On the other hand, PD-L1 status is a prognostic

biomarker for progression-free survival, but not for overall survival, in patients with urothelial carcinoma treated with anti-PD-1/PD-L immunotherapy. Therefore, the clinical utility of determining PD-L1 expression in urothelial bladder carcinoma remains limited at present, and the need for reliable predictive biomarkers is increasing given the growing number of novel antitumor immunotherapeutic agents.

2. Overview of the Dissertation

The presented Dissertation is written on 136 pages and includes: Title page (1 page), Table of Contents (3 pages), List of abbreviations used (1 page), Introduction (2 pages), Literature review (48 pages), Aims and objectives (1 page), Materials and methods (10 pages), Results and Discussion (41 pages), Summary (3 pages), Conclusions (1 page), Contributions (1 page), Bibliography (18 pages), List of publications and participations (2 pages). It is illustrated with 10 color figures (in the "Literature review"), 39 combined panels of microscopic photographs in the "Results and discussion" section) and 8 tables (5 in the "Literature review", 1 in "Materials and methods" and 1 in "Results and discussion").

The introduction reveals the motives that provoked interest in the topic of the dissertation, its relevance and significance. The literature review sequentially examines the main points of the modern concept of the immunology of malignant tumors and antitumor immunotherapy - tumor antigens, antitumor immune response, the role of apoptosis. The stages in the historical development of immunotherapy in medical oncology are indicated, as well as the types of immunotherapy in oncological diseases. The biological function of PD-1 and PD-L1, the PD-1/PD-L1 signaling pathway, its role in neoplasms and the mechanism of action of therapeutic inhibitors of immune checkpoints are described in great detail. A thorough and detailed analysis of the epidemiological and clinical-morphological characteristics of bladder carcinoma has been conducted with a very topical emphasis on the wide range of risk factors - smoking and other newly spreading harmful habits, environmental factors, occupational hazards, use of medications, previous illnesses, dietary habits and the influence of the bladder and intestinal microbiome. The doctoral student has analyzed the currently used heterogeneous pathomorphological classifications and especially the Consensus Molecular Biology Classification of Urothelial Carcinomas, in which a variety of characteristics are used to characterize the histological variants and specific morphological patterns of urothelial carcinomas: presentation of tumor cells and their

microenvironments (immune cells, fibroblasts and smooth muscle cells); differentiation scale, showing characteristics related to the gradient from luminal to basal and neuroendocrine differentiation; consideration of oncogenic mechanisms, mutations, stromal infiltrate, immune infiltrate, histology, clinical characteristics and average overall survival.

Aim and objectives. The aim is clearly and precisely formulated. The tasks (7 in number) are described in detail and consistently structure the planned research activity. The section "Materials and Methods" contains a detailed description of the applied methodologies and work protocols, as well as the diagnostic antibodies and kits used for visualization of the described antigen-antibody reactions.

The results meet the set goals and objectives. They are presented in detail and excellently illustrated by figures from very high-quality microphotographs, tables and diagrams, which facilitate their perception. The discussion of the obtained results is placed sequentially together with their presentation in the structure of the dissertation, which facilitates the assessment of their significance.

In the preparation of the dissertation, tumor tissues from 110 diagnosed urothelial carcinoma of the urinary bladder, 1 sarcomatoid variant of bladder carcinoma, 5 metastatic lymph nodes and 9 bone and soft tissue metastases were examined. Original data were obtained on the distribution of urothelial carcinomas by gender and age, by degree of differentiation and depth of tumor invasion in primary tumors and in relapses, stage of the disease at the time of diagnosis. Immunohistochemically, high levels of PD-L1 expression were found in poorly differentiated urothelial carcinomas, in metastatic lymph nodes and most clearly in osteolytic bone metastases. In the course of work on the dissertation, the doctoral student Dr. Rossen Spasov logically concluded that determining the PD-L1 status before proceeding with immunotherapy with immune checkpoint inhibitors is essential, but not sufficient. Therefore, the doctoral student expands the scope of research on putative predictive and prognostic biomarkers with other histological characteristics – the presence of tumor-infiltrating lymphocytes (TILs), CD8⁺ T-lymphocytes, tumor-associated macrophages (TAMs) and the formation of metastatic foci. The original results obtained enrich our knowledge of the possibilities for precise determination of the individual benefit for each specific patient from the new immunotherapeutic and targeted treatment strategies being introduced and the need to apply molecular biological and genetic methods, building on the

established morphological and immunohistochemical biomarkers and revealing new diagnostic, prognostic and therapeutic possibilities.

Conclusions and contributions - based on the results obtained, 7 conclusions have been systematized, which I fully accept. I agree with the 2 original contributions, 3 confirmatory contributions and 1 applied contribution, highlighting the innovative elements in the dissertation work.

The cited literature covers a total of 224 titles (1 in Cyrillic, the rest in Latin), with 196 of them (~87%) being published in the last 10 years.

3. Publications and participation in scientific forums of the doctoral student on the topic of the dissertation work

On the topic of the dissertation work, 3 articles have been published in publications, 2 of which are in refereed and indexed in world-renowned databases for scientific information (Scopus, Web of Science). 21 citations have been noted so far. The results obtained are presented in 3 communications at 3 scientific forums, 1 of which is with international participation.

4. Critical notes and comments, recommendations, questions to the doctoral student

I have no substantive remarks. I noticed technical errors in the arrangement of the cited literary sources. I would like to ask the following question to the doctoral student Dr. Rossen Spasov: Will he continue his work in this field and in what direction?

5. Biographical data and personal impressions of the doctoral student

Dr. Rossen Alexandrov Spasov was born on September 14, 1966 in the city of Sofia. He graduated from secondary education at the District Mathematical High School Kyustendil. In 1996, he graduated from the Medical University - Sofia, majoring in Medicine. He has been working as a resident physician in the Department of Clinical Pathology of the "Dr. N. Vasiliev" Hospital in Kyustendil since 1997. He obtained a specialty in "General and Clinical Pathology" in 2008. After a competition, he was appointed Head of the Department of Clinical Pathology of the "Dr. N. Vasiliev" Hospital in Kyustendil, a position he holds to this day. At the same time, during the period 2012-2019, he performed biopsy, cytological and express intraoperative diagnostics as a resident physician in the Department of Clinical Pathology of the Sofia Hospital for Oncology and

acquired expert knowledge and skills in the pathomorphological diagnostics of tumors of the breast and gastrointestinal tract.

In 2016, he was elected as an assistant professor at the Department of Anatomy and Histology, Pathology and Forensic Medicine at the Faculty of Medicine of Sofia University "St. Kliment Ohridski" after a successful presentation in a competition. As an assistant, Dr. Rossen Spasov participates in the teaching work at the Department of Anatomy and Histology, Pathology and Forensic Medicine - he conducts practical classes in general and clinical pathology with students of medicine, pharmacy and occupational therapy. He is actively involved in the diagnostic process of the University Hospital "Lozenets" by applying all the basic methods of pathomorphological diagnosis and shows a deep interest in tumor pathology and lesions with borderline malignant potential of the urogenital and gastrointestinal tract. From 2019 to the present, Dr. Rossen Spasov is the Head of the Department of Clinical Pathology at the Specialized Oncology Hospital "St. Mina" in Blagoevgrad and a consultant in the Department of Clinical Pathology at the University Hospital "Lozenets" in Sofia. His many years of diagnostic experience are supported by certified courses and trainings - Digital Breast Pathology (2022), Academy of Molecular Uropathology (2021), Academy of Personalized Molecular Pathology and Oncology - Agnostic Malignant Tumors (2020), PD-L1 Immunohistochemistry - principles of scoring (2017, Cassel, Germany).

As a part-time doctoral student in the scientific specialty Morphology in the Pathology section of IEMPAM-BAS, Dr. Rossen Spasov is preparing for defense a dissertation on the topic "Study on the expression of PD-L1 in urothelial carcinoma of the urinary bladder as a basis for a therapeutic strategy".

He is a co-author of 9 publications and participates in the team of 5 research projects.

Dr. Rossen Spasov is a member of the Bulgarian Medical Union, the Bulgarian Society of Pathology and the Bulgarian Anatomical Society.

6. Conclusion

The presented dissertation is dedicated to a topical and significant topic, the goals and objectives set in it have been fully achieved, at a high professional level, and the original results obtained are an undeniable contribution to the field of pathomorphology and oncology.

During the preparation, the doctoral student has added to his diagnostic and medical knowledge a rich set of modern methods and techniques, he has grown as a capable and promising scientist. The

new results obtained during the implementation of the dissertation contribute to the fight against one of the leading medical and social challenges of the 21st century - malignant diseases and, more specifically, urothelial carcinoma of the urinary bladder. The efforts made by the doctoral student in the preparation of the dissertation work and the final product obtained deserve high praise.

All this gives me reason to believe that the presented dissertation work fully complies with the Act on the Development of the Academic Staff in the Republic of Bulgaria, as well as the Regulations for its application in IEMPAM - BAS for awarding the educational and scientific degree "Doctor".

I confidently give my positive assessment of the dissertation work prepared by Dr. Rossen Alexandrov Spasov and support before the participants in the scientific jury the acquisition of the educational and scientific degree "Doctor" in the scientific specialty "Morphology", code 01.06.26, professional direction "Biological Sciences" 4.3., field of higher education 4. "Natural Sciences, Mathematics and Informatics".

18.03. 2025 г.

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/ Prof. Dr. Galina Kurteva, MD /