

REVIEW

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part of the scientific jury according to the Decision with Protocol № 1 from the IEMPAM SC meeting of 15.01.2025 and by Order No. NO-05-05 - 2 of 21.01.2025 of the Director of IEMPAM and appointed to write an opinion on

the dissertation work of **Dr. Rossen Alexandrov Spasov**, part-time PhD student at the Section of Pathology of the IEMPAM of the Bulgarian Academy of Sciences on the topic „**Study on PD-L1 expression in urothelial carcinoma of the bladder as a basis for therapeutic strategy**“, submitted for the award of the educational and scientific degree "DOCTOR" in the scientific specialty 01.06.26. Morphology, in the field of higher education 4. Natural Sciences, Mathematics and Informatics, in the professional field 4.3 Biological Sciences

The submitted set of materials on paper and electronic media is in accordance with the procedure for the acquisition of the educational and scientific degree "PhD" at the IEMPAM of BAS.

1. Information about the author

Dr. Rossen Alexandrov Spasov, born on September 14, 1966 in Sofia, graduated in medicine with a master's degree in 1996. From 1997 to the present time he has worked successively and simultaneously as a physician-coordinator and head of the Department of Clinical Pathology of the Hospital "Dr. N. Vasiliev" in Kyustendil, physician-coordinator, assistant and consultant physician in the Department of Clinical Pathology of the University Hospital "Lozenets", head of the Department of Clinical Pathology of the Hospital of Oncology "St. Mina" in Blagoevgrad. The present work was submitted by him as a part-time PhD student in the Section of Pathology of the Institute of Pathology of the Bulgarian Academy of Sciences.

2. General characteristics of the dissertation

The dissertation contains 136 pages. They are distributed as follows: introduction - 3 pages; literature review - 50 pages; aim and tasks – 1 page; materials and methods – 11 pages; results and discussion – 40 pages; summary – 3 pages; conclusions – 1 page; contributions – 1 page; scientific asset – 2 pages; bibliography including 224 references, of which 1 in Cyrillic and 223 in Latin. The illustration is by 52 figures (10 diagrams, 28 light microscopic photographs, 13 histograms, 1 graphic scale) and 8 tables. The requirement that the own results be the largest part of the dissertation is not met, as even with the discussion they are less than the very large literature review.

The chosen topic "Study on PD-L1 expression in urothelial carcinoma of the bladder as a basis for a therapeutic strategy" seems very ambitious and modern. It is unquestionably and quite relevant in view of the prospect of an increasingly broad and targeted application of immunotherapy for the treatment of cancer. This is evident from the fact that of the 224 titles cited in the bibliography, 163 are of publications after 2015, i.e. in the last ten years. The subject of the study, namely bladder cancer (BC), was well chosen. It is the second most common malignancy of the urinary tract worldwide, causing 549 000 new cases and approximately 200 000 deaths per year. This reflects the PhD student's long-standing interest in tumour morphology and in particular the wide range of its changes in different clinical cases of bladder cancer. The idea to specifically study the expression of the PD-L1 ligand of the programmed cell death receptor PD-1 in bladder carcinoma tissue is also very good. This is related to the clear need for modern immunological treatments of cancer to target immune checkpoint inhibitors on tumor cells. The PhD student entered this field at the beginning of his work in the Laboratory of General and Clinical Pathology and Forensic Medicine of the University Hospital "Lozenets". Subsequently, he deepened these studies as a part-time PhD student at the Section of Pathology of the Institute of Experimental Morphology, Pathology and Anthropology with Museum at the Bulgarian Academy of Sciences. The results have been published and repeatedly presented at specialized anatomical congresses and conferences.

However, the well-placed title of the paper does not fully correlate with the research done in it, as the study on PD-L1 expression actually represents only a small part of the actual results.

The review of the literature is thematically divided into subchapters and topics, covering quite extensively information on membrane proteins PD-1 and PD-L1, apoptosis, carcinogenesis, tumor antigens and immunotherapy, epidemiological and clinicomorphological characteristics of bladder cancer, as well as current therapeutic strategies and immune checkpoint inhibitors in bladder cancer. On the one hand, this shows the doctoral student's good literary awareness of all aspects of this complex clinical-morphological problem. On the other hand, however, the literature review is overstretched in view of the overall length of the dissertation. A very large part of it, for example at least the topic "Risk factors and bladder cancer", which is 11 pages, could easily be removed from the dissertation. It has no specific relevance to the research conducted, nor is it used further for any discussion.

The aim of the thesis is clearly stated. Seven main tasks requiring qualitative and quantitative research are outlined to fulfil the aim. Tasks 1 and 3, however, overlap. Task 7 has no stated clear link to the dissertation objective.

The chapter "Materials and Methods" presents detailed information on the processing of tissue samples to obtain the light microscopic preparations and their qualitative and quantitative analysis. The study subject, tumor tissues from 110 diagnosed urothelial carcinomas of the bladder, 1 sarcomatoid variant of bladder carcinoma, 5 metastatic lymph

nodes, 9 bone and soft tissue metastases, and cells from tumor cell lines, was appropriately selected in view of the study. Immunohistochemical methods were applied to visualize the expression of PD-L1, CD8, CD68, Ki67, as well as an assay to determine the proliferative activity of treated cells. Statistical analysis of the frequency of tumor findings was also performed.

3. Evaluation of the results obtained and their discussion

The dissertation has a common chapter "Results and Discussion". In it, the actual results are distributed as follows: 8 pages are devoted to frequency distribution of bladder carcinoma, 12 pages to tumor invasion, 8 pages to PD-L1 expression study, 9 pages to CD8 and CD68 expression, 3 pages to DNA isolation from tumor tissues. These last three pages on DNA isolation are devoted to a methodical description of DNA isolation and should therefore be transferred to the end of the Materials and Methods chapter.

There are gaps in the designation of the illustrative material and the bibliography. Table 5 is missing and there are two tables numbered 6. Some of the figures are not cited in the text - e.g. figures 38, 40 - 47. Many of the bibliographic sources cited are unevenly cited, some with one, two or more of the required elements missing. Their citation in the text is also patchy, mostly with only the name of the first author and the year, regardless of the number of authors.

Discussion in the usual sense of comparing one's own results with those of other authors is lacking. In the entire common chapter Results and Discussion there are only 5 sentences with authors cited that can be considered as a comparison. This is strange in view of what is written at the end of the literature review "Despite the many studies conducted, challenges remain in using PD-L1 as a biomarker for ICIs in bladder cancer."

The "Summary" chapter outlines the search for more successful therapeutic approaches for the treatment of bladder cancer, including immunotherapy and relevant clinicopathological methods to evaluate their effectiveness. In this regard, the present study on the expression of the transmembrane protein of programmed cell death PD-1, its ligand PD-L1 and their role in the evasion of the antitumor immune response is important. PD-L1 expression is heterogeneous in tumor and shows differences between primary tumor and metastasis. Therefore, a thorough pathomorphological evaluation of tumors is important as the basis of a successful personalized treatment strategy. This makes the obtained data important for practice.

4. Evaluation of the contributions received

As a result of the research conducted, 2 original scientific contributions and 3 other contributions of a confirmatory nature were derived. A collection of tumor tissues from 110 bladder carcinomas with varying degrees of differentiation and depth of infiltrative growth in muscle layers, metastatic lymph nodes, and bone metastases was established. Statistical data were obtained on the distribution by age, sex, degree of differentiation, degree of tumor

invasion, and stage of disease of primary and recurrent bladder carcinomas diagnosed in the Department of Clinical Pathology of Lozenets University Hospital for the period 2016-2020.

5. Evaluation of the scientific asset

In accordance with the Regulation for the Conditions and Procedure for the Acquisition of Scientific Degrees and for Occupation of Academic Positions at IEMPAM - BAS in connection with the dissertation are attached 3 scientific publications, one in Bulgarian source, the other two in foreign ones. In one publication Dr. Spasov is the first author. Three contributions to scientific forums are also presented.

6. Evaluation of the abstract

The abstract is in usual length, appropriately structured and well formatted. It summarizes the main sections of the thesis.

7. Conclusion

The presented dissertation of Dr. Rossen Alexandrov Spasov entitled "Study on PD-L1 expression in urothelial carcinoma of the bladder as a basis for therapeutic strategy" is a contemporary and topical study of an important problem both for clinical practice and for basic morphological science. This work has a logical structure and a clear aim. Despite the many disadvantages and gaps listed, there are results obtained and documented. They present two original and three confirmatory scientific contributions. These facts, as well as the fulfilled requirements for the necessary qualitative and quantitative criteria under the Academic Staff Development Act, its Regulations, and the Regulations for the Conditions and Procedure for the Acquisition of Scientific Degrees and for Occupation of Academic Positions at IEMPAM - BAS give me grounds to recommend to the Honorable Scientific Jury to award Dr. Rossen Alexandrov Spasov the educational and scientific degree "DOCTOR".

18.03.2025

Prepared by



/ Prof. Dr. Lachezar Surchev, DSc /