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REVIEW

by Prof. MVD Emil Ivanov Sapundzhiev, PhD - in the specialty "Morphology" (01.06.26.) at the University of Forestry, Faculty of Veterinary Medicine, Department of "Anatomy, Physiology and Animal Sciences"

Concerning: The dissertation developed by the part-time doctoral student Rosen Alexandrov Spasov, at the Section "Pathology" of IEMPAM- BAS on the topic: "Study on the expression of PD-L1 in urothelial carcinoma of the urinary bladder as a basis for a therapeutic strategy", supervised by Prof. Dr. Radostina Ivaylova Alexandrova, for the award of the scientific and educational degree "DOCTOR" (PhD), in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, in the scientific specialty "Morphology" (01.06.26.).

By order of the Director of IEMPAM-BAS No. NO-05-05-2/21.01.2025, I was appointed a member of the Scientific Jury and at its first meeting held on 04.02.2025, I was elected as a reviewer in the ongoing procedure for the defense of the aforementioned Dissertation.

1. Compliance with the procedure.

The set of materials provided to me is in accordance with the list of documents required for the defense of a dissertation for the degree "Doctor" (PhD), according to the RASRB Law - Art. 5 (1), and the Regulations for its implementation - Art. 33 (1), and is in correlation with the Regulations of IEMPAM-BAS. The application contains a total of 9 items with the relevant documents, of which 1 item - the printed dissertation work, 2 and 3 items - bibliography of published scientific articles on the topic of the dissertation, as well as copies of the publications themselves, 4 item - list of participations with scientific communications in scientific forums, 5 item - a report on successfully passed exams and the credits received from them, according to the evaluation system of the Training Center - BAS, 6 item - a report on the fulfillment of the minimum requirements for the acquisition of the degree "Doctor" (PhD) of IEMPAM-BAS, 7 item - protocols and certificates for successfully passed exams according the individual training plan in the specialty, additional specialized training courses, language training and computer skills, in item 8 - opinions of habilitated persons, however, I do not find attachments, which I attribute both to a technical omission and to understated control of the scientific supervisor and the committee for accepting documents, and in the last 9 item - indicated other materials, the CV according to the template, the diploma for completed higher education in medicine from MU Sofia No. 01560 and the diploma for acquired medical specialty in "General and Clinical Pathology" with No. 012395/2009 from MU Sofia are completed.

The available documentation does not contain any signals or objections regarding the deadlines for the procedural steps, the topic of the dissertation, or any other reason for its development and defense, which is why the opinion is that the documents comply with the legal basis, as well as the moral and ethical norms for implementing the procedure for defending the developed dissertation work.

2. Brief biographical and career data about postdoctoral student.

The humane doctor Rosen Alexandrov Spasov studied at the Medical University of Sofia and completed his higher education as a master in 1996. He worked as a resident physician in the Department of Clinical Pathology of the "Dr. N. Vasiliev" Hospital in the town of Kyustendil for almost 10 years (1997-2008). Simultaneously with acquiring a specialty in "General and Clinical Pathology" from the beginning of 2009, he also became the head of the "Clinical Pathology" department at the same workplace and has been so far. He also worked at a second workplace at the Specialized Hospital for Active Treatment and Oncology (SBALO) "Prof. I. Chernozemski" in Sofia from 2012 to 2019, with the main activities being profiled related to biopsy, geffry, cytological and autopsy diagnostics. At the same time, for the period 2016-2019, he also participated as a lecturer - assistant professor in "General and Clinical Pathology" at the Faculty of Medicine of the University Hospital "Lozenets" in Sofia. Then, from 2019 to the present, he is simultaneously a consultant physician in the Department of Clinical Pathology, Biopsy, Geffry and Cytological Diagnostics of the same hospital, as well as Head of the Department of Clinical Pathology, Biopsy, Geffry and Cytological Diagnostics at the "St. Mina" General Hospital in the city of Blagoevgrad.

According to the credit system report at the Bulgarian Academy of Sciences, he has accumulated a total of 338 credits from exams taken, participation in scientific events and printed publications during his scientific development.

3. Relevance of the dissertation topic and appropriateness of the goals and objectives of the work.

The developed dissertation work is thematically related to bladder carcinoma (BC), which is a significant public health problem and one of the leading causes of high mortality in oncological diseases of the urinary system not only in our country, but also worldwide. Its results shed additional light on the oncological issue, to establish the morphological and immunohistochemical characteristics of the expression of the inhibitor ligand - PD-L1, which binds to the transmembrane protein of programmed cell death PD-1, in urothelial carcinomas of the urinary bladder and to establish their dependence on the degree of differentiation in antitumor therapy, as well as the prognosis for rational decisions in treatment with antibodies in combination with other treatment methods, and with various prognostic markers.

In this regard, the choice of the topic is relevant and gives high theoretical and applied value not only for the morphological direction of science, but also for clinical medical, pathological, and especially oncological practice. The relevance of the developed dissertation work also stems from the method of immunotherapy of malignant neoplasias, which has become increasingly popular in the last decade, using inhibitors of immune checkpoints and, accordingly, the treatment of bladder carcinoma, in which urothelial carcinoma, appearing as a heterogeneous disease, with its characteristic histological types and corresponding different molecular characteristics, leads to diverse specifically resulting clinical results.

4. General presentation of the dissertation topic, knowledge and mastery of the issues.

The structure of the presented manuscript is in accordance with the accepted norms for this category of scientific works, there is a good balance between the individual sections, and it is presented in an understandable way in an accessible and literary language, even for a wider circle of specialists. The topic is precisely formulated and points to the essence of the researched scientific problem. The individual parts of the dissertation are appropriately separated and titled. The theoretical part of the dissertation prevails over the empirical part in volume, which is related to the need to analyze a large number of literary sources and review a wide range of issues of theoretical and practical significance. The text, together with 49 figures and 8 tables, is presented on 136 computer-formatted pages and includes the main sections of the content: introduction, literature review, goal and objectives, materials and methods, results and discussion, summary, conclusions, contributions, list of cited sources, list of publications on the topic of the dissertation, and participation in scientific forums.

The literature review is well constructed and formatted, and includes a total of 224 sources, the first of which is a normative document presented in Cyrillic, and all the others are foreign scientific publications and are presented in Latin. Of these, 92 (41.2%) are from the last 5 years, and the others are before that, but these data show that the issues of the dissertation topic are particularly relevant, and at the same time timeless corresponds with the morphological studies in norm and pathology. I have no information that data from the indicated and other sources that are subject to foreign copyright have been used incorrectly. However, I also found about a dozen citations that have incomplete bibliographies and I have marked them for verification in the copy provided to me. The review purposefully introduces the essence and significance of the problem under development, then sheds light on related issues of apoptosis, carcinogenesis and antitumor therapy and tumor antigens, antitumor immune response, types of immunotherapy, expression, biological function, signaling pathway and the role of the PD-1 and PD-L1 combination in neoplasia, as well as checkpoint inhibitors, pathomorphological classification and heterogeneity of bladder tumors, epidemiological and clinico-morphological characteristics, prognosis and recurrence, modern therapeutic strategies and immune checkpoint inhibitors in bladder carcinoma. The data is handled freely and competently, and their interpretation is in connection with the morpho-functional nature of the problem and has the corresponding clinical and diagnostic significance.

This dissertation part is the fruit of conscientious work and demonstrates the broad professional and theoretical culture of the postdoctoral student, who operates adequately with scientific literature, is able to cite sources and present and analyze the various author's opinions. The literary data are examined objectively, with understanding and insight into them, whereby the style of the presentation is completely subordinate to the logic of the theoretical study.

The review ends with the logical conclusion and hope that the initially established PD-1/PD-L1 blockade therapy in malignant oncological diseases of the urinary tract will become

the main method of immunotherapy in the coming years. This determines the need for in-depth studies of this signaling pathway, which also explains the need for permanent research into oncological diseases and, respectively, the personalization of therapeutic strategies, and is an impeccable contribution to the morphological specialty and clinical medical practice.

5. Research methodology.

The goal of the developed dissertation is clearly and specifically formulated, it is related to the study of the morphological and immunohistochemical characteristics of PD-L1 expression in urothelial carcinomas of the urinary bladder and the establishment of their dependence on the degree of differentiation. In connection with its achievement, seven main tasks have been logically set. They are implemented sequentially, with the necessary scientific precision, and provide additional clarification of the developed problem.

The selection of biopsy and resection surgical tumor tissue samples used in the studies was appropriately made, including a total of 110 diagnosed urothelial bladder carcinomas, 1 sarcomatoid variant of bladder carcinoma, 5 metastatic lymph nodes and 9 bone and soft tissue metastases. They are distributed for the relevant histological and clinical studies and are consistent with the tasks and possibilities for statistical processing and reliability of the data. The classical methods used, such as routine histology and staining with hematoxylin and eosin, as well as innovative approaches for inverted and fluorescent microscopy, immunohistochemistry, cell line cultivation, DNA isolation, are adequate for the derivation of the tasks on the topic. The description of the methods used is correct and consistent and gives the impression that the doctoral student has mastered them and can work independently using them, as well as process the obtained data and include them in publications. However, I do not establish data on the period of time over which the histological samples were collected and processed, which would have important informative value, especially in statistical analysis.

6. Assessment of the results of the dissertation.

Traditionally, the Pathology section of IEMPAM works in the field of human pathology, and in particular oncogenesis and antitumor studies, through the scientific supervisor and relevant collaborators. The available capacity is further developed with this topic, adding data for rational diagnosis and therapy of urothelial carcinoma of the urinary bladder in humans, which gives additional weight and importance to the dissertation work.

The own results are presented in it in a clear and understandable way on 37 pages, and simultaneously with their interpretation, the discussion is also conducted. The scientific material is presented in understandable Bulgarian and the data from the obtained results are validated with rich photographic material, which successfully demonstrates the facts of the research process and can be used to illustrate training procedures. The representative quality of the attached figures and the attractively prepared graphs make a good impression. The research was carried out according to the required regulations and the obtained results were subjected to statistical analysis. I find in the presented facts that the qualitative

analysis of the data on the morphological findings when reading the histological preparations is more complete than the quantitative one. The results show that since the attempts to apply antitumor therapy with antibodies in combination PD-1/PD-L1 are still expensive and experimental in nature, there must be a reliable and accurate accompanying diagnostic criterion and protocol, through which the immunological interaction between the tumor and the patient's body can be fully taken into account, including the morphological finding of the tumor-infiltrating immune cells and the degree of invasion of the bladder wall. This reinforces the importance of the in-depth pathomorphological assessment of tumors as the basis for a successful personalized treatment strategy and the perspective for its further development. The results obtained and their significance from the dissertation work are in accordance with the set topic, goals and objectives, and the methodology of implementation.

I would like to share in a critical context that the indicated magnifications of some pictures, in my opinion, are placed inadequately and puzzle the attentive reader about the indicated value, especially when comparing neighboring objects. It is also striking that in places the discussion is poorly represented and even absent, in which the possibility of comparability of the results with the known facts in the literature on the problem under consideration is not used, thus losing the connection for coincidence or difference with the obtained data. I also recommend that the terms from Nomina histologica be used adequately and for their intended purpose. Usually, at the end of each developed dissertation work, a synthesized summary of its essence and implementation is presented, which I do not find in the manuscript version provided to me.

7. Discussion of the conclusions, and contributions of the dissertation.

From the obtained results, 7 conclusions have been formulated, which I accept in essence, since they are directly related to the hypotheses of the study. A gender-separated and age-related correlation of patients with BC is established, and a unified methodological classification has been used. In the oncological finding, moderately differentiated urothelial carcinomas with deep invasion of the subepithelial connective tissue plate of the mucosa - *lamina propria mucosae*, as well as the mucous muscle plate - *lamina muscularis mucosae*, predominate. It was established immunohistochemically that the expression of PD-L1 is up to 25% of the tumors, with high levels of PD-L1 expression being reported mainly in poorly differentiated urothelial carcinomas, in metastatic lymph nodes and most clearly in osteolytic bone metastases. In tumor cell lines from human colorectal carcinoma (HT29), human cervical adenocarcinoma (HeLa), and transplantable rat sarcoma induced with Rous sarcoma virus (RST), no expression of the PD-L1 receptor ligand was detected upon induced programmed cell death and treatment with the cytostatic agent - cisplatin.

The derived contributions have a certain scientific and applied value, with 2 being original, 3 being confirmatory and 1 being applied. They are related and build on the existing framework of knowledge on the topic of the dissertation and can be used for future scientific developments and routine morphological work.

8. Personal impressions.

I have not personally known our colleague Dr. Rosen Spasov up to now. From the brief contact I had with him during the presentation of the materials, it is impressive that the postdoctoral student is an opened, communicative, informed and modern person with developed social skills, correct in his relations with others and dedicated to his professional development. He also presents himself as having very good manual skills and with a strong interest in technical means and equipment for working in a biological environment. As a result of his career development and after implementation in practice, he shows motivation and aspiration for scientific research and teaching work, moreover in one of the most difficult medical disciplines - "Pathology", and the accompanying clinical and diagnostic methods related to the morphology of the organism.

9. Evaluation of the abstract and publications for the dissertation.

The abstract of the dissertation is designed according to the requirements in terms of structure, content and technical aspects, but the summary is missing, which I have already established in the manuscript. It presents the main components of the dissertation and the most important results of the research in a sufficiently comprehensive form. The abstract adequately reflects the dissertation content and allows the scientific community to briefly familiarize themselves with the most important in the developed scientific work, although it also lacks a summary in Bulgarian and English, respectively.

The postdoctoral student has three publications listed in the dissertation work related to his topic, two of which are from the quadrilles Q2 and Q4 and carry 32 points respectively. The report on the implementation of the educational program lists eight printed communications and two full-text reports, and this publication activity satisfies the need for initial scientific appearances of Dr. Rosen Spasov and meets the requirements for doctoral training for the third level of higher education.

10. CONCLUSION:

The dissertation scientific work submitted to me for review on the topic: "Study on the expression of PD-L1 in urothelial carcinoma of the urinary bladder as a basis for a therapeutic strategy", elaborated by part-time postdoctoral student Rosen Alexandrov Spasov, supervised by Prof. Dr. Radostina Ivaylova Aleksandrova, from the Section "Pathology" of IEMPAM- BAS, is a current and complex scientific study, and fully corresponds of the criteria of the ZRASRB and the Regulations for its application. In this regard and with the presented overall assessment, I confidently give a positive opinion on the procedure for defending the dissertation and awarding the humane doctor - Master Rosen Alexandrov Spasov, the educational and scientific degree "DOCTOR" (PhD), in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences, in the scientific specialty "Morphology" (01.06.26.).

The review was submitted on: 14 March 2025 Reviewer's signature: .....

(Prof. DVM Emil Sapundzhiev, PhD, DSc)