

STANDPOINT

under the procedure for acquisition of educational and scientific degree "Doctor"

by Prof. Dr. Ivan Yankov Dechev, PhD, Head of Department of Urology, University Hospital "St. George" Plovdiv, appointed by order № P-19 / 10.01.2021 of the Rector of MU - Plovdiv as a member of the scientific jury

Author: Dr. Milena Gulinac, Department of General and Clinical Pathology at the Faculty of Medicine, Medical University - Plovdiv

Topic: "IMMUNOMORPHOLOGICAL ASPECTS OF UROTHELIAL CARCINOMAS OF THE BLADDER"

Supervisor: Assoc. Prof. Dr. Dorian Ivanchev Dikov, PhD

1. Relevance of the problem developed in the dissertation study in scientific and scientific-applied terms

Bladder cancer (BC) is in the top 10 of solid malignancies in men and women with unpredictably changing tumor biology, making it a serious problem for the global cancer community. According to the distribution of new cases for 2018, it is the fifth most common cancer in both genders and all age groups after malignant diseases of the colon, prostate, lung and breast (Bulgaria Globocan 2018). That is why it continues to arouse unceasing scientific interest due to the proven alarming facts - BC remains one of the main causes of disability and premature cancer mortality, which define it as a significant medical and social problem and a real scourge for the middle-aged human population. Justifiably, nowadays it deserves more attention from the general public, the state and scientists. The desire to reduce local recurrences and disease progression today is becoming more real as a result of significant and profound advances in knowledge of its pathology, epidemiology and genetics. Therefore, Dr. Gulinac' idea to examine in depth the immunomorphological aspects of urothelial bladder carcinomas deserves admiration. The need for serious and in-depth research to assess and analyze morphological diversity in bladder epithelial cells and heterogeneity of urothelial carcinoma in parallel with the expression and activity of a panel of new diagnostically reliable tumor biomarkers suitable for controversial, questionable and not always solvable with conventionally applied pathohistological methods is an innovative, challenging and ambitious task. Judged from the position of the above arguments, the dissertation is characterized by a strong focus, relevance and practical benefit. As far as I

know, in practice we lack such current and complex developments, which determines the need for this study and it is undoubtedly a contribution to our medical science.

2. Knowledge of the problem and literature review

The literature review as a structure and content deserves high praise (useful and cognitive in nature) and shows the excellent awareness of the doctoral student on the research problems. There is a strong emphasis on the epidemiological aspects of BC in the world and in our country, its pathohistological features according to The *2016 WHO Classification of tumors of the urinary system and male genital organs - part II: prostate and bladder tumors* (contains new histological types and subtypes with special morphological signs), which reveal great heterogeneity with a pronounced tendency for different (divergent) differentiation and of urothelial neoplasms with mixed histological characteristics, which makes their diagnosis a modern challenge. In a synthesized form and comparative aspect, the typical histological characteristics of papillary high-grade and low-grade and invasive urothelial carcinomas of the bladder are presented in a concise way - a diverse group of tumors with diverse and nonspecific pathohistological picture and marked terminological disagreement. The pathological staging of urothelial carcinoma of the bladder is presented with generally accepted and internationally accepted criteria for depth of infiltration and degree of malignancy of the primary tumor, positioned in the new stage and pathohistological WHO classifications. The current concept of the interaction between the PD-1 programmed cell death protein and its PD-L1 ligand in normal and cancer cells and that the expression of PD-L1 by tumor cells can suppress the antitumor immune response by inactivating cytotoxic T is presented analytically and accurately. Special attention is paid to the immunotherapy and study of PD-L1 in urothelial carcinoma of the bladder and especially its importance for antitumor immunotherapy with Checkpoint Inhibitors. The current concept of giant cell stromal changes in the bladder mucosa in norm and pathology, morphological findings and confusing terminology, proving them with modern diagnostic methods (histological and immunomorphological) are key issues and theoretical basis of this study and will have important practical application. As a natural and logical conclusion of the literature review is the summary, which explicitly emphasizes that currently the detailed study of BC in morphological terms in the context of current changes in the histological classification of WHO and the latest version of TNM classification is key to adequate diagnosis and treatment.

I find the selected references adequate in quantity and content to outline the current knowledge and unresolved issues in the field of dissertation study. I definitely believe that the dissertation of Dr. Gulinać, dedicated primarily to solving problems related to the diagnosis of urothelial BC is one of the serious challenges of medical science and clinical practice given their problems, diagnostic difficulties and the urgent need to develop an effective approach to mastering them. An indisputable fact is the free and competent handling of oncological terminology, demonstration of wide mastery of the material and creative use of the cited literature, allowing correct and thorough interpretation of the available and received data.

3. Correspondence of the chosen research methodology with the set aim and tasks of the dissertation study

The balanced literature review and the demonstrated competence with which the available results are analyzed have allowed the author to formulate clearly and correctly the purpose and specific tasks of the dissertation, which achieves a good balance in terms of future application of scientific contributions. The 5 set tasks are in accordance with the defined aim and it really helps to achieve it. The expediency is clearly motivated by the formulation of one's own research. The used clinical material is sufficient in volume, well documented, which guarantees the reliability of the results and drawing correct and safe conclusions. In methodological terms, the dissertation study is built correctly and classically. Special and serious attention is paid to the ***protein for programmed cell death PD-1 and the expression of its ligand PD-L1 in the tumor parenchyma and stroma and histological and immunohistochemical study of tertiary lymphoid structures in the stroma of invasive and non-invasive carcinomas of the bladder the relationship between the presence of tertiary lymphoid structures and the expression of PD-L1 in the urothelial BC was considered***. In light of the experimentally revealed relationships between the studied markers and the local tumor stage and grading, their expression is commented, which significantly contributes to the complete formulation of the histological diagnosis of urothelial bladder cancer. The main aim in studying these specific tumor biomarkers is to improve the accuracy in formulating the pathohistological diagnosis of urothelial BC and to avoid diagnostic errors. The difficulties in pathohistological diagnosis are caused by the histological heterogeneity of this malignant neoplasm, the wide range of urothelial lesions with variable biological behavior and the diverse morphological and not always clear and accurate pathomorphological criteria for reliable differentiation of low-grade and high-grade papillary urothelial carcinomas. Through the diversity and definiteness of the obtained results, the set aim (***complex immunomorphological study of urothelial bladder carcinoma with emphasis on the expression of PD-L1 and the immunomorphology of giant cells in the tumor stroma***) was achieved, as well as the main task of a dissertation study. I find the 5 set tasks of the dissertation correctly defined, located on the scientifically "free white fields", corresponding to the tasks set and they really help to achieve it.

The accumulated evidence in the scientific literature, as well as the obtained own results convincingly show that PD-L1 is expressed in 28.8% of cases of urothelial bladder cancer, more often in men between 50 and 80 years and the expression of PD-L1 is more common in invasive high-grade BC and those with squamous cell differentiation. The hypothesis that giant cells in the stroma of the urothelial BC are found in 35.6% of cases with high frequency in high-grade and muscle-invasive carcinomas has been stated and confirmed. This makes these biomarkers key in the diagnosis of BC and they can reasonably be added to the routine immunohistochemical panel. The dissertation study provides new information about their diagnostic significance and reliability and respectively further supports and improves the degree of detection of BC. A significant part of the work is devoted to the study of the main morphological parameters of urothelial carcinoma of the bladder (histological variant and tumor stage), which can be directly applied in clinical practice and can guarantee a scientific approach to this malignant localization. Another expression of the author's competence is the derivation of a differential diagnostic algorithm

from histological, histochemical and IHC criteria for the diagnosis of multinucleated giant cells observed in the stroma of urothelial bladder cancer. Through the diversity and certainty of the results obtained, both the set aim and the implementation of the main tasks have been achieved. The 7 summarized conclusions are convincing, logical and useful.

The discussion of the results obtained by the doctoral student illustrates mastery in performing scientific analysis.

4. Contributions to the dissertation study

The contributions are presented on pages 61 - 63 in the abstract and I agree with them. I believe that the presentation of the tasks and their stratification as original contributions is an original idea of the author and they are entirely of clinical application.

5. Evaluation of the publications on the dissertation study

The list of publications and scientific activity of the PhD student is in accordance with the requirements, thematically all of them are related to the issues of the developed dissertation study and reflect the results of their own research. The dissertation study is based on 3 publications in specialized scientific journals, 2 of which are published in prestigious international journals with IF. The PhD student has 2 participations in scientific forums in our country. Thus, the dissertation study covers (even exceeds) the mandatory scientometric indicators for obtaining the educational and scientific degree "Doctor" of the Medical University - Plovdiv.

6. Opinions, recommendations and notes

I have no significant remarks on the dissertation in terms of composition, method and interpretation. My constructive recommendations from the previous opinions are taken into account in its final formation. My personal impressions from our joint work are that Dr. Gulinac is a highly qualified young specialist with high academic potential. The dissertation leaves a good impression with its graphic design and the scientific language in which it is written. In general, the text is well illustrated with figures and tables that help to better perceive and make sense of the results.

7. Conclusion

Based on the analysis of the dissertation study (developed very competently and with a high degree of originality) in terms of relevance and relevance of the topic, in-depth analysis of the results, the nature of the contributions of their own research, which enrich and further develop clinical oncology. I think Dr. Gulnac has fulfilled the mission in both educational and scientific aspects. Dr. Gulnac demonstrates in-depth theoretical knowledge and professional skills. At the same time, this study is in full compliance with the requirements of the Law on the Protection of the Rights of Persons with Disabilities, the Regulations for its application and the Plovdiv Law on Public Administration. The stated considerations give me grounds to give a completely **positive assessment** of the developed dissertation and **I propose to the esteemed scientific jury to award the educational and scientific degree "Doctor"** of Dr. Milena Gulnac in the field of higher education: 7. Health and sports, professional field: 7.1 Medicine and scientific specialty 03.01.03 "Pathoanatomy and cytopathology".

15.01.2021

Plovdiv

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(Prof. Dr. Ivan Dechev, PhD)

