



To
The chairman of the scientific jury,
Determined by Order № P - 19 / 08.01.2021
of the Rector of the Medical University - Plovdiv
V. Aprilov Blvd. A15A
4002 Plovdiv

I present: Review

under the procedure for acquisition of educational and scientific degree "Doctor"
in the professional field "Pathoanatomy and Cytopathology"

Reviewer: Assoc. Prof. Dr. Julian Rumenov Ananiev, MD, PhD

Scientific specialty "Pathoanatomy and cytopathology"

Institution: Faculty of Medicine, Trakia University

Address and contacts:

Postal address: 6000 Stara Zagora, 11 Armeyska Str

Email: julian.r.ananiev@gmail.com

Phone: 0035942664200

REVIEW

From **Assoc. Prof. Julian Rumenov Ananiev, MD, PhD**

Dean of Medical Faculty, Trakia University, Department of General and Clinical Pathology,
Forensic Medicine and Deontology, and Dermatovenereology,
Faculty of Medicine, Trakia University

of dissertation study for acquisition of educational and scientific degree "PhD"
professional field: **7.1. Medicine "Pathoanatomy and Cytopathology"**

Author: **Milena Gulinac, MD**

Type of doctoral studies: **free doctoral studies**

Department: **General and Clinical Pathology**

Topic: **IMMUNOMORPHOLOGICAL ASPECTS OF UROTELIAL CARCINOMAS OF THE BLADDER**

Supervisor: **Assoc. Prof. Dorian Ivanchev Dikov, MD, PhD**

1. Brief biographical data of the candidate

Dr. Milena Gulinac was born on April 30, 1986 in town of Kumanovo, North Macedonia. She received her secondary education in her hometown. In the year of 2011 she graduated at the Medical University of Plovdiv. From 2013 to 2020 she worked as a resident doctor at St. George Hospital, Plovdiv. In 2016 she started working as an assistant professor in the Department of General and Clinical Pathology at the Medical University of Plovdiv. After acquiring a specialty in 2016, Dr. Gulinac started working at Hospital "Asenovgrad"- Asenovgrad. She is fluent in English and Serbian.

2. Relevance of the topic and problem

Neoplasms of the urogenital system and in particular those of the bladder, are an essential part of human oncopathology. Urothelial carcinoma continues to be among the most controversial in terms of diagnosis, staging, therapy, and prognosis. There are number of studies focusing on the relationship between differentiation and stage on one hand, and prognosis and response to therapy on the other. In the last few years, data related to the use of checkpoint inhibitors in the treatment of neoplastic diseases have been accumulated, including in UC of the bladder. There are also sporadic publications looking for a link between cellular and molecular factors determining the prognosis and development of urothelial cancer.

3. Characteristics of the dissertation study

The dissertation of Dr. Milena Gulinac contains a total of 153 standard pages. It is evident from the content that it consists of the following chapters: introduction, literature review, aim and tasks, materials and methods, results, discussion, conclusions, contributions, bibliography and publications and participation in scientific forums.

At the beginning Dr. Gulinac makes a literature review on the epidemiology, etiology and pathogenesis, clinical, morphological and histological characteristics of urothelial carcinoma. Of particular interest is the comparative characteristics of the systems for histological grading and staging, which is a key point in the construction of some of the subsequent aim and tasks of the study. This is followed by a modern description of the concept of oncogenesis and the influence of the immune system, the interaction between the PD-1 programmed cell death protein and its PD-L1 ligand, and then a description of the role of morphology and significance for clinical response and progression-free survival after administration of neoadjuvant chemotherapy and anti-tumor immunotherapy with checkpoint inhibitors. In a subchapter on the concept of giant cell stromal changes in the bladder mucosa in pathological changes and norm, the author emphasizes the role of "giant cell structures" by comparing with the data found in the literature so far. The chapter ends with five conclusions from the literature review.

This is followed by the chapter "Aims and tasks", in which Dr. Gulinac aims to study the epidemiological, morphological and immunohistochemical features of urothelial carcinoma, focusing on the expression of PD-L1 and the immunomorphology of giant cells in the tumor stroma, as well as to find the diagnostic and prognostic value of these indicators and to compare them with the histological differentiation and the tumor stage. In order to achieve the aim, five tasks directly related to that are described in detail.

In the chapter "Materials and methods" Dr. Gulinac visually describes the retrospective research and current urological material from the bladder, including biopsies and TURs from the biopsy

array of the Department of Clinical Pathology of St. George Hospital - Plovdiv and the Department of Pathology of Grand Hôpital de l'Est Francilien, Jossigny, France, for a period of 4 years. Dr. Gulnac divided the cases into two groups: one group of 28 cases with non-invasive bladder UC, and second group of 77 cases with invasive low-grade and high-grade bladder UC. A control group of patients was also added. Conventional histological and immunohistochemical methods, including and excluding criteria, principles and rules for IHC diagnosis of PD-1 and PD-L1 in UC and others are described in detail. Standard protocols and monoclonal studies for giant stromal cells in the bladder mucosa in urothelial carcinoma are also presented. At the end of the chapter, the statistical processing methods, the significance level of the null hypothesis rejection, and the analysis software are listed.

The chapter "Results" follows, in which the results obtained for each of the set tasks are described, including tables and figures. The results of task 1. present an analysis of the frequency of UC in the Bulgarian and French populations; it has been established that UC is observed mostly in the age decade 60-69 years and more often in men. The presented results show that no statistically significant difference was found in terms of one of the main epidemiological indicators - age, in patients with bladder cancer, depending on the geographical distribution of this type of malignant neoplasia: Eastern Europe / Western Europe. In the results of task 2. it is established that the most common is the classical variant of UC, followed by that with squamous cell differentiation, glandular differentiation, microcystic differentiation, micropapillary differentiation; among UC of the bladder, the largest share is occupied by tumors with invasion of the lamina propria, followed by non-invasive UC; when performing a correlation analysis in search of an association between the degree of TNM invasion and, respectively, gender and age of patients with bladder UC, it was found that there is no statistically significant relationship between them. In the results of task 3. it is evident that in 42/105 cases (40%) it is a bladder cancer with a low degree of malignancy, and in 63/105 cases (60%) - for a cancer with a high degree of malignancy; among the primary diagnosed UC of the bladder, moderate and highly differentiated UC (G2 and G3) according to the WHO classification 1973, respectively those with a high degree of malignancy (HG) according to the WHO classification 2016 occupy a predominant place; the comparison between the three-stage and the two-stage system for assessment of malignancy of tumors under WHO 1973 and under WHO 2016 establishes a pronounced correlation between them; there is a correlation between the age of the patients and the three-level scale for assessing malignancy according to the 1973 WHO; the performed correlation analysis between the histological grading of the UC of the bladder, according to WHO 1973 and WHO 2016 and the TNM staging system, shows a moderately significant statistical association.

The results of tasks 4. and 5. - examination of the IHC expression of PD-L1 in the parenchyma of the bladder UC and search for correlation of the results with the gender and age of patients, TNM stage, histological type and grade of carcinoma, as well as histological and IHC examination of giant cells in the stroma of the bladder, with general pathological and prognostic significance in comparison with the tumor stage, with the histological type and grading of

bladder urothelial carcinoma, are illustrated with many, well selected and high quality photos supported by representative tables and diagrams. At the end of the results of task 5 is presented a generalized algorithm of histological, histochemical and IHC criteria for the diagnosis of multinucleated giant cells observed in the stroma of the UC of the bladder.

In the "Discussion" chapter, within 25 pages, Dr. Gulinac makes an extensive analysis and comparison of the data from her results, comparing them with those found in the literature. Each subchapter here contains an up-to-date and comprehensive literature reference, visually compared with the significant results of the research, and an attempt is made to demonstrate the differences and applicability of the author's own results.

The chapters "Conclusions" and "Contributions" follow, in which Dr. Gulinac presents seven conclusions, as well as numerous theoretical contributions; contributions of a confirmatory nature and scientific-applied and methodological contributions.

The dissertation ends with a "References" containing 157 authors, of which 8 in Cyrillic and 149 in Latin. In Latin large percentage are published in refereed and / or indexed journals.

The work is illustrated with a total: 10 tables, 25 figures and 37 high-quality high-resolution photos.

4. Evaluation of the abstract and publications

To the obligatory attributes of the dissertation study, Dr. Milena Gulinac attaches an abstract - generalized version of the dissertation containing the "essential" parts of the work, presented in an appropriate form and supported by the same figures, tables and photographs.

Dr. Gulinac presents 3 more publications and 2 participations in scientific forums directly related to the topic of the dissertation.

5. Critical remarks

Most of my critical remarks given at the previous stages in the work on the dissertation have been complied with and corrected in the present version.

CONCLUSION

In conclusion, I will summarize that the dissertation study presented in this way is distinguished by its relevance and applicability. The set aim and tasks, as well as their skillful solution and description, highly demonstrate the ability of Dr. Milena Gulinac to analyze, summarize and draw conclusions about the relationship between major morphological and immunomorphological indicators, and their role in the diagnosis, prediction and follow-up urothelial carcinoma.

Based on the above, I believe that the dissertation of Dr. Milena Gulinac is a fully completed scientific product with scientifically applicable and diagnostically significant contributions.

I give my **positive assessment and I will vote for acquisition of educational and scientific degree "PhD"** in the professional field 7.1. Medicine, doctoral program: "Pathoanatomy and Cytopathology" of **Milena Gulinac, MD**, and I call on the other members of the esteemed Scientific Jury to vote positively.

15/01/2021
Stara Zagora



Assoc. Prof. Julian Rumenov Ananiev, MD, PhD