To: The Chairman of the Scientific Jury,

Appointed by Order R-109-131 from 20.02.2025,

By Rector Prof. Dimitar Raykov, MD, PhD,

Medical University –Varna.

STATEMENT

From: **Prof. Elitsa Petkova Encheva-Mitsova, MD, PhD**

Department of Nuclear medicine, Methabolic therapy and Radiotherapy

Head of the Educational Sector "Radiotherapy"

Medical University "Prof. Dr. Paraskev Stoyanov"- Varna

Head of the Radiotherapy Clinic, University Hospital "St. Marina"- Varna

In reference to: Defense of the PhD thesis of **Temenuzhka Rumenova Radeva-Petkova, MD** with title of the thesis "***INVESTIGATING THE EXPRESSION OF NECROTIC CELL DEATH MARKERS AND THEIR PREDICTIVE VALUE IN NEOADJUVANT CHEMORADIOTHERAPY OF LOCALLY ADVANCED RECTAL CARCINOMA"***, with PhD tutor Prof. Elitsa Petkova Encheva-Mitsova, MD, PhD at the Department of Nuclear Medicine, Methabolic therapy and Radiotherapy for obtaining the educational and scientific degree "PhD" in the area of higher education 7. Healthcare and Sports, in the professional direction 7.1. "Medicine", and the PhD program "Medical Radiology and Roentgenology speciality (including the use of radioactive isotopes)

Information about the Procedure

By Order No. R-109-131 from 20.02.2025, of rector Prof. Dimitar Raykov, MD, PhD, Medical University –Varna, I was appointed as a member of the scientific jury and participate with a statement.

The submitted documents comply with the regulations for obtaining a PhD degree, in accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria (dated 19.07.2022) and the Rregulations for the Development of Academic Staff at the Medical University- Varna (dated 08.07.2024).

Biographical Data of the PhD student

Dr. Temenuzhka Rumenova Radeva-Petkova graduated Medical University - Varna in 2013 with obtaining degree of Medical Doctor. Since January 2015, she has been working as a physician at the Radiotherapy Clinic of University Hospital "St. Marina," Varna, and since 2016, she has been an assistant professor at the Department of „Nuclear Medicine, Metabolic Therapy, and Radiotherapy" at the Medical University "Prof. Dr. Paraskev Stoyanov". She teaches classes for medical and dental students in Bulgarian and English. In 2023, she pass the state exam and become specialist in radiotherapy. On April 24th 2017, she become a Ph.D. student in Medical Radiology and Roentgenology specialty (including the use of radioactive isotopes) at the Department of "Imaging Diagnostics, Interventional Radiology and Radiotherapy at Medical University, Varna. On May 13, 2023, she was allowed to undergo PhD diffence.

Structure of the PhD thesis

The PhD thesis of Dr. Temenuzhka Rumenova Radeva-Petkova, is written on 140 pages, structured according to standard requirements, including the following chapters: Introduction— 2 pages; Literature Review of the topic— 47 pages; Aim and tasks— 1 pages; Materials and Methods — 13 pages; Results— 41 pages; Discussion — 7 pages; Conclusions — 1 pages; Contributions of PhD thesis — 1 pages. The PhD thesis contains 16 tables and is illustrated with 57 figures. The references list includes 262 papers, of which 2 in Bulgarian and 260 in English.

Evaluation of the Relevance of the Topic

The topic of the PhD thesis is highly relevant, considering that rectal carcinoma (RC) is one of the leading causes of death among cancer patients. The management of RC is a clinical challenge. The current standard of treatment of locally advanced rectal carcinoma (LARC) is a multimodal approach, including neoadjuvant radiotherapy combined with chemotherapy (nCCRT), followed by total mesorectal excision (TME) and adjuvant chemotherapy (ACT) based on fluoropyrimidines. The therapeutic response among patients varies significantly. The identification of biomarkers associated with necrotic cell death induced by ionizing radiation may help predicting the individual therapeutic response. Studying the expression of these markers could allow for a more personalized treatment strategy and help identify patients who are most likely to benefit

from nCCRT. Necrosis, as a form of cell death, is related to several processes such as cell proliferation, autophagy, inflammation, and immune response. The study of specific markers could reveal new mechanisms of tumor response to treatment, which in turn could enable the prediction of therapeutic outcomes and the adaptation of treatment. Research in this field is significant for both clinical practice and scientific studies aimed at improving therapeutic results and the quality of life for patients.

Evaluation of the Literature Review of the topic

The literature review is in-depth, comprehensive, and its structure aligns with the stated aim and tasks. The literature review of the dissertation is presented over 47 pages, where the author thoroughly analyzes the epidemiology of RC, the etiology, risk factors, histological types, imaging methods for tumor staging, treatment of LARC, and predictive markers for the response to nCCRT in LARC. The imaging methods for staging and evaluating the response after nCCRT are discussed in depth. A detailed analysis is provided on the various types of cell death, markers of necrotic cell death, and their predictive and prognostic roles in different tumor diseases. The review also extensively covers HMGB1 (a marker for necrotic cell death), its secretion, structure, role in carcinogenesis, and its predictive and prognostic significance. A total of 262 scientific papers are discussed, including 2 in Bulgarian, highlighting the existence of a few studies and a limited amount of summerized data on the role of HMGB1 in RC. This demonstrates Dr. Radeva's extensive knowledge in the field and her ability to analyze the available scientific literature. The PhD thesis is one of the few existing reports that investigate and analyze the role of HMGB1 in nCCRT for LARC in a significant number of patients.

Aim and Tasks

Nine main tasks are clearly and precisely formulated, logically following and corresponding to the defined aim.

Materials and Methods

The materials and methods are appropriately selected to achieve the PhD thesis aim and objectives. The prospective study included a total of 65 patients with LARC. It was conducted at the Radiotherapy Clinic of University Hospital "St. Marina" in Varna

between 2015 and 2021. Venous blood samples were obtained from all patients to measure the serum concentration of HMGB1 both before the start of nCCRT and in the end of the treatment. An ELISA kit was used to determine HMGB1 levels in human plasma. Detailed clinical data for each patient are presented. The radiotherapy method is described in depth, encompassing the full range of modern imaging techniques used to define the target volumes, such as MRI and PET/CT, along with the application of advanced planning and RT techniques: VMAT (Volumetric Modulated Arc Therapy), IGRT (Image-Guided Radiation Therapy), and SIB (Simultaneous Integrated Boost).

Appropriate modern statistical methods were applied for data analysis.

Results and Discussion

The results obtained after the statistical analysis of the data are adequately and systematically presented over 48 pages, illustrated with extended tables and graphs. When comparing the average HMGB1 concentrations before and after nCCRT, it has been observed that the mean HMGB1 concentration in the serum of patients after nCCRT (M = 9.94, SD = 4.60) was significantly higher than that before nCCRT (M = 6.61, SD = 2.25), with a p-value of <0.0001. When patients has been grouped based on complete and partial responses versus stable disease and disease progression, a significant difference was observed in the HMGB1 concentration values after treatment (p = 0.030). The concentration values are notably higher in patients with stable disease and disease progression. ROC analysis reveals that HMGB1 concentration values above 7.73 ng/ml after nCCRT may potentially serve as a predictor of poor treatment response (AUC = 0.657, 95% CI: 0.524–0.790, p = 0.034), with a sensitivity of 74% and specificity of 50%. ROC analysis reveals that an increase in HMGB1 concentration by 2.02 ng/ml after nCCRT may potentially serve as a negative predictor for an unfavorable therapeutic response (AUC = 0.727, 95% CI: 0.603–0.851, p = 0.02), with a sensitivity of 77.4% and specificity of 56.9%. The applied regression analysis shows that an increase in the difference in HMGB1 concentration before and after nCCRT is also associated with a poor response to the treatment (odds ratio = 1.254, 95% CI = 1.068–1.474, p = 0.006).

The discussion is thorough and consistent, comparing the extensive results of Dr. Radeva's PhD thesis work with the current scientific knowledge in the field. The achieved results are in concordance with those reported in the literature and contribute to the global research knowledge in that field. The discussion clearly demonstrates that the author has

a detailed understanding of the studied subject, with profound clinical approach and excellent professional background. Future work in this research field is planned.

Conclusions

The 8 main conclusions are clearly and precisely formulated, optimally reflecting the results achieved in the tasks and objectifying the aim of the PhD thesis.

The conclusions indicate that the average HMGB1 concentration in the serum of patients after nCCRT is significantly higher compared to the levels before nCCRT. An important finding is that there is a significant difference in the HMGB1 concentration values after the completion of therapy, depending on the therapeutic response, with higher concentrations observed in patients with stable disease and disease progression. It is found that the increase in HMGB1 concentration, over time, may potentially indicate a poor response to the treatment. The results demonstrate the predictive significance of serum HMGB1 levels after nCCRT and their dynamics in relation to the therapeutic response, with higher HMGB1 levels after treatment being associated with an unfavorable therapeutic outcome. It logically follows that an increase in the difference in HMGB1 concentrations before and after nCCRT is also associated with a negative treatment response. Additionally, SUVmax values greater than 8.7 before nCCRT may potentially serve as indicators of a poor response to the treatment. All patients have excellent tolerance to the nCCRT, modern RT techniques (IMRT, VMAT, IGRT) and oral capecitabine

Contributions

The contributions presented in the PhD thesis reflect the objective achievements of Dr. Temenuzhka Radeva. The contributions of the PhD thesis have an original, scientific-theoretical, and applied scientific character. The formulated contributions are based on the author's own data from the scientific study. The most important of them are:

1. This is the first study in Bulgaria to examine and investigate the serum concentration levels of HMGB1 in patients with LARC.
2. For the first time in Bulgaria is reported the potential role of serum HMGB1 concentration levels and their dynamics as a predictive marker indicating the effect of nCCRT.

3. This is the first report in Bulgaria about the potential role of serum HMGB1 concentration levels and their dynamics as a predictive marker for the effectiveness of nCCRT.

4. This is the first report in Bulgaria about the potential role of SUVmax as a predictor of response to neoadjuvant chemoradiotherapy (NCRT) in patients with rectal carcinoma (RC).

5. For the first time in Bulgaria, a standardized group of patients with LARC has been reported, staged using modern and high-tech imaging methods (MRI, PET/CT), meeting the global standard of radiotherapy practice.

6. This is the first report in Bulgaria on the implementation of advanced radiotherapy techniques (IMRT, VMAT, IGRT, and SIB – Simultaneous Integrated Boost) in nCCRT for locally advanced rectal cancer (LARC).

7. A standardized contouring protocol based on MRI and PET/CT data is applied for the first time in Bulgaria.

8. The patients follow-up included colonoscopy and imaging methods to determine the therapeutic response after nCCRT.

9. Worldwide, this is one of the few studies investigating the role of HMGB1 in nCCRT for LARC, and it includes a significant number of patients.

Publication Activity

In relation with the PhD thesis, the PhD student has published 3 publications and 4 participation in a scientific forum in the country with a presentation were made.

Abstract of the PhD thesis

The abstract of the PhD thesis accurately reproduces the content of the PhD thesis and is composed according to the requirements. It is presented over 72 pages and includes 42 figures and 14 tables.

Personal Impressions

I have known Dr. Radeva for 10 years. She possesses the necessary theoretical knowledge and professional skills as a radiation oncologist and demonstrates the qualities and abilities required for conducting independent scientific research, representing her as a promising young medical doctor and scientist.

Conclusion

The PhD thesis of Dr. Temenuzhka Rumenova Radeva- Petkova is of high scientific and practical value on a significant topic in radiotherapy practice. This topic has significant clinical and scientific importance worldwide. In oncology, there is an increasing need on personalized treatment strategies. If markers for necrotic cell death can predict the efficacy of nCCRT, it could lead to adaptive therapies, reduced unnecessary toxicity, and improved survival rates.

I consider that the presented PhD thesis, abstract of the PhD thesis and published scientific papers meet the scientometric criteria of the Law for the Development of Academic Staff in the Republic of Bulgaria and the Regulations for the Development of Academic Staff of Medical University Varna for awarding the educational and scientific degree "PhD".

As a PhD tutor and member of the Scientific Jury, I give my positive assessment and recommend to the honored members of the Scientific Jury to award Dr. Temenuzhka Rumenova Radeva- Petkova the educational and scientific degree "PhD".

Заличено на основание чл. 5, §1, б. „В“ от Регламент (ЕС) 2016/679

Varna, 07.04.2025 Pr[redacted]D, PhD