Peer Review

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Accredited in professional direction 7.1
Appointed by Order No. R-109-131 from 20.02.2025, as an external member of the Scientific Jury, by Decision of the Scientific Jury in accordance with Protocol No. 1/27.02.2025 to prepare peer reviews in Bulgarian and English

To

Temenuzhka Rumenova Radeva- Petkova, MD

Author of a dissertation thesis for the acquisition of the educational and scientific degree "PhD" in the area of higher education 7. Healthcare and Sports, Professional Direction 7.1 Medicine, Scientific Specialty "Medical radiology and Roentgenology speciality (includinguse of radioactive isotopes)

Title of the dissertation thesis:

"INVESTIGATING THE EXPRESSION OF NECROTIC CELL DEATH MARKERS AND THEIR PREDICTIVE VALUE IN NEOADJUVANT CHEMORADIOTHERAPY OF LOCALLY ADVANCED RECTAL CARCINOMA"

Ph.D tutor

Prof. Elitsa Petkova Encheva- Mitsova, MD, PhD

Varna, 2025

The dissertation thesis was discussed, approved, and forward for defense by the Departmental Council of the Department of "Nuclear Medicine, Metabolic Therapy and Radiotherapy", Faculty of Medicine, at the Medical University "Prof. Dr. Paraskev Stoyanov"-Varna.

1. **General Overview of the Procedure and Documents:**

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 Rules for the Development of Academic Staff at the Medical University- Varna (dated 08.07.2024).

2. Relevance of the Dissertation Topic, Aim and Tasks

Dr Radeva's dissertation has an important role for oncology and radiotherapy practice. The study of an important social contingent of patients with advanced rectal cancer (LARC) is essential for the establishment of standard practice in Bulgaria - neoadjuvant concurrent chemoradiation (nCCRT) and the evaluation of the method for the quality of life of the patient depending on the time of administration after (nCCRT) and the volume of surgery. Molecular biomarkers are being widely investigated as potential predictors of treatment response, in the case of nCCRT, because of the alternative possibility of total neoadjuvant chemotherapy or other therapy. So far, no biomarker has been validated for such routine use in clinical practice. The scientific hypothesis underlying the dissertation is an attempt to demonstrate the predictive and prognostic value of HMGB1 (High Mobility Group Box 1 (HMGB1)). Its localization in the nucleus is associated with the regulation of DNA damage repair caused by RT and CT, while outside the nucleus, it is associated to cell proliferation, autophagy, inflammation, and immunity.

The current dissertation aims to investigate the key markers of necrotic cell death and their predictive value in assessing the response to administered nCCRT treatment in LARC.

The tasks are clearly and specifically formulated to address the objective.

3. Dissertation structure:

The dissertation is written on 140 standard pages and includes 16 tables and 57 figures. References include 262 literature sources, of which two are in Bulgarian and 260 are in English. The dissertation thesis is well structured, the proportions of the separate sections are balanced and it meets the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria.

4. Literature Review:

The literature review is analytical and focused on identifying the problems that require a solution.

5. Materials and Methods:

The applied clinical methods are accurately and precisely described, utilizing the full range of modern imaging techniques for tumor volume assessment, such as MRI and PET/CT. RT for LARC has been performed according to global standards, characteristic of one of the leading radiation oncology clinics in Bulgaria.

A comprehensive statistical framework has been applied, which the PhD candidate handles competently and skillfully in this challenging task of identifying statistically significant results.

A significant group of 65 patients has been treated, and for the first time, the results of modern nCCRT have been published with a detailed analysis of DFS and OS- an important contribution to Bulgarian clinical practice, aligning with global outcomes. The author critically evaluates the study, highlighting the non-standard surgical approach, independent of the radiation oncologist, where the full effect of CRT is not awaited for 6–

8 weeks (recently established at 10 weeks), especially when aiming to avoid mutilating surgery.

6. Results and Conclusions:

The PhD candidate conducts a detailed statistical analysis to determine whether the concentration of HMGB1 before and after CRT in different groups follows a normal distribution. However, I do not see a biological sense for this approach. Following her scientific hypothesis, she classifies the patients into those who achieve a complete or partial clinical response to the administered therapy and those with stable disease or disease progression. In these two groups, she examines the concentration of the studied biomarker in an attempt to identify a statistically significant correlation. She succeeds by applying the Jonckheere-Terpstra and Mann-Whitney tests, demonstrating that in cases with poor response to CRT, HMGB1 levels after treatment are significantly higher ($p = 0.030$). This suggests that a larger proportion of HMGB1 secreted into the extracellular environment plays a role in tumor progression. Other studies reveal that this occurs through the RAGE receptor. Through ROC analysis, a cutoff value of HMGB1 above 7.73 ng/mL after CRT is identified as a potential predictor of poor response to treatment (AUC = 0.657, 95% CI: 0.524–0.790, $p = 0.034$). This finding aligns with other precise experimental studies, revealing a novel mechanism by which HMGB1, released from dying cells during RT or CT, can stimulate the proliferation of viable tumor cells. Furthermore, inhibition or genetic ablation of HMGB1 suppresses tumor cell proliferation, highlighting a potential opportunity for targeted therapy. An interesting finding is that higher SUVmax values above 8.7 on FDG PET-CT may potentially serve as negative predictors and be associated with a poor therapeutic response. This is an additional finding, as the method is primarily used for staging and further treatment planning in radiotherapy. However, according to literature data, most studies do not find a significant correlation between SUVmax values and disease-free survival, overall survival, local recurrences, or the clinicopathological characteristics of patients. An important stage lies ahead for the PhD candidate and the team at the Radiation Therapy

Clinic - to conduct a long-term assessment of treatment outcomes. This includes evaluating overall survival, disease-free survival, and the effectiveness of the non-surgical "watch and wait" approach in cases of low rectal cancer where anus praeter would otherwise be necessary. The conclusions can be summarized into three main points:

1. The concentration levels of HMGB1 in cases of stable disease and disease progression are significantly higher than in cases with complete or partial response after nCCRT for LARC.

2. An increase in HMGB1 concentration by 2.02 ng/mL may potentially serve as a negative predictive marker of poor response to treatment ($p= 0.02$), with a sensitivity of 77.4% and specificity of 56.9%. This leads to the conclusion that higher HMGB1 concentrations after CRT are associated with a poor therapeutic response (odds ratio= 1.129, 95% CI= 1.006–1.267, $p= 0.039$). This finding could be utilized for the implementation of total neoadjuvant therapy or other individualized approaches, particularly in low rectal tumors.

3. A pre-treatment SUVmax value above 8.7 on FDG PET-CT may potentially serve as a negative predictive marker of poor response to treatment ($p= 0.03$).

7. I evaluate the contributions of the dissertation in two aspects:

Scientific Contribution:

1. For the first time in Bulgaria, the serum concentration of HMGB1 and its dynamics according to the response to nCCRT for rectal cancer (RC) are studied as a predictive marker for treatment individualization. Globally, this is one of the few studies conducted on a significant cohort of 65 patients, investigating the role of this biomarker in this specific treatment approach for RC.

Practical Contribution:

1. Advancements in High-Tech Radiotherapy – For the first time in Bulgaria, the application of modern planning and irradiation techniques such as VMAT (Volumetric Modulated Arc Therapy), IGRT (Image-Guided Radiotherapy), and SIB (Simultaneous Integrated Boost) are reported and thoroughly described in the dissertation's abstract. This has an educational significance for young Bulgarian radiation oncologists.

2. It is worth mentioning that the PhD candidate fluently manipulates and applies statistical methods and tools, this allows identification of subtle differences in clinical parameters.

3. In FDG PET/CT, a statistically significant SUVmax value above 8.7 before rectal cancer treatment has been identified as a potential negative predictive marker of poor response to preoperative CRT.

8. Publications on the Dissertation:

In accordance with the Regulations for the Development of Academic Staff the PhD candidate has published PhD thesis results in three reports- in two publications as the first author and one publication as a co-author. She has participated in four scientific forums, including one poster presentation abroad at ESMO.

The dissertation summary is presented in a volume of 72 pages and contains the main chapters of the dissertation thesis, into 42 figures and 14 tables.

9. Personal Contribution of the PhD Candidate:

Dr. Radeva has independently collected, processed, and structured the results and conclusions, which form the basis of the scientific and practical contributions I have outlined. This is considered her personal achievement, supported by her PhD tutor, Prof. E. Encheva.

10. Critical Remarks and Recommendations:

The conclusions should be more concise to clearly highlight how the dissertation achieves its objective.

Similarly, the proposed contributions should be optimized - from nine down to four, with one scientific and three practical contributions.

A crucial clinical phase lies ahead for the PhD candidate and the Radiation Therapy Clinic team- a long-term follow-up on the effects of CRT regarding overall survival, disease-free survival, and the impact of a non-surgical ("watch and wait") approach, particularly in low rectal cancer cases requiring anus praeter.

Conclusion

The documents presented by Temenuzhka Radeva, MD meet the requirements set forth in the Law on the Development of Academic Staff in the Republic of Bulgaria (2022) and the Rules for the Development of Academic Staff at the Medical University of Varna (2024).

The dissertation is original, contributing to the clarification of an interesting and previously unexplored concept. It presents a large dataset, which has been analyzed using various statistical methods. The conclusions demonstrate both scientific and practical significance, and the number of publications is sufficient to support the research findings. This gives the arguments for my positive review, and I recommend to the esteemed scientific jury to award Temenuzhka Rumenova Radeva- Petkova, MD with the educational and scientific degree "Ph.D." in the scientific specialty "Medical Radiology and Roentgenology, including the use of radioactive isotopes".

25.03.2025

Prof. Tatiana Dimitrova Hadjieva, MD, PhD, DSc