To

The Chair of the Scientific Jury

as announced by Order No. P-109-42/16. 01. 2026

of the Rector of MU – Varna

REVIEW

by Prof. Dr. Krum Sotirov Katsarov, MD, DSc

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Regarding: Dissertation on the topic**: "The Role of Endoscopy in Assessing Response Following Neoadjuvant Chemoradiotherapy in Rectal Cancer" for the award of the academic and scientific degree of "Doctor"** in the scientific specialty "Gastroenterology" – 7.1.

Author: Dr. Alexander Dimitrov Trifonov, full-time doctoral student at the Second Department of Internal Medicine, Faculty of Medicine, Medical University of Varna, with training based at the Gastroenterology Clinic, University Hospital "St. Marina" EAD – Varna.

Scientific supervisors: Assoc. Prof. Dr. Alexander Kanev Zlatarov, MD

1. General presentation of the procedure and the doctoral student

The set of materials and documents submitted by Dr. Alexander Trifonov in both hard copy and electronic format complies with the requirements of the Law on the Development of Academic Staff of the Republic of Bulgaria, The

Regulations for its Implementation, the Higher Education Act, and the Regulations on the Development of the Academic Staff at MU Varna, namely:

- Dissertation
- Abstract
- List of scientific publications
- Curriculum vitae, diplomas, orders, minutes, etc.

The doctoral student has published 1 (one) paper on the topic.

Dr. Alexander Trifonov was enrolled as a regular doctoral student by Order No. P-109-552/03.12.2021 of the Rector of MU-Varna, in the doctoral program "Gastroenterology," professional field 7.1 Medicine in the higher education field 7. Healthcare and Sports.

At a meeting of the Departmental Council No. 102-3328/19.12.2025 and by Decision of the Faculty Council of the Faculty of Medicine—Minutes No. 103-291/14.01.2026, the doctoral student was granted a one-year extension with the right to defend his dissertation.

The set of documents meets the requirements, and after reviewing the procedure for the development, presentation, and implementation of the doctoral program, I consider them to be in compliance with the regulatory framework. The submitted documents demonstrate the legality and completeness of the training and procedure conducted.

2. Brief biographical information about the doctoral candidate

Dr. Alexander Trifonov was born in 1994. He graduated with honors with a Master's degree in Medicine from the Medical University of Varna in 2019. From 2019 to 2020, he worked as a physician in the emergency department at St. Marina University Hospital in Varna. In 2020, he won a competitive selection for the position of assistant professor at the Second Department of Internal Medicine, Medical University of Varna. He completed his residency in gastroenterology in 2024. He currently works in the Department of Gastroenterology and Endoscopic Diagnostics at St. Marina University Hospital, Varna.

3. The dissertation comprises 139 pages and contains all required sections, distributed in an optimal proportion in terms of volume, illustrated with 21 figures, 28 tables, and 5 appendices. One publication related to the dissertation is presented.

Significance of the problem:

Malignant diseases of the rectum are a significant health and social problem due to the clear trend of increasing incidence, the lack of a national screening program, and a clear strategy for evaluating the effect of treatment. On the other hand, following the introduction of advanced surgical strategies, neoadjuvant chemoradiotherapy, and total neoadjuvant therapy, a significant improvement has been achieved in the rate of organ-preserving treatment, local control, and survival in these patients. Literature data indicate that with strict patient selection, total neoadjuvant therapy can provide oncological outcomes comparable to standard surgery. In this regard, endoscopic evaluation is an essential part of the diagnostic workup assessing the presence of recurrence, the need for surgical intervention, and subsequent clinical follow-up.

4. The literature review (LR) is 56 pages long, written in clear and accessible language, and provides a critical presentation and analysis of the data presented. A total of 113 references are cited, all directly related to the subject of the study. It consists of 11 chapters.

The first and second chapters are devoted to the analysis of data related to the epidemiology and etiopathogenesis of rectal carcinoma. Other key issues related to screening, the diagnostic algorithm, surgical strategy, adjuvant and neoadjuvant therapy, organ-preserving strategies, as well as algorithms for monitoring the treatment are also discussed. In other words, the first and second chapters are "foundational" and structure the subsequent analysis of information related to the problem under consideration.

Chapter 3 is devoted to the place and role of neoadjuvant chemoradiotherapy (NCRT) and total neoadjuvant chemotherapy (TNT) in the treatment algorithm for rectal carcinoma. The principles and possible variants of the cited methods are discussed in detail, as well as the sequence of the applied methods, patient selection, and possible side effects. Special attention is given to systems for assessing tumor response and regression. The role of these two methods in the current standard of care for rectal cancer is presented, as well as future therapeutic strategies based on these methods.

Chapter Four is devoted to methods for assessing therapeutic response. The author presents the main prognostic criteria used to evaluate treatment outcomes—pathological complete response and histopathological assessment of tumor regression. The author also presents the standardized criteria for imaging assessment of therapeutic response—RECIST and PERCIST—based on CT, MRI, and PET criteria. The role of endorectal sonography in local imaging assessment is also examined as a method for evaluating the effect of the administered treatment.

Special attention is given to the standardization and limitations in the assessment of therapeutic response and the key role of the multimodal approach in these cases.

Chapter Five is pivotal, as it presents an analysis of information relevant to the author's research interests in the dissertation—the role of endoscopy in the diagnostic and evaluation phases of treatment and in monitoring outcomes following treatment for rectal carcinoma. Dr. A. Trifonov discusses in detail the various stages—initial diagnosis, options for endoscopic treatment of early rectal carcinoma (EMR, ESD, and eFTR), as well as transanal surgical endoscopic techniques (TEM and TAMIS). He outlines the timing and setting for performing endoscopy as the primary method for assessing therapeutic response in rectal carcinoma. The three categories used to classify the endoscopic response are presented, as well as the role of magnifying chromoendoscopy and fluorescence endoscopy as technologies that enhance assessment capabilities. The author has analyzed the diagnostic and prognostic relationship between endoscopy and imaging studies—specifically MRI with the advanced capabilities of DWI-MRI—emphasizing the critical importance of a multimodal approach in the evaluation of these patients.

The following chapters are devoted to the limitations of the diagnostic capabilities of the methods used to assess therapeutic response and their comparative efficacy.

Chapters 7, 8, and 9 are devoted to the so-called "watch and wait" strategy in the treatment of advanced rectal carcinoma. The author examines the concept, related studies, and meta-analyses to outline the place of this method within the overall therapeutic regimen. The criteria for patient selection and prognostic factors for achieving a complete clinical response are discussed, as well as the monitoring protocols employed. It is emphasized that these are based on a multimodal approach integrating endoscopy, MRI, and, more recently, ctDNA as prognostic

criteria. Surgery for local recurrence and the role of distant metastases as a prognostic factor determining survival in these patients are discussed. Survival and quality of life are compared with those of patients undergoing standard surgical techniques. Issues related to the standardization of patient selection and follow-up, as well as the cost-effectiveness of the method, are analyzed.

The last two chapters are devoted to future directions and expected outcomes related to the development of personalized medicine and the introduction of new imaging technologies and biomarkers associated with the biology of rectal carcinoma.

5. The objective of this study is clearly defined and based on the results of the literature review: "To conduct a retrospective, observational, single-center cohort study in patients over 18 years of age with rectal carcinoma who underwent neoadjuvant chemoradiotherapy, aimed at determining the role of endoscopy in assessing the response to the treatment."

Six tasks were correctly formulated to address all aspects of the problem.

6. Materials and Methods. The study is based on a retrospective analysis of 157 patients over 18 years of age who underwent neoadjuvant chemoradiotherapy between 2019 and 2024. The study is defined as a retrospective, observational, single-center cohort study of patients over 18 years of age with rectal carcinoma treated with neoadjuvant chemoradiotherapy at the Department of Radiation Oncology at UMHAT "Sveta Marina" EAD – Varna, during the period 2019–2024. Inclusion and exclusion criteria were defined based on:

Disease stage—patients with rectal carcinoma in non-metastatic stage II/III (T2–4; N0–2; M0) according to the TNM classification. The stage of the disease was determined via pelvic MRI, CT with intravenous contrast enhancement of the chest and abdomen, and 18F-FDG PET/CT.

Endoscopic assessment of tumor location and extent—based on distance from the anal verge (three groups) and circumferential extent (four groups). Serum CEA tumor marker levels were measured in all enrolled patients as part of the routine clinical evaluation prior to initiation of neoadjuvant therapy and during follow-up after its completion.

MRI tumor characteristics—local tumor characteristics were assessed using T2-weighted MRI (T2-MRI) and DWI-MRI.

Endorectal ultrasound and PET were also included in the diagnostic evaluation algorithm.

After applying exclusion criteria, 103 patients were identified as eligible for the study and subsequently divided into three groups based on endoscopically assessed clinical response.

Neoadjuvant chemoradiotherapy.

Two standard regimens were administered to the patients:

- 28 fractions of 1.8 Gy to a total tumor dose of 50.4 Gy
- 25 fractions of 2.0 Gy to a total tumor dose of 50 Gy

Chemotherapy - All patients received concurrent chemotherapy with Capecitabine alongside radiation therapy; the dosage was as per protocol, 825 mg/m^2 twice daily, taken orally on radiation days throughout the entire course of radiation therapy.

Endoscopic biopsy and response scales - Endoscopic assessment of the therapeutic response was performed within 6–8 weeks after the end of nCRT. According to the endoscopic classification introduced by the MSKCC for assessing response after neoadjuvant therapy in rectal cancer, three groups are distinguished: clinical complete response (cCR), nearly complete clinical response (nCR), and incomplete clinical response (iCR), in accordance with the relevant criteria.

MRI assessment of post-therapeutic tumor regression—magnetic resonance imaging (MRI) assessment of tumor regression is performed within 6–8 weeks after completion of neoadjuvant chemotherapy (nCRT). The MRI scan is performed on the day following the endoscopic examination. The degrees of tumor response are defined in five groups—mrTRG1–5.

Pathological evaluation of the surgical specimen—the degree of tumor infiltration after nCRT—is assessed according to the TNM criteria: urT 0–urT4b. UrN and the degree of tumor regression according to Dworak (TRG 0–4) are also determined.

Statistical methods—analyses were performed using two specialized statistical software packages—Jamovi version 2.6.3 and IBM SPSS Statistics version 25. Jamovi was used primarily for descriptive and comparative analyses, χ^2 tests, nonparametric comparisons, correlations, and graphical visualizations of categorical distributions. SPSS was used for Kaplan–Meier survival analyses, modeling of time-dependent relationships, and creating graphical representations of survival curves.

7. The results and discussion are presented on 32 pages, with the results organized into eight subgroups corresponding to the six research objectives formulated by the author. They are well illustrated with 28 tables and 21 figures, which, together with their detailed descriptions, facilitate the understanding of the

presented material. The data obtained are a consequence of the research vectors established by the author. The summarized results show that:

Demographic indicators reveal a slight predominance of men, a mean age of approximately 67 years, and a high prevalence of concomitant cardiovascular and metabolic diseases. More than half of the tumors are located in the lower rectum, and circumferential involvement is ≥50% in a significant proportion of patients. EMVI-positivity and MRI-confirmed lymph node involvement are observed in a large proportion of patients, reflecting an advanced local stage at diagnosis, and to some extent differ from patients in the cited more selective clinical trials.

In the majority of patients, an endoscopically incomplete clinical response is observed, with the frequency of endoscopically confirmed complete clinical response (cCR) being 5.8%, and near-complete response (nCR) occurring in approximately 10%. Pathological complete response (pCR, Dworak 4) is observed in approximately one-tenth of the operated patients. Most patients remain at stage ypT2–3 and ypN0–1, indicating that in a significant proportion of patients, neoadjuvant therapy leads to partial, but not complete, tumor regression. The pCR rate in the analyzed cohort falls at the lower end of the published range for standard long-course neoadjuvant chemoradiotherapy, in which pCR typically ranges between 10% and 25% depending on the treatment regimen, stage distribution, and interval to surgery. Similar results have been reported in classic studies of preoperative chemoradiotherapy and in some cohorts treated with conventional regimens without escalation of systemic therapy. The lower incidence of cCR in the study population likely reflects the more unfavorable initial tumor profile (high proportion of EMVI+, positive lymph nodes, large circumferential extent), as well as the fact that the data are from routine practice rather than a strictly standardized clinical protocol.

Analysis of prognostic factors for endoscopically confirmed complete clinical response reveals: smaller maximum tumor diameter, lower baseline CEA levels, as well as less frequent presence of EMVI and compromised mesorectal fascia in patients with cCR. Due to the small number of cases with cCR, these trends do not reach statistical significance, but they outline a profile of a patient with locally confined disease and favorable tumor characteristics.

A comparative analysis between the groups with good (cCR+nCR) and incomplete endoscopically confirmed clinical response (iCR) demonstrated a higher frequency of favorable response in distally located tumors (0–6 cm) compared to more proximal locations, albeit with a limited number of observations.

Regarding the impact of the interval between the end of neoadjuvant chemoradiotherapy and the first follow-up endoscopy on the pathological response, a median of 61 days was observed, with a wide range of variation. The incidence of an unfavorable pathological response remains relatively constant during the first 6–8 weeks after completion of therapy.

Data evaluating the prognostic value of clinical, endoscopic, and imaging indicators for achieving a complete pathological response indicate that, in the multivariate logistic regression model, only endoscopically confirmed cCR remains an independent, statistically significant predictor associated with an approximately 15-fold increase in the probability of pCR. A negative biopsy and a CEA level $\leq$5 ng/mL show a trend toward prognostic value but do not reach statistical significance, while PET/CT findings do not demonstrate an independent predictive role.

The results of the present study confirm endoscopy as the primary method for assessing response after neoadjuvant therapy in rectal cancer. The presence of

endoscopic cCR, defined by strict morphological criteria, is associated with a significantly higher probability of pCR and may serve as a basis for discussing organ-preserving strategies. At the same time, partial responses (nCR) and especially incomplete responses (iCR) require standard management—surgical treatment or, under certain circumstances, endoscopic or transanal local interventions.

The association between EMVI-positivity and an incomplete endoscopically confirmed clinical response classifies these patients as high-risk, with a low probability of true pCR; therefore, aggressive local treatment (radical resection) remains the preferred standard of care. Similarly, a larger circumferential tumor extent and higher T-stages should guide the clinician toward a more conservative assessment of organ-preservation options, even in the presence of an apparently favorable endoscopic finding.

7. I agree with the six conclusions formulated by the author.

8. The contributions of the dissertation fall into two groups:

- Scientific – the first systematic analysis in Bulgaria of endoscopically confirmed clinical response following neoadjuvant chemoradiotherapy in patients with locally advanced rectal cancer, including an analysis of the results from a multimodal assessment approach incorporating MRI, ERUS, PET/CT, endoscopic biopsy, serum CEA, and pathological indicators (ypT, ypN, Dworak).

- The scientific and applied contributions relate to: practical criteria for determining the degrees of endoscopic response following neoadjuvant chemoradiotherapy and criteria for selecting patients with a favorable profile for the application of organ-preserving therapeutic strategies.

9. Conclusion

The dissertation is written in clear language, with precisely and clearly formulated conclusions and contributions. It reflects the author's unique experience in the field of endoscopy and its role in the multimodal strategy for assessing response following neoadjuvant chemoradiotherapy for rectal cancer.

All of this gives me reason to propose to the members of the distinguished scientific jury that they award Dr. Alexander Dimitrov Trifonov the academic and scientific degree of "Doctor" in the scientific specialty of "Gastroenterology."

January 20, 2026

Sofia

Prof. Krum Katsarov, MD, DSc