

Immunohistochemical Markers P53, Ki-67, and VEGF as Predictors of Response to Neoadjuvant Chemoradiotherapy in Locally Advanced Rectal Cancer

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Abstract **Relevance.** Rectal cancer is often diagnosed at locally advanced stages, where pretreatment risk stratification is crucial. **Objective.** To evaluate the prognostic significance of p53, Ki-67, and VEGF expression in patients with locally advanced rectal cancer who received neoadjuvant combined therapy. **Materials and Methods.** A total of 58 patients were analyzed (2019–2024, Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology). Immunohistochemistry of biopsy samples was performed for p53, Ki-67, and VEGF; findings were compared with MRI staging and clinico-morphological parameters. **Results.** Complete response was observed in 19% of cases. High Ki-67 expression was associated with better tumor regression and survival, whereas abnormal p53 and high VEGF levels correlated with poor prognosis. **Conclusion.** The combined p53/Ki-67/VEGF panel together with MRI staging increases prognostic accuracy and can be applied for therapy personalization.

Keywords Rectal cancer, Neoadjuvant therapy, p53, Ki-67, VEGF

1. Relevance

Rectal cancer is one of the most pressing issues in modern oncology due to its high incidence, significant mortality, and frequent diagnosis at locally advanced stages. According to Sung et al. (2021), colorectal cancer ranks third in global cancer incidence and second in cancer-related mortality, accounting for approximately 10% of all cancer cases and deaths [7, *CA Cancer J Clin*, 2021].

In Asian countries, including the Central Asian region, there is a persistent upward trend in incidence. Wiley (2022) notes that more than 50% of new colorectal cancer cases and nearly half of cancer-related deaths are registered in Asia, with the disease increasingly being diagnosed among people of working age [8].

Analysis of the presented map indicates significant geographical differences in the incidence of rectal cancer (Fig. 1). The highest rates (9.5–17.2 per 100,000 population) are observed in Europe, North America, Australia, and Russia. Intermediate levels (5.4–9.5 per 100,000) are typical for several countries of South America and East Asia. The lowest rates (<2.1 per 100,000) are predominantly registered in African countries and some regions of South Asia. In Central Asia, including Uzbekistan, incidence rates fall within the moderate range (2.1–3.6 per 100,000), which

underlines the growing medical and social significance of this pathology in the region.

The presented epidemiological overview demonstrates that the highest mortality rates from rectal cancer (3.6–9.0 per 100,000 population) are observed in Eastern Europe, Russia, Mongolia, as well as in several countries of South America and Australia (Fig. 2). Moderate values (2.7–3.6 per 100,000) are characteristic of Western Europe, Canada, China, and South Korea. The lowest mortality rates (<1.4 per 100,000) are registered in Africa and Southeast Asia.

For the Central Asian region, including Uzbekistan, rectal cancer mortality rates fall within the moderate category (2.0–2.7 per 100,000). This underscores the need for early detection, the implementation of screening programs, and the application of prognostic biomarkers to optimize treatment strategies.

The development of rectal cancer is driven by complex interactions of genetic, epigenetic, and microenvironmental factors. Key molecular events include alterations in the APC/ β -catenin, KRAS/NRAS/BRAF, and TP53 signaling pathways, which lead to dysregulation of the cell cycle, apoptosis, and DNA repair mechanisms. According to Chen et al. (2011), p53 inactivation is associated with increased resistance of tumor cells to cytotoxic agents and radiotherapy [1]. Simultaneously, activation of the VEGF-mediated angiogenic cascade contributes to neoangiogenesis, hypoxia, and the development of radioresistance [11].

Age-Standardized Rate (World) per 100 000, Incidence, Both sexes, in 2022
Rectum

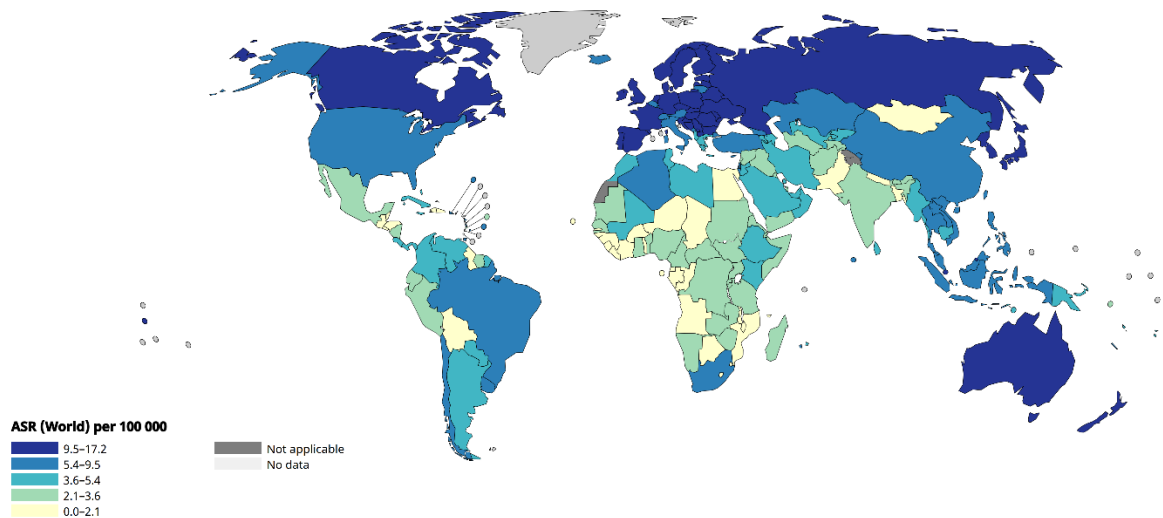


Figure 1. Global incidence of rectal cancer (age-standardized rates per 100,000 population, both sexes, 2022, according to GLOBOCAN/IARC)

Age-Standardized Rate (World) per 100 000, Mortality, Both sexes, in 2022
Rectum

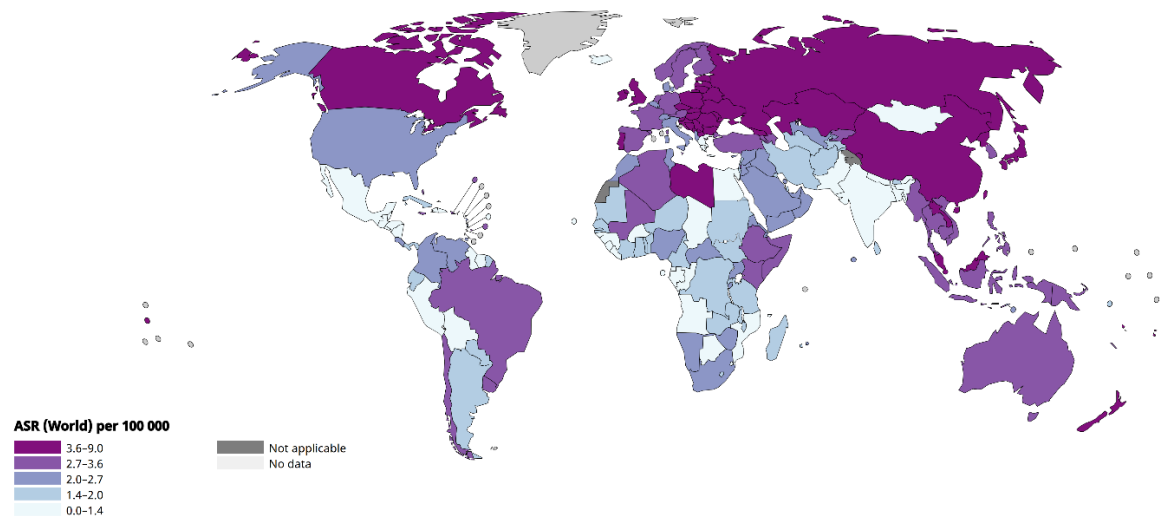


Figure 2. Global mortality from rectal cancer (age-standardized rates per 100,000 population, both sexes, 2022, according to GLOBOCAN/IARC)

The current staging algorithm is based on pelvic magnetic resonance imaging. According to ESMO (2024) and NCCN (2025) guidelines, the assessment of tumor invasion depth (T), regional lymph node status (N), signs of extramural vascular invasion, and the minimal distance to the mesorectal fascia are mandatory. These parameters determine the choice of neoadjuvant therapy, the feasibility of organ-preserving interventions, and the risk of local recurrence [3,6].

Immunohistochemical (IHC) studies provide detailed insights into tumor biology and can predict therapeutic

efficacy. Ki-67 reflects proliferative activity. According to Jakob *et al.* (2008), a high Ki-67 index is associated with a greater likelihood of pathological complete response following neoadjuvant chemoradiotherapy [5]. p53 serves as a surrogate marker for TP53 mutations. Chen *et al.* (2011) demonstrated that aberrant p53 expression is linked with lower tumor regression rates and worse survival outcomes [1]. VEGF plays a central role in angiogenesis. Yu *et al.* (2019) showed that elevated VEGF levels before treatment predict a lower probability of complete response and a higher

risk of local recurrence [10].

Recent studies emphasize that combined biomarker evaluation is more valuable than isolated assessment. In particular, Dalle Fratte et al. (2022) proposed an IHC biomarker panel (including Ki-67 and VEGF), which significantly improves the accuracy of predicting therapeutic response compared to single-marker evaluation [2].

Objective

To determine the prognostic significance of pretreatment expression of p53, Ki-67, and VEGF in comparison with clinical and radiological parameters in patients with locally advanced rectal cancer who received standard combined treatment.

2. Materials and Methods

This retrospective study was based on the analysis of clinical, morphological, radiological, and therapeutic data of 58 patients with histologically verified adenocarcinoma of locally advanced rectal cancer, who underwent treatment at the Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology (RSSPMCOR) between January 2019 and June 2024.

The inclusion criteria for the study were: patient age 18 years and older at the time of diagnosis; presence of histologically confirmed rectal adenocarcinoma located no higher than 12 cm from the anal verge; disease stage T3–T4 and/or N+ according to pretreatment pelvic magnetic resonance imaging of the pelvis; somatic status of 0–2 points on the ECOG scale; completion of a course of neoadjuvant chemoradiotherapy followed by radical surgery in the form of total mesorectal excision. The exclusion criteria included: presence of distant metastases (M1) at the time of diagnosis; previous radiotherapy to the pelvic region; synchronous or previously treated malignant tumors; absence of complete medical documentation or loss of paraffin blocks for immunohistochemical examination; as well as refusal of treatment or deviation from standard protocols. Within the framework of the study, demographic characteristics (age, sex), clinical parameters (somatic status, tumor location), pretreatment pelvic magnetic resonance imaging data with detailed staging according to T, N, presence of vascular invasion, and distance to the mesorectal fascia were analyzed. Morphological analysis included the histological variant of the tumor, degree of differentiation, and pathomorphological response to therapy. Immunohistochemical analysis was performed on pretreatment biopsies to assess the expression of p53, Ki-67, and VEGF. Therapeutic data included chemoradiotherapy regimens (total dose 50.4 Gy/28 fractions, IMRT/VMAT technique with concurrent capecitabine 825–900 mg/m², interval to surgical intervention, and surgical volume. Treatment outcomes were assessed by the rate of achieving pathological complete response (absence of invasive carcinoma and lymph node metastases), degree of tumor regression, frequency of local recurrences,

and recurrence-free survival indicators. For systematization of information, a unified coding table was used, which enabled the creation of cross-tabulation tables and multivariate analysis. Statistical processing of the data was carried out using the R software package. Descriptive statistical methods with calculation of absolute and relative indicators were applied. To analyze the relationship of categorical variables, Pearson's χ^2 test and Fisher's exact test were used. To evaluate factors associated with the probability of achieving pathological complete response, logistic regression was applied with calculation of odds ratios and 95% confidence intervals. Statistical significance was established at $p < 0.05$.

3. Results

A total of 58 patients with locally advanced rectal cancer were included in the study. Analysis of baseline clinical and magnetic resonance characteristics showed that the median age of the patients was 59 years (interquartile range 51–66 years), with a predominance of males (62.1%). In almost half of the patients, the tumor was located in the lower ampullary section of the rectum (≤ 5 cm from the anal verge — 44.8%). According to pre-treatment pelvic MRI, stage T3 was detected in 70.7% of patients, and stage T4 in 29.3%. Regional lymph node involvement was noted in 63.8% of cases. Signs of extra-organ vascular invasion were observed in 34.5% of patients. A threatened circumferential resection margin (≤ 1 mm) was recorded in 27.6% of patients, with the median distance to the mesorectal fascia being 2.4 mm (interquartile range 1.6–3.6 mm).

Table 1. Baseline clinical and radiological characteristics of patients (n=58)

Parameter	Value
Age, years, median (interquartile range)	59 (51–66)
Sex, n (%)	Male — 36 (62.1); Female — 22 (37.9)
Performance status (Eastern Cooperative Oncology Group, ECOG), n (%)	0 — 12 (20.7); 1 — 38 (65.5); 2 — 8 (13.8)
Distance from the lower tumor edge to the anal verge ≤ 5 cm, n (%)	26 (44.8)
Tumor invasion depth by MRI (T-category), n (%)	T3a–b — 22 (37.9); T3c–d — 19 (32.8); T4a — 10 (17.2); T4b — 7 (12.1)
Regional lymph node status by MRI (N-category), n (%)	N0 — 21 (36.2); N+ — 37 (63.8)
Presence of extramural vascular invasion by MRI, n (%)	Positive — 20 (34.5); Negative — 38 (65.5)
Distance to mesorectal fascia (circumferential resection margin), n (%)	≤ 1 mm — 16 (27.6); >1 mm — 42 (72.4); median — 2.4 mm (1.6–3.6)

In the immunohistochemical analysis of pre-treatment rectal biopsies, an abnormal p53 expression pattern was identified in 28 out of 58 patients (47.6%), characterized either by a complete absence of tumor cell staining or by diffuse intense nuclear expression in $\geq 60\%$ of cells. A high Ki-67 proliferation index ($\geq 30\%$) was recorded in 32 patients (55.2%). A high level of VEGF angiogenesis factor expression (H-score ≥ 150 points) was found in 25 patients (43.1%).

A combined assessment of the three markers made it possible to distinguish conditional “triad phenotypes”: a favorable phenotype (wild-type p53, high Ki-67, low VEGF) — 10 patients (17.2%); intermediate combinations — 34 patients (58.6%); unfavorable phenotype (abnormal p53 and/or high VEGF with low Ki-67) — 14 patients (24.1%).

Table 2. Distribution of p53, Ki-67 and VEGF expression, as well as triad phenotypes (n=58)

Marker	Expression level	Number of patients, n (%)
p53	Wild type	30 (52.4)
	Abnormal pattern	28 (47.6)
Ki-67	Low proliferation index (<30%)	26 (44.8)
	High proliferation index ($\geq 30\%$)	32 (55.2)
VEGF	Low expression (<150 H-score)	33 (56.9)
	High expression (≥ 150 H-score)	25 (43.1)
Triad phenotypes	Favorable (wild-type p53, high Ki-67, low VEGF)	10 (17.2)
Intermediate combinations		34 (58.6)
Unfavorable (abnormal p53 and/or high VEGF with low Ki-67)		14 (24.1)

According to the results of the morphological analysis of the surgical material, a pathological complete response,

defined as the absence of invasive carcinoma in the bowel wall and metastases in the regional lymph nodes, was achieved in 11 out of 58 patients (19.0%). A favorable degree of tumor regression according to the Dworak scale (grades 1–2) was observed in 32 patients (55.2%). According to the Mandard scale, an intermediate level of regression (grades 2–3) was most frequently recorded, whereas a complete response (grade 1) was documented in 11 patients (19.0%).

R0 resection (absence of tumor growth at the resection margins) was achieved in 52 patients (89.7%), while R1 resection (presence of tumor cells at the resection line) was noted in 6 patients (10.3%). The median distance from the tumor or tumor infiltration to the circumferential resection margin was 2.6 mm (interquartile range 1.8–3.8 mm).

Sphincter-preserving surgeries were performed in 36 patients (62.1%), while abdominoperineal resection was required in 18 cases (31.0%).

Table 3. Pathological response, degree of regression, resection margin status, and radicality of surgery (n=58)

Parameter	Results, n (%)
Pathological complete response*	11 (19.0)
Regression grade by Dworak scale	Grade 1 — 18 (31.0); Grade 2 — 14 (24.1); Grade 3 — 15 (25.9); Grade 4 — 11 (19.0)
Regression grade by Mandard scale	Grade 1 — 11 (19.0); Grade 2 — 13 (22.4); Grade 3 — 16 (27.6); Grade 4 — 12 (20.7); Grade 5 — 6 (10.3)
Distance to circumferential resection margin, mm	Median 2.6 (1.8–3.8)
Resection radicality	R0 — 52 (89.7); R1 — 6 (10.3)
Type of surgery	Sphincter-preserving — 36 (62.1); Abdominoperineal resection — 18 (31.0); Hartmann’s procedure — 4 (6.9)

Table 4. Associations of p53, Ki-67, and VEGF expression with pathological complete response and degree of tumor regression (n=58)

Marker	Expression level	Complete pathological response, n/N (%)	Odds ratio (95% confidence interval)	Significance level (p)	Favorable regression grade (Dworak 1–2), n/N (%)	Statistical measure	Significance level (p)
Ki-67	High	8/32 (25,0)	3,2 (1,02–9,9)	0,045	21/32 (65,6)	$\chi^2 = 6,4$	0,011
	Low	3/26 (11,5)	1.0 (reference level)	—	11/26 (42,3)	—	—
p53	Abnormal pattern	3/28 (10,7)	0,36 (0,12–1,03)	0,055	13/28 (46,4)	Odds ratio = 0.43	0,027
	Anomalous pattern	8/30 (26,7)	1.0 (reference level)	—	19/30 (63,3)	1.0 (reference level)	—
VEGF	high	3/25 (12,0)	0,40 (0,13–1,26)	0,109	9/25 (36,0)	$\chi^2 = 6,9$	0,009
	low	8/33 (24,2)	1.0 (reference level)	—	23/33 (69,7)	1.0 (reference level)	—

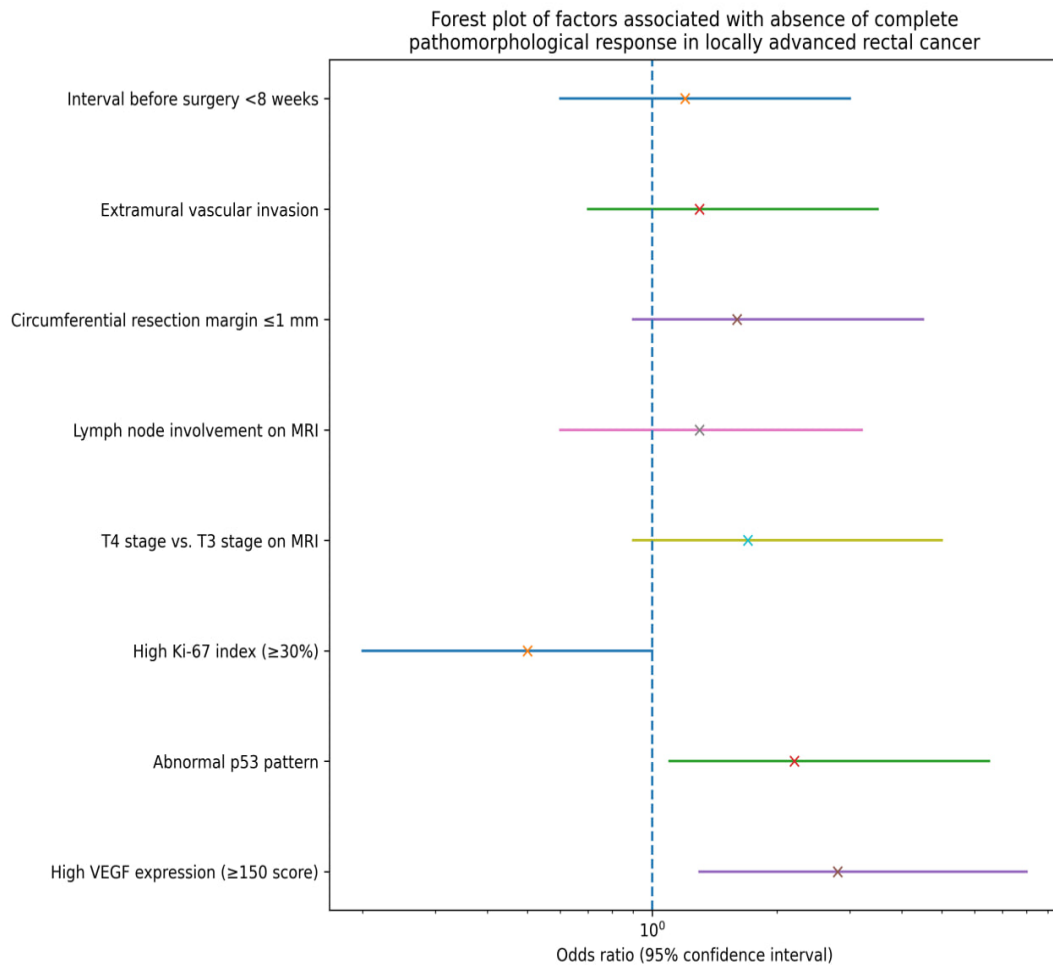


Figure 3. Factors associated with the absence of a complete pathological response in patients with locally advanced rectal cancer

The association of immunohistochemical markers with response to therapy showed that a high Ki-67 proliferation index was significantly associated with an increased frequency of pathological complete response (25.0% vs. 11.5%) and a favorable degree of tumor regression (65.6% vs. 42.3%). An abnormal pattern of p53 expression was accompanied by a decreased likelihood of complete response (10.7% vs. 26.7%) and an increase in the proportion of unfavorable regression grades. High VEGF expression was also associated with a low frequency of favorable regression (36.0% vs. 69.7%) and a trend toward incomplete response. Thus, Ki-67 demonstrated prognostic significance as a favorable marker, whereas p53 and VEGF acted as unfavorable markers.

We also performed a multivariate analysis of the impact of clinicomorphological and immunohistochemical factors on the probability of achieving a pathological complete response. Figure 3 presents a visualization in the form of a forest plot: high VEGF expression and an abnormal p53 pattern significantly increased the risk of non-response, whereas a high Ki-67 index was associated with a higher probability of a favorable outcome. Radiological characteristics (T4 stage, lymph node involvement, extramural vascular

invasion, threatened circumferential resection margin) showed only a tendency toward an unfavorable influence, without statistical significance.

Based on our observations, the median follow-up duration was 34 months, which made it possible to reliably assess the long-term treatment outcomes. We found that the three-year disease-free survival differed significantly depending on the expression levels of the studied markers. Specifically, in patients with a high Ki-67 proliferation index, it reached 68% compared to 52% in those with low expression ($p = 0.038$), indicating a paradoxical protective role of this marker under conditions of neoadjuvant chemoradiotherapy.

At the same time, high VEGF expression was associated with an unfavorable prognosis: the three-year disease-free survival decreased to 49% compared to 71% in patients with low VEGF expression ($p = 0.030$). Similarly, the presence of an abnormal p53 pattern was accompanied by a trend toward worse outcomes (51% versus 66% in cases with wild-type p53, $p = 0.081$). The multivariate Cox model confirmed the independent prognostic significance of VEGF and p53 as unfavorable factors, whereas high Ki-67 retained a significant value as a protective predictor (hazard ratio 0.64; $p = 0.041$). It should be emphasized that a threatened

circumferential resection margin and the presence of extramural vascular invasion showed a trend toward worse survival outcomes; however, they did not reach statistical significance.

Thus, our results revealed that the immunohistochemical markers VEGF, p53, and Ki-67 possess prognostic potential in locally advanced rectal cancer and may be used for risk stratification and the individualization of treatment strategies.

4. Discussion

Our study demonstrated that the immunohistochemical markers p53, Ki-67, and VEGF possess prognostic value in locally advanced rectal cancer. We found that a high Ki-67 proliferation index was associated with a higher likelihood of achieving a complete pathological response and with improved disease-free survival rates. In contrast, an abnormal p53 pattern and high VEGF expression emerged as independent unfavorable factors, linked to lower tumor regression rates and an increased risk of recurrence.

Similar findings have been reported in the international literature. Jakob *et al.* (2008) showed that elevated Ki-67 correlates with greater tumor sensitivity to neoadjuvant chemoradiotherapy. Chen *et al.* (2011) noted that abnormal p53 predicts poorer treatment response and reduced survival [1,5]. The role of VEGF as a factor of radioresistance and as a prognostic biomarker has been confirmed by Zlobec *et al.* (2008) and Yu *et al.* (2019). More recent studies emphasize the value of combined panels incorporating multiple markers, which significantly improve prognostic accuracy [2,10,11].

Our observations are also consistent with epidemiological data from GLOBOCAN (Sung *et al.*, 2021), which report colorectal cancer as the third most common and the second most deadly malignancy worldwide. In Asian countries, including Central Asia, a steady increase in incidence rates has been observed (Wiley, 2022), underlining the importance of risk stratification for treatment optimization [4,7,8].

It should be noted that MRI-based staging (T, N), as recommended by the ESMO (2024) and NCCN (2025) guidelines, in our cohort showed only a trend toward unfavorable impact without reaching statistical significance [3,6]. Nevertheless, in clinical practice, these parameters remain crucial for surgical planning.

Our findings support that the use of an IHC panel (p53, Ki-67, VEGF) in combination with radiological characteristics may improve prognostic assessment and facilitate the individualization of treatment strategies in patients with locally advanced rectal cancer.

5. Conclusions

In a single-center cohort of 58 patients with locally advanced rectal cancer, a high pre-treatment Ki-67 proliferation index was associated with a higher frequency of complete pathological response and improved disease-free

survival. In contrast, an abnormal p53 pattern and high VEGF expression predicted incomplete regression, reduced oncological radicality, and an increased risk of unfavorable outcomes. In multivariate models, VEGF and p53 retained their independent adverse prognostic roles, whereas Ki-67 demonstrated a protective effect. The addition of pre-treatment magnetic resonance imaging characteristics (depth of invasion, regional lymph nodes, extramural vascular invasion, and distance to the mesorectal fascia) enhanced the discriminatory power of the combined model. Joint pre-treatment assessment of p53, Ki-67, and VEGF together with standardized MRI staging may be recommended for personalized risk stratification and for guiding the intensity of neoadjuvant and surgical treatment.

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