RESEARCH ARTICLE

Combined COX-2 and HER-2 Biomarker Profiling to Predict Neoadjuvant Chemoradiotherapy Response in Locally Advanced Rectal Cancer

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Abstract

BACKGROUND: Locally advanced rectal cancer (LARC) is commonly treated with neoadjuvant chemoradiotherapy (nCRT), but highly variable response limits outcomes and highlights the need for predictive biomarkers. Cyclooxygenase-2 (COX-2) and human epidermal growth factor receptor 2 (HER-2) are overexpressed in a subset of colorectal cancers and are mechanistically linked to radioresistance. Both pathways are therapeutically targetable and exhibit molecular crosstalk, suggesting that combined assessment may improve prediction of nCRT response, but their combined predictive value in LARC remains unexplored. Therefore, this study was conducted to evaluate the association between COX-2 and HER-2 expression and radiotherapy response in patients with LARC.

METHODS: This observational retrospective cohort study included 59 patients with stage II–III rectal adenocarcinoma treated with standardized nCRT. COX-2 and HER-2 expressions on pretreatment biopsies were assessed by immunohistochemistry, and radiologic response 4–8 weeks post nCRT dichotomized into good and poor responses using RECIST 1.1.

RESULTS: High COX-2 expression was present in 67.8% of tumors and was associated with poor response (p<0.001; OR=10.08; 95% CI: 2.92–34.78). HER-2 positivity (32.2% of cases) was also associated with poor response (p=0.039; OR=4.28; 95% CI: 1.16–15.79). In multivariate analysis, high COX-2 (adjusted OR=0.110; p=0.002) and HER-2 positivity (adjusted OR=0.197; p=0.049) remained independent predictors of poor response. Tumors with combined COX-2 low/HER-2 negative and COX-2 high/HER 2 positive profiles showed good response rates of 86.7% and 13.3%, respectively, representing a 73.4% absolute difference.

CONCLUSION: Since Low COX-2 expression and HER-2 negativity is mostly associated with good radiotherapy response, hence COX-2 and HER-2 might be independent molecular predictors of radiotherapy response in LARC, and combined biomarker profiling provides robust risk stratification that may guide treatment intensification or de escalation strategies.

KEYWORDS: COX-2, HER-2, rectal cancer, radiotherapy response, biomarker, personalized medicine

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Introduction

Rectal cancer is a major global health burden, with an estimated 704,376 new cases and 311,900 deaths annually. (1) Locally advanced rectal cancer (LARC), defined as stage II–III disease (T3–4N0 or any T/N1–2M0), accounts for approximately 40–50% of new rectal cancer diagnoses and carries a 5 year overall survival of only 60–70% despite advances in multimodal therapy.(2,3)

Neoadjuvant chemoradiotherapy (nCRT) followed by total mesorectal excision has become the standard of care

for LARC, improving local control and survival compared with surgery alone.(4) However, responses to nCRT are heterogeneous: 15–27% of patients achieve a pathological complete response, while 20–30% show minimal regression or progression, underscoring the need for predictive biomarkers to individualize treatment.(5)

Cyclooxygenase 2 (COX-2) is an inducible enzyme that converts arachidonic acid into prostaglandins and has major effects on inflammation, angiogenesis, and tumor progression in the colorectal cancer microenvironment.(6) COX-2 is overexpressed in 50–80% of colorectal cancers and correlates with advanced stage, increased angiogenesis, and resistance to chemotherapy and radiotherapy.(7,8) COX-2–derived prostaglandins promote a pro angiogenic and hypoxic milieu, thereby contributing to radioresistance and more aggressive tumor behavior.(9,10)

Human epidermal growth factor receptor (HER)-2, or also known as ERBB2, is a receptor tyrosine kinase whose overexpression or amplification drives aggressive tumor biology through sustained activation of growth and survival signaling pathways.(11) Although HER-2 amplification is less frequent in colorectal cancer (5–10%) than in breast or gastric cancer, it has been associated with poor prognosis, distant metastasis, and reduced benefit from conventional chemotherapy and radiotherapy.(12,13) In a colorectal cancer, HER-2 overexpression has been linked to enhanced DNA damage tolerance, pro angiogenic signaling, and more invasive tumor behavior in experimental models, features that are consistent with resistance to chemoradiotherapy. (14,15)

While COX-2 and HER-2 have each been independently implicated in colorectal cancer progression and treatment resistance, and their individual prognostic significance in rectal cancer is well-established, the combined use of COX-2 and HER-2 as an integrated dual-biomarker panel specifically to predict radiotherapy response in neoadjuvant chemoradiotherapy for LARC remains unexplored. Critically, emerging evidence reveals molecular crosstalk between these pathways; Prostaglandin E2 (PGE2) transactivates HER-2 through E-prostanoid (EP) receptor signaling, and HER-2 reciprocally induces COX-2 expression via mitogen-activated protein kinase (MAPK)-dependent nuclear factor-kappaB (NF-κB) activation, creating a positive feedback loop that theoretically amplifies radioresistance beyond either biomarker alone. (16) This synergistic pathway interaction and the potential for combined biomarker-guided treatment stratification (treatment intensification versus de-escalation) in LARC patients represents the novel clinical context that justifies systematic evaluation of their combined predictive value. Therefore, this study was to investigate whether COX-2 and HER-2 expression, individually and in combination, predict radiotherapy response in patients with LARC treated with standardized nCRT protocols.(17)

Methods

Study Design and Subjects Recruitment

An observational retrospective cohort study was conducted at the Department of Surgery, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia. The study population comprised of patients with histologically confirmed rectal adenocarcinoma who underwent nCRT between January 2022 and December 2024. Inclusion criteria were subjects with stage II–III LARC (T3–4N0 or any T/N1–2M0); completion of standardized nCRT (Gy long-course or 25 Gy short-course with concurrent 5 fluorouracil or capecitabine-based chemotherapy); had available pre-treatment tumor tissue for immunohistochemistry; and completed pre- and post-treatment imaging for response assessment. Exclusion criteria included non adenocarcinoma histology (*e.g.*, signet ring or mucinous), distant metastasis at presentation, prior pelvic radiotherapy or systemic chemotherapy, multiple malignancies, inadequate tissue for biomarker analysis, major comorbidities precluding reliable response evaluation, incomplete follow up data, or non compliance with treatment.

Sample size calculation for COX-2 was based on an expected 60% good response in COX-2 low versus 20% in COX-2 high tumors (effect size 0.40), with α=0.05 and β=0.20, yielding a minimum of 53 patients; HER-2 analysis required at least 49 patients. To allow for drop outs and multivariate modeling, in this study, 59 patients were included. The study protocol was approved by the Institutional Review Board and Ethics Committee of Dr. Hasan Sadikin General Hospital (No. DP.04.03/D.XIV.6.5/489/2025). All procedures conformed to institutional and national ethical standards.

Immunohistochemistry for COX-2 and HER-2

Formalin-fixed, paraffin-embedded (FFPE) tumor tissue sections of 4 μm thickness were prepared for immunohistochemical analysis. Epitope retrieval was achieved by heating tissue sections in citrate buffer (pH 6.0) to 95°C for 20 minutes. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide. Tissue sections

were then exposed to primary antibodies against COX-2 (monoclonal antibody, clone AFHD-16, Cat. No. M00084-1; Boster Biological Technology, Pleasanton, CA, USA) and HER-2 (monoclonal antibody, clone E2-4001; Biocare Medical, Pacheco, CA, USA) applied at concentrations specified by the manufacturers for 60 minutes at ambient temperature. Following rinse steps, sections were treated with horseradish peroxidase-conjugated secondary antibody for 30 minutes, followed by colorimetric detection using 3,3'-diaminobenzidine (DAB). Nuclear counterstaining was performed with hematoxylin, and slides were mounted for microscopic examination. Each immunohistochemistry run incorporated appropriate positive and negative control specimens to verify assay performance and specificity.

The COX-2 expression was semiquantitatively assessed using the immunoreactive score (IRS): intensity (0–3) multiplied by the percentage of positive tumor cells (0–4), yielding a total score of 0–12. Tumors with IRS 0–4 were categorized as low COX-2 and IRS 5–12 as high COX-2.(18)

Meanwhile, HER-2 expression was scored according to HERACLES criteria adapted for colorectal cancer: 0 (no membrane staining in ≥10% of cells), 1+ (weak incomplete membrane staining), 2+ (weak complete or moderate/strong incomplete staining), and 3+ (moderate/strong complete membrane staining in ≥10% of cells). Cases with 2+ staining underwent fluorescence *in situ* hybridization (FISH) or silver-enhanced *in situ* hybridization (SISH) for ERBB2 amplification (ratio ≥2.0 considered positive). HER-2 scores 0–1+ were defined as negative, and 2+ or 3+ as positive.(19)

Radiotherapy Response Evaluation

Radiologic response was evaluated 4–8 weeks after completion of nCRT using RECIST 1.1 based on CT and/or MRI. Complete response (CR) was defined as disappearance of all target lesions, partial response (PR) as ≥30% decrease in the sum of target lesion diameters, stable disease (SD) as insufficient change to qualify as PR or PD, and progressive disease (PD) as ≥20% increase in sum of diameters. For analysis, CR and PR were grouped as "good response," and SD and PD as "poor response."(20)

Statistical Analysis

Categorical variables were expressed as frequencies and percentages, and continuous variables as mean±standard deviation or median with interquartile range as appropriate. Associations between biomarker expression and radiotherapy response were tested using Chi-square (χ^2) tests with odds ratios (ORs) and 95% confidence intervals (CIs). Variables with $p<0.20$ in bivariate analysis and clinically relevant factors (age, stage, histologic grade) were entered into a multivariate binary logistic regression model to identify independent predictors of good response. Combined biomarker profiles were defined as: COX-2 low/HER-2 negative, COX-2 low/HER-2 positive, COX-2 high/HER-2 negative, and COX-2 high/HER-2 positive. Analyses were conducted using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA), with $p<0.05$ considered statistically significant.

Results

Subjects Characteristics

Total 59 subjects were recruited in this study. The subjects were predominantly female cohort (61.0%) with advanced-stage disease. Mean age of the subjects was 50.0±11.2 years old, with range of 30–70 years old. Histologic differentiation was mostly in dominated by the moderate tumor category (40.7%) followed by poor tumor category (37.3%). Pre-treatment staging showed that most subjects were in tumor stage IIIB (44.1%) followed by stage IIA (32.2%) (Table 1).

High COX-2 Expression Predominated, while HER-2 Positivity Occurs in One-third of the Subjects

High COX-2 expression (IRS 5–12) was observed in 67.8% tumor subjects, while 32.2% subjects had low expression (IRS 0–4). Strong (3+) cytoplasmic staining was present in 57.6% subjects, while moderate (2+) in 10.2% subjects, and weak (1+) in 32.2% subjects (Table 2, Figure 1).

HER-2 positivity (2+ or 3+) was found in 32.2% subjects, while 67.8% of subjects were negative. The HER-2 scores were 3+, 2+, 1+, and 0 in 13.6%, 18.6%, 15.3%, and 52.5% subjects, respectively (Table 2, Figure 2).

Radiotherapy Response Distribution

Overall, 37.3% subjects achieved a good response, comprising 11.9% subjects with complete response (CR) and 25.4% subjects with partial response (PR), while 62.7% subjects demonstrated a poor response, including 49.2% subjects with stable disease (SD) and 13.6% subjects with progressive disease (PD) (Table 3). Good response was defined as either disappearance of all target lesions (CR) or ≥30% reduction in the sum of target lesion diameters (PR) on imaging 4–8 weeks after completion of nCRT, as per RECIST 1.1 criteria. In contrast, poor response encompassed tumors showing <30% reduction (SD) or ≥20% increase

Table 1. Study subjects characteristics.

Variable	n (%)
Gender	
Female	36 (61.0)
Male	23 (39.0)
Histological Differentiation	
Well	13 (22.0)
Moderate	24 (40.7)
Poor	22 (37.3)
Pre-Radiotherapy Stage	
IIA	19 (32.2)
IIB	2 (3.4)
IIIA	3 (5.1)
IIIB	26 (44.1)
IIIC	9 (15.3)
Total	**59 (100.0)**

Table 2. Distribution of COX-2 and HER-2 expressions (n=59).

Variable	n (%)
COX-2 Score	
3 (Strong Positive)	34 (57.6)
2 (Moderate Positive)	6 (10.2)
1 (Weak Positive)	19 (32.2)
0 (No Expression)	0 (0)
Combined COX-2 Category	
Low (0-1)	19 (32.2)
High (2-3)	40 (67.8)
HER-2 Score	
3 (Strong Positive)	8 (13.6)
2 (Moderate Positive)	11 (18.6)
1 (Weak Positive)	9 (15.3)
0 (Negative)	31 (52.5)
Combined HER-2 Category	
Negative (0-1)	40 (67.8)
Positive (2-3)	19 (32.2)

in target lesion diameters (PD). The predominance of poor radiotherapy response in this cohort underscores the heterogeneity of nCRT effectiveness and highlights the clinical need for predictive biomarkers to identify patients at risk of treatment failure.

COX-2 Low Expression and HER-2 Negativity were Independently Associated with Improved Radiotherapy Response

Good response was observed in 14 of 19 (73.7%) COX-2 low tumors compared with 8 of 40 (20.0%) COX-2 high tumors (χ^2=13.663; p=0.001; OR=10.08; 95% CI: 2.92–34.78) (Table 4). Among HER-2 negative subjects, 47.5% subjects achieved good response versus 3 of 19 (15.8%) in the HER-2 positive group (χ^2=4.266; p=0.039; OR=4.28; 95% CI: 1.16–15.79) (Table 4).

Combined COX-2 Low/HER-2 Negative Profile Predicts Superior Response

Combined biomarker profiling showed that COX-2 low/HER-2 negative tumors (n=15) had an 86.7% good response rate, while COX-2 high/HER-2 positive tumors (n=15) had only 13.3% good response. Intermediate profiles, COX-2 high/HER-2 negative (24.0% good response) and COX-2 low/HER-2 positive (25.0% good response), showed intermediate risk (Table 5).

In multivariate logistic regression, high COX-2 expression (adjusted OR=0.110; 95% CI: 0.027–0.447; p=0.002) and HER 2 positivity (adjusted OR=0.197; 95% CI: 0.039–0.993; p=0.049) remained independent predictors of poor response, while age and gender were not significant (Table 6, Table 7).

Discussion

This study demonstrates that COX-2 and HER-2 expression are independent molecular predictors of radiotherapy response in LARC, with combined biomarker profiling substantially improving risk stratification. The findings are consistent with clinical data implicating these pathways in

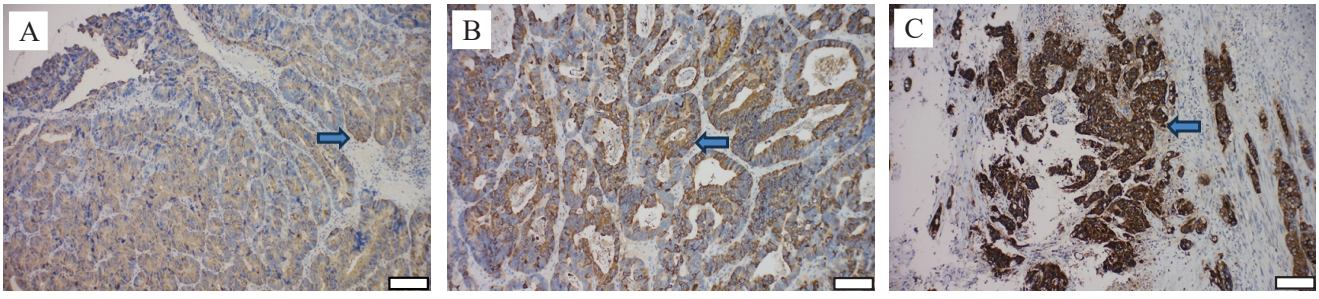

Figure 1. COX-2 expression evaluation using immunohistochemistry. A: Weak Positive (1); B: Moderate positive (2); C: Strong positive (3). Blue arrow: COX-2 expression. White bar: 100 µm.

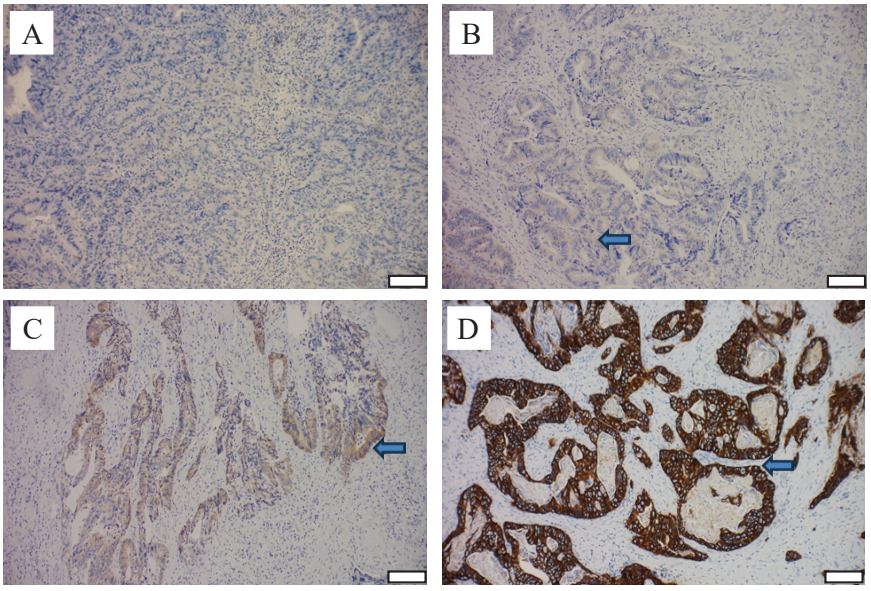

Figure 2. HER-2 intrepetation (score) evaluation using immunohistochemistry . A: Negative (1); B: Weak Positive (2); C: Moderate positive (3); D: Strong positive (4). Blue arrow: HER-2 expression. White bar: 100 µm.

treatment resistance and support integration of molecular markers into precision oncology approaches.

The strong association between elevated COX-2 expression and poor radiotherapy response is in line with evidence linking COX-2 overexpression to advanced stage, angiogenesis, and unfavorable prognosis in colorectal cancer. COX-2–derived PGE2 activates EP receptors and downstream phosphoinositide 3-kinases (PI3K)/Akt/mammalian target of rapamycin (mTOR), MAPK/extracellular signal-regulated kinase (ERK), and Wnt/β catenin signaling, attenuating radiation-induced apoptosis and promoting tumor survival under genotoxic stress. PGE2 also induces hypoxia-inducible factor (HIF) 1α and pro angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF), which remodel the vasculature and generate hypoxic microenvironments that diminish radiotherapy efficacy. COX-2 expression is also correlated with VEGF, supporting the concept that COX-2 drives angiogenesis and aggressive behavior via VEGF mediated pathways.(21-23)

COX-2 further modulates the inflammatory and immune milieu by activating NF-κB, which upregulates anti apoptotic genes, pro inflammatory cytokines, and additional angiogenic mediators. PGE2 signaling promotes the accumulation and suppressive function of myeloid-derived suppressor cells and regulatory T cells, which inhibit tumor infiltrating lymphocytes and foster an immunosuppressive microenvironment. Neoadjuvant chemotherapy has been shown to alter Forkhead box P3 $(FOXP3)^{+}$ tumor infiltrating lymphocytes in invasive breast cancer, illustrating how cytotoxic treatment can interact with the immune contexture and potentially mirror similar interactions in rectal cancer under chemoradiotherapy. In this context, the minority of COX-2 high tumors that nevertheless respond well to nCRT may harbor genetic or epigenetic alterations that counterbalance COX-2–mediated survival signaling and warrant further molecular characterization.(24-26)

Table 3. Radiotherapy response distribution according to RECIST criteria (n=59).

Response Category	Definition	n (%)
Good Response		
Complete Response (CR)	ypT0N0M0	7 (11.9)
Partial Response (PR)	≥30% dimension reduction	15 (25.4)
Total Good Response	CR + PR	22 (37.3)
Poor Response		
- Stable Disease (SD)	<30% dimension change	29 (49.2)
- Progressive Disease (PD)	≥20% dimension increase	8 (13.6)
Total Poor Response	SD + PD	37 (62.7)

Table 4. COX-2 and HER-2 expressions and radiotherapy response.

Variable	Good Response [n (%)]	Poor Response [n (%)]	Total [n]	χ^2	*p*-value	OR	95% CI
COX-2 Expression							
Low (0-1)	14 (73.7)	5 (26.3)	19	13.663	0.001	10.08	2.92-34.78
High (2-3)	8 (20.0)	32 (80.0)	40				
HER-2 Expression							
Negative (0-1)	19 (47.5)	21 (52.5)	40	4.266	0.039	4.28	1.16-15.79
Positive (2-3)	3 (15.8)	16 (84.2)	19				
Total	**22**	**37**	**59**				

HER-2 positivity was also associated with poor radiotherapy response, reflecting its role in aggressive tumor behavior and treatment resistance. Although HER-2 amplification is relatively uncommon in colorectal cancer, it is clinically important when present, conferring worse prognosis and resistance to standard therapies. HER-2–driven radioresistance involves enhanced DNA damage recognition and repair, prolonging cell cycle arrest and allowing efficient repair of radiation induced DNA damage.(27) HER-2 signaling also promotes epithelial–mesenchymal transition (EMT) through repression of E cadherin and upregulation of mesenchymal markers such as N cadherin and vimentin, a pattern that has been shown to correlate with more invasive behavior in colorectal cancer cell lines. EMT supports acquisition of stem like traits, increased motility, and resistance to apoptosis, all of which contribute to radioresistance. In addition, HER-2 enhances angiogenesis via VEGF and related mediators and activates PI3K/AKT dependent anti apoptotic signaling while facilitating p53 inactivation, further consolidating treatment resistance.(28,29)

The combined COX-2/HER-2 analysis revealed a steep gradient of response: patients with COX-2 low/HER-2 negative tumors had an 86.7% good response rate, whereas those with COX-2 high/HER-2 positive tumors had only 13.3% good response, a 73.4% absolute difference that exceeds the predictive power of either marker alone. This suggests additive or synergistic effects, likely because both pathways converge on MAPK/ERK and PI3K/AKT, jointly regulating survival signaling, DNA damage responses, and EMT. Experimental data indicate that PGE2 can transactivate HER-2 through EP receptor–mediated PI3K/AKT/Src activation, while HER 2 signaling can upregulate COX-2 via MAPK dependent NF-κB activation, forming a positive feedback loop that reinforces both pathways. COX-2 and HER-2 also contribute to angiogenic remodeling and immune suppression; concurrent activation is therefore expected to amplify neovascularization, hypoxia adaptation, and immune evasion, creating a tumor microenvironment highly resistant to chemoradiotherapy. Additional work in colorectal adenocarcinoma showing that FGF receptor (FGFR)2 expression correlates with tumor infiltrating lymphocyte grade further underscores the importance of integrating receptor driven signaling and immune context in biomarker panels for prognosis and treatment prediction. (21,29,30)

Compared with anatomical TNM staging, which primarily reflects tumor extent, biomarker based stratification offers complementary biological information about resistance mechanisms. The independent predictive value of COX-2 and HER-2 in multivariate analysis, even after adjusting for stage and other clinicopathologic variables, supports the incorporation of molecular profiles into clinical decision making for LARC.(31)

From a clinical perspective, the present findings support a biomarker guided, personalized approach to nCRT

Table 5. Combined COX-2/HER-2 biomarker profile and radiotherapy response.

Biomarker Profile	Total n	Good Response	Poor Response	Risk
COX-2 Low + HER-2 Negative	15	13 (86.7%)	2 (13.3%)	Low
COX-2 Low + HER-2 Positive	4	1 (25.0%)	3 (75.0%)	Intermediate
COX-2 High + HER-2 Negative	25	6 (24.0%)	19 (76.0%)	Intermediate
COX-2 High + HER-2 Positive	15	2 (13.3%)	13 (86.7%)	High
Total	**59**	**22 (37.3%)**	**37 (62.7%)**	—

Table 6. Bivariate analysis results for all variables.

Variable	*Chi-square*	*p*-value	OR	95% CI	Status
COX-2 (High *vs.* Low)	13.663	0.000	10.08	2.92-34.78	Highly Significant***
HER-2 (Positive *vs.* Negative)	4.266	0.039	4.28	1.16-15.79	Significant*
Age (≥50 *vs*. <50 years)	1.552	0.213	2.22	0.77-6.43	Not Significant
Gender (Male *vs.* Female)	2.884	0.089	3.02	0.96-9.53	Not Significant
Stage (III *vs.* II)	0.000	1.000	1.06	0.36-3.12	Not Significant
Differentiation (Moderate/Poor *vs.* Well)	0.180	0.672	1.60	0.48-5.37	Not Significant

in LARC. Double high risk patients (COX-2 high/HER-2 positive) show low response rates and may benefit from treatment intensification strategies, such as adding HER-2 directed monoclonal antibodies or COX-2 inhibitors, using more intensive systemic regimens, or exploring rational combinations with immunotherapy in selected molecular backgrounds. Double low risk patients (COX-2 low/HER-2 negative), who have a high probability of good response, may be candidates for standard intensity nCRT and, in future trials, for cautious de escalation of treatment intensity to reduce toxicity while maintaining excellent local control. Intermediate risk patients with discordant biomarker profiles require individualized therapeutic decisions incorporating additional molecular findings and patient factors.(21,29,30,32,33)

The retrospective single center design and modest sample size (n=59) may limit external generalizability; prospective multicenter studies with larger cohorts are needed to validate these findings across diverse populations. Radiologic response assessment by RECIST 1.1, while widely used, represents a surrogate endpoint; longer follow up is essential to confirm whether COX-2 and HER-2 expression independently predicts disease free survival and recurrence. Immunohistochemistry, though practical and standardized, remains semi quantitative; digital image analysis may enhance reproducibility and reduce interobserver variability. Systematic evaluation of potential confounders, including nonsteroidal anti inflammatory drug use, hormonal status, and concurrent molecular alterations, would strengthen interpretation of biomarker effects on treatment response. To establish combined COX-2 and HER-2 profiling as a robust predictor of nCRT response in LARC, future prospective studies should incorporate: 1) multi-marker panels combining COX-2 and HER-2 with other molecular, immune, and radiologic features; 2) integration with genomic data, and radiomics-derived features; and 3) standardized protocols for biomarker assessment and response evaluation across multiple centers. Such comprehensive biomarker integration will likely be necessary to develop a clinically applicable prediction model for nCRT response in LARC.

Conclusion

This study demonstrates that COX-2 and HER-2 expression are independent molecular predictors of radiotherapy response in locally advanced rectal carcinoma. Combined COX-2 and HER-2 biomarker profiling provides robust risk stratification, with tumors expressing low COX-2 and negative HER-2 achieving 86.7% good response rates compared to only 13.3% in those with high COX-2 and positive HER-2 expression. These findings support the clinical utility of dual biomarker assessment for personalized treatment planning in patients with LARC, enabling identification of high-risk patients who may benefit from

Table 7. Multivariate logistic regression analysis results.

Variable	Coefficient (B)	Standard Error	*p*-value	Adjusted OR	95% CI
Constant	1.914	0.713	0.007	6.782	1.68-27.42
COX-2 (High)	-2.207	0.715	0.002	0.110	0.027-0.447
HER-2 (Positive)	-1.624	0.825	0.049	0.197	0.039-0.993
Age (≥50 years)	-0.827	0.695	0.234	0.437	0.11-1.71
Gender (Male)	-0.485	0.745	0.515	0.615	0.14-2.65
Model: Pseudo R^2 = 0.291 | Log-Likelihood = -27.644 | LLR *p* <0.001 | AIC = 65.29					

treatment intensification and low-risk patients who might be candidates for de-escalation strategies.

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Authors Contribution

TS conceived the study, recruited patients, acquired data, interpreted the results and drafted the manuscript. KL and AP supervised the study and critically revised the manuscript. MSU designed the radiotherapy protocol and contributed to clinical data interpretation. EP performed histopathological evaluation and immunohistochemistry interpretation. All authors contributed to data interpretation, reviewed the manuscript, and approved the final version.

Conflict of Interest

The authors declare no conflicts of interest and no specific funding for this study.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68(6): 394-424.
2. van Gijn W, Marijnen CA, Nagtegaal ID, Kranenbarg EM, Putter H, Wiggers T, *et al.* Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer: 12-year follow-up of the multicentre, randomised controlled TME trial. Lancet Oncol. 201; 12(6): 575-82.
3. Sauer R, Becker H, Hohenberger W, Rödel C, Wittekind C, Fietkau R, *et al.* Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med. 2004; 351(17): 1731-40.
4. Bosset JF, Calais G, Mineur L, Maingon P, Radosevic-Jelic L, Daban A, *et al.* Fluorouracil-based adjuvant chemotherapy after preoperative chemoradiotherapy of rectal cancer. N Engl J Med. 2004; 351(17): 1731-40.
5. Fokas E, Elloumi F, Gardyan M, Fragkandrea I, Köhler A, Rütten H, *et al*. Tumor regression grading after preoperative chemoradiotherapy as a prognostic factor and definition of response criteria. J Clin Oncol. 2014; 32(15): 1554-62.
6. Wang D, DuBois RN. Role of prostanoids in gastrointestinal cancer. J Clin Invest. 2018; 128(7): 2732-42.
7. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994; 107(4): 1183-8.
8. Liu B, Qu L, Yan S. Cyclooxygenase-2 promotes tumor growth and suppresses tumor immunity. Cancer Cell Int. 2015; 15: 106. doi: 10.1186/s12935-015-0260-7.
9. Nakanishi M, Rosenberg DW. Multifaceted roles of PGE2 in inflammation and cancer. Semin Immunopathol. 2013; 35(2): 123-37.
10. Wang D, DuBois RN. Eicosanoids and cancer. Nat Rev Cancer. 2010; 10(3): 181-93.
11. Hynes NE, MacDonald G. ErbB receptors and signaling pathways in cancer. Curr Opin Cell Biol. 2009; 21(2): 177-84.
12. Takegawa N, Yonesaka K. HER2 as an emerging oncotarget for colorectal cancer treatment after failure of anti-epidermal growth factor receptor therapy. Clin Colorectal Cancer. 2017; 16(4): 247-51.
13. Yamada T, Yamamoto Y, Moriwaki T, Hyodo I. Is serum HER2 ECD a predictive biomarker for response to trastuzumab in advanced gastric cancer? J Gastroenterol. 2016; 5 1(5): 506-7.
14. Takegawa N, Yonesaka K, Sakai K, Ueda H, Watanabe S, Nonagase Y, *et al.* HER2 genomic amplification in circulating tumor DNA from patients with cetuximab-resistant colorectal cancer. Oncotarget. 2016; 7(3): 3453-60.
15. Raghav KPS, Moasser MM. Molecular pathways and mechanisms of HER2 in cancer therapy. Clin Cancer Res. 2023; 29(13): 2351-61.
16. Castellone MD, Teramoto H, Gutkind JS. Cyclooxygenase 2 and colorectal cancer chemoresistance: the intricate relationship. Cancer Res. 2006; 66(10): 4889-94.
17. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med. 2002; 8(3): 289-93.
18. Xiong B, Sun TJ, Hu WD, Cheng FL, Mao M, Zhou YF. Expression of cyclooxygenase-2 in colorectal cancer and its clinical significance. World J Gastroenterol. 2005; 11(8): 1105-8.
19. Albarello L, Pecciarini L, Doglioni C. HER2 testing in gastric cancer. Adv Anat Pathol. 2011; 18(1): 53-9.
20. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, *et al.* New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009; 45(2): 228-47.
21. Wu QB, Sun GP. Expression of COX 2 and HER 2 in colorectal cancer and their correlation. World J Gastroenterol. 2015; 21(20): 6206-14.
22. Berbecka M, Forma A, Baj J, Furtak Niczyporuk M, Maciejewski R, Sitarz R. A systematic review of the cyclooxygenase 2 (COX 2) expression in rectal cancer patients treated with preoperative radiotherapy or radiochemotherapy. J Clin Med. 2021; 10(19): 4443.
23. Rahaju P, Wirattami AT, Sandra F, Kurniawan S, Nisa K, Soehartono, *et al.* A pilot study on immunohistochemical expressions of NF κB, Cyclin D1, VEGF, and COX 2 in advanced stage laryngeal

carcinoma. Indones Biomed J. 2021; 13(3): 350-4.
24. Chen EP, Smyth EM. COX 2 and PGE2 dependent immunomodulation in breast cancer. Prostaglandins Other Lipid Mediat. 2011; 96(1 4): 14-20.
25. Rustamadji P, Wiyarta E, Pramono M, Maulanisa SC. Response to neoadjuvant chemotherapy in invasive breast cancer predicted by CD4+, CD8+, and FOXP3+ tumor infiltrating lymphocytes. Asian Pac J Cancer Prev. 2024; 25(5): 1607-13.
26. Rianti AM, Cangara MH, Yamin A, Dahlan H, Ilyasa MR, Miskad UA. FGFR2 as a prognostic and predictive marker in colorectal adenocarcinoma based on TILs grade. Indones Biomed J. 2025; 17(3): 188-96.
27. Zheng Lin B, Bekaii Saab TS. Treatment options for HER2 expressing colorectal cancer: Updates and recent approvals. Ther Adv Med Oncol. 2024; 16: 17588359231225037. doi: 10.1177/17588359231225037.
28. Luminturahardjo W, Kurniawati Y, Sandra F. N cadherin as an important marker in colorectal cancer: An investigation of β catenin and cadherin expressions of SW 480 and HCT 116 cell lines. Indones Biomed J. 2021; 13(3): 230-5.
29. Mann M, Sheng H, Shao J, Williams CS, DuBois RN, Pisacane PI, *et al.* Targeting cyclooxygenase 2 and HER 2/neu pathways inhibits colorectal carcinoma growth. Gastroenterology. 2001; 120(7): 1713-9.
30. El Agy F, Bahja S, El Abkari M, Ibrahimi SA, El Rhazi K, Chbani L, *et al.* The implications of COX 2 and HER2 protein expression for the prognosis of colorectal cancer patients: an exploratory study from the North African region. Rev Esp Patol. 2025; 58(3): 100840. doi: 10.1016/j.patol.2025.100840.
31. Wang J, Liu Y, Jiang W, Zhang D, Cheng C, Liu C, *et al.* Predictive value of the systemic inflammation grade for overall survival in patients with colorectal cancer after surgery: outperforming NLR and mGPS. Front Oncol. 2025; 15: 1529670. doi: 10.3389/fonc.2025.1529670.
32. Weis SM, Cheresh DA. Tumor angiogenesis: Molecular pathways and therapeutic targets. Nat Med. 2011; 17(11): 1359-70.
33. Wijaya T, Akmal AAM, Herman N, Hasan AA, Hafiz A, Widyastuti H. Apoptotic effects sulfated polysaccharides of Caulerpa racemosa extract on colorectal cancer cells through Caspase-3. Mol Cell Biomed Sci. 2025; 9(3): 171-8.