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Neoadjuvant Treatment Modalities for Rectal Cancer: Independent Correlates of Tumor Response and Sphincter Preservation in a Real-World Cohort

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Abstract

Purpose: To compare outcomes across three neoadjuvant treatment modes for rectal adenocarcinoma in routine practice and to assess whether pathological response and sphincter preservation should be interpreted separately.

Methods: We conducted a retrospective cohort study at West China Hospital, Sichuan University, including consecutive patients with rectal adenocarcinoma who received neoadjuvant therapy between November 2008 and June 2023 followed by radical resection. The analytic cohort comprised patients with baseline M0 disease (n = 298; CRT 74, NACT 128, TNT 96). The primary efficacy endpoint was ypStage 0–I, and the primary surgical endpoint was sphincter preservation. Adjusted comparisons were performed using inverse-probability-of-treatment weighting and multivariable Firth logistic regression.

Results: Baseline disease was more advanced in the CRT and TNT groups. After adjustment, treatment mode was not independently associated with ypStage 0–I (NACT vs CRT: adjusted OR 1.19, 95% CI 0.60–2.35; TNT vs CRT: adjusted OR 0.65, 95% CI 0.31–1.35). In contrast, NACT was associated with higher odds of sphincter preservation than CRT (adjusted OR 3.75, 95% CI 1.39–10.15). The association between treatment mode and sphincter preservation differed from that for pathological response ($P = 1.7 \times 10^{-4}$). Exploratory analyses suggested age-related heterogeneity in tumor regression.

Conclusion: In this real-world cohort, CRT, NACT and TNT showed no clear adjusted difference in pathological response, whereas NACT was associated with higher sphincter preservation. Pathological efficacy and anatomical preservation should therefore be evaluated separately.

Keywords: Rectal cancer; Neoadjuvant therapy; Total neoadjuvant therapy; Sphincter preservation; Pathological response; Tumor regression grade

Introduction

Locally advanced rectal cancer is increasingly managed with diverse neoadjuvant strategies [1-3]. While neoadjuvant chemoradiotherapy (CRT) followed by total mesorectal excision remains a cornerstone, total neoadjuvant therapy (TNT) has expanded by shifting systemic chemotherapy into the preoperative period to optimize compliance and enhance systemic disease control [1, 2]. At the same time, neoadjuvant chemotherapy alone (NACT) has attracted attention as a possible approach to omit pelvic radiotherapy in selected patients, motivated by concerns about radiotherapy-related toxicity and long-term quality-of-life [3, 4]. With these options in practice, a pressing issue is how treatment choice balances tumor control with the goal of sphincter preservation.

A key challenge in translating comparative evidence to real-world practice is that treatment selection is rarely random. Differences across CRT, TNT, and NACT may reflect baseline clinical selection and planning factors such as tumor height, circumferential resection margin risk, anticipated surgical feasibility, and institutional practice over time [5, 6]. This has particular implications for sphincter preservation. Unlike pathological response endpoints for example, complete response or downstaging, sphincter preservation is strongly influenced by baseline anatomy and operative decision-making [7]. Therefore, pathological efficacy and anatomical surgical preservation may not move in parallel. Disentangling these domains is essential to interpret observational comparisons among contemporary neoadjuvant modes.

In addition, whether neoadjuvant response differs by age at onset remains uncertain. Early-onset colorectal cancer is increasingly common and is thought to represent a distinct clinical and potentially biological subset [8]. However, existing evidence is limited by small subgroup sizes, incomplete molecular characterization, and confounding by treatment selection, leaving unanswered whether early-onset tumors show different regression patterns with CRT, NACT, or TNT [9, 10].

In this retrospective real-world cohort from West China Hospital, Sichuan University, we compared three neoadjuvant treatment modes (CRT, NACT, TNT) in baseline M0 rectal

adenocarcinoma patients who received classifiable neoadjuvant therapy followed by radical resection. We defined postoperative pathological stage 0–I (ypStage 0–I) as the primary efficacy endpoint and sphincter preservation as the primary surgical endpoint (interpreted anatomically, given limited surgical outcome data). The study aims to clarify how pathological response and anatomical preservation relate across treatment modes, and whether early-onset disease modifies associations with tumor regression—generating hypotheses rather than causal treatment effects.

Materials and Methods

Study Design and Population

This retrospective cohort study was conducted at West China Hospital, Sichuan University. Consecutive patients with rectal adenocarcinoma who had records of neoadjuvant therapy between November 2008 and June 2023 were identified from institutional medical and pathology records; repeated hospitalizations were consolidated, yielding 710 patients. The study period spanned multiple eras of rectal cancer management, and therefore treatment mode should be interpreted as a real-world institutional treatment strategy rather than as a randomized or protocol-mandated intervention. The study was approved by the Ethics Committee of West China Hospital, Sichuan University (No. 2023-958), and the requirement for individual informed consent was waived owing to the retrospective design.

Eligible patients had histologically confirmed rectal adenocarcinoma, received neoadjuvant therapy followed by radical resection at our institution, and had available baseline clinical staging and postoperative pathological records. Patients were excluded stepwise for the following reasons (Figure 1): no documented evidence of administered neoadjuvant therapy (planned but not delivered; n = 195); a regimen not classifiable into the three predefined treatment modes (composition inconsistent with the definitions, or insufficient documentation; n = 66); an incomplete or unconfirmable neoadjuvant course (documented discontinuation, or fewer recorded cycles than planned; n = 39); no radical resection (n = 22); and no complete postoperative pathological record (n = 46). Because these exclusions may have selected for patients with more complete documentation and completed multimodality treatment, the analytic cohort should be interpreted as a treated surgical cohort rather than as an inception cohort of all patients initially considered for neoadjuvant therapy. After these exclusions, 342 patients remained; those with baseline M0 disease (n = 298; CRT, 74; NACT, 128; TNT, 96) formed the analytic cohort, and those with M1 disease (n = 44) were summarized descriptively.

Neoadjuvant Treatment Modes

Three neoadjuvant treatment modes were compared. Treatment-mode assignment followed the recorded multidisciplinary treatment plan and was based on the role of each treatment component: (1) neoadjuvant chemoradiotherapy (CRT): pelvic radiotherapy delivered with concurrent chemotherapy (fluoropyrimidine-based, with or without oxaliplatin) as a radiosensitizing component of the radiation course; (2) total neoadjuvant therapy (TNT): pelvic radiotherapy plus multi-agent systemic chemotherapy, the chemotherapy being completed in the preoperative phase as a full-intent treatment component rather than solely as a concurrent radiosensitizing course; and (3) neoadjuvant chemotherapy alone (NACT): multi-agent chemotherapy without radiotherapy.

Regimens that could not be classified into these three definitions were not assigned to any mode and were excluded during screening (Figure 1). Thus, the comparisons in this study reflect broad real-world treatment modes rather than uniform trial-style regimens. Because radiotherapy was completely collinear with treatment mode in the M0 cohort, the NACT-versus-CRT comparison was interpreted as a treatment-mode comparison, reflecting the contemporary question of selective omission of radiotherapy, and not as a causal estimate of the effect of radiotherapy itself. Accordingly, observed differences between modes may reflect both treatment components and clinical selection into those modes. Induction-versus-consolidation sequencing within TNT could not be determined because treatment dates were unavailable, and the neoadjuvant cycle count was treated as part of the administered treatment rather than a covariate. Detailed time-dependent treatment characteristics, including exact treatment sequencing and interval from completion of neoadjuvant therapy to surgery, were therefore not incorporated into the main models.

Data Collection and Pathological Assessment

Clinical and pathological variables were extracted from the records, including demographics,

tumor distance from the anal verge, clinical T and N categories and TNM stage (AJCC, 8th edition) [11], tumor differentiation (WHO grading), baseline CRM and EMVI status, neoadjuvant cycles, the definitive surgical procedure, postoperative stage, TRG, and recurrence. Baseline staging variables were extracted from the available institutional clinical, imaging, endoscopic, and pathological documentation.

Immunohistochemistry for mismatch repair proteins (MLH1, Zhongshan Golden Bridge, Beijing, China, #ZM-0154; MSH2, #ZA-0622; MSH6, #ZA-0541; PMS2, #ZA-0542) and Ki67 (#ZM-0167) followed institutional protocols, with all stains interpreted independently by two pathologists in consensus; loss of one or more MMR proteins defined deficient MMR (dMMR). MMR status was available in 224 patients (including four dMMR tumors), and KRAS, NRAS, BRAF, and HER2 results (availability 8 – 31%) were not analyzed inferentially. CRM, EMVI, and differentiation had substantial missingness (50.0%, 65.1%, and 25.2%, respectively) and were handled as three-level factors with unknown as an explicit category. This approach preserved cohort size but cannot fully eliminate bias from non-random missingness in clinically important MRI-derived variables. Early-onset disease was defined as diagnosis before age 50 years.

Outcome Definitions

The primary efficacy endpoint was postoperative pathological stage 0 – I (ypStage 0 – I). This endpoint was selected a priori as a clinically interpretable composite of primary tumor and nodal pathological response and was expected to provide a larger number of events than pCR in this real-world cohort. Pathological complete response (pCR) was defined as ypT0N0. Tumor regression was graded by the modified Ryan scheme (College of American Pathologists protocol, version 4.4.0.1, Note J) as TRG 0 to 3 ; because TRG assesses the primary tumor bed only and does not incorporate nodal status, a ypT0 tumor with residual nodal disease may achieve TRG 0 without meeting the pCR definition [12]. Favorable response was defined as TRG 0 – 1. T-category and overall-stage downstaging were defined

relative to baseline clinical stage. The neoadjuvant rectal (NAR) score was calculated as [5 × ypN − 3 × (cT − ypT) + 12]^2 /9.61 and categorized as low (<8), intermediate (8 – 16), or high (>16) [13].

The primary surgical endpoint was sphincter preservation. Sphincter-preserving surgery comprised all low anterior resection subtypes and local excision, and non-preserving surgery comprised abdominoperineal resection (APR) and Hartmann procedure; the APR rate served as the strictly anatomical measure. Because postoperative bowel function, continence, quality of life, and permanent stoma status were not uniformly available, sphincter preservation was interpreted as an anatomical surgical endpoint rather than as a direct measure of surgical outcome.

Postoperative recurrence was adjudicated from surveillance imaging and clinical assessment at postoperative readmission; without a uniform follow-up cutoff, it was analyzed as a binary variable. Therefore, recurrence analyses were considered exploratory and were not interpreted as formal time-to-event estimates of disease-free survival or oncologic safety.

Statistical Analysis

Primary between-mode comparisons

Continuous variables are summarized as median (interquartile range) and compared with the Kruskal – Wallis test; categorical variables with the chi-squared or Fisher's exact test; between-group balance with standardized mean differences (SMD > 0.10 indicating residual imbalance) [14]. No sample size calculation was performed because of the retrospective design. All analyses should therefore be interpreted as observational and hypothesis-generating, especially for subgroup and exploratory endpoints. The primary endpoints were compared between modes by two prespecified approaches: inverse-probability-of-treatment weighting (IPTW; multinomial logistic propensity model for the above covariates with unknown as a category, stabilized weights truncated at the 1st/99th

percentiles, weighted binomial regression with HC0 robust standard errors), and multivariable Firth-penalized logistic regression (events-per-variable 8.1 and 4.0 for the efficacy and surgical models) to reduce small-sample bias and address separation [14-17]. Results are reported as odds ratios (ORs) with 95% confidence intervals (CIs). Because some outcomes were common, odds ratios were interpreted as measures of association rather than risk ratios.

Given the non-randomized allocation of treatment mode, adjusted associations were not interpreted as causal treatment effects.

Model evaluation and decoupling

Discrimination was assessed by the area under the receiver operating characteristic curve (AUC) with DeLong confidence intervals and bootstrap-corrected optimism (1000 resamples); calibration by the Hosmer – Lemeshow test; collinearity by variance inflation factors [18, 19]. Whether the NACT preservation advantage was independent of tumor regression was tested with a generalized estimating equation (binomial family, exchangeable working correlation) fitted to each patient's paired responses (favorable response; sphincter preservation), using a treatment-mode-by-endpoint interaction and its joint Wald test (2 degrees of freedom) as the decoupling statistic [20]. This analysis tested whether treatment-mode associations differed between the tumor-regression and surgical-preservation domains; it did not establish biological mediation or causal independence. The two-pathway analysis (anatomic vs biologic) was associative, not formal causal mediation.

Recurrence, quadrants, and the early-onset module

Recurrence was modeled in preoperative (treatment mode, clinical N category, distance from the anal verge) and postoperative (treatment mode, NAR score, TRG 0 – 1) Firth specifications. Because recurrence was analyzed as a binary outcome without uniform follow-up duration, these models were exploratory and primarily descriptive. patients were cross-classified into response-by-preservation quadrants, with pairwise Fisher's exact tests

and Bonferroni adjustment for quadrant-wise recurrence.

For the early-onset module (prespecified endpoint TRG 0 – 1), mode-by-onset interaction was estimated by Firth logistic regression with joint Wald tests under three prespecified specifications, complemented by within-stratum exact tests, ordinal TRG comparisons, a complete age-cutpoint scan (35 – 65 years), a continuous age-by-mode interaction, and a repeat analysis excluding the four dMMR tumors. Because of small subgroup sizes and multiple exploratory evaluations, the early-onset module was interpreted as hypothesis-generating even when nominal or false-discovery-rate-adjusted significance was observed.

Sensitivity analyses and multiplicity. Six sensitivity analyses were performed: extended adjustment with CRM and EMVI; exclusion of seven patients undergoing multivisceral resection (n = 6) or Hartmann procedure (n = 1), whose surgical decision reflected non-anatomic considerations; an 8-cm distance threshold; untruncated weights; exclusion of one patient with more than eight cycles; and a propensity model without CRM and EMVI. Multiplicity was controlled by a prespecified gatekeeping hierarchy (primary surgical hypothesis and decoupling test protected first; all remaining analyses exploratory); within the early-onset module, the ten module tests were the three mode-by-onset joint Wald specifications, the continuous age-by-mode interaction, the four within-stratum mode comparisons, the ordinal TRG comparison within the CRT group, and the repeat interaction test excluding dMMR tumors, with the primary interaction remaining significant after Benjamini – Hochberg correction ($q \approx 0.03$) [21]. All tests were two-sided, with $P < 0.05$ considered statistically significant. Analyses were performed using R version 4.3.1 [22].

Results

Study Population and Baseline Characteristics

Of 710 unique patients with rectal adenocarcinoma identified through neoadjuvant-therapy records, 368 were excluded in a stepwise manner (Figure 1), leaving 342 patients who received neoadjuvant therapy followed by radical resection with complete pathological records. The final cohort therefore represents patients with documented completion of classifiable neoadjuvant therapy and subsequent radical surgery at the study institution. The analytic M0 cohort comprised 298 patients (CRT, 74; NACT, 128; TNT, 96); 44 patients with baseline M1 disease were analyzed descriptively.

Baseline characteristics differed across modes in a pattern consistent with clinical selection (Table 1). Median age was 50.0 years (IQR, 44.2–60.0) in the CRT group, 56.0 years (48.0–65.2) in the NACT group, and 53.0 years (46.0–60.0) in the TNT group (P = 0.022). CRT and TNT patients presented with more locally advanced disease than NACT patients: cT4 tumors in 48.6% and 47.9% versus 22.7% (P < 0.001), cN2 disease in 47.3% and 45.8% versus 21.1% (P < 0.001), and baseline CRM positivity in 45.9% and 34.4% versus 16.4% (P < 0.001). These imbalances indicate substantial confounding by indication and support cautious interpretation of between-mode comparisons. Sex, BMI, distance from the anal verge, tumor differentiation, EMVI, and Ki67 were distributed comparably across modes (all P > 0.10). The number of neoadjuvant cycles differed by design (P = 0.003). Early-onset disease (age <50 years) accounted for 47.3%, 32.0%, and 37.5% of patients in the CRT, NACT, and TNT groups, respectively (P = 0.097).

Primary Efficacy Endpoint: ypStage 0-I

ypStage 0–I was achieved in 81 of 298 patients (27.2%). After IPTW, effective sample sizes were 55, 94, and 83 for the CRT, NACT, and TNT groups, respectively. Weighted balance was generally adequate; residual SMDs were 0.103, 0.119, and 0.172 for clinical T category,

clinical N category, and baseline CRM, respectively—the first two being covered by the prespecified covariate adjustment in the Firth models, and the last reflecting its 50% missing proportion and addressed in sensitivity analysis SA6. Nevertheless, residual imbalance in baseline disease severity remained possible.

Weighted ypStage 0–I rates were 25.1%, 28.6%, and 18.5%, respectively. In the weighted comparison, neither NACT (OR, 1.19; 95% CI, 0.57–2.51; P = 0.641) nor TNT (OR, 0.68; 95% CI, 0.30–1.53; P = 0.347) differed significantly from CRT (Table 2).

The Firth multivariable model was concordant: adjusted ORs for ypStage 0–I were 1.19 (95% CI, 0.60–2.35; P = 0.614) for NACT and 0.65 (95% CI, 0.31–1.35; P = 0.249) for TNT versus CRT (Table 3, Model A; Figure 2). Treatment mode was not independently associated with ypStage 0–I in this observational cohort. Among baseline covariates, cT4 disease was inversely associated with ypStage 0–I (OR, 0.27; 95% CI, 0.08–0.96; P = 0.043, borderline), and poor differentiation showed a nonsignificant trend (OR, 0.47; 95% CI, 0.21–1.03; P = 0.059). Model discrimination was moderate (apparent AUC 0.693, 95% CI 0.630–0.757; bootstrap-corrected AUC 0.641; Hosmer–Lemeshow P = 0.759; maximum VIF 7.26 for the clinical T-category dummies) (Figure 3A). These confidence intervals do not establish equivalence of the modes, as no non-inferiority margin was prespecified.

Secondary Efficacy Endpoints

pCR was infrequent, occurring in 12 of 298 patients (4.0%), and did not differ significantly across modes: 2.7%, 3.1%, and 6.2% for CRT, NACT, and TNT, respectively (P = 0.491). Because of the small number of pCR events, multivariable pCR modeling was not performed, and pCR comparisons were interpreted descriptively. Favorable tumor regression (TRG 0–1) occurred in 43 of 298 patients (14.4%; 17.6%, 10.9%, and 16.7% by mode; P = 0.326). T-category downstaging was achieved in approximately half of the patients in every mode (48.6%, 48.4%, and 51.0%; P = 0.920). Overall-stage downstaging showed a nonsignificant

trend toward lower rates in the TNT group (54.1%, 53.1%, and 38.5%; P = 0.055). NAR score distributions were virtually identical across modes (median 15.0 in all three; P = 0.601). Exploratory analyses showed a recurrence gradient across NAR categories (5.3%, 9.9%, and 14.4%; P = 0.143) and no association of Ki67 with either response endpoint (Table 4).

Primary Surgical Endpoint: Sphincter Preservation

Sphincter preservation was achieved in 250 of 298 patients (83.9%); APR was performed in 47 patients (15.8%). Preservation rates were 81.1% for CRT, 93.8% for NACT, and 72.9% for TNT, and APR rates were 17.6%, 6.2%, and 27.1%, respectively.

In the Firth multivariable model, NACT was associated with higher odds of sphincter preservation than CRT (OR, 3.75; 95% CI, 1.39–10.15; P = 0.009), whereas TNT showed no significant difference (OR, 0.52; 95% CI, 0.23–1.19; P = 0.121) (Table 3, Model B; Figure 2). Greater distance from the anal verge was strongly associated with preservation (OR per cm, 1.62; 95% CI, 1.36–1.94; P < 0.001); age (OR per year, 0.98; 95% CI, 0.95–1.00; P = 0.092) and female sex (OR, 1.92; 95% CI, 0.86–4.29; P = 0.111) were not significant. Discrimination was good (apparent AUC 0.860, 95% CI 0.803–0.917; bootstrap-corrected AUC 0.816; Hosmer–Lemeshow P = 0.907; maximum VIF 7.32) (Figure 3B).

Because sphincter preservation is strongly influenced by tumor height, sphincter involvement, surgeon judgment, patient preference, and baseline anatomical feasibility, this association should not be interpreted as proof that NACT itself caused surgical preservation. The NACT association was robust across sensitivity analyses (Table 5): extended adjustment with CRM and EMVI (OR, 3.69; P = 0.010), exclusion of seven patients undergoing multivisceral resection (n = 6) or Hartmann procedure (n = 1), whose surgical decision reflected non-anatomic considerations (OR, 4.07; P = 0.008), and an 8-cm distance threshold (OR, 3.01; P = 0.019); efficacy-side sensitivity analyses left the efficacy conclusions unchanged (untruncated weights: NACT OR, 1.13; P = 0.757; propensity model without CRM/EMVI:

OR, 1.15; P = 0.690; exclusion of the single patient with more than eight cycles: OR, 1.18; P = 0.636). Among patients achieving TRG 0–1, surgery remained sphincter-preserving in the large majority of cases in every mode (Supplementary Table S8).

Decoupling of the Surgical Preservation and Efficacy Domains

Because NACT was associated with higher sphincter preservation without a corresponding increase in ypStage 0–I, we examined whether treatment-mode associations differed between the tumor-regression and surgical-preservation domains. In the GEE model fitted to the paired responses, the mode-by-endpoint interaction was significant for NACT (OR, 6.07; P = 0.002) but not for TNT (OR, 0.67; P = 0.423), and the joint Wald test confirmed significant decoupling ($\chi^2 = 17.317$, 2 df, $P = 1.7 \times 10^{-4}$).

These findings support cohort-level decoupling between anatomical surgical preservation and pathological tumor regression, rather than proving mechanistic independence. The associative mechanistic decomposition was internally consistent: preservation was dominated by anatomic factors (distance from the anal verge, OR per cm 1.56, 95% CI 1.33–1.84, P < 0.001; cT4, OR 0.27, 95% CI 0.13–0.53, P < 0.001), whereas individual tumor response contributed (TRG 0–1, OR 5.78; 95% CI, 1.10–30.34; P = 0.038) without bridging the case-mix differences between modes (Supplementary Table S3). These pathway models are associative and do not constitute causal mediation.

Postoperative Recurrence and the Response-Preservation Quadrants

Postoperative recurrence was documented in 33 of 298 patients (11.1%). Because follow-up duration was not uniform and recurrence was analyzed as a binary variable, recurrence results are exploratory and should not be interpreted as formal disease-free survival estimates. In the preoperative Firth model, TNT showed a non-significant trend toward higher recurrence odds versus CRT (OR, 1.99; 95% CI, 0.81–4.89; P = 0.135); NACT did not differ (OR, 0.73; 95% CI, 0.27–2.01; P = 0.545). Adding the NAR score and TRG 0–1 left mode estimates

essentially unchanged (NAR OR per unit, 1.02; 95% CI, 0.99–1.04; P = 0.153). Discrimination was limited (bootstrap-corrected AUC 0.582 and 0.611).

Cross-classification by ypStage 0–I and sphincter preservation yielded four quadrants: full benefit (n = 77; recurrence 3.9%), response without preservation (n = 4; 25.0%), preservation without response (n = 173; 11.6%), and neither (n = 44; 20.5%). The full-benefit quadrant had significantly lower recurrence than the double-negative quadrant (Fisher P = 0.008; Bonferroni-adjusted P = 0.0495). Given the small cell size, especially for the response-without-preservation quadrant, quadrant-wise recurrence findings were interpreted descriptively. The four-patient decoupled quadrant is reported descriptively (Tables 6 and 7).

Baseline M1 Case Series

Among 44 patients with baseline M1 disease, all five CRT patients received radiotherapy; preservation was achieved in 60.0%, 100%, and 85.7%, and documented recurrence occurred in 60.0%, 44.0%, and 57.1% of CRT, NACT, and TNT patients, respectively. Given the small numbers, these data are descriptive only (Supplementary Table S1). No inferential comparison was attempted in the M1 subgroup.

Early-Onset Heterogeneity in Tumor Regression

Early-onset (age <50 years) and late-onset patients were clinically indistinguishable at baseline except for a higher proportion of female patients among early-onset cases (46.4% versus 28.5%; P = 0.003); all other clinicopathological variables were comparable (Table 8).

The treatment-mode ranking of TRG 0–1 rates was reversed in early-onset patients. Among early-onset patients, TRG 0–1 was achieved in 31.4% (11/35) of CRT versus 4.9% (2/41) of NACT patients (P = 0.004) and 13.9% (5/36) of TNT patients (P = 0.094); among late-onset

patients, no significant mode differences were observed (5.1% versus 13.8%, P = 0.223; versus 18.3%, P = 0.071) (Table 9; Figure 4). The mode-by-onset interaction was significant under all three specifications (joint Wald $\chi^2 = 8.82$, P = 0.012 unadjusted; $\chi^2 = 9.40$, P = 0.009 adjusted; $\chi^2 = 8.52$, P = 0.014 sex-adjusted), remained significant after Benjamini–Hochberg correction across the ten module tests ($q \approx 0.03$), and was unchanged after excluding the four dMMR tumors ($\chi^2 = 8.167$, P = 0.017). On the ordinal TRG scale, the early-onset–late-onset difference was specific to CRT (Mann–Whitney P = 0.024) and absent in NACT (P = 0.374) and TNT (P = 0.746). The signal was concentrated in the 45–49-year band, where TRG 0–1 reached 50.0% (8/16) with CRT versus 8.7% (2/23) with NACT (Figure 4A).

The complete age-cutpoint scan (35–65 years) showed that statistical significance was confined to a narrow band around the prespecified threshold (P = 0.012, 0.021, and 0.025 at cutpoints 50, 51, and 52 years, respectively; P = 0.061 at 49 years; P = 0.596 at the <45-year probe) (Figure 5), and a continuous age-by-mode interaction showed a supportive but non-significant trend ($\chi^2 = 5.353$, P = 0.069). By contrast, the corresponding interaction for ypStage 0–I was null ($\chi^2 = 0.255$, P = 0.880), indicating that the heterogeneity resides in the degree of tumor regression rather than in the stage-0–I milestone.

These interaction findings should be regarded as hypothesis-generating. In this non-randomized cohort, the pattern cannot distinguish greater radiosensitivity of early-onset tumors from preferential selection of radiosensitive young patients into CRT. The small subgroup sizes, the concentration of the signal near the 45–49-year band, incomplete molecular characterization, and the borderline continuous age interaction further limit

mechanistic interpretation. External validation with molecular data is required before any biological conclusion can be drawn.

Discussion

In this retrospective cohort, neoadjuvant treatment mode was not independently associated with ypStage 0–I after adjustment, whereas NACT was associated with higher sphincter preservation than CRT. The main implication is that pathological response and sphincter preservation should not be assumed to represent the same treatment domain in rectal cancer. In routine practice, these outcomes may diverge because they are driven by overlapping but non-identical factors.

The absence of a clear difference in ypStage 0–I across CRT, NACT and TNT should be interpreted in the context of both the published literature and the design of the present study [5, 6]. Randomized trials have defined roles for CRT and TNT in selected populations, but real-world treatment allocation is influenced by local stage, MRI risk features, surgical feasibility and institutional preference [1-3, 5, 6]. In our cohort, patients treated with CRT and TNT had more locally advanced disease at baseline, consistent with confounding by indication [6, 14]. It is therefore plausible that any true differences in pathological efficacy were attenuated, or obscured, by case mix. The similarly low pCR rates and the lack of clear separation in other regression-related endpoints support a cautious interpretation of efficacy comparisons between models in observational data.

By contrast, the association between NACT and sphincter preservation was stronger and remained robust in sensitivity analyses. This finding is clinically relevant, but it should not be interpreted as evidence that NACT itself improves preservation. Sphincter-preserving surgery is determined not only by treatment response but also by tumour height, sphincter complex involvement, pelvic anatomy, surgeon assessment and patient preference [7]. Previous studies of radiotherapy omission have mainly focused on oncological outcomes, whereas the surgical implications of selective omission have been less clearly examined [3]. Our findings suggest that, in practice, patients selected for NACT may also be those with more favourable anatomical conditions for preservation.

This interpretation is reinforced by the decoupling analysis. The association of treatment mode with sphincter preservation was not mirrored by a corresponding association with

pathological response, supporting the view that preservation should not be used as a surrogate for tumour regression in non-randomized comparisons. Although response may facilitate less extensive surgery in individual patients, preservation at the cohort level remains strongly constrained by baseline anatomy. This distinction is especially relevant when interpreting real-world studies of NACT or selective radiotherapy omission.

The exploratory early-onset analysis raises a separate question. Interest in early-onset colorectal cancer has increased substantially, and differences in biology and treatment sensitivity remain under investigation [8, 10]. In this cohort, the interaction between age at onset and treatment mode for TRG suggests possible heterogeneity in local regression, particularly for CRT. However, this signal was not seen for ypStage 0–I, was concentrated in a narrow age range, and was derived from a non-randomized dataset with limited molecular data. It should therefore be regarded as hypothesis-generating only.

The limitations of this study are mostly those inherent to retrospective observational analyses. Residual confounding is likely despite weighting and multivariable adjustment. Treatment groups represented broad real-world modes rather than standardized regimens, and details such as TNT sequencing and interval to surgery were unavailable. Missingness in CRM and EMVI limited complete baseline risk adjustment. The study cohort was restricted to patients who completed classifiable neoadjuvant therapy and underwent radical resection with complete pathology, and therefore reflects a treated surgical cohort rather than all patients considered for neoadjuvant treatment. In addition, sphincter preservation was assessed anatomically, without uniform data on function, stoma status or quality of life. Recurrence analyses were also exploratory because follow-up was not standardized.

The study nevertheless has several strengths. It reflects contemporary real-world practice across three clinically relevant neoadjuvant approaches and explicitly separates pathological and surgical endpoints, which are often conflated in the literature [1, 6]. This distinction may be useful in interpreting comparative studies where favorable surgical outcomes do not necessarily indicate superior tumor response [5, 6].

In conclusion, in this real-world cohort of rectal adenocarcinoma treated with neoadjuvant therapy and radical resection, CRT, NACT and TNT showed no clear adjusted difference in ypStage 0–I, whereas NACT was associated with higher sphincter preservation. These findings support separate evaluation of pathological efficacy and anatomical preservation when comparing neoadjuvant strategies. The age-related heterogeneity observed for tumor regression requires external validation. Future prospective studies should incorporate detailed anatomical staging, surgical outcomes and molecular profiling to better define which patients may benefit from selective radiotherapy omission.

Abbreviations

AJCC, American Joint Committee on Cancer;

APR, abdominoperineal resection;

AUC, area under the receiver operating characteristic curve;

BMI, body mass index;

CI, confidence interval;

CRM, circumferential resection margin;

CRT, chemoradiotherapy;

dMMR, deficient mismatch repair;

EMVI, extramural vascular invasion;

ESS, effective sample size;

GEE, generalized estimating equation;

HL, Hosmer–Lemeshow (test);

IHC, immunohistochemistry; IQR, interquartile range;

IPTW, inverse-probability-of-treatment weighting;

MMR, mismatch repair;

NACT, neoadjuvant chemotherapy;

NAR, neoadjuvant rectal (score);

OR, odds ratio; pCR, pathological complete response;

SA, sensitivity analysis;

SMD, standardized mean difference;

TNT, total neoadjuvant therapy;

TRG, tumor regression grade;

VIF, variance inflation factor.

Declarations

Ethical approval and Consent to participate

This study was reviewed and approved by the Ethics Committee of West China Hospital, Sichuan University (No. 2023-958). The requirement for informed consent was waived by the Ethics Committee of West China Hospital, Sichuan University due to the retrospective study design and the use of de-identified data.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data in any form that could identify the patient.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

MMH, SL, ZM, YW and XDZ contributed to the conceptualization and methodology of the study. MMH, SL, ZM, DT, YS, SC, YMC and MS performed the formal analysis and investigation. MMH, SL, ZM and XDZ wrote the original draft of the manuscript. YW and XDZ reviewed and edited the manuscript. ZM, XDZ and YW provided resources. XDZ and YW supervised the study. All authors read and approved the final manuscript.

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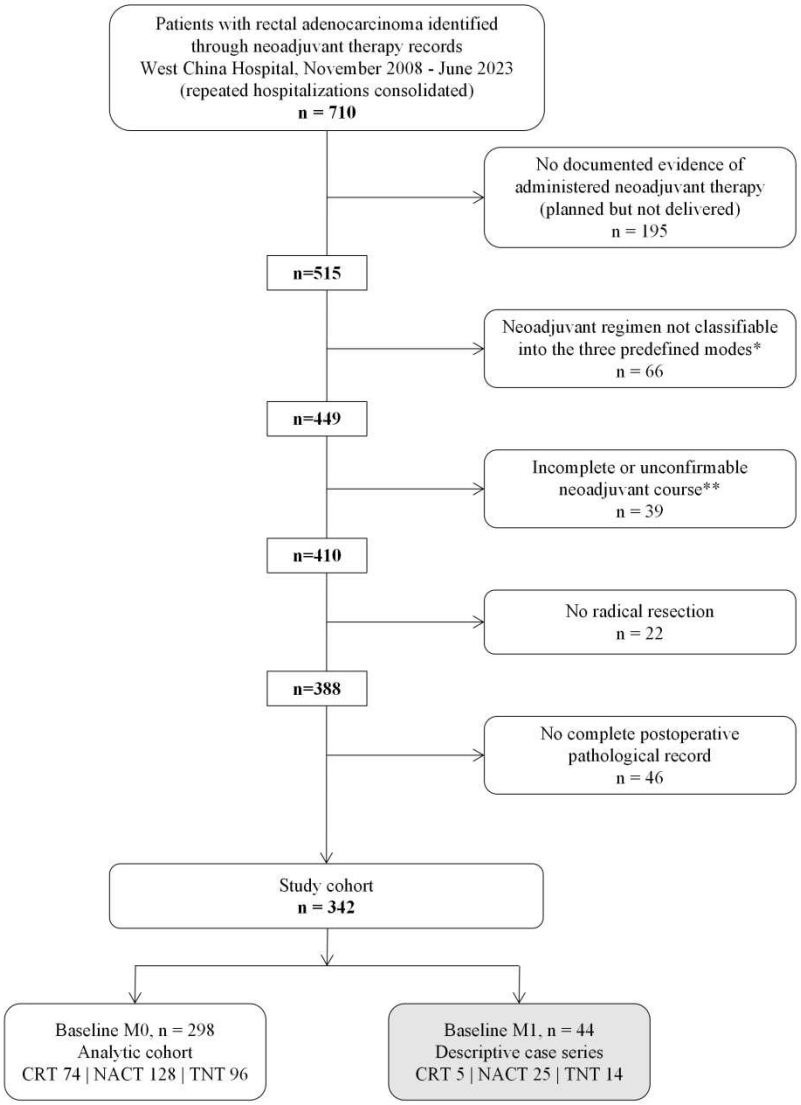

Figure 1. Flow diagram of patient selection and cohort assembly. Of 710 unique patients with rectal adenocarcinoma identified through neoadjuvant-therapy records at West China Hospital between November 2008 and June 2023 (repeated hospitalizations consolidated), 368 were excluded in a stepwise manner. The remaining 342 patients who received neoadjuvant therapy followed by radical resection with complete postoperative pathological records were stratified by baseline distant metastasis status into the M0 analytic cohort (n = 298; CRT, n = 74; NACT, n = 128; TNT, n = 96) and the M1 descriptive case series (n = 44). CRT, chemoradiotherapy; NACT, neoadjuvant chemotherapy; TNT, total neoadjuvant therapy.

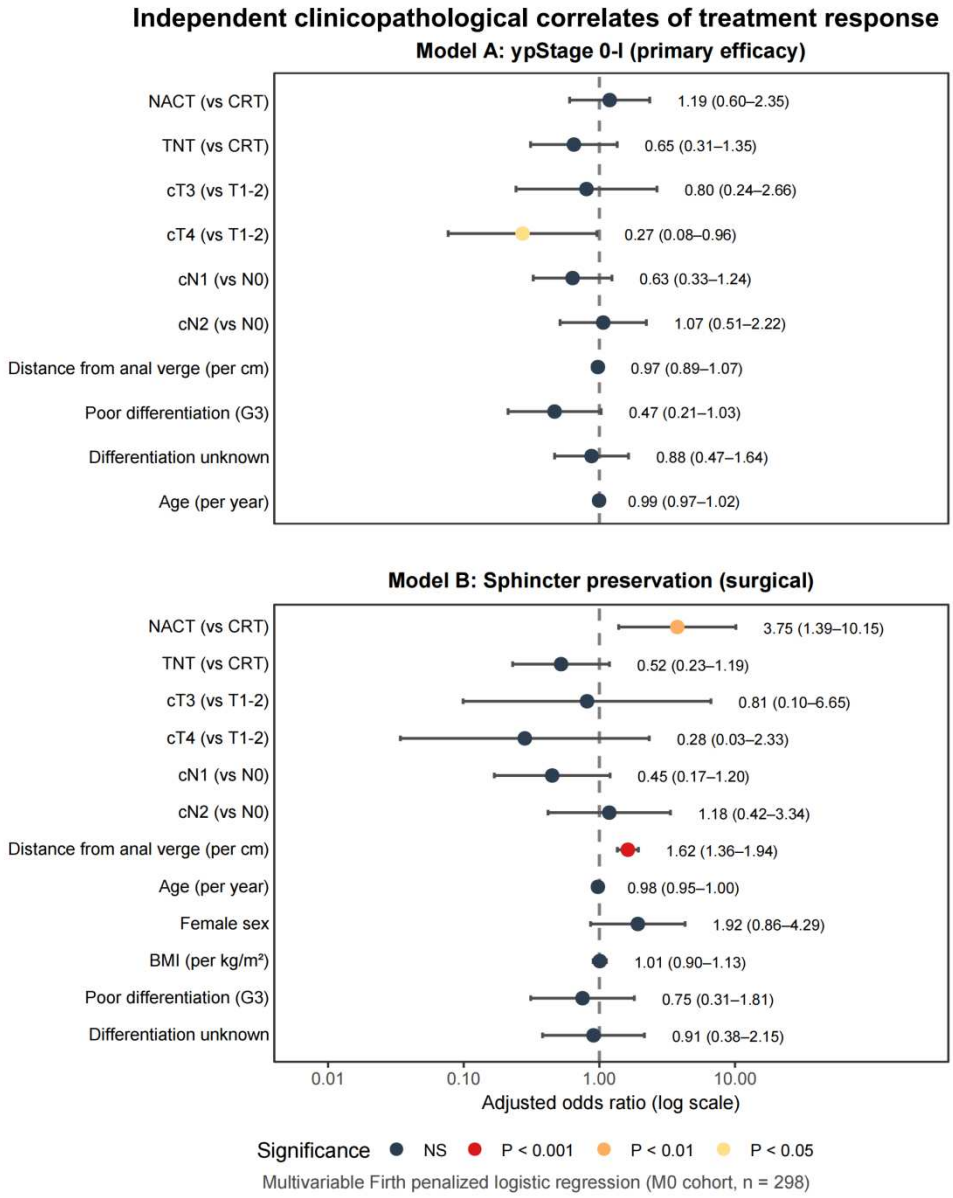

Figure 2. Independent clinicopathological correlates of treatment response and sphincter preservation. Forest plot of the two multivariable Firth-penalized logistic regression models in the M0 cohort (n = 298). (A) Model A: ypStage 0 – I (primary efficacy endpoint, 81 events). (B) Model B: sphincter preservation (primary surgical endpoint, 48 non-preservation events). Odds ratios (squares) with 95% confidence intervals (horizontal lines) are shown on a logarithmic scale; the dashed line marks an odds ratio of 1. Points are colored according to statistical significance: red, $P < 0.001$; orange, $P < 0.01$; yellow, $P < 0.05$; dark gray, not significant. Distance from the anal verge reached $P < 0.001$ in Model B. NACT was independently associated with sphincter preservation (OR 3.75, 95% CI 1.39 – 10.15, $P = 0.009$) but not with ypStage 0 – I; no covariate reached $P < 0.001$ in Model A. CRT, chemoradiotherapy; NACT, neoadjuvant chemotherapy; TNT, total neoadjuvant therapy; OR, odds ratio; CI, confidence interval.

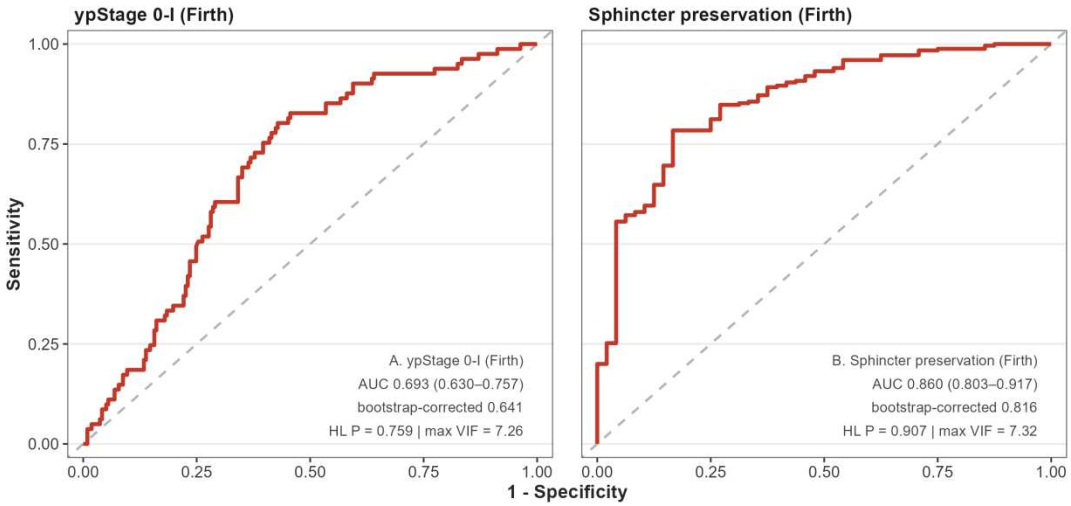

Figure 3. Discrimination of the multivariable Firth models. Receiver operating characteristic curves for (A) the ypStage 0 – I model and (B) the sphincter-preservation model in the M0 cohort (n = 298). Apparent AUC with 95% confidence intervals (DeLong), bootstrap-corrected AUC (1000 resamples), Hosmer – Lemeshow goodness-of-fit P values, and maximum variance inflation factors are shown within each panel. AUC, area under the receiver operating characteristic curve; HL, Hosmer – Lemeshow; VIF, variance inflation factor.

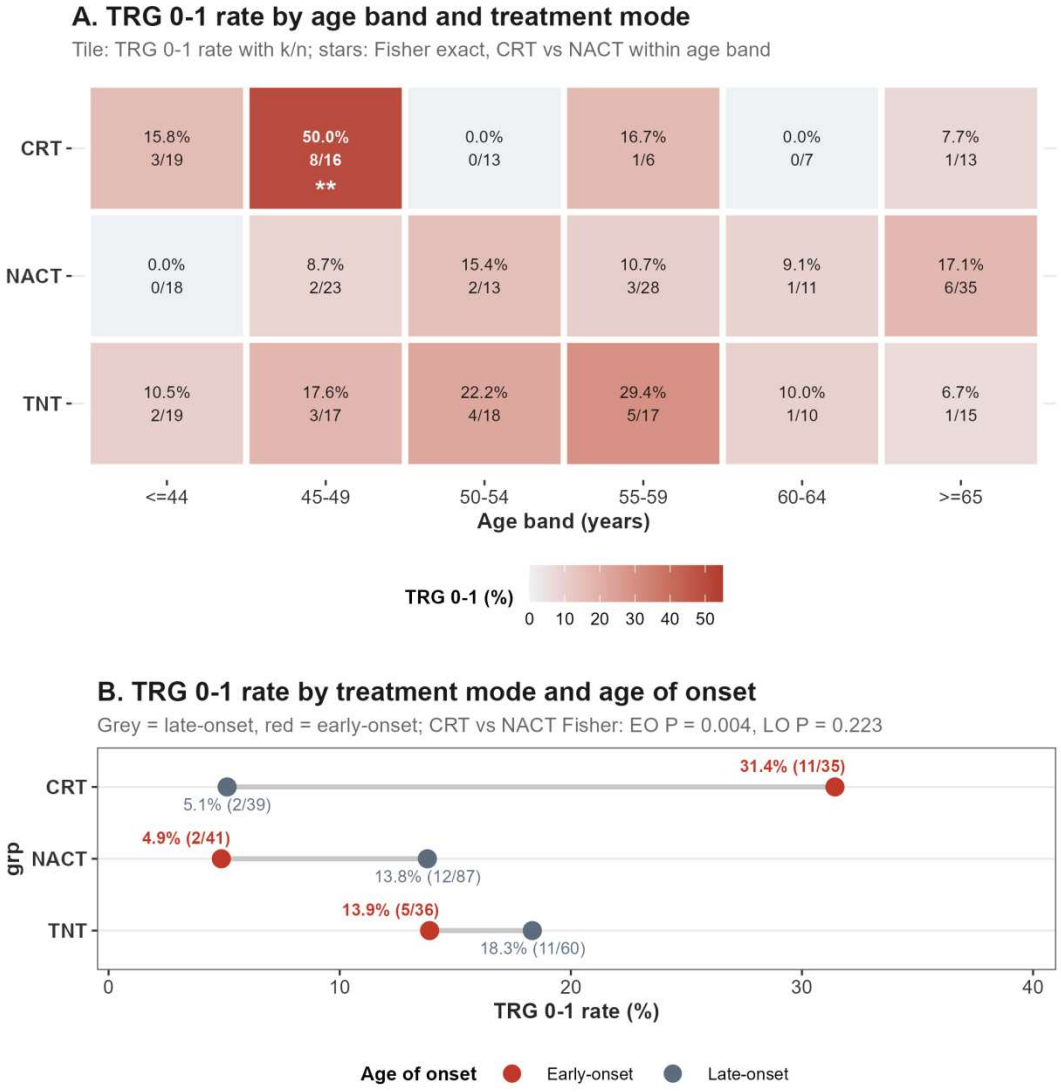

Figure 4. Tumor regression by age at onset and treatment mode. (A) Heat map of TRG 0–1 rates across age bands (≤44, 45–49, 50–54, 55–59, 60–64, and ≥65 years) and treatment modes; each tile shows the rate with k/n, and asterisks denote Fisher exact comparisons of CRT versus NACT within the age band (*P < 0.05). The reversal of the treatment-mode ranking among early-onset patients was concentrated in the 45–49-year band (CRT 50.0% vs NACT 8.7%). (B) Dumbbell plot of TRG 0–1 rates by treatment mode and age at onset; red dots indicate early-onset (age <50 years) and gray dots late-onset patients, with group sizes shown as k/n. Within early-onset patients, TRG 0–1 was significantly more frequent with CRT than with NACT (31.4% vs 4.9%, P = 0.004), whereas no significant mode differences were observed among late-onset patients. TRG, tumor regression grade; CRT, chemoradiotherapy; NACT, neoadjuvant chemotherapy; TNT, total neoadjuvant therapy.

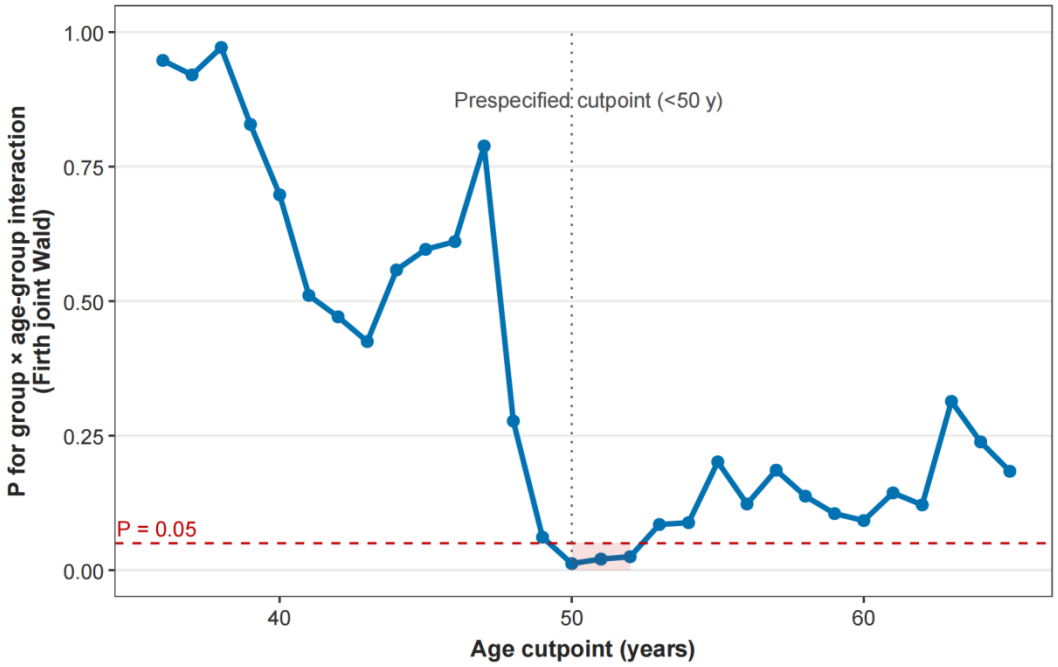

Figure 5. Age-cutpoint scan of the treatment-mode-by-age-group interaction. P values (Firth joint Wald test, 2 degrees of freedom) for the interaction between treatment mode and dichotomized age are plotted for every candidate cutpoint from 35 to 65 years in the M0 cohort (cutpoints yielding a stratum of fewer than 15 patients were not evaluated). The red dashed line marks P = 0.05. Statistical significance was confined to a narrow band around the prespecified cutpoint of 50 years (P = 0.012, 0.021, and 0.025 at cutpoints 50, 51, and 52 years, respectively; P = 0.061 at 49 years; P = 0.596 at the <45-year probe), supporting the prespecified <50-year dichotomization while underscoring the hypothesis-generating nature of this signal.

Table 1. Baseline clinicopathological characteristics by neoadjuvant treatment mode (M0 cohort, n = 298)

	CRT (n = 74)	NACT (n = 128)	TNT (n = 96)	P value
Age, years	50.0 (44.2-60.0)	56.0 (48.0-65.2)	53.0 (46.0-60.0)	**0.022**
BMI, kg/m²	22.8 (21.5-25.2)	22.9 (21.0-25.0)	22.5 (20.9-24.9)	0.514
Distance from anal verge, cm	5.0 (4.0-7.0)	5.0 (4.0-8.0)	5.0 (3.0-8.0)	0.917
Neoadjuvant cycles,	4.0 (2.8-6.0)	4.0 (2.0-4.0)	4.0 (3.0-6.0)	**0.003**
Ki67, %	70.0 (55.0-80.0)	70.0 (40.0-80.0)	60.0 (50.0-80.0)	0.399
Sex				0.269
Male	51 (68.9)	86 (67.2)	56 (58.3)	
Female	23 (31.1)	42 (32.8)	40 (41.7)	
Age at onset				0.097
Early-onset	35 (47.3)	41 (32.0)	36 (37.5)	
Late-onset	39 (52.7)	87 (68.0)	60 (62.5)	
BMI category				0.614
<18.5 kg/m²	7 (9.5)	5 (3.9)	6 (6.2)	
18.5-23.9 kg/m²	36 (48.6)	76 (59.4)	57 (59.4)	
24-27.9 kg/m²	23 (31.1)	36 (28.1)	26 (27.1)	
≥28 kg/m²	8 (10.8)	11 (8.6)	7 (7.3)	
Clinical T category				**<0.001**
T1-2	2 (2.7)	5 (3.9)	4 (4.2)	
T3	36 (48.6)	94 (73.4)	46 (47.9)	
T4	36 (48.6)	29 (22.7)	46 (47.9)	
Clinical N category				**<0.001**
N0	11 (14.9)	37 (28.9)	17 (17.7)	
N1	28 (37.8)	64 (50.0)	35 (36.5)	
N2	35 (47.3)	27 (21.1)	44 (45.8)	
Baseline AJCC stage				0.099
I	0 (0.0)	3 (2.3)	1 (1.0)	
II	11 (14.9)	34 (26.6)	16 (16.7)	
III	63 (85.1)	91 (71.1)	79 (82.3)	
Baseline CRM status				**<0.001**
CRM-negative	10 (13.5)	38 (29.7)	13 (13.5)	
CRM-positive	34 (45.9)	21 (16.4)	33 (34.4)	

Unknown	30 (40.5)	69 (53.9)	50 (52.1)	
Baseline EMVI status				0.182
EMVI-negative	11 (14.9)	12 (9.4)	7 (7.3)	
EMVI-positive	23 (31.1)	27 (21.1)	24 (25.0)	
Unknown	40 (54.1)	89 (69.5)	65 (67.7)	
Tumor differentiation				0.106
G1-G2	38 (51.4)	81 (63.3)	44 (45.8)	
G3	17 (23.0)	19 (14.8)	24 (25.0)	
Unknown	19 (25.7)	28 (21.9)	28 (29.2)	

Data are median (interquartile range) or n (%). P values were calculated using the Kruskal-Wallis test for continuous variables and the chi-squared or Monte Carlo Fisher exact test for categorical variables. CRT, chemoradiotherapy; NACT, neoadjuvant chemotherapy; TNT, total neoadjuvant therapy; BMI, body mass index; CRM, circumferential resection margin; EMVI, extramural vascular invasion; AJCC, American Joint Committee on Cancer.

Table 2. Covariate balance and weighted comparison of ypStage 0-I (IPTW, M0 cohort)

A. Covariate balance before and after weighting

Covariate	SMD, unweighted	SMD, weighted
Age, years	0.324	0.093
Sex	0.221	0.060
BMI	0.152	0.055
Clinical T category	0.564	0.103
Clinical N category	0.575	0.119
Distance from anal verge	0.069	0.070
CRM status	0.673	0.172
EMVI status	0.323	0.042
Tumor differentiation	0.356	0.078

B. Weighted ypStage 0-I rates and weighted between-mode comparison

Group	Weighted ESS	Weighted rate, %	OR (95% CI) vs CRT	P value
CRT	55	25.1	Reference	-
NACT	94	28.6	1.19 (0.57-2.51)	0.641
TNT	83	18.5	0.68 (0.30-1.53)	0.347

IPTW used a multinomial logistic propensity model (age, sex, BMI, clinical T and N categories, distance from the anal verge, CRM, EMVI, and tumor differentiation; the latter three with unknown as a category), stabilized weights truncated at the 1st/99th percentiles, and weighted binomial regression with HC0 robust standard errors. SMD, standardized mean difference; ESS, effective sample size; other abbreviations as in Table 1.

Table 3. Multivariable Firth-penalized logistic regression models for the two primary endpoints (M0 cohort, n = 298)

Variable	Model A: ypStage 0-I OR (95% CI)	P value	Model B: sphincter preservation OR (95% CI)	P value
NACT vs CRT	1.19 (0.60-2.35)	0.614	3.75 (1.39-10.15)	**0.009**
TNT vs CRT	0.65 (0.31-1.35)	0.249	0.52 (0.23-1.19)	0.121
cT3 vs cT1-2	0.80 (0.24-2.66)	0.720	0.81 (0.10-6.65)	0.845
cT4 vs cT1-2	0.27 (0.08-0.96)	**0.043**	0.28 (0.03-2.33)	0.240
cN1 vs cN0	0.63 (0.33-1.24)	0.183	0.45 (0.17-1.20)	0.110
cN2 vs cN0	1.07 (0.51-2.22)	0.861	1.18 (0.42-3.34)	0.751
Distance from anal verge, per cm	0.97 (0.89-1.07)	0.586	1.62 (1.36-1.94)	**<0.001**
Age, per year	0.99 (0.97-1.02)	0.668	0.98 (0.95-1.00)	0.092
Female vs male	-	-	1.92 (0.86-4.29)	0.111
BMI, per kg/m²	-	-	1.01 (0.90-1.13)	0.907
Poor differentiation (G3) vs G1-G2	0.47 (0.21-1.03)	0.059	0.75 (0.31-1.81)	0.524
Differentiation unknown vs G1-G2	0.88 (0.47-1.64)	0.679	0.91 (0.38-2.15)	0.821

Odds ratios (ORs) with 95% confidence intervals (CIs) from Firth-penalized logistic regression. Model A (ypStage 0-I, 81 events): apparent AUC 0.693 (95% CI 0.630-0.757), bootstrap-corrected AUC 0.641; Hosmer-Lemeshow P = 0.759; maximum VIF 7.26. Model B (sphincter preservation, 48 non-preservation events): apparent AUC 0.860 (95% CI 0.803-0.917), bootstrap-corrected AUC 0.816; Hosmer-Lemeshow P = 0.907; maximum VIF 7.32 (cT dummy variables). Bold P values indicate statistical significance. AUC, area under the receiver operating characteristic curve; VIF, variance inflation factor; other abbreviations as in Table 1.

Table 4. Secondary efficacy endpoints by treatment mode (M0 cohort, n = 298)

Endpoint	CRT (n = 74)	NACT (n = 128)	TNT (n = 96)	P value
Pathological complete response	2 (2.7)	4 (3.1)	6 (6.2)	0.491
Favorable tumor regression (TRG 0-1)	13 (17.6)	14 (10.9)	16 (16.7)	0.326
T-category downstaging	36 (48.6)	62 (48.4)	49 (51.0)	0.920
Overall-stage downstaging	40 (54.1)	68 (53.1)	37 (38.5)	0.055
NAR score, median	15.0 (8.4-30.1)	15.0 (8.4-30.1)	15.0 (8.4-30.1)	0.601

Data are n (%) or median (interquartile range [IQR]). P values: Fisher exact test (pathological complete response); chi-squared test (TRG 0-1, downstaging); Kruskal-Wallis test (NAR). Recurrence increased across NAR categories (5.3%, 9.9%, and 14.4% for low, intermediate, and high NAR; P = 0.143, exploratory). Between-group NAR distributions did not differ pairwise (Bonferroni-adjusted P > 0.05). Ki67 was not associated with either response endpoint (Supplementary Table S9). NAR, neoadjuvant rectal score; TRG, tumor regression grade; other abbreviations as in Table 1.

Table 5. Sensitivity analyses for the treatment-mode associations

Sensitivity analysis	Endpoint	NACT vs CRT, OR (95% CI)	P value	TNT vs CRT, OR (95% CI)	P value
Primary model	ypStage 0-I	1.19 (0.60-2.35)	0.614	0.65 (0.31-1.35)	0.249
Primary model	Sphincter preservation	3.75 (1.39-10.15)	**0.009**	0.52 (0.23-1.19)	0.121
SA1: extended adjustment (CRM, EMVI)	ypStage 0-I	1.14 (0.57-2.29)	0.711	0.62 (0.30-1.30)	0.207
SA1: extended adjustment (CRM, EMVI)	Sphincter preservation	3.69 (1.36-10.00)	**0.010**	0.50 (0.22-1.14)	0.099
SA2: exclusion of 7 multivisceral/Hartmann cases	Sphincter preservation	4.07 (1.45-11.40)	**0.008**	0.53 (0.23-1.21)	0.129
SA3: 8-cm distance threshold	Sphincter preservation	3.01 (1.20-7.56)	**0.019**	0.58 (0.27-1.22)	0.153
SA4: untruncated weights	ypStage 0-I	1.13 (0.53-2.42)	0.757	0.66 (0.29-1.50)	0.317
SA5: exclusion of >8 cycles	ypStage 0-I	1.18 (0.60-2.33)	0.636	0.64 (0.31-1.34)	0.240
SA6: propensity model without CRM/EMVI	ypStage 0-I	1.15 (0.57-2.34)	0.690	0.65 (0.30-1.43)	0.282

All models were Firth-penalized logistic regressions except SA4 and SA6 (weighted binomial regression with HC0 robust standard errors). SA2 excluded seven patients undergoing multivisceral resection (n = 6) or Hartmann procedure (n = 1), whose surgical decision reflected non-anatomic considerations. OR, odds ratio; CI, confidence interval; other abbreviations as in Table 1.

Table 6. Firth-penalized logistic regression models for postoperative recurrence (M0 cohort, 33 events)

Variable	Preoperative model OR (95% CI)	P value	Postoperative model OR (95% CI)	P value
NACT vs CRT	0.73 (0.27-2.01)	0.545	0.73 (0.27-1.97)	0.531
TNT vs CRT	1.99 (0.81-4.89)	0.135	1.95 (0.79-4.83)	0.148
cN1 vs cN0	1.15 (0.43-3.03)	0.781	-	-
cN2 vs cN0	1.10 (0.40-3.01)	0.849	-	-
Distance from anal verge, per cm	0.94 (0.83-1.06)	0.328	-	-
NAR score, per unit	-	-	1.02 (0.99-1.04)	0.153
TRG 0-1 vs TRG 2-3	-	-	1.02 (0.34-3.07)	0.973

Preoperative model: treatment mode, clinical N category, distance from the anal verge. Postoperative model: treatment mode, NAR score, TRG 0-1. Apparent AUC 0.645 (bootstrap-corrected 0.582) and 0.657 (bootstrap-corrected 0.611), respectively. Bold P values indicate statistical significance. Abbreviations as in Tables 1 and 4.

Table 7. Cross-classification by ypStage 0-I and sphincter preservation: distribution and recurrence

A. Distribution by treatment mode, n

Quadrant	CRT	NACT	TNT	Total
Full benefit (ypStage 0-I and preserved)	17	43	17	77
Response without preservation	2	1	1	4
Preservation without response	43	77	53	173
Neither	12	7	25	44

B. Recurrence by quadrant

Quadrant	n	Recurrence
Full benefit	77	3 (3.9)
Response without preservation	4	1 (25.0)
Preservation without response	173	20 (11.6)
Neither	44	9 (20.5)

Pairwise comparisons (Fisher exact test, Bonferroni-adjusted): full benefit vs neither, $P = 0.008$ (adjusted $P = 0.0495$); full benefit vs preservation without response, $P = 0.059$ (adjusted $P = 0.356$); all other pairs $P > 0.10$. The response-without-preservation quadrant contained only four patients and is reported descriptively.

Table 8. Baseline characteristics by age at onset (M0 cohort, n = 298)

Characteristic	Early-onset (n = 112)	Late-onset (n = 186)	P value
BMI, kg/m²	22.8 (20.5-25.0)	22.8 (21.3-25.1)	0.814
Distance from anal verge, cm	5.0 (3.0-7.2)	5.0 (4.0-8.0)	0.417
Neoadjuvant cycles	4.0 (2.0-4.0)	4.0 (2.0-4.0)	0.671
Ki67, %	70.0 (50.0-80.0)	70.0 (50.0-80.0)	0.908
Sex			**0.003**
Male	60 (53.6)	133 (71.5)	
Female	52 (46.4)	53 (28.5)	
Clinical T category			0.133
T1-2	5 (4.5)	6 (3.2)	
T3	58 (51.8)	118 (63.4)	
T4	49 (43.8)	62 (33.3)	
Clinical N category			0.124
N0	22 (19.6)	43 (23.1)	
N1	42 (37.5)	85 (45.7)	
N2	48 (42.9)	58 (31.2)	
Tumor differentiation			0.178
G1-G2	54 (48.2)	109 (58.6)	
G3	24 (21.4)	36 (19.4)	
Unknown	34 (30.4)	41 (22.0)	
Baseline CRM status			0.479
CRM-negative	27 (24.1)	34 (18.3)	
CRM-positive	32 (28.6)	56 (30.1)	
Unknown	53 (47.3)	96 (51.6)	
Baseline EMVI status			0.351
EMVI-negative	11 (9.8)	19 (10.2)	
EMVI-positive	33 (29.5)	41 (22.0)	
Unknown	68 (60.7)	126 (67.7)	
Baseline AJCC stage			0.562
I	2 (1.8)	2 (1.1)	
II	20 (17.9)	41 (22.0)	
III	90 (80.4)	143 (76.9)	
BMI category			0.335
<18.5 kg/m²	9 (8.0)	9 (4.8)	
18.5-23.9 kg/m²	60 (53.6)	109 (58.6)	
24-27.9 kg/m²	30 (26.8)	55 (29.6)	
≥28 kg/m²	13 (11.6)	13 (7.0)	
Clinical outcomes			

ypStage 0-I	33 (29.5)	48 (25.8)
TRG 0-1	18 (16.1)	25 (13.4)
Sphincter preservation	95 (84.8)	155 (83.3)
Postoperative recurrence	11 (9.8)	22 (11.8)

Data are median (interquartile range) or n (%). P values were calculated using the Wilcoxon rank-sum test for continuous variables and the chi-squared or Monte Carlo Fisher exact test for categorical variables. Clinical outcome rows are descriptive (no formal testing). Abbreviations as in Table 1.

Table 9. Treatment-mode-by-onset interaction for TRG 0-1 and within-stratum comparisons (M0 cohort)

A. Firth logistic regression: mode-by-onset interaction (joint Wald test)

Specification	NACT × early-onset OR (95% CI)	P value	TNT × early-onset OR (95% CI)	P value	Joint Wald χ^2 (df)	P value
Unadjusted	0.05 (0.01-0.41)	**0.005**	0.11 (0.02-0.67)	**0.017**	8.82 (2)	**0.012**
Adjusted for distance, cT, cN	0.05 (0.01-0.37)	**0.003**	0.11 (0.02-0.66)	**0.016**	9.40 (2)	**0.009**
Sex-adjusted	0.06 (0.01-0.43)	**0.006**	0.11 (0.02-0.70)	**0.019**	8.52 (2)	**0.014**

B. TRG 0-1 rates within onset strata (Fisher exact tests)

Stratum	Comparison	First group	Second group	P value
Early-onset	CRT vs NACT	31.4% (11/35)	4.9% (2/41)	**0.004**
Early-onset	CRT vs TNT	31.4% (11/35)	13.9% (5/36)	0.094
Late-onset	CRT vs NACT	5.1% (2/39)	13.8% (12/87)	0.223
Late-onset	CRT vs TNT	5.1% (2/39)	18.3% (11/60)	0.071

The ordinal TRG comparison between early-onset and late-onset patients was significant within the CRT group (Mann-Whitney P = 0.024) but not within NACT (P = 0.374) or TNT (P = 0.746). The complete age-cutpoint scan and supporting interaction analyses are provided in Supplementary Tables S6 and S7. TRG, tumor regression grade; other abbreviations as in Table 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ESM1.pdf