Experimental Research

INTERNATIONAL JOURNAL OF SURGERY

OPEN

Efficacy and safety of combining short-course neoadjuvant chemoradiotherapy with envafolimab in locally advanced rectal cancer patients with microsatellite stability: a phase II PRECAM experimental study

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Background: Conventional neoadjuvant chemoradiotherapy (nCRT) yields a pathologic complete response (pCR) rate of 15–30% for locally advanced rectal cancer (LARC). This study ventures to shift this paradigm by incorporating short-course nCRT with immunotherapy, specifically Envafolimab, to achieve improved treatment efficacy and possibly redefine the standard of care for LARC.

Materials and methods: The PRECAM study is a prospective, single-arm, phase 2 clinical trial for LARC in patients with microsatellite stable (MSS) tumors. Participants received short-course radiotherapy (25Gy/5f), followed by two cycles of CAPEOX chemotherapy and six weekly doses of Envafolimab, a PD-L1 antibody, before total mesorectal excision surgery. The primary endpoint was the pCR rate.

Results: From April to December 2022, 34 patients were enrolled, of whom 32 completed the study, each diagnosed with an MSS rectal adenocarcinoma. All patients underwent preoperative CRT combined with Envafolimab. Remarkably, a pCR rate of 62.5% (20/32) was attained, and a significant pathologic response rate of 75% (24/32) was achieved. Additionally, 21 of 32 participants achieved a neoadjuvant rectal (NAR) score below 8, suggesting an effective treatment response. Common adverse events included tenesmus (78.1%), diarrhea (62.5%), and leukocyte decrease (40.6%). Two Grade 3 adverse events were noted, one related to liver function abnormality and the other to a decrease in platelet count. Surgical procedures were performed in all cases, with minor complications, including ileus, infections, and anastomotic leakage. As of this report, there have been no reported cases of recurrence or death during the follow-up period, ranging from 12 to 20 months.

Conclusion: In LARC patients exhibiting MSS tumors, combining short-course nCRT with Envafolimab demonstrated favorable efficacy, leading to a significant pCR rate. Minor adverse effects and surgical complications were observed. These preliminary but promising results underscore the potential of this approach and call for further exploration and validation through a randomized controlled trial.

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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International Journal of Surgery (2025) 111:334–345

Received 12 April 2024; Accepted 6 July 2024

Supplemental Digital Content is available for this article. Direct URL citations are provided in the HTML and PDF versions of this article on the journal's website, www.lww.com/international-journal-of-surgery.

Published online 2 August 2024

http://dx.doi.org/10.1097/JS9.0000000000001960

Keywords: envafolimab, immune checkpoint inhibitor, locally advanced rectal cancer, microsatellite stability, pathologic complete response, preoperative neoadjuvant therapy, short-course radiotherapy

Introduction

Colorectal cancer continues to pose a considerable global health challenge, with rectal cancer constituting approximately one-third of all diagnosed cases[1]. Despite significant advances in medical technology and various treatment modalities, locally advanced rectal cancer (LARC) remains particularly problematic, often resulting in compromised patient survival due to its high propensity for local recurrence and distant metastasis[2,3].

Historically, long-course neoadjuvant chemoradiotherapy (nCRT) has been the standard of care for LARC, utilizing radiation doses ranging from 1.8 to 2 Gy over 25–28 sessions[4]. While effective in early downstaging and improving resection rates, this approach is fraught with limitations, including substantial costs, pronounced adverse effects, disease progression, elevated risk of bowel obstruction or perforation requiring emergency surgical intervention, and potential long-term organ impairment[5–8]. Furthermore, this conventional treatment's pathologic complete response (pCR) rate lingers at a suboptimal 10–20%, failing to significantly extend overall patient survival[9–11]. In contrast, short-course nCRT, characterized by 5 Gy doses administered over five sessions, is emerging as a favorable alternative[12]. It offers reduced short-term side effects equivalent to local control, with no significant differences in survival or incidence of distant metastases[13,14]. Its patient-centric design has also fostered improved patient acceptance. Notwithstanding its advantages, this approach has challenges, such as inadequate tumor downstaging prior to surgery[15] and a relatively modest initial pCR rate[16], indicating that the quest for the optimal treatment regimen persists.

The emergence of immune checkpoint inhibitors (ICIs) has revolutionized the approach to cancer treatment[17]. ICIs have demonstrated encouraging therapeutic outcomes across a spectrum of malignancies, including but not limited to melanoma[18], nonsmall cell lung cancer[19], esophageal cancer[20], and advanced colorectal cancer with high microsatellite instability (MSI-H)[21]. In a prospective phase II study conducted by Cercek *et al*., 100% (12/12) of patients diagnosed with MSI-H LARC had clinically complete responses after receiving 6 months of anti-PD-1 (dostarlimab) therapy. The responses were sustained without any evidence of residual tumor on serial biopsies for a duration of $\geq$6 months[22]. Nevertheless, the utility of immunotherapy is limited to 5% of colorectal cancer patients with MSI-H status, with minor therapeutic responses reported in the predominant microsatellite stable (MSS) population[23]. In response to the limited effectiveness of immunotherapy in MSS tumors, our research specifically targets MSS-type LARC. In this context, Envafolimab, a novel, humanized single-domain anti-PD-L1 antibody fused to an Fc fragment, offers a potential advancement[24]. Administered subcutaneously, it provides a less invasive and more convenient modality, potentially expanding the applicability of PD-L1 targeted therapies[25].

A few studies have explored the ICIs combined with short-course nCRT in neoadjuvant therapy for LARC. Studies such as a Phase II trial in China utilizing short-course nCRT with camrelizumab[26], the Averectal study in Lebanon and Jordan incorporating short-course nCRT with avelumab[27], and the TORCH study introducing an innovative short-course nCRT-based total neoadjuvant therapy (TNT) model[28] have all reported encouraging outcomes. In comparative terms, these trials have outperformed those employing long-course nCRT, where pCR rates are generally no higher than 30%[29–32]. These findings enrich our understanding of tumor response dynamics and indicate that the amalgamation of short-course nCRT and immunotherapy could potentially represent a new frontier in LARC management.

Nonetheless, it is imperative to exercise caution when interpreting these optimistic findings. Current studies are compromised by limitations such as small sample sizes, limited follow-up durations, and heterogeneity in treatment protocols, potentially affecting the validity of their conclusions. Additionally, the potential lymphocyte-mediated adverse effects induced by concurrent radiotherapy may confound the accurate assessment of treatment efficacy. These inherent complexities accentuate the existing challenges and highlight opportunities for further scientific exploration.

In light of the limitations of current preoperative neoadjuvant therapy for LARC patients with MSS tumors, this study aims to explore the feasibility and efficacy of a new model that combines short-course nCRT and Envafolimab. This innovative approach seeks to improve pCR rates and minimize adverse effects, and aspires to formulate a more patient-centric treatment paradigm. Notably, our study has achieved the highest pCR rate reported in

HIGHLIGHTS

- Targeted MSS Population: The PRECAM study specifically targets the microsatellite stable (MSS) population of locally advanced rectal cancer (LARC), historically considered less responsive to immunotherapy.
- Unprecedented pCR Rate: Among the MSS LARC population, the PRECAM study achieves a groundbreaking pathologic complete response (pCR) rate of 62.5%—the highest among all similar studies to date.
- Revolutionizing Treatment Paradigms: This milestone achievement challenges conventional perceptions by demonstrating the potential efficacy of combining short-course neoadjuvant chemoradiotherapy (nCRT) with Envafolimab immunotherapy in MSS LARC, previously considered poorly responsive.
- Expanding Research Horizons: The success of this innovative treatment model not only sets a new standard but also opens new avenues for research and clinical practice in MSS LARC. The exceptional pCR rate observed in this study underscores the transformative impact of this treatment approach.
- Broad Clinical Implications: With its potential to redefine the standard of care for MSS LARC, this study heralds a new era in the management of rectal cancer. The implications extend beyond efficacy, offering hope for improved outcomes and quality of life for patients facing this formidable disease.

any relevant study to date, without incurring exceedingly high side effects and surgical complications. By focusing on the MSS population, which is generally less responsive to immunotherapy, our study provides valuable insights into treatment effectiveness in this subgroup. This paper elucidates the preliminary findings of our clinical trial, offering critical insights into the viability of this novel approach as a potential new standard of care for LARC.

Methods

Study overview

The PRECAM study is designed as an open-label, single-arm, prospective, interventional phase II clinical trial. Our study aimed to investigate the efficacy and safety of short-course nCRT in combination with Envafolimab as a neoadjuvant treatment modality for patients diagnosed with LARC harboring MSS tumors. After the neoadjuvant treatment, all enrolled subjects underwent total mesorectal excision (TME) surgery. The primary endpoint of the current study phase is the pathological complete response (pCR) rate. Ethical approval for this study was provided by the Ethical Committee of Sir Run Run Shaw Hospital on 11 January 2022. Trial Registration Number and Date of Registration: ClinicalTrials.gov NCT05216653, January 14th, 2022.

Sample size

The pCR rates for nCRT with and without the administration of PD-L1 monoclonal antibody have been reported to be ~30 and 10%, respectively. We hypothesize a 20% improvement in the pCR rate due to our treatment intervention (from 10 to 30%). The sample size was calculated based on Simon's optimal two-stage design. Initially, 10 patients were enrolled. If one or no pCR were observed in the first stage, the study would be terminated; otherwise, an additional 19 patients would be enrolled in the second stage. This sample size provides a one-sided alpha level of 0.05 and a statistical power of 80%. Considering a dropout rate of 20%, the final estimated sample size is 35 patients.

Patient selection

The selection of participants for this study was meticulously designed to ensure a homogeneous patient population with progressive low and intermediate rectal cancer. The study included patients with histologically, and NGS confirmed MSS-type rectal adenocarcinoma who were willing to accept neoadjuvant therapy. The tumor should be ≤ 12 cm from the anal verge and was clinically staged as cT2N+, cT3/T4aN_{any} by pelvic enhanced CT and pelvic high-resolution MRI with no evidence of distant metastases. Additionally, patients were required to have an ECOG physical fitness score of 0–1, no prior antitumor or immunotherapy, and laboratory tests that met specific criteria. Voluntary participation and signing of the informed consent form were also mandatory.

We excluded patients with a history of other malignancies within the past 5 years or those with metastases to other sites. Patients with certain clinical stages, emergency surgical needs due to intestinal complications, or known hypersensitivity to study drugs were also excluded. Other exclusion factors included specific pathological conditions, unstable systemic disease, active autoimmune disease, a history of HIV or AIDS, use of immunosuppressive agents, or recent participation in another interventional clinical trial.

Therapeutic schemes

The study intervention involved a combination regimen of neoadjuvant radiotherapy, chemotherapy, immunotherapy, and TME surgery for enrolled patients with MSS-type progressive rectal cancer. The procedure began with short-course radiotherapy, delivering a tumor dose of 25 Gy to the primary tumor and high-risk areas. Following radiation, patients underwent a combination regimen of immunotherapy with Envafolimab and two cycles of CAPEOX chemotherapy. Two weeks after the completion of this neoadjuvant therapy, TME surgery was performed.

The radiotherapy program adhered to clinical practice, utilizing three-dimensional conformal or intensity-modulated radiotherapy techniques, with a dose split of 5 Gy/f and a total dose of 25 Gy/5f, irradiated within 7 days. The chemotherapy regimen followed the CAPEOX regimen: oxaliplatin (130 mg/m^2, ivgtt, d1) and capecitabine (1000 mg/m^2, po, d1-14). In conjunction with the CAPEOX regimen, Envafolimab was given by subcutaneous injection at 150 mg weekly for 6 weeks.

The surgical procedure involved TME surgery, which could be performed open, laparoscopically, or robotically, depending on the patient. All TME procedures were videotaped, and specimens were photographed and processed by the Department of Pathology at the research institution.

Data collection

Data acquisition was rigorously orchestrated at various stages of the therapeutic intervention. Baseline metrics, encompassing comprehensive physical examinations, multimodality imaging (including thoracic CT, hepatic MRI, and abdominopelvic CT or MRI), and laboratory assays, were amassed before initiating nCRT. Two independent physicians reviewed all the imaging reports. Before the administration of the CAPEOX regimen combined with Envafolimab, a similar data set was gathered, with an augmented focus on physiological health and laboratory parameters. After neoadjuvant treatment, the same data were collected and supplemented by ECG, echocardiogram, and toxic reaction evaluation.

Peripheral venous blood was drawn at five pivotal points throughout the treatment course to quantify cytokine expression levels and immune cell subset composition. Biopsy tissues harvested before nCRT were subjected to next-generation sequencing (NGS) to assess gene mutation profiles and calculate tumor mutational burden (TMB). NGS also verified the tumors' microsatellite stable status, and the results showed a constant 100% concordance with the tumors' histological mismatch repair (MMR) status. In addition, these biopsy samples were employed for single-cell RNA sequencing (scRNA-seq) analyses at distinct research stages, thereby enabling a dynamic longitudinal characterization of alterations within the tumor microenvironment.

Upon surgical intervention through TME, the surgical specimens were meticulously examined in the pathology department. This evaluation was overseen by two experienced pathologists who thoroughly assessed tumor staging and regression metrics. We also systematically collected colonoscopy, MRI, and pathology images before and after treatment to evaluate

comprehensively rectal mucosa, anatomical structures, and tissue-level changes. These images played a vital role in assessing treatment responses and enhancing the depth of our study.

Outcome assessment

The primary endpoint of this study was designated as the pCR rate, defined stringently as the microscopic absence of neoplastic cells or locoregional lymphatic metastases following TME surgery after neoadjuvant therapy.

Secondary endpoints included pathological tumor regression grade (TRG) and magnetic resonance-based TRG (MR-TRG), neoadjuvant rectal (NAR) score, treatment adverse effects, and surgical complications. The NAR Score, a recognized assessment tool for tumor response, was incorporated in a post-hoc analysis to quantify downstaging[33]. This score is derived from a blend of clinical and pathological staging, with lower scores suggesting a more favorable therapy response.

$$NAR = \frac{[5pN - 3(cT - pT) + 12]^2}{9.61}$$

Toxicities of neoadjuvant therapy were evaluated according to the common terminology criteria for adverse events (CTCAE) criteria (version 5.0), with follow-up assessments conducted during the treatment duration and 30 days after the end of the last cycle. Postoperative complications were assessed using the Clavien–Dindo classification system, which categorizes complications based on their severity and the type of intervention required.

Local recurrence or distal metastatic events were rigorously investigated upon the emergence of clinical symptomatology, escalations in carcinoembryonic antigen (CEA) levels, or the identification of radiographic abnormalities. Disease-free survival (DFS) and overall survival (OS) were also important aspects of

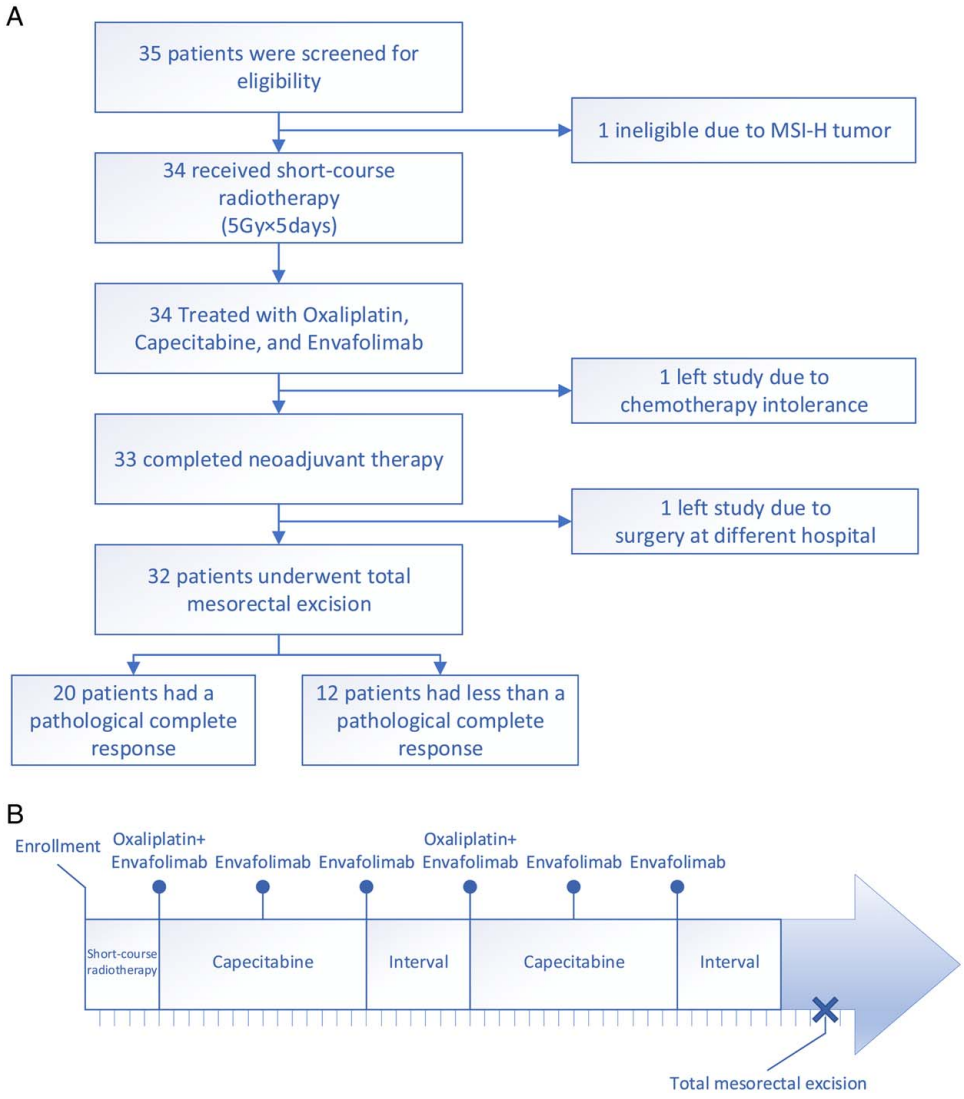

Figure 1. Workflow of the study. Flowchart in Figure 1A details the process from patient enrollment to the implementation of our treatment protocol. Figure 1B delineates the treatment strategy employed in this study.

the study, but their assessments will be addressed in subsequent research.

Genomic characterization

Genomic DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumoral specimens procured from 31 subjects. These were subjected to hybrid-capture-based next-generation sequencing (NGS) employing a panel of 990 genes executed on the Illumina HiSeq4000 platform. The genomic analysis recorded single-nucleotide variants (SNVs), copy number alterations (CNAs), and TMB, calculated per standard criteria and reported in mutations per megabase of the sequenced genomic region. TMB was categorized into high and low strata, with a cut-off value of eight mutations per megabase. Concurrently, single-cell RNA sequencing (scRNA-seq) was executed on samples acquired at various research phases to investigate dynamic changes in the tumor microenvironment. The intricate analyses and outcomes pertaining to scRNA-seq will be elaborated upon in subsequent articles. Patients participating in these comprehensive molecular analyses provided additional written informed consent specific to those analyses.

Statistical analysis

Efficacy analyses were conducted on a complete analysis set. The nonparametric Mann–Whitney *U* test will be employed to compare continuous variables. Fisher's exact test or the χ^2 test will contrast categorical variables. The Wilcoxon Signed-Rank Test will be invoked for analyses involving paired datasets encompassing numeric and non-numeric variables. A *P*-value threshold of less than 0.05 will be adopted to infer statistical significance. Categorical data will be described using frequencies and percentages, while continuous variables will be elucidated via means and 95% CI. Statistical computations will be conducted utilizing R (Version 4.2.0) and Python (Version 3.6.13). In addition to the methods described, detailed procedural information can be found in the Supplementary Protocol File (see Supplementary Data, Supplemental Digital Content 1, http://links.lww.com/SCS/G573).

Results

Overview of participants

Between April 2022 and December 2022, 35 patients were screened for eligibility, of which 34 were enrolled. A single patient was excluded due to the identification of an MSI-H tumor to maintain the focus of our analysis on the MSS population. Subsequent dropouts included a patient who withdrew owing to chemotherapy intolerance and another who opted for surgical treatment at a different facility. This led to a final cohort of 32 patients, as illustrated in Figure 1.

Clinical and pathological characteristics

The baseline demographic characteristics of the patient cohort are elucidated in Table 1. The cohort predominantly comprised male patients (71.9%) aged over 60 (81.3%), each of whom was histopathologically and NGS confirmed to have MSS rectal adenocarcinoma. Specifically, a preponderance of participants was observed to be in advanced T stage III (71.9%), accompanied by a negative circumferential resection margin (CRM) status in 87.5% of cases. Furthermore, 34.4% of the cohort exhibited a positive extramural venous invasion (EMVI) status, an important prognostic factor for local recurrence and survival. Lymph node status was diversified, with distribution across N stage 0 (40.6%), N stage I (40.6%), and N stage II (18.8%). Baseline tumor dimensions averaged 42.1 ± 11.0 mm, and these neoplasms were situated at an average distance of 60.2 ± 26.6 mm from the anal verge.

Concerning tumor biomarkers, ~53.1% displayed carcinoembryonic antigen (CEA) levels within the normative ranges (< 5 ng/ml), whereas an overwhelming 96.9% demonstrated normal CA19-9 levels (< 37 IU/ml). The diverse and intricate clinical characteristics of this patient population offered a comprehensive perspective for evaluating the therapeutic strategy's efficacy and safety, thereby enhancing the generalizability of our findings and laying a solid foundation for future research in this field.

Treatment efficacy

According to the pathologic assessment by more than two independent pathologists, we categorized the remaining patients based on the TRG system. In our study of 32 patients, 20 (62.5%) achieved TRG 0, indicating a pCR to the treatment without detectable neoplastic cells in the resected tissue. Additionally, four patients (12.5%) were classified as TRG 1, indicating minimal residual cancer. These two categories, designated as the major pathologic response (MPR), accounted for 75% of the total cohort. A residual of seven patients (21.9%) fell under TRG 2, with a single case (3.1%) allocated to TRG 3 (Fig. 2A). These classifications represent different levels of tumor regression following the treatment and provide nuanced insights into the efficacy of our therapeutic approach.

In parallel with pathological evaluation, our treatment efficacy was substantiated using a comprehensive set of radiographic

Table 1

Baseline patient demographic and clinical characteristics.

Characteristic	Subcharacteristic	Number and (%)
Age (years)	< 50	3 (9.4%)
	50–59	3 (9.4%)
	60–69	15 (46.9%)
	≥ 70	11 (34.4%)
Sex	Male	23 (71.9%)
	Female	9 (28.1%)
Tumor stage	Stage II	5 (15.6%)
	Stage III	23 (71.9%)
	Stage IV	4 (12.5%)
Lymph node status	Stage 0	13 (40.6%)
	Stage I	13 (40.6%)
	Stage II	6 (18.8%)
Distal location	cT3-4 and ≤ 5 cm	13 (40.6%)
	Others	19 (59.4%)
CRM status[a]	Positive	4 (12.5%)
	Negative	28 (87.5%)
EMVI status[b]	Positive	11 (34.4%)
	Negative	21 (65.6%)
CEA level	< 5 ng/ml	17 (53.1%)
	> 5 ng/ml	15 (46.9%)
CA19-9 level	< 37 IU/ml	31 (96.9%)
	> 37 IU/ml	1 (3.1%)

[a]CRM: circumferential resection margin.
[b]EMVI: extramural venous invasion.

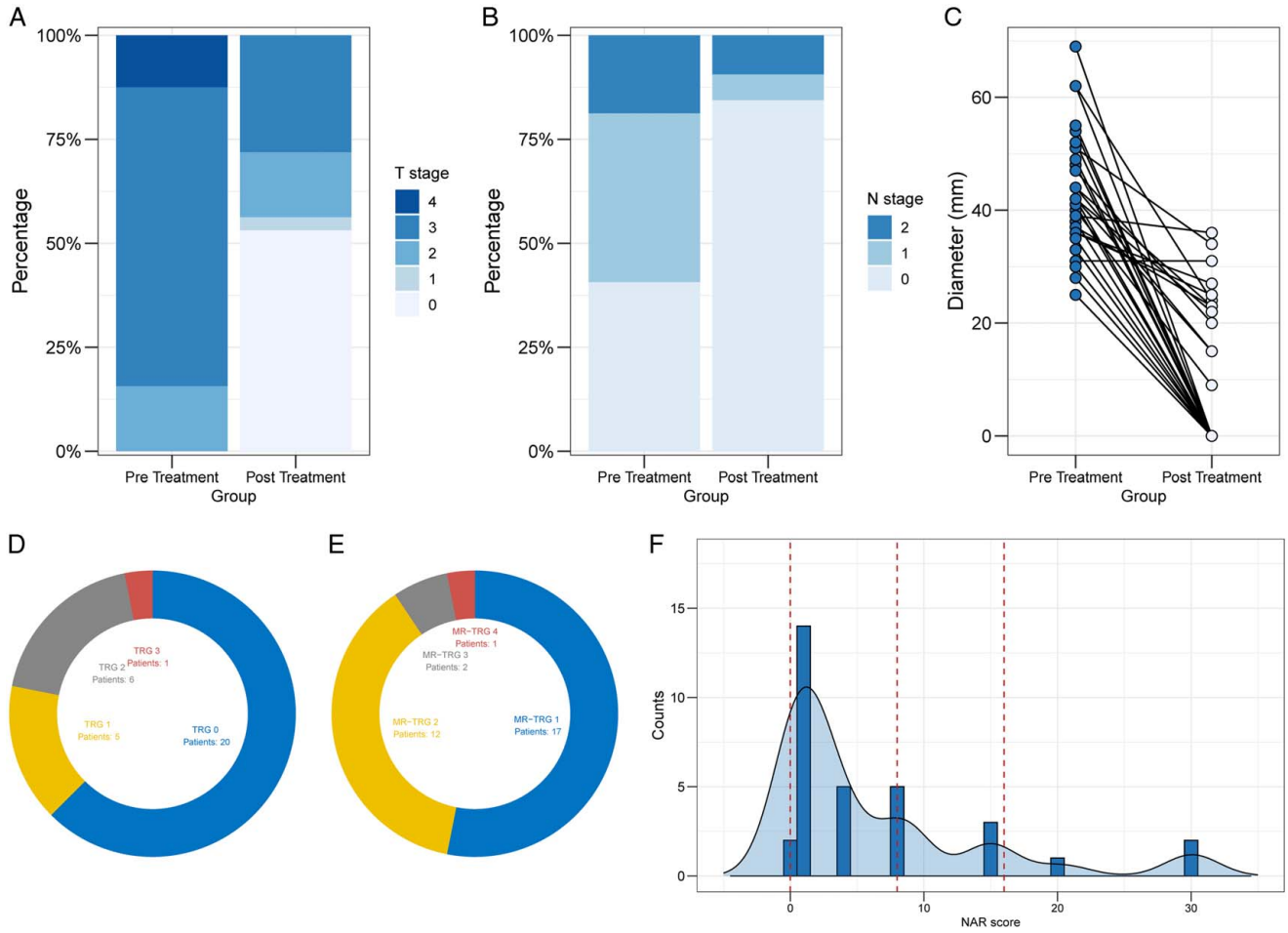

Figure 2. Comprehensive analysis of treatment response in MSS-type LARC. Figure 2A illustrates the changes in T-stage before and after the treatment. Figure 2B depicts the shifts in N-stage as a result of the treatment. Figure 2C compares the tumor diameters measured on MRI images before and after the treatment. Figure 2D categorizes patients based on their post-treatment pathological TRG. Figure 2E focuses on the MR-TRG grouping post-treatment. Figure 2F displays the distribution of NAR scores for each sample, quantifying the degree of response to the neoadjuvant therapy.

indicators obtained from pretreatment and post-treatment pelvic MRIs. The changes in preoperative and postoperative T and N stages were congruent with the efficacy of the neoadjuvant treatment, as a significant number of patients exhibited downstaging (Fig. 2B, C). Moreover, as 19 cases became too small to be measured after treatment, statistical analyses using the Wilcoxon Signed-Rank Test confirmed a significant diminution in tumor diameters pretreatment and post-treatment (Fig. 2D). Additional metrics, such as the distance between the lower end of the tumor and the anal verge, improved, and alterations were observed in the relationship between the tumor and the peritoneal reflection. In-depth assessments of the anal complex, circumferential resection margin (CRM), and extramural vascular invasion (EMVI) were congruent with the overall efficacy of our intervention. Finally, the MR-TRG was determined, providing a comprehensive evaluation harmonizing well with the elevated pCR rates noted in the study (Fig. 2E).

The NAR score is a prognostic tool used to predict the pathological response of rectal cancer to neoadjuvant therapy. Lower NAR scores indicate more favorable treatment outcomes and are thus associated with improved prognoses. In this study, the preponderance of patients (21 out of 32) exhibited an NAR score below 8, indicating an excellent response to the neoadjuvant therapy. The distribution of patients' NAR scores is graphically elucidated in Figure 2F.

In Figure 3, we present a comprehensive analysis of treatment-induced changes in patients with LARC, utilizing a curated collection of colonoscopy images, MRI, and pathology images for each TRG group. This visual compilation allows for a detailed examination of the morphological alterations before and after nCRT.

Treatment compliance and toxicity

Adverse events observed during the study are delineated in Table 2. A spectrum of adverse events was registered among the study participants. Most prominently, tenesmus affected a substantial proportion of patients (78.1%), followed by diarrhea (62.5%) and leukocyte count reduction (40.6%). Importantly, none of the recorded adverse events reached Grade 3. Many patients reported liver function abnormality and platelet decrease (40.6 and 37.5%, respectively), with each adverse event having a single case (3.1%) of Grade 3 severity. Less frequent adverse

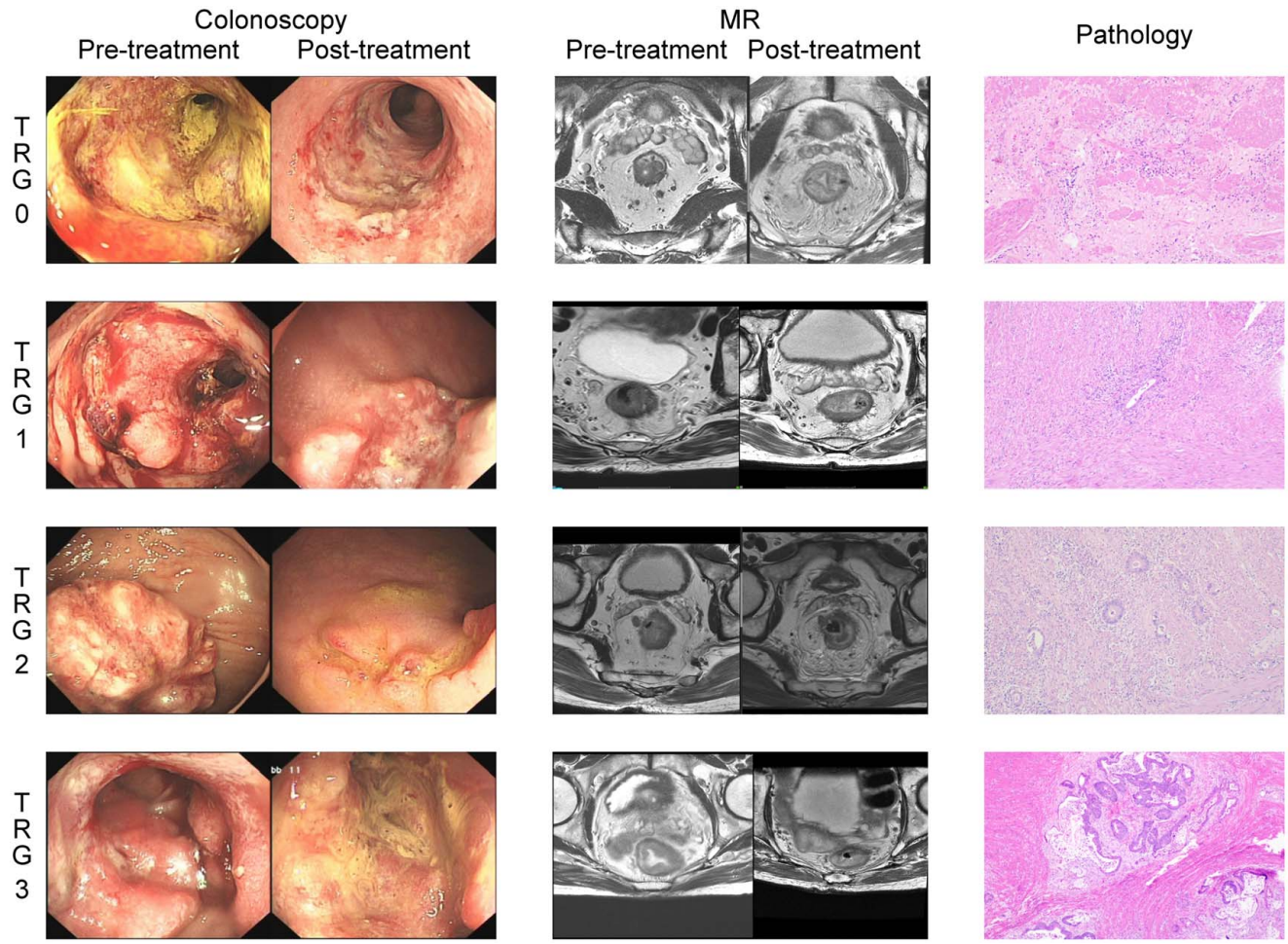

Figure 3. Evolution of endoscopic, radiographic, and pathological responses in representative cases across TRG groups. This figure provides a detailed visual representation for each TRG subgroup, illustrating the treatment's impact. For each subgroup, we present a set of images: endoscopic images and MR images taken before and after treatment, alongside corresponding postsurgical pathological findings.

events encompass abdominal pain, rash, urinary irritation, and fever.

Most participants completed the designated therapeutic regimen despite these adverse events regarding treatment compliance. The adverse events were manageable and did not markedly hinder patient compliance with the treatment protocol.

Table 2

Adverse event since the initiation of therapy.

Adverse event	Grade 1–2	Grade 3
Tenesmus	25 (78.1%)	0
Diarrhea	20 (62.5%)	0
Abdominal pain	13 (40.6%)	0
Leukocyte decrease	13 (40.6%)	0
Liver function abnormal	12 (37.5%)	1 (3.1%)*
Platelet decrease	11 (34.4%)	1 (3.1%)*
Rash	7 (21.9%)*	0
Urinary irritation	4 (12.5%)	0
Fever	2 (6.3%)	0

Events are listed according to category of toxicity. Toxicities were evaluated according to the common terminology criteria for adverse events (CTCAE) criteria (version 5.0).
Items marked with *indicate immune-related adverse events (irAEs).

Surgery and complications

The primary surgical procedures performed on the cohort of 32 patients included low anterior resection (LAR) in 28 cases, Hartmann's procedure in three cases, and a single instance of Miles' procedure. These procedures represent standard interventions for the treatment of rectal adenocarcinoma and align with the severity and location of the tumor in each patient. Postoperatively, a spectrum of complications was observed (Table 3). Ileus was the most frequently occurring complication, documented in five instances (15.6%). Intra-abdominal and respiratory tract infections were reported in four (12.5%) and three (9.4%) patients, respectively. Anastomotic leakage and urinary retention each occurred in two cases (6.3%), as did venous thrombosis. Sepsis, lymphatic leak, and enteritis were observed individually (3.1%). The comprehensive details of the surgical procedures conducted and the quality assessment of the surgical specimens are delineated in Table 4.

Genetic landscape and tumor mutation burden

In the genetic mutation landscape of the patient cohort, an oncoplot was generated to provide a comprehensive overview of

Table 3

Complications after surgery.

Complication	Number of cases (Proportion)	Clavien–Dindo classification (Grade > 3 cases)
Ileus	5 (15.6%)	0
Intra-abdominal infection	4 (12.5%)	0
Respiratory tract infection	3 (9.4%)	0
Anastomotic leakage	2 (7.1%)	0
Urinary retention	2 (6.3%)	0
Venous thrombosis	2 (6.3%)	0
Sepsis (caused by Acinetobacter baumannii)	1 (3.1%)	0
Lymphatic leak	1 (3.1%)	0
Enteritis	1 (3.1%)	0

gene mutations in the study cohort (Fig. 4A). Notably, mutations in the KRAS gene were frequently identified and present in 20 out of 31 patients, of which 13 achieved pCR. TMB was also quantified for each participant: three patients were classified as TMB-High, while the remainder fell into the TMB-Low category. A statistical assessment failed to demonstrate any significant correlation between either KRAS mutation status or TMB level and the probability of achieving pCR ($P > 0.05$ for both variables).

A comprehensive statistical evaluation was also conducted to examine the relationship between all gene mutations and pCR outcomes (Fig. 4B). The analysis revealed that no individual gene mutation displayed a statistically significant association with the achievement of pCR ($P > 0.05$ for all tested genes). Despite the higher incidence of pCR in patients with KRAS mutations and the variable TMB statuses, neither these nor any other specific gene mutations had a statistically significant impact on pCR rates in this investigation phase.

Discussion

Recent advances have positioned immunotherapy as a cornerstone in oncology, notably for various malignant tumors[34]. Patients with MSI-H traits, marked by elevated TMB and pronounced tumor-infiltrating lymphocytes (TILs), demonstrate a particular receptivity to such therapies[35,36]. In a recent investigation assessing the efficacy of the anti-PD1 antibody dostarlimab as a neoadjuvant treatment for individuals with MSI-H LARC, noteworthy outcomes were observed, with all 12 patients achieving complete remission[22]. The pivotal Keynote-177 study further endorsed pembrolizumab monotherapy as the primary treatment modality for mismatch repair deficient (dMMR)/MSI-H colorectal cancer[37]. However, the prevalence of dMMR/MSI-H tumors is limited to ~5% of all colorectal cases. The quest for improved therapeutic regimens remains a paramount research objective for the predominant MSS tumors, which demonstrate reduced susceptibility to immunotherapy. Moreover, while related articles in the field often explore the use of PD-1 monoclonal antibodies in conjunction with nCRT, this study is the pioneering investigation into the PD-L1 monoclonal antibody.

Table 4

Surgical parameters and the parameters of the surgical specimen.

Procedures performed and surgical specimen parameters	Number/Value
Surgical procedure	
Low anterior resection, LAR	28
Hartmann	3
Miles	1
Surgical platform	
da Vinci surgical system Xi	20
Laparoscopy	12
Operation duration (mins)	171.88 ± 39.51
Intraoperative blood loss (ml)	48.13 ± 7.38
Mesorectal integrity	93.75%
Circumferential margin positive rate	0%
Proximal margin (cm)	9.61 ± 2.94
Distal margin (cm)	2.07 ± 1.8
Distance from anastomosis to anal verge (cm)	2.90 ± 1.54
Tumor size (cm)	1.67 ± 1.19
Number of lymph bodes examined	17.88 ± 7.11

In this investigation into the management of LARC, the combination of short-course nCRT with the PD-L1 antibody, Envafolimab, demonstrated a distinct therapeutic edge. Compared to conventional neoadjuvant strategies, we observed a pronounced increase in pCR rates, accompanied by acceptable mild adverse events, thereby emphasizing the crucial role of the PD-L1 antibody in bolstering the combined treatment's efficacy and safety. Traditionally, preoperative radiotherapy in tandem with chemotherapy has resulted in tumor downstaging and a drop in local recurrence. The pCR has typically materialized in only 10–30% of rectal cancer patients[38]. In stark contrast, our research, with a dedicated focus on MSS tumor patients, reported a pCR rate of 62.5%. This unprecedented rate highlights the synergistic potential of combining short-course nCRT with Envafolimab, suggesting a prospective paradigm shift in therapeutic strategies for rectal cancer.

Many clinical studies have provided preliminary results on the combination of nCRT with immunotherapy, with most being prospective stage I-II trials with limited sample sizes. Whereas the majority of these trials, including Voltage-A[29], NSABP FR-2[30], and PANDORA[39], focus on long-course nCRT and yield pCR rates hovering around 30–35%, trials employing short-course nCRT have demonstrated superior outcomes. For example, the Averectal study from Lebanon and Jordan involved 44 LARC patients treated with short-course radiotherapy, mFOLFOX6, and avelumab[27]. The reported pCR rate was 37.5%. A separate Chinese phase II study combined short-course radiotherapy with XELOX and carrelizumab treatments[26]. Among the 27 operated patients, the overall pCR rate stood at 48%, with subgroup rates of 46% for pMMR/MSS and 100% for dMMR/MSI-H. In the TORCH phase II trial, utilizing the CRT-based TNT model, results disclosed at the 2023 ESMO Annual Meeting showcased a noteworthy tumor regression[28]. The pCR rate was an impressive 49.2% (29/59) among those who underwent TME surgery. In light of these clinical studies, our trial distinctly stands out, achieving the highest pCR rate, which shows the substantial effectiveness of our treatment strategy compared to other approaches in the area.

The elevated pCR rate may be attributed to a confluence of mechanisms. Firstly, radiotherapy induces immunogenic cell death (ICD) in tumor cells[40], releasing proinflammatory signals like neoantigens[41] and damage-associated molecular patterns (DAMPs)[42]. This, in turn, bolsters the activation of antitumor T cells and augments the congregation of TILs[43]. Notably, the integration of radiotherapy with immunotherapy has showcased

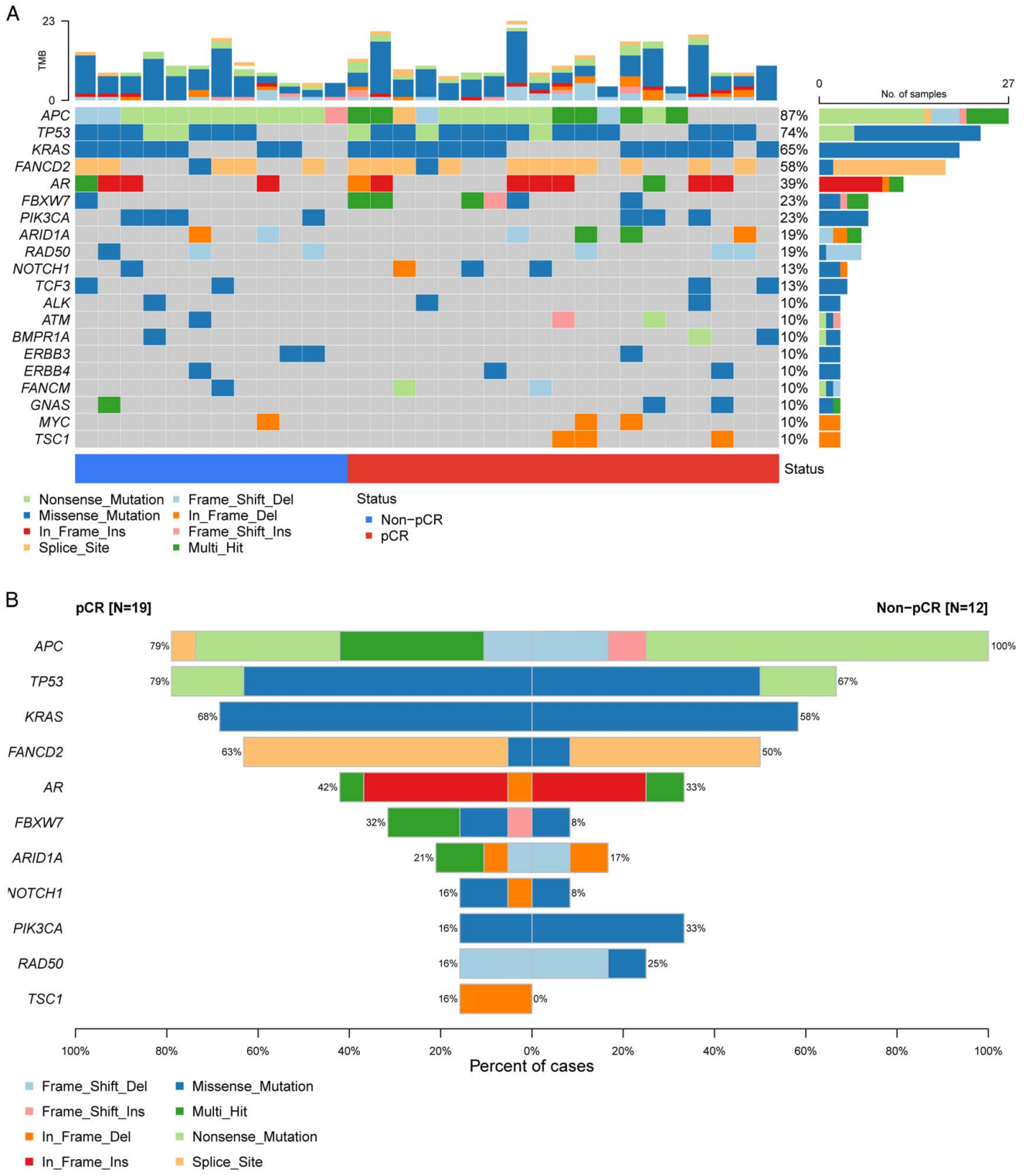

Figure 4. Genetic mutation profiles in study samples. Figure 4A offers a comprehensive overview of the mutations identified across all samples, illustrating the range and frequency of genetic alterations. Figure 4B presents a focused comparison of mutations between the pCR and non-pCR groups.

the 'abscopal effect', wherein irradiation of a tumor in one location precipitates significant regression in a distal, non-irradiated tumor, likely resulting from a systemically activated immune response[44]. Moreover, radiotherapy has been observed to enhance PD-L1 expression within tumor tissues, thereby amplifying their susceptibility to immunotherapy[45]. To optimize this effect, we administered chemotherapy and immunotherapy 2 days postradiation. This timing leverages the peak period of

immune activation induced by radiotherapy, which diminishes over time, reverting to an immunosuppressive state. In synergy, radiotherapy and PD-L1 antibody interventions recalibrate the tumor microenvironment, tempering its immunosuppressive tendencies and intensifying T-cell-derived antitumor cytokine production[46–48]. Thus, the amalgamation of nCRT and immunotherapy via a cooperative sensitization mechanism fosters synergy between localized and systemic interventions, culminating in enhanced tumor regression[49].

Among the few studies exploring the use of PD-L1 inhibitors in combination with nCRT, our research stands out in demonstrating their superior efficacy compared to PD-1 inhibitors in this specific clinical setting. This distinction is rooted in the unique mechanisms through which PD-L1 inhibitors interact with the immune system. By directly targeting PD-L1 on tumor cells, these inhibitors not only prevent the cells from evading immune detection but also potentially engage a broader spectrum of immune cells, including those like natural killer cells, which mostly do not express PD-1[50]. This wider scope of immune activation, especially in tandem with the ICD induced by radiotherapy, presents a significant advantage. Furthermore, the influence of PD-L1 inhibitors on the altered hypoxic status of tumors post-nCRT may contribute to their enhanced effectiveness[51,52]. These differences in modes of action and specific interactions within the tumor microenvironment and immune system underpin the observed superior outcomes of PD-L1 inhibitors in our study, underscoring their potential over PD-1 inhibitors in this treatment context.

The reduced adverse events observed in our study align with prior evidence suggesting that short-course nCRT, owing to its shorter treatment duration and decreased radiation dose, might have a diminished risk of radiation-induced toxicity compared to long-course nCRT[6]. This bolsters the argument for short-course nCRT as a potentially safer rectal cancer treatment alternative. We observed only two significant side effects of immunotherapy: one case of grade 3 liver function abnormality and one case of grade 3 platelet decrease. These results suggest that while serious side effects can occur, their incidence is low, indicating that the side effects of immunotherapy are generally manageable. Furthermore, surgical interventions inherently carry a degree of risk, especially in the context of advanced rectal adenocarcinomas, where disease severity and patient comorbidity can contribute to complications[53]. However, the postoperative complications in our study, spanning from ileus to intra-abdominal infections and venous thrombosis, do not surpass those generally anticipated with traditional preoperative therapies. This suggests that our novel approach maintains an acceptable safety profile without introducing new risks.

As we pivot to the genomics landscape of our patient cohort, a noteworthy observation emerges. The incidence of KRAS mutations in our cohort was significantly higher, at 64.5%, compared to the ~40% commonly observed in general LARC populations[54]. While KRAS mutations are generally considered poor prognostic indicators for neoadjuvant therapy in rectal cancer, our study presents an intriguing contrast[55–57]. Despite a high incidence of KRAS mutations within our cohort, our trial demonstrated remarkable pCR rates. One plausible hypothesis for this counterintuitive finding could be a synergistic interaction between nCRT and immunotherapy in our treatment regimen. It is conceivable that the immunotherapeutic agents used might modulate the tumor microenvironment or alter immune checkpoint mechanisms in a way that mitigates the adverse effects typically associated with KRAS mutations. This offsetting effect might allow for the encouraging pCR outcomes observed in our study.

Nonetheless, we must highlight that our data failed to show a statistically significant association between KRAS mutations, TMB, and other gene mutation-related indicators with pCR rates. This absence of statistical correlation could be attributed to our limited sample size. More extensive research in larger, diverse cohorts is necessary to elucidate these relationships definitively.

The potential clinical implications of our findings are significant. If validated in larger studies, the combination of short-course nCRT and immunotherapy could become a new standard of care for rectal cancer patients, offering improved efficacy and safety compared to current treatment modalities. However, it is important to acknowledge several limitations of our study. These include a small sample size, potential biases, and a short follow-up period, which hinder the comprehensive assessment of longer-term outcomes such as recurrence rates and overall survival. Therefore, increasing sample sizes, implementing appropriate control groups, and conducting longer follow-up periods would be essential to address these limitations and validate our findings. Additionally, if the efficacy is confirmed, exploring the possibility of organ preservation strategies would be worthwhile. Future research should focus on optimizing the treatment regimen and identifying biomarkers to predict response to this combined approach.

Conclusion

In summary, the PRECAM study represents a prospective, phase II, single-arm trial evaluating the efficacy of short-course radiotherapy in conjunction with CAPEOX and Envafolimab as a neoadjuvant therapy for LARC patients. The primary objective is to ascertain if integrating immunotherapy with nCRT enhances tumor regression, exhibits favorable tolerability, and offers improved prognosis. Future research is essential to validate these preliminary outcomes and delve into this therapeutic approach's long-term implications.

Ethical approval

Ethical approval for this study was provided by the Ethical Committee of Sir Run Run Shaw Hospitals, Hangzhou, China on 11 January 2022 (No. 20220111-8).

Consent

Written informed consent was obtained from the patient (or, where applicable, the patient's guardian or next of kin) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Source of funding

Financial support and sponsorship: This study was supported by grants from 2022 Zhejiang Provincial Key R&D Program (No. 2022C03032), 2023 Provincial-Ministerial Joint Key Project (No. WKJ-ZJ-2313), and National Natural Science Foundation of China (No. 81703076 and No. 82072628).

Author contribution

F.W.: led the project administration, curated data, and oversaw both conceptualization and methodology, while also supervising the team and contributing to the writing and review process; C.X.L.: was pivotal in conceptualization, methodology, data curation, and investigation, and also took part in the writing review; Y.M.L.: focused on methodology, developed software, conducted formal analysis, and contributed to visualization, resources management, and the original draft writing; F.X.Z.: was involved in investigation, data curation, and managing resources; L.M.S.: contributed to investigation, data curation, and validation; Y.F.W.: worked on data curation, formal analysis, validation, and software development; Y.B.S.: engaged in data curation, validation, and investigation; L.N.X.: was responsible for software development, validation, visualization, and contributed to writing and editing; P.H. and W.T.: contributed to validation, visualization, data curation, and methodology; D.Y.X.: focused on data curation and resources; G.Y.C. and L.N.S.: provided resources and were involved in data curation; X.Y.J. and Y.Y.C.: were engaged in data curation; Y.Y.C.: also contributing to visualization and writing review; D.W.L., D.W., and W.F.L.: were dedicated to writing review and editing; H.C.G.: acquired funding, supervised aspects of the project, and contributed to data curation and writing review; X.N.S.: oversaw supervision and methodology; X.F.H. and S.D.: were involved in project administration, conceptualization, formal analysis, and investigation; X.F.H.: contribute to the original draft and resource management; S.D.: acquiring funding and resources.

Conflicts of interest disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Research registration unique identifying number (UIN)

Trial Registration Number and Date of Registration: ClinicalTrials.gov NCT05216653, 14th January 2022.

Guarantor

Fei Wang, Yiming Lv, and Sheng Dai are designated as the guarantors for this study. They accept full responsibility for the work and the conduct of the study, had access to the data, and controlled the decision to publish.

Data availability statement

In compliance with the regulatory frameworks and patient confidentiality agreements upheld by Sir Run Run Shaw Hospital, and in alignment with external data governance standards, dissemination of both aggregate and individual-level patient data from this study is not permissible. Researchers with academic interest in this domain are encouraged to initiate scholarly engagement through communication with the corresponding author.

Provenance and peer review

This paper was not invited.

Acknowledgement

Assistance with the study: none.
Presentation: none.

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