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Ultrasound-targeted microbubble destruction combined with chemoimmunotherapy in locally advanced MSS/pMMR rectal cancer (UNIC): an exploratory phase 2 study

Zhenqiang Sun

fccsunzq@zzu.edu.cn

The First Affiliated Hospital of Zhengzhou University https://orcid.org/0000-0002-9098-7296

Baojing Tuo

Zhengzhou University First Affiliated Hospital

Bo Xiong

Zhengzhou University First Affiliated Hospital

Lin Gan

Zhengzhou University First Affiliated Hospital

Bang Du

Zhengzhou University First Affiliated Hospital

Ming Yue

Zhengzhou University First Affiliated Hospital

Xiaofei Duan

Zhengzhou University First Affiliated Hospital

Suhong Lin

The First Affiliated Hospital of Zhengzhou University

Shuochen Gao

Zhengzhou University First Affiliated Hospital

Guixian Wang

The First Affiliated Hospital of Zhengzhou University

Yugui Lian

Zhengzhou University First Affiliated Hospital

Jianning Yao

Zhengzhou University First Affiliated Hospital

Tengyu Li

Zhengzhou University First Affiliated Hospital

Shuang Xie

Zhengzhou University First Affiliated Hospital

Shengyun Hu
The First Affiliated Hospital of Zhengzhou University
Junhong Hu
The First Affiliated Hospital of Zhengzhou University
Yang Fu
The First Affiliated Hospital of Zhengzhou University https://orcid.org/0000-0002-8221-3354
Yan Zhang
Zhengzhou University First Affiliated Hospital

Article

Keywords:

Posted Date: May 15th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9701606/v1

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Additional Declarations: There is **NO** Competing Interest.

Ultrasound-targeted microbubble destruction combined with chemoimmunotherapy in locally advanced MSS/pMMR rectal cancer (UNIC): an exploratory phase 2 study

Baojing Tuo[1,2#], Bo Xiong[3#], Lin Gan[1#], Bang Du[1,2#], Ming Yue[1,2], Xiaofei Duan[1], Suhong Lin[1], Shuochen Gao[4], Guixian Wang[1], Yugui Lian[1], Jianning Yao[5], Tengyu Li[1], Shuang Xie[6], Shengyun Hu[1], Junhong Hu[1*], Yang Fu[7*], Yan Zhang[3*], Zhenqiang Sun[1,2*]

1. Department of Colorectal Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, 450052, China.
2. Henan Institute of Interconnected Intelligent Health Management, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, Henan, China.
3. Department of Ultrasound, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, Henan, China.
4. Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, Henan, China.
5. Department of Gastroenterology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China.
6. Department of General Surgery, The 988th Hospital of PLA Joint Logistics Support Force, Zhengzhou, 450052, Henan, China.
7. Department of Gastrointestinal Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052, China.

[#] These authors contributed equally to this work and should be considered co-first authors.

* Corresponding authors.

Abstract

The development of neoadjuvant strategies that preserve normal tissue while maintaining tumour control remains a central challenge in locally advanced rectal cancer. Radiotherapy can enhance local control but causes pelvic tissue injury and long-term functional sequelae, whereas most rectal cancers are microsatellite-stable or mismatch repair-proficient (MSS/pMMR) and respond poorly to immune checkpoint blockade alone. We developed UNIC, a non-ionising ultrasound-based strategy integrating ultrasound-targeted microbubble destruction (UTMD), CAPOX chemotherapy and PD-1 inhibition. In this single-centre exploratory phase 2 study, 18 patients with previously untreated MSS/pMMR locally advanced rectal cancer received UNIC with response-adapted treatment duration guided by real-time ultrasound. Of nine patients who completed neoadjuvant treatment and underwent surgery, four achieved pathological complete response (44.4%), and all achieved R0 resection with sphincter preservation. The objective response rate was 100% among ten imaging-evaluable patients. No grade 3 or higher treatment-related adverse events occurred, and patient-reported outcomes showed maintained quality of life and no major LARS among evaluable patients. These data introduce UTMD as a clinically feasible physical therapeutic modality in gastrointestinal cancer and provide a rationale for non-radiotherapy, tissue-preserving neoadjuvant treatment strategies.

Main

Locally advanced rectal cancer (LARC) is currently treated with neoadjuvant chemoradiotherapy (CRT) followed by total mesorectal excision (TME) as the standard approach[1,2]. Although long-course synchronous chemoradiotherapy reduces the risk of local recurrence, it is associated with significant acute and long-term toxicities, including radiation-induced enteritis, dermatitis, myelosuppression, and chronic bowel dysfunction[3-6]. Furthermore, radiation-induced pelvic oedema and fibrosis can complicate surgery, impair pelvic nerve protection, increase the risk of anastomotic leakage, and further compromise postoperative bowel, urinary, and sexual function, significantly affecting patients' quality of life[7-9]. Notably, even with standard treatment, the pathological complete response (pCR) rate in LARC remains limited, typically ranging from 11-15%, and the risk of distant metastasis is not sufficiently controlled[10,11]. Therefore, reducing radiation-related damage while ensuring tumour control remains a critical challenge in LARC treatment.

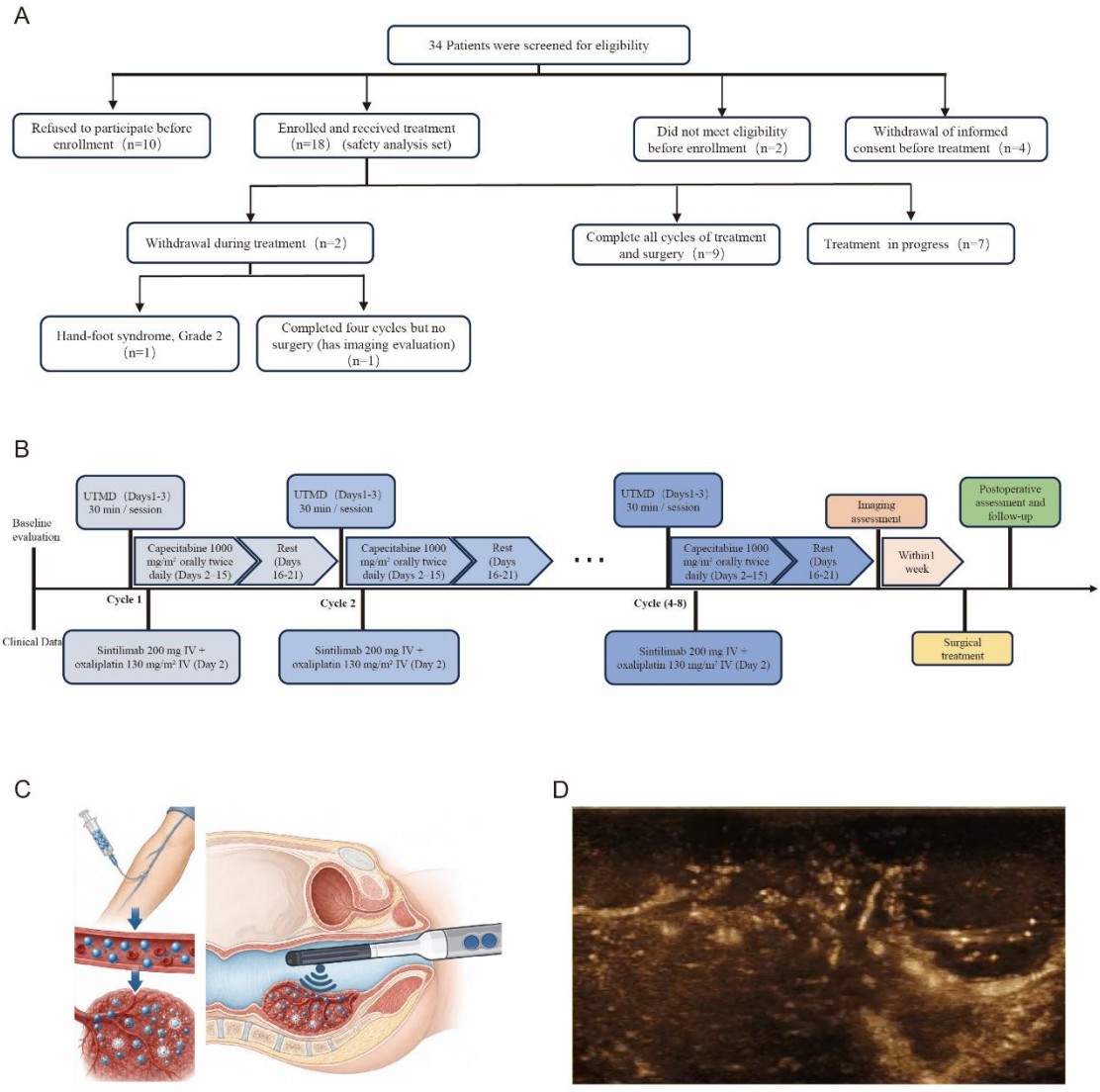
In recent years, immune checkpoint inhibitors have shown remarkable efficacy in various solid tumours, particularly in microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) cancers, where they induce durable antitumour responses[12,13]. However, the majority of rectal cancer cases (approximately 95%) are microsatellite stable or mismatch repair proficient (MSS/pMMR), rendering them largely resistant to monotherapy with immune checkpoint inhibitors[14-16]. To enhance the efficacy of neoadjuvant treatment in MSS/pMMR LARC, multiple combination strategies have been explored[17,18]. Among these, short-course radiotherapy followed by chemotherapy and immunotherapy has emerged as a promising approach, with some clinical studies reporting higher pCR rates[19,20]. However, these strategies still rely on radiotherapy, inheriting the associated acute and long-term toxicities.

Ultrasound-targeted microbubble destruction (UTMD) is a novel physical treatment technique that uses low-frequency ultrasound to induce microbubble oscillation and rupture, generating local mechanical effects that act on tumour tissue and its microenvironment, without damaging normal bowel or surrounding tissues[21,22]. Preclinical studies have suggested that UTMD may increase tumour vascular permeability, enhance drug delivery, and potentially improve local antitumour effects[23]. Unlike radiotherapy, UTMD does not rely on ionizing radiation, and its biological effects are primarily mechanical, theoretically reducing damage to surrounding normal tissues. Although promising results have been reported in animal models[21,24,25], its clinical application in gastrointestinal tumours has yet to be explored.

Given these considerations, we designed this single-center, open-label, exploratory phase 2 study (UNIC), which is the first clinical application of this innovative treatment strategy in gastrointestinal malignancies. The study aims to assess the preliminary efficacy and safety of UTMD combined with CAPOX chemotherapy and PD-1 inhibition (UNIC treatment regimen) in patients with initially diagnosed MSS/pMMR LARC, with the goal of achieving tumour control while preserving the anus and its functions, reducing treatment-related adverse effects, and improving patients' quality of life, ultimately providing a potential non-radiotherapy-dependent neoadjuvant treatment option for clinical MSS/pMMR LARC.

Results

From March 2025 to March 2026, 34 patients with LARC were screened for eligibility. Of these, 18 patients met the inclusion criteria, provided written informed consent, and received at least one cycle of study treatment (safety analysis set). The trial profile is shown in figure 1A-D.



Among the 18 treated patients, one discontinued therapy during the treatment period owing to grade 2 hand-foot syndrome. No patient met the prespecified criterion for early treatment termination based on absence of tumour shrinkage on two consecutive ultrasound assessments. Reasons for cessation of therapy were either completion of the maximum prespecified number of cycles (8 cycles; n=1) or attainment of individualised stopping criteria—defined as tumour regression reaching a plateau or radiographic disappearance following consecutive shrinkage—whichever occurred first (n=9). The remaining patients continue to receive study treatment. In the nine patients who completed neoadjuvant therapy and proceeded to surgery, the actual number of treatment cycles received was as follows: 4 cycles in five patients, 6 cycles in three patients, and 8 cycles in one patient. No cases of premature treatment discontinuation due to insufficient tumour response were

observed. Nine patients completed the full planned course of neoadjuvant therapy and underwent curative-intent resection for rectal cancer (per-protocol efficacy analysis set). One patient declined surgery after completing four cycles of treatment, and an additional seven patients remain on active therapy.

Baseline demographic and clinicopathological characteristics of the 18 treated patients are summarised in table 1. All patients were Asian, with a median age of 60 years (range 40-74), and nine (50%) were female. Clinical staging was as follows: cT2 in 1 patient (6%), cT3 in 16 patients (89%), cT4 in 1 patient (6%); cN1 in 9 patients (50.0%), and cN2 in 9 patients (50.0%). The median distance from the anal verge was 3.1 cm (range 1.5-5.0).

Table 1. Baseline Demographic and Clinicopathological Characteristics of the Patients		
Characteristic	Total Patients (N=18)	Notes
Demographics		
Age (years)	60 (40-74)	
Sex, n (%)		
Male	9 (50%)	
Female	9 (50%)	
ECOG Performance Status, n (%)		
0	12 (67%)	
1	6 (33%)	
Tumour Characteristics		
Clinical T stage (cT), n (%)*		Assessed by baseline MRI.
cT2	1 (6%)	
cT3	16 (89%)	
cT4	1 (6%)	
Clinical N stage (cN), n (%)		Assessed by baseline MRI.
cN0	0	
cN1	9 (50%)	
cN2	9 (50%)	
Distance from Anal Verge (cm)	3.1 (1.5-5.0)	
Histological Type		
Adenocarcinoma	18 (100%)	
Microsatellite Status		Key inclusion criterion.
MSS/pMMR (Confirmed by IHC/PCR)	18 (100%)	
Key Laboratory Values		
CEA Level (ng/mL)	Median (Range)	
≤5 (Normal)	13 (72%)	
>5 (Elevated)	5 (28%)	

*Percentages might not sum to 100% because of rounding.

Among patients who completed neoadjuvant therapy and proceeded to surgery, a high pCR rate was observed (44.4%, 4/9). Given that several patients remain on active treatment or are awaiting surgery,

intention-to-treat analysis outcomes are not yet mature. All patients who underwent surgery achieved R0 resection (100%, 9/9) and sphincter preservation (100%, 9/9). An additional patient achieved a complete response in the primary tumour (ypT0) but had residual nodal involvement (ypN1); therefore, the primary tumour complete response rate (ypT0) was 55.6% (5/9). The lymph node negativity rate (ypN0) was 88.9% (8/9). Intraoperative macroscopic assessment revealed no evidence of oedema in the bowel wall or perirectal tissues in any of the nine patients, with clear pelvic anatomical planes and complete preservation of autonomic nerves (hypogastric plexus and pelvic splanchnic nerves). Within 30 days postoperatively, only one patient (11.1%) experienced a Clavien-Dindo grade I hypoalbuminaemia event; no complications of grade II or higher were recorded. Specifically, the incidences of anastomotic leakage, surgical site infection, intestinal obstruction, haemorrhage, and urinary retention were all 0%.

Among 10 evaluable patients with complete baseline assessment and at least one post-treatment radiographic evaluation-comprising 9 patients who underwent surgery and 1 patient who completed 4 cycles of treatment without undergoing surgery-the ORR per RECIST version 1.1 was 100% (10 of 10), consisting of 6 complete responses and 4 partial responses (Figure 2A-D; Table 2).

Regarding functional preservation outcomes, urinary function and quality-of-life scores were available for all 9 patients who underwent surgery. The median International Prostate Symptom Score (IPSS) was 1 (range 0-7), and all patients fell within the mild symptom range (0-7), indicating a low burden of urinary symptoms. Quality of life was assessed using the EQ-5D-5L instrument, with results reported as the EQ visual analogue scale (VAS). The median EQ VAS scores at baseline and upon completion of all neoadjuvant therapy were 80 (range 70–82) and 85 (range 80–90), respectively, suggesting no deterioration in overall quality of life during neoadjuvant treatment and a numerical trend toward improvement.

For 4 patients remained under protective ileostomy at the time of follow-up and had not yet resumed transanal defecation, Low Anterior Resection Syndrome (LARS) scores were assessed in only 5 evaluable patients. Among these patients, the median LARS score was 25 (range 16–29); 1 patient (20%) was classified as having no LARS, 4 (80%) had minor LARS, and no patient experienced major LARS. Given that LARS evaluation occurred relatively early after stoma reversal, these findings primarily reflect short-term bowel functional outcomes.

As of the data cutoff date (March 31, 2026), the median follow-up for the 9 patients who underwent surgery was 5 months (range 0.6-6.9). During the follow-up period, no patient has experienced disease recurrence or died. Owing to the limited duration of follow-up, data for DFS and OS remain immature; long-term follow-up is ongoing.

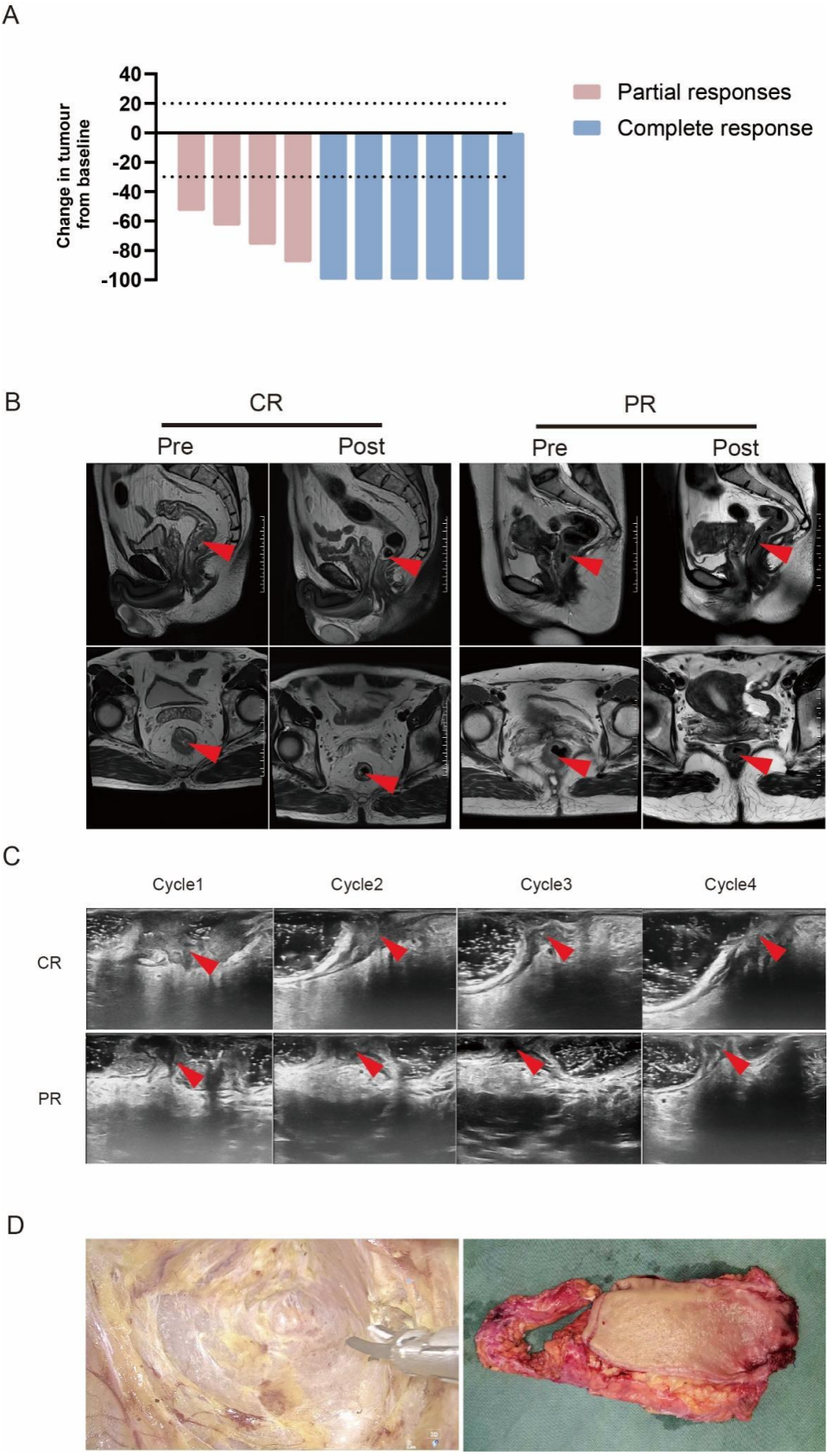


Figure 2 Imaging assessment of tumour response after neoadjuvant treatment.
Percentage change in tumour size from baseline as assessed by local investigator (A) and magnetic resonance imaging (B) and transrectal ultrasound findings (C) in patients with complete response

and partial response. (D) Representative intraoperative image showing clear pelvic anatomical planes and absence of obvious perirectal tissue oedema, together with the postoperative gross specimen after radical rectal cancer surgery.

Table 2. Surgical, pathological, imaging, safety, and patient-reported outcomes	
Outcome	**Value**
Surgical outcomes(n=9)	
R0 resection rate, n (%)	9 (100%)
Sphincter preservation rate, n (%)	9 (100%)
Absence of bowel wall oedema on intraoperative inspection, n (%)	9 (100%)
Pathological outcomes(n=9)	
Pathologic complete response (pCR), n (%)	4 (44.4%)
ypT0 (primary tumour complete response), n (%)	5 (55.6%)
ypN0 (lymph node negative), n (%)	8 (88.9%)
Radiographic outcomes (n=10)	
Objective response rate (ORR), n (%)	10 (100%)
Complete response (CR), n (%)	6 (60%)
Partial response (PR), n (%)	4 (40%)
Postoperative complications (n=9)	
Any complication, n (%)	1(11.1%)
Grade I (Clavien-Dindo I), n (%)	1(11.1%)
Grade II (Clavien-Dindo II), n (%)	0
Grade ≥III (Clavien-Dindo III–V), n (%)	0
Hypoalbuminemia, n (%)	1(11.1%)
Anastomotic leakage, n (%)	0
Surgical site infection, n (%)	0
Ileus, n (%)	0
Patient-reported and functional outcomes	
LARS score in evaluable patients (n=5), median (range)*	25 (16-29)
No LARS (0-20), n (%)	1 (20%)
Minor LARS (21-29), n (%)	4 (80%)
Major LARS (30-42), n (%)	0
IPSS score (n=9), median (range)	1(0-7)
Mild IPSS (0–7), n (%)	9 (100%)
EQ VAS score at baseline (n=9), median (range)	80 (70–82)
EQ VAS score after neoadjuvant treatment, before surgery (n=9), median (range)	85 (80–90)

*LARS was assessed only in patients who had undergone stoma reversal and resumed transanal defecation at the time of follow-up.

All 18 enrolled patients were included in the safety analysis. Overall, the combination of UTMD with CAPOX and sintilimab (UNIC treatment regimen) was well tolerated. No treatment-related deaths occurred, and no adverse events of grade 3 or higher or serious adverse events were reported. The UTMD procedure itself demonstrated a favourable safety profile, with no procedure-related

adverse events and no complications such as rectal perforation, major bleeding, or severe pain. Patients tolerated the transrectal ultrasound procedure well, and no additional analgesia or sedation was required.

Treatment-related adverse events were predominantly consistent with the expected toxicity profile of the CAPOX chemotherapy regimen and immune-related adverse events associated with PD-1 inhibition. The vast majority of events were grade 1-2 in severity and resolved spontaneously or with supportive care. Regarding haematological toxicity, thrombocytopenia was the most frequently reported adverse event, occurring in 38.9% of patients (7/18)—grade 1 in 5 patients and grade 2 in 2 patients. Leukopenia occurred in 33.3% of patients (6/18), all of which were grade 1 events. No grade 3-4 haematological toxicities, febrile neutropenia, or events requiring transfusion or growth factor support were observed. Gastrointestinal reactions included nausea in 50.0% of patients (9/18), all grade 1, and vomiting in 5.6% (1/18), also grade 1. All gastrointestinal events resolved spontaneously or were managed with antiemetic therapy; no grade 3-4 gastrointestinal toxicity was reported. Hepatic function abnormalities consisted of ALT or AST elevations in 11.1% of patients (2/18) and total bilirubin elevation in 5.6% (1/18); all events were grade 1. Neurotoxicity, primarily manifesting as peripheral numbness of the hands and feet, was noted in 33.3% of patients (6/18). All cases were grade 1 and did not interfere with activities of daily living. Dermatological toxicity included 1 patient who developed grade 2 hand-foot syndrome, characterised by desquamation, fissuring, and pain involving the palms, soles, and genital region, which was considered an adverse reaction to fluoropyrimidine therapy. This event led to study discontinuation after completion of 3 cycles of treatment. No other dermatological toxicity events were reported. With respect to immune-related adverse events, 1 patient experienced a grade 1 rash that resolved spontaneously without corticosteroid intervention. No other immune-related adverse events were observed.

Table 3. Treatment-related adverse events

Adverse Event	**Any Grade (N=18)**	**Grade 1–2**	**Grade 3–4**
Any adverse event	14 (77.8%)	14 (77.8%)	0
Haematological			
Thrombocytopenia	7 (38.9%)	7 (38.9%)	0
Leukopenia	6 (33.3%)	6 (33.3%)	0
Anaemia	0	0	0
Gastrointestinal			
Nausea	9 (50.0%)	9 (50.0%)	0
Vomiting	1 (5.6%)	1 (5.6%)	0
Diarrhea	0	0	0
Hepatic			
ALT/AST elevation	2 (11.1%)	2 (11.1%)	0
Total bilirubin elevation	1(5.6%)	1(5.6%)	0
Neurologic			
Peripheral neuropathy	6 (33.3%)	6 (33.3%)	0
Dermatologica			
Hand-foot syndrome	1 (5.6%)	1 (5.6%)	0
Rash	1 (5.6%)	1 (5.6%)	0

Immune-related			
Rash (immune-related)	1 (5.6%)	1 (5.6%)	0
Inflammatory events			
Enteritis	0	0	0
Cystitis	0	0	0
Dermatitis	0	0	0

Discussion

At present, radiotherapy remains a core component of neoadjuvant treatment for approximately 95% of patients with MSS/pMMR-LARC; however, this strategy is constrained by a notable trade-off between tumour control and treatment-related morbidity. Previous studies have shown that standard chemoradiotherapy yields (pCR) rates typically in the range of 11-15%, indicating considerable room for improvement in antitumour activity[4,14,26]. Concurrently, radiotherapy is associated with well-documented acute and chronic toxicities, including radiation proctitis, and exerts lasting adverse effects on pelvic organ function-namely, defecatory, urinary, and sexual dysfunction—that persistently impair patients' quality of life[27]. In recent years, intensified approaches such as short-course radiotherapy combined with chemotherapy or immunotherapy have achieved higher pCR rates in selected studies; nevertheless, for these regimens remain radiotherapy-based, the fundamental issues of toxicity and functional impairment have not been substantially ameliorated[14,17,19,20,28]. Consequently, attaining effective tumour control without reliance on radiotherapy while mitigating treatment-related burden has emerged as a key priority for optimising neoadjuvant therapeutic strategies.

In this study, the combination of UTMD with CAPOX and a PD-1 inhibitor-a novel therapeutic strategy (UNIC regimen) applied for the first time in clinical gastrointestinal oncology-demonstrated preliminary yet clinically meaningful antitumour activity in patients with MSS/pMMR-LARC Among patients who completed neoadjuvant therapy and underwent surgery, the pCR rate reached 44.4%, and an ORR of 100% was observed in evaluable patients. Moreover, the regimen was generally well tolerated, with no grade 3 or higher treatment-related adverse events reported.

With respect to patient-reported functional outcomes, among evaluable patients who had undergone stoma reversal, no cases of major low anterior resection syndrome (LARS) were documented, and short-term bowel function outcomes consisted predominantly of minor LARS, contrasting with the high LARS burden reported in the published literature[29]. It should be noted that LARS scores were obtained relatively early after stoma reversal. Previous research indicates that LARS symptoms are typically more pronounced during the initial months following surgery or stoma closure and may improve gradually over time, suggesting that subsequent LARS scores could decline further[30]. The overall burden of urinary symptoms was low, and EQ VAS scores showed no deterioration from baseline, with a numerical trend toward improvement-a change that may reflect symptom relief during the course of therapy. Collectively, these preliminary findings suggest that this strategy exhibits both antitumour activity and a relatively low treatment-related burden, supporting its further

evaluation as a potential radiotherapy-free neoadjuvant approach.

From a mechanistic standpoint, UTMD may confer tumour-sensitising effects akin to those of radiotherapy through non-ionising physical mechanisms. Under low-frequency ultrasound exposure, microbubbles undergo oscillation and collapse, generating shear stress, microstreaming, and local pressure fluctuations that act upon tumour cells and their microenvironment[21,31,32]. Preclinical studies, conducted exclusively in animal models, have suggested that UTMD may enhance tumour vascular permeability, facilitate intratumoural drug distribution, temporarily augment cellular drug uptake via altered membrane permeability, and exert modulatory effects on the tumour microenvironment, thereby potentiating the antitumour efficacy of systemic therapy[33,34]. Notably, these effects are primarily mediated by mechanical rather than ionising radiation, theoretically enabling antitumour activity in the absence of radiotherapy[35]. The relatively high pCR rate and favourable functional outcomes observed in the present study provide clinical context for this mechanistic hypothesis, offering a plausible explanation for how the strategy achieves meaningful efficacy signals while circumventing radiotherapy-associated toxicities. Nonetheless, these mechanistic inferences are largely extrapolated from prior basic investigations and the preclinical observations reported herein; direct human mechanistic data are lacking, and further confirmatory studies are warranted.

Clinically, no manifestations of classic radiotherapy-related toxicities-such as proctitis, dermatitis, or cystitis-were encountered in this study. Intraoperative findings revealed no discernible pelvic tissue oedema, and pelvic anatomical planes remained clearly defined. Notably, this therapeutic technique not only avoided induction of proctitis but also appeared to facilitate repair of tumour-associated rectal inflammation, an outcome consistent with the absence of anastomotic leakage. Furthermore, the overall toxicity profile was largely consistent with the established safety characteristics of CAPOX combined with immunotherapy, and the UTMD procedure itself was not associated with any appreciable procedure-related adverse events, underscoring the clinical feasibility of this approach. Taken together, these results indicate that the regimen may mitigate a portion of the treatment-related burden inherent to conventional radiotherapy-based pathways while preserving antitumour activity.

In summary, UNIC—a combination of UTMD, CAPOX, and PD-1 inhibition—showed preliminary antitumour activity with a favourable safety profile in patients with MSS/pMMR-LARC. The strategy also suggested potential benefits in sphincter preservation, functional outcomes, and treatment-related quality of life, supporting further evaluation as a non-radiotherapy neoadjuvant approach. This strategy thus holds promise as a potential radiotherapy-free alternative for neoadjuvant therapy. Nonetheless, the present data derive from a single-centre, early-phase exploratory investigation with a relatively modest sample size and limited duration of follow-up. Further validation through expanded cohorts and multicentre studies is required.

Methods

Study design and participants

UNIC (Ultrasound-based Novel technology combined with Immunotherapy and Chemotheray) is a

single-center, open-label, exploratory phase 2 clinical study aimed at evaluating the preliminary efficacy of UTMD combined with CAPOX chemotherapy and PD-1 inhibition (UNIC treatment regimen) in patients with newly diagnosed LARC (MSS/pMMR). The patients were recruited from the First Affiliated Hospital of Zhengzhou University. The primary endpoint was pathological complete response (pCR). Key secondary endpoints included objective response rate (ORR), safety, R0 resection rate, sphincter preservation rate, survival outcomes, and patient-reported outcomes.

This study was conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University (Approval No. S2024-K011-001, S2024-K011-002). All patients provided written informed consent. The trial was registered with the Chinese Clinical Trial Registry (Registration No. ChiCTR2400090149).

Eligible patients were aged 18-75 years, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and histologically confirmed rectal adenocarcinoma. MSI testing or immunohistochemistry confirmed MSS or pMMR status. Clinical staging was locally advanced, defined as T3-4N0M0 or T1-4N+M0 according to high-resolution MRI, with distant metastasis excluded by chest and abdominal CT. Major organ function was required to meet the criteria for neoadjuvant treatment (detailed criteria are provided in the appendix). Key exclusion criteria included prior treatment with any antitumour therapies (chemotherapy, radiotherapy, targeted therapy, immunotherapy, or surgery), known allergies or contraindications to study drugs (SONAZOID ultrasound contrast agent, PD-1 inhibitor [sintilimab], capecitabine, oxaliplatin), active infections (including hepatitis B, hepatitis C, HIV), pregnancy or breastfeeding.

Procedures

Each treatment cycle consisted of 3 consecutive days of UTMD therapy. For each UTMD session, oxybuprocaine gel was applied to the anal canal, and 100 mL of ultrasound coupling agent was instilled into the rectal lumen through a rectal catheter. The ultrasound probe was then inserted into the rectum and positioned in direct contact with the tumour surface. SONAZOID (16 μL) was dissolved in 2 mL saline and administered intravenously in five divided doses at 6-min intervals during the 30-min ultrasound exposure. Low-frequency, high-mechanical-index ultrasound was applied to the tumour site for 30 min using the VINNO ULTIMUS 9E ultrasound system. On day 2 of each cycle (UTMD day 2), patients received chemotherapy and immunotherapy, including 200 mg of PD-1 inhibitor intravenously, 130 mg/m² of oxaliplatin intravenously, and capecitabine 1000 mg/m² orally twice daily from days 2 to 15 of each 21-day cycle. After finishing oral treatment, the patient stopped taking the medication and rested for 6 days, then came to the hospital for the next cycle of treatment.

During neoadjuvant treatment, patients were monitored each cycle with physical exams, electrocardiograms, and laboratory tests (including complete blood count, liver and renal function, electrolytes, thyroid function, and tumour markers).

In the event of adverse events, the treating physician assessed the causative drug and adjusted the dose accordingly. If adverse events were related to oxaliplatin or capecitabine, the chemotherapy

dose was appropriately reduced, while the PD-1 inhibitor dose remained unchanged. If adverse events did not improve to grade 0–1 or baseline levels after dose reduction, the treatment was permanently discontinued. All adverse events were monitored weekly during treatment, with follow-up monitoring every 3 months after treatment. In cases of unresolved adverse events despite active management, the study principal investigator could approve discontinuation of treatment. Patients had the right to withdraw from the study at any time for any reason.

Baseline data included medical history, physical examination, lung function analysis, electrocardiogram, and laboratory tests. Blood and urine samples were collected at baseline, the start of each treatment cycle, at the end of treatment, and each safety follow-up for clinical laboratory analysis. Quality of life was assessed using the EQ-5D-5L scale at baseline and after completion of all neoadjuvant treatment, with EQ VAS scores also reported.

The duration of neoadjuvant treatment was individualised according to tumour response assessed by real-time ultrasound. Tumour size was measured before each UTMD session. When tumour response reached a plateau, defined as no further shrinkage on two consecutive ultrasound assessments, or when the tumour was no longer visible on ultrasound, patients proceeded to preoperative imaging assessment and then radical rectal cancer surgery within 1 week, rather than receiving additional neoadjuvant cycles. The maximum duration of neoadjuvant treatment was eight cycles. Imaging assessments (abdominal/pelvic contrast-enhanced CT and pelvic MRI) were performed every 4 cycles. After surgery, adjuvant treatment was determined according to pathological findings and clinical guidelines. When indicated, patients received CAPOX as adjuvant chemotherapy to complete a total of eight cycles of oxaliplatin-based chemotherapy, including the neoadjuvant CAPOX cycles. UTMD and PD-1 inhibition were not continued after surgery. Follow-up began after completion of adjuvant chemotherapy, with visits every 3 months for 2 years, including chest/abdominal CT, tumour marker assessments, and routine laboratory tests (complete blood count and liver function). After 2 years, follow-up continued every 6 months with the same assessments. Colonoscopy was scheduled within 1 year after surgery; if preoperative colonoscopy had been incomplete because of tumour obstruction, colonoscopy was performed 3–6 months after surgery. Subsequent colonoscopy was planned every 5 years if no colorectal lesions were detected, or earlier according to endoscopic and pathological findings. During follow-up, urinary symptoms were assessed using the IPSS questionnaire, and bowel function was assessed using the LARS score, with evaluation limited to patients who had undergone stoma reversal and restoration of bowel continuity.

Outcomes

The primary endpoint was pCR, defined as the absence of residual tumour cells in the primary tumour and all regional lymph node specimens following radical surgery (ypT0N0). Pathological evaluation was performed independently by two experienced pathologists, with discrepancies resolved through consensus. Tumour regression was graded using the modified Ryan criteria.

Secondary endpoints included the ORR, defined as the proportion of patients achieving complete or partial response according to RECIST 1.1, with all responses confirmed by imaging at least 4 weeks after initial assessment. Other secondary endpoints were disease control rate (DCR), defined

as the proportion of patients with complete response, partial response, or stable disease (lasting at least 6 weeks); R0 resection rate, defined as the proportion of patients with microscopically negative surgical margins; disease-free survival (DFS), defined as the time from surgery to disease recurrence (local recurrence or distant metastasis) or any cause of death; overall survival (OS), defined as the time from the start of treatment to any cause of death; and safety, defined as the incidence and severity of adverse events (AEs).

Patient-reported outcomes were assessed, including anal and bowel function using the LARS score, urinary symptoms using the IPSS questionnaire, and quality of life using the EQ-5D-5L scale, with EQ VAS as a reported indicator.

Statistical analysis

This is an exploratory, single-centre phase 2 trial designed to provide a preliminary assessment of the antitumour activity and safety of UTMD combined with chemotherapy and immunotherapy (UNIC regimen) in the neoadjuvant treatment of MSS/pMMR LARC. Given that this treatment strategy consists of widely adopted chemotherapy and immunotherapy regimens augmented by a physical intervention, with no dose escalation or exposure to novel pharmaceutical agents, the trial did not incorporate a conventional phase 1 dose-finding design. Instead, preliminary efficacy was evaluated concurrently with continuous safety monitoring. As an exploratory study, a formal sample size calculation was not prespecified. The enrolment target was determined pragmatically based on the capacity to detect an initial efficacy signal, the requirement for rigorous safety surveillance, and the actual accrual capabilities of the single participating centre. The findings reported herein represent a preliminary analysis of data collected up to March 31, 2026; enrolment and follow-up are ongoing.

The primary endpoint of pCR rate was assessed in the per-protocol efficacy analysis set, which comprised patients who completed neoadjuvant therapy and underwent definitive surgery. ORR, a secondary endpoint, was evaluated in patients with a complete baseline assessment and at least one post-treatment radiographic evaluation. All patients who received at least one dose of any component of the study treatment were included in the safety analysis set. Owing to the exploratory nature of this phase 2 trial and the limited sample size, all analyses are descriptive, and confidence intervals were not calculated. Continuous variables are presented as median (range), and categorical variables as frequency and percentage. Binary endpoints, including pCR, ORR, R0 resection rate, and DCR, are reported as percentages. DFS and OS are intended to be estimated using the Kaplan-Meier method; however, due to the short duration of follow-up (median follow-up 5.0 months), survival data remain immature. Accordingly, only event counts and median follow-up duration are reported at this time, without estimation of median survival times, calculation of confidence intervals, or application of log-rank tests. Adverse events, laboratory findings, and vital signs are summarised using descriptive statistics. The data cutoff for this analysis was March 31, 2026.

Role of the funding source

This study was funded by the National Health Commission of the People's Republic of China, the Henan Academy of Medical Sciences, and the First Affiliated Hospital of Zhengzhou University. Additional support was provided by Innovent Biologics, Inc., which supplied sintilimab; GE

Healthcare AS, which provided Sonazoid; and VINNO, which supplied the ultrasound diagnostic and therapeutic system. The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Contributors

B. Tuo, B. Xiong, L. Gan, and B. Du contributed equally to this work as co-first authors. Z. Sun, J. Hu, Y. Zhang, and Y. Fu contributed equally as co-corresponding authors.

Author contributions: Z. Sun, J. Hu, Y. Zhang, and Y. Fu conceived and designed the study. B. Xiong, L. Gan, B. Du, M. Yue, X. Duan, S. Lin, G. Wang, Y. Lian, W. Zhang, T. Li, S. Xie, and S. Hu performed the clinical interventions and data collection. B. Tuo, B. Xiong, L. Gan, B. Du, M. Yue, S. Gao, and J. Yao analysed the data. B. Tuo, B. Xiong, L. Gan, and B. Du drafted the manuscript. All authors reviewed, edited, and approved the final manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Data collected for the study, including individual participant data, de-identified participant data, and a data dictionary, can be requested by qualified researchers and will be assessed by a scientific review board as well as the trial sponsor. Requests should be sent to the corresponding author(fccsunzq@zzu.edu.cn).

Acknowledgments

This study was supported by the Excellent Young Physicians Program of the National Health Commission of the People's Republic of China (2024), the Henan Clinical Medical Scientist Program of the Henan Academy of Medical Sciences (No. HNCMS202431), and the Scientific Research and Innovation Team Program of the First Affiliated Hospital of Zhengzhou University (No. ZYCXTD2023017). We also gratefully acknowledge Innovent Biologics, Inc. for providing sintilimab, GE Healthcare AS for supplying Sonazoid, and VINNO for providing the ultrasound diagnostic and therapeutic system. The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Figure legends

Figure 1: Patient enrolment flowchart and treatment schema of the UNIC study.

(A) Patient screening, enrolment, and analysis populations. (B) Treatment schema, including UTMD, CAPOX plus PD-1 inhibition, imaging assessment, surgery, and postoperative follow-up.

(C) Schematic illustration of the UTMD procedure: intravenously administered SONAZOID microbubbles enter the tumour vasculature, and a transrectal ultrasound probe delivers lateral ultrasound exposure to the rectal tumour through coupling agent within the rectal lumen. (D) Representative ultrasound system interface during UTMD treatment, showing real-time monitoring of microbubble cavitation at the tumour site.

Figure 2 Imaging assessment of tumour response after neoadjuvant treatment.
Percentage change in tumour size from baseline as assessed by local investigator (A) and magnetic resonance imaging (B) and transrectal ultrasound findings (C) in patients with complete response and partial response. (D) Representative intraoperative image showing clear pelvic anatomical planes and absence of obvious perirectal tissue oedema, together with the postoperative gross specimen after radical rectal cancer surgery.

Supplementary Files

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

- UTMD.mp4
- Protocol.docx
- surgery.mp4