Single-Arm, Phase II Clinical Study on Short-Course Radiotherapy Combined with Neoadjuvant Chemotherapy and PD1 Inhibitor in the Treatment for Locally Advanced Rectal Cancer

Institution Responsible for the Clinical Study:	**Union Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology**
Responsible Person and Tel.:	Zhang Tao +86-27-85871982
Version No.:	**V2.0**
Version Date:	**May 20, 2020**

This clinical trial protocol is a confidential document, intended for use only by the investigators and the ethics committee of the study institution, and must not be distributed

Lin Z, *et al. J Immunother Cancer* 2021; 9:e003554. doi: 10.1136/jitc-2021-003554

Table of Contents

Protocol Summary

Study title:	Single-Arm, Phase II Clinical Study on Short-Course Radiotherapy Combined with Neoadjuvant Chemotherapy and PD1 Inhibitor in the Treatment for Locally Advanced Rectal Cancer
Study phase	Phase II
Study objective:	To evaluate the efficacy and safety of preoperative short-course radiotherapy (SCRT) combined with subsequent chemotherapy (capecitabine and oxaliplatin) and PD1 inhibitor camrelizumab in patients with locally advanced rectal cancer (LARC).
Endpoints	**Primary endpoint:** Pathological complete response (pCR) rate **Secondary endpoint:** 3-year event-free survival rate R0 resection rate 3-year Overall survival rate Surgical complication rate Safety Quality of life
Sample size:	A total of 30 patients are planned to be enrolled in this study. The specific algorithm is as follows: this study will employ the single-arm design, with pCR rate as the primary endpoint. This study will adopt PASS 15 software, use tests for one proportion, and set a=0.025 (one side) and power=0.80. The pCR rate of neoadjuvant therapy for locally advanced colorectal cancer was 15% previously, which is expected to reach 40% in this study. It is expected that 24 patients will need to be enrolled. Considering the drop-out rate of 20%, a total of 30 subjects should be enrolled
Study design	Patients with locally advanced rectal cancer (T3, T4a or 4b/N+) who are to receive preoperative neoadjuvant therapy: will receive preoperative local pelvic short-course radiotherapy. Radiotherapy will employ intensity-modulated radiation therapy, with a dose of 25 Gy/5 Fracions /1 week. One week later, a 3-week regimen of CAPOX chemotherapy combined with camrelizumab will be performed for 2 cycles. One week later, radical resection of rectal carcinoma will be performed. Postoperative adjuvant chemotherapy regimen will be determined by the doctor
Inclusion criteria:	(1) Age 18-75 years, male or female; (2) Histologically confirmed T3-4N0M0 or T1-4N+M0 rectal adenocarcinoma (AJCC/UICC TNM staging (8th Edition, 2017); (3) with initial treatment (untreated with surgery, radiotherapy, chemotherapy or targeted therapy); (4) inferior margin ⩽ 10 cm from the anal verge, (5) ECOG performance status score is 0-1; (6) With no severe hematologic disorder, cardiac, pulmonary, hepatic or renal dysfunction, or immunodeficiency; (7) Hemoglobin (Hb) ≥ 9 g/dL; white blood cell (WBC) ≥ 3×10^9/L; neutrophil (ANC) ≥ 1.5×10^9/L; platelet (Pt) ≥ 100×10^9/L; bilirubin <1.5 times the upper limit of normal value; aspartate aminotransferase (AST) & alanine aminotransferase (ALT) ≤2.5 times the upper limit of normal value; serum creatinine ≤1.5 times the upper limit of normal value or creatinine clearance rate ≥50 mL/min; thyroid stimulating hormone (TSH) ≤ ULN (If abnormal, T3 and T4 levels should be referred to simultaneously. If T3 and T4 are normal, the patient can also be enrolled); (8) Males or females with reproductive ability who are willing to use contraception in the trial; Women of childbearing potential who consent to practicing contraception during the period from giving informed consent to at least 23 weeks after the last dose of therapy; Male patients who consent to practicing contraception during the period from giving informed consent to at least 31 weeks after the last dose of the study drug; (9) Patients or their family members agree to participate in the study and sign the informed consent form; (10) No other severe cardiopulmonary diseases.
Exclusion criteria:	(1) Previous exposure to any anti-PD-1 or anti-PD-L1 antibody; (2) Lactating, pregnant women or women preparing for pregnancy;

	(3) Patients who need to be treated with corticosteroid (dose equivalent to prednisone of >10 mg/day) or other immunosuppressive agents within 2 weeks prior to study drug administration; (4) Patients with concurrent autoimmune disease or a history of chronic or recurrent autoimmune disease; (5) Patients with a history of thyroid dysfunction; (6) Patients with a history or finding of cardiovascular risk; (7) Patients with a known history of allogeneic organ transplantation and allogeneic hematopoietic stem cell transplantation; patients infected with HIV, or with active hepatitis B or C (reference for active hepatitis B: HBV DNA $\geq 10^4$ copies/mL; reference for active hepatitis C: HCV RNA $\geq 10^3$ copies/mL); (8) Patients with a history of pneumonitis or interstitial lung disease (ILD, including previous and current medical history), such as interstitial pneumonia and pulmonary fibrosis, or have evidence of ILD on baseline chest CT or MRI; (9) Patients who have allergic constitution or are allergic to multiple drugs; (10) Patients with recurrent rectal cancer or a history of pelvic radiation; (11) Patients with a history of inflammatory bowel disease; (12) Patients complicated with severe infection; (13) Patients with significant unstable mental diseases or other medical diseases that may interfere with the safety of the subjects, obtaining informed consent, or compliance with the procedures for the clinical study; (14) Patients who participated in other clinical trials within 30 days before enrollment; (15) Patients who are not suitable for participation in clinical trials in the opinion of the investigator.
Discontinuation criteria:	Trial discontinuation means the clinical trial is not completed according to protocol and is prematurely discontinued. The main purpose of trial discontinuation is to protect the rights and interests of the subjects, ensure the quality of the trial, and avoid unnecessary economic losses. (1) If serious safety issues occur during the trial, the trial should be discontinued in time; (2) During the trial, major errors are found in the clinical trial protocol that make evaluation of drug efficacy difficult; or major deviations from the well-designed protocol are found in the implementation that make evaluation of drug efficacy difficult if the trial is to be continued; (3) The sponsor requests to discontinue the trial (because of economic or management factors or others); (4) The administrative department in charge rescinds the trial.
Dose design and treatment regimen:	Patients will receive local rectal short-course radiotherapy. Radiotherapy will employ conformal or intensity-modulated radiation therapy, with a pelvic irradiation dose of 25 Gy/5 Fractions/1 week. Then rest for 1 week after radiotherapy and begin to receive neoadjuvant chemotherapy CAPOX and PD1 inhibitor for immunotherapy, for 2 cycles. One week after the completion of neoadjuvant therapy, a second evaluation on the primary lesions will be performed, and the surgical method is total mesorectal excision. Postoperative adjuvant therapy will be started 3-4 weeks after surgery, and the treatment regimen will be determined by the investigator. The efficacy of neoadjuvant therapy on postoperative specimens will be evaluated in accordance with Rectal Cancer Regression Grade. Treatment-related toxic and side effects will be evaluated as per CTCAE 5.0 toxic and side effects evaluation criteria.
Statistical method:	This study will employ SPSS 16.0 statistical software package for analysis. EFS and OS will be analyzed using the Kaplan-Meier method and log-rank test.
Trial schedule:	Estimated start time of the trial: Oct. 2019 Estimated time of enrollment completion: Oct. 2021 Estimated end time of the trial: Dec. 2025
Version No.:	V2.0

List of Abbreviations

Abbreviations and statistics descriptions (English)

dMMR	Deficient mismatch repair protein
MSI	Microsatellite instability
mCRC	Metastatic Colorectal Cancer
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death ligand 1
MSS	Microsatellite stability
CTLA-4	Cytotoxic T lymphocyte-associated protein 4
OS	Overall survival
pCR	Pathological complete response
DNA	Deoxyribonucleic acid
WBC	White Blood Cell
RBC	Red Blood Cell
HGB	Hemoglobin
PLT	Platelet
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
Cr	Creatinine
BUN	Blood urea nitrogen
ESR	Erythrocyte sedimentation rate
AE	Adverse event
SAE	Serious adverse event
CRF	Case report form

1. Study Background

Rectal cancer is one of the most common malignant tumors in China, and its incidence rate is increasing year by year. Due to the atypical early symptoms of this disease, most of the patients are already in the locally advanced stage when they visit the doctor. The neoadjuvant chemoradiotherapy-based multidisciplinary synthetic therapy is the main treatment method for locally advanced rectal cancer at present. Preoperative radiotherapy can employ long-course regimen, in which conventional fraction radiotherapy and concurrent chemotherapy are used, or short-course radiotherapy with the dose of 25 Gy/5 Fractions. The former has a high tumor response rate, and the latter has low toxic and side effects and good tolerance. No difference is found in the local control rate between them. Delaying surgery for 4-8 weeks after short-course radiotherapy further increases the tumor response rate compared to surgery within 7 days. Recent studies have found that short-course delayed radiotherapy combined with neoadjuvant chemotherapy and delayed surgery can further improve the pathological complete response rate of tumor, and even show a better trend than the long-course concurrent radiochemotherapy, which have become a hotspot of current studies.

In 2015, it was first observed that metastatic colorectal cancer (mCRC) with molecular phenotype of deficient mismatch repair protein (dMMR) or microsatellite instability-high (MSI-H) could benefit significantly from monoclonal antibody immunotherapy with immune checkpoint inhibitor (programmed cell death receptor PD-1), which ushers in a MSI era in CRC immunotherapy. However, MSS/pMMR type colorectal cancer is not sensitive to immunotherapy, and even PD-L1 monoclonal antibody combined with MEK inhibitor or with antiangiogenic drugs does not bring clinical benefit. The Canadian study CCTG Co.26, reported in 2019 ASCO GI, is the first large phase II clinical study with positive results in MSS type mCRC patients, in which CTLA-4 monoclonal antibody combined with PD-L1 monoclonal antibody prolonged the OS of patients with refractory MSS type mCRC. Further, a study reported in 2019 ASCO combined nivolumab monoclonal antibody with standard long-course radiotherapy for preoperative treatment. The overall pCR rate was as high as 30%, and PD-L1 expression and the ratio of CD8+ lymphocytes/ regulatory T cells (CD8/Treg) could predict the efficacy of nivolumab monoclonal antibody. This study suggests that the immune checkpoint inhibitors are of great significance in neoadjuvant therapy for rectal cancer, especially their broad application prospect in MSS patients in the majority.

Radiotherapy can not only directly induce lethal DNA damage of tumor cells, making tumor cells acquire immunogenicity and producing anti-tumor immune response, but also generates remote effect through immune response. Mole first proposed and defined "abscopal" as the distant tissue with the same structure as the irradiated site in 1953. Since then, a large number of experiments have confirmed the existence of abscopal effect, especially the hypofractionated radiation therapy, which is more likely to trigger the abscopal effect. Considering that short-course hypofractionated delayed radiotherapy has equivalent efficacy with the long-course chemoradiotherapy in neoadjuvant therapy for rectal cancer and the potential immune activation effects brought by hypofractionated radiation therapy, we have reasons to believe that the short-course hypofractionated radiation therapy combined with neoadjuvant chemotherapy and immune checkpoint inhibitors may achieve better results in neoadjuvant therapy for rectal cancer.

2. Study Objective

Primary objective: The primary objective is to evaluate the efficacy of preoperative short-course radiotherapy combined with neoadjuvant chemotherapy and PD1 inhibitor in the treatment for locally advanced rectal cancer

Secondary objective: The secondary objective is to evaluate the safety of preoperative short-course radiotherapy combined with neoadjuvant chemotherapy and PD1 inhibitor in the treatment for locally advanced rectal cancer

Exploratory objective: To evaluate the relation of biomarkers (e.g. PD-L1 and MSI) in tumor tissues and/or blood to PD1 efficacy and pCR

3. Study Design

A prospective, single-arm, phase II clinical trial.

4. Selection and Withdrawal of Subjects

4.1 Inclusion criteria

1) Age 18-75 years, male or female;
2) Rectal cancer diagnosed by histology, with initial treatment (untreated with surgery, radiotherapy, chemotherapy or targeted therapy);
3) Locally advanced rectal lesion: T3, T4a or 4b/N+;

4) With no severe hematologic disorder, cardiac, pulmonary, hepatic or renal dysfunction, or immunodeficiency;
5) Hemoglobin (Hb) ≥9 g/dL; white blood cell (WBC) ≥ 3×10^9/L; neutrophil (ANC) ≥ 1.5×10^9/L; platelet (Pt) ≥ 100×10^9/L; bilirubin <1.5 times the upper limit of normal value; aspartate aminotransferase (AST) & alanine aminotransferase (ALT) ≤2.5 times the upper limit of normal value; serum creatinine ≤1.5 times the upper limit of normal value or creatinine clearance rate ≥50 mL/min; thyroid stimulating hormone (TSH) ≤ ULN (If abnormal, T3 and T4 levels should be referred to simultaneously. If T3 and T4 are normal, the patient can also be enrolled);
6) Males or females with reproductive ability who are willing to use contraception in the trial; ECOG performance status score is 0-1;
7) Patients or their family members agree to participate in the study and sign the informed consent form;
8) No other severe cardiopulmonary diseases.

4.2 Exclusion criteria

1) Previous exposure to any anti-PD-1 or anti-PD-L1 antibody;
2) Lactating, pregnant women or women preparing for pregnancy;
3) Patients who need to be treated with corticosteroid (dose equivalent to prednisone of >10 mg/day) or other immunosuppressive agents within 2 weeks prior to study drug administration;
4) Patients with active, known, or suspected autoimmune disease or a history of such disease within the past 2 years (patients with vitiligo, psoriasis, alopecia, or Grave's disease who do not require systemic treatment within 2 years, those with hypothyrodism who require only thyroid hormone replacement therapy, or those with type I diabetes who require only insulin replacement therapy can be enrolled);
5) Patients with a known history of allogeneic organ transplantation and allogeneic hematopoietic stem cell transplantation; patients infected with HIV, or with active hepatitis B or C (reference for active hepatitis B: HBV DNA ≥10^4 copies/mL; reference for active hepatitis C: HCV RNA ≥10^3 copies/mL);
6) Patients with interstitial lung disease (ILD, including previous and current medical history), such as interstitial pneumonia and pulmonary fibrosis, or have evidence of ILD on baseline chest CT or MRI;
7) Patients who have allergic constitution or are allergic to multiple drugs;
8) Patients with severe cardiac, pulmonary, hepatic or renal dysfunction, such as patients with decompensated heart, lung, kidney, liver or other major organs dysfunction, failure or poor glycemic control, or patients who are intolerant of chemotherapy;
9) Previous pelvic radiotherapy history;
10) Patients complicated with severe infection;
11) Patients with cognitive disorder, or poor compliance to chemotherapy as determined by the investigator;
12) Patients who participated in other clinical trials within 30 days before enrollment;
13) Patients who are not suitable for participation in clinical trials in the opinion of the investigator.

4.3 Removal criteria

Subjects who do not meet the inclusion criteria but are enrolled should be rejected, including: (1) misdiagnosis; (2) mistaken enrollment; (3) having not taken the medication; (4) having no evaluation records.

The reason of rejection should be explained for all rejected subjects, and their medical history in the study should be reserved for future reference. However, subjects who have received at least one treatment and have at least one safety record can be included in the safety analysis.

4.4 Drop-out (withdrawal) criteria

(1) Adverse events; (2) lack of efficacy; (3) trial protocol deviation (including poor compliance); (4) trial discontinuation is determined to be necessary for subjects by the investigator due to medical concerns; (5) the patients themselves request to withdraw the trial.

When a patient drops out, the investigators must fill in the reasons for dropout in the case report form, try their best to contact the patient, and complete as much of evaluation items and related examinations. The investigators should fill in the pathography in the last time the patient is contacted, including the patient's last medication time and efficacy. Those who drop out due to adverse events, whether investigational product-related or not, should be recorded in the case report form.

4.5 Criteria for trial discontinuation

Trial discontinuation means the clinical trial is not completed according to protocol and is prematurely discontinued. The main purpose of trial discontinuation is to protect the rights and interests of the subjects, ensure the quality of the trial, and avoid unnecessary economic losses.

1) If serious safety issues occur during the trial, the trial should be discontinued in time;
2) During the trial, major errors are found in the clinical trial protocol that make evaluation of drug

efficacy difficult; or major deviations from the well-designed protocol are found in the implementation that make evaluation of drug efficacy difficult if the trial is to be continued;

3) The sponsor requests to discontinue the trial (because of economic or management factors or others);
4) The administrative department in charge rescinds the trial.

5. Therapeutic Drug and Consolidation Radiotherapy Regimen

5.1 Investigational product

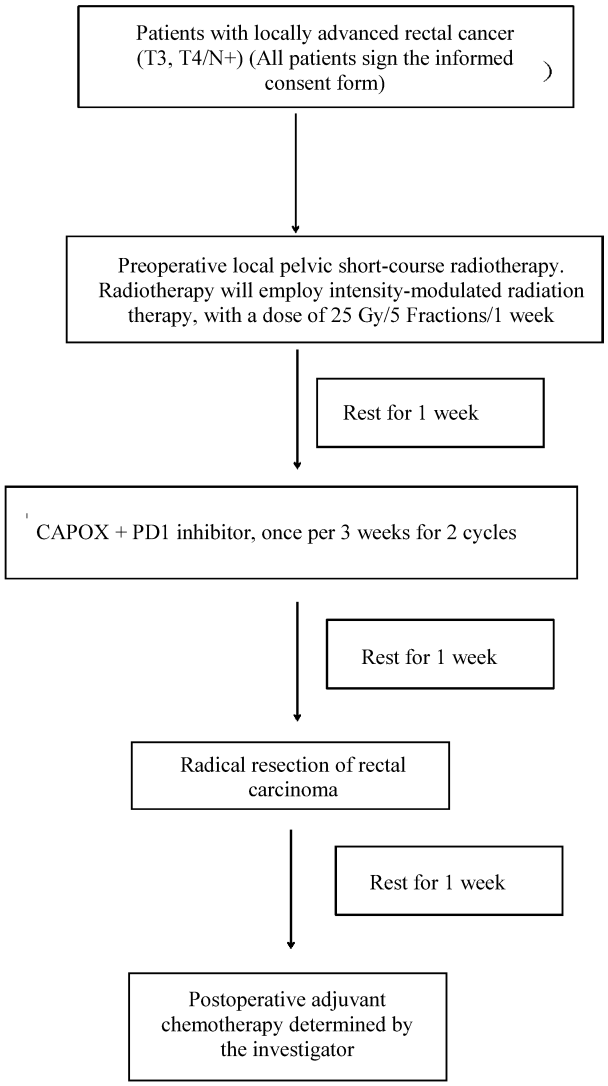
Protocol, short-course radiotherapy + neoadjuvant chemotherapy combined with immunotherapy (CAPOX + PD1 inhibitor)

5.2 Administration method

CAPOX： Oxaliplatin 130 mg/m2 iv d1
Capecitabine 1000 mg/m2 po bid, d1-14, q3w;

PD1 inhibitor camrelizumab: 200 mg iv drip, q3w;

5.3 Technical routes



5.4 Precautions

(1) Palpitation, chest tightness, dyspnea, cold limbs, and lowered blood pressure during chemotherapy should be observed closely, which indicate that the patient may have allergic reaction. Therefore, the treatment should be stopped immediately and the condition should be actively treated as allergic reaction.

(2) The dose-limiting toxicity of oxaliplatin is neurotoxicity, mainly in peripheral sensory nerves, manifested as sensory disturbance or/and paresthesia in the extremities. With or without algospasm, which is often cold-induced. These symptoms occur in 85-95% of patients receiving treatment. The symptoms usually decrease in treatment intervals, but gradually worsen with the increase of treatment cycles. Dysfunction includes an inability to perform fine movements, which is related to sensory disturbance. Evaluation with Levi sensory neurotoxicity classification criteria: 1) grade 0: no; 2) grade 1: paresthesia or dysesthesia (cold-induced), can be resolved within 1 week; 3) grade 2: paresthesia or dysesthesia, can be completely resolved within 21 days; 4) grade 3: paresthesia or dysesthesia, cannot be completely resolved within 21 days; 5) grade 4: paresthesia or dysesthesia, accompanied with dysfunction. The duration of the patient's symptoms and severity of pain and/or dysfunction are the indications for dose adjustment, and sometimes even treatment discontinuation is needed. When the treatment is discontinued, the neurological symptoms usually may improve.

(3) Camrelizumab is a PD-1 monoclonal antibody inhibitor and is an immune checkpoint inhibitor drug. PD-1 receptor inhibitors can block negative regulatory signals from T cells to relieve immunosuppression, which enhances the anti-tumor effect of T cells and may also abnormally enhance normal immune response, leading to immune tolerance imbalance. When PD-1 accumulates in normal tissues, the autoimmune-like inflammatory response appears, which is called immune-related adverse events (irAEs) and involves in multiple organs such as the skin, gastrointestinal tract, liver, endocrine, and lung. With reference to the clinical medication safety information in the package inserts of similar varieties nivolumab and pembrolizumab that have been listed abroad, camrelizumab may cause the following common adverse reactions: rash, pruritus, fatigue, weakness, fever, diarrhea, constipation, nausea, vomiting, decreased appetite, dyspnea, cough/productive cough, upper respiratory tract infection, musculoskeletal pain, joint pain, back pain, etc. In addition, camrelizumab is an antibody biomacromolecule. Vital signs, color of face and sweating of subjects should be closely monitored in clinical study, especially during the first administration, so as to detect signs of infusion reactions early. Combined with the results of preclinical animal experiments and clinical trials, camrelizumab has good safety and similar main drug-related adverse reactions to competitors. The investigator should closely monitor subjects for the above symptoms throughout the clinical trial, prepare appropriate medications and equipment, educate and encourage subjects to conduct self-monitoring. Possible common and severe adverse reactions caused by the investigational product camrelizumab should be closely monitored.

5.5 Concomitant medications

(1) During the clinical trial, except systemic intravenous chemotherapy according to the protocol, patients should stop using other drugs related to systemic treatment of tumor, including other chemotherapy drugs, targeted therapy, immunomodulators, Chinese patent medicine and other drugs that affect the efficacy;

(2) If there is a definite infection, or if the body temperature ≥ 38°C and infectious fever cannot be excluded, antibiotics can be used and recorded in the CRF. The use of antifungal or antiviral drugs should also be recorded in the CRF.

(3) The subjects who receive concomitant medications that cause the difficulty of correct judgment of efficacy and safety should be treated as rejected subjects.

6. Medical Ethics Requirements

(1) The clinical trial protocol shall be approved by the ethics committee of the participating institution before it can be implemented. The approval document of the ethics committee on the clinical trial protocol will be copied to each clinical trial institution for the record.

(2) Informed consent form: The investigator should explain the objectives and process of the study to the enrolled subjects, and the informed consent form will be signed after obtaining the consent of the subjects.

This clinical trial will be conducted in accordance with the Declaration of Helsinki (2013 edition) and relevant Chinese Good Clinical Practice. The protocol should be reviewed and approved by the ethics committee of the hospital before the start of the trial. Any necessary modifications to the trial protocol during the clinical study should be reported to the ethics committee for the record.

7. Outcome Measures and Laboratory Tests

7.1 Medical history and physical examination:

Patients should be inquired by the doctor about their present and previous medical history in detail (including histories of allergy, cardiovascular disease, endocrine disease, respiratory system disease, and medication) before

being enrolled in the study. Systematic physical examinations and related laboratory tests will be performed before the trial and after the end of treatment. The medical history, physical examinations and laboratory tests results will be recorded in the CRF.

7.2 Laboratory Tests:

The enrolled patients will receive the following tests before treatment and after the end of the trial:

1) Blood routine test (HB + RBC + WBC), and coagulation function test;
2) Routine urine test (protein, RBC, WBC, and urine glucose), and stool routine;
3) Liver function (ALT and AST), renal function (BUN and Cr), electrolytes, and blood glucose;
4) Electrocardiogram, lung CT, liver MRI, abdominal and pelvic CT or MRI;
5) Blood tumor markers;
6) Urine pregnancy test in women of childbearing age (only before enrollment);
7) Thyroid function test.

All above tests on the subjects prior to treatment will be carried out in qualified laboratories certified by the clinical trial center, and the test results will be recorded in the CRF. Subjects with abnormality found in reexamination after the end of treatment should be followed up and reexamined, and the relation between the abnormality and the investigational product will be determined.

7.3 Observation of adverse events

(1) Clinical adverse events

All subjects should be observed for any adverse events that occur during the clinical trial. The clinical manifestations, severity, occurrence time, duration, treatment methods and prognostic measures of adverse events should be recorded in time, and the correlation between adverse events and the investigational product should be determined.

(2) Abnormal laboratory tests results

Subjects with abnormal results of the above tests after medication should be closely followed up and observed until they return to normal or stable, and the correlation between abnormality and the investigational product should be determined.

8. Efficacy Evaluation

8.1 Safety evaluation

Changes in patients' subjective symptoms (nausea, vomiting, poor appetite, alopecia, etc.), KPS and PS scores before and after treatment; changes in indexes of blood routine, urine routine and stool routine tests, electrocardiogram, changes in physiological indexes (body temperature, blood pressure, heart rate, respiration) during medication, and comparison of hepatic and renal function before and after treatment.

8.2 Efficacy evaluation

Study endpoints

The primary endpoint was pathological complete response (pCR) rate, pCR was defined as the absence of viable tumour cells in the resected primary tumour specimen and all sampled regional lymph nodes (ypT0N0).

Secondary endpoints were 3-year event-free survival rate (defined as the percentage of patients without disease recurrence or progression or death due to any cause after 3-year follow-up), R0 resection rate (defined as the rate of negative margin microscopically), 3-year OS rate (defined as the percentage of patients alive after 3-year follow-up), complication rate, safety, and quality of life. Adverse events (AEs), duration of which collection was from the time the patient signed the informed consent form to 90 days after surgery, were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0.

Biomarker analysis

Tissue samples or paraffin sections must be provided as a patient was enrolled. Immunohistochemistry was performed to detect the expression of PD-L1 and DNA mis-match repair (MMR) proteins (MSH6, MSH2, MLH1 and PMS2). PD-L1 combined positive score (CPS) was evaluated using PD-L1 IHC 22C3 pharmDx (Agilent Technologies Inc., California, USA), defined as the number of PD-L1 positive cells (tumour cells, lymphocytes, macrophages) as a proportion of the total number of tumour cells multiplied by 100, Positive PD-L1 expression was considered when CPS was 1 or more. Comprehensive genomic profiling was conducted using targeted gene capture-based next-generation sequencing technology. Briefly, for formalin-fixed paraffin-embedded (FFPE) tissues, hematoxylin and eosin staining (H&E staining) was performed, and the stained sections were evaluated by a pathologist to ensure the tumor cells of ≥ 20%. DNA was extracted from the tumor tissues of patients using standard methods. A panel of 418 genes was captured and then sequenced through the Illumina NovaSeq6000 platform (Illumina, San Diego, USA) with 2 × 150 bp paired-end reads. The average of sequencing depth of tumor

tissues was ≥ 1000X. Genomic alterations including tumor mutation burden (TMB), single nucleotide variants (SNV), short and long insertions and deletions (INDELs), copy number variants (CNV), and gene fusions were assessed.

Statistical analysis

According to the previous studies, the pCR rate after preoperative CRT in patients with LARC is approximately 15%. We expected that the regimen of SCRT combined with subsequent chemotherapy and camrelizumab could increase the pCR rate from 15% to 40%. A sample size of 24 patients was required to provide at least 80% power to detect this estimated improvement, in one-sided $\chi 2$-test with a significance level of 2.5%, and a 20% dropout was considered, resulting in that a total sample size of 30 patients was planned for this study.

Statistical analyses were conducted using SAS® software (version 9.2, SAS Institute Inc, Cary, USA). Continuous variables were summarized using medians and ranges, and categorical variables were described using the frequency and percentage. Baseline and safety analyses were performed for all enrolled patients (intention-to-treat [ITT] population), and efficacy analyses were conducted for those who administrated at least 1 dose of camrelizumab (full analysis set [FAS] population). P value of less than 0.05 was considered statistically significant.

The exploratory objective is: to evaluate the relation of biomarkers (e.g. PD-L1 and MSI) in tumor tissues and/or blood to PD1 efficacy and pCR. Biomarker detection will be carried out with the treated tissue and/or blood obtained prior to treatment.

Continuous variables that conform to normal distribution will be expressed as mean +/- standard deviation, minimum value and maximum value, and those do not conform to normal distribution will be expressed as median; categorical variables will be described in frequency and percentage, and 95% confidence interval will be calculated if necessary. Mean values between the two groups will be compared by t test, and the rates between the two groups will be compared by chi-square test. Survival analysis will be performed by the Kaplan-Meier method. Comparison between groups will be performed by bilateral log-rank test. All tests are two-sided, and 0.05 is taken as the level of the tests.

9. Adverse Event Monitoring

9.1 Observation of adverse events

1) Clinical adverse reactions: All subjects should be observed carefully for any adverse events that occur during the clinical study. The clinical manifestations, severity, occurrence time, duration, treatment methods and prognosis of adverse events should be recorded in time, and the correlation between adverse events and the investigational product should be determined.
2) Abnormal laboratory tests results: Subjects with abnormal results of the above tests after medication should be closely followed up and observed until they return to normal or stable, and the correlation between abnormality and the investigational product should be determined.

9.2 Criteria for determining adverse event (AE) severity

1) Mild: Complain of discomfort, and not requiring symptomatic treatment or drug discontinuance;
2) Moderate: Complain of discomfort, requiring symptomatic treatment, not requiring drug discontinuance but requiring reduction of the drug dose by 25%;
3) Severe: Complain of significant discomfort, requiring symptomatic treatment and drug discontinuance.

9.3 Criteria for determining the relationship between adverse event (AE) and the investigational product

According to the criteria for determining the causality between the drug and AEs, the correlation between AEs and the investigational product is divided into five grades: definitely related, probably related, possibly related, possibly unrelated, and definitely unrelated. The three conditions of definitely related, probably related, and possibly related are classified as adverse reactions caused by the investigational product. The total number of subjects with investigational product-related adverse reactions is taken as the numerator, and the number of all enrolled subjects for adverse reaction evaluation is taken as the denominator to calculate the incidence rate of adverse reaction. Please refer to Table 2 for specific determination.

Table 2. Criteria for determining the causality of adverse drug reactions

Indicator	Definitely related	Probably related	Possibly related	Possibly unrelated	Definitely unrelated
Reasonable chronological sequence	Yes	Yes	Yes	Yes	No
Conforming to the known reaction type of the investigational product	Yes	Yes	Yes	No	No
Improving after the discontinuance of the investigational product	Yes	Yes	Yes or No	Yes or No	No
Recurring after reusing the investigational product	Yes	?	?	?	No
Possible other explanation for the drug-induced reaction	No	No	Yes	Yes	Yes

Note: ? means that the medicine is not allowed to be administrated again due to medical ethics. "Possibly unrelated" indicates that further observation is needed for evaluation.

9.4 Severe adverse event handling and reporting methods

The severe adverse events (that is, death or life threatening, disability, or loss of partial living ability, and prolongation of length of stay) occurred during the trial, whether or not related to the trial and the investigational product, should be treated promptly and actively based on symptoms to minimize the loss of patients, be immediately reported to the principal investigator, and be notified to the trial sponsor within 24 hours.

10. Data management

10.1 CRF data management

The investigators should record the data in the case report form in an accurate, complete, clear and timely manner according to the original observation records of subjects.

The clinical research associate (CRA) should monitor the trial according to the trial protocol. And confirm that all CRFs are filled correctly and completely and are consistent with the original data. If there are any errors and omissions, the investigators should correct them in time. For any modifications, the original records should be clearly visible, and the corrections should be signed and dated by the investigator.

After inspection by the CRA, the CRFs should be checked and signed by the CRA, and submitted to the clinical trial data manager in time. For the transmission of the completed CRFs among investigators, CRA and data managers, there should be special records. The records should have corresponding signatures when receive and be kept properly.

The data manager should check again after data entry, and inform the CRA in time if any problems are found, and ask the investigators to answer them. All kinds of questions and answers between them should be exchanged in the form of question table, which should be kept for future reference.

10.2 Data entry and management

Before data entry, the data manager should understand the content and coding of each item in the observation form, and record the coding process in the code book for preservation. The database should be named in a standardized, easy-to-read, easy-to-find, correct, safe and confidential manner.

Data should be inputted twice using the EpiData 3.02 database system by two data entry clerks. If any problems or unforeseen circumstances are found in the process of data entry, they should be recorded well and reported in time so as to be handled quickly. After the end of data entry, partial observation forms should be checked randomly to evaluate the input quality, analyze and deal with the existing problems.

The data manager and the principal investigator should develop a data range check and logical check together based on the range and correlation of the values of various indicators in the CRF. And they should also write the corresponding computer programs to control incorrect data entry before entry, and find out the cause and correct. All errors and corrections should be recorded and properly kept.

The original CRFs should be archived and stored in order of the subject codes after completion of data entry and review, and the retrieval catalog should be filled in for review later on. Electronic data files, including databases, checking program, analysis program, analysis results, code books and supporting papers, should be classified and properly archived in multiple backups on different disks or record media to prevent damage. All original files should be archived in accordance with the prescribed time limit in the Good Clinical Practice of China.

11. Statistical analysis

11.1 Data analysis set

Full analysis set (FAS): includes those who were administrated at least one dose of camrelizumab. When a primary efficacy indicator is missing, the previous result should be carried forward according to the principle of an intention to treat analysis. The missing values of comparability analysis and secondary efficacy indicators should not be subjected to data-carry-forward, and should be analyzed based on the actual data.

Per protocol set (PPS): includes subjects who meet the inclusion criteria, do not meet the exclusion criteria and have completed the treatment regimen, that is, those who meet the trial protocol, have good compliance and have filled all required sections of the CRF are included in the PPS analysis. In this trial, subjects who drop out after half of the treatment course due to aggravation should be included as invalid subjects in the PPS analysis.

Safety set (SS): subjects who have received at least one treatment and have actual safety assessment data. The missing safety data should not be subjected to data-carry-forward; some rejected subjects who are evaluable can be included, such as those with ages beyond the inclusion criteria, but excluding those who cannot be evaluated for safety due to the use of prohibited drugs. The number of subjects in the SS will be used as the denominator for the incidence of ARs.

11.2 Statistical analysis plan

Statistical analysis should employ the two-sided test, and $P \leq 0.05$ indicates a statistically significant difference (unless otherwise specified).

The Continuous data should be statistically described by mean, median, standard deviation, maximum value, and minimum value; the categorical data or ranked data should be statistically described by frequency.

The test should be used to compare the Continuous data between the groups, and the $\chi 2$ test or rank sum test should be used to compare the categorical data between the groups.

Efficacy evaluation method: the evaluation of primary efficacy evaluation indicator: the overall response rate between the experimental group and the control group should be compared by the superiority test. Hypothesis test is

$$H_0: \pi_T=\pi_C, H_1: \pi_T>\pi_C$$

The 95% CI of the difference in overall response rate between the experimental group and the control group should be calculated, and the lower limit >0 can be considered as the establishment of superiority. Meanwhile, CMH-$\chi 2$ with central effect should be used for comparison between groups.

Safety evaluation methods: the $\chi 2$ test/Fisher's precision probability test should be used to compare the incidence rates of all adverse events, severe adverse events and adverse reactions between the two groups, and the normal/abnormal changes in laboratory test results before and after treatment should be described.

SAS 9.2 software should be employed for statistical analysis, and the analysis process should be all programmed.

12. Quality control of the clinical trial

(1) The preparation of test vesicle packaged chemotherapeutic drugs provided by the sponsor should conform to the relevant regulations and conditions and be subject to strict quality control.

(2) In the process of clinical study, the clinical research associate assigned by the sponsor should visit the study hospital regularly and faithfully make the inspection records to ensure that the study protocol is strictly followed and all CRFs can be filled in accurately.

(3) The laboratory tests should be conducted by study sites in accordance with the standard operating procedures (SOP). The testing methods and quality control of different study sites should be unified.

(4) The clinical laboratory of the study site should carry out internal quality control according to relevant regulations and obtain the quality assessment certificate from National Center for Clinical Laboratories.

13. Case Report Form and Statistical Report

(1) The case report form (CRF) in duplicate should be filled out (with pen or water-based black pen) by the physician in charge during clinical observation and shall not be altered at will. After verification, the physician should cross out the contents to be corrected in pen, clearly write the correct data, sign and indicate the date of correction in a responsible manner. The test reports should be attached to the CRF, and the CRF should be signed by the doctor in charge, verified and signed by the principal investigator of the institution.

(2) The statistical analysis plan should be formulated by the medical statistician according to the clinical trial protocol, and the statistical analysis report should be completed by the medical statistician according to the Biostatistics Guidelines for Clinical Trials.

14. Data Collection

During the clinical trial, the investigator should archive all original data (including laboratory test reports). The study site should regularly collect the second copy of CRF that has completed the clinical trial (copy of the clinical trial participating institution) to the trial sponsor. After the sponsor collects the second copy of all

observation forms and the clinical research associate carefully reviews and confirms that the data in the CRF meet the requirements of the trial protocol, the second copy of all observation forms should be sent to the data statistics unit for processing.

15. Summary of Reports

After the end of the trial, the data statistics center should conduct data analysis in accordance with the Statistics Guidelines for Clinical Trials. The clinical trial sponsor and participating institutions should write the clinical trial summary and sub-center summary reports based on the statistical analysis reports and the guidelines for clinical trials.

16. Investigator Responsibilities

The principal investigator and participating investigators should conduct the trial according to the clinical study protocol and in accordance to the *Declaration of Helsinki*, relevant Chinese laws and regulations and current GCP guidelines.

17. Trial Funding

A separate contract is signed for the trial to state the specific funding provision.

18. Provision for Early Termination of the Clinical Trial

The trial sponsor has the right to terminate the clinical trial at any time for management or other reasons. Once the clinical trial is terminated prematurely, the sponsor must bear all costs incurred thereby.

19. Document Archival

The case report form (CRF) should be filled in with black pen in duplicate, one for the sponsor and one for the participating institution. All original data, statistical data and summary reports should be archived by the clinical trial institution for at least 5 years after the end of the trial.