Phase II, single-arm trial of preoperative short-course radiotherapy followed by chemotherapy and camrelizumab in locally advanced rectal cancer

Zhanyu Lin, Ming Cai, Peng Zhang, Gang Li, Tao Liu, Xin Li, Kailin Cai, Xu Nie, Jing Wang, Junli Liu, Hongli Liu, Weikang Zhang, Jingbo Gao, Chuanqing Wu, Linfang Wang, Jun Fan, Lan Zhang, Zheng Wang, Zhiguo Hou, Chi Ma, Kunyu Yang, Gang Wu, Kaixiong Tao, Tao Zhang

ABSTRACT

Background In locally advanced rectal cancer (LARC), preoperative short-course radiotherapy (SCRT) with delayed surgery has been shown to be as effective as long-course chemoradiotherapy, with only modest benefits. This study aimed to evaluate the efficacy and safety of preoperative SCRT combined with subsequent CAPOX (capecitabine and oxaliplatin) and the anti-PD-1 antibody camrelizumab in patients with LARC.

Methods This was a prospective, single-arm, phase II trial. Treatment-naive patients with histologically confirmed T3-4N0M0 or T1-4N+M0 rectal adenocarcinoma received 5×5 Gy SCRT with two subsequent 21-day cycles of CAPOX plus camrelizumab after 1 week, followed by radical surgery after 1 week. The primary endpoint was pathological complete response (pCR) rate. Biomarker analysis was performed to identify a potential predictor of pCR to treatment.

Results From November 7, 2019 to September 14, 2020, 30 patients were enrolled, and 27 patients received at least one dose of CAPOX plus camrelizumab. Surgery was performed in 27 (100%) patients. The pCR (ypT0N0) rate was 48.1% (13/27), including 46.2% (12/26) for proficient mismatch repair (MMR) tumors and 100% (1/1) for deficient MMR tumors. Immune-related adverse events were all grade 1–2, with the most common being reactive cutaneous capillary endothelial proliferation (81.5%). No grade 4/5 adverse events occurred. Biomarker analysis showed patients without FGR1–3 deletions had a better tendency for pCR.

Conclusions SCRT combined with subsequent CAPOX plus camrelizumab followed by delayed surgery showed a favorable pCR rate with good tolerance in patients with LARC, especially in the proficient MMR setting. A randomized controlled trial is ongoing to confirm these results.

Trial registration number ClinicalTrials.gov identifier: NCT04231552.

INTRODUCTION

Rectal cancer, which accounts for more than one-third of colorectal cancer (CRC) cases, has been challenging in terms of treatment and organ preservation, especially for locally advanced rectal cancer (LARC), because of the complex anatomical structures and high rates of postoperative complications and local recurrence. Currently, multidisciplinary therapy based on neoadjuvant long-course chemoradiotherapy (LC-CRT) with delayed surgery or short-course radiotherapy (SCRT) with immediate surgery is the predominant treatment strategy for LARC. The Stockholm III trial of patients with LARC was the first to demonstrate that SCRT with delayed surgery was superior to SCRT with immediate surgery and long-course radiotherapy (LCRT) with delayed surgery in terms of pathological complete response (pCR) and tumor downstaging. Recently, a novel treatment combination for LARC patients, namely, neoadjuvant short-course hypofractionated radiotherapy combined with subsequent chemotherapy followed by delayed surgery, led to a greater pCR and had a lower 3-year disease-related treatment failure rate than LC-CRT followed by delayed surgery. However, unfortunately, the pCR rate is consistently below 30%, which is not good in terms of meeting treatment needs or enabling organ preservation for patients with LARC, therefore, there is a need to explore new neoadjuvant treatment options.

In 2015, immunotherapy was shown to provide significant clinical benefits to patients with metastatic CRC with a deficient mismatch repair (dMMR)/microsatellite instability-high (MSI-H) status, whereas no response was observed in patients with a proficient mismatch repair (pMMR)/microsatellite stable (MSS) status, which accounts for approximately 96%–98% of metastatic CRCs and 85% of all CRCs. Of
note, compared with patients with advanced-stage CRC, early-stage patients have higher CD8+ T-cell infiltration, an increase that could enhance the response to immune checkpoint inhibitors, as previously reported in melanoma.10–11 In addition, the preliminary data in the NICHE study demonstrated that 13% of patients with early-stage pMMR colon cancer could benefit from neoadjuvant dual immunotherapy, consistent with reports in other solid tumors that early-stage disease may be more responsive to immunotherapy, particularly as neoadjuvant therapy.12–13

Numerous studies have demonstrated that the combination of checkpoint blockade immunotherapy and radiotherapy could generate synergistic antitumor effects against local and distant tumors.14–16 Recently, the clinical efficacy and safety of preoperative LC-CRT and subsequent nivolumab monotherapy in the treatment of rectal cancer were described in the VOLTAGE-A study, which enrolled and assessed 37 patients with MSS LARC.17 18 A pCR rate of 30% and a major pathologic response rate of 38% were observed, with tolerable toxicities. Given previous studies suggesting that short-course hypofractionated radiotherapy combined with subsequent chemotherapy is comparable with LC-CRT in terms of the effects of neoadjuvant therapy in rectal cancer and that the immune response was increased by hypofractionated radiotherapy plus programmed death 1 (PD-1) blockade,19 we hypothesized that neoadjuvant short-course hypofractionated radiotherapy combined with subsequent chemotherapy and immune checkpoint inhibitors followed by delayed surgery would afford clinical benefits for LARC.

We report the short-term results from a single-arm, single-center, phase II trial evaluating the efficacy and safety of preoperative short-course radiotherapy combined with subsequent chemotherapy (capcitabine and oxaliplatin) and the anti-PD-1 antibody camrelizumab in patients with LARC.

METHODS

Study design and participants

This was a prospective, single-arm, single-center, phase II trial performed at the Union Hospital affiliated to Tongji Medical College of Huazhong University of Science and Technology in China.

Eligible patients were aged 18 to 75 years, with histologically confirmed T3-4N0M0 or T1-4N+M0 rectal adenocarcinoma, inferior margin of ≤10 cm from the anal verge, treatment naive, an Eastern Cooperative Oncology Group performance status of 0–1, and no severe hematologic, cardiac, pulmonary, hepatic, or renal functional abnormalities or immunodeficiency diseases. Laboratory tests were required to meet the show the following: hemoglobin level ≥9 g/dL; white blood cell count ≥3×10^9/L; absolute neutrophil count ≥1.5×10^9/L; platelet count ≥100×10^9/L; bilirubin level ≤1.5× the upper limit of normal (ULN); aspartate aminotransferase and alanine aminotransferase (AST) levels ≤2.5× ULN; serum creatinine level ≤1.5× ULN or creatinine clearance ≥50 mL/min; thyroid-stimulating hormone level ≤1× ULN or T3 and T4 levels within normal limits. Patients were recruited regardless of their programmed death ligand 1 (PD-L1) expression level. Key exclusion criteria included previous exposure to any anti-PD-1 or anti-PD-L1 antibody, a history of pelvic radiation, treatment with corticosteroids or other immunosuppressive agents within 14 days prior to study drug administration, presence of autoimmune disease, and known interstitial lung disease.

Procedures

Eligible patients received SCRT (5×5 Gy over 5 days), followed 1 week later by two subsequent 21-day cycles of CAPOX (oxaliplatin 130 mg/m² intravenously, day 1; capcitabine 1000 mg/m² oral twice daily, days 1–14) plus camrelizumab (200 mg intravenous drip, day 1), followed by radical surgery after 1 week. Surgery was done according to total mesorectal excision principles.

Low anterior resection was performed for middle and low rectal cancers with distal margins of more than 1 cm, and abdominoperineal resection was conducted by the surgeon for extremely low tumors.

Patients received a baseline assessment including collection of information on demographics, medical history, and disease characteristics before enrollment, and they underwent systematic physical examination and relevant laboratory and imaging (chest CT, liver MRI, abdominal and pelvic CT or MRI) tests before and after treatment. Patients discontinuing treatment for reasons other than progressive disease were followed up every 3 months until disease relapse or death.

Surgically resected specimens were processed and examined as previously reported.20 The extent of the residual tumor in the resected specimens was classified as per the 8th edition of the International Union Against Cancer TNM staging system. All resected specimens (including the whole tumor bed and surrounding tissues) were sampled and cross-sectioned consecutively. If no residual viable tumor was identified after examination, additional three-level sections were carried out. All sampled lymph nodes were examined based on standard procedures. All sections were evaluated independently by two senior pathologists. A pCR was defined as the absence of any remaining viable cancer cells in the resected primary tumor specimen and all sampled regional lymph nodes (ypT0N0). The tumor regression grade (TRG) was assessed according to the criteria proposed by Ryan et al.24

Outcomes

The primary endpoint was pCR rate, defined as the proportion of patients who had a pCR. Secondary endpoints included 3-year event-free survival rate (defined as the percentage of patients without disease recurrence or progression or death due to any cause at the 3-year follow-up), R0 resection rate (defined as the rate of negative margins microscopically), 3-year overall survival rate (defined as the percentage of patients alive at the 3-year follow-up), complication rate, safety, and
quality of life. Adverse events (AEs), recorded during the period when patients signed their informed consent forms 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 were required to be provided when patients were enrolled. Immunohistochemistry was performed to detect the expression of PD-L1, DNA mismatch repair (MMR) proteins (MSH6, MSH2, MLH1, and PMS2), CD4, and CD8. The PD-L1 combined positive score (CPS) was evaluated using the PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies, Santa Clara, CA, USA), defined as the number of PD-L1 positive cells (tumor cells, lymphocytes, macrophages) as a proportion of the total number of tumor cells multiplied by 100. Positive PD-L1 expression was considered when the 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 tissues, H&E staining was performed, and the stained sections were evaluated by a pathologist to ensure tumor cells ≥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 NovaSeq 6000 platform (Illumina, San Diego, CA, USA) with 2×150 bp paired-end reads. The average sequencing depth of tumor tissues was ≥1000×. Genomic alterations, including tumor mutation burden (TMB), single nucleotide variants, short and long insertions and deletions, copy number variants, and gene fusions, were assessed.

**Table 1** Patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, median (range)</td>
<td>57 (31–73)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17 (56.7)</td>
</tr>
<tr>
<td>Female</td>
<td>13 (43.3)</td>
</tr>
<tr>
<td>ECOG performance status, n (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>13 (43.3)</td>
</tr>
<tr>
<td>1</td>
<td>17 (56.7)</td>
</tr>
<tr>
<td>Clinical T category, n (%)</td>
<td></td>
</tr>
<tr>
<td>cT3</td>
<td>22 (73.3)</td>
</tr>
<tr>
<td>cT4</td>
<td>8 (26.7)</td>
</tr>
<tr>
<td>Clinical N category, n (%)</td>
<td></td>
</tr>
<tr>
<td>cN0</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>cN1</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>cN2</td>
<td>10 (33.3)</td>
</tr>
<tr>
<td>Clinical disease stage, n (%)</td>
<td></td>
</tr>
<tr>
<td>Stage II (cT3-4N0)</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Stage III (cT1-4N1-2)</td>
<td>26 (86.7)</td>
</tr>
<tr>
<td>CRM, n (%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>21 (70.0)</td>
</tr>
<tr>
<td>Negative</td>
<td>9 (30.0)</td>
</tr>
<tr>
<td>EMVI, n (%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>12 (40.0)</td>
</tr>
<tr>
<td>Negative</td>
<td>18 (60.0)</td>
</tr>
<tr>
<td>Distance from primary tumor to anal verge, n (%)</td>
<td></td>
</tr>
<tr>
<td>Median (range), cm</td>
<td>4.7 (1.9–9.0)</td>
</tr>
<tr>
<td>&lt;5</td>
<td>15 (50.0)</td>
</tr>
<tr>
<td>5–10</td>
<td>15 (50.0)</td>
</tr>
<tr>
<td>Length of tumor lesion, cm</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>5.5 (1.7)</td>
</tr>
<tr>
<td>Median (range)</td>
<td>5.4 (2.1–10.0)</td>
</tr>
<tr>
<td>Mismatch repair status, n (%)</td>
<td></td>
</tr>
<tr>
<td>dMMR</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>pMMR</td>
<td>28 (93.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>PD-L1 expression, CPS, n (%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>20 (66.7)</td>
</tr>
<tr>
<td>Positive (≥1)</td>
<td>6 (20.0)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Tissue-based TMB (mut/Mb), n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td>7 (23.3)</td>
</tr>
<tr>
<td>≥10</td>
<td>7 (23.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>16 (53.3)</td>
</tr>
<tr>
<td>Baseline CEA level, n (%)</td>
<td></td>
</tr>
<tr>
<td>Normal (&lt;5 ng/mL)</td>
<td>17 (56.7)</td>
</tr>
<tr>
<td>Abnormal (≥5 ng/mL)</td>
<td>13 (43.3)</td>
</tr>
</tbody>
</table>

CEA, carcinoembryonic antigen; CPS, combined positive score; CRM, circumferential resection margin; dMMR, deficient mismatch repair; ECOG, Eastern Cooperative Oncology Group; EMVI, extramural vascular invasion; PD-L1, programmed death ligand 1; pMMR, proficient mismatch repair; TMB, tumor mutation burden.
According to previous studies, the pCR rate after preoperative CRT in patients with LARC is approximately 15%.22 23 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 a one-sided χ² test with a significance level of 2.5%, and a 20% dropout was estimated, resulting in a total sample size of 30 patients planned for this study.

Statistical analyses were conducted using SAS software (V.9.2; SAS Institute, Cary, NC, USA). Continuous variables were summarized using medians and ranges, and categorical variables were described using frequencies and percentages. Baseline and safety analyses were performed for all enrolled patients (intention-to-treat (ITT) population), and efficacy analyses were conducted for those who were administered at least one dose of camrelizumab (full analysis set). Comparisons between the groups were performed using Fisher’s exact test or the χ² test. P values <0.05 were considered statistically significant.

RESULTS
Patient characteristics and compliance
From November 7, 2019 to September 14, 2020, the target number of eligible patients was enrolled (n=30). Three patients were not treated with CAPOX plus camrelizumab for the reasons given in figure 1 and were excluded from the efficacy analysis. Patient baseline and disease characteristics were summarized in table 1. Approximately half of the patients had at least one high-risk factor, including cT4 disease in 26.7% (8/30), cN2 disease in 33.3% (10/30), extramural vascular invasion in 40.0% (12/30), and tumors located within <5 cm from the anal verge in 50.0% (15/30).

During the treatment period, all patients received the full irradiation dose of 25 Gy (100%, 30/30), and 27 patients (90.0%, 27/30) received at least one cycle of CAPOX plus camrelizumab, of whom three patients did not complete the second cycle of CAPOX plus camrelizumab as specified in the protocol due to the impact of the COVID-19 pandemic. The median intervals from the completion of preoperative SCRT to receiving subsequent CAPOX plus camrelizumab and from the last dose of CAPOX plus camrelizumab to surgery were 12 (range, 4–18) days and 14 (range, 5–141) days, respectively.

Surgery and pathology
A total of 27 patients (100%, 27/27) underwent surgery in the full analysis set, with an R0 resection rate of 100% (27/27) and anal preservation rate of 88.9% (24/27). A total of four patients developed postoperative complications, including infection in three patients (11.1%, 3/27) and bleeding in one patient (3.7%, 1/27). No other

<table>
<thead>
<tr>
<th>pCR (ypT0N0), n (%)</th>
<th>Total (n=27)</th>
<th>pMMR (n=26)</th>
<th>dMMR (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T category, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ypT0</td>
<td>13 (48.1)</td>
<td>12 (46.2)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>ypTis</td>
<td>1 (3.7)</td>
<td>1 (3.8)</td>
<td>0</td>
</tr>
<tr>
<td>ypT2</td>
<td>5 (18.5)</td>
<td>5 (19.2)</td>
<td>0</td>
</tr>
<tr>
<td>ypT3</td>
<td>8 (29.6)</td>
<td>8 (30.8)</td>
<td>0</td>
</tr>
<tr>
<td>N category, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ypN0</td>
<td>19 (70.4)</td>
<td>18 (69.2)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>ypN1</td>
<td>6 (22.2)</td>
<td>6 (23.1)</td>
<td>0</td>
</tr>
<tr>
<td>ypN2</td>
<td>2 (7.4)</td>
<td>2 (7.7)</td>
<td>0</td>
</tr>
</tbody>
</table>

Pathological stage, n (%)

<table>
<thead>
<tr>
<th>Pathological stage, n (%)</th>
<th>Total (n=27)</th>
<th>pMMR (n=26)</th>
<th>dMMR (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14 (51.9)</td>
<td>13 (50.0)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5 (18.5)</td>
<td>5 (19.2)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8 (29.6)</td>
<td>8 (30.8)</td>
<td>0</td>
</tr>
</tbody>
</table>

Tumor regression grade, n (%)

<table>
<thead>
<tr>
<th>Tumor regression grade, n (%)</th>
<th>Total (n=27)</th>
<th>pMMR (n=26)</th>
<th>dMMR (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13 (48.1)</td>
<td>12 (46.2)</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>1</td>
<td>5 (18.5)</td>
<td>5 (19.2)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>7 (25.9)</td>
<td>7 (26.9)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>2 (7.4)</td>
<td>2 (7.7)</td>
<td>0</td>
</tr>
</tbody>
</table>

dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.
postoperative complications or treatment-related deaths occurred. The median interval from surgery to hospital discharge was 8 (range, 7–16) days.

The pCR (ypT0N0) rate was 48.1% (13/27), including 46.2% (12/26) for patients with pMMR and 100% (1/1) for those with dMMR. Negative nodes (ypN0) were reported in 19 (70.4%) of the 27 patients, 18 of whom were in the pMMR patient subset (69.2%, 18/26; table 2). As an example, imaging of patient 7 (a 63-year-old woman) with cT4N2bMx pMMR rectal cancer showed a visible tumor at baseline, the volume of which was significantly reduced after two cycles of treatment, and the histopathologic specimen revealed complete regression of the primary tumor, that is, pCR (figure 2A). Moreover, an obvious increase in CD4+ and CD8+ T-cell infiltration was observed in posttreatment tumor samples compared with pretreatment samples in patient 7 but not in patient 12 who did not achieve pCR (figure 2B). Notably, all

![Figure 2](image-url)
patients with PD-L1 CPS ≥1 were reported to be ypN0. The pCR of patients with PD-L1 CPS ≥1 was numerically higher than that of patients with CPS <1 (66.7%, 4/6 vs 45.0%, 9/20, p=0.2422).

Safety
Treatment-emergent AEs and immune-related AEs are summarized in Table 3. At the data cut-off (January 15, 2021), the most common treatment-emergent AEs of any grade were leukopenia (80.0%, 24/30), reactive cutaneous capillary endothelial proliferation (73.3%, 22/30), and radiation proctitis (70.0%, 21/30). The most common grade 3 treatment-emergent AEs were neutropenia (13.3%, 4/30) and anemia (13.3%, 4/30). Immune-related AEs were all grade 1–2, and the most common was reactive cutaneous capillary endothelial proliferation, which occurred in 22 (81.5%) of 27 patients. Five patients experienced serious treatment-emergent AEs, among whom four were hospitalized or had prolonged hospitalization due to grade 2 radiation enteritis and one was hospitalized due to abdominal pain. No grade 4 or 5 AE occurred during the study.

Results of biomarker analysis
Next-generation sequencing was carried out in 53.8% (14/26) of patients with pMMR in this study; the remainder were not performed due to delivery of unqualified samples or patient refusal. As shown in Figure 3A, the most frequently altered genes at baseline were TP53, APC, and KRAS. Our small-sample analysis did not identify any genes with significant differences in the frequency of genomic alterations between the pCR and non-pCR groups (Figure 3B). Compared with patients with TMB <10, those with TMB ≥10 had a better tendency for pCR (42.9%, 3/7 vs 28.6%, 2/7, p=0.3671). Interestingly, according to baseline copy number variant, none of the five patients with FGFR1-3 deletions achieved pCR, while more than half of the nine patients without FGFR1-3 deletions achieved, although no statistically significant difference was observed (55.6%, 5/9 vs 0%, 0/5, p=0.086).

Discussion
To the best of our knowledge, our study is the first to propose a new neoadjuvant therapy regimen of short-course hypofractionated radiotherapy combined with subsequent chemotherapy and anti-PD-1 antibody. In addition, this study provides preliminary evidence that the addition of camrelizumab to neoadjuvant SCRT followed by the CAPOX chemotherapy regimen results in a remarkable pCR for patients with LARC and is well tolerated, without new or unexpected safety issues.

As shown in previous studies, preoperative radiotherapy combined with chemotherapy resulted in tumor downstaging and reduced local recurrence, whereas pCR was observed only in 15%–30% of patients with rectal cancer. In this study, our pCR rate of 48.1% is encouraging, meaning that our innovative preoperative combination therapy strategy provides more opportunities for sphincter-preserving surgery and also raises the prospect that more patients with LARC, especially those with low rectal cancer, may achieve a clinical complete response and have a watch-and-wait strategy of nonsurgical treatment implemented to improve their quality of life.

Immunotherapy is generally ineffective in the pMMR/MSS tumors that constitute the majority of CRCs, which could be attributed to insufficient lymphocytic infiltration. Preclinical data have shown that radiotherapy can sensitize refractory tumors to PD-1/PD-L1 blockade by modulating the immunogenicity of tumor cells, enhancing antigen-specific CD8+ T-cell responses, and increasing PD-L1 expression on tumor cells and immune cells in the tumor microenvironment; in addition, chemotherapy can also upregulate PD-L1 on dendritic cells and increase immune-cell infiltration. Based on these rationales, immunotherapy strategies combined with chemoradiotherapy are being explored in patients
Figure 3  Genetic analysis. (A) Overall frequency of gene alterations at baseline. (B) Frequency of genomic alterations between the pCR and non-pCR groups. pCR, pathological complete response.
with pMMR/MSS rectal cancer, especially in LARC setting. In the VOLTAGE study, the pCR rate was 30% in patients with MSS LARC receiving preoperative LC-CRT and sequential nivolumab.17 In our study, the pCR rate was 46.2% for patients with pMMR disease. In addition, the recently reported pCR rate was 37.5% among patients with locally advanced rectal adenocarcinoma receiving SCRT followed by mFOLFOX-6 plus avelumab (an anti-PD-L1 antibody) as neoadjuvant therapy in the Averest study.31 When comparing the results of our study and the Averest study, differential N staging was noted between the enrolled patients, with stage N1 patients being predominant in our study (53.3%) but stage N2 in the Averest study (75.0%).34 In addition, a meta-analysis of randomized trials has indicated the superior efficacy of anti-PD-1 antibody over anti-PD-L1 antibody in solid tumors, regardless of monotherapy or combination strategies.32 Although the course of neoadjuvant immunotherapy plus chemotherapy is inconclusive, a short course of chemotherapy has been shown to improve the early efficacy of immunotherapy, as reported by Checkmate 9LA.35 Results from the NRG-G1002 study demonstrated neoadjuvant concurrent pembrolizumab and LC-CRT after FOLFOX induction did not significantly improve the short-term clinical outcomes of patients with LARC compared with FOLFOX and LC-CRT alone, with pCR rate of 31.9% in pembrolizumab group versus 29.4% in control group (p=0.75).34 Although cross-study comparisons should be made with caution, it is interesting that the pCR rate of 48.1% observed in our study compared favorably with the NRG-G1002 study. The differences may be explained by different neoadjuvant therapeutic strategies with the differential timing of anti-PD-1 antibody administration.

A time interval of at least 7 days after SCRT has been reported to be probably required to achieve a favorable immune response, suggesting that the choice of the timing of immunotherapy after SCRT is important.35-37 The PACIFIC trial reported that the initiation of durvalumab within 2 weeks after chemoradiotherapy (rather than ≥2 weeks) was linked to a higher clinical benefit.36 Therefore, we have reasons to believe that our choice of timing for the initiation of immune checkpoint inhibitor camrelizumab in this study (median 12 days) is appropriate.

The previous VOLTAGE-A study showed that positive PD-L1 expression and high TMB are good predictors of neoadjuvant efficacy from LC-CRT followed by nivolumab.18 Similarly, a trend toward a better pCR rate was observed in patients with PD-L1 CPS ≥1 or with TMB ≥10 in our phase II trial, although the differences were not statistically significant. Given that ours was an exploratory analysis with a small sample, we recommend that this finding be interpreted with caution. In addition, patients without FGFR1-3 deletions appear to be more likely to achieve clinical benefit from our neoadjuvant strategy than those with FGFR1-3 deletions (55.6% vs 0%). In urothelial carcinoma, patients with FGFR mutations have been reported to show a worse response to immunotherapy than those without FGFR mutations.37 A similar result was demonstrated in metastatic gastric adenocarcinoma, showing that the patient harboring MSI-H and FGFR2 alterations was resistant to anti-PD-1 monotherapy.38 If positively validated in the subsequent larger study that we plan to conduct, the presence of FGFR1-3 deletions may be a potent predictor of response to neoadjuvant immunotherapy in rectal cancer.

The limitations of this study consist of the small sample size of patients, lack of a control group, and insufficient postoperative follow-up time. Furthermore, whether improvement in pCR in our study can contribute to better survival needs further exploration. A large multicenter randomized phase III study is going to confirm the value of preoperative short-course hypofractionated radiotherapy combined with subsequent chemotherapy and camrelizumab regimen (NCT04928807).

In conclusion, SCRT combined with subsequent CAPOX plus camrelizumab followed by delayed surgery showed a favorable pCR rate with manageable toxicities, especially in the pMMR setting. This suggests that neoadjuvant SCRT combined with subsequent CAPOX plus camrelizumab is a promising feasible strategy and is expected to provide more opportunities for surgery to be delayed or avoided among patients with middle-low LARC and for organ function to be preserved in the future.

Author affiliations
1Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
2Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
3Department of Digestive Surgical Oncology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
4Department of Radiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
5Department of Pathology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
6Department of Medical Affairs, Jiangsu Hengrui Pharmaceuticals Co., Ltd, Shanghai, China

Acknowledgements We thank the patients and their families as well as all other participants who made the study possible. We thank Haihong Wang for her contribution to the collection and management of specimens, Yali Kong (a former employee of Jiangsu Hengrui Pharmaceuticals Co., Ltd.) for her input in data analysis, and Xinzhai Gao (Jiangsu Hengrui Pharmaceuticals Co., Ltd.) for her careful revision of the manuscript. We also thank Yanhua Xu (a medical writer at Jiangsu Hengrui Pharmaceuticals Co., Ltd.) for the medical writing assistance in accordance with Good Publication Practice guidelines.

Contributors KT, TZ, ZH, and CM conceived and designed this study. ZL, MC, PZ, GL, TL, XC, JW, JL, HL, WZ, JG, CW, LW, JF, LZ, and ZW enrolled patients and collected the data. TZ, ZL, MC, PZ, and XN participated in the data curation. XL, XN, JF, and LZ analyzed the data, and all authors participated in data interpretation. XL, JF, KY, and GW provided the administrative support. KT, ZL, MC, and PZ drafted the manuscript and all authors reviewed. KT and TZ had full access to all the data in the study and had final responsibility for the decision to submit for publication. The final version was approved by all authors. KT and TZ are guarantors of the work.

Funding This work was supported by Ministry of Science and Technology of China (2018YFC1313302), 2018 National Natural Science Foundation of China (81874061), and Jiangsu Hengrui Pharmaceuticals Co., Ltd. (no grant number).
REFERENCES

19. Orcid
20. Tazhong Hao http://orcid.org/0000-0003-4018-3393

This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iD

Tao Zhang http://orcid.org/0000-0003-4018-3393

J Immunother Cancer: first published as 10.1136/jitc-2021-003354 on 1 November 2021. Downloaded from by guest. Protected by copyright.


36 Falivre-Finn C, Spigel DR, Senan S, et al. Efficacy and safety evaluation based on time from completion of radiotherapy to randomization with durvalumab or placebo in pts from PACIFIC. *Annals of Oncology* 2018;29:viii488.


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.
Table of Contents

TABLE OF CONTENTS ..................................................................................................................................... 2
PROTOCOL SUMMARY .................................................................................................................................. 4
LIST OF ABBREVIATIONS .............................................................................................................................. 6
1. STUDY BACKGROUND .............................................................................................................................. 7
2. STUDY OBJECTIVE ....................................................................................................................................... 7
3. STUDY DESIGN .............................................................................................................................................. 7
4. SELECTION AND WITHDRAWAL OF SUBJECTS ......................................................................................... 7
  4.1 INCLUSION CRITERIA ................................................................................................................................. 7
  4.2 EXCLUSION CRITERIA ................................................................................................................................ 8
  4.3 REMOVAL CRITERIA ................................................................................................................................. 8
  4.4 DROP-OUT (WITHDRAWAL) CRITERIA ...................................................................................................... 8
  4.5 CRITERIA FOR TRIAL DISCONTINUATION ................................................................................................. 8
5. THERAPEUTIC DRUG AND CONSOLIDATION RADIOTHERAPY REGIMEN ............................................. 9
  5.1 INVESTIGATIONAL PRODUCT .................................................................................................................. 9
  5.2 ADMINISTRATION METHOD .................................................................................................................... 9
  5.3 TECHNICAL ROUTES .............................................................................................................................. 9
  5.4 PRECAUTIONS ......................................................................................................................................... 10
  5.5 CONCOMITANT MEDICATIONS .............................................................................................................. 10
6. MEDICAL ETHICS REQUIREMENTS ........................................................................................................ 10
7. OUTCOME MEASURES AND LABORATORY TESTS ................................................................................... 10
  7.1 MEDICAL HISTORY AND PHYSICAL EXAMINATION: ........................................................................ 10
  7.2 LABORATORY TESTS: ............................................................................................................................ 11
  7.3 OBSERVATION OF ADVERSE EVENTS ................................................................................................ 11
8. EFFICACY EVALUATION .......................................................................................................................... 11
  8.1 SAFETY EVALUATION ............................................................................................................................. 11
  8.2 EFFICACY EVALUATION ......................................................................................................................... 11
9. ADVERSE EVENT MONITORING ............................................................................................................ 12
  9.1 OBSERVATION OF ADVERSE EVENTS ................................................................................................. 12
  9.2 CRITERIA FOR DETERMINING ADVERSE EVENT (AE) SEVERITY ..................................................... 12
  9.3 CRITERIA FOR DETERMINING THE RELATIONSHIP BETWEEN ADVERSE EVENT (AE) AND THE
       INVESTIGATIONAL PRODUCT ................................................................................................................ 12
  9.4 SEVERE ADVERSE EVENT HANDLING AND REPORTING METHODS .................................................. 13
10. DATA MANAGEMENT .............................................................................................................................. 13
  10.1 CRF DATA MANAGEMENT ....................................................................................................................... 13
  10.2 DATA ENTRY AND MANAGEMENT ............................................................................................................ 13
11. STATISTICAL ANALYSIS ........................................................................................................................ 13
  11.1 DATA ANALYSIS SET ................................................................................................................................ 13
  11.2 STATISTICAL ANALYSIS PLAN ................................................................................................................. 14
12. QUALITY CONTROL OF THE CLINICAL TRIAL .............................................................................. 14
13. CASE REPORT FORM AND STATISTICAL REPORT ........................................................................ 14
14. DATA COLLECTION ................................................................................................................................ . 14
15. SUMMARY OF REPORTS ......................................................................................................................... 15
16. INVESTIGATOR RESPONSIBILITIES ................................................................................................... 15
17. TRIAL FUNDING ........................................................................................................................................ 15
18. PROVISION FOR EARLY TERMINATION OF THE CLINICAL TRIAL ......................................... 15
19. DOCUMENT ARCHIVAL .......................................................................................................................... 15
Clinical Study Protocol

Version No.: V2.0

Protocol Summary

<table>
<thead>
<tr>
<th><strong>Study title:</strong></th>
<th>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</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study phase:</strong></td>
<td>Phase II</td>
</tr>
<tr>
<td><strong>Study objective:</strong></td>
<td>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).</td>
</tr>
</tbody>
</table>
| **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 Fractions /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 x 10^9/L; neutrophil (ANC) ≥ 1.5 x 10^9/L; platelet (Pt) ≥ 100 x 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 ≥10^4 copies/mL; reference for active hepatitis C: HCV RNA ≥10^4 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)

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

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+;
Clinical Study Protocol

Version No.: V2.0

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 (Plt) ≥ 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 hypothyroidism 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 ≥10^6 copies/mL; reference for active hepatitis C: HCV RNA ≥10^5 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
Capcitabine 1000 mg/m2 po bid, d1-14, q3w;

PD1 inhibitor camrelizumab: 200 mg iv drip, q3w;

5.3 Technical routes

Patients with locally advanced rectal cancer (T3, T4/N+) (All patients sign the informed consent form).

Preoperative local pelvic short-course radiotherapy. Radiotherapy will employ intensity-modulated radiation therapy, with a dose of 25 Gy/5 Fractions/1 week

Rest for 1 week

CAPOX + PD1 inhibitor, once per 3 weeks for 2 cycles

Rest for 1 week

Radical resection of rectal carcinoma

Rest for 1 week

Postoperative adjuvant chemotherapy determined by the investigator
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 algesias, 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 $\geq 38^\circ C$ 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

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Definitely related</th>
<th>Probably related</th>
<th>Possibly related</th>
<th>Possibly unrelated</th>
<th>Definitely unrelated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reasonable chronological sequence</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Conforming to the known reaction type of the investigational product</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Improving after the discontinuance of the investigational product</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes or No</td>
<td>Yes or No</td>
<td>No</td>
</tr>
<tr>
<td>Recurring after reusing the investigational product</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>Possible other explanation for the drug-induced reaction</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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
Clinical Study Protocol

Version No.: V2.0

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≤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 χ^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

\[ H_0: \pi_1 = \pi_2 \quad \text{vs} \quad H_1: \pi_1 > \pi_2 \]

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-χ^2 with central effect should be used for comparison between groups.

Safety evaluation methods: the χ^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.