# CLINICAL STUDY PROTOCOL

A Phase II Single-Arm Study of High-Bioavailability Curcumin as Neoadjuvant Chemoradiotherapy in Mid-to-Low Rectal Cancer: Integrated Clinical and Translational Analysis of Tumor Tissue

(BCMRECRAD)

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INVESTIGATORS

Shu-Huan Huang, Jeng-Fu You, Sum-Fu Chiang, Cheng-En Hsieh

SITES

Division of Colon and Rectal Surgery, Chang Gung Memorial Hospital, Linkou, Taiwan

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# 1. Synopsis

**Protocol Title：**

A Phase II Single-Arm Study of High-Bioavailability Curcumin as Neoadjuvant Chemoradiotherapy in Mid-to-Low Rectal Cancer: Integrated Clinical and Translational Analysis of Tumor Tissue

**Study Objectives：**

This study aims to investigate the anti-inflammatory and anti-cancer properties of high-bioavailability curcumin in patients with mid-to-low rectal cancer undergoing neoadjuvant chemoradiotherapy. Curcumin is generally considered to have a high safety profile, and its oral formulation has been classified by the U.S. Food and Drug Administration (FDA) as **"Generally Recognized as Safe" (GRAS)** (GRAS Notice [GRN] No. 686). Reports of adverse effects related to curcumin are rare and primarily involve interference with bile secretion and iron metabolism.[1, 2] However, despite numerous molecular studies demonstrating curcumin's anti-cancer and anti-inflammatory mechanisms, as well as its excellent oral safety, its oral bioavailability remains extremely low due to its lipophilic nature. Previous human studies have shown that even with oral administration of 12 grams of curcumin, the serum concentration only reaches 0.051 mg/mL[3], and approximately 75% of orally administered curcumin is excreted via feces.[4] The primary objective is to evaluate whether curcumin can reduce radiotherapy-associated adverse effects, such as radiation-induced enteritis, and improve the pathological complete response (pCR) rate to chemoradiotherapy. In addition, translational research will be conducted on tumor tissue samples collected at different time points to elucidate the underlying molecular mechanisms.

**Investigational product(s)：**

High-Bioavailability Curcumin (BCM95 curcumin)

**Development Phase：** ☐ I ■II ☐III ☐IV ☐其它_____ ☐不適用

**Study Design：**

1. ■ Experimental Group
   ☐ Control Group ： ☐ Placebo
   ☐ Study Drug (Name、Dose、Usage) _____
   ☐ Other _____
2. Blinding：■ Open ☐ Evaluator-blind ☐ Single-blind(patient) ☐ Double-blind(patient+PI)
   ☐ Double Dummy ☐ Other _____
3. Randomization: ☐ Yes ■ No
4. ☐ Parallel design ☐ Crossover design ■ Other __single arm__ ☐ Not applicable
5. Treatment Period：5 months ☐ Not applicable
6. Study Period: 3 years (or From 2026/01/01 to 2029/12/31)
6. Dose adjustment：☐ Mandatory ■ Selectively ☐ No ☐ Not applicable
7. Study location：■ Single ☐ Multi-center ☐ Global

**Endpoints (Outcome measure)：**

1. Primary endpoint:
   1. **Grade 3 enteritis/proctitis** or grade 3 GI AE: evaluated by National Cancer Institute Common

Terminology Criteria for Adverse Events Version 5.0 (NCI CTCAE v. 5.0) and the criteria of the Radiation Therapy Oncology Group (RTOG) and European Organisation for Research and Treatment of Cancer (EORTC). The cumulative incidence GI symptoms (ie, abdominal pain, loose stools, bloody stools, tenesmus, and nausea).

2. Secondary endpoints:
   1. Colonoscopy evaluation of proctitis by Vienna Rectoscopy Score: baseline, 2±1 weeks and 12 ±1 weeks after radiation therapy completed.
   2. Any grade III adverse events during the neoadjuvant therapy: evaluated by National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (NCI CTCAE v. 5.0) and the criteria of the Radiation Therapy Oncology Group (RTOG) and European Organisation for Research and Treatment of Cancer (EORTC)
   3. Clinical complete response rate of the primary tumor after completion of the neoadjuvant treatment: evaluated by Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST v1.1)
   4. Pathological or clinical complete response rate of the primary tumor after completion of the neoadjuvant treatment: defined as no residual cancer cells in the resected specimen after curative resection
   5. Patient's tolerability and completion rate of the neoadjuvant therapy

3. Exploratory endpoints (if any):
   1. Three-year disease-related treatment failure rate: defined as locoregional recurrence, distal metastasis or cancer-related death from the neoadjuvant treatment to 3-year follow-up after the surgery
   2. Five-year overall survival death: defined as the death rate during the treatment and follow-up period
   3. Compare the molecular marker alterations in rectal tumors and adjacent tissues following chemoradiotherapy with those documented in existing molecular databases

**Inclusion/Exclusion Criteria：**

**Inclusion criteria:**

(A) Males and females more than 20 years of age

(B) Signed informed consent

(C) Patients with a pathologically proven rectal adenocarcinoma located less than 10 cm to the anus.

(D) Clinical staging (AJCC 8th ed.): T2-4 N0 M0 or T any N1-2 M0

(E) Distal metastasis has been excluded by imaging study: by chest-to-pelvic computed tomography or Positron Emission Tomography

(F) Preoperative pelvic staging by pelvic Magnetic Resonance Imaging (preferred) or trans-rectal ultrasound

(G) Patients with WHO/ECOG performance scale 0 or 1

**Exclusion criteria:**

(A) Refuse to sign the informed consent
(B) Distal metastasis revealed by the imaging study
(C) Patients does not receive radiotherapy
(D) Unable to receive further curative resection
(E) Patients receive tumor resection before the neoadjuvant treatment
(F) Patients have history of more than 5 Gy of pelvic radiation
(G) Patients in pregnancy or lactation status
(H) Patients have allergic history to curcumin, 5-fluouracil or oxaliplatin
(I) Patients of childbearing potential can not cooperate with appropriate contraceptive method (oral, injectable, or implantable hormonal contraceptive; tubal ligation; intra-uterine device; barrier contraceptive with spermicide; or vasectomized partner)
(J) Patients with any concurrent malignancy; patients with history of malignancy should be cancer-free for more than 5 years
(K) Patients with New York Heart Association (NYHA) class III or IV heart failure, unstable angina pectoris, unstable cardiac arrhythmia or tachycardia (heart rate > 100 beats/minute)
(L) Patients have concurrent uncontrolled medical conditions, such as illness ongoing or requiring IV antibiotics, severe chronic renal failure (eGFR <30 mL/min/1.73m$^2$) or severe active hepatitis(AST/ALT>3x upper normal limit)、Total Bilirubin>2 mg/dl
(M) Patients with previous or current drug abuse
(N) Patients underwent major surgery within 28 days of study enrollment (except diverting colostomy)
(O) Patients have Familial Adenomatosis Polyposis Coli (FAP), Hereditary Non-Polyposis Colorectal Cancer (HNPCC), active Crohn's disease or active ulcerative Colitis
(P) Patients have known dipyrimidine dehydrogenase deficiency (DPD)
(Q) Patients with congenital iron metabolic or hematopoietic diseases
(R) Patients with synchronous colon cancer
**(S) The Patients with hematologic abnormalities (INR > 1.5, white blood cell (WBC) count < 3,000/μL, absolute neutrophil count (ANC) < 1,500/μL, platelet count < 100,000/μL, hemoglobin < 9.0 g/dL not caused by tumor treatment) or known hematologic diseases (aplastic anemia, myelodysplastic syndrome (MDS), leukemia, malignant lymphoma, multiple myeloma, hereditary hematologic diseases such as thalassemia, sickle cell anemia, etc.).**
(T) The patient has diabetes mellitus
**(U) The patient is taking the immunosuppressants Cyclosporine, Tacrolimus, Sirolimus, and Everolimus and antigoagulants (warfarin、NOACs、aspirin)**
(V) Patients with The medical, psychological, or social condition that, in the opinion of the investigator, may increase the patient's risk or limit the patient's adherence with study

requirements

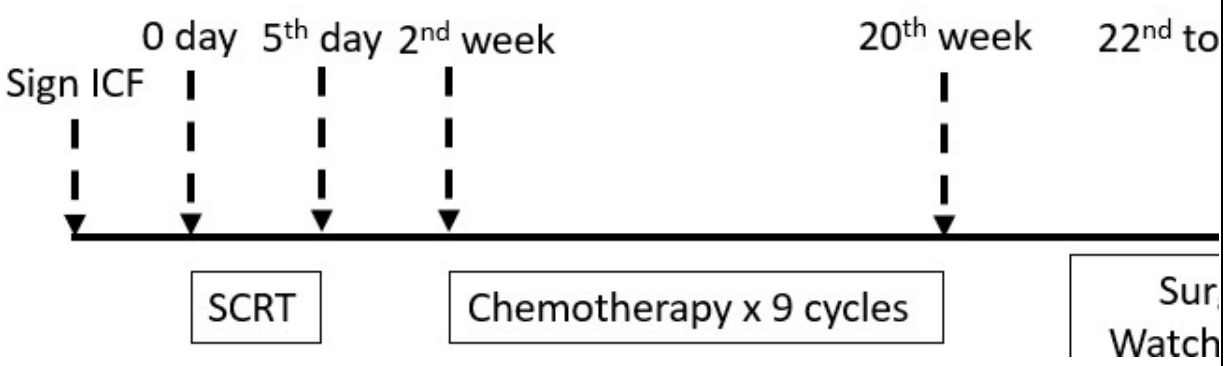
**Study Procedures：**

This single-arm study is aimed to recruit 72 mid-to-low locally advanced rectal cancer patients who need neoadjuvant chemotherapy and radiotherapy. The pre-operative evaluation, including completed colonoscopy, neck-to-abdomen computed tomography, pelvis *magnetic resonance imaging*, and serum carcinoembryonic antigen, are arranged as a current practice guideline. After the patients agree to participate, a daily dose of 3g of high bioavailability curcumin is given as the chemotherapy starts. The curcumin therapy is continued during the whole neoadjuvant chemotherapy and radiation therapy. A complete blood count is checked during each cycle of chemotherapy.

**During the treatment period, participants will undergo biweekly (every 2 weeks) routine blood tests and adverse event assessments from the start of CRT until the completion of neoadjuvant therapy and subsequent surgery (approximately 3–4 months).**Moreover, additional biochemical examinations with liver and renal function and adverse events (CTCAE version 5) and the criteria of the Radiation Therapy Oncology Group (RTOG) and European Organisation for Research and Treatment of Cancer (EORTC) are examined every 3 cycles of chemotherapy. The curcumin treatment is ended along with the completion of chemotherapy. An additional colonoscopy will be arranged 2 to 3 weeks after the radiation therapy completed to evaluate acute proctitis by Vienna Rectoscopy Score. After completion of the chemoradiation, colonoscopy and pelvis magnetic resonance imaging are conducted to evaluate the tumor response. **Tumor response will be evaluated concurrently based on imaging and clinical findings. The total neoadjuvant CRT course lasts approximately 19 weeks.** Based on each surgeon's clinical judgment, the patient receives either curative surgery or a watch-and-wait strategy. Colonoscopy and tissue biopsies of both tumor and adjacent normal rectal tissue will be performed at three designated time points: prior to treatment, upon completion of radiotherapy, and following the completion of chemoradiotherapy. The obtained specimens will be subjected to molecular analysis and compared with established databases to evaluate treatment-related molecular changes.

**For translational analyses, only residual clinical specimens (e.g., a few millimeters of surplus tumor tissue) obtained after standard diagnostic or therapeutic procedures will be utilized. The sampling volume is minimal and will not interfere with diagnostic interpretation. All specimens will be de-identified and coded in accordance with ethical and regulatory standards and will be used exclusively for translational and biomarker studies.**

Sample size calculation is performed using one proportion equality test using the exact test to achieving an 80% power at the 5% level of significance. With the expectation of an -15% of improvement of acute radiation proctitis (30% to 15%), the total estimated patients number in a single-arm trial required is 64. Consider the patient with incomplete data and drop-out rate of 10%, the total estimated number of sample size is 72. Sample size calculation was performed using PASS software (Power Analysis and Sample Size version 2021, NCSS, Kaysville, Utah, USA).




**Concomitant Treatments：** ☐不適用

**1.** Concomitant Therapy：

1.1 TEGAFOX(one cycle)：

*Oxaliplatin 85 mg/m2 in D5W 250 ml IVF 2 hrs, D1, every 2 wks
*Tegafur-uracil 300mg/$m^2$/day and leucovorin 90mg/day PO, given on days 1 to 5 and 8 to 12 days, every 2 weeks

1.2 FOLFOX(one cycle)：

*Oxaliplatin 85 mg/m2 in D5W 250 ml IVF 2 hours, D1
*Calcium Folinate 100 - 400 mg/m2 in D5W 250ml IVF 2 hours, D1
*5-FU 400 mg/m2 IV bolus on D1 *5-FU (2400 mg/m2) IVF 46 hrs, Repeat every 2 weeks

1.3 UFUR (one cycle)

*Tegafur 300-350 mg/$m^2$/day and leucovorin 90mg/day PO on day 1 to day 5, then rest for 2 days for 2 weeks

1.4 The radiation therapy modules are as follows：

Short-Course (hypofractionated, 25 Gy/5 fractions) Radiotherapy（SCRT）

**2.** Prohibited Therapy：cell therapy, immunotherapy

**Statistical Methods：**

**1.** Main study Hypothesis：■ Equality ☐ Superiority ☐ Non-inferiority ☐ Equivalence ☐ Other

**2.** Estimated Sample Size：整個試驗預計納入人數_72_，整個試驗可評估人數_64_
本中心預計納入人數__72_，本中心可評估人數_64_

**3.** Efficacy assessment group：■ Intent-to-treat (ITT) ☐ Per-Protocol (**PP)** ☐ Other _____

附註：意圖治療：Intent-to-treat (ITT)；依計畫書：Per-Protocol (**PP)**

**4.** Interim analysis：☐ Yes ■ No

**5.** Statistical methods：descriptive statistics, Kaplan-Meier method, T-test

**6.** Handling of Missing Data：Listwise deletion

# 2. Introduction and Rationale

The primary treatment method for non-metastatic locally advanced rectal cancer is TME (Total mesorectal excision). In recent years, neoadjuvant chemotherapy and radiation therapy have been proven to decrease local recurrence and increase the possibility of organ preservation. [5, 6] However, radiation proctitis is a joint adverse event in one-third of patients one week after radiation treatment is complete; [7] and severe acute toxicity was observed in 4.2% of patients. [8]

Curcumin (diferuloylmethane) is a hydrophobic polyphenol derived from the rhizome of Curcuma longa. It has been proven an anti-inflammatory agent as an NF-kB inhibitor [9] and has numerous intrinsic and extrinsic anti-neoplastic pathways. [10] The murine model showed that oral curcumin could alleviate irinotecan-induced intestinal injury via attenuation of NF-κB activation, oxidative stress and endoplasmic reticulum stress. [11] One phase I clinical trial showed the oral form curcumin can be safe even up to 8 gram per day.[12] Moreover, the USFDA has designated oral curcumin as a "generally recognized as safe" agent. (US FDA; per 21 CFR 182.10, 182.20) However, due to its hydrophobic feature, poor bioavailability limited its clinical application.[13]

BCM-95® contains an extract characterized as a 95% curcuminoid complex (curcumin, demethoxycurcumin, and bisdemethoxycurcumin in their natural ratios) along with turmeric essential oil. The bioavailability of BCM-95® has been proven to be 6.93-fold compared to regular curcumin. [14] Therefore, the application of BCM-95® may improve radiation-related inflammation and chemotherapy-induced mucosal injury. Moreover, its anti-neoplastic effect may improve the tumor response to current neoadjuvant therapy and therefore improve the long-term outcome of rectal patients.

## 2.1 Investigational product(s)

BCM-95® curcumin 3 gram per day

## 2.2 Animal and preclinical study data

Guo et al: curcumin can promote Bax, TP53 gene, activated the caspase-3 and caspase-9 in CRC cell line (LoVo), which can induce cancer cell apoptosis. [15]
Mosieniak et al.: curcumin can activate senescence and autophagy via p53 and p21 overexpression. [16]

NF-κB Ionizing radiation (IR) is well recognized for activating the transcription factor NF-κB, a key mediator of both cancer cell resistance to treatment and radiation-induced normal tissue toxicity [17]. In cancer cells, NF-κB drives the activation of pro-survival signaling pathways, facilitating tumor growth and progression. Additionally, NF-κB regulates the expression of numerous cytokines and chemokines with strong pro-inflammatory properties, which can exacerbate tissue injury following IR exposure [18].

Curcumin has been shown to inhibit the proliferation and post-irradiation clonogenic survival of multiple colorectal cancer cell lines. While ionizing radiation activates NF-κB in a dose- and time-dependent fashion, curcumin effectively counteracts this by preventing the phosphorylation and degradation of IκBα, inhibiting IκB kinase activity, and reducing Akt phosphorylation. It also suppresses the expression of key NF-κB-regulated proteins, including Bcl-2, Bcl-xL, IAP-2, COX-2, and cyclin D1, thereby enhancing the therapeutic efficacy of radiotherapy [19].

In addition to its radiosensitizing effects, curcumin also demonstrated protective activity in normal intestinal cells exposed to Irinotecan. It blocked NF-κB signaling and mitigated Irinotecan-induced apoptosis by upregulating molecular chaperones such as GRP78, P4HB, and PRDX4, while downregulating pro-apoptotic proteins like CHOP and cleaved caspase-3. These protective effects are mediated through curcumin's inhibition of NF-κB activation, along with its ability to reduce oxidative stress and endoplasmic reticulum stress, ultimately alleviating intestinal mucosal injury caused by chemotherapy [11].

## 2.3 Clinical data

Howells et al.: Curcumin is a safe and tolerable adjunct to FOLFOX chemotherapy in metastatic colorectal cancer patients with potential benefits of overall survival. [20]
Carroll et al: Curcumin 4g per day can reduce 40% of aberrant crypt foci in the rectum of healthy smokers [21]

## 2.4 Risks / benefits Assessment

Benefit: the anti-inflammation and anti-neoplastic effect of curcumin may alleviate the side effect or improved the tumor response to neoadjuvant chemotherapy and radiation therapy.

Risk: the limited case reports of curcumin on hepatitis and iron metabolism may effect patient with relative underlying disease such as active hepatitis or iron metabolic diseases.[1, 22]

## 2.5 Regulatory

This study will be conducted in compliance with the protocol approved by the Institutional Review Board, and according to Good Clinical Practice standards. No deviation from the protocol will be implemented without the prior review and approval of the IRB except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the IRB as soon as possible.

# 3. Objectives and Endpoints

*3.1 Study Objectives:*

*3.1.1 Primary objective:* the anti-inflammation effect of curcumin on the radiation proctitis, adverse events and completion of neoadjuvant radiation therapy and chemotherapy

*3.1.2 Secondary objectives:* the anti-neoplastic effect of curcumin on the treatment of rectal cancer in adjuvant with radiation therapy and chemotherapy

*3.2 Study endpoints:*

*3.2.1 Primary endpoint:*

1. **Grade 3 enteritis/proctitis**: evaluated by National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (NCI CTCAE v. 5.0) and colonoscopy evaluation 2 weeks after radiation therapy completed

*3.2.2 Secondary endpoints:*

1. Any grade III adverse events during the neoadjuvant therapy: evaluated by National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (NCI CTCAE v. 5.0)

2. Clinical complete response rate of the primary tumor after completion of the neoadjuvant treatment: evaluated by Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST v1.1)
3. Pathological or clinical complete response rate of the primary tumor after completion of the neoadjuvant treatment: defined as no residual cancer cells in the resected specimen after curative resection
4. Patient's tolerability and completion rate of the neoadjuvant therapy

# 4. Study Design

## 4.1 Overall Design

This phase II single-arm study is aimed to recruit 72 mid-to-low locally advanced rectal cancer patients who need neoadjuvant chemotherapy and radiotherapy. The pre-operative evaluation, including completed colonoscopy, neck-to-abdomen computed tomography, pelvis *magnetic resonance imaging*, and serum carcinoembryonic antigen, are arranged as a current practice guideline. After the patients agree to participate, a daily dose of 3g of BCM-95® curcumin is given as the chemotherapy starts. The curcumin therapy is continued during the whole neoadjuvant chemotherapy and radiation therapy. A complete blood count is checked during each cycle of chemotherapy.

Moreover, additional biochemical examinations with liver and renal function and adverse events (CTCAE version 5) and the criteria of the Radiation Therapy Oncology Group (RTOG) and European Organisation for Research and Treatment of Cancer (EORTC) are examined every 3 cycles of chemotherapy. The curcumin treatment is ended along with the completion of chemotherapy. An additional colonoscopy will be arranged 2 to 3 weeks after the radiation therapy completed to evaluate acute proctitis by Vienna Rectoscopy Score. After completion of the chemoradiation, colonoscopy and pelvis magnetic resonance imaging are conducted to evaluate the tumor response. Based on each surgeon's clinical judgment, the patient receives either curative surgery or a watch-and-wait strategy.

**Run-in Cohort (n=6) Design**

**Study Design**

**This trial will begin with a run-in cohort of 6 patients receiving high-bioavailability curcumin (BCM-95) in combination with standard neoadjuvant chemoradiotherapy (nCRT) for mid-/low-rectal cancer. The purpose of the run-in phase is to confirm safety, tolerability, and feasibility prior to expanding enrollment to the full Phase II cohort.**

**Objectives**

- **Primary Objective (Run-in cohort):**
  **To evaluate the incidence of dose-limiting toxicities (DLTs), particularly hepatotoxicity and gastrointestinal adverse events (AEs), during curcumin administration with concurrent chemoradiotherapy.**
- **Secondary Objectives:**
  - **To confirm feasibility of oral curcumin administration and patient adherence.**
  - **To monitor preliminary gastrointestinal protective effects (e.g., radiation proctitis, diarrhea).**
  - **To validate safety monitoring and study logistics prior to full expansion.**

**Patient Population**

- **6 patients with stage II–III mid-/low-rectal adenocarcinoma scheduled for standard nCRT will be enrolled.**
- **Patients must meet all eligibility criteria, including adequate hematologic and hepatic function.**

**Treatment**

- **Investigational Product: BCM-95 curcumin, administered orally throughout the course of nCRT.**
- **Concomitant Therapy: Standard long-course pelvic radiotherapy (50.4 Gy/28 fractions) plus concurrent capecitabine.**

**Safety Monitoring**

- **DLT Evaluation Window: Entire course of concurrent nCRT (6 weeks).**
- **DLT Criteria (adapted for this study):**
  - **Grade ≥3 non-hematologic toxicity (CTCAE v5.0), excluding expected alopecia and transient nausea/vomiting.**
  - **ALT or AST >3× ULN confirmed on repeat test, or ALT/AST >5× ULN at any time.**
  - **Total bilirubin >2× ULN with concurrent ALT/AST elevation.**
  - **Grade 4 hematologic toxicity lasting >7 days.**
  - **Any toxicity requiring treatment interruption >2 weeks.**
- **Monitoring Plan:**
  - **CBC, renal, and liver function tests weekly during nCRT.**
  - **Symptom assessment (diarrhea, proctitis, fatigue) using CTCAE v5.0 and RTOG scoring.**

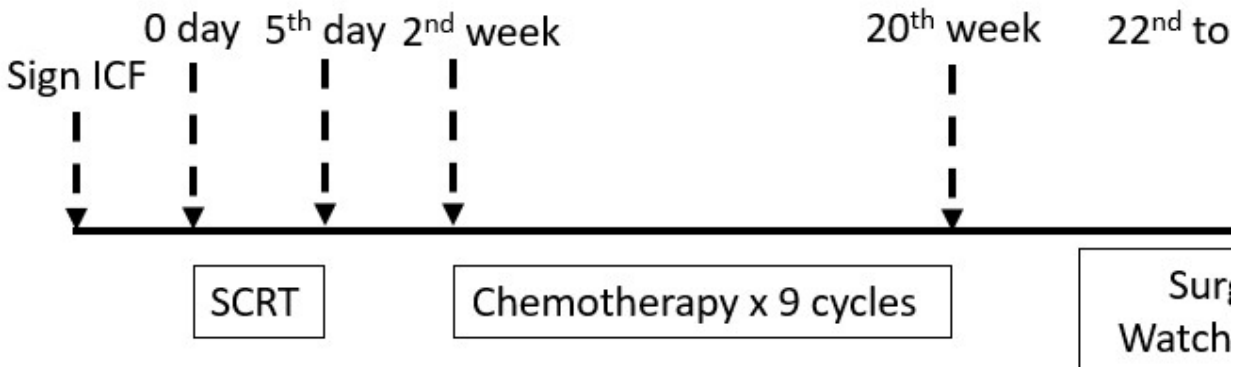
**Decision Rules**

- **If ≤1 of 6 patients develops a DLT, the study will proceed to Phase II enrollment (planned n=72).**
- **If ≥2 of 6 patients develop a DLT, the Principal Investigator (PI) and Co-Principal Investigator (Co-PI) will review safety data, and dose adjustment, protocol amendment, or early termination will be considered before further enrollment.**

**Transition to Full Cohort**

- **Upon the confirmation of safety and IRB notification, the trial will expand to the Phase II cohort.**
- **The same treatment regimen will be maintained unless other safety issues come into concern.**

## Flow Chart (試驗流程圖)：


Sign ICF
0 day
5th day
2nd week
20th week
22nd to
SCRT
Chemotherapy x 9 cycles
Sur
Watch

## 4.2 Number of Patients

Sample size calculation is performed using one proportion equality test using the exact test to achieving an 80% power at the 5% level of significance. With the expectation of an -15% of improvement of acute radiation proctitis (30% to 15%), the total estimated patients number in a single-arm trial required is 64. Consider the patient with incomplete data and drop-out rate of 10%, the total estimated number of sample size is 72. Sample size calculation was performed using PASS software (Power Analysis and Sample Size version 2021, NCSS, Kaysville, Utah, USA).

## 4.3 Schedule of Activities

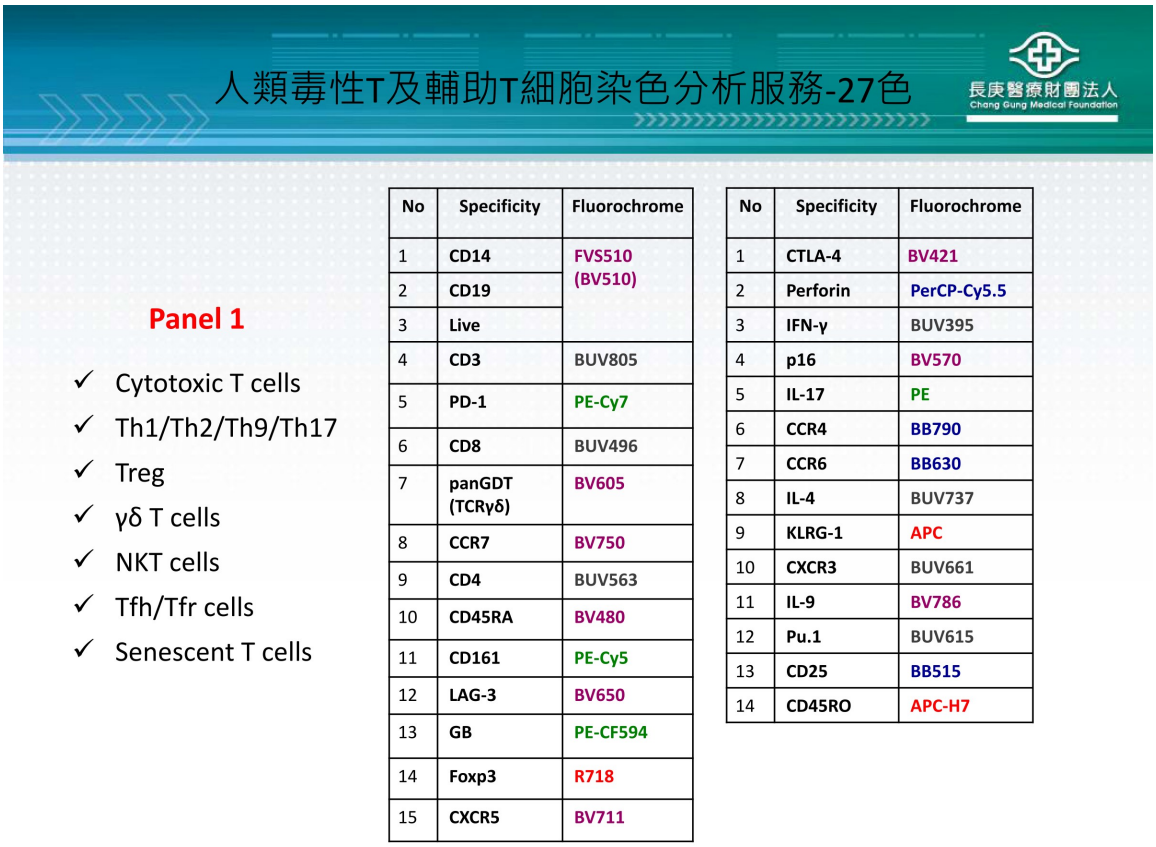
### Time-Event scheme(評估時程表):

| Phase | Screening | | Treatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Screening/Baseline | Short-course radiation therapy | Chemotherapy | | | | | | | | | Surgery watch-and wait |
| Week | 0 | 1 | 3 ± 1 | 5 ± 1 | 7±1 | 9 ± 1 | 11 ± 1 | 13 ± 1 | 15 ± 1 | 17 ± 1 | 19 ± 1 | 22-24 |
| Clinic or ward visit | X | X | X | X | X | X | X | X | X | X | X | X |
| Screening/Efficacy | | | | | | | | | | | | |
| ICF/ assent form | X | | | | | | | | | | | |
| Inclusion/Exclusion criteria | X | | | | | | | | | | | |
| Medical and surgical history | X | | | | | | | | | | | X |
| Physical exam (with digital exam) | X | | X | | | | X | | | | X | |
| Colonoscopy (Vienna Rectoscopy Score) | X | | X | | | | X | | | | X | |
| Colonoscopy biopsy (tumor and normal tissue) | X | | X | | | | X | | | | X | |
| Pelvis MRI (or transrectal ultrasound) | X | | | | | | | | | | X | |
| Computed tomography | X | | | | | | | | | | X | |
| Biopsy and tissue proof | X | | | | | | | | | | | |
| Tumor analysis | X | | X | | | | X | | | | X | X |
| Adverse event evaluation | | | X | X | X | X | X | X | X | X | X | |
| Peripheral blood analysis | X | | X | | X | | | X | | | X | |
| Complete blood count/ WBC differentiation | X | | X | X | X | X | X | X | X | X | X | |
| Serum carcinoembryonic antigen/ CA 19-9 | X | | | | X | | | X | | | X | |

## Immune profiling

### ➢ Peripheral blood

The presence of tumors is often linked to a significant reduction in both effector and memory T cell populations, a shift from type 1 to type 2 immune responses, and diminished effector T cell proliferation. Preclinical studies have shown that curcumin can counteract these immunosuppressive effects by preserving T cell populations, expanding central memory (TCM) and effector memory (TEM) T cell subsets, reversing the type 2 immune bias, and restoring T cell proliferation in tumor-bearing hosts. Curcumin also suppresses regulatory T cell (Treg) function by downregulating the secretion of immunosuppressive cytokines such as TGF-β and IL-10. Importantly, it enhances the cytotoxic activity of effector T cells against cancer cells. These findings highlight curcumin's potential as an immunomodulatory agent capable of mitigating tumor-induced suppression of cell-mediated immunity and augmenting antitumor immune responses [23]. To determine whether these findings are applicable to patients, we plan to use BD FACSymphony high-parameter flow cytometry to assess T effector cell function before and after combinatorial treatment. This will be done using a validated antibody panel available at Chang Gung Memorial Hospital (Table 1).




| No | Specificity | Fluorochrome |
|---|---|---|
| 1 | CD14 | FVS510 (BV510) |
| 2 | CD19 | |
| 3 | Live | |
| 4 | CD3 | BUV805 |
| 5 | PD-1 | PE-Cy7 |
| 6 | CD8 | BUV496 |
| 7 | panGDT (TCRγδ) | BV605 |
| 8 | CCR7 | BV750 |
| 9 | CD4 | BUV563 |
| 10 | CD45RA | BV480 |
| 11 | CD161 | PE-Cy5 |
| 12 | LAG-3 | BV650 |
| 13 | GB | PE-CF594 |
| 14 | Foxp3 | R718 |
| 15 | CXCR5 | BV711 |

| No | Specificity | Fluorochrome |
|---|---|---|
| 1 | CTLA-4 | BV421 |
| 2 | Perforin | PerCP-Cy5.5 |
| 3 | IFN-γ | BUV395 |
| 4 | p16 | BV570 |
| 5 | IL-17 | PE |
| 6 | CCR4 | BB790 |
| 7 | CCR6 | BB630 |
| 8 | IL-4 | BUV737 |
| 9 | KLRG-1 | APC |
| 10 | CXCR3 | BUV661 |
| 11 | IL-9 | BV786 |
| 12 | Pu.1 | BUV615 |
| 13 | CD25 | BB515 |
| 14 | CD45RO | APC-H7 |

**Table 1.** Tentative antibody panel for BD FACSymphony high-parameter immunophenotyping. (The antibody panel is subject to modification based on the experimental conditions.)

To evaluate whether curcumin can suppress pro-inflammatory cytokines during radiotherapy and chemotherapy, we plan to employ a cytokine array to comprehensively assess cytokine levels before and after total neoadjuvant treatment. Additionally, we aim to monitor circulating tumor DNA (ctDNA) dynamics—a promising non-invasive

biomarker—by measuring ctDNA levels pre- and post-treatment. This approach will allow us to explore potential correlations between ctDNA fluctuations and treatment response or clinical outcomes, supporting the use of liquid biopsy for real-time monitoring and assessment of therapeutic efficacy. As a control group, we will include blood samples from patients with chronic liver disease but without HCC, collected from the Human Tissue Bio-bank at Chang Gung Memorial Hospital.

*We will use the Milliplex cytokine array (Human Cytokine/Chemokine Magnetic Bead Panel IV; Catalog #HCYP4MAG-64K), which includes targets such as BAFF, Blys, BRAK, CXCL14, CCL28, CXCL16, HCC-4, CCL16, HMGB1, IFNβ, IL-14, IL-19, IL-24, IL-28B, IL-32α, IL-34, IL-35, IL-36β, IL-1F8, IL-37, IL-1F7, IL-38, IL-1F10, MIP-4, MPIF-1, CCL23, and YKL40.
(Note: The panel may be adjusted depending on experimental needs.)

- **Tumor biopsy**

To investigate whether curcumin enhances the antitumor efficacy and mitigates normal tissue toxicity of radiotherapy and chemotherapy, we will employ multiplex immunohistochemistry to perform high-dimensional spatial profiling of both tumor and adjacent normal colon mucosal tissues from colorectal cancer (CRC) patients. This cutting-edge multiplex imaging technique allows for simultaneous detection of dozens of protein markers on formalin-fixed paraffin-embedded (FFPE) samples, preserving spatial context and cellular interactions within the tumor microenvironment [24].

Using multiplex immunohistochemistry, we will comprehensively characterize immune cell infiltration patterns, including CD4+ and CD8+ T cells, regulatory T cells (Tregs), macrophages (M1 and M2 phenotypes), and dendritic cells, as well as assess expression levels of immune checkpoint molecules such as PD-1, PD-L1, CTLA-4, and CD47 on both immune and tumor cells. We will also evaluate stromal components, cytokine-associated markers, and markers of tissue damage or repair to assess treatment-induced changes in both tumor and normal mucosa. By comparing immune cell composition, activation states, and checkpoint expression in tissues from curcumin-treated versus untreated cohorts, we aim to delineate how curcumin modulates the immunological landscape of CRC. This will help determine whether curcumin not only amplifies local and systemic antitumor immunity but also confers a protective effect against treatment-induced inflammation and injury in adjacent normal tissues [25].

# 5. Study Population

## 5.1 Inclusion Criteria

***Teeatment group***
*Inclusion criteria:*

(A) Males and females more than 20 years of age

(B) Signed informed consent

(C) Patients with a pathologically proven rectal adenocarcinoma located less than 10 cm to the anus.

(D) Clinical staging (AJCC 8th ed.): T2-4 N0 M0 or T any N1-2 M0

(E) Distal metastasis has been excluded by imaging study: by chest-to-pelvic computed tomography or Positron Emission Tomography

(F) Preoperative pelvic staging by pelvic Magnetic Resonance Imaging (preferred) or trans-rectal ultrasound

(G) Patients with WHO/ECOG performance scale 0 or 1

## 5.2 Exclusion Criteria

***Treatment group***

*Exclusion criteria:*

(A) Refuse to sign the informed consent

(B) Distal metastasis revealed by the imaging study

(C) Patients does not receive radiotherapy

(D) Unable to receive further curative resection

(E) Patients receive tumor resection before the neoadjuvant treatment

(F) Patients have history of more than 5 Gy of pelvic radiation

(G) Patients in pregnancy or lactation status

(H) Patients have allergic history to curcumin, 5-fluouracil or oxaliplatin

(I) Patients of childbearing potential can not cooperate with appropriate contraceptive method (oral, injectable, or implantable hormonal contraceptive; tubal ligation; intra-uterine device; barrier contraceptive with spermicide; or vasectomized partner)

(J) Patients with any concurrent malignancy; patients with history of malignancy should be cancer-free for more than 5 years

(K) Patients with New York Heart Association (NYHA) class III or IV heart failure, unstable angina pectoris, unstable cardiac arrhythmia or tachycardia (heart rate > 100 beats/minute)

(L) Patients have concurrent uncontrolled medical conditions, such as illness ongoing or requiring IV antibiotics, severe chronic renal failure (eGFR <30 mL/min/1.73m$^2$) or severe active hepatitis(AST/ALT>3x upper normal limit)、Total Bilirubin>2 mg/dl…etc.

(M) Patients with previous or current drug abuse

(N) Patients underwent major surgery within 28 days of study enrollment (except diverting colostomy)

(O) Patients have Familial Adenomatosis Polyposis Coli (FAP), Hereditary Non-Polyposis Colorectal Cancer (HNPCC), active Crohn's disease or active ulcerative Colitis

(P) Patients have known dipyrimidine dehydrogenase deficiency (DPD)

(Q) Patients with congenital iron metabolic or hematopoietic diseases

(R) Patients with synchronous colon cancer

**(S) The Patients with hematologic abnormalities (INR > 1.5, white blood cell (WBC) count < 3,000/μL, absolute neutrophil count (ANC) < 1,500/μL, platelet count <**

**100,000/μL, hemoglobin < 9.0 g/dL not caused by tumor treatment) or known hematologic diseases (aplastic anemia, myelodysplastic syndrome (MDS), leukemia, malignant lymphoma, multiple myeloma, hereditary hematologic diseases such as thalassemia, sickle cell anemia, etc.).**

(T) The patient has diabetes mellitus

**(U) The patient is taking the immunosuppressants Cyclosporine, Tacrolimus, Sirolimus, and Everolimus and Everolimus and antigoagulants (warfarin、NOACs、aspirin)**

(V) Patients with The medical, psychological, or social condition that, in the opinion of the investigator, may increase the patient's risk or limit the patient's adherence with study requirements

## 5.3 Withdrawal criteria

Subject should be withdrawn from the study due to any of the following conditions:
1. The subject voluntarily decides to withdraw her/his consent
2. The subject dies or is lost to follow-up
3. The subject is pregnancy
4. The subject develops disease progression (metastasis or enlargement confirmed)
5. Any clinical adverse event, laboratory abnormality, intercurrent illness, or any occurrence or change in the subject's physically and/or psychologically status giving indication to the investigator that further participation in the study may not be the best interest of the subject
6. The subject does not receive, or is unable to receive, the study intervention, does not keep appointments, or otherwise does not adhere to protocol requirements
7. The subject was enrolled by error and/or it is discovered that the subject does not meet the eligibility criteria after enrollment
8. The study is terminated prematurely by the investigator, research institution, sponsor, IRB or regulatory authorities

For a subject who is withdrawn due to progress confirmed, CT (or MRI) and colonoscopy could be waived at the Early Termination Visit per the investigator's judgments. If a subject is discontinued due to an AE, every effort will be made to follow the event until event resolves or stabilizes at a level acceptable to the investigator.

# 6. Treatments

## 6.1.Treatment Administration

A daily dose or high bioavailability curcumin (BCM-95 curcumin) 3g per day is given orally along with the neoadjuvant short-course radiation therapy and chemotherapy.

## 6.2. Concomitant Therapy

**1.** Concomitant Therapy：

1.1 TEGAFOX(one cycle)：

*Oxaliplatin (own expense) 85 mg/m2 in D5W 250 ml IVF 2 hrs, D1, every 2 wks
*Tegafur-uracil 300mg/m2/day and leucovorin 90mg/day, given on days 1 to 5 and 8 to 12 days, every 2 weeks

1.2 FOLFOX(one cycle)：

*Oxaliplatin (own expense) 85 mg/m2 in D5W 250 ml IVF 2 hours, D1
*Calcium Folinate 100 - 400 mg/m2 in D5W 250ml IVF 2 hours, D1
*5-FU 400 mg/m2 IV bolus on D1 *5-FU (2400 mg/m2) IVF 46 hrs, Repeat every 2 weeks
1.3 UFUR (one cycle)
*Tegafur 300-350 mg/$m^2$/day and leucovorin 90mg/day PO on day 1 to day 5, then rest for 2 days for 2 weeks

1.4 The radiation therapy modules are as follows：

Short-Course (hypofractionated, 25 Gy/5 fractions) Radiotherapy (SCRT)

**2.** Prohibited Therapy：cell therapy, immunotherapy

# 7. Efficacy Assessments

1. **Grade 3 enteritis/proctitis**: evaluated by National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (NCI CTCAE v. 5.0) and the criteria of the Radiation Therapy Oncology Group (RTOG) and European Organization for Research and Treatment of Cancer (EORTC). The sign or symptoms of radiation proctitis (diarrhea, rectal bleeding, rectal pain) is evaluated and recorded by our research nurse 2 to 4 weeks after radiation therapy completed. Colonoscopy evaluation for Vienna Rectoscopy Score will take place 2 to 3 weeks after the radiation therapy is completed.

2. Any grade III adverse events during the neoadjuvant therapy: evaluated by clinicans according to National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (NCI CTCAE v. 5.0) every cycles of chemotherapy. An additional evaluation and record will be recorded by the research nurse every 3 cycles of chemotherapy.

3. Clinical complete response rate of the primary tumor after completion of the neoadjuvant treatment: evaluated by Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST v1.1) after neoadjuvant chemotherapy and radiation therapy completed

4. Pathological or clinical complete response rate of the primary tumor after completion of the neoadjuvant treatment: defined as no residual cancer cells in the resected specimen after curative resection

5. Patient's tolerability and completion rate of the neoadjuvant therapy

# 8. Safety Assessments

The complete blood count is evaluated on each admission for chemotherapy. The adverse events is evaluated according to National Cancer Institute Common Terminology Criteria for Adverse Events Version 5.0 (NCI CTCAE v. 5.0). Serum creatinine, electrolyte, AST, ALT, total and direct bilirubin is examined on each 3 cycle of chemotherapy.
**If ALT or AST exceeds three times the upper limit of normal (3×ULN), curcumin administration will be suspended. This 3×ULN threshold is a commonly used warning indicator for drug-induced liver injury. In such cases, we will monitor liver function tests weekly until the values recover to <1.5×ULN or return to baseline.**

# 9. Adverse event reporting

*Dr. Shu-Huan Huang will report SAEs to the IRB of Chang Gung Medical Foundation according to the Serious Adverse Event Reporting Procedures and Guidelines as posted in the Clinical Trials Resource on the website of Chang Gung Medical Foundation IRB. SAE reports to the IRB should include the following information when calling the Medical Monitor:*

- *Date and time of the SAE*
- *Date and time of the SAE report*
- *Name of reporter*
- *Call back phone number*
- *Affiliation/Institution conducting the study*
- *Protocol number*
- *Title of protocol*
- *Description of the SAE, including attribution to drug and expectedness*

## 9.1 Definitions and reports of Adverse Events

*All adverse events that occur after the informed consent is signed (including run-in) must be recorded on the adverse event CRF (paper and/or electronic) whether or not related to study agent. AE Data Elements including:*

- *AE reported date*
- *AE Verbatim Term*
- *CTCAE Term (v 5.0)*
- *Event onset date and event ended date*
- *Severity grade*
- *Attribution to study agent (relatedness)*
- *Whether or not the event was reported as a Serious Adverse Event (SAE)*
- *Action taken with the study agent*
- *Outcome of the event*
- *Comments*

*Identify the adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed.*

*AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to their impact on the participant's ability to perform daily activities as follows:*

| ***Grade*** | ***Severity*** | ***Description*** |
|---|---|---|
| *1* | *Mild* | • *Barely noticeable, does not influence functioning*<br>• *Causing no limitations of usual activities* |
| *2* | *Moderate* | • *Makes participant uncomfortable, influences functioning*<br>• *Causing some limitations of usual activities* |
| *3* | *Severe* | • *Severe discomfort, treatment needed*<br>• *Severe and undesirable, causing inability to carry out usual activities* |
| *4* | *Life threatening* | • *Immediate risk of death*<br>• *Life threatening or disabling* |

| | | |
|---|---|---|
| *5* | *Fatal* | • *Causes death of the participant* |

*The possibility that the adverse event is related to study drug will be classified as one of the following: not related, unlikely, possible, probable, definite.*

***DEFINITION of Serious Adverse Events**: ICH Guideline E2A and GCP of Taiwan define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:*

- *Results in death*
- *Is life threatening (Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).*
- *Requires inpatient hospitalization or prolongation of existing hospitalization*
- *Results in persistent or significant disability/incapacity*
- *Is a congenital abnormality/birth defect*
- *Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner.*

## 9.2 Adverse event follow-up

*All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such. Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the IRB of Chang Gung Medical Foundation of SAE form in the appropriate format. Follow-up information should be sent to Chang Gung Medical Foundation IRB as soon as possible according to IRB's Serious Adverse Event Reporting Procedures and Guidelines.*

# 10. Criteria for the termination of the trial

1. The drug's lack of efficacy is proven early for failure to obtain the expected benefit. Treatment does not lead to an equal or better result than the treatment against which is compared.
2. Be shown prematurely that the drug is harmful, resulting in a risk for patients enrolled in the trial.
3. Logistics issues for being unable to recruit the expected number of subjects. It is important to publish this type of trial indicating problems observed and the causes leading to early termination since they can help other investigators avoid the same problems and repeating the same mistakes.

# 11. Statistical Considerations

Quantitative variables will be presented as mean±standard deviation (SD) and categorical variables will be presented as proportions. The Student t-test, Mann-Whitney U-test and Spearman rank correlation test will be used to analyze differences between groups of variables.

## 11.1 Sample size Determination

Sample size calculation is performed using one proportion equality test using the exact test to achieving an 80% power at the 5% level of significance. With the expectation of an -15%

of improvement of acute radiation proctitis (30% to 15%), the total estimated patients number in a single-arm trial required is 64. Consider the patient with incomplete data and drop-out rate of 10%, the total estimated number of sample size is 72. Sample size calculation was performed using PASS software (Power Analysis and Sample Size version 2021, NCSS, Kaysville, Utah, USA).

## 11.2 Planned Statistical methods of analysis

Categorical variables were compared using Pearson's chi-squared test or Fisher's exact test, while continuous variables were compared using the Mann–Whitey U test. Survival curves were generated using the Kaplan–Meier method and compared between the two groups using the log-rank test. The index date of survival analysis was the day the patient started radiotherapy. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS), version 24 (IBM Corp., New York, NY, USA). Differences were considered statistically significant at a two-sided p-value of $< 0.05$.

### 11.2.1 Efficacy analysis

Adverse events are assessed by the clinician on each cycle of admission, and additional record of adverse events (CTCAE version 5) by our research nurse on every 3 cycles of chemotherapy.
Clinical complete remission is evaluated on each colonoscopy examination after the neoadjuvant chemotherapy and radiation therapy completed according to Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST v1.1). In addition, the pathologist evaluates pathological complete response and tumor regression grade after curative resection.

### 11.2.2 Safety analysis

The adverse events and complete blood count is evaluated on each admission for chemotherapy. Serum creatinine, electrolyte, AST, ALT, total and direct bilirubin is examined on each 3 cycle of chemotherapy. Adverse events are assessed by the clinician on each cycle of admission, and additional evaluation of adverse events (CTCAE version 5) by our research nurse on every 3 cycles of chemotherapy, which is the standard follow-up scheduled of the chemotherapy.

### 11.2.3 Additional analysis

Not applicable

### 11.2.4 The level of significance

A p-value less than 0.05 was considered significant and was denoted by *.

### 11.2.5 Analysis Population

The outcome of this single arm clinical trial is compared with other clinical trials and our previous retrospective neoadjuvant chemoradiation data of CGMH.

#### 11.2.5.1 Procedure for accounting for missing, unused and spurious data

The incomplete clinical data and sequencing data with poor quality will be excluded in the study.

### 11.2.5.2 Procedures for reporting any deviation(s) from the original statistical plan

Investigators permit IRB to access to the raw data of experiment for trial-related monitoring, audits and regulatory inspection if additional statistical plan is required.

# 12. Direct access to source data/documents

Investigators permit IRB to access to the source data of experiment for trial-related monitoring, audits and regulatory inspection.

# 13. Ethical considerations

This study will be conducted according to Taiwan and international standards of Good Clinical Practice for all studies. Applicable government regulations and Chang Gung Medical Foundation research policies and procedures will also be followed.

This protocol and any amendments will be submitted to the Chang Gung Medical Foundation Institutional Review Board (IRB) for formal approval to conduct the study. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

# 14. Data handling and keeping

Clinical samples will be collected in Chang Gung Medical Foundation. The sequencing data will be stored in computers of laboratory with an electronic encryption. The clinical and source data can only be assessed by clinical doctors and investigators of the study.

**Data and Safety Monitoring Plan (DSMP)**

This section outlines the Data and Safety Monitoring Plan (DSMP) for the clinical study. The purpose of the DSMP is to ensure the safety of study participants and the integrity of the study data. The monitoring activities will be performed regularly and will be adapted based on study progress and emerging safety signals.

**1. Monitoring Plan**

- Enrollment monitoring will be conducted tri-monthly by the Principal Investigator (PI).
- Serious Adverse Events (SAEs) will be reported immediately and reviewed quarterly by the Safety Officer.
- Protocol deviations will be recorded and reviewed monthly by the Clinical Research Coordinator (CRC).
- Data quality will be assessed on an ongoing basis by the Data Manager.

**2. Reporting**

All safety monitoring activities and findings will be reported to the Sponsor and PI.

# 15. Financing and Insurance

This study is supported by Chang Gung Medical Foundation (CGMF). The main research fund is aimed to be supported by Chang Gung Medical Foundation.

# 16. Privacy and Confidentiality Protection

A research code will be used to represent the patient's identity, and this code will not display the patient's name, ID number, or address. The principal investigator will maintain a strict confidentiality stance regarding the results and diagnoses of the patient's visit. If research findings are published, the patient's identity will still be kept confidential.

By signing the consent form, the patient is agree that his or her visit records may be directly reviewed by monitors, auditors, research ethics committees, and regulatory authorities to ensure that the research process and data comply with relevant laws and regulations. The individuals mentioned above also pledge never to violate the confidentiality of the patient's identity.

According to the regulations of the United States or the European Union for pharmaceutical management, trial results will be published on the public clinical trial information website: Clinicaltrials.gov (for the United States). However, the patient's personal information will remain confidential, and the website will only have a summary of the trial results.

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