Project Name

Observation of MRD Detection for General Risk Stage II Colorectal Cancer of preoperative sensitivity and postoperative positive rate

NCT ID not yet assigned

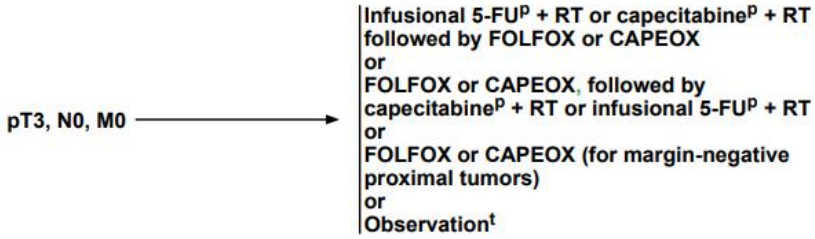
Date: February 25, 2023

1. Project approval basis and research status

Colorectal cancer is the third malignant tumor with the highest incidence rate in the world, and its mortality rate is the second [1]. The incidence rate and mortality of colorectal cancer in China rank the third and fifth in all malignant tumors, respectively, and the incidence rate has shown a significant upward trend in recent years [2].

The adjuvant treatment for colorectal cancer after surgery is currently based on postoperative pathological staging. According to the "NCCN Guidelines for Rectal Cancer 2022. V1", for rectal cancer with T3N0M0, there is also a choice between postoperative chemotherapy and observation. Similarly, the requirements for "follow-up observation" are: highly differentiated or moderately differentiated, involving<2mm of the mesorectum, without lymphatic or vascular invasion, located in the upper rectum. In China's "CSCO Guidelines for the Diagnosis and Treatment of Colorectal Cancer (2022)", for patients with pMMR (90% of colorectal cancer are pMMR patients), if they are of general risk type, adjuvant chemotherapy can be performed after surgery, and follow-up observation can also be considered. High risk factors include: T4 stage, poor histological differentiation (poorly differentiated or undifferentiated), vascular infiltration, neural infiltration, preoperative intestinal obstruction or tumor site perforation, positive or unknown surgical margin, insufficient safety distance from the surgical margin, and

less than 12 lymph nodes submitted for examination. In other words, when the patient is a colorectal cancer T3N0M0 patient without high risk factors, doctors have difficulty selecting adjuvant treatment after surgery.



Figer 1 excerpted from page REC-4 of the "NCCN Guidelines for Rectal Cancer 2022. V1"

Although the article published in the New England Journal in June 2022 has already been applied to Phase II colorectal cancer with Signature@ MRD detection product, and has proven its role in guiding postoperative chemotherapy[3]. However, there are still two problems: 1. There is no independent distinction between the common risk types of stage II colorectal cancer, and there is no answer to the question of the postoperative positive rate of MRD in patients with stage II colorectal cancer common risk types; 2. There are no similar studies in the Asian population.

Therefore, we designed an observational study to observe the sensitivity and postoperative positive rate of this method in patients with general risk stage II colorectal cancer, with the aim of answering the positive rate of MDR testing in patients with general risk stage II

colorectal cancer after surgery, and laying a foundation for exploring the guidance of this method for postoperative adjuvant treatment.

Reference:

[1] Bray, F., et al. (2018). "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries." CA Cancer J Clin 68(6): 394-424.
[2] Deng, Y. (2017). "Rectal Cancer in Asian vs. Western Countries: Why the Variation in Incidence?" Curr Treat Options Oncol 18(10): 64.
[3] Jeanne Tie, et al. “Circulating Tumor DNA Analysis Guiding Adjuvant Therapy in Stage II Colon Cancer.” N Engl J Med, 2022 Jun 16;386(24):2261-2272.

2. Objects and contents

2.1 Objective

To observe the sensitivity of Huajian micro MRD detection in preoperative diagnosis of patients with general risk stage II colorectal cancer, and to study its positive rates at one and three months after surgery.

2.2 Contents

Preoperative venous blood and intraoperative tumor tissue samples were reserved for patients with colorectal cancer who were estimated as stage II general risk through pelvic magnetic resonance imaging. Patients with colorectal cancer who were confirmed as stage II general risk type by postoperative pathological reports began to be enrolled. To observe the sensitivity of preoperative Huajian micro MRD detection and the postoperative positive rate of this type of patients.

2.3 Research steps

1) Patients who meet the inclusion and exclusion criteria for preoperative examinations, especially pelvic magnetic resonance imaging, are enrolled.

2) Informed consent signed by patients or family members before surgery.

3) Before surgery, 20 ml of venous blood was collected, and the dedicated anticoagulant tube was provided and stored by BGI Gene

manufacturer.

4) During the operation, tissue samples, about the size of soybeans, were kept in vitro for 30 minutes, and were routinely fixed with formalin before being handed over to BGI Gene manufacturer.

5)Due to the storage by the manufacturer, the samples shall be disposed according to the sample destruction procedure after testing.

6) After the postoperative pathological report is released, the patient is evaluated as a general risk type II colorectal cancer patient, and the BGI Gene manufacturer is notified for testing.

7) Complete exon sequencing and preoperative MRD detection.

8) Peripheral blood MRD testing was performed one month and three months after surgery under the same conditions as 5).

2.4 Evaluation indicators

Key point:

Sensitivity of preoperative MRD detection (sensitivity=number of truly positive patients/pathologically diagnosed as general risk stage II colorectal cancer)

Secondary points:

Positive rate of MRD testing at 1 month after surgery

Positive rate of MRD testing at 3 months after surgery

DFS of 2-years in patients with two MRD outcomes

2.5 Follow up

1) Implement a unified follow-up plan: All patients will be followed up every 3 months for the first 2 years after surgery; Observation end point is PFS of 2 years. Follow up every six months from the second to fifth year after surgery; Thereafter, a follow-up visit will be conducted annually. The follow-up includes: Finger rectal examination, blood routine, biochemical routine, tumor markers, pelvic MRI, chest and abdominal CT, endoscopy, etc.

2) Follow up time nodes: 3, 6, 9, 12, 18, and 24 months after surgery.

3) Outside the time node for the return visit, a dedicated person will conduct the follow-up by phone, letter questionnaire, email, etc

Sample size estimation

2.6 Sample number estimation

According to existing literature, the positive rate of MRD detection is 85%, and the expected sensitivity is 80% $\alpha = 0.05$， $1-\beta = 0.90$, the statistical difference can be obtained by calculating 48 cases. 50 cases were planned to be enrolled.

3. Inclusion and exclusion criteria

3.1 Inclusion criteria

- Age 18-75;
- Pathologically confirmed rectal adenocarcinoma;
- Pathological differentiation is highly or moderately differentiated;
- The pathological stage was pT3N0, that is, invasion of the proper muscular layer of the intestinal wall without lymph node metastasis;
- No new adjuvant treatment before operation
- There was no vascular infiltration and nerve infiltration in postoperative pathology;
- No preoperative intestinal obstruction or tumor site perforation;
- No postoperative pathological margin was positive or unknown;
- The distance between the pathological cutting edge and the tumor was more than 1 cm;
- Invasion of mesentery of intestinal wall ≤ 2mm;
- More than 12 lymph nodes were submitted for examination;
- Nonlocal recurrence and distant metastasis;
- No multiple primary carcinoma of colon and rectum;
- Physical condition score PS ≤ 2 points;
- Patients and their families can understand and are willing to participate in this study and provide written informed consent.

3.2 Exclusion criteria

- Multiple intestinal carcinomatosis
- Previous history of malignant tumor,
- There are concurrent malignant tumors in the whole body except for colorectal cancer
- Colorectal cancer with preoperative anti-tumor treatment
- Pregnant or lactating women
- There are serious complications during or after operation, affecting the prognosis
- Hepatitis B or Hepatitis C antibody positive
- HIV antibody positive
- Other diseases considered by the research doctor to affect the prognosis and survival
- Other conditions that the research doctor believes are not consistent with this study.

3.3 Termination criteria

Termination of study treatment does not represent withdrawal from the study. Subjects who terminate the study must continue to complete the remaining tasks according to the protocol requirements

Research visits. The study treatment must be terminated if the subject meets any of the following criteria:

- Subjects voluntarily withdraw from the study, terminate testing, or withdraw their informed consent;

- Subjects have poor compliance and cannot be tested on time;
- The subject is missing a visit or has a pregnancy event;
- Other situations where the researcher believes it is necessary to end the research and testing.