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Evaluation of tyrosine kinase inhibitors combined with antiprogrammed cell death protein 1 antibody in tyrosine kinase inhibitor-responsive patients with microsatellite stable/proficient mismatch repair metastatic colorectal adenocarcinoma: protocol for open-label, single-arm trial

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ABSTRACT

Introduction The prognosis of patients with advanced metastatic colorectal adenocarcinoma (mCRC) after multiple-line therapy remains poor due to the high tumour load, high level of malignancy and strong drug resistance. The application of programmed cell death protein 1 (PD-1) blockade alone for patients with microsatellite stable/proficient mismatch repair (MSS/pMMR) mCRC is ineffective. PD-1 blockade combined with antiangiogenic therapy has synergistic effects and has initially shown therapeutic effects. The aim of this trial is to explore the efficiency and safety of tyrosine kinase inhibitors (TKIs) combined with PD-1 blockade therapy in patients with mCRC with MSS/pMMR.

Methods and analysis The screening phase of the trial will involve administering one cycle of TKIs (fruquintinib or regorafenib). Patients will be divided into three arms—arm A (obvious response to TKIs), arm B (general response to TKIs) and arm C (poor response to TKIs)—according to their response to TKIs, as determined by significant changes in imaging findings. Patients in arm A will then receive TKIs in combination with anti-PD-1 antibody, patients in arm C will withdraw from the study, and those in arm B will continue to take TKIs for another one further cycle. Next, patients with obvious response to TKIs will be reallocated to arm A, those with general response to TKIs will stay in arm B and will continue to take TKIs, and patients with poor response to TKIs will withdraw from the study. Administration of arm A or arm B will last until disease progression or intolerable toxicity. Anti-PD-1 antibody can be administered for up to 2 years. This trial will provide necessary data to improve the prognosis of patients with MSS/pMMR mCRC.

Trial registration number NCT04483219; Pre-results.

Strengths and limitations of this study

- This is the first study to try to evaluate the efficacy and safety of tyrosine kinase inhibitors (TKIs) in combination with antiprogrammed cell death protein 1 (anti-PD-1) antibody in TKI-responsive patients with microsatellite stable/proficient mismatch repair (MSS/pMMR) metastatic colorectal adenocarcinoma (mCRC).
- The results will provide valuable data for precision immunotherapy in patients with MSS/pMMR mCRC.
- The novelty of the present study lies in the screening phase of the study design, which could help screen patients who could benefit more from PD-1 blockade combined with antiangiogenic therapy and could also provide a safe dose for the following combined therapy.
- The initial screening of the study is based on response to TKIs, which will be determined based on imaging data (eg, tumour shrinkage, appearance of cavity or reduction in density).
- The study setting, criteria, inclusion, interventions and outcomes are based on a pragmatic approach to ensure external validity.
- Study limitations include its single-arm design, which has no comparator, making it hard to assess internal validity.

INTRODUCTION

Metastatic colorectal cancer (mCRC) is one of the main causes of cancer-related deaths worldwide.[1] Approximately 40% of patients with mCRC are diagnosed at an advanced stage, which could only receive palliative treatment.[2 3] Chemotherapy combined with targeted therapy has significantly improved the prognosis of patients with mCRC.[4–10] However, the prognosis of patients with mCRC after multiple-line therapeutic strategies

remains poor due to the high tumour load, high level of malignancy and strong drug resistance. At present, irinotecan combined with cetuximab, regorafenib, fruquintinib or trifluridine, and tipiracil hydrochloride tablets (TAS-102) are the regimens for palliative therapy after second-line treatment.[11–15] However, overall therapeutic efficacy remains unsatisfactory (the progression-free survival (PFS) is about 4 months and the overall survival (OS) fluctuates between 6 and 9 months).[11–15] Thus, exploring palliative therapies after second-line treatment is urgently required.

Over the recent years, immunotherapy has provided a new opportunity to treat solid tumours.[16 17] To date, studies have mainly concentrated on assessing the efficacy of combined therapies and the superiority of screening of population. It has been discovered that approximately 90% of patients with mCRC have microsatellite stable/proficient mismatch repair (MSS/pMMR) tumours. Yet the results of early trials on programmed cell death protein 1 (PD-1) blockade for patients with MSS/pMMR mCRC remain unsatisfactory, highlighting an urgent need for strategies to enhance the immune response to cancer therapy.[18–21]

PD-1 blockade combined with antiangiogenic therapy has shown to be effective against some types of cancer (eg, hepatocellular carcinoma).[22 23] Antiangiogenic drugs can have an important role in transforming the microenvironment. They can promote the normalisation of tumour blood vessels, which can enhance tissue perfusion and infiltration of immune cells to the tumour, thereby enhancing the effects of immunotherapy. Moreover, the activation/reprogramming of immune cells can influence tumour blood vessels.[20] Tumour vascular normalisation and immune reprogramming can improve the tumour microenvironment via a benign cycle of mutual enhancement.[24] Therefore, combining immunotherapy with antiangiogenesis is supposed to have a synergistic effect in converting the tumour immune microenvironment from an immunosuppressive status to a state of immune promotion.

Fruquintinib and regorafenib are multitargeted tyrosine kinase inhibitors (TKIs) mainly applied for antiangiogenesis. They can induce tumour necrosis, release several new antigens, improve the immunosuppressive microenvironment and induce tumour vascular normalisation. In addition to killing tumour cells, fruquintinib and regorafenib can also convert the immune-suppressive properties of the tumour microenvironment, which can sensitise PD-1 blockade, ultimately improving the prognosis of patients with MSS/pMMR mCRC.[25 26] A phase Ib trial from Japan (REGONIVO) assessed the safety and efficacy of regorafenib combined with nivolumab in the treatment of mCRC, obtaining an objective response rate (ORR) of 33% for MSS mCRC.[22] Another study examined 52 cases of patients with mCRC who were treated with sintilimab combined with fruquintinib and reported an ORR of 15.38%, a disease control rate (DCR) of 57.6% and a median PFS of 108 days.[27] At our centre, we attempted to use regorafenib or fruquintinib combined with anti-PD-1 antibody to treat patients with MSS/pMMR mCRC who did not respond well to standard treatment. Among five cases, four had a stable disease (SD) and the DCR was 80%. In one of the cases, efficiency was maintained for more than 12 months. Also, remarkable changes, including tumour shrinkage, massive cavitation of solid pulmonary metastatic lesions and decrease in the density of liver metastatic targeted lesions, were recorded during treatment in four patients. These data imply that TKI in combination with anti-PD-1 antibody may have a potential antitumour effect in patients with MSS/pMMR mCRC, with significant changes in imaging findings. However, before starting treatment, it is essential to optimise the administration mode of combined therapies and indicate which group of patients can obtain the most significant clinical benefit of combined therapies. We hypothesised that patients with effective initial treatment of TKIs could obtain the highest clinical significance of subsequently combined therapies due to the following characteristics: (1) the antiangiogenic effects of TKI: TKI favours the induction of cancer cell death inside the tumour, which in turn reduces tumour burden and enhances immune response to tumour-associated antigens, and during this time imaging changes are visible (tumour shrinkage, appearance of cavity or reduction in density); and (2) TKI induces tumour vessel normalisation, causing more tumour-infiltrating lymphocytes to infiltrate the tumour microenvironment. Consequently, TKI combined with PD-1 blockade enhances antitumour effects on residual tumour cells, reducing the risk of tumour progression. Therefore, we hypothesised that patients with effective initial treatment of TKIs can benefit the highest clinical significance of subsequent combined therapies. We also attempted to evaluate the efficacy of initial TKI treatment by imaging examinations. Tumour shrinkage is the most significant outcome of TKI treatment. Angiogenesis inhibitors can inhibit tumour angiogenesis and shrink immature blood vessels, which prevents nutrition and oxygen from reaching the tumour tissue, affecting tumour metabolism and leading to tissue necrosis.[28 29] This process, that is, the corresponding changes in perfusion parameters and metabolic status, such as the appearance of the cavity or reduction in density, can be observed on imaging.[30]

Both fruquintinib and regorafenib are regimens for palliative therapy after second-line treatment in patients with mCRC in China. The recommended dose of fruquintinib is 5 mg once a day for 3 consecutive weeks, followed by a 7-day pause. In the initial exploratory phase of the REGONIVO trial, enrolled patients received regorafenib plus nivolumab in a dose-dependent manner to estimate the maximum tolerated dose. Additional patients were enrolled in a dose-expansion manner. Besides, 80–160 mg regorafenib were administered once a day for 21 days, followed by a 7-day pause, with 3 mg/kg nivolumab every 2 weeks.[22] The combination of 80–120 mg regorafenib plus nivolumab showed a manageable safety profile and

encouraged antitumour activity in patients with mCRC. In China, toripalimab (anti-PD-1 antibody) received its first approval for second-line treatment of metastatic melanoma on 17 December 2018 and was commercially launched in February 2019. The recommended dose is a fixed dose of 240 mg once every 3 weeks by intravenous injection. In the clinical study of combined therapy, 240 mg toripalimab showed controllable safety.

Herein, we designed a prospective, single-arm, multicentre, phase II clinical trial to evaluate the efficacy and safety of TKIs (fruquintinib or regorafenib) in combination with anti-PD-1 antibody in TKI-responsive patients with MSS/pMMR mCRC.

METHODS AND ANALYSIS

Objectives

The objectives of the present study are the following: (1) to evaluate the efficacy and safety of TKIs in combination with anti-PD-1 antibody in patients with MSS/pMMR mCRC experiencing disease progression or intolerable toxicity after standard treatment; and (2) to explore biomarkers that can predict therapeutic efficacy and prognosis.

Study design

The screening phase of the trial will involve administering one cycle of TKIs (fruquintinib or regorafenib). According to their response to TKIs, patients will be divided into three arms: (1) arm A: an obvious response to TKIs, including reduction in the diameter of target lesions, to complete response (CR), partial response (PR) or shrunken SD, according to the revised Response Evaluation Criteria in Solid Tumors (RECIST) guidelines (version 1.1), cavitation of solid metastatic lung lesions or decrease in the density of metastatic liver lesions ≥15% (based on Choi *et al*'s criteria[31]); (2) arm B: general response to TKIs, including enlarged SD (based on RECIST version 1.1 guidelines); (3) arm C: poor response to TKIs, including progressive disease (based on RECIST version 1.1 guidelines). TKIs in combination with an anti-PD-1 antibody are administered in arm A. Study patients in arm C may withdraw from the trial, while those in arm B may continue to receive TKIs for another one further cycle. Next, patients with obvious response to TKIs will be reallocated to arm A, those with general response to TKIs will stay in arm B and will continue to take TKIs, and patients with poor response to TKIs will withdraw from the study. Administration of TKIs in arm A or arm B will last until disease progression or intolerable toxicity. Anti-PD-1 antibody can be administered for up to 2 years. The study design is shown in figure 1.

The following are the regimens to be used in this trial: patients may be given fruquintinib/regorafenib and toripalimab. Fruquintinib (5 mg/day) is administered orally for 21 days, followed by a 7-day pause; regorafenib (120 mg/day) is administered orally for 21 days, followed by a 7-day pause; and toripalimab (240 mg) is given once intravenously every 3 weeks. During the study period, the dose of the regimens can be adjusted according to drug-related toxic and side effects, and the dose adjustment scheme shall be carried out according to the recommendations in the drug instructions. Toripalimab is allowed to be delayed for up to 12 weeks without reducing the dose. A diary card is used to record patients' medication. If patients experience any discomfort, they can contact the research doctor for timely examination and treatment. According to the researcher's judgement, corresponding concomitant treatment can be given considering the interests of the patients'. Concomitant medication and concomitant treatment of patients 30 days before and during the study shall be recorded on the case report form (CRF) in strict accordance with the requirements of Good Clinical Practice (GCP). During the study period, antitumour treatment prohibited by the protocol will not be allowed. Palliative and supportive care will depend on the investigator's judgement and relevant guidelines. Patients can be given the best supportive care during treatment, and complications and various adverse reactions shall be actively treated.

Study population

The study population consists of patients with MSS/pMMR mCRC who experienced disease progression or intolerable toxicity after standard treatment. The selection of patients is based on the following inclusion and exclusion criteria:

Inclusion criteria

The study includes the following: (1) patients willing to voluntarily participate in the study and sign the written informed consent form and those who can comply with

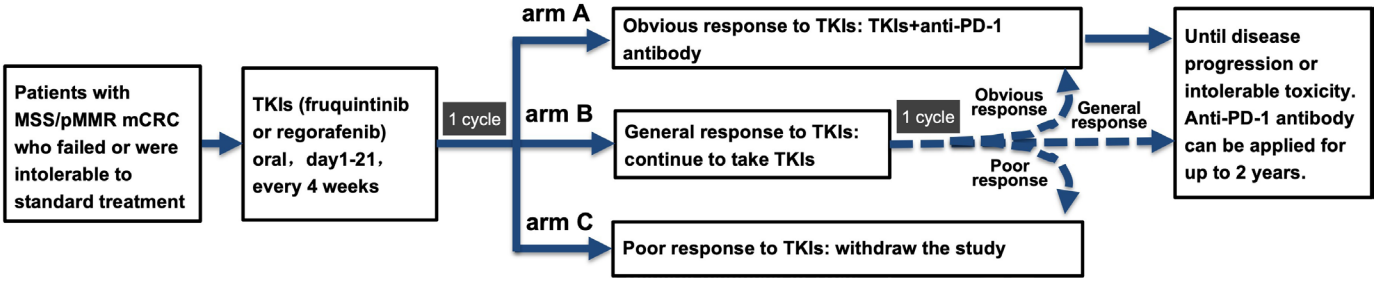

Figure 1 Study design and flow chart. mCRC, metastatic colorectal adenocarcinoma; MSS/pMMR, microsatellite stable/proficient mismatch repair; PD-1, programmed cell death protein 1; TKI, tyrosine kinase inhibitors.

the protocol of the study; (2) male or female patients aged 18–75 years; (3) patients with histopathologically confirmed colorectal adenocarcinoma and with locally advanced (unresectable) or mCRC; (4) patients who underwent standard antitumour therapies (fluorouracil, oxaliplatin and irinotecan were used, with or without administration of bevacizumab, ramucirumab, aflibercept, cetuximab and/or panitumumab); (5) patients with MSS/pMMR mCRC (diagnosed based on immunohistochemistry, PCR or next-generation sequencing); (6) all adverse reactions associated with drug use or surgery reduced to grade 0–1 (according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0) or to a level required by the protocol criteria; (7) presence of at least one measurable lesion by CT or MRI; (8) Eastern Cooperative Oncology Group performance status score ≤1; (9) patients with life expectancy ≥12 weeks; and (10) adequate important organ functions: bone marrow function (neutrophil count $\geq 1.5\times10^9$/L; platelet $\geq 80\times10^9$/L; haemoglobin ≥90 g/L), liver function (serum albumin ≥28 g/L; total bilirubin ≤1.5× the upper limit of normal (ULN); alanine aminotransferase and aspartate aminotransferase ≤3× ULN; or ≤5× ULN if liver metastases are present), renal function (serum creatinine ≤1.5× ULN or creatinine clearance ≥40 mL/min, using the Cockcroft-Gault formula; urine protein <2+; 24-hour urinary protein content <1.0 g/24-hour if urinary protein ≥2+), coagulation function (international normalised ratio or activated partial thromboplastin time ≤2× ULN) and thyroid function (thyrotropin ≤1× ULN).

Exclusion criteria

The study excludes the following: (1) patients with known microsatellite instability-high mCRC; (2) participation in another study with intervention or drugs within the past 4 weeks; (3) patients who underwent surgery and had incomplete recovery within the past 4 weeks; (4) patients with active autoimmune diseases or with related history—patients with controlled type I diabetes or hypothyroidism with substitution therapy may be included for further screening; (5) patients with any conditions requiring corticosteroids (>10 mg per day of prednisone or equivalent) or immunosuppressive drugs as systemic treatment within the past 1 week; (6) other active malignancy within the past 5 years, except for the cured limited cancer (such as basal cell carcinoma, carcinoma in situ of the prostate or cervix, etc); (7) patients with a history of hepatic encephalopathy or confirmed metastases to the central nervous system; (8) patients with non-infectious pneumonia under steroid treatment within the past 6 months; (9) patients suffering from chronic or active infections, fever (≥38.5°C) within the past 1 week or white cell count $>15\times10^9$/L, requiring systemic anti-infective treatment during the screening period, except for viral hepatitis; (10) patients with any other abnormal condition that is inconsistent with the study medication, or that may increase the risk of the patient according to the investigator's judgement; (11) congenital or acquired immunodeficiency (such as HIV); (12) patients with active hepatitis B virus (HBV) (HBV surface antigen-positive and HBV-DNA >2000 IU/mL) or hepatitis C virus (HCV) (HCV antibody and HCV-RNA-positive); (13) patients who received a live attenuated vaccine within the past 4 weeks or with vaccination planned during anti-PD-1 antibody treatment or within 5 months after the last treatment; (14) more than mild pericardial effusion, massive pleural or/and peritoneal effusions requiring puncture and drainage during the screening period; (15) patients with symptomatic heart and cerebrovascular diseases: heart failure (New York Heart Association class III or IV, left ventricular ejection fraction <50%), uncontrolled hypertension or arrhythmias, and serious cardiovascular and cerebrovascular events (acute coronary syndrome, stroke, thromboembolism, etc) within the past 6 months; (16) known allergies to targeted drugs; (17) pregnant or lactating women or those planning to get pregnant during the trial; and (18) patients with any other conditions judged by the investigator as ground for exclusion.

Measurement points

The study population includes patients receiving TKIs followed by TKIs in combination with anti-PD-1 antibody. The primary endpoint is 9-month PFS rate (from the date of the first dose of treatment to the first of either disease progression, relapse or death from any cause); the secondary endpoints are ORR, duration of response, DCR, OS, PFS, safety and health-related quality of life (HRQOL).

In addition, the following exploratory biomarkers are used: PD-1/PD-1 ligand 1, tumour-infiltrating lymphocytes, T lymphocyte subsets from peripheral blood samples, granulocyte to lymphocyte ratio, tumour mutational burden, circulating tumour DNA, exosomes, dynamic changes in serum levels of protein tumour markers, etc.

Outcome measurements

The first two imaging evaluations should be performed every 4 weeks from the beginning of the administration to evaluate response to TKIs, and then once every 6 weeks, until the end of treatment or the patient's withdrawal or death. Response to TKIs is assessed according to the RECIST criteria (version 1.1) and significant changes in imaging findings (cavitation in metastatic lung lesions or decrease in the density of liver metastatic targeted lesions ≥15%). In addition, the efficacy of TKIs followed by TKIs in combination with anti-PD-1 antibody is assessed based on the immune-related RECIST (iRECIST) criteria (version 1.1).

Safety evaluation is carried out according to the CTCAE (version 5.0). HRQOL is assessed using the Chinese version of the EuroQol 5-Dimension 5-Level (EQ-5D-5L) questionnaire and the European Organisation for Research and Treatment of Cancer Quality-of-Life Questionnaire.

Sample size calculation

This is a single-arm phase II clinical trial, with 9-month PFS rate as the primary endpoint. A Simon's two-staged design is used to calculate the sample size.

The null hypothesis is that the true 9-month PFS rate is 27.1%, which was reported by the FRESCO trial. The 9-month PFS rate of the TKI+anti-PD-1 antibody (arm A) group is supposed to be 55%, yielding a type 1 error rate of 0.05 and power of 0.90. The simon2stage package of STATA software (version 15.0) is used to obtain the sample size. The sample size of arm A is 25 cases. Sixteen evaluable patients are recruited in the first stage. If more than four patients can reach 9 months according to iRECIST 1.1 criteria, nine additional patients will be added to the second stage (for a total of 25 patients). The null hypothesis will be rejected if more than 10 patients achieve 9-month PFS in 25 patients.

According to the FRESCO study, the proportion of patients with CR+PR+shrunken SD in the fruquintinib group was 52.9% after 8-week treatment. Hence, to ensure that there are 25 patients in arm A, 48 patients should be selected at the initial stage. Considering a 10% dropout rate, we need to recruit 53 patients in this trial.

Recruitment

The recruitment advertisement of the clinical trial has passed ethical review.

Data collection and management

After enrolment, each patient will be assigned a unique identifier. Data will be entered in the CRF by the researchers. Data quality control measures include queries to identify missing data, outliers and discrepancies. Only researchers will be allowed to view the CRF. Laboratory report, CT or MRI, ECG record, patient diary card, etc must include the patient number and operation data. The corresponding medical evaluation will be carried out as follows: both the name and date shall be signed by the researcher. The researcher must keep all research records and original documents according to relevant regulations and guidelines. If the investigator withdraws from the study, the records are handed over to a mutually designated investigator. The original medical records shall be kept as complete as the original documents of clinical trials. The data on the CRF come from original documents such as original medical records and laboratory examination reports and shall be consistent with the original documents.

Statistical analysis

Continuous data are represented by mean and SD and analysed by Mann-Whitney U test. Categorical data are represented by frequency and percentage distribution and analysed by Fisher's exact test. Correlation is analysed by Spearman's correlation test. The PFS and OS are estimated by the Kaplan-Meier survival method, and the survival distribution is compared by the log-rank (Mantel-Cox) test. Two-sided p values of <0.05 are considered statistically significant.

Data monitoring

The study is a phase II exploratory test without major safety problems; there is no external data monitoring committee. The principal investigator and study staff will monitor data internally and will meet in person or by phone on a weekly basis to ensure the study is proceeding as intended.

Study period

The start date was July 2020 and the expected end date is July 2022.

Management of adverse events

The severity of adverse events is judged according to CTCAE version 5.0 standard. During the test, the adverse event record form is filled, including the occurrence time, severity, correlation with the study drug, duration, measures taken and outcome of the adverse event. If the adverse event is judged as a serious adverse event, it is reported according to the corresponding process. All adverse events and serious adverse events are followed until disappearance, remission to baseline level or less than grade 1, reaching a stable state, or reasonable explanation (such as loss of follow-up and death).

Protocol amendments

Amendments to the protocol will be according to GCP requirements.

Confidentiality of patient information

Patient information is kept in strict confidentiality by the researcher, participating researchers, sponsor and agents. Confidentiality also covers biological samples and genetic tests in addition to the patient's clinical information. All relevant research or data information shall not be disclosed to any unauthorised third party without written approval.

ETHICS AND DISSEMINATION

The research protocol has been approved by the Research Ethics Committee of the Liaoning Tumor Hospital & Institute (approval number 20200702), and signed informed consent is collected from all participants before enrolment. The data set used during the study will be available from the corresponding author on reasonable request. The results will be disseminated in peer-reviewed journals and will be presented in academic conferences as well.

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