ARTICLE DETAILS

**TITLE (PROVISIONAL)**
Evaluation of tyrosine kinase inhibitors combined with anti-programmed cell death protein 1 antibody in tyrosine kinase inhibitors responsive patients with microsatellite stable/proficient mismatch repair metastatic colorectal adenocarcinoma: protocol for open-label single-arm trial

**AUTHORS**
Dong, Qian; Diao, Yanwen; Sun, Xin; Zhou, Yang; Ran, Jialing; Zhang, Jingdong

GENERAL COMMENTS

The rationale of the protocol is interesting, because the treatment of patients with pretreated metastatic colorectal cancer, with MSS/pMMR is an unmet need. However, I have some comments for the authors.

1. The idea of keeping in treatment with single agent TKI those patients who will show an "obvious" response to the initial treatment is reasonable. However, it is not clear to me the calculation done for the sample size. Authors state that, according to FRESCO study, CR + PR + SD at 8 weeks is 52.9%, so they plan to enrol 48 patients to expect 25 will be eligible for arm A (obtaining an obvious response to TKI). However, if I understand well, patients with stable disease are not considered "obvious responders" so the proportion of responders eligible for arm A could be substantially lower than 52.9%. Please explain better.

2. Sample size has been calculated according to Simon two-stage design. Authors choose the progression-free rate at 9 months. Applying a two-stage design to a 9-month endpoint means that the study could be necessarily stopped for many months between the first and second stage. Did the authors consider this issue?

3. I have tried to replicate the calculation of sample size (http://www.cancer.unc.edu/biostatistics/program/ivanova/SimonsTwoStageDesign.aspx). It seems the authors used a two-stage "minimax" design, with 16 patients at first stage and 25 patients overall. However, authors state "If 4 or more patients can reach 9-month according to iRECIST 1.1 criteria, then, 9 additional patients will be added into the second stage for a total of 25 patients. The null hypothesis will be rejected if
10 or more patients achieve 9-month PFS in 25 patients. Check the numbers, because according to my calculations MORE THAN 4 successes (and not "4 or more") at the first stage and MORE THAN 10 (and not "10 or more") at the end are needed to define the study positive. Please check.

4. Please check carefully the spelling of drugs (regorafenib, fruquintinib, etc.) throughout the paper.

Minor comment
1. Patients with another tumor in the previous 5 years are excluded. Is this restriction really needed, considering that patients have a life expectancy of 6-9 months and a pretreated metastatic colorectal cancer? I suppose many previous tumors could not impact on the prognosis. A different time-frame (eg 2 or 3 years) could be considered?

REVIEWER
Paolo Sammartino
Umberto I Policlinico di Roma, Pietro Valdoni

REVIEW RETURNED
04-Jun-2021

GENERAL COMMENTS
My first concern is including patients (inclusion criteria point 3) with colorectal adenocarcinoma with local advanced disease (unresectable..) or metastatic as if it were overlapping clinical situations. Second it is not clear if we are talking of resected patients with metachronous metastatic involvement or patients with synchronous colorectal cancer and metastatic involvement. Third in colorectal cancer lung, liver or peritoneal metastatic involvement had a different outcome and this study which is certainly of scientific interest should be calibrated on a more selected group of patients.

VERSION 1 – AUTHOR RESPONSE

Reply to the reviewer 1:

Prof. Massimo Di Maio, Università degli Studi di Torino
Reviewer 1
Comments to the Author:
The rationale of the protocol is interesting, because the treatment of patients with pretreated metastatic colorectal cancer, with MSS / pMMR is an unmet need. However, I have some comments for the authors.
Comment 1. The idea of keeping in treatment with single agent TKI those patients who will show an "obvious" response to the initial treatment is reasonable. However, it is not clear to me the calculation done for the sample size. Authors state that, according to FRESCO study, CR + PR + SD at 8 weeks is 52.9%, so they plan to enrol 48 patients to expect 25 will be eligible for arm A (obtaining an obvious response to TKI). However, if I understand well, patients with stable disease are not considered "obvious responders" so the proportion of responders eligible for arm A could be substantially lower than 52.9%. Please explain better.
Answer: Thank you for your kindly comments. I’m so sorry that there is a slip of the pen in the article that affects your understanding. It should be shrunken SD instead of SD. According to the FRESCO study, the proportion of CR + PR + shrunken SD patients in the Fruquintinib group was 52.9% after 8-week treatment. The patients with shrunken SD are also considered “obvious responders”. We have corrected the mistake in the revised manuscript.
Comment 2. Sample size has been calculated according to Simon two-stage design. Authors choose the progression-free rate at 9 months. Applying a two-stage design to a 9-month endpoint means that the study could be necessarily stopped for many months between the first and second stage. Did the authors consider this issue?
Answer: Thank you for your kindly comments. The problems you mentioned will indeed affect the progress of the study. Our study began in July 2020, and the first subject signed the informed consent on July 31, 2020. Due to the reasons for COVID-19 epidemic in China, the speed of entry was much slower than expected. By September 2021, we had 13 subjects in arm A. However, it is exciting that the therapeutic effect of group A is better than expected. At present, 4 subjects have achieved the progression free survival of 9 months. Another subject started the first dose of treatment on February 3, 2021 and has been used for nearly 8 months so far. The imaging evaluation was shrunken SD. Moreover, the performance status and quality of life of the subject are good and is still in treatment. At present, it is fortunate that this design does not affect the progress of the study. However, thank you for your valuable suggestions, which will be very helpful in the design and development of our future clinical research.

Comment 3. I have tried to replicate the calculation of sample size (http://www.cancer.unc.edu/biostatistics/program/ivanova/SimonsTwoStageDesign.aspx). It seems the authors used a two-stage "minimax" design, with 16 patients at first stage and 25 patients overall. However, authors state "If 4 or more patients can reach 9-month according to iRECIST 1.1 criteria, then, 9 additional patients will be added into the second stage for a total of 25 patients. The null hypothesis will be rejected if 10 or more patients achieve 9-month PFS in 25 patients". Check the numbers, because according to my calculations MORE THAN 4 successes (and not "4 or more") at the first stage and MORE THAN 10 (and not "10 or more") at the end are needed to define the study positive. Please check.

Answer: Thank you very much for your kindly comments and your careful verification. Our calculation results, like yours, are more than 4 at the first stage and more than 10 at the end. It is our inaccurate translation that causes the writing errors in the manuscript. I’m so sorry for that. We have corrected the mistakes in the revised manuscript. However, the Chinese version of the protocol that we are carrying out is the correct version, so it does not affect the progress and accuracy of study. Thank you again for your careful verification.

Comment 4. Please check carefully the spelling of drugs (regorafenib, fruquintinib, etc.) throughout the paper.

Answer: Thank you for your kindly comments. According to the advice, we have carefully checked the spelling of drugs (regorafenib, fruquintinib, etc.) throughout the paper. We have corrected the mistakes in the revised manuscript. Thank you very much for your careful verification.

Minor comment

Comment 1. Patients with another tumor in the previous 5 years are excluded. Is this restriction really needed, considering that patients have a life expectancy of 6-9 months and a pretreated metastatic colorectal cancer? I suppose many previous tumors could not impact on the prognosis. A different time-frame (eg 2 or 3 years) could be considered?

Answer: Thank you for your kindly comments. The question you mentioned is very reasonable. Considering that patients have a life expectancy of 6-9 months and a pretreated metastatic colorectal cancer, another tumor in the previous 5 years may have little effect. However, it is difficult for Chinese patients with metastatic colorectal cancer to accept biopsy after disease progression, and another active malignancy may relapse and metastasize again within 5 years. Therefore, to avoid the impact on the efficacy evaluation, we listed “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.)” as one of the exclusion criteria.

I am expected to receive detailed suggestions if the reply might not satisfy you.

Reply to the reviewer 2:
Dr. Paolo Sammartino, Umberto I Policlinico di Roma
Reviewer 2
Comments to the Author:
My first concern is including patients (inclusion criteria point 3) with colorectal adenocarcinoma with local advanced disease (unresectable) or metastatic as if it were overlapping clinical situations.

Second it is not clear if we are talking of resected patients with metachronous metastatic involvement or patients with synchronous colorectal cancer and metastatic involvement. Third in colorectal cancer lung, liver or peritoneal metastatic involvement had a different outcome and this study which is certainly of scientific interest should be calibrated on a more selected group of patients.

Comment 1. My first concern is including patients (inclusion criteria point 3) with colorectal adenocarcinoma with local advanced disease (unresectable) or metastatic as if it were overlapping clinical situations.

Answer: Thank you for your kindly comments. What you mentioned above is very reasonable. It does exist in clinical practice. Generally, after the first two lines of treatment, most locally advanced (unresectable) cases will develop into mCRC. But theoretically, there are also very few patients, such as T4bN+, who invade the surrounding important organs and tissue structures and have multiple regional lymph node metastases, after first-line and second-line treatment, they may have poor response to treatment or poor tolerance to the treatment, resulting in the patients receiving third-line treatment before the tumor has distant metastasis. Of course, this situation is rare in clinical practice, and we have not encountered it during the study. Anyway, this situation has not affected the scientific development of the research. According to the advice, we will modify the text expression in the next protocol modification.

Comment 2. Second it is not clear if we are talking of resected patients with metachronous metastatic involvement or patients with synchronous colorectal cancer and metastatic involvement.

Answer: Thank you for your kindly comments. The question you mentioned above is very reasonable. Synchronous or metachronous metastasis may reflect the biological behavior of tumor and may affect the survival outcome of mCRC patients. We enrolled subjects with unresectable mCRC patients after multi-line therapeutic strategies without the opportunity of transformation. However, this study focuses on the later-line treatment, aiming to explore the clinical significance of TKIs combined anti-PD-1 antibody according to response to TKIs. Therefore, we did not consider the matter of synchronous or metachronous metastasis. However, the problem you mentioned is an important factor affecting the prognosis. According to the advice, we will analyze the impact of this factor on the prognosis and combined immunotherapy in the subsequent analysis in the future.

Comment 3. Third in colorectal cancer lung, liver or peritoneal metastatic involvement had a different outcome and this study which is certainly of scientific interest should be calibrated on a more selected group of patients.

Answer: Thank you for your kindly comments. The question you mentioned above is very meaningful. As you mentioned, different metastatic sites may affect the prognosis of patients with mCRC. This study is a phase II exploratory study. We will explore the impact of metastasis site on treatment efficacy and prognosis in subsequent analysis. Moreover, according to the valuable and helpful comments, we will focus on a more selected group of patients in the subsequent study.

VERSION 2 – REVIEW

| REVIEWER                  | Paolo Sammartino          |
|---------------------------|---------------------------|
|                           | Umberto I Policlinico di Roma, Pietro Valdoni |

| REVIEW RETURNED          | 09-Oct-2021               |

| GENERAL COMMENTS         | The main objection concerns the fact that this type of approach on MSS advanced colon cancer combining Regorafenib and Toripalimab as third therapeutic line has already been adopted in China (Nanchang University) Journal of Oncology 2021 doi 10.1155/2021/9959946 with extremely modest objective response rate (12%) so I don't think that the results could be very different if not modifying the research plane in some way. |
GENERAL COMMENTS

This is a prospective, single-arm, open-label, multicenter phase II study to verify the efficacy and safety of tyrosine kinase inhibitors (TKI) and programmed cell death protein 1 (PD-1) combination therapy. It is a significant study to confirm whether the combined use of TKI and PD-1 is effective for microsatellite stable/proficient mismatch repair (MSS/pMMR) colorectal cancer. In particular, the form of additional administration of PD-1 after confirming the effect of TKI is unique and exciting. There was almost no problem with the paper’s content, but minor revise is necessary for the following points.

1) There is a misspelling (fowllowed) on page 7 and line 14, so it needs to be fixed.
2) The word “Thus” is used repeatedly on line 5 and line 6 on page 10, so it needs to be fixed.
3) Paragraphs starting with line 5 on Page 11 are not needed for the introduction and should be deleted.
4) For Choi Criteria on line 7 of Page 12, it is necessary to specify the citation.
5) In which Arm will be the case with adverse events incorporated in the screening phase? How do you treat it as a dropout?
6) Line 4 on Page 14 describes bevacizumab and cetuximab as pretreatments, but it should also describe how other drugs, such as ramucirumab, aflibercept, and panitumumab, were treated.
7) The sentence on Page 17, line 3, is hard to read. Consider rephrasing as a following one; The secondary endpoints are ORR, duration of response (DOR), DCR, OS, PFS, safety, and health-related quality of life (HRQOL).

VERSION 2 – AUTHOR RESPONSE

Reply to the reviewer 2:
Dr. Paolo Sammartino, Umberto I Policlinico di Roma

Reviewer 2

Comments to the Author:

The main objection concerns the fact that this type of approach on MSS advanced colon cancer combining Regorafenib and Toripalimab as third therapeutic line has already been adopted in China (Nanchang University) Journal of Oncology 2021 doi 10.1155/2021/9959946 with extremely modest objective response rate (12%) so I don't think that the results could be very different if not modifying the research plane in some way.

Answer: Thank you for your kindly comments. The question you mentioned is very reasonable. Your comment is the major issue of greatest concern at the beginning of research design. PD-1 blockade combined with antiangiogenic therapy has synergistic effect and has achieved promising results in some types of cancer (e.g., hepatocellular carcinoma). However, the overall efficacy of this type of approach on MSS/pMMR mCRC patients is still unsatisfactory. As you mentioned, combining
Regorafenib and Toripalimab as third therapeutic line has already been adopted in China with extremely modest objective response rate (ORR, 12%). Another study included 52 cases of mCRC patients, who were treated with Sintilimab combined with Fruquintinib. It was reported that the ORR was 15.38%. Therefore, it is highly essential to optimize the administration mode of combined therapies and to indicate which group of patients can benefit the highest clinical significance of combined therapies. Our mode of TKIs followed by TKIs in combination with PD-1 blockade may work via the following steps: (1) Antiangiogenic effects of TKIs. TKIs favor the induction of cancer cells death inside the tumor, leading to reduce tumor burden and enhance immune responses to tumor-associated antigens; meanwhile, imaging changes will appear (tumor shrinkage, appearance of cavity, or reduction of density); (2) TKIs induce tumor vessel normalization, causing more tumor-infiltrating lymphocytes infiltrate into tumor microenvironment. Then, TKI combined with PD-1 blockade can enhance antitumor effects on residual tumor cells, reducing the risk of tumor progression. Therefore, we hypothesized that the 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 initials TKIs treatment by imaging examinations. Tumor shrinkage is the most significant outcome of TKIs treatment. In addition, angiogenesis inhibitors can inhibit tumor angiogenesis and shrink immature blood vessels. Thus, the tumor tissue cannot obtain enough nutrition and oxygen, and the tumor metabolism may be inhibited, resulting in tissue necrosis. Thus, there will be corresponding changes of perfusion parameters and metabolic status in imaging findings, such as appearance of cavity, or reduction of density. According to the mechanism of drug action and clinical experience, the obvious effective response to TKIs will be determined by significant changes in imaging findings, including tumor shrinkage, appearance of cavity, or reduction of density. Therefore, we designed the current prospective, single-arm, multicenter, phase II clinical trial to evaluate the efficacy and safety of TKIs (fruquintinib or regorafenib) in combination with anti-PD-1 antibody for TKIs-responded patients with MSS/pMMR mCRC.

The study design is as follows:

One cycle of TKIs (fruquintinib or regorafenib, chosen by the investigator) is administered in the screening phase. According to response to TKIs, study subjects are divided into three arms: arm A (an obvious response to TKIs), including reduction of 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 criteria); (2) arm B (general response to TKIs), including enlarged SD (based on RECIST guidelines (version 1.1)); (3) arm C (poor response to TKIs), including progressive disease (PD) (based on RECIST guidelines (version 1.1)). TKIs in combination with anti-PD-1 antibody are administered into arm A. Study subjects in arm C may withdraw the trial, and those in arm B may continue to receive TKIs for another one cycle. Then subjects with obvious response to TKIs will be involved in arm A, those with general response to TKIs will stay in arm B and will continue to take TKIs, and subjects with poor response to TKIs will withdraw the study. The administration of TKIs into arm A or arm B will last until disease progression...
or intolerable toxicity, and anti-PD-1 antibody can be applied for up to 2 years. The study design is shown in Figure 1.

![Study design and flow chart](image)

**Figure 1** The study design and flow chart. TKIs: tyrosine kinase inhibitors; mCRC: metastatic colorectal adenocarcinoma; PD-1: programmed cell death protein 1; microsatellite stable/proficient mismatch repair (MSS/pMMR).

At present, the research is in progress smoothly and has seen preliminary results. Our study began in July 2020, and the first subject signed the informed consent on July 31, 2020. Due to the reasons for COVID-19 epidemic in China, the speed of entry was much slower than expected. By December 2021, we had 13 subjects in arm A. However, it is exciting that the therapeutic effect of group A is better than expected. At present, 4 subjects have achieved the progression free survival of 9 months. Another two subjects have been treated for nearly 9 months so far. Tumor evaluation of these two subjects showed that the treatment was still effective. Moreover, the performance status and quality of life of the subjects are good and is still in treatment. Thank you for your valuable suggestions, which will be very helpful in the design and development of our future clinical research.

I am expected to receive detailed suggestions if the reply might not satisfy you.

**Reply to the reviewer 3:**
Dr. MICHIO NAKAMURA, Sapporo City General Hospital

**Reviewer 3**
Comments to the Author:

This is a prospective, single-arm, open-label, multicenter phase II study to verify the efficacy and safety of tyrosine kinase inhibitors (TKI) and programmed cell death protein 1 (PD-1) combination therapy. It is a significant study to confirm whether the combined use of TKI and PD-1 is effective for microsatellite stable/proficient mismatch repair (MSS/pMMR) colorectal cancer. In particular, the form of additional administration of PD-1 after confirming the effect of TKI is unique and exciting. There was almost no problem with the paper’s content, but minor revise is necessary for the following points.

**Answer:** Thank you for your kindly comments. According to the valuable and helpful comments concerning our manuscript, we have revised and improved our paper.
Comment 1. There is a misspelling (followed) on page 7 and line 14, so it needs to be fixed.

Answer: Thank you for your kindly comments. I’m so sorry that there is a slip of the pen in the article. We have corrected the mistake in the revised manuscript.

Comment 2. The word “Thus” is used repeatedly on line 5 and line 6 on page 10, so it needs to be fixed.

Answer: Thank you for your kindly comments. According to the helpful comments concerning our manuscript, we have revised our paper.

Comment 3. Paragraphs starting with line 5 on Page 11 are not needed for the introduction and should be deleted.

Answer: Thank you for your kindly comments. According to the helpful comments concerning our manuscript, we have deleted the paragraphs on treatment of adverse reactions in the introduction section of the revised manuscript.

Comment 4. For Choi Criteria on line 7 of Page 12, it is necessary to specify the citation.

Answer: Thank you for your kindly comments. According to the advice, we have specified the citation of the Choi Criteria in the revised manuscript.

Comment 5. In which Arm will be the case with adverse events incorporated in the screening phase? How do you treat it as a dropout?

Answer: Thank you for your kindly comments. In the screening phase, we will record adverse events in all subjects. However, as a secondary endpoint, the adverse events of Arm A subjects (including subjects receiving TKIs followed by TKIs in combination with anti-PD-1 antibody) will be statistically analyzed. The subjects with adverse events in other Arms will be analyzed as descriptive results.

Comment 6. Line 4 on Page 14 describes bevacizumab and cetuximab as pretreatments, but it should also describe how other drugs, such as ramucirumab, aflibercept, and panitumumab, were treated.

Answer: Thank you for your kindly comments. According to the advice, we have added ramucirumab, aflibercept, and panitumumab in the paragraph in the revised manuscript. However, these drugs have not been approved by China Food and Drug Administration.

Comment 7. The sentence on Page17, line 3, is hard to read. Consider rephrasing as a following one; The secondary endpoints are ORR, duration of response (DOR), DCR, OS, PFS, safety, and health-related quality of life (HRQOL).
Answer: Thank you for your kindly comments. According to the helpful comments concerning our manuscript, we have rephrased the paragraph in the revised manuscript.

**VERSION 3 – REVIEW**

| REVIEWER | Paolo Sammartino  
| Umberto I Policlinico di Roma, Pietro Valdoni |
| REVIEW RETURNED | 16-Jan-2022 |

| GENERAL COMMENTS | In recent years the possibility of the immunotherapy in metastatic colorectal cancer (mCRC) is on the agenda in the research landscape in this topic. Considering that about 90% of mCRC are microsatellite stable/mismatch repair-proficient it is quite evident that this problem is prevalent given the poor therapeutic responses obtained from the combination of chemotherapy with biological drugs. The proposed study appears well conceived and well articulated in its stages of development so it will be of great interest to await the results. |

| REVIEWER | MICHIO NAKAMURA  
| Sapporo City General Hospital |
| REVIEW RETURNED | 08-Jan-2022 |

| GENERAL COMMENTS | I think the authors respond appropriately to the comments of each reviewer. |