PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Study protocol for a multicenter single-arm phase II trial evaluating the safety and efficacy of panitumumab and irinotecan in patients with NeoRAS wild-type metastatic colorectal cancer (C-PROWESS trial)
AUTHORS	Osumi, Hiroki; Ishizuka, Naoki; Takashima, Atsuo; Kumekawa, Yosuke; Nakano, Daisuke; Shiozawa, Manabu; Denda, Tadamichi; Sawada, Ryoichi; Ouchi, Kota; Wakatsuki, Takeru; Narikazu, Boku; Kato, K; Yamaguchi, Kensei; Shinozaki, Eiji

VERSION 1 – REVIEW

REVIEWER	Daniele Rossini
REVIEW RETURNED	10-Apr-2022

GENERAL COMMENTS	The study proposal is quite interesting, we have a strong need for a clarification of the NeoRAS status and the role. I suggest to consider enrollment just for TAS102/Regorafenib pretretreated patients due to ethical concerns about the role of anti-EGFRS in RAS mutated patiens. I suggest also clarifying the statistical design of the study and, moreover, a deeper insight into the rationale of the study: in this context an insight on CRICKET and CHRONOS studies is necessary. Please add limitations of the study. It would be useful also to clarify why BRAF wt were not considered (see. NCCN guidelines on rechallenge). Moreover, a native language revision is necessary, Page 4 Line 10 and Line 48 Modify in "single arm, phase II" Page 4 Line 35-36 Consider to eliminate the statement: "Results of this [...] journal". Page 4 Line 50 "However [...]" Consider to add a sparate point for this statament. Page 5 Line 7-8 Eliminate "Protocol" Page 6 Line 8 I suggest to use mutated instead of "MTs" Page 6 Line 16 "Therefore [....] clinically". This phrase is ambiguous, consider to eliminate o rephrase. Page 6 Line 22 Please give a reference. Page 6 Line 41 I suggest to consider also the CHRONOS study in your introduction. Page 7 Line 14 Please consider to clarify the concept of "refractory" for chemotherapy Page 9 Line 26 Please use the term tipiracil/trifluridine instead of TAS102. Page 9 Line 28 Please specify according to which sample size design. Page 9 Line 33-34 "All the [...] (SAS Institute)". Consider to eliminate

	this phrase, is not usefull at the moment.

REVIEWER	Marc Peeters Antwerp Univ Hosp
REVIEW RETURNED	03-May-2022

GENERAL COMMENTS	This manuscript describes the study protocol for a trial in which patients with initially RAS MT mCRC will be treated with panitumumab and irinotecan based on the results of a liquid biopsy (NeoRAS WT). The authors aim to study the response rate in this group of NeoRAS WT mCRC patients. I want to congratulate the authors for designing this interesting study and drafting this in a concise manuscript. One of the strengths of this study is the collection of tissue and liquid biopsy samples before start of therapy and at progression. One of the limitations of this study is the fact that there is no comparator arm included in this study. It might be of interest to add this to the study. Kindly find below several minor comments I would like to raise: - The manuscript could benefit of editorial assistance, particularly with regard to the use of English language. The text is sometimes difficult to understand and some sentences/paragraphs should be simplified. The authors should consider proofreading by a native English speaker. - The authors sometimes use “NeoRAS” and other times “Neo RAS”. Please be consistent in your spelling. - What does “C-PROWESS” stand for? If this is an acronym, please add this to the protocol because this helps readers remember your trial. - Please add the date of when the study has started or will start. - Is it correct that the RAS mutation is detected in the tissue biopsy that is taken at initial diagnosis, as standard of care? And that another tissue biopsy will be taken before inclusion in this trial and at progression? So all patients still have a lesion that is suitable for tissue biopsies? - How will the authors evaluate whether the response of patients is due to therapy with irinotecan or panitumumab? - Can the authors elaborate on why they have chosen a mutant allele frequency of ≤0.1% as cut-off value? And is the presence of ctDNA confirmed in case no RAS mutation is detected? (Cfr. Moati E, Blons H, Taly V, et al. Plasma clearance of RAS mutation under therapeutic pressure is a rare event in metastatic colorectal cancer. Int J Cancer. 2020;147(4):1185-1189. doi:10.1002/ijc.32657) - In the “Outcomes and statistical considerations” section, the authors mention that complete or partial response will be assessed by the investigator. Can the authors elaborate on why they have opted for this approach? And not for example based on RECIST measurements by an independent radiologist? - Why did the authors choose to only use the Guardant360 at baseline? And not at end of study? - In the “trial organization” section, the authors 8 Japanese centres participated in the trial. Does this mean that the trial is already finished? Otherwise, it might be better to write “will participate”. - The “Clinical questions” section is difficult to read. Since this one of the most important parts of the manuscript, I would suggest rewriting it. Try to simplify the sentences to improve readability. - The last sentence of the summary is rather vague. If you want to discuss the potential implications of your research maybe you can

	add this in another paragraph of the manuscript. Try to keep the summary concise but clear.

VERSION 1 – AUTHOR RESPONSE

Response to Comment 1:

I confirmed that the description of all outcome measures for our trial in our protocol article, as well as the primary and secondary outcome measures, are consistent between our protocol article and the trial registry. Thank you.

(Page 8, lines 8–21, Page 9, lines 1–2)

2.Please include the planned start and end dates for the study in the methods section.

Response to Comment 2:

Following the comment by the reviewer, we added the following description in the Methods section.

(Page 9, lines 1–2)

Participant enrolment started on 1 February 2022 and will end on 31 January 2023.

3.Please ensure that the main text contains an ethics and dissemination section as per our instructions

for authors: https://bmjopen.bmj.com/pages/authors/#protocol

Response to Comment 3:

Following the comment by the reviewer, we added the following description in the Methods section.

(Page 9, lines 26–31)

Ethics and dissemination

This study was approved by the certified review board of National Cancer Center Hospital (jRCT, s031210565. Registered date, January 20, 2022.). The main results of the trial will be presented in international meetings and in medical journals.

4.Along with your revised manuscript, please include a copy of the SPIRIT checklist indicating the page/line numbers of your manuscript where the relevant information can be found (http://www.spirit-statement.org/)

Response to Comment 4:
Following the comment by the reviewer, we added a copy of the SPIRIT checklist indicating the page/line numbers of our manuscript.

5.Along with your revised manuscript, please provide an example of the patient consent form as a 'Supplemental Material' file, as per item #32 of the SPIRIT checklist.

Response to Comment 5:
Following the comment by the reviewer, we added an example of the patient consent form of our trial.

Formatting Amendments (where applicable):
6.Table should be in editable format. Please make sure that your table is editable and in table tools format.

Response to Comment 6:
Following the comment by the reviewer, we revised and added a table in an editable format in our manuscript.

Reviewer: 1 Daniele Rossini
Comments to the Author:
The study proposal is quite interesting, we have a strong need for a clarification of the NeoRAS status and the role.
1. I suggest to consider enrollment just for TAS102/Regorafenib pretretreated patients due to ethical concerns about the role of anti-EGFRS in RAS mutated patiens.

Response to Comment 1:

Thank you for your compliment. Previous reports showed that the incidence of NeoRAS mCRC for the front line is higher than those for the later lines (Moati E, et al. *Int J Cancer* 2020;147:1185–9). Furthermore, taking into account the feasibility of the study, we decided to set the trial in the third-line setting. The CETIDYL trial (NCT04189055) is already ongoing for the same line.

2.I suggest also clarifying the statistical design of the study and, moreover, a deeper insight into the rationale of the study: in this context an insight on CRICKET and CHRONOS studies is necessary.

Response to Comment 2:

Thank you for your suggestion. A rationale for the re-challenge of EGFR inhibitors has been added to the Introduction section. The study population is completely different between NeoRAS and re-challenge of EGFR inhibitors, as eligible for this study are patients with tissue RAS mutations who convert to RAS wild-type after treatment.

(Page 5, lines 12–19)

Recent advances in diagnostic technology for the detection of genetic mutations by liquid biopsy, especially circulating tumor DNA (ctDNA), have made minimally invasive, simple, and repeatable testing possible.[14-16] It is well known that RAS status can change before and after treatment. First reported was the identification of RAS MTs in ctDNA in EGFR inhibitor-resistant RAS WT mCRC patients.[17,18] This involved acquired resistance to EGFR inhibitors, and several clinical trials have reported that re-measuring the RAS status before treatment is an important predictor of treatment efficacy when considering EGFR inhibitor re-challenge.[19, 20, 21]

3.Please add limitations of the study. It would be useful also to clarify why BRAF wt were not considered (see. NCCN guidelines on rechallenge).

Response to Comment 3:

Thank you for your suggestion. The limitations of this study are a small sample size and having no control arm. mCRC patients with tissue BRAF MT were excluded from this study, and the study population is completely different between NeoRAS and re-challenge of EGFR inhibitors.

(Page 10, lines 27-28)

The limitations of this study are a small sample size lack of a control arm.

4.Moreover, a native language revision is necessary,
Response

Thank you for your suggestion. Our revised manuscript has been checked by a native English speaker.

Page 4 Line 10 and Line 48 Modify in "single arm, phase II"
Response

We modified these sentences; thank you.

Page 4 Line 35-36 Consider to eliminate the statement: "Results of this [...] journal".

Response

Thank you for your suggestion. However, this sentence was left as it is required per the Journal's guidelines.

Page 4 Line 50 "However [...]" Consider to add a separate point for this statament.
Response

We added a separate point for that statement. Thank you.

Page 5 Line 7-8 Eliminate "Protocol"
Response

We deleted that sentence; thank you.

Page 6 Line 8 I suggest to use mutated instead of "MTs"

Response

Thank you for this suggestion. However, some of the literature already reported translates mutations as MTs, so we have left the term as it is.

Page 6 Line 16 "Therefore [....] clinically". This phrase is ambiguous, consider to eliminate o rephrase.

Response

We deleted this sentence; thank you.

Page 6 Line 22 Please give a reference.

Response

Is this a reference to this statement?

There have been some reports that RAS MT observed at the initial diagnosis converted to a RAS WT after treatment.

We added a reference; thank you.

Page 6 Line 41 I suggest to consider also the CHRONOS study in your

Response

Thank you for your suggestion. A rationale for the re-challenge of EGFR inhibitors has been added to the Introduction of our manuscript.

Page 7 Line 14 Please consider to clarify the concept of "refractory" for chemotherapy

Response

"Refractory" defined resistance to 5-FU, oxaliplatin, and irinotecan.

Page 9 Line 26 Please use the term tipiracil/trifluridine instead of TAS102.

Response

We modified that sentence; thank you.

Page 9 Line 28 Please specify according to which sample size design.

Response

As already stated, the RR threshold is set at 4%, according to the results of previous clinical trials with tipiracil/trifluridine (+ bevacizumab) or regorafenib. The required sample size is 30, whereas an RR of 15% is deemed to be promising (one-sided $\alpha = 0.10$; $\beta = 0.2$).

Page 9 Line 33-34 "All the [...] (SAS Institute)". Consider to eliminate this phrase, is not usefull at the moment.

Response to Comment 4:

We deleted this sentence. Thank you.

Reviewer: 2

Dr. Marc Peeters, Antwerp Univ Hosp

Comments to the Author:
This manuscript describes the study protocol for a trial in which patients with initially RAS MT mCRC will be treated with panitumumab and irinotecan based on the results of a liquid biopsy (NeoRAS WT). The authors aim to study the response rate in this group of NeoRAS WT mCRC patients.
I want to congratulate the authors for designing this interesting study and drafting this in a concise manuscript.
One of the strengths of this study is the collection of tissue and liquid biopsy samples before start of therapy and at progression.
One of the limitations of this study is the fact that there is no comparator arm included in this study. It might be of interest to add this to the study.

Kindly find below several minor comments I would like to raise:
1 The manuscript could benefit of editorial assistance, particularly with regard to the use of English language. The text is sometimes difficult to understand and some sentences/paragraphs should be simplified. The authors should consider proofreading by a native English speaker.

Response to Comment 1:

Thank you for your compliments. Our manuscript has been revised and checked by a native speaker.

2 The authors sometimes use “NeoRAS” and other times “Neo RAS”. Please be consistent in your spelling.

Response to Comment 2:

Thank you for noticing this. Following the comment by the reviewer, we unified the spelling to *NepRAS*.

3 What does “C-PROWESS” stand for? If this is an acronym, please add this to the protocol because this helps readers remember your trial.

Response to Comment 3:

Following the comment by the reviewer, we added the following description in the METHODS AND ANALYSIS section.

(Page 6, lines 7–10)

This trial is a multicenter, single-arm, phase II trial to investigate the safety and effi**C**acy of **P**anitumumab and i**R**in**O**tecan in NeoRAS **W**ild type m**E**ta**S**tatic colorectal cancer patient**S** (C-PROWESS trial).

4 Please add the date of when the study has started or will start.

Response to Comment 4:

In accordance with the comment by the reviewer, we added the following description in the METHODS AND ANALYSIS section.

(Page 9, lines1-2)

Participant enrolment started on 1 February 2022 and will end on 31 January 2023.

5 Is it correct that the RAS mutation is detected in the tissue biopsy that is taken at initial diagnosis, as standard of care? And that another tissue biopsy will be taken before inclusion in this trial and at progression? So all patients still have a lesion that is suitable for tissue biopsies?

Response to Comment 5:

Confirmation of RAS mutations using pre-treatment tissue samples is performed as the standard of care. In this study, the collection of tissue samples is not mandatory. Therefore, it is possible to enroll patients for whom it is difficult to collect tissue samples.

6 How will the authors evaluate whether the response of patients is due to therapy with irinotecan or panitumumab?

Response to Comment 6:

The primary endpoint is response rate. Tumor shrinkage should be determined per the RECIST guidelines (version 1.1).

7 Can the authors elaborate on why they have chosen a mutant allele frequency of ≤0.1% as cut-off value? And is the presence of ctDNA confirmed in case no RAS mutation is detected? (Cfr. Moati E, Blons H, Taly V, et al. Plasma clearance of RAS mutation under therapeutic pressure is a rare event in metastatic colorectal cancer. Int J Cancer. 2020;147(4):1185-1189. doi:10.1002/ijc.32657)

Response to Comment 7:

We followed the RAS positive criteria for the Oncobeam™ RAS CRC kit. This has been used in another clinical trial (e.g., the PERSUIT trial). Although we do not know if ctDNA is detected or not using the Oncobeam™ RAS CRC kit, we believe that the use of G360 in the accompanying study will compensate for its limitation.

8 In the “Outcomes and statistical considerations” section, the authors mention that complete or partial response will be assessed by the investigator. Can the authors elaborate on why they have opted for this approach? And not for example based on RECIST measurements by an independent radiologist?

Response to Comment 8:

The primary endpoint is response rate. An off-site review should be conducted and any discrepancies with the attending physician's judgment should be discussed and the results recorded.

9 Why did the authors choose to only use the Guardant360 at baseline? And not at end of study?

Response to Comment 9:

As funds are currently limited, pre-treatment samples will be measured by G360, and post-treatment samples will be measured by the Oncobeam™ RAS CRC kit in daily clinical practice. We plan to

secure the remaining ctDNA and re-measure it (post-treatment samples) with G360 after obtaining funding.

10 In the “trial organization” section, the authors 8 Japanese centres participated in the trial. Does this mean that the trial is already finished? Otherwise, it might be better to write “will participate”.

Response to Comment 10:

Following the comment by the reviewer, we revised the following description in the METHODS AND ANALYSIS section.

(Page 9, lines 25)

Eight core high-volume centers in Japan will participate in this trial.

11 The “Clinical questions” section is difficult to read. Since this one of the most important parts of the manuscript, I would suggest rewriting it. Try to simplify the sentences to improve readability.

Response to Comment 11:

Following the comment by the reviewer, we rewrote the “Clinical questions” section to improve readability.

12 The last sentence of the summary is rather vague. If you want to discuss the potential implications of your research maybe you can add this in another paragraph of the manuscript. Try to keep the summary concise but clear.

Response to Comment 12:

Following the comment by the reviewer, we modified the last sentence as follows:

(Page10, lines 30–32)

Our trial will evaluate the efficacy of panitumumab and irinotecan in patients with NeoRAS WT mCRC to develop personalized therapeutic regimens based on the ctDNA results.

VERSION 2 – REVIEW

REVIEWER	Daniele Rossini
REVIEW RETURNED	06-Jul-2022

GENERAL COMMENTS	The paper is now ready to be accepted.