PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Determination of biomarkers associated with neoadjuvant treatment response focusing on colibactin-producing Escherichia coli in patients with mid or low rectal cancer: a prospective clinical study protocol (MICARE)
AUTHORS	Taoum, Christophe; Carrier, Guillaume; Jarlier, Marta; Roche, Gwenaelle; Gagniere, Johan; Fiess, Catherine; De forges, Helene; Chevarin, Caroline; Colombo, Pierre-Emmanuel; Barnich, Nicolas; Rouanet, Philippe; Bonnet, Mathilde

VERSION 1 – REVIEW

REVIEWER	Shiba, Satoshi National Cancer Center Hospital, Outpatient Treatment Center
REVIEW RETURNED	19-Apr-2022

GENERAL COMMENTS	MICARE study has a well designed protocol and then objectives are also properly defined. Therefore, it is expected that a novel evidence will be established in the field of rectal cancer. However, following points are needed to be confirmed in conducting this hopeful research. 1. On 181 line of page 9, what is a regimen of neoadjuvant CRT in accordance with the French national guidelines? Moreover, will all-enrolled patients undergo the same regimen? The authors should describe the detail content of neoadjuvant treatment in this manuscript. 2. On 214 line of page 11, the authors describe "All adverse events will be reported following the study sponsor's pharmacovigilance procedures" in safety analysis. What objective criteria are used for evaluation when adverse events are collected in this study? Besides, are the authors going to analyze the association between adverse events and gut microbiota? If this analysis is conducted, its content should be described in the secondary objectives.

REVIEWER	Kobberøe Søgaard, Kirstine Aarhus Universitetshospital, Department of Clinical Epidemiology
REVIEW RETURNED	18-May-2022

GENERAL COMMENTS	I read with great interest the study protocol.The proposal and methodology are sound. I look very much forward to follow their work with clarifying the potential role of colibactin in tumor response to chemoradiotherapy in CRC.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Satoshi Shiba, National Cancer Center Hospital

Comments to the Author:
MICARE study has a well designed protocol and then objectives are also properly defined. Therefore, it is expected that a novel evidence will be established in the field of rectal cancer. However, following points are needed to be confirmed in conducting this hopeful research.
Authors thank the reviewer for his comment.

1. On 181 line of page 9, what is a regimen of neoadjuvant CRT in accordance with the French national guidelines? Moreover, will all-enrolled patients undergo the same regimen? The authors should describe the detail content of neoadjuvant treatment in this manuscript.
Done. The following sentence has been added as requested by the reviewer: “The recommended regimen is a concomitant oral chemotherapy (5-FU/CAPECITABINE) and 50 Grey radiotherapy. Despite PRODIGE 23 and RAPIDO trials, it is highly recommended to add a systemic chemotherapy (FOLFIRINOX or FOLFOX) to the RCT in locally advanced rectal cancer. ” (lines 183-186)

2. On 214 line of page 11, the authors describe "All adverse events will be reported following the study sponsor’s pharmacovigilance procedures" in safety analysis. What objective criteria are used for evaluation when adverse events are collected in this study? Besides, are the authors going to analyze the association between adverse events and gut microbiota? If this analysis is conducted, its content should be described in the secondary objectives.
The objective criteria used for evaluation of the adverse events are listed in Supplementary file 3 (line 219). Association between adverse events and gut microbiota will be studied. This is already included in the secondary objectives. Indeed, clinical data described on line 126, also included adverse events.

Reviewer: 2
Dr. Kirstine Kobberøe Søgaard, Aarhus Universitetshospital, Aalborg Universitetshospital

Comments to the Author:
I read with great interest the study protocol. The proposal and methodology are sound.
I look very much forward to follow their work with clarifying the potential role of colibactin in tumor response to chemoradiotherapy in CRC.
Authors thank the reviewer for his comment.

VERSION 2 – REVIEW

REVIEWER	Shiba, Satoshi National Cancer Center Hospital, Outpatient Treatment Cente
REVIEW RETURNED	25-Aug-2022

GENERAL COMMENTS	Thank you for responding reviewers' comments. Concerning about measuring adverse events in this study, supplementary file 3 (line 219) the authors mentioned shows whether each adverse event exists or not (YES or NO). According to reference 5, including "Hofheinz RD, et al. Lancet Oncol

	2012;13:579–88", and 12 the authors offered, adverse events are graded using "Common Terminology Criteria for Adverse Events" from National Cancer Institute in both clinical trials. The authors should add how to evaluate adverse events in this document.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Dr. Satoshi Shiba, National Cancer Center Hospital

Comments to the Author:

Concerning about measuring adverse events in this study, supplementary file 3 (line 219) the authors mentioned shows whether each adverse event exists or not (YES or NO). According to reference 5, including "Hofheinz RD, et al. Lancet Oncol 2012;13:579–88", and 12 the authors offered, adverse events are graded using "Common Terminology Criteria for Adverse Events" from National Cancer Institute in both clinical trials. The authors should add how to evaluate adverse events in this document.

Authors thank the reviewer for his comment.

The primary objective of the study is to assess the correlation between tumor response to neoadjuvant chemo-radiotherapy (CRT) and CoPEC presence in stool samples. The Dworak classification will be used in pathologic analysis of surgical specimens to evaluate the tumour regression grade. In this context, we thought that gradation of adverse events of the RCT does not necessary for the main purpose of our clinical study.

The aim is not to assess a correlation between side effects of CRT and microbiota. Surgical events will be evaluated using the Clavien Dindo classification.

The correlation between clinical data and microbiota composition modulation induced by CRT is one of the secondary objectives. Therefore, we decided not to use adverse events gradation of the CRT. However, if our results suggest an association between some microbial factors and the presence of adverse events, it will be possible to find data and retrospectively grade the side effects in patient records. It would be based on "Common Terminology Criteria for Adverse Events" from National Cancer Institute.

Despite the fact that it would be interesting, evaluation of adverse events should be a primary objective of a new specific study. Indeed, this study would need multiple stool samples during CRT, not only at the beginning and at the end.