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Keywords:	CHEMOTHERAPY, Gastrointestinal tumours < GASTROENTEROLOGY, Gastrointestinal tumours < ONCOLOGY

Study Protocol

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)

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ABSTRACT

Introduction: A new concept of "NeoRAS wild-type (WT)" which means conversion of RAS status from RAS mutant to RAS WT after the treatment has been reported. Previous observational and proof-of-concept studies have demonstrated the efficacy of epidermal growth factor receptor inhibitors in patients with NeoRAS WT metastatic colorectal cancer (mCRC). Moreover, post hoc biomarker analyses of these studies have suggested that not only the RAS status in the circulating tumor DNA (ctDNA) but also other gene mutational status may be useful as biomarkers of epidermal growth factor receptor inhibitors for NeoRAS WT mCRC.

Methods and analysis: This trial is a multicenter, single-arm phase II trial to assess the efficacy and safety of panitumumab plus irinotecan therapy for NeoRAS mCRC patients. The key eligibility criteria include RAS mutant mCRC initially proven in tumor tissue refractory or intolerant to fluoropyrimidine, oxaliplatin, and irinotecan; RAS WT in ctDNA (defined as plasma mutant allele frequencies of all RAS ≤ 0.1%) within 28 days prior to enrollment; and Eastern Cooperative Oncology Group performance status ≤2. The primary endpoint is a response rate. The target sample size is 30 patients. Biomarker analyses are planned to be performed using next-generation sequencing-based ctDNA analysis.

Ethics and dissemination: This study was approved by the certified review board of national cancer center hospital. Results of this study will be submitted for publication in a peer-reviewed journal.

Trial registration: jRCT, s031210565. Registered date, January 20, 2022.

Keywords: Metastatic colorectal cancer, Circulating tumor DNA, Liquid biopsy, NeoRAS, EGFR inhibitor

Strengths and limitations of this study

- The C-PROWESS trial is a prospective multicenter, single-arm phase II trial.
- The trial is designed to assess the efficacy and safety of panitumumab plus irinotecan therapy in patients with NeoRAS; however, no comparator is a limitation.

- Translational Research of biomarkers using liquid biopsies at baseline and after discontinuation of the treatment is planned to be performed protocol.

INTRODUCTION

RAS mutations (MTs) induce the activation of the protein kinase pathway and promote carcinogenesis and cancer growth.[1] Patients with RAS MT metastatic colorectal cancer (mCRC) have a poorer prognosis than those with RAS wild-type (WT) mCRC.[2, 3] Epidermal growth factor receptor (EGFR) inhibitors (i.e., cetuximab and panitumumab), which are key drugs for mCRC, are ineffective for patients with RAS MT mCRC (KRAS/NRAS exons 2, 3, and 4).[4-10] Therefore, more treatment options for patients with RAS-MT mCRC are warranted clinically.

International guidelines recommend RAS genetic testing prior to the administration of an EGFR inhibitor in patients with mCRC.[11-13] Repeat biopsies are not performed in routine clinical practice to monitor the RAS MT status,[11-13]; the consistency of the RAS MT status before and after chemotherapy remains unclear.

There have been some reports that RAS MT observed at the initial diagnosis converted to a RAS WT after treatment.[14] These cases have been called as “Neo RAS WT” mCRC.[14] The incidence of Neo RAS WT mCRC has been reported to range from 10.7% to 40% when assessed in tumor tissue samples,[15, 16] and from 18.8% to 83.3% in the circulating tumor DNA (ctDNA).[17-23]

There have been several reports of the use of EGFR inhibitors in patients with Neo RAS WT. Mohamed et al., in a proof-of-concept study of EGFR inhibitors in patients with Neo RAS WT,[23] reported that the objective response rate was 55.6% and progression-free survival (PFS) was 9 months in Neo RAS WT mCRC patients treated with fluorouracil, folinic acid, irinotecan, and cetuximab.[23] This result suggested that EGFR inhibitors may be effective in Neo RAS WT mCRC patients. Although retrospective analyses and proof-of-concept studies have indicated the potential efficacy of EGFR inhibitors in NeoRAS WT mCRC,[14, 23] the safety and efficacy have not been validated prospectively. Furthermore, the definition of NeoRAS WT mCRC has not been established.

Therefore, this trial will evaluate the efficacy of panitumumab and irinotecan in NeoRAS WT mCRC patients confirmed in ctDNA after prior treatment.

METHODS AND ANALYSIS

Trial design

The C-PROWESS trial is a multicenter, single-arm phase II trial assessing the efficacy and safety of panitumumab and irinotecan in NeoRAS WT mCRC patients. The overall trial scheme is illustrated in Fig. 1.

Patient

Key eligibility criteria are: 1) RAS MT (KRAS/NRAS exon 2,3,4) and BRAF V600E WT mCRC initially diagnosed in the tumor tissue; 2) refractory or intolerant to fluoropyrimidine, oxaliplatin, and irinotecan; 3) RAS WT in ctDNA (mutant allele frequencies of all RAS ≤ 0.1%) within 28 days prior to enrollment; 4) an Eastern Cooperative Oncology Group performance status ≤ 2; and 5) a preserved organ function. Details of the eligibility criteria are presented in Table 1.

Table 1 Eligibility criteria for the C-PROWESS trial
Inclusion criteria
1) Histologically proven diagnosis of colorectal adenocarcinoma
2) Advanced or recurrent colorectal cancer (excluding appendix and anal canal cancer)
3) RAS mutation (MT) (KRAS/NRAS exon 2, 3, or 4 MT) confirmed by tumor histology prior to first-line chemotherapy
4) Confirmation of refractory or intolerant to previous treatments with chemotherapies including fluoropyrimidines, oxaliplatin, or irinotecan (irinotecan is applied to refractory only), regardless of prior treatment with trifluridine tipiracil hydrochloride, regorafenib, or angiogenesis inhibitors
5) Confirmation of the RAS WT within 28 days from test result date by ctDNA analysis using the OncoBEAM (TM) RAS CRC KIT
6) At least one measurable lesion according to RECIST version 1.1 criteria evaluated by CT or MRI wi thin 28 days before registration
7) Eastern Cooperative Oncology Group (ECOG) performance status <= 2
8) Age >= 20 years
9) Adequate organ functions (bone marrow, liver, renal functions) as defined by the following laborat ory values obtained within 15 days prior to the stu dy enrollment: a.Absolute neutrophil count >= 1500/ mm3 b.Platelets >= 75000/mm3 c.Serum total bilirubin <= 1.5 mg/dL d.Serum AST(GOT) and ALT(GPT) <= 100 U/L(exc ept for patients with tumor involvement of the liver who must have AST and ALT <= 200 U/L)
10) A life expectancy of at least 60 days
11) Written Informed consent
Exclusion criteria
1) Evidence of BRAF V600E MT by tumor histology
2) Treated with blood transfusion, blood products, or hematopoietic factor products such as Granulo cyte Colony Stimulating Factor within 7 days prior to enrollment in this study
3) A history of severe drug hypersensitivity or severe drug allergy
4) Active infection (fever of 38 degree or higher du e to infection)
5) Ascites, pleural effusion, or pericardial effusion requiring continuous drainage
6) Uncontrolled diabetes mellitus
7) Uncontrolled hypertension
8) Patients who have been treated with any of the following treatments prior to starting study drug: a.Extensive surgery <= 4 weeks prior to starting stu dy drug (e.g., surgical treatment with organ resection, excluding colostomy) b.Proctocolectomy <= 2 weeks prior to starting the study drug c.Any chemotherapy <= 2 weeks prior to starting the study drug d.Radiotherapy <= 2 weeks prior to starting the study drug
9) Clinically significant electrocardiographic abnor mality or clinically significant cardiovascular accidents within 6 months prior to study enrollment, including myocardial infarction, severe unstable angina, or New York Heart Association functional class ification class III or IV congestive heart failure
10) Patients with severe lung disease (interstitial pneumonia, pulmonary fibrosis, severe emphysema)
11) History of clinically significant mental disorder or central nervous system disorder
12) Symptomatic brain metastasis or clinically suspected brain metastasis upon symptoms
13) Diarrhea that interferes with daily life
14) Intestinal paralysis, intestinal obstruction
15) Co-existing active malignancies
16) Pregnant or lactating women; women of childbearing potential or men with women partners of childbearing potential who are unwilling to use a highly effective method of contraception or avoid intercourse during and upon completion of the study
17) Patients who have been assessed by the site physician as inappropriate for this study
18) Patients who have been treated with EGFR inhibitors prior to starting the study drug

Treatment

Patients will receive panitumumab at 6 mg/kg and irinotecan at 150 mg/m^2 biweekly until progressive disease, unacceptable toxicity, withdrawal of informed consent, or death. The starting dose of irinotecan can be reduced to 120 mg/m^2 according to UGT1A1 status (homozygosity/double heterozygosity).

Outcomes and statistical considerations

The primary endpoint of the C-PROWESS trial is a response rate (RR), defined as the proportion of patients who achieve a complete or partial response by the investigator's assessment. The secondary endpoints include PFS, overall survival (OS), a disease control rate, and incidences of adverse events. The response is evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, using computed tomography at 6 and 12 weeks after the start of the protocol treatment and repeated every 8 weeks thereafter. The RR threshold is set at 4%, according to the results of previous clinical trials with TAS102 (+ bevacizumab)[24-26] or regorafenib.[27] The required sample size is 30, whereas an RR of 15% is deemed to be promising (one-sided $\alpha = 0.10$; $\beta = 0.2$). The primary endpoint is planned to be analyzed in all the patients that receive at least one dose of the protocol treatment and who satisfy all the inclusion and exclusion criteria. All the statistical analyses will be performed using the SAS software, version 9.2 (SAS Institute).

Biomarker analysis

Samples for liquid biopsies are planned be collected at baseline and after discontinuation of the protocol treatment (Fig. 1). The ctDNA will be analyzed using a highly sensitive digital polymerase chain reaction method, the OncoBEAM RAS CRC kit, and targeted next-generation sequencing, Guardant360. The OncoBEAM RAS CRC kit, which has been approved in Japan to detect RAS mutations in the ctDNA derived from mCRC, detects 34 mutations in KRAS/NRAS codons 12, 13, 59, 61, 117, and 146 in plasma.[28] Guardant360, which is a hybrid capture-based next-generation sequencing panel of the ctDNA developed by Guardant Health, detects other gene alterations.[29] Exploratory analyses will be performed to identify the proportion of patients without RAS and other mutations related to resistance to anti-EGFR inhibitors (defined as "True Neo RAS WT"). The clinical outcomes (RR, PFS, OS, and disease control rate) of true Neo RAS WT patients receiving panitumumab and irinotecan combination therapy will be compared to

those of patients without true Neo RAS WT and the overall study population. Moreover, the clinical outcomes will be compared according to the presence or absence of each genetic and epigenetic abnormality using exome sequencing and immunohistochemical staining in tissue samples and in ctDNA analysis before and after treatment with the protocol treatment using NGS, to explore the relationship with clinical outcomes and the mechanism of resistance.

Trial organization

Eight core high volume centers in Japan participated in this trial.

Clinical Questions

The clinical questions to be addressed in this study will be the definition of NeoRAS WT mCRC and the therapeutic effect of EGFR inhibitors on NeoRAS WT mCRC.

First, because “Neo RAS WT” mCRC is currently defined by RAS MT status evaluated only in the tissue, it is not possible to determine whether the RAS MT has completely disappeared or missed. Therefore, we will perform more sensitive analysis using an NGS of the ctDNA before administering the protocol treatment to confirm the incidence of Neo RAS patients. If the proportion of true Neo RAS WT mCRC cases is clarified, they will be a target of new treatment strategy with EGFR inhibitors.

Second, our trial will evaluate the relationship between gene mutations other than RAS in ctDNA and the efficacy of EGFR inhibitors in NeoRAS WT mCRC. The ORR, PFS, and OS may differ according to the presence or absence of some genetic abnormality that may lead to primary resistance to EGFR inhibitors such as EGFR extracellular domain, Erb-B2 Receptor Tyrosine kinase 2, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit alpha, among others, in the ctDNA (NGS) prior to protocol treatment.

In addition, we plan to measure the ctDNA RAS again after the administration of the protocol treatment. This will allow us to understand how frequently RAS MT reappears after treatment and whether the same RAS MT reappear or different variants of RAS MT newly appear. If the same RAS increases after the administration of treatment, it may be due to tissue heterogeneity in which dominant clones with/out RAS mutation in tumors is changed by an EGFR inhibitor. Furthermore, the mechanism of resistance will be clarified.

Summary

Our trial will evaluate the efficacy of panitumumab and irinotecan in patients with NeoRAS WT mCRC. Moreover, developing personalized therapeutic regimens based on the ctDNA results will be a breakthrough in developing treatments for other cancers.

Patient and public involvement statement

No patient involved.

Acknowledgements

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Authors' contributions

HO, AT, KK, NB, KY, and ES, as task managers, participated in the coordination of this trial, the design and writing of the protocol, data collection, data analysis, data interpretation, and writing of the manuscript. AT, YK, DN, MS, DT, KO, RS, KO, and TW as the protocol preparation committee, participated in this trial, including design and writing of the protocol, data collection, data analysis, data interpretation, and preparation of the manuscript. NI, as the chief of statistical analysis, participated in the statistical setting of the trial, design, and data analysis. All the authors have reviewed and approved the final manuscript.

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Competing interests:

Dr. Kensei Yamaguchi has received honoraria from Daiichi-Sankyo.

Availability of data and materials

Not applicable.

References

1 Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nat Rev Cancer* 2011;11:761–74.

2 Richman SD, Seymour MT, Chambers P, et al. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol* 2009;27:5931–7.

3 Cremolini C, Loupakis F, Antoniotti C, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. *Lancet Oncol* 2015;16:1306–15.

4 Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;360:1408–17.

5 Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008;359:1757–65.

6 Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010;28:4697–705.

7 Loupakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer* 2009;101:715–21.

8 Allegra CJ, Rumble RB, Hamilton SR, et al. Extended ras gene mutation testing in metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy: American Society of Clinical Oncology provisional clinical opinion Update 2015. *J Clin Oncol* 2016;34:179–85.

9 De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010;11:753–62.

10 Schirripa M, Cremolini C, Loupakis F, et al. Role of NRAS mutations as prognostic and predictive markers in metastatic colorectal cancer. *Int J Cancer*

2015;136:83–90.
11 Benson AB, 3rd, Venook AP, Cederquist L, et al. *Colon cancer*, version 1.2017, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2017;15:370–98.
12 Yoshino T, Arnold D, Taniguchi H, et al. Pan-Asian adapted ESMO consensus guidelines for the management of patients with metastatic colorectal cancer: a JSMO-ESMO initiative endorsed by CSCO, KACO, MOS, SSO and TOS. *Ann Oncol* 2018;29:44–70.
13 Hashiguchi Y, Muro K, Saito Y, et al. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2019 for the treatment of colorectal cancer. *Int J Clin Oncol* 2020;25:1–42.
14 Gazzaniga P, Raimondi C, Urbano F et al. EGFR inhibitor as second-line therapy in a patient with mutant RAS metastatic colorectal cancer: circulating tumor DNA to personalize treatment. *JCO Precis Oncol* 2018;2:1–6.
15 Sugimachi K, Sakimura S, Kuramitsu S, et al. Serial mutational tracking in surgically resected locally advanced colorectal cancer with neoadjuvant chemotherapy. *Br J Cancer* 2018;119:419–23.
16 Li W, Qiu T, Guo L et al. Major challenges related to tumor biological characteristics in accurate mutation detection of colorectal cancer by next-generation sequencing. *Cancer Lett* 2017;410:92–9.
17 Raimondi C, Nicolazzo C, Belardinilli F, et al. Transient disappearance of RAS mutant clones in plasma: A counterintuitive clinical use of EGFR inhibitors in RAS mutant metastatic colorectal cancer. *Cancers (Basel)* 2019;11.
18 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;147:1185–9.
19 Henry J, Willis J, Parseghian CM, et al. NeoRAS: incidence of RAS reversion from RAS mutated to RAS wild type. *J Clin Oncol* 2020;38:180.
20 Fernández Montes A, Martinez Lago N, De la Cámara Gómez J, et al. FOLFIRI plus panitumumab as second-line treatment in mutated RAS metastatic colorectal cancer patients who converted to wild type RAS after receiving first-line FOLFOX/CAPOX plus bevacizumab-based treatment: Phase II CONVERTIX trial. *Ann Oncol* 2019;30:iv23–4.
21 Sunakawa Y, Nakamura M, Ishizaki M, et al. RAS mutations in circulating tumor DNA and clinical outcomes of rechallenge treatment with anti-EGFR antibodies in patients with metastatic colorectal cancer. *JCO Precis Oncol* 2020;4:898–911.
22 Nicolazzo C, Barault L, Caponnetto S, et al. Circulating methylated DNA to

monitor the dynamics of RAS mutation clearance in plasma from metastatic colorectal cancer patients. *Cancers (Basel)* 2020;12.

23 Bouchahda M, Saffroy R, Karaboué A, et al. Undetectable RAS-mutant clones in plasma: possible implication for anti-EGFR therapy and prognosis in patients with RAS-mutant metastatic colorectal cancer. *JCO Precis Oncol* 2020;4:1070–9.

24 Mayer RJ, Van Cutsem E, Falcone A, et al. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N Engl J Med* 2015;372:1909–19.

25 Kuboki Y, Nishina T, Shinozaki E, et al. TAS-102 plus bevacizumab for patients with metastatic colorectal cancer refractory to standard therapies (C-TASK FORCE): an investigator-initiated, open-label, single-arm, multicentre, phase 1/2 study. *Lancet Oncol* 2017;18:1172–81.

26 Pfeiffer P, Yilmaz M, Möller S, et al. TAS-102 with or without bevacizumab in patients with chemorefractory metastatic colorectal cancer: an investigator-initiated, open-label, randomised, phase 2 trial. *Lancet Oncol* 2020;21:412–20.

27 Grothey A, Van Cutsem E, Sobrero A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013;381:303–12.

28 Diehl F, Schmidt K, Durkee KH, et al. Analysis of mutations in DNA isolated from plasma and stool of colorectal cancer patients. *Gastroenterology* 2008;135:489–98.

29 Odegaard JI, Vincent JJ, Mortimer S, et al. Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. *Clin Cancer Res* 2018;24:3539–49.

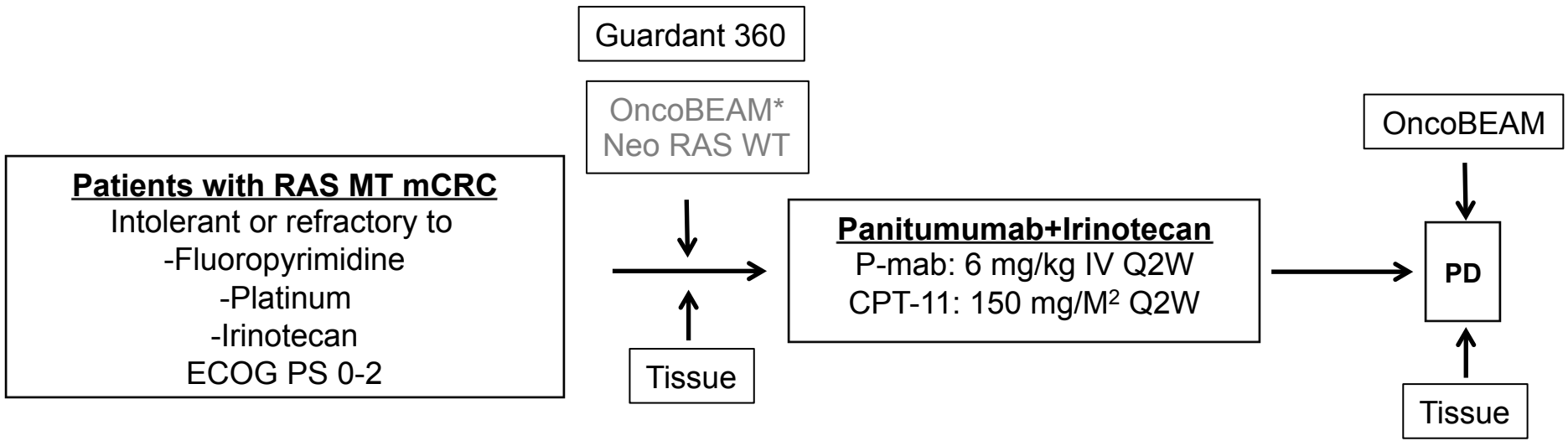
Figure legend

Fig. 1 Overall trial scheme.

Liquid biopsies for the OncoBEAM RAS CRC kit and Guardant360 will be performed at baseline, and the OncoBEAM RAS CRC kit will be used after the discontinuation of the protocol treatment.

OncoBEAM: OncoBEAM RAS CRC kit; *Substitution of results immediately before enrollment.

Overall Trial Scheme



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Ethics and dissemination: This study was approved by the certified review board of National Cancer Center Hospital. The main results of the trial will be presented in international meetings and in medical journals.

Trial registration: jRCT, s031210565. Registration date, January 20, 2022.

Keywords: Metastatic colorectal cancer, Circulating tumor DNA, Liquid biopsy, NeoRAS, EGFR inhibitor

Strengths and limitations of this study

- The C-PROWESS trial is a prospective multicenter, single-arm, phase II trial.
- The trial is designed to assess the efficacy and safety of panitumumab plus irinotecan therapy in patients with NeoRAS
- However, no comparator is a limitation.

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RAS mutations (MTs) induce the activation of the protein kinase pathway and promote carcinogenesis and cancer growth.[1] Patients with RAS MT metastatic colorectal cancer (mCRC) have a poorer prognosis than those with RAS wild-type (WT) mCRC.[2,3] Epidermal growth factor receptor (EGFR) inhibitors (such as cetuximab and panitumumab), which are key drugs for mCRC, are ineffective for patients with RAS MT mCRC (KRAS/NRAS exons 2, 3, and 4).[4-10]

International guidelines recommend RAS genetic testing prior to the administration of an EGFR inhibitor in patients with mCRC.[11-13] Repeat biopsies are not performed in routine clinical practice to monitor the RAS MT status;[11-13] the consistency of the RAS MT status before and after chemotherapy remains unclear.

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]

On the other hand, there have been some reports that RAS MT observed at the initial diagnosis converted to RAS WT after treatment.[22] These cases have been called “NeoRAS WT” mCRC.[22] The incidence of NeoRAS WT mCRC has been reported to range from 10.7% to 40% when assessed in tumor tissue samples,[23, 24] and from 18.8% to 83.3% in the circulating tumor DNA (ctDNA).[25-31]

There have been several reports of the use of EGFR inhibitors in patients with NeoRAS WT. Mohamed et al., in a proof-of-concept study of EGFR inhibitors in patients with NeoRAS WT,[31] reported that the objective response rate was 55.6% and progression-free survival (PFS) was 9 months in patients with NeoRAS WT mCRC treated with fluorouracil, folinic acid, irinotecan, and cetuximab.[31] This result suggested that EGFR inhibitors may be effective in patients with NeoRAS WT mCRC.

Although retrospective analyses and proof-of-concept studies have indicated the potential efficacy of EGFR inhibitors in NeoRAS WT mCRC,[22, 31] the safety and efficacy have not been validated prospectively. Furthermore, the definition of NeoRAS WT mCRC has not been established.

Therefore, this trial will evaluate the efficacy of panitumumab and irinotecan in patients with NeoRAS WT mCRC confirmed in ctDNA after prior treatment.

METHODS AND ANALYSIS

Trial design

This trial is a multicenter, single-arm, phase II trial to investigate the safety and effi**C**acy of Panitumumab and i**R**in**O**tecan in NeoRAS **W**ild type m**E**ta**S**tatic colorectal cancer patient**S** (C-PROWESS trial). The overall trial scheme is illustrated in Fig. 1.

Patients

The key eligibility criteria are as follows: 1) RAS MT (KRAS/NRAS exon 2,3,4) and BRAF V600E WT mCRC initially diagnosed in the tumor tissue; 2) refractory or intolerant to fluoropyrimidine, oxaliplatin, and irinotecan; 3) RAS WT in ctDNA (mutant allele frequencies of all RAS ≤ 0.1%) within 28 days prior to enrollment; 4) an Eastern Cooperative Oncology Group performance status ≤ 2; and 5) preserved organ function. The details of the eligibility criteria are presented in Table 1.

Table 1 Eligibility criteria for the C-PROWESS trial

Inclusion criteria
1) Histologically proven diagnosis of colorectal adenocarcinoma 2) Advanced or recurrent colorectal cancer (excluding appendix and anal canal cancer)
3) RAS mutation (MT) (KRAS/NRAS exon 2, 3, or 4 MT) confirmed by tumor histology prior to first-line chemotherapy

4) Confirmation of refractoriness or intolerance to previous treatments with chemotherapy, including fluoropyrimidines, oxaliplatin, or irinotecan (irinotecan is applied to refractory disease only), regardless of prior treatment with trifluridine tipiracil hydrochloride, regorafenib, or angiogenesis inhibitors

5) Confirmation of the RAS WT within 28 days from the test result date by ctDNA analysis using the OncoBEAM^{TM} RAS CRC KIT

6) At least one measurable lesion according to the RECIST version 1.1 criteria evaluated by CT or MRI within 28 days before registration

7) Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2

8) Age ≥ 20 years

9) Adequate organ function (bone marrow, liver, renal function) as defined by the following laboratory values obtained within 15 days prior to enrollment in the study:
a.Absolute neutrophil count ≥ 1500/ mm^3 b.Platelets ≥ 75000/mm^3
c.Serum total bilirubin ≤ 1.5 mg/dLd; Serum AST(GOT) and ALT(GPT) ≤ 100 U/L (except for patients with tumor involvement of the liver who must have AST and ALT ≤ 200 U/L)

10) A life expectancy of at least 60 days

11) Written informed consent

Exclusion criteria

1) Evidence of BRAF V600E MT by tumor histology

2) Treated with blood transfusion, blood products, or hematopoietic factor products such as Granulocyte Colony Stimulating Factor within 7 days prior to enrollment in this study

3) A history of severe drug hypersensitivity or severe drug allergy

4) Active infection (fever of 38°C or higher due to infection)

5) Ascites, pleural effusion, or pericardial effusion requiring continuous drainage

6) Uncontrolled diabetes mellitus

7) Uncontrolled hypertension

8) Patients who have been treated with any of the following treatments prior to starting the study drug: a.Extensive surgery ≤ 4 weeks prior to starting the study drug (e.g., surgical treatment with organ resection, excluding colostomy)
b.Proctocolectomy ≤ 2 weeks prior to starting the study drug
c.Any chemotherapy ≤ 2 weeks prior to starting the study drug
d.Radiotherapy ≤ 2 weeks prior to starting the study drug

9) Clinically significant electrocardiographic abnormality or clinically significant cardiovascular accidents within 6 months prior to study enrollment, including myocardial infarction, severe unstable angina, or New York Heart Association functional classification class III or IV congestive heart failure
10) Patients with severe lung disease (interstitial pneumonia, pulmonary fibrosis, or severe emphysema)
11) History of clinically significant mental disorder or central nervous system disorder
12) Symptomatic brain metastasis or clinically suspected brain metastasis upon symptoms
13) Diarrhea that interferes with daily life
14) Intestinal paralysis, intestinal obstruction
15) Co-existing active malignancies
16) Pregnant or lactating women; women of childbearing potential or men with female partners of childbearing potential who are unwilling to use a highly effective method of contraception or avoid intercourse during and upon completion of the study
17) Patients who have been assessed by the site physician as inappropriate for this study
18) Patients who have been treated with EGFR inhibitors prior to starting the study drug

Treatment

Patients will receive panitumumab at 6 mg/kg and irinotecan at 150 mg/m^2 biweekly until progressive disease, unacceptable toxicity, withdrawal of informed consent, or death. The starting dose of irinotecan can be reduced to 120 mg/m^2 according to UGT1A1 status (homozygosity/double heterozygosity).

Outcomes and statistical considerations

The primary endpoint of the C-PROWESS trial is response rate (RR), defined as the proportion of patients who achieve complete or partial response by the investigator's assessment. The secondary endpoints include PFS, overall survival (OS), disease control rate, incidences of adverse events, and the ratio of Neo RAS WT mCRC after failure of fluoropyrimidines, oxaliplatin, and irinotecan. The response is evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, using computed tomography at 6 and 12 weeks after the start of the protocol treatment and repeated every 8 weeks thereafter. The RR threshold is set at 4%,

according to the results of previous clinical trials with tipiracil/trifluridine (+ bevacizumab)[32-34] or regorafenib.[35] The required sample size is 30, whereas an RR of 15% is deemed to be promising (one-sided α = 0.10; β = 0.2). The primary endpoint is planned to be analyzed in all the patients that receive at least one dose of the protocol treatment and who satisfy all the inclusion and exclusion criteria. Participant enrolment started on 1 February 2022 and will end on 31 January 2023.

Biomarker analysis

Samples for liquid biopsies are planned to be collected at baseline and after discontinuation of the protocol treatment (Fig. 1). The ctDNA will be analyzed using a highly sensitive digital polymerase chain reaction method, the OncoBEAM RAS CRC kit, and targeted next-generation sequencing, Guardant360. The OncoBEAM RAS CRC kit, which has been approved in Japan to detect RAS mutations in the ctDNA derived from mCRC, detects 34 mutations in KRAS/NRAS codons 12, 13, 59, 61, 117, and 146 in plasma.[36] Guardant360, a hybrid capture-based next-generation sequencing panel of the ctDNA developed by Guardant Health, detects other gene alterations.[37] Exploratory analyses will be performed to identify the proportion of patients without RAS and other mutations related to resistance to anti-EGFR inhibitors (defined as “True NeoRAS WT”). The clinical outcomes (RR, PFS, OS, and disease control rate) of patients with true NeoRAS WT receiving panitumumab and irinotecan combination therapy will be compared to those of patients without true NeoRAS WT and the overall study population. Moreover, the clinical outcomes will be compared according to the presence or absence of each genetic and epigenetic abnormality using exome sequencing and immunohistochemical staining in tissue samples and in ctDNA analysis before and after treatment with the protocol treatment using NGS, to explore the relationship with clinical outcomes and the mechanism of resistance.

Trial organization

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

Ethics and dissemination

This study was approved by the certified review board of the national cancer center hospital (jRCT, s031210565; Registration date, January 20, 2022.). The main results of the trial will be presented in international meetings and in medical journals.

Clinical Questions

The clinical questions to be addressed in this study will be the definition of NeoRAS WT mCRC and the therapeutic effect of EGFR inhibitors on NeoRAS WT mCRC.

First, when “NeoRAS WT” mCRC is defined by RAS MT status only, it is not possible to determine whether the RAS MT has completely disappeared or has not been measured due to a low volume of ctDNA. Therefore, we will perform NGS analysis using ctDNA before administering the protocol treatment to confirm the incidence of “True NeoRAS WT” mCRC patients. “True NeoRAS WT” mCRC is defined as the disappearance of RAS and the detection of other genetic mutations after treatment. If the proportion of “True NeoRAS WT” mCRC patients is clarified, it may be even more useful in the enrichment of the population that will respond to treatment with EGFR inhibitors.

Second, our trial will evaluate the relationship between gene mutations other than RAS in ctDNA and the efficacy of EGFR inhibitors in NeoRAS WT mCRC. The ORR, PFS, and OS may differ according to the presence or absence of some genetic abnormality that may lead to primary resistance to EGFR inhibitors, such as EGFR extracellular domain, Erb-B2 Receptor Tyrosine kinase 2, Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit alpha, among others, in the ctDNA (NGS) prior to protocol treatment.

In addition, we plan to measure the ctDNA RAS again after the administration of the protocol treatment. This will allow us to understand how frequently RAS MT reappears after treatment and whether the same RAS MT reappears or different variants of RAS MT newly appear. If the same RAS variant allele frequency increases after the administration of treatment, it may be due to tissue heterogeneity in which dominant clones with/out RAS mutation in tumors are changed by an EGFR inhibitor. Furthermore, the mechanism of resistance to EGFR inhibitors will be clarified.

Limitations

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

Summary

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.

Patient and public involvement statement

No patient involvement.

Acknowledgments

We thank the independent data monitoring committee (Atsushi Sato, Yasuo Hamamoto, and Hidekazu Kuramochi) and the data managers (Yumiko Izoe, Tomoko Sanada, Hitomi Hannan, and Yuki Horiike).

Authors' contributions

HO, AT, KK, NB, KY, and ES, as task managers, participated in the coordination of this trial, the design and the writing of the protocol, data collection, data analysis, data interpretation, and writing of the manuscript. AT, YK, DN, MS, DT, KO, RS, KO, and TW, as the protocol preparation committee, participated in this trial, including design and writing of the protocol, data collection, data analysis, data interpretation, and preparation of the manuscript. NI, as the chief of statistical analysis, participated in the statistical setting of the trial, design, and data analysis. All the authors have reviewed and approved the final manuscript.

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Competing interests:

Dr. Kensei Yamaguchi has received honoraria from Daiichi-Sankyo.

Availability of data and materials

Not applicable.

References

[1] Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. *Nat Rev Cancer*. 2011; 11: 761-74.

[2] Richman SD, Seymour MT, Chambers P, et al. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol*. 2009; 27: 5931-7.

[3] Cremolini C, Loupakis F, Antoniotti C, et al. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. *Lancet Oncol*. 2015; 16: 1306-15.

[4] Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med*. 2009; 360: 1408-17.

[5] Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*. 2008; 359: 1757-65.

[6] Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol*. 2010; 28: 4697-705.

[7] Loupakis F, Ruzzo A, Cremolini C, et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer.* 2009; 101: 715-21.

[8] Allegra CJ, Rumble RB, Hamilton SR, et al. Extended RAS Gene Mutation Testing in Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015. *J Clin Oncol*. 2016; 34: 179-85.

[9] De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol.* 2010; 11: 753-62.

[10] Schirripa M, Cremolini C, Loupakis F, et al. Role of NRAS mutations as prognostic and predictive markers in metastatic colorectal cancer. *Int J ancer*. 2015; 136: 83-90.

[11] Benson AB, 3rd, Venook AP, Cederquist L, et al. Colon Cancer, Version 1.2017, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network* : JNCCN. 2017; 15: 370-98.

[12] Yoshino T, Arnold D, Taniguchi H, et al. Pan-Asian adapted ESMO consensus guidelines for the management of patients with metastatic colorectal

cancer: a JSMO-ESMO initiative endorsed by CSCO, KACO, MOS, SSO and TOS. *Ann Oncol.* 2018; 29: 44-70.
[13] Hashiguchi Y, Muro K, Saito Y, et al. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2019 for the treatment of colorectal cancer. *Int J Clin Oncol.* 2020; 25: 1-42.
[14] Siravegna G, Marsoni S, Siena S, Bardelli A. Integrating liquid biopsies into the management of cancer. *Nat Rev Clin Oncol*. 2017; 14: 531-48.
[15] Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nature reviews Cancer.* 2017; 17: 223-38.
[16] Osumi H, Shinozaki E, Yamaguchi K, Zembutsu H. Clinical utility of circulating tumor DNA for colorectal cancer. *Cancer Sci.* 2019; 110: 1148-55.
[17] Misale S, Yaeger R, Hobor S, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature.* 2012; 486: 532-6.
[18] Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med.* 2015; 21: 795-801.
[19] Cremolini C, Rossini D, Dell'Aquila E, et al. Rechallenge for Patients With RAS and BRAF Wild-Type Metastatic Colorectal Cancer With Acquired Resistance to First-line Cetuximab and Irinotecan: A Phase 2 Single-Arm Clinical Trial. *JAMA Oncol.* 2018.
[20] Sartore-Bianchi A, Pietrantonio F, Lonardi S, et al. Phase II study of anti-EGFR rechallenge therapy with panitumumab driven by circulating tumor DNA molecular selection in metastatic colorectal cancer: The CHRONOS trial. *J Clin Oncol*. 2021; 39: 3506.
[21] Osawa H, Shinozaki E, Nakamura M, et al. Phase II study of cetuximab rechallenge in patients with ras wild-type metastatic colorectal cancer: E-rechallenge trial. *Annals of Oncology*. 2018; 29: viii161.
[22] Gazzaniga P, Raimondi C, Urbano F, Cortesi E. EGFR Inhibitor as Second-Line Therapy in a Patient With Mutant RAS Metastatic Colorectal Cancer: Circulating Tumor DNA to Personalize Treatment. *JCO Precis Oncol.* 2018: 1-6.
[23] Sugimachi K, Sakimura S, Kuramitsu S, et al. Serial mutational tracking in surgically resected locally advanced colorectal cancer with neoadjuvant chemotherapy. *Br J Cancer*. 2018; 119: 419-23.
[24] Li W, Qiu T, Guo L, Ying J. Major challenges related to tumor biological characteristics in accurate mutation detection of colorectal cancer by next-generation sequencing. *Cancer Lett.* 2017; 410: 92-9.
[25] Raimondi C, Nicolazzo C, Belardinilli F, et al. Transient Disappearance of RAS Mutant Clones in Plasma: A Counterintuitive Clinical Use of EGFR Inhibitors in RAS Mutant Metastatic Colorectal Cancer. *Cancers (Basel).* 2019; 11.
[26] 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: 1185-9.

[27] Henry J, Willis J, Parseghian CM, et al. NeoRAS: Incidence of RAS reversion from RAS mutated to RAS wild type. *J Clin Oncol.* 2020; 38: 180.
[28] Fernández Montes A, Martinez Lago N, De la Cámara Gómez J, et al. FOLFIRI plus panitumumab as second-line treatment in mutated RAS metastatic colorectal cancer patients who converted to wild type RAS after receiving first-line FOLFOX/CAPOX plus bevacizumab-based treatment: Phase II CONVERTIX trial. *Ann Oncol.* 2019; 30: iv23-iv4.
[29] Sunakawa Y, Nakamura M, Ishizaki M, et al. RAS Mutations in Circulating Tumor DNA and Clinical Outcomes of Rechallenge Treatment With Anti-EGFR Antibodies in Patients With Metastatic Colorectal Cancer. *JCO Precis Oncol.* 2020: 898-911.
[30] Nicolazzo C, Barault L, Caponnetto S, et al. Circulating Methylated DNA to Monitor the Dynamics of RAS Mutation Clearance in Plasma from Metastatic Colorectal Cancer Patients. *Cancers (Basel).* 2020; 12.
[31] Bouchahda M, Saffroy R, Karaboué A, et al. Undetectable RAS-Mutant Clones in Plasma: Possible Implication for Anti-EGFR Therapy and Prognosis in Patients With RAS-Mutant Metastatic Colorectal Cancer. *JCO Precis Oncol.* 2020: 1070-9.
[32] Mayer RJ, Van Cutsem E, Falcone A, et al. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N Engl J Med.* 2015; 372: 1909-19.
[33] Kuboki Y, Nishina T, Shinozaki E, et al. TAS-102 plus bevacizumab for patients with metastatic colorectal cancer refractory to standard therapies (C-TASK FORCE): an investigator-initiated, open-label, single-arm, multicentre, phase 1/2 study. *Lancet Oncol.* 2017; 18: 1172-81.
[34] Pfeiffer P, Yilmaz M, Möller S, et al. TAS-102 with or without bevacizumab in patients with chemorefractory metastatic colorectal cancer: an investigator-initiated, open-label, randomised, phase 2 trial. *Lancet Oncol.* 2020; 21: 412-20.
[35] Grothey A, Van Cutsem E, Sobrero A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet*. 2013; 381: 303-12.
[36] Diehl F, Schmidt K, Durkee KH, et al. Analysis of mutations in DNA isolated from plasma and stool of colorectal cancer patients. Gastroenterology. 2008; 135: 489-98.
[37] Odegaard JI, Vincent JJ, Mortimer S, et al. Validation of a Plasma-Based Comprehensive Cancer Genotyping Assay Utilizing Orthogonal Tissue- and Plasma-Based Methodologies. *Clin Cancer Res*. 2018; 24: 3539-49.

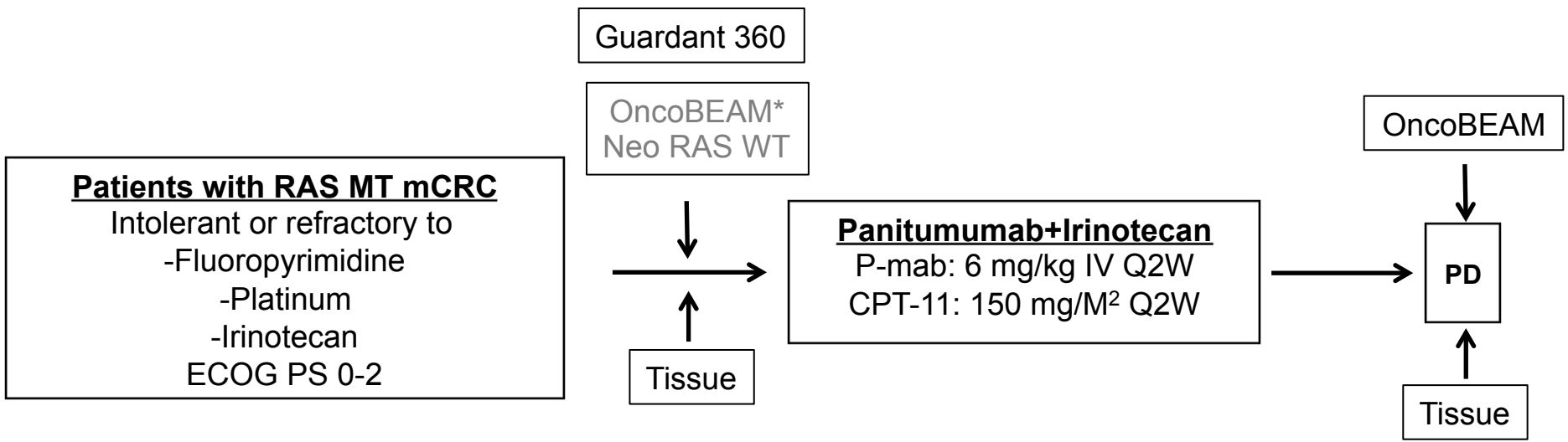
Figure legend

Fig. 1 Overall trial scheme.

Liquid biopsies for the OncoBEAM RAS CRC kit and Guardant360 will be performed at baseline, and the OncoBEAM RAS CRC kit will be used after the discontinuation of the protocol treatment.

OncoBEAM: OncoBEAM RAS CRC kit; *Substitution of results immediately before enrollment.

Overall Trial Scheme



SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym (Page1, Line 2-4)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry (Page3, Line 23)
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support (Page11, Line 18-19)
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors (Page11, Line 9-16)
	5b	Name and contact information for the trial sponsor (No involvement)
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities (No involvement)
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) (Page11, Line 5-7)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention (Page 5, Line 2-33, Page 6, Line 1-4)
	6b	Explanation for choice of comparators (No comparator)
Objectives	7	Specific objectives or hypotheses (Page 6, Line 3-4)

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) (Page 6, Line 8-11)
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained (Page 9, Line 25)
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) (Table1)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended (Page 8, Line 9-21, Page 9, Line 1)
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) (Page 9, Line 1-2)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations (Page 9, Line 17-20)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol (Page 8, Line 20-21)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) (Page 9, Line 5-23)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval (Page 9, Line 28-29)
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) (Table1)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site (Page 11, Line 22)
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates (Supplemental)
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.