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Utility of circulating tumour DNA for prognosis and prediction of therapeutic effect in locally recurrent rectal cancer: study protocol for a multi-institutional, prospective observational study (JCOG1801A1, CAP-LR study)

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ABSTRACT

Introduction In locally recurrent rectal cancer (LRRC), surgery is a standard treatment for resectable disease. However, short-term and long-term outcomes are unsatisfactory due to the invasive nature of surgical procedures and the high proportion of local recurrence. Consequently, the identification of reliable prognostic and predictive biomarkers to guide treatment decisions may improve outcomes. The presence of circulating tumour DNA (ctDNA) in plasma after surgery may signify the presence of minimal residual disease (MRD) in various cancers. Therefore, we have launched a multi-institutional prospective observational study of ctDNA for MRD detection in conjunction with JCOG1801, a randomised, controlled phase III trial evaluating the efficacy of preoperative chemoradiotherapy (pre-CRT) compared with up-front surgery for LRRC (jRCTs031190076, NCT04288999).

Methods and analysis JCOG1801A1 is the first correlative study that assesses ctDNA in LRRC patients enrolled in JCOG1801. Patients randomised to up-front surgery will provide whole blood samples at three time points (prior to surgery, after surgery and after postoperative chemotherapy); those to pre-CRT will provide at five time points (prior to pre-CRT, after pre-CRT, prior to surgery, after surgery and after postoperative chemotherapy). Cell-free DNA will be extracted from plasma and analysed by Guardant Reveal, a tumour tissue–agnostic assay that assesses both genomic alterations and methylation patterns to determine the presence or absence of ctDNA. We will compare the prognosis and treatment response of patients according to their ctDNA status after surgery and at other time points.

Ethics and dissemination The study protocol received approval from the Institutional Review Board of National Cancer Center Hospital East on behalf of the participating institutions in February 2023. The study is conducted in accordance with the precepts established in the Declaration of Helsinki and Ethical Guidelines for Medical and Biological Research Involving Human Subjects. Written informed consent will be obtained from all eligible patients prior to registration.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first multi-institutional prospective study to assess the clinical utility of circulating tumour DNA (ctDNA) as a biomarker for patients with resectable locally recurrent rectal cancer (LRRC), concurrent with the randomised phase III trial (JCOG1801).
⇒ Blood samples will be obtained according to a pre-established protocol schedule in JCOG1801, thus ensuring reliable results for clinical application.
⇒ The analysis of plasma samples will be performed using Guardant Reveal, which can detect ctDNA with a high level of sensitivity, obviating the need for specific molecular information from tissue samples.
⇒ This study has the potential to offer insights into the utility of ctDNA as a prognostic factor or to predict treatment response in LRRC patients.
⇒ The limitations of this study include its observational design and lack of robust statistical power due to the relatively small sample size.

INTRODUCTION

Rectal cancer, ranked as the eighth-leading cause of cancer-related deaths worldwide, is initially treated through a combination of surgical resection, chemotherapy and chemoradiotherapy according to the extent of the disease.[1 2] Nevertheless, the incidence of recurrence after curative resection is alarmingly high, with a proportion of 24.3%, frequently manifesting as local recurrence (8.8%).[3] In Japan, up-front surgical resection is the mainstay of treatment for locally recurrent rectal cancer (LRRC). Preoperative

chemoradiotherapy (pre-CRT) has been proposed as an alternative, given favourable results from previous studies.[4 5] Thus, JCOG1801, a randomised, controlled phase III trial evaluating the efficacy of pre-CRT in comparison with up-front surgery for LRRC, was launched in August 2019 (jRCTs031190076, NCT04288999).[6] However, the invasiveness of surgical procedures and the high proportion of local recurrence emphasise the urgent need for the development of predictive biomarkers to inform treatment decisions that can optimise outcomes for LRRC patients.

Circulating tumour DNA (ctDNA) is a rapidly developing blood-based biomarker that is available for use in clinical practice for determining minimal residual disease (MRD) after surgical intervention in a variety of cancers.[7] In colorectal cancer, several studies have indicated that patients deemed ctDNA-positive after surgical resection are more prone to recurrence than those classified as ctDNA-negative.[8–11] Previous research on ctDNA in rectal cancer has demonstrated that patients undergoing preoperative treatment exhibit a reduction or clearance of ctDNA and tend to be more likely to achieve pathological complete response (pCR) compared with those with residual ctDNA.[12–15] The DYNAMIC trial demonstrated the utility of postoperative ctDNA assessment by minimising the proportion of patients with stage II colon cancer who received chemotherapy without negatively impacting long-term outcomes.[16] These findings suggest that ctDNA analysis could serve as a biomarker for guiding treatment adaptation, detecting MRD or early recurrence, and monitoring treatment response in various forms of cancer.[17]

Although numerous studies have explored the potential of ctDNA analysis in colorectal cancer, no investigations have been performed in LRRC, rendering the utility of ctDNA uncertain. Hence, this study was designed to assess the ctDNA status of patients enrolled in JCOG1801 using Guardant Reveal, a tumour tissue–agnostic liquid biopsy system developed by Guardant Health (Redwood City, California, USA).[18] In participants diagnosed with LRRC, there are often challenges associated with obtaining a sufficient quantity of tissue samples through pretreatment biopsy. Additionally, it is common for the initial surgery for the primary tumour to be performed at a different hospital that is not involved in this study. Therefore, we have chosen to use the Guardant Reveal, which offers the advantage of not requiring tissue collection. This assay can detect ctDNA with high sensitivity, analysing DNA methylation and genomic mutations without the need for tissue sampling. The results of this study may enable the prediction of the therapeutic effect of pre-CRT, leading to informed decisions regarding the necessity of additional treatments or invasive surgery in LRRC patients. Furthermore, the detection of MRD through ctDNA evaluation may guide decisions regarding postoperative adjuvant chemotherapy. Additionally, ctDNA status after postoperative adjuvant chemotherapy may serve as a predictor of recurrence and influence the intensity of follow-up.

This is the first multi-institutional prospective study to evaluate the utility of ctDNA as a predictive marker of prognosis and therapeutic effect in LRRC patients. The confirmation of ctDNA's role in this study has the potential to inform individualised treatment strategies, including decisions regarding supplementary interventions or optimal adjuvant chemotherapy. The Japan Clinical Oncology Group (JCOG) Protocol Review Committee approved the study protocol in January 2023. The study protocol was approved by an institutional review board in each participating institution. Patient enrolment will begin in April 2023.

METHODS AND ANALYSIS

Overall study design

JCOG1801A is a correlative study associated with JCOG1801, a randomised phase III clinical trial exploring the efficacy of pre-CRT for LRRC (jRCTs031190076, NCT04288999).

Eligibility criteria

Patients must fulfil the previously reported eligibility criteria for JCOG1801 and provide written informed consent to participate in this correlative study.[6] Whole blood samples will be collected at various prespecified time points. The main eligibility criteria for JCOG1801 are as follows: age 20–80 years, Eastern Cooperative Oncology Group performance status 0–1, LRRC after initial treatment with R0/1 by surgery or ER0/1 by endoscopic resection, the main tumour located in the pelvis as diagnosed on contrast-enhanced CT and MRI imaging, no distant metastasis, resectable LRRC but not amenable to endoscopic resection and adequate organ function. In this study, biopsy prior to registration is not mandatory. Confirmation of local recurrence requires the implementation of any of the subsequent techniques after treatment for the initial rectal cancer; histopathological diagnosis of the recurrent lesion, diagnosis based on at least two modalities such as contrast-enhanced CT, contrast-enhanced MRI, or positron emission tomography (PET), or the observation of chronological lesion progression on more than one modality including contrast-enhanced CT, MRI or PET.

Study schema and plasma sampling schedule

In JCOG1801, patients are randomised in a 1:1 ratio to receive either up-front surgery or pre-CRT. In the up-front surgery arm, patients undergo curative resection followed by 24 weeks of adjuvant chemotherapy, either CAPOX (a combination of capecitabine and oxaliplatin) or modified FOLFOX6 (a combination of fluorouracil, leucovorin and oxaliplatin). Patients with poor performance status or peripheral neuropathy will receive capecitabine or 5-FU plus leucovorin. In the pre-CRT arm, patients receive pre-CRT, consisting of capecitabine (1650 mg/m^2/day for 28 days) and radiotherapy (50.4 Gy/28 Fr), followed by curative surgery and adjuvant chemotherapy as in the

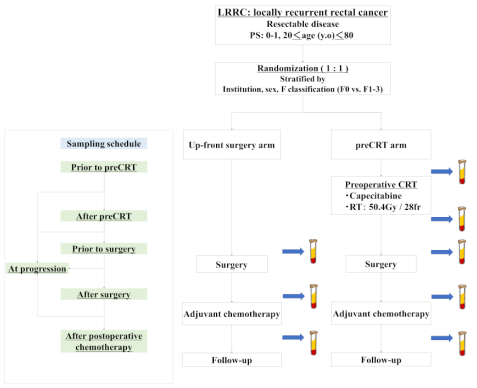

Figure 1 Study scheme and sampling schedule of JCOG1801A1. Blood sampling points in the up-front surgery arm will occur prior to surgery, after surgery and after completion of postoperative chemotherapy. Blood collection in the pre-CRT arm will occur prior to pre-CRT, after completion of pre-CRT, prior to surgery, after surgery and after completion of postoperative chemotherapy. If patients experience progressive disease during the study period, blood samples will be collected at the time of progression, and patients will be excused from further blood sampling. The blood samples will be shipped to a central laboratory to analyse ctDNA status (Guardant Health, Redwood City, California, USA). ctDNA, circulating tumour DNA; pre-CRT, preoperative chemoradiotherapy.

up-front surgery arm. In this correlative study, peripheral blood samples (40 mL) will be collected into four 10 mL Streck Cell-Free DNA BCT tubes at three time points in the up-front surgery arm and five time points in the pre-CRT arm. Out of the 40 mL blood samples collected in this study, 20 mL undergo immediate processing, while the remaining 20 mL are stored for potential repeat analysis, if required. The study schema and sampling points are described in figure 1. Blood sampling points in the up-front surgery arm will occur prior to surgery, after surgery and after completion of postoperative chemotherapy. Blood collection in the pre-CRT arm will occur prior to pre-CRT, after completion of pre-CRT, prior to surgery, after surgery and after completion of postoperative chemotherapy. If patients experience progressive disease during the study period, blood samples will be collected at the time of progression, and patients will be excused from further blood sampling. The blood samples will be shipped to a central laboratory (Guardant Health).

Evaluation of ctDNA

Plasma is separated from each blood sample via centrifugation and preserved at −80°C until cell-free DNA (cfDNA) extraction. The cfDNA is extracted from plasma and subjected to analysis using Guardant Reveal. After extraction, the cfDNA is enriched in its targeted regions, followed by sequencing. The sequencing data are analysed through a bioinformatics software pipeline designed to detect ctDNA originating from tumour cells on the basis of a multitude of analytical features including genomic variation (single nucleotide variants and Indel: insertions/deletions) and epigenomic variation (DNA methylation). The results of the analysis are reported as either ctDNA-positive or ctDNA-negative, determined through an algorithm originally trained and developed using normal samples, tumour samples and other patient background information related to epigenomic alterations. A sample exhibiting positivity for epigenomic alterations is considered ctDNA-positive and will also report any detected genomic somatic mutations, whereas a sample that is epigenomic-alteration negative is classified as ctDNA-negative. In this study, samples will be preserved after collection and subsequently subjected to retrospective analysis for the identification of ctDNA. The analysis will not be conducted synchronously with the longitudinal course of the study, nor will the findings be disclosed to the physician or the patient; thus, the assay shall not be used to determine a therapeutic approach.

Outcome measures/endpoints

Comparison of clinical endpoints, such as relapse-free survival (RFS), local relapse-free survival (LRFS), overall survival (OS), the proportion of local relapse and the proportion of distant relapse, will be conducted based on the ctDNA status after surgery. Similar to the definitions outlined in JCOG1801, RFS is defined as the time from registration in the trial to either the first incidence of relapse or death from any cause and censored at the last date of contact for a living patient. LRFS is defined as the period from registration in the trial to either the first event of local relapse or death from any cause and censored at the last date of contact for a living patient. OS is defined as the time from registration in the trial to death from any cause and censored at the last date of contact for a living patient. The treatment response end points, including the proportion of R0 resection, the response rate of pre-CRT and the pCR rate, will be compared only in the pre-CRT arm and determined through the ctDNA status. The Kaplan-Meier method will be employed to calculate cumulative survival curves, and the Cox proportional-hazards model will be used to estimate HRs, along with their confidence intervals, for RFS, LRFS and OS. The Greenwood formula will be employed to construct the CI for the annual RFS, LRFS and OS. Categorical data-type end points including proportions of local relapse, distant relapse, pathological R0 resection, pre-CRT response rate and pathological complete response rate in the pre-CRT will be analysed by the Fisher's exact test. Additionally, patients after surgery will be divided into those who have received adjuvant chemotherapy and those who have not, and the interaction of the treatment effect between both groups and ctDNA status will be evaluated using a Cox proportional-hazards model. The interaction p value for the proportions of local rerecurrence and distant recurrence will be calculated using a logistic regression model.

Sample size and power calculation

The planned sample size of JCOG1801 is 110 participants, with approximately 40 expected to be enrolled in this correlative study. This is because the present investigation

is a prospective observational study, with approximately 60 patients already enrolled at its inception, we have established a maximum sample size of 40 patients based on eligibility and the availability of blood samples. At the time of the primary analysis, JCOG1801 anticipates 56 events for LRFS, which equates to approximately 21 events among participants in JCOG1801A1. Based on the results from the GALAXY study,[8] an observational study evaluating the association of ctDNA dynamics with clinical outcomes for patients with colorectal cancer in CIRCULATE-Japan, we conjectured that the proportion of ctDNA-positive patients would be 0.2–0.3 and an HR for LRFS comparing ctDNA-positive to ctDNA-negative would be 0.1–0.3. With the assumption of a one-sided alpha of 0.1, the estimated statistical power is within the range of 0.80–0.99, and it is anticipated to remain at or above 0.8.

The analyses of this study, comparing the ctDNA status and clinical end points such as the proportion of patients with a pathological R0 resection, response rate of pre-CRT, and pCR rate in the pre-CRT arm, will be conducted prior to the primary analysis of JCOG1801. The analysis is intended to use follow-up data collected after March 2025, subject to the approval of the JCOG Data and Safety Monitoring Committee. Conversely, the prognostic end points will be analysed following the completion of the primary analysis of JCOG1801.

Patient and public involvement

JCOG data centre regularly organises patient and public involvement conferences to facilitate the exchange of opinions regarding protocol concepts. Regarding this protocol, patients and general public were not involved in the design, conduct, reporting or dissemination plans.

Ethics and dissemination

Ethics approval and consent for participation

Most sites participating in JCOG1801 will also contribute to this study. The JCOG Protocol Review Committee approved the study protocol in January 2023. The study protocol has received approval from the Institutional Review Board of National Cancer Center Hospital East on behalf of the participating institutions in February 2023. Subsequently, the study protocol was approved by an institutional review board in each participating institution. The study is conducted in accordance with the precepts established in the Declaration of Helsinki and Ethical Guidelines for Medical and Biological Research Involving Human Subjects. All patients will receive information for decision-making to participate in this study. Consent to publication includes the general consent form, and each participant's data will be handled anonymously. All participants' information will be stored in the JCOG Data Centre.

Dissemination

The primary results of this study will be published in an article in English.

Individual participant data that underlie the results reported in this article will not be shared because the follow-up of the patients will continue until August 2029. After publication, individual participant data that underlie the results, after deidentification, will be shared if investigators whose proposed use of the data is approved by the Colorectal Cancer Study Group of JCOG identified for this purpose. The data will be available for achieving aims in the approved proposal.

Study status

Patient accrual will start in April 2023, and it is expected to be completed by August 2029.

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Contributors All authors were involved in the conceptualisation of this study. JM and HF was responsible for data curation. TH, YT, MI, KK, JM and HF conducted the formal analysis, applying statistical or mathematical techniques to analyse the data. YT, MI, ST, AT, YK and HF was involved in funding acquisition. JM and HF developed the methodology, designing the methods and procedures used in the research. KK, JM and HF managed the project administration. ST, AT, YT, HF and YK provided key resources. HF was responsible for the software. ST, YT, JM and HF provided supervision, overseeing the research team and ensuring the research was conducted appropriately. TH and YT contributed to visualisation. TH and YT drafted the original manuscript. All authors reviewed and edited the paper and approved the submitted version.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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