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REPORT

DENEB: Development of new criteria for curability after local excision of pathological T1 colorectal cancer using liquid biopsy

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Abstract

According to the current international guidelines, high-risk patients diagnosed with pathological T1 (pT1) colorectal cancer (CRC) who underwent complete local resection but may have risk of developing lymph node metastasis (LNM) are recommended additional intestinal resection with lymph node dissection. However, around 90% of the patients without LNM are exposed to the risk of being overtreated due to the insufficient pathological criteria for risk stratification of LNM. Circulating tumor DNA (ctDNA) is a noninvasive biomarker for molecular residual disease and relapse detection after treatments including surgical and endoscopic resection of solid tumors. The CIRCULATE-Japan project includes a large-scale patient-screening registry of the GALAXY study to track ctDNA status of patients with stage II to IV or recurrent CRC that can be completely resected. Based on the CIRCULATE-Japan platform, we launched DENEB, a new prospective study, within the GALAXY study for patients with pT1 CRC who underwent complete local resection and were scheduled for additional intestinal resection with lymph node dissection based on the standard

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pathologic risk stratification criteria for LNM. The aim of this study is to explore the ability of predicting LNM using ctDNA analysis compared with the standard pathological criteria. The ctDNA assay will build new evidence to establish a noninvasive personalized diagnosis in patients, which will facilitate tailored/optimal treatment strategies for CRC patients.

KEYWORDS
circulating tumor DNA, colorectal cancer, local resection, lymph node metastasis, pathological T1

1 | INTRODUCTION

Developments in endoscopic devices and techniques have enabled curative resection through endoscopic mucosal resection or endoscopic submucosal dissection for patients with T1 colorectal cancer (CRC). Patients with the potential risk of developing lymph node metastasis (LNM) are recommended additional intestinal resection with lymph node dissection based on the histopathological examination of the resected specimen. The indication criteria for additional surgical resection after en bloc local resection of pathological T1 (pT1) CRC with a negative margin in the US, European, and Japanese guidelines include submucosal invasion (>1000 μm), lymphovascular invasion, high-grade tumor budding, and poorly differentiated histology.[1-3] However, the rate of LNM indicated by these guidelines is only 6%-16%.[4] Therefore, around 90% of the patients without LNM are exposed to the risk of being overtreated with a recommendation of additional intestinal resection because of the insufficient/unclear pathological criteria for risk stratification of LNM. To reduce these unnecessary additional surgical resections, a new high-quality marker for determining the risk of LNM is necessary.

Circulating tumor DNA (ctDNA) is a noninvasive biomarker for molecular residual disease (MRD) and relapse detection after treatments including surgical and endoscopic resection of solid tumors.[5] Signatera™ (Natera, Inc) is an innovative, custom-built ctDNA monitoring assay for MRD detection via a personalized blood test tailored to fit the unique signature of somatic single-nucleotide variants found in the individual's tumor.[6] Signatera has been utilized for ctDNA detection in a prospective, multicenter cohort study for stage I to III CRC, wherein, preoperatively, ctDNA was detected in 108 of 122 samples with a sensitivity of 90% in patients with stage III CRC, and at postoperative day 30, ctDNA-positive patients were observed to be seven times more likely to relapse than ctDNA-negative patients, suggesting ctDNA as an effective biomarker that enables stratification of patients with high risk of recurrence.[6] Furthermore, the utility of ctDNA is currently being evaluated in a large platform called CIRCULATE-Japan project. Circulate-Japan encompasses an observational (GALAXY study) and two randomized phase III trials (VEGA and ALTAIR).[7] Based on the CIRCULATE-Japan platform, we launched DENEB, a new prospective study, within the GALAXY study for patients with pT1 CRC who underwent complete local resection and were scheduled for additional intestinal resection with lymph node dissection based on the standard pathologic risk stratification criteria for LNM. The DENEB study will determine the ability of ctDNA to help predict the risk of LNM in patients diagnosed with pT1 CRC after complete local resection compared with the standard pathological criteria.

2 | METHODS/DESIGN

2.1 | Study design

The DENEB study, in which a total of 200 patients will be enrolled, is a prospectively conducted nationwide registry designed to evaluate the relationship between preoperative ctDNA status and pathological factors, especially LNM for patients with pT1 CRC after complete local resection (Figure 1). The method of local resection may be

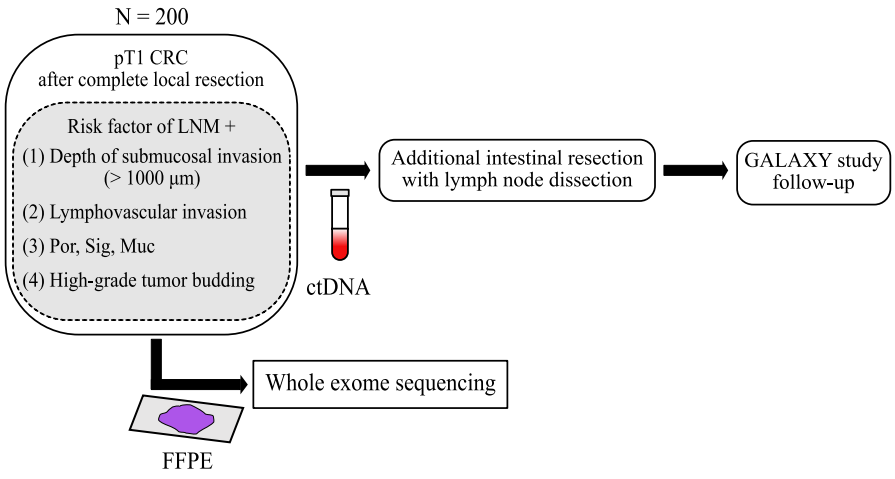

FIGURE 1 DENEB study design. CRC, colorectal cancer; ctDNA, circulating tumor DNA; FFPE, formalin-fixed, paraffin-embedded; LNM, lymph node metastasis; Muc, mucinous carcinoma; Por, poorly differentiated adenocarcinoma; pT1, pathological T1; Sig, signet-ring cell carcinoma

either endoscopic resection or surgical local resection. Key eligibility criteria are based on the GALAXY study: patients aged over 20 years with colorectal adenocarcinoma and Eastern Cooperative Oncology Group performance status of 0 or 1. Additionally, patients with histologically confirmed pT1 CRC who underwent complete local resection with a negative margin and have additional surgical resection scheduled based on the pathologic criteria for risk stratification of LNM will be included (Table 1). The indication criteria for additional surgical resection are (a) depth of submucosal invasion (>1000 μm); (b) lymphovascular invasion; (c) poorly differentiated adenocarcinoma, signet-ring cell carcinoma, or mucinous carcinoma; and (d) high-grade tumor budding (BD2/3) at the site of deepest invasion according to the Japanese guideline.[1] In principle, additional surgery is performed within 3 months after local resection. Locally resected tumor tissue will be collected from registered patients as formalin-fixed, paraffin-embedded sample, and blood samples will be taken within 4 weeks before additional intestinal resection, followed by a personalized, tumor-informed ctDNA assay of Signatera™. A follow-up will be performed according to the protocol of the GALAXY study.

2.2 | Endpoints and statistical analyses

The endpoints of this study include concordance rates of diagnosis of ctDNA for the presence or absence of LNM in additional intestinal resection as well as sensitivity, specificity, false positive rate, and false negative rate of ctDNA. These endpoints will be summarized using the proportions and 95% exact (Clopper-Pearson) confidence interval. For the diagnostic concordance rate, a binomial test with a threshold of 80% will be performed at a one-sided significance level of 2.5%.

3 | DISCUSSION

The CIRCULATE-Japan project includes a large-scale patient-screening registry of the GALAXY study for patients with clinical stage II to IV or recurrent CRC and two ctDNA-guided phase III trials: VEGA and ALTAIR.[7] The launch of the DENEB study within the GALAXY study, evaluating the ctDNA status of patients with pT1 CRC will help stratify high-risk patients who may benefit from additional treatment/intestinal resection, sparing low-risk patients from being exposed to unnecessary treatment. This will be achieved by examining the relationship between the locally resected specimens, the additional resected specimens, and ctDNA analysis. In the United States, Natera, Inc received Medicare coverage for Signatera™ MRD Test in Stage II-III CRC for its effectiveness in guiding adjuvant therapy decisions, detecting recurrence earlier than standard diagnostic tools, and monitoring treatment response.[6] In the DENEB study, we will further demonstrate the clinical usefulness of ctDNA for detecting MRD after local excision of CRC.

TABLE 1 Patient inclusion and exclusion criteria

Inclusion criteria

1. Histopathologically diagnosed with adenocarcinoma
2. The primary location of the tumor is the colon (cecum, colon, and rectosigmoid) or rectum (excluding appendix and anal canal cancer)
3. Histologically confirmed pT1 colorectal cancer after complete local resection with a negative margin
4. Additional intestinal resection with lymph node dissection planned due to the following risk factors of lymph node metastasis:
   (a) Depth of submucosal invasion (>1000 mm)
   (b) Presence of lymphovascular invasion
   (c) Poorly differentiated adenocarcinoma, signet-ring cell carcinoma, or mucinous carcinoma
   (d) High-grade tumor budding (BD2/3) at the site of deepest invasion
5. The age at the time of acquisition of informed consent is 20 y or older
6. Eastern Cooperative Oncology Group Performance Status is 0 or 1
7. The subject has given a written informed consent for participation in the study

Exclusion criteria

1. Two or more synchronous colorectal cancer (multiple cancer)*
   *Patients with clinical stage Tis or T1a colorectal cancer judged to be cured by local treatment may be included in this study only when it is confirmed that the cancer has been completely resected by local treatment
2. Active double cancer*
   *However, patients with a relapse-free survival period of 5 y or longer or patients with skin basal cell carcinoma, spinocellular carcinoma, superficial bladder cancer, or cervical cancer which has been considered cured by local treatment; carcinoma in situ (intraepithelial cancer) or lesions equivalent to intramucosal cancer that can be treated endoscopically; or nonmetastatic prostate cancer that does not require systemic treatment may be enrolled
3. History of surgery, chemotherapy, immunotherapy, or radiotherapy within 6 mo before enrollment with clinical stage II or III colon cancer (cecum, colon, rectum sigmoid)
4. Pregnant or breastfeeding women
5. Serious complication
6. Positive for hepatitis B surface (HBs) antigen or positive for hepatitis C virus (HCV) antibody
7. Human immunodeficiency virus (HIV) antibody positive (a patient may enroll even if HIV antibody has not been tested)
8. Active novel coronavirus infection (COVID-19) is present*
   *Patients with positive SARS-CoV-2 PCR or suspected COVID-19 based on clinical symptoms; patients with confirmed negative SARS-CoV-2 PCR or other tests and no symptoms of COVID-19 may be included in this study. However, if the physician deems that the patients will affect the evaluation of this study, the patients are ineligible (COVID-19 testing is not required)
9. The study doctor deemed that the patient is ineligible for this study

The recommendations for surgical resection after local resection of pT1 CRC are indicated in much the same way across different guidelines (Japan, United States, Europe, and Korea). Several studies as outlined by the guidelines indicate lymphovascular invasion, poorly differentiated histology, high-grade tumor budding, and depth of submucosal invasion as main risk factors associated with LNM in patients with pT1 CRC.[4] However, LNM has only been identified in about 10% of cases, and the assessment based on the established pathological risk factors is limited due to high interobserver variability between pathologists.[8] More recently, the effectiveness of artificial intelligence using clinicopathological factors and the transcriptomic panel based on microRNAs and messenger RNAs in the blood for identification of LNM have been explored; however, none of these methodologies are well established and have sufficient evidence.[9,10] To our knowledge, this is the first study to explore the predictability of LNM using the established ctDNA assay from a different perspective than the pathological standpoint, in which prediction of LNM has been discussed for many years.

It will be possible to follow up pT1 patients with negative ctDNA after complete local resection and to evaluate the prognosis, provided a sufficient diagnostic concordance rate can be obtained. Reducing unnecessary surgery could contribute to the avoidance of postsurgical complications and preservation of patients' quality of life by preventing deterioration of their defecation function and avoiding stoma in cases of rectal cancer. The ctDNA assay will build new evidence to establish a noninvasive personalized diagnosis that will help to avoid unnecessary treatment and guide appropriate treatment for patients with CRC.

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DISCLOSURE

Alexey Aleshin, Paul R. Billings, and Matthew Rabinowitz are from Natera Inc. Takeshi Kato received lecture fees from Taiho and Chugai. Yoshiaki Nakamura received research funds from Taiho and Chugai. Hiroya Taniguchi received lecture fees from Taiho and Chugai. Kentaro Yamazaki received lecture fees from Taiho and Chugai, and research funds from Taiho and Chugai. Masahito Kotaka received lecture fees from Taiho and Chugai. Daisuke Kotani received lecture fees from Taiho and Chugai. Eiji Oki received lecture fees from Taiho and Chugai. Masaki Mori received lecture fees from Taiho and Chugai, and research funds from Taiho and Chugai. Takayuki Yoshino received lecture fees from Taiho and Chugai, and research funds from Taiho and Chugai. The rest of the authors do not have conflict of interest.

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