Development of circulating tumour DNA analysis for gastrointestinal cancers

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ABSTRACT

Comprehensive genomic profiling using next-generation sequencing (NGS) enables the identification of multiple genomic biomarkers established in advanced gastrointestinal (GI) cancers. However, tissue-based NGS has limitations, such as long turnaround time and failure to detect tumour heterogeneity. Recently, the analysis of circulating tumour DNA (ctDNA) using polymerase chain reaction-based or NGS-based methods has demonstrated the capability to detect genomic alterations with high accuracy compared with tumour tissue analysis with short turnaround time and identify heterogeneous resistance mechanisms. Furthermore, ctDNA analysis can be repeatedly performed on disease progression to clarify resistant clones. Clinical trials that test the outcome of a selected targeted therapy based on a ctDNA result are ongoing to prospectively evaluate the clinical utility of ctDNA analysis. Furthermore, the improvement of ctDNA analysis beyond current technical limits of mutation-based ctDNA detection methods has expanded the potential for detecting the presence of tumours in patients with no clinically evident disease, such as minimal residual disease and early cancer. Although a careful understanding of the advantages and limitations are required and further prospective studies are needed, the ctDNA analysis has the potential to overcome several challenges in the treatment of various types of cancers at all stages, including GI cancers.

INTRODUCTION

Preclinical studies and clinical trials involved in the treatment of solid tumours, including gastrointestinal (GI) cancers, have established several predictive genomic biomarkers, such as RAS and BRAF mutations for metastatic colorectal cancer (mCRC), HER2 (ERBB2) amplification for metastatic gastro-oesophageal cancer, germline BRCA mutations for metastatic pancreatic cancer and microsatellite instability (MSI) and NTRK fusions for advanced solid tumours.1–7 Furthermore, advances in next-generation sequencing (NGS) technologies have enabled large-scale genomic profiling in GI cancers and have elucidated potentially targetable alterations. Tumour genomic profiling using NGS testing is now widely used in clinical practices for patients with advanced GI cancers to identify genomic alterations that can be therapeutically targeted.

Tissue-based NGS, however, has inherent limitations. For example, the long turn-around time between the receipt of tissue samples and reporting results may delay the start of treatment. Furthermore, recent studies have revealed that multiple genomic changes can arise because of clonal evolution under treatment pressure in cancer, resulting in acquired secondary resistance.8 Therefore, the longitudinal surveillance of clonal evolution is required to identify secondary resistance or adequately select subsequent treatments. However, repeat tissue biopsies are sometimes challenging to perform because of the inherent risk of complications. Moreover, biopsies or tissue sections represent only a single snapshot of the tumour in time and space; therefore, they often fail to detect intratumoural genetic heterogeneity, which is a great challenge for the optimal treatment selection for GI cancers.

Recent technical advances have enabled the analysis of tumour materials obtained from the blood or other body fluids. These liquid biopsies can be used to assess intratumoural genetic heterogeneity and overcome the limitations of tissue analyses. Specifically, the analysis of circulating tumour DNA (ctDNA), which is tumour-derived fragmented DNA released into the bloodstream, has been suggested to have clinical utility in detecting genomic alterations in various types of cancers. Furthermore, evolving technologies of ctDNA analysis using NGS-based methods have expanded the potential of ctDNA analysis for genomic profiling as an alternative for tissue-based NGS and for detecting the presence of a tumour in patients with no clinically evident disease. In this review, we focus on the utility of ctDNA analyses for the identification of predictive genomic biomarkers for GI cancers and the potential for detecting minimal residual disease (MRD) and early cancers with no clinically evident disease.
Comparison between tumour and ctDNA analysis for validated biomarkers in advanced GI cancers

Assays available for ctDNA analysis can be categorised into two general classes: those targeted for a single or small number of variants and those aimed at a broader coverage.9 Targeted assays generally use one of several polymerase chain reaction (PCR)-based strategies, such as BEAMing (beads, emulsion, amplification and magnetics) or droplet digital PCR (ddPCR) method, for the detection of specific known variants, often at very low levels within the circulating cell-free DNA (cfDNA) composed of germline DNA from normal cells.10 11 On the contrary, broad-coverage assays use NGS-based approaches and have the capability of detecting a larger number of variants in multiple genes, often examining parts of >50 genes to be applied to multiple different tumour types. Modified NGS-based approaches incorporating deep sequencing coverage, molecular barcoding methods and error-suppression algorithms have improved the limits of detection.12–15 Currently available PCR-based or NGS-based clinical ctDNA assays are listed in table 1.

Table 1: Currently available PCR or NGS-based clinical ctDNA assays

| Method       | Assay                        | Cancer     | Gene                                      | Company                        |
|--------------|------------------------------|------------|-------------------------------------------|--------------------------------|
| PCR-based    | Cobas EGFR Mutations Test v2 | NSCLC      | EGFR del19, EGFR L858R and EGFR T790M    | Roche Molecular Diagnostics    |
|              | Therascreen EGFR QG Plasma   | NSCLC      | EGFR del19 and EGFR L858R                | Qiagen Inc                     |
|              | PCR kit                      |            |                                           |                                |
|              | AmoyDx Super-ARMS EGFR      | NSCLC      | EGFR del19, EGFR L858R and EGFR T790M    | AmoyDx                         |
|              | mutations test               |            |                                           |                                |
|              | OncoBEAM RAS CRC Kit         | CRC        | KRAS and NRAS mutations                   | Sysmex Inostics                |
|              | Idylla ctKRAS Mutation Test  | CRC        | KRAS, NRAS and BRAF mutations             | Biocartis, Inc.                |
|              | and Idylla ctNRAS-BRAF Mutation Test |           |                                           |                                |
| NGS-based    | Guardant360                  | Solid tumours | 74 genes and MSI                         | Guardant Health                |
|              | FoundationOne Liquid         | Solid tumours | 70 genes and MSI                         | Foundation Medicine Inc.       |
|              | PlasmaSELECT                 | Solid tumours | 64 genes and MSI                         | Personal Genome Diagnostics    |
|              | Oncomine Lung cfDNA Assay    | NSCLC      | 11 genes                                  | ThermoFisher Scientific        |
|              | Reveals ctDNA 28 Kit         | Solid tumours | 28 genes                                 | ArcherDX                      |
|              | OptiSeq NGS Pan-Cancer Panel | Solid tumours | 65 genes                                 | DiaCarta                       |

CRC, colorectal cancer; ctDNA, circulating tumour DNA; MSI, microsatellite instability; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction.

Mutations in RAS are negative predictive biomarkers that have been validated in prospective–retrospective or retrospective analyses in randomised studies with anti-epidermal growth factor receptor (EGFR) antibodies for mCRC. Several studies on PCR-based ctDNA assays have detected RAS mutations with high accuracy compared with validated tissue RAS testing.16–20 In addition to retrospective studies that compared with the progression-free survival (PFS) of patients with mCRC treated with an anti-EGFR therapy using tissue RAS versus ctDNA, RAS testing results indicated a similar PFS following first-line19 and second/third-line treatments.21 The OncoBEAM™ RAS CRC Kit, which detects RAS mutations in ctDNA derived from mCRC by a BEAMing method, is the only ctDNA assay approved in Europe and Japan for ctDNA genomic biomarker testing for GI cancers.

Recently, NGS-based ctDNA assays have demonstrated a high correlation with ddPCR assays for the measurement of mutant allele fractions (MAF) of genes, including RAS mutations in mCRC.22 23 Furthermore, multiple NGS-based ctDNA assays have been shown to detect genomic alterations in advanced GI cancers, such as RAS and BRAF mutations in CRC and HER2 amplification in gastroesophageal cancer, with high accuracy compared with tissue NGS analysis (table 2)25–28. One of these investigations showed that the turnaround time was 18 days and 7 days for tumour tissue and ctDNA analysis in this study, respectively.25

MSI is a predictive biomarker of the response to immune checkpoint inhibitors in unresectable or metastatic solid tumours, including GI cancers.6 29 MSI is the archetypical manifestation of a defective DNA mismatch repair, which leads to dramatically increased mutation loads throughout the genome, including the gain and/or loss of nucleotides within repeating motifs, known as microsatellite tracts. Currently, MSI testing is most commonly performed via PCR and/or immunohistochemistry (IHC) analysis of tumour tissue samples, and NGS can also accurately characterise MSI status in tumours by assessing the microsatellite length, allowing for a comprehensive profiling of targetable genomic biomarkers and MSI status via a single NGS testing.30–33 Recent publications have demonstrated that ctDNA-based NGS testing can assess the MSI status in various types of cancers, including GI cancers, with a high concordance with tissue-based testing.34 35
Germline BRCA mutations are associated with the efficacy of the poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitors in patients with ovarian or breast cancer. A randomised phase III trial, POLO, demonstrated that patients who had a germline BRCA1 mutation and metastatic pancreatic cancer that had not progressed during first-line platinum-based chemotherapy had significantly longer PFS with maintenance olaparib than with placebo. Although germline mutations are generally tested through the analysis of genomic DNA extracted from whole blood, broad NGS-based approaches also incidentally identify germline variants. Tissue-based NGS testing cannot definitively distinguish germline from somatic mutations without a comparison with healthy tissues. However, ctDNA-based NGS testing may identify germline mutations at approximately 50% of the MAF, which are distinguishable from somatic mutations that typically occur at lower MAFs in ctDNA. Data from more than 10000 patients with advanced solid tumours who underwent Guardant360, an NGS-based ctDNA assay receiving Food and Drug Administration (FDA) breakthrough device designation, showed suspected hereditary cancer mutations in 1.4% of the total population. In this study, putative germline BRCA1/2 mutations were found in 8 out of 332 (2.4%) patients with pancreatic cancer.

**UTILITY OF CTDNA ANALYSIS FOR ASSESSING INTRATUMOURAL GENOMIC HETEROGENEITY**

Intratumoural genomic heterogeneity has been shown in various types of cancers and can contribute to treatment failure and drug resistance. As ctDNA is shed from tumour cells throughout the body, ctDNA analysis can potentially identify multiple concurrent heterogeneous resistance mechanisms in individual patients that single-lesion tumour biopsies may miss. Pectasides et al revealed that 42% of mutations and 67% of gene amplifications were discordant between primary tumours and synchronous metastasis in metastatic gastro-oesophageal cancer. Of note, NGS analyses of matched metastatic lesions and ctDNA showed 87.5% concordance for genomic alterations in samples for which discordance between the primary tumour and the metastases were observed.

Most recently, Parikh et al directly compared genomic alterations between ctDNA and the tumour biopsy in a prospective cohort of patients with advanced GI cancers. NGS analyses of matched ctDNA and biopsy specimens of the brain, liver and subcutaneous metastases from patients with BRAFV600E mCRC showed that only subclonal alterations were detected in each metastasis, such as KRAS and NRAS mutations, and EGFR amplification was represented in ctDNA. Similarly, multiple FGFR2 mutations detected in 17 autopsy specimens of a patient with FGFR2 fusion-positive metastatic gastric cancer who was treated with an FGFR inhibitor were also detected by ctDNA analysis.

These findings suggest that ctDNA analysis may be more useful in identifying heterogeneous clinically relevant subclones than a single-lesion tumour biopsy, although treatment strategies have not been established for subclones with alterations identified by ctDNA analysis but not by tissue analysis.

**EVALUATION OF CTDNA-BASED PATIENT SELECTION IN PROSPECTIVE CLINICAL TRIALS**

Recently, the association between the efficacy of targeted agents and genomic alterations in ctDNA has been evaluated in prospective clinical trials, in which ctDNA was retrospectively analysed using blood samples collected at baseline. Sym004 is a mixture of two monoclonal antibodies, futuximab and modotuximab, that bind to nonoverlapping epitopes on the EGFR extracellular domain (ECD) III. In a randomised phase II trial comparing Sym004 with the investigator’s choice of treatments for patients with KRAS exon2 wild-type mCRC,
the significant difference in overall survival (OS) was not shown, but in a subgroup by ctDNA (RAS/BRAF/EGFR ECD-mutation negative), the OS was longer in Sym004-treated patients compared with those in the investigator’s choice of treatment.41

As the plasma copy number of gene amplifications in ctDNA is often misleadingly low in samples with a low tumour fraction, the plasma copy number needs to be adjusted by the ctDNA amount. NGS-based ctDNA analyses allow adjustments to the plasma copy number using the maximum MAF as a surrogate for the tumour content. The HERACLES study of trastuzumab plus lapa-
tinib for HER2-positive mCRC showed that an adjusted plasma copy number of HER2 amplification in pre-treatment ctDNA was correlated with the best objective response and PFS.42 A Korean umbrella trial, VIKTORY, also showed that an increased adjusted MET plasma copy number was significantly associated with prolonged PFS on savolitinib to a significantly greater degree than the tissue NGS MET copy number.43 These findings have indicated that the adjustment of plasma copy number assessed by NGS-based ctDNA assays can be more predictive for the efficacy of targeted agents than the absolute plasma copy number.

Although these studies suggest that genomic profiling by ctDNA analysis can identify patients who may benefit from an optimal targeted therapy, clinical trials that prospectively test the outcome of selected targeted therapy based on a ctDNA result are very limited. A molecular profiling programme, TARGET, matched patients with advanced cancers to early phase clinical trials based on an analysis using a 641 cancer-associated gene panel in a single ctDNA assay.44 Actionable mutations were identified in 41 of 100 patients, and 11 of these patients received a matched therapy with some tumour responses. We are now conducting an umbrella/basket project, the GOZILA study (UMIN000029315), for patients with advanced solid malignancies, including GI cancer. In the GOZILA study, genomic alterations in the ctDNA have been analysed using Guardant360 for 4000 patients with advanced solid malignancies, and clinical trials have concurrently been conducted for rare fractions of patients with advanced solid malignancies (Figure 1). Patients with metastatic GI cancers having specific genomic alterations are enrolled in organ-specific trials. Tumour-agnostic basket trials are also conducted for rare gene alterations identified in various types of cancers, such as FGFR alterations. Most recently, we reported an interim result of a clinical trial.
Previous studies have identified the emergence of ongoing mutagenesis in cancer during anti-EGFR therapy.47 Longitudinal ctDNA surveillance potentially monitors genomic alterations of each tumour change over time as a result of the Darwinian clonal evolution imposed by selective pressures, including targeted therapies.48–51 Acquired resistance to anti-EGFR therapy in clinical practice has been suggested to emerge from the selection of RAS mutant clones in ctDNA before the absence of KRAS mutant clones in ctDNA.52–55 Furthermore, a longitudinal surveillance of ctDNA using an NGS-based ctDNA assay after anti-EGFR therapy indicated the emergence of acquired RAS mutations and alterations in other genes, including MET, ERBB2, FLT3, EGFR and MEK.56 Interestingly, the temporal withdrawal of an anti-EGFR antibody resulted in a decline in KRAS-mutant alleles, suggesting that the sensitivity to anti-EGFR antibody was restored.52–57

Trastuzumab, in combination with chemotherapy, significantly improved OS in patients with HER2-positive gastro-oesophageal cancer in a randomised phase III trial, the ToGA study.4 However, the unsatisfactory gain of the median PFS suggests acquired resistance to HER2-targeted therapies. Wang et al conducted a longitudinal surveillance of serial plasma samples using an NGS-based ctDNA assay from 24 patients with HER2-positive metastatic gastro-oesophageal cancer who were treated with trastuzumab plus chemotherapy.60 In this study, NF1 mutation was newly identified in ctDNA at disease progression and was confirmed to be related to the resistance to trastuzumab in vitro and in vivo.

The potential of ctDNA to identify various resistance mechanisms has now been used in the development of new drugs. In early clinical trials, the emergence of FGFR2 mutations in patients with FGFR2 fusion-positive cholangiocarcinoma treated with FGFR inhibitors,61 62 BRCA reverse mutations in patients with BRCA-mutated cholangiocarcinoma have been suggested to emerge from the selection of pre-existing FGFR2-mutant subclones and also as a result of ongoing mutagenesis in cancer during anti-EGFR therapy.9 Previous studies have identified the emergence of RAS mutations by the analysis of plasma collected for the HER2-amplified mCRC, TRIUMPH, conducted as an arm in the GOZILA study.48 In the TRIUMPH study, patients with treatment-refractory RAS wild-type mCRC with HER2 amplification confirmed by tissue or ctDNA analysis were treated with the combination of trastuzumab and pertuzumab. The primary endpoint of the confirmed objective response rate was 35% in the tissue-positive group and 33% in the ctDNA-positive group, respectively. This response rate suggested that ctDNA analysis can equally identify patients with HER2-amplified mCRC who benefit from the dual HER2-targeted therapy as the tissue analysis. For advanced gastro-oesophageal cancer, a randomised phase III trial to evaluate the efficacy of the addition of bemarituzumab, a monoclonal antibody against FGFR2b, to modified FOLFOX6 for disease with FGFR2 amplification by ctDNA analysis or FGFR2b overexpression by IHC analysis is ongoing.46 Other ongoing ctDNA-based clinical trials for advanced GI cancers are listed in Table 3.

**LONGITUDINAL CTDNA SURVEILLANCE TO IDENTIFY ACQUIRED RESISTANCE MECHANISMS**

Genomic alterations of each tumour change over time as a result of the Darwinian clonal evolution imposed on cancer cells by selective pressures, including targeted therapy. Longitudinal ctDNA surveillance potentially interrogates the clonal evolution with minimal invasiveness. RAS mutant clones have been identified as drivers of acquired resistance to anti-EGFR therapy in clinical and preclinical studies.47–51 Acquired KRAS mutations have been suggested to emerge from the selection of pre-existing KRAS-mutant subclones and also as a result of ongoing mutagenesis in cancer during anti-EGFR therapy.9 Previous studies have identified the emergence of RAS mutations by the analysis of plasma collected

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**Table 3** Ongoing clinical trials incorporating ctDNA analysis for patient selection in advanced GI cancer

| Trial identifier (Title acronym) | Phase | Estimated no. of patients | Criteria for patient selection | Intervention | Primary endpoint | Study location |
|----------------------------------|-------|---------------------------|--------------------------------|--------------|-----------------|---------------|
| NCT03343301 (FIGHT)              | I/III | 10±548                    | Metastatic gastro-oesophageal cancer with FGFR2 amplification by ctDNA analysis or FGFR2b overexpression by IHC analysis | mFOLFOX6 + bevacizumab vs mFOLFOX6 | Safety and OS | Global         |
| NCT02980510 (PANIRINOX)          | II    | 209                       | RAS/BRAF wild-type mCRC       | mFOLFOX6 + panitumumab vs FOLFIRINOX + panitumumab | CR rate in FOLFIRINOX + Panitumumab | France         |
| NCT03227926 (CHRONOS)           | II    | 129                       | mCRC with subsequent decay of RAS mutant clones | Rechallenge with panitumumab | ORR | Italy         |
| NCT03087071                      | II    | 84                        | mCRC according to RAS/BRAF/EGFR mutation status | Panitumumab vs panitumumab + trametinib | ORR | USA           |
| UMIN000027887 (TRIUMPH)         | II    | 25                        | ERBB2-amplified mCRC          | Trastuzumab + pertuzumab | ORR | Japan         |

CR, complete response; CRC, colorectal cancer; ctDNA, circulating tumour DNA; IHC, immunohistochemistry; ORR, objective response rate; OS, overall survival.

for the HER2-amplified mCRC, TRIUMPH, conducted as an arm in the GOZILA study.48 In the TRIUMPH study, patients with treatment-refractory RAS wild-type mCRC with HER2 amplification confirmed by tissue or ctDNA analysis were treated with the combination of trastuzumab and pertuzumumab. The primary endpoint of the confirmed objective response rate was 35% in the tissue-positive group and 33% in the ctDNA-positive group, respectively. This response rate suggested that ctDNA analysis can equally identify patients with HER2-amplified mCRC who benefit from the dual HER2-targeted therapy as the tissue analysis. For advanced gastro-oesophageal cancer, a randomised phase III trial to evaluate the efficacy of the addition of bemarituzumab, a monoclonal antibody against FGFR2b, to modified FOLFOX6 for disease with FGFR2 amplification by ctDNA analysis or FGFR2b overexpression by IHC analysis is ongoing.46 Other ongoing ctDNA-based clinical trials for advanced GI cancers are listed in Table 3.

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pancreatic cancer treated with PARP inhibitors, and NTRK mutations in patients with TRK fusion-positive solid tumours, including GI cancers, treated with TRK inhibitors were identified as secondary resistance mechanisms by longitudinal ctDNA surveillance.

**LIMITATIONS OF CTDNA ANALYSIS**

Although the findings reviewed above suggest that ctDNA analysis can be a potential surrogate for standard-of-care tumour tissue analysis, some limitations for ctDNA analysis have been observed. First, preanalytical variables for ctDNA, such as sample collection, handling, transport, processing and storage conditions, may affect ctDNA analysis. However, limited data are available regarding the effect of preanalytical variables on ctDNA results. Patient-related factors, including diurnal or other biological influences, smoking, pregnancy, exercise and numerous non-malignant disorders, such as inflammatory conditions, anaemia, heart disease, metabolic syndrome and autoimmune disorders, may also contribute to the release of cfDNA. Future studies using blood samples with well-documented preanalytical variables are required to address which variables affect the quality of the samples and results of the ctDNA analysis.

Second, the release of ctDNA may also be affected by tumour-associated factors. For example, the ctDNA concentration varies with the tumour type. Most patients with advanced solid tumours harbour detectable levels of ctDNA, whereas those with primary brain tumours have only low levels of ctDNA probably owing to the presence of the blood–brain barrier. Among GI malignancies, mCRC tends to have higher ctDNA levels. In patients with mCRC, undetectable ctDNA is associated with several factors, such as those related to low tumour burden, including primary tumour resection and metachronous metastases, the absence of liver metastases, presence of lung metastases or peritoneal carcinomatosis, low leucocyte counts, low lactate dehydrogenase, alkaline phosphatase, CA19-9 and CEA levels and high albumin levels.

Third, healthy haematopoietic cells accumulate somatic mutations during ageing, which can drive clonal expansions of haematopoietic cells in the absence of dysplasia. These mutations are referred to as clonal haemapoiesis of indeterminate potential (CHIP). Although no definitive method can discriminate these somatic mutations in healthy cells released into ctDNA and ctDNA mutations, CHIP does not affect the care of advanced cancers because the allele frequencies are generally low compared with those of ctDNA in advanced cancers. However, CHIP may cause false-positive ctDNA results in cases where high sensitivity is required, such as MRD or early cancer detection.

**NEW APPLICATION OF CTDNA ANALYSIS: MRD AND EARLY CANCER DETECTION**

Analysis of ctDNA evaluates biomarkers as an alternative for tissue analysis and potentially detects the presence of tumours in patients with no clinically evident disease. This potential capability may be useful for the detection of MRD after surgery or screening in the early detection of new cancers, which can be achieved with high sensitivity assays. In a landmark study, Diehl et al identified somatic mutations of resected tumours in 18 patients with CRC by conventional Sanger sequencing and then analysed ctDNA using a BEAMing method to measure allele fractions as low as 0.01% of a given gene that was identified in each tumour. Most patients in whom ctDNA was detectable at the first follow-up visit (13–56 days after the surgery) had a recurrence, while no patients with undetectable ctDNA had a recurrence (p=0.006). In a prospective study involving 178 patients with stage II CRC who did not receive adjuvant chemotherapy, the postoperative ctDNA levels assessed by measuring allele fractions of mutations identified in the primary tumour using an NGS-based method (Safe-SeqS) were significantly associated with an 18 times higher risk of recurrence in patients with positive ctDNA than in those with negative ctDNA. Most recently, Reinert et al and Wang et al corroborated the poor prognostic implications of ctDNA positivity in patients with resected stages I–III CRC, respectively. In Reinert’s study, Signatera assay, a custom-designed ctDNA assay tracking 16 tumour-specific variants, which received FDA breakthrough device designation, was used. All studies mentioned above used a tumour-directed identification of somatic mutations to personalise ctDNA probes and determine the ctDNA status. This approach substantially reduces confounders, such as CHIP, although additional complexity exists by requiring the sequencing of the resected tumour before generating probes specific for the individual patient. Assays with a fixed-gene panel that analyse only plasma and do not require tissue analysis have also been studied as another approach to assess the MRD. The DYNAMIC study (ACTRN1261500381583) is an ongoing Australian trial of stage II colon cancer that randomly assigned patients to usual care versus ctDNA-informed adjuvant therapy. The NRG Oncology/National Clinical Trials Network phase II/III trial NRG GI-005 (COBRA) will randomise US and Canadian patients with resected low-risk stage II colon cancer to standard surveillance or adjuvant therapy in accordance with ctDNA results.

Implementation of ctDNA analysis for cancer screening requires a degree of analytical sensitivity beyond current technical limits of mutation-based ctDNA detection methods. A blood test, called CancerSEEK, which simultaneously evaluates levels of cancer proteins and the presence of cancer gene mutations from circulating DNA in the blood, was applied to 1005 patients with non-metastatic colorectal, gastric, oesophageal, pancreatic, liver, lung, breast and ovarian cancers. The test was positive in a median of 70% of the 1005 patients with sensitivities ranging from 33% for breast cancer to 98% for ovarian cancer and a specificity of greater than 99%. An analysis of tumour-specific methylation in ctDNA has been conducted to identify ctDNA in previous studies. A
recent study using immunoprecipitation-based profiling of methylation patterns in ctDNA examined 388 blood samples from patients with early and late-stage cancer of different tumour types and demonstrated receiver operating characteristic area under the curve values of 0.97 in detecting lung cancer, 0.92 for pancreatic cancer and 0.96 for healthy controls. Fragmentation patterns of ctDNA are also known to be different between healthy individuals and patients with cancer. Cristiano et al revealed various fragment sizes at different genomic regions in patients with cancer using a low-coverage whole-genome sequencing method. Incorporating this method with mutation-based ctDNA analysis demonstrated a sensitivity of 91% and a specificity of 98% for cancer detection.

CONCLUSION
ctDNA analysis has emerged as a potential tool for evaluating the complicated biological processes involved in GI cancers, represented by intratumoral genomic heterogeneity and clonal evolution, given the limited availability of precision therapy for patients with advanced GI cancers. The practical advantages, such as short turnaround time, enable medical oncologists to guide patients to an optimal therapy promptly and survey the potential therapeutic resistance using multiple sampling to implement changes during therapy. In addition, improvement in the limit of detection by evolving technologies has demonstrated the potential of ctDNA analysis to stratify adjuvant chemotherapy through the detection of postoperative MRD and identification of early cancers in patients with no clinically evident disease. Although a careful understanding of the advantages and limitations are required and further prospective study is needed, ctDNA analysis has the potential to overcome challenges for all stages in various types of cancers, including GI cancers.

Patients with advanced solid malignancies, including GI cancers, and those who were not previously treated or do not have disease progression after anticancer therapy were screened by an NGS-based ctDNA assay.

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