Circulating Tumor DNA Analysis Detects *FGFR2* Amplification and Concurrent Genomic Alterations Associated with FGFR Inhibitor Efficacy in Advanced Gastric Cancer

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ABSTRACT

Purpose: *FGFR2* amplification is associated with poor prognosis in advanced gastric cancer and its subclonal heterogeneity has been revealed. Here, we examined whether circulating tumor DNA (ctDNA) was useful for detecting *FGFR2* amplification and co-occurring resistance mechanisms in advanced gastric cancer.

Experimental Design: We assessed genomic characteristics of *FGFR2*-amplified advanced gastric cancer in a nationwide ctDNA screening study. We also analyzed *FGFR2* amplification status in paired tissue and plasma samples with advanced gastric cancer. In addition, we examined patients with *FGFR2*-amplified advanced gastric cancer identified by ctDNA sequencing who received FGFR inhibitors.

Results: *FGFR2* amplification was more frequently detected by ctDNA sequencing in 28 (7.7%) of 365 patients with advanced gastric cancer than by tissue analysis alone (2.6%–4.4%). *FGFR2* amplification profiling of paired tissue and plasma revealed that *FGFR2* amplification was detectable only by ctDNA sequencing in 6 of 44 patients, which was associated with a worse prognosis. Two patients in whom *FGFR2* amplification was detected by ctDNA sequencing after tumor progression following previous standard chemotherapies but not by pretreatment tissue analysis had tumor responses to FGFR inhibitors. A third patient with *FGFR2* and *MET* co-amplification in ctDNA showed a limitation of benefit from FGFR inhibition, accompanied by a marked increase in the *MET* copy number.

Conclusions: ctDNA sequencing identifies *FGFR2* amplification missed by tissue testing in patients with advanced gastric cancer, and these patients may respond to FGFR inhibition. The utility of ctDNA sequencing warrants further evaluation to develop effective therapeutic strategies for patients with *FGFR2*-amplified advanced gastric cancer.

Introduction

Gastric cancer remains an important cancer, being the fifth most frequently diagnosed cancer and the third leading cause of cancer death worldwide (1). Despite the advance in systemic therapy, the prognosis of patients with advanced gastric cancer is still poor with median survival time of approximately 1 year. Molecularly targeted therapeutic strategies have been attempted for patients with advanced gastric cancer, but have frequently failed to improve overall survival (OS) due to its nature of molecular heterogeneity (2–10).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Clin Cancer Res 2021;27:5619–27

doi: 10.1158/1078-0432.CCR-21-1414

Translational Relevance

The efficacy of FGFR inhibition has not been clear for *FGFR2*-amplified advanced gastric cancer with significant genomic heterogeneity, because adequate testing to detect *FGFR2* amplification for this population has not been established before FGFR treatment. In this study, we demonstrated that the utility of circulating tumor DNA (ctDNA) to evaluate targetable genomic alterations including *FGFR2* amplification, and to guide targeted therapy in advanced gastric cancer. In addition, we also suggested that ctDNA sequencing may be useful for assessing other concurrent genomic alterations including resistance alterations for guiding the management of this type of cancer.

FGFRs (FGFR1, FGFR2, FGFR3, and FGFR4) are transmembrane receptor tyrosine kinases, and FGF/FGFR signaling can be aberrantly activated by altered *FGFR* genes in cancers (11). Approximately 5% of patients with gastric cancer have *FGFR2* amplification (12), which is associated with poor prognosis (13–15). The relevance of high-level *FGFR2* amplification in gastric cancer to the response to FGFR inhibitors has been suggested in preclinical studies (16–18); however, a randomized phase II trial (SHINE) failed to demonstrate improved progression-free survival (PFS) with the pan-FGFR tyrosine kinase inhibitor (TKI) AZD4547 compared with paclitaxel in the second-line treatment of advanced gastric cancer with *FGFR2* amplification confirmed by tissue testing (19).

The analysis of circulating tumor DNA (ctDNA) has been demonstrated to be able to detect genomic alterations in tumor cells throughout the body and has been suggested as a method to assess heterogeneous resistance mechanisms (20, 21). A translational study of patients with *FGFR2*-amplified advanced gastric cancer treated with AZD4547 indicated that ctDNA sequencing identified high-level *FGFR2* amplifications in responders (16). A utility of ctDNA sequencing for identifying heterogeneous *FGFR2* amplification within spatially distinct regions of the primary tumor and distant metastases has also been suggested (22). In addition, previous studies suggested the utility of ctDNA sequencing for identifying genomic resistance mechanisms in advanced gastric cancer harboring *ERBB2, MET*, and *EGFR* amplifications (23–26). These observations suggest that ctDNA sequencing may be useful in guiding therapy for *FGFR2*-amplified advanced gastric cancer by detecting *FGFR2* amplification, including cases missed by single-lesion tumor biopsies, and by identifying heterogeneous resistance mechanisms. Indeed, recently, a randomized phase II trial reported that the addition of bemarituzumab, a mAb against FGFR2b, to chemotherapy improved survival in patients with FGFR2b-overexpressing or *FGFR2*-amplified gastric cancer identified by tissue or ctDNA analysis (NCT03343301; ref. 27).

Here, we evaluated the utility of ctDNA sequencing compared with tissue analysis for detecting *FGFR2* amplification in advanced gastric cancer as well as other genomic alterations, including resistance alterations, and for guiding the management of this type of cancer. The ctDNA sequencing revealed that some patients with *FGFR2*-amplified advanced gastric cancer in ctDNA may benefit from FGFR inhibition but cannot be identified by current tissue testing practices and clarified resistance mechanisms.

Materials and Methods

GI-SCREEN and GOZILA study design and patient selection

SCRUM-Japan GI-SCREEN is a nationwide tumor tissue cancer genomic profiling study involving 26 core cancer institutions in Japan (28), which aims to characterize the genomic landscape for all gastrointestinal cancers and accelerate development and improve care in this area by matching patients to suitable clinical trials. The key inclusion criteria included the following: (i) histopathologically confirmed unresectable or metastatic gastrointestinal cancer, (ii) receipt (or planned receipt) of systemic therapy, (iii) age ≥20 years, (iv) an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–1, (v) adequate organ function, and (vi) available tumor tissue. Eligible patients provided written informed consent. The genotyping of archival or fresh tumor tissue samples from enrolled patients was performed using the Oncomine Comprehensive Assay (OCA; Thermo Fisher Scientific), which is described in more detail below. This study was initiated in February 2015 and completed enrollment in April 2019.

GOZILA is a nationwide plasma genomic profiling study in Japan based on the SCRUM-Japan GI-SCREEN platform, which, like GI-SCREEN, aims to effectively identify patients with gastrointestinal cancers who might benefit from targeted therapy, with 31 institutions including the above 26 sites (28). The key inclusion criteria were similar to those of GI-SCREEN: (i) histopathologically confirmed unresectable or metastatic gastrointestinal cancer, (ii) age ≥20 years, and (iii) a life expectancy of at least 12 weeks. To avoid the suppression of ctDNA shedding due to chemotherapy, patients were included only if they showed disease progression during systemic chemotherapy and had not started the subsequent therapy at the time of blood sampling. Eligible patients provided written informed consent, and ctDNA genotyping was performed using Guardant360 (Guardant Health, Inc.), which is described in additional detail below. This study was launched in January 2018.

Both studies were conducted in accordance with the Declaration of Helsinki and the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects. Each study protocol was approved by the institutional review board of each participating institution and registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (protocol IDs UMIN000016344 for GI-SCREEN and UMIN000029315 for GOZILA).

FGFR2 profiling concordance study design and patient selection

A retrospective study was performed to evaluate the concordance of *FGFR2* amplification between tissue and plasma in patients with advanced gastric cancer between January 2015 and December 2018 at the National Cancer Center Hospital East. On the basis of the recommended formalin-fixed, paraffin-embedded (FFPE) sample storage period from our previous study (29), the tissue samples collected within 4 years were used. Patients who met the following criteria were included: (i) presence of histologically confirmed gastric adenocarcinoma, (ii) receipt of systemic treatment for advanced disease, and (iii) an available plasma sample collected near the time of tumor biopsy and before the initiation of systemic treatment. Patients with tumors previously known to harbor *FGFR2* amplification in GI-SCREEN or GOZILA who had available matched plasma and tissue samples were preferentially included. Tumor responses were assessed according to RECIST v1.1 (30).

This study was conducted in accordance with the Declaration of Helsinki and the Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects. The study protocol was approved by the Institutional Review Board at the National Cancer Center (UMIN000041008). Written informed consent was obtained from patients who were alive at the time of the study. For deceased patients and their relatives, we disclosed the study design on the website of the National Cancer Center and gave the families a chance to express the will of the decedents.

Tissue-based next-generation sequencing (NGS) analysis

For patients enrolled in GI-SCREEN and the retrospective concordance study, the NGS analysis of tumor tissue was performed using OCA v1 and OCA v3 at the Life Technologies Clinical Services Lab, a Clinical Laboratory Improvement Amendments (CLIA)-certified, College of American Pathologists (CAP)-accredited laboratory, as described previously (31). These assays examined 143 (OCA v1) and 161 (OCA v3) cancer-related genes and detected relevant single-nucleotide variants (SNV), copy-number variations, gene fusions, and indels in one streamlined workflow. Briefly, tumor DNA and RNA were isolated from FFPE sections, and DNA/RNA libraries were prepared. Purified libraries were sequenced using Ion Torrent PGM (Thermo Fisher Scientific). Sequence reads were aligned to the hg19 assembly and were called using Ion Reporter Software version 4.4 (for OCA v1) and v5.0 (for OCA v3) to detect alterations.

ctDNA-based NGS analysis by Guardant360

For patients enrolled in GOZILA and the retrospective concordance study, the NGS analysis of ctDNA was performed using Guardant360 at Guardant Health, a CLIA-certified, CAP-accredited, New York State Department of Health–approved laboratory, as described previously (32). Guardant360 detects SNVs, indels, fusions, and copy-number alterations in 74 genes with a reportable range of ≥0.04%, ≥0.02%, ≥0.04%, and ≥2.12 copies, respectively. For GOZILA patients, 2 × 10-mL whole-blood samples were collected from enrolled patients in Streck Cell-Free DNA blood collection tubes (BCT; Streck, Inc) and sent to Guardant Health. For the other patients, 3 mL of frozen plasma prepared from whole blood collected in EDTA tubes was sent for analysis. Five to 30 ng of cell-free DNA (cfDNA) isolated from plasma was labeled with nonredundant oligonucleotides ("molecular barcoding"), enriched using targeted hybridization capture, and sequenced on an Illumina NextSeq 550 platform (Illumina, Inc.). Base call files generated by Illumina's RTA software version 2.12 were demultiplexed using bcl2fastq version 2.19 and processed as described previously (32). Somatic cfDNA alterations were identified using a proprietary bioinformatics pipeline.

IHC

FGFR2 IHC was performed using a rabbit anti-FGFR2 polyclonal antibody (18601; IBL) at Geneticlab, and MET IHC was performed using a rabbit anti-c-MET mAb (790–4430; Ventana) at National Cancer Center (Kashiwa, Japan). FGFR2 IHC results were scored according to the intensity and percentage of positively stained carcinoma cells, as follows: 0, no positive cells; 1, weak staining and ≥10%; 2, strong staining and <10%; 3, strong staining and 10% to 49%; and 4, strong staining and ≥50%.

FISH

The assessment of *FGFR2* amplification by FISH was conducted using an *FGFR2*/*CEP10* probe (Geneticlab; Supplementary Table S1) for 20 tumor nuclei per sample at Geneticlab. *FGFR2* amplification was defined as an *FGFR2* copy number ≥4.0 signals per cell and *FGFR2*/*CEP10* ratio ≥2.0. If the *FGFR2* copy number was ≥4.0 signals per cell and the *FGFR2*/*CEP10* ratio was <2.0, the case was defined as polysomy.

Statistical analysis

Associations of the *FGFR2* status with clinicopathological factors and the variant allelic frequency (VAF) were analyzed using Fisher exact test or Mann–Whitney *U* test. OS was defined as the interval from the first day of the first-line chemotherapy to the day of death or the most recent follow-up visit. Kaplan–Meier curves were constructed, and statistical significance was determined using the log-rank test. A P value of <0.05 was considered significant. JMP software (ver. 14.0; SAS Institute Inc.) was used to perform statistical analyses.

Results

ctDNA sequencing detects *FGFR2* amplification and a unique genomic profile in patients with *FGFR2*-amplified advanced gastric cancer

To evaluate the utility of ctDNA compared with tissue samples for detecting *FGFR2* amplification in advanced gastric cancer, we reviewed ctDNA sequencing results of advanced gastric cancer from the GOZILA and GI-SCREEN studies as well as from publicly available tissue-based databases [The Cancer Genome Atlas (TCGA) and Memorial Sloan Kettering Cancer Center (MSKCC) databases]. *FGFR2* amplification was detected in 28 (7.7%) of 365 patients with advanced gastric cancer enrolled in the GOZILA study between January 2018 and January 2020 (**Fig. 1A**). This prevalence was significantly higher than that detected by tissue sequencing in GI-SCREEN and publicly available tissue-based databases (GI-SCREEN: 3.4%, $P = 0.00080$; TCGA: 4.4%, $P = 0.049$; and MSKCC: 2.6%, $P = 0.0027$; **Fig. 1A**), which is consistent with a previous study reporting higher incidences of amplification of receptor tyrosine kinase genes in ctDNA than tissue (33). Of 365 patients from GOZILA study, no ctDNA alterations were detected in 54 (14.8%) patients. Very weak correlation between the *FGFR2* plasma copy number (pCN) and ctDNA maximum VAF (max VAF) was observed ($r^2 = 0.15$; $P = 0.041$; **Fig. 1B**). These findings indicated that ctDNA sequencing may identify *FGFR2* amplification that cannot be detected by conventional tissue analysis.

Next, we evaluated if ctDNA could be used to identify other genomic features in *FGFR2*-amplified advanced gastric cancer. To this end, we compared concurrent genomic alterations between patients with *FGFR2*-amplified and nonamplified advanced gastric cancer. In *FGFR2*-amplified advanced gastric cancer, co-occurring amplifications of *PIK3CA, MYC, CDK6, CCND1, BRAF*, and *CDK4* and mutations in *ARID1A, BRCA2*, and *RHOA* were detected at a significantly higher frequency than in gastric cancer without *FGFR2* amplification (**Fig. 1C**). The co-occurring amplification of *ERBB2, MET*, and *EGFR* was detected in 1 (3.6%), 3 (10.7%), and 6 (21.4%) of 28 patients with *FGFR2* amplification, respectively. These suggest that ctDNA *FGFR2*-amplified advanced gastric cancer has a distinct profile of concurrent genomic alterations.

ctDNA can detect *FGFR2* amplification missed by tissue analyses

The increased prevalence of *FGFR2* amplification in ctDNA suggests that ctDNA sequencing may detect heterogeneous *FGFR2* amplification that tissue biopsy fails to identify. To assess whether ctDNA analysis can identify *FGFR2* amplification missed by tissue

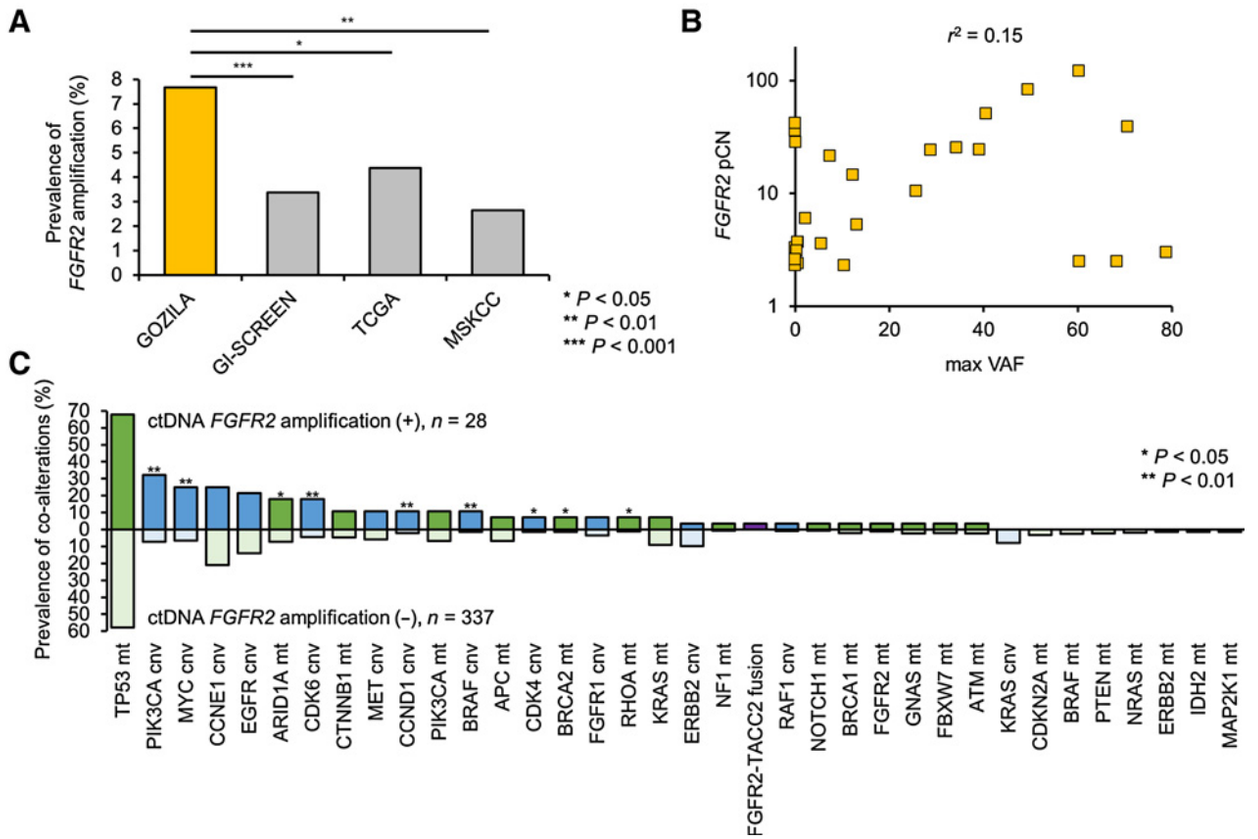


Figure 1.

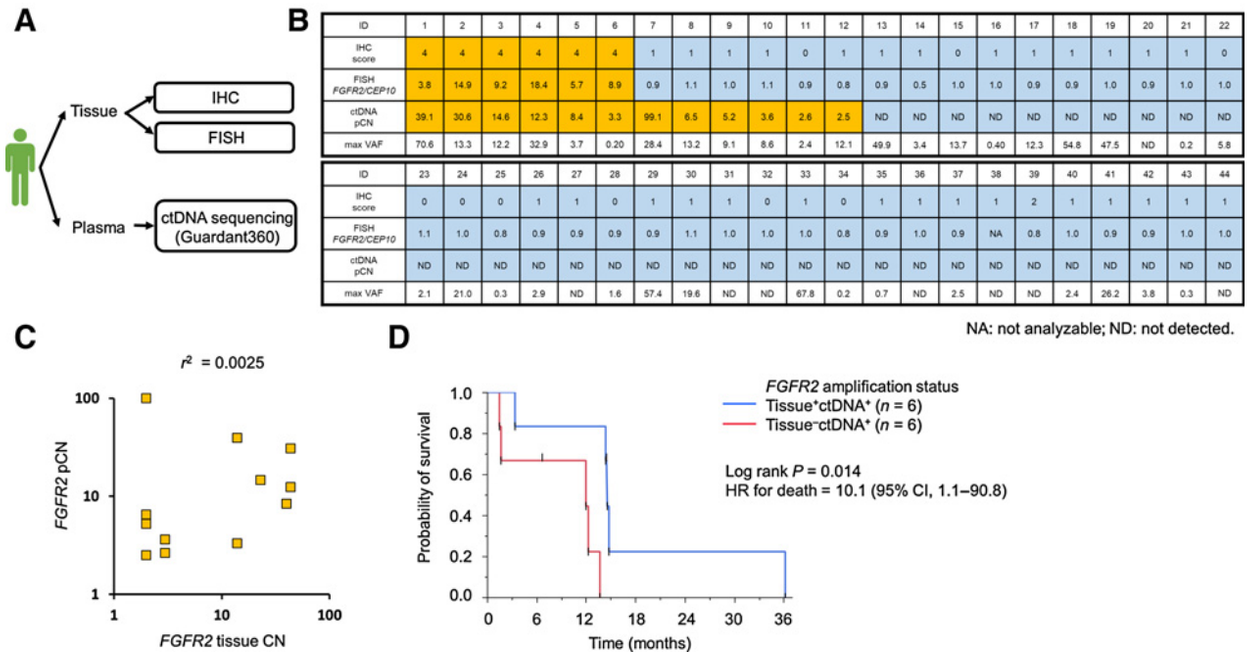
Genomic characteristics of advanced gastric cancer with *FGFR2* amplification based on ctDNA analysis. **A,** Prevalence of *FGFR2* amplification in advanced gastric cancer in GOZILA, GI-SCREEN, and the TCGA and MSKCC databases. **B,** Correlations [coefficient of determination (r^2)] between *FGFR2* plasma copy number (pCN) and max VAF in *FGFR2*-amplified samples from GOZILA ($n = 28$). **C,** Prevalence of co-alterations in *FGFR2*-amplified ($n = 28$) versus nonamplified patients ($n = 337$) in GOZILA. Green and blue bars indicate prevalence of mutations and copy-number variations co-altered with *FGFR2* amplification, respectively. Prevalence in patients without *FGFR2* amplification is highlighted in light colors. cnv, copy-number variation; max VAF, maximum variant allele frequency; mt, mutation.

analysis, we determined *FGFR2* amplification in pretreatment tissue biopsy samples by IHC and FISH and in paired plasma samples obtained near the time of tissue biopsy (median 2 days, interquartile range 1–4 days) by ctDNA sequencing in 44 patients with advanced gastric cancer (**Fig. 2A**). No ctDNA genomic alteration was identified in 4 (9.1%) patients. *FGFR2* amplification was detected by both tissue and ctDNA analysis in 6 patients and ctDNA analysis detected *FGFR2* amplification in 6 additional patients, whereas no *FGFR2* amplification was detected by tissue analysis only (**Fig. 2B**). The pCN was not significantly different between tissue$^+$ctDNA$^+$ versus tissue$^-$ctDNA$^+$ ($P = 0.18$; **Fig. 2B**). No correlation of the CN detected in tissue and ctDNA analysis was observed in tissue$^+$ctDNA$^+$ and tissue$^-$ctDNA$^+$ patients ($r^2 = 0.0025$; $P = 0.88$; **Fig. 2C**). Patients with *FGFR2* amplification in ctDNA (tissue$^+$ctDNA$^+$ or tissue$^-$ctDNA$^+$) had a significantly shorter OS than those without *FGFR2* amplification [tissue$^-$ctDNA$^-$; median, 13.7 months vs. 27.8 months; HR = 2.2; 95% confidence interval (CI), 1.0–4.9; $P = 0.047$; Supplementary Fig. S1]. The OS of tissue$^-$ctDNA$^+$ patients was significantly shorter than that of tissue$^+$ctDNA$^+$ patients (median, 12.0 months vs. 14.6 months; HR = 10.1; 95% CI, 1.1–90.8; $P = 0.014$; **Fig. 2D**). All of these patients received standard systemic chemotherapy, but not FGFR-targeted therapy. No statistically significant differences were observed in the clinicopathological characteristics among tissue$^+$ctDNA$^+$ and tissue$^-$ctDNA$^+$ patients (Supplementary Table S2). In addition, the median max VAF was 12.8 in the tissue$^+$ctDNA$^+$ and 10.6 in the tissue$^-$ctDNA$^+$ groups with no significant difference ($P = 0.52$; Supplementary Fig. S2). These findings suggest that ctDNA can identify *FGFR2* amplification missed by tissue analyses, which is associated with a poorer prognosis.

Patients with *FGFR2* amplification identified by ctDNA analysis can benefit from FGFR inhibition therapy

Previous studies had shown contradictory results regarding the efficacy of FGFR inhibitor therapy in patients with *FGFR2*-amplified advanced gastric cancer; because our results had shown that *FGFR2* amplification might be missed by analysis of single-lesion tumor biopsies probably due to tumor heterogeneity, we next examined whether patients with *FGFR2* amplification detected in ctDNA could achieve clinical benefit from an FGFR inhibitor. To this end, we highlight 2 patients who had *FGFR2* amplification not detected by tissue analysis but identified by ctDNA sequencing and received an FGFR inhibitor.

Patient 1 was a 54-year-old man who had recurrent advanced gastric cancer with multiple lymph node metastases. NGS analysis of a tissue sample collected before primary tumor resection identified only a mutation in the *TP53* gene. This patient was treated with



ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
IHC score	4	4	4	4	4	4	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0
FISH *FGFR2/CEP10*	3.8	14.9	9.2	18.4	5.7	8.9	0.9	1.1	1.0	1.1	0.9	0.8	0.9	0.5	1.0	1.0	0.9	0.9	1.0	0.9	1.0	1.0
ctDNA pCN	39.1	30.6	14.6	12.3	8.4	3.3	99.1	6.5	5.2	3.6	2.6	2.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
max VAF	70.6	13.3	12.2	32.9	3.7	0.20	28.4	13.2	9.1	8.6	2.4	12.1	49.9	3.4	13.7	0.40	12.3	54.8	47.5	ND	0.2	5.8

ID	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
IHC score	0	0	0	1	1	0	1	1	1	0	1	0	1	1	1	1	2	1	1	1	1	1
FISH *FGFR2/CEP10*	1.1	1.0	0.8	0.9	0.9	0.9	0.9	1.1	1.0	1.0	1.0	0.8	0.9	1.0	0.9	NA	0.8	1.0	0.9	0.9	1.0	1.0
ctDNA pCN	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
max VAF	2.1	21.0	0.3	2.9	ND	1.6	57.4	19.6	ND	ND	67.8	0.2	0.7	ND	2.5	ND	ND	2.4	26.2	3.8	0.3	ND

NA: not analyzable; ND: not detected.

Figure 2.

Comparative analysis of paired tissue and plasma samples in patients with advanced gastric cancer. **A,** Schematic depicting analyses of paired synchronous primary tissue and plasma samples in 44 patients with advanced gastric cancer. **B,** *FGFR2* amplification status based on IHC (score), FISH (*FGFR2/CEP10* ratio), and Guardant360 (pCN). Yellow boxes indicate high FGFR2 expression for tissue IHC score and *FGFR2* amplification for tissue FISH or ctDNA sequencing. Low FGFR2 expression and no *FGFR2* amplification are indicated by blue boxes. **C,** Correlations [coefficient of determination (r^2)] between *FGFR2* pCN and tissue CN for patients with *FGFR2* amplification detected in ctDNA. **D,** OS based on the Kaplan–Meier method for patients with *FGFR2* amplification detected in tissue$^+$ctDNA$^+$ versus in tissue$^-$ctDNA$^+$. max VAF, maximum variant allele frequency.

tegafur/gimeracil/oteracil (S-1) plus cisplatin as first-line, nanoparticle albumin-bound (nab)-paclitaxel plus ramucirumab as second-line, nivolumab as third-line, and irinotecan as fourth-line treatment. At the time of progression on nivolumab, ctDNA analysis using Guardant360 in GOZILA identified *FGFR2* amplification with a pCN of 24.2 as well as lower-level amplifications of *ERBB2, CDK4, CCND1*, and *CCNE1*, a subclonal *FGFR2-TACC2* fusion, and mutations in *NF1* and *TP53* (Supplementary Table S3). Accordingly, following progression on irinotecan, the patient received an FGFR TKI based on the results of the ctDNA analysis. The patient achieved a −73.6% response with shrinkage of the metastatic cervical and axillary lymph nodes as target lesions (**Fig. 3A**). The treatment was continued for 3 months, at which point disease progression occurred.

Patient 2 was a 57-year-old man who had unresectable gastric cancer with lymph node metastases and peritoneal dissemination. A pretreatment biopsy of the primary tumor showed only a *PIK3CA* mutation. This patient was treated with S-1 plus oxaliplatin as first-line, paclitaxel as second-line, and irinotecan as third-line treatment. The analysis of ctDNA using Guardant360 at the time of progression on irinotecan detected *FGFR2* amplification with a pCN of 6.0 and mutations in *APC* and *ARID1A* (Supplementary Table S3). After progression on irinotecan, he received an FGFR TKI, which lead to the shrinkage of the thickened gastric wall and peritoneal dissemination with a decrease in carbohydrate antigen 19–9 (CA 19–9; **Fig. 3B**). To investigate changes in *FGFR2* amplification patterns during chemotherapy, we retrospectively performed IHC and FISH analysis of tissue samples taken before treatment and after progression on S-1 plus oxaliplatin. Although *FGFR2* amplification was not detected in the pretreatment biopsy, the IHC and FISH analysis of the tissue sample obtained after S-1 plus oxaliplatin showed the emergence of high FGFR2 expression (score 4) and *FGFR2* amplification with a CN of 32.9 (Supplementary Fig. S3), suggesting that *FGFR2* amplification emerged during the treatment or was missed by the initial single-site tissue biopsy in this case and was successfully identified by ctDNA-based sequencing.

These clinical responses to FGFR inhibitors suggest that patients with *FGFR2* amplification identified by ctDNA sequencing but not detected by tissue analysis due to heterogeneity potentially benefit from treatment with FGFR inhibitors.

Concurrent *MET* amplification limited the clinical efficacy of FGFR inhibition therapy

Concurrent genomic alterations in *FGFR2*-amplified advanced gastric cancer shown in our study may be associated with the resistance to FGFR inhibition. We next report 1 patient with *FGFR2* amplification and concurrent *MET* amplification in ctDNA who was treated with an FGFR inhibitor.

Patient 3 was a 64-year-old woman with unresectable gastric cancer with lymph node metastases and pleural and peritoneal dissemination. This patient was treated with 5-fluorouracil/leucovorin plus oxaliplatin as first-line and nab-paclitaxel plus ramucirumab as second-line treatment. ctDNA sequencing using Guardant360 in GOZILA at the time of progression on nab-paclitaxel plus ramucirumab detected *FGFR2* amplification with a pCN of 14.6 with concurrent *MET* amplification and mutations in *TP53, CTNNB1*, and *ARID1A* (Supplementary Table S3). On the basis of ctDNA analysis, she received an FGFR TKI. However, a CT scan at the first evaluation revealed not only progressive lymph node enlargement (+13.3%)

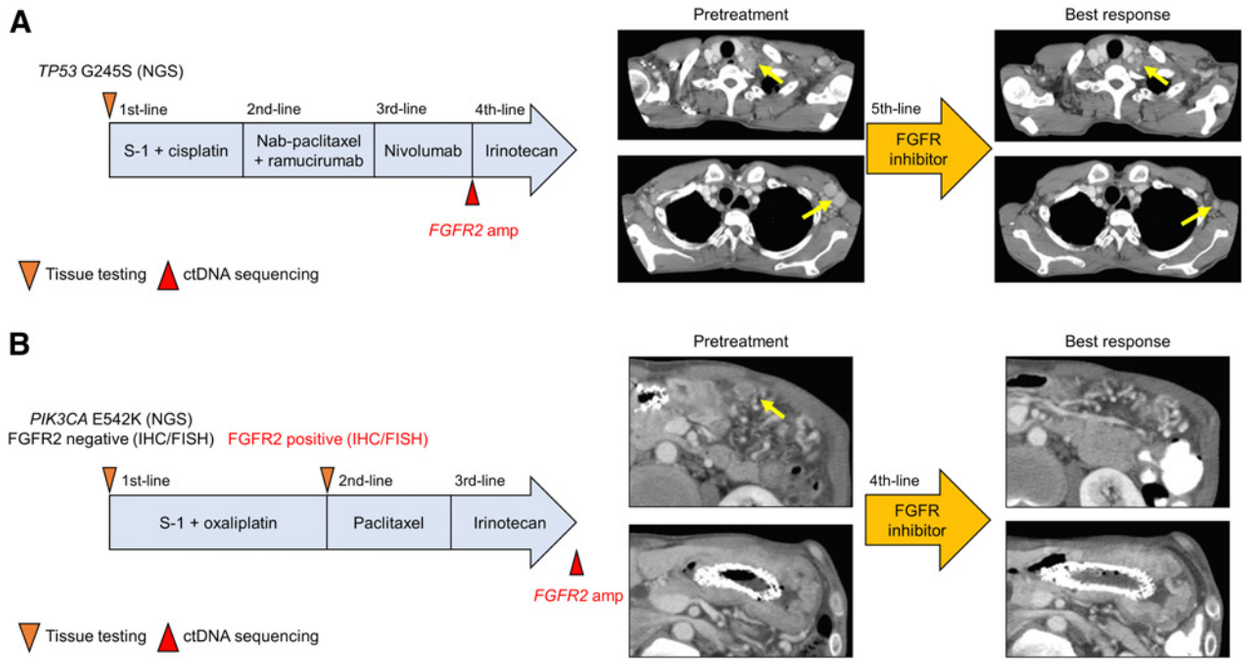


Figure 3.
Treatment history and tumor evaluation by CT before treatment and best response in patients with *FGFR2* amplification detected only by ctDNA who had tumor responses to FGFR inhibition with the shrinkage of target lesions (yellow arrows). **A,** Patient 1. **B,** Patient 2.

but also the emergence of pleural effusion and a new brain metastasis within 30 days after the initiation of the investigational drug (**Fig. 4A** and **B**). The tumor marker CA 19–9 increased from 476 to 937 U/mL during this period (**Fig. 4B**). To identify potential genomic mechanisms of resistance, pre- and postprogression tissue and postprogression plasma were analyzed using OCA and Guardant360, respectively. Compared with the pretreatment pCNs, the pCN of *FGFR2* amplification decreased after FGFR inhibitor therapy, falling from 14.6 to 7.3, whereas the pCN of *MET* amplification markedly increased from 3.0 to 15.7 (**Fig. 4C**; Supplementary Table S3). Paired tissue NGS analysis also showed similar decreases in the *FGFR2* CN and the emergence of *MET* amplification (**Fig. 4D**). These dynamic changes in *FGFR2* and *MET* amplification were confirmed by the IHC analysis of protein expression in the paired tissue samples (**Fig. 4E**). Thus, in the patients with concurrent *MET* amplification, the clinical benefit of FGFR inhibitors may be limited by the outgrowth of *MET*-amplified clones that are not sensitive to FGFR inhibition.

Discussion

The efficacy of FGFR inhibition has not been clear for *FGFR2*-amplified advanced gastric cancer with significant genomic heterogeneity, because more effective testing to detect *FGFR2* amplification for this population has not been established before FGFR treatment. This study reveals that ctDNA sequencing can more frequently detect *FGFR2* amplification than tissue analysis by identifying *FGFR2* amplifications that may be missed by conventional tissue analysis. In addition, some patients with *FGFR2* amplification identified only by ctDNA sequencing responded to treatment with an FGFR inhibitor. To our knowledge, this is the first report to show the efficacy of FGFR inhibition for advanced gastric cancer with amplified *FGFR2* detected only by ctDNA sequencing.

Patients with *FGFR2* amplification that is detected in ctDNA but undetectable by tissue analysis in our study might have *FGFR2*-amplified tumor cells in distant metastatic organs or primary tumors missed by single-lesion biopsy. These patients with tissue^{-}ctDNA^{+} for *FGFR2* amplification tended to have poorer prognosis than those with *FGFR2* amplification detectable in tissue, despite no statistically significant differences in the clinicopathologic characteristics and max VAF among these groups. This is consistent with previous studies showing an association between genomic heterogeneity in gastric cancer and poor prognosis (34), although it remains possible that ctDNA shedding due to the tumor burden contributed to this effect. These findings support that the utility of ctDNA sequencing in advanced gastric cancer for identifying genomic heterogeneity reported previously (22) can be applied for *FGFR2*-amplified disease.

In our case series, ctDNA detected *FGFR2* amplification that was not detected with tissue testing, which may have occurred due to the acquisition of *FGFR2* amplification in the interim between tissue and ctDNA testing and/or intratumoral heterogeneity that can be missed by single-lesion biopsies. Interestingly, the retrospective testing of a previously untested postprogression tissue sample in patient 2 confirmed the ctDNA finding, suggesting that *FGFR2* amplification arose in this patient after initial chemotherapy or was missed by the initial single-lesion biopsy and successfully identified by ctDNA analysis, which integrates tumor cells throughout the body. This finding underscores the importance of genomic profiling of patients with advanced gastric cancer at each instance of disease progression, which can be achieved by ctDNA sequencing with minimal invasiveness in clinical practice.

In patient 3 in our study, ctDNA detected the concurrent amplification of *FGFR2* and, to a lesser degree, *MET*. At primary progression

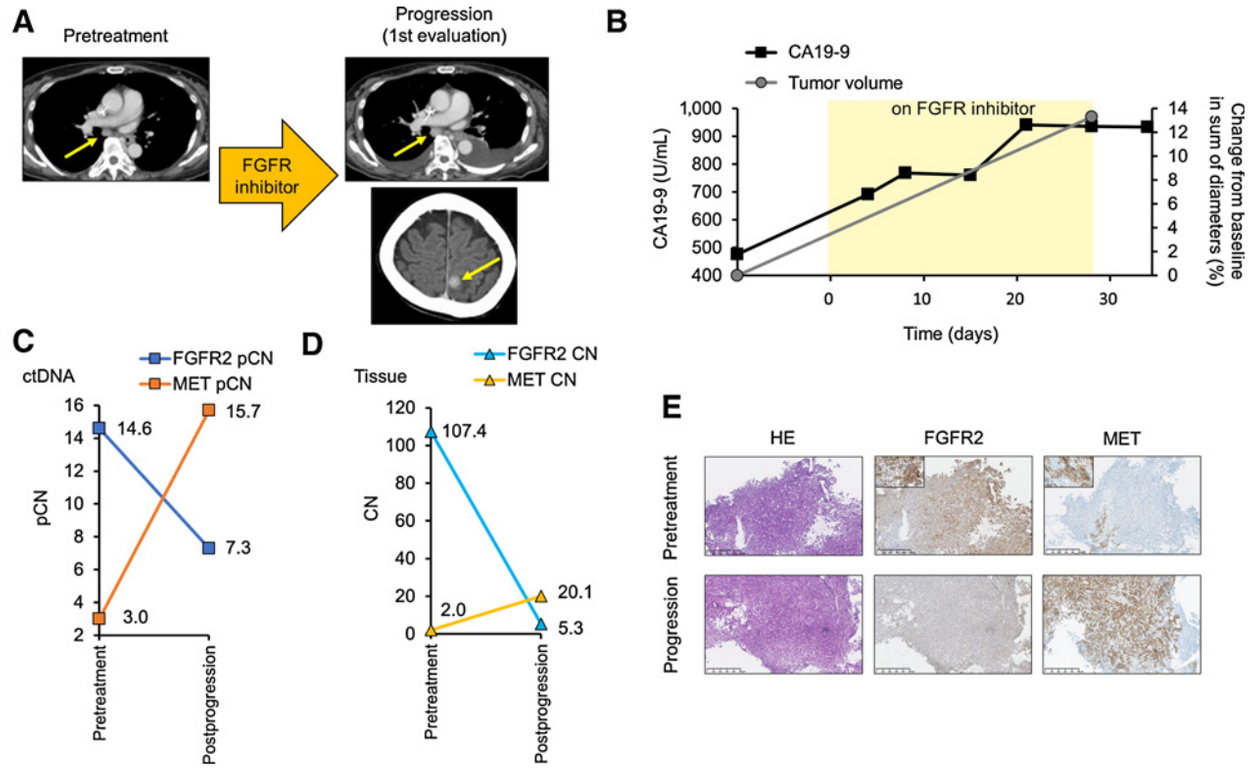


Figure 4.

Clinical presentation. **A,** Tumor evaluation by CT at pretreatment and progression on an FGFR inhibitor with progressive lymph node enlargement and the emergence of a new brain metastasis (yellow arrows) in patient 3. **B,** Changes in CA 19-9 and sum of diameters of target lesions by CT following treatment with an FGFR inhibitor. **C,** Change in the ctDNA pCN of *FGFR2* and *MET* amplification before treatment and at progression on an FGFR inhibitor. **D,** Change in the tissue CN of *FGFR2* and *MET* amplification before treatment and at progression on FGFR inhibitor. **E,** Hematoxylin and eosin–stained and IHC-stained images with anti-FGFR2 and MET antibodies of biopsy specimens of the primary gastric cancer before treatment and at progression on an FGFR inhibitor.

on an FGFR inhibitor, the *MET* pCN was markedly elevated from baseline, while *FGFR2* pCN was reduced, suggesting that two coexisting tumor cell subpopulations driven by different oncogenes responded to FGFR inhibition in opposing manners. Tumor tissue analysis and IHC confirmed that the dominant FGFR2-expressing tumor cell population was replaced by MET-expressing clones upon progression. These findings strongly indicate the relevance of *MET* amplification in limiting the clinical efficacy of FGFR inhibition. This observation has particular relevance in light of our ctDNA genomic profiling, which revealed the frequent co-occurrence of *FGFR2* amplification with other alterations, including *ERBB2, EGFR*, and *MET* amplifications. Others have similarly suggested that spatial intratumoral heterogeneity and concurrent genomic alterations in downstream molecules or other signaling pathways could act as resistance mechanisms to targeted therapies in advanced gastric cancer (23–26), leading to the frequent failure of targeted therapies. As such, ctDNA sequencing may also be useful for assessing concurrent genomic alterations to guide treatments.

An important caveat is that our findings were restricted to a Japanese population, although the frequency of tissue *FGFR2* amplification in GI-SCREEN was consistent with that in the TCGA or MSKCC data sets. The analysis of paired tissue and plasma was conducted with a limited sample size due to the availability of plasma samples from the same timepoint as tissue collection. Furthermore, the frequency of *FGFR2* amplification of this analysis was much higher than previously reported because this population included the patients previously known to have *FGFR2* amplification. In addition, ctDNA genotyping potentially underestimated the frequency of *FGFR2* amplification because some patients with gastric cancer have insufficient ctDNA shedding to detect genomic alterations in ctDNA, although no patients in our study had *FGFR2* amplification only in tissue, suggesting the prevalence of *FGFR2* amplification missed by ctDNA analysis due to the low amount of ctDNA may be limited. The efficacy of an FGFR inhibitor for *FGFR2* amplification in ctDNA was also shown only in 2 patients. The utility of ctDNA sequencing in detecting *FGFR2* amplification in advanced gastric cancer needs to be investigated in prospective studies.

In conclusion, we report the utility of ctDNA sequencing for the detection of *FGFR2* amplification that is missed by tissue analysis. Patients with such *FGFR2* amplifications have a poor prognosis when treated with standard nontargeted therapies but may benefit from FGFR inhibitor treatment. We also found that concurrent *MET* amplification detected by ctDNA sequencing was associated with limited clinical efficacy of FGFR inhibition, suggesting that combined FGFR and MET inhibition may be indicated in such cases. Taking advantage of the ability of ctDNA sequencing to detect *FGFR* alterations, we are currently conducting a phase II basket trial of futibatinib, an irreversible FGFR TKI, for patients with solid tumors harboring *FGFR* alterations confirmed by Guardant360 (JapicCTI-194624; ref. 35). This trial will provide more validated evidence regarding

the utility of ctDNA sequencing for identifying *FGFR* alterations for FGFR-targeted therapy.

Authors' Disclosures

Y. Nakamura reports grants from Taiho Pharmaceutical Co., Ltd., Chugai Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Guardant Health, Inc., Genomedia, Inc., and Seagen, Inc. outside the submitted work. K. Shitara reports grants and personal fees from Astellas Pharma, Eli Lilly and Company, Ono Pharmaceutical, Daiichi Sankyo, Taiho Pharmaceutical, and Merck Pharmaceutical; personal fees from Bristol-Myers Squibb, Takeda Pharmaceuticals, Pfizer Inc., Novartis, AbbVie Inc., Yakult, GlaxoSmithKline, Amgen, and Boehringer Ingelheim; and grants from Dainippon Sumitomo Pharma, Chugai Pharma, Medi Science, and Eisai outside the submitted work. H. Bando reports personal fees from Eli Lilly Japan and Taiho Pharmaceutical outside the submitted work. H. Yasui reports grants from MSD, Ono Pharmaceutical; grants and personal fees from Daiichi Sankyo; and personal fees from Taiho Pharmaceutical, Chugai Pharma, Bristol-Myers Squibb Japan, TERUMO, Eli Lilly Japan, Merck Biopharma, and Yakult Honsha outside the submitted work. T. Esaki reports grants and personal fees from MSD, Ono, Daiichi Sankyo, and Merck Serono; grants from Novartis, Dainippon Sumitomo, Astellas, AstellasAmgenBiopharma, BeiGene, Pierre Fabre Medicament, Ignyta, and Array BioPharma; and personal fees from Bayer, Eli Lilly, Taiho, Chugai, Sanofi, and Takeda outside the submitted work. T. Satoh reports grants and personal fees from Ono Pharmaceutical, Chugai Pharmaceutical, Yakult Honsha, Eli-Lilly, MSD, Daiichi-Sankyo, and Taiho Pharmaceutical, as well as grants from Gilead, BeiGene, and Astellas outside the submitted work. T. Nishina reports grants and personal fees from Taiho Pharmaceutical Co., Chugai Pharmaceutical Co., Ono Pharmaceutical Co., Bristol-Myers Squibb, and Lilly Pharma, as well as grants from MSD, Sumitomo Dainippon Pharma Co., and AstraZeneca outside the submitted work. Y. Sunakawa reports grants and personal fees from Takeda, Sanofi, Chugai Pharmaceutical, and Taiho Pharmaceutical; personal fees from Bristol-Myers Squib, Merck Biopharma, and Lilly Japan; and grants from Otsuka Pharmaceutical outside the submitted work. H. Hara reports grants from Astellas, AstraZeneca, Eisai, Elevar, GSK, Incyte, Pfizer, and BeiGene; grants and personal fees from Bayer, Boehringer Ingelheim, Chugai, Daiichi Sankyo, Dainippon Sumitomo, Merck, MSD, Ono, and Taiho; and personal fees from BMS, Kyowa Hakko Kirin, Sanofi, Takeda, and Yakult outside the submitted work. E. Oki reports other support from Taiho Pharm during the conduct of the study, as well as other support from Eli Lilly, Bayer, Chugai Pharm, and Ono Pharm outside the submitted work. T. Ohta reports personal fees from Bristol-Myers Squibb Co., Ltd., Eli Lilly Japan K.K., Novartis Pharma K.K., Chugai Pharmaceutical Co., Ltd., Teijin Pharma Ltd., Takeda Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co. Ltd, and Eisai Co., Ltd. outside the submitted work. T. Kawakami reports personal fees from Bayer, Taiho Pharmaceutical, Takeda, Ono Pharmaceutical, and Bristol-Myers Squibb outside the submitted work. N. Okano reports personal fees from Taiho Pharmaceutical, Eli Lilly Japan, Kyowa Hakko Kirin, Eisai, Bayer Yakuhin, Chugai, J-Pharma, Ono Pharmaceutical, Takeda, and GSK outside the submitted work. T. Yamada reports personal fees from Johnson and Johnson, Taiho, Ono, and Nippon Kayaku outside the submitted work. A. Tsuji reports personal fees from Takeda Pharmaceutical Company Limited, Sanofi Corporation, and Pfizer Japan Inc.; grants and personal fees from Merck Serono Co., Ltd., Chugai Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd., Eli Lilly Japan Co., Ltd., Ono Pharmaceutical Co., Ltd., Kyowa Hakko Kirin Co., Ltd., MSD Corporation, Toray Medical Co., Ltd., Daiichi Sankyo Co., Ltd., and Bristol-Myers Squibb Corporation; and grants from Eisai Co., Ltd. outside the submitted work. J.I. Odegaard reports employment with Guardant Health. T. Doi reports grants from Lilly, Merck Serono, Pfizer, BMS, IQVIA, and Eisai; grants and personal fees from MSD, Daiichi Sankyo, Sumitomo Dainippon, Taiho, Novartis, Janssen Pharma, Boehringer Ingelheim, and AbbVie; and personal fees from Takeda, Chugai Pharma, Bayer, Rakuten Medical, Otsuka Pharma, BMS, Ono Pharma, Oncolys Bio Pharma, Amgen, and Astellas Pharma outside the submitted work. T. Yoshino reports grants from Taiho Pharmaceutical, Sumitomo Dainippon Pharma, Ono Pharmaceutical, Chugai Pharmaceutical, Amgen, PAREXEL International, MSD, Daiichi Sankyo, and Sanofi outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

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Acknowledgments

We would like to thank the Translational Research Support Section of the National Cancer Center Hospital East for study management and data center support; Geneticlab Co., Ltd. and Thermo Fisher Scientific, Inc. for data analysis; and Mari Takahashi and Yuka Nakamura for their excellent technical assistance. This work was supported by grants from the Japan Agency for Medical Research and Development (20ck0106447h0002 to T. Yoshino).

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Received April 17, 2021; revised June 26, 2021; accepted August 4, 2021; published first August 10, 2021.

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