

Mechanisms of Acquired Resistance to Savolitinib, a Selective MET Inhibitor in *MET*-Amplified Gastric Cancer

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PURPOSE Some gastric cancers harbor *MET* gene amplifications that can be targeted by selective MET inhibitors to achieve tumor responses, but resistance eventually develops. Savolitinib, a selective MET inhibitor, is beneficial for treating patients with MET-driven gastric cancer. Understanding the resistance mechanisms is important for optimizing postfailure treatment options.

PATIENTS AND METHODS Here, we identified the mechanisms of acquired resistance to savolitinib in 3 patients with gastric cancer and *MET*-amplified tumors who showed a clinical response and then cancer progression. Longitudinal circulating tumor DNA (ctDNA) is useful for monitoring resistance during treatment and progression when rebiopsy cannot be performed.

RESULTS Using a next-generation sequencing 100-gene panel, we identified the target mechanisms of resistance *MET* D1228V/N/H and Y1230C mutations or high copy number *MET* gene amplifications that emerge when resistance to savolitinib develops in patients with *MET*-amplified gastric cancer.

CONCLUSION We demonstrated the utility of ctDNA in gastric cancer and confirmed this approach using baseline tumor tissue or rebiopsy.

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INTRODUCTION

Gastric cancer is the fifth most common cancer worldwide and the third leading cause of cancer-related death.¹ Gastric cancer is a heterogenous disease, the drivers of which remain unclear, making it difficult to treat these patients. Pembrolizumab was approved for treating GI malignancies with mismatch repair deficiencies and for third-line programmed death ligand 1–positive gastric cancer based on its recently observed promising antitumor activity.^{2,3} However, it remains urgent to identify a subset of patients with gastric cancer who may benefit from molecularly targeted agents. One such potential target is *MET* amplification, which is observed in approximately 5% of patients with gastric cancer.³⁻⁹

Savolitinib (AZD6094, HMPL-504, volitinib) is a potent small-molecule MET kinase inhibitor that inhibits MET kinase at a half-maximal inhibitory concentration (IC₅₀) of 4 nM and MET phosphorylation in tumor cells. Savolitinib was found to inhibit cell growth in vitro against tumors with *MET* gene amplification in the absence of hepatocyte growth factor stimulation, with IC₅₀ generally < 10 nM. The combination of savolitinib and osimertinib is being tested in patients with

non–small-cell lung cancer, and savolitinib monotherapy is being studied in patients with metastatic papillary renal cell carcinoma (PRCC).¹⁰ Phase II trial results from 44 patients with MET-driven PRCC revealed 8 confirmed partial responders.¹⁰ On the basis of this trial, the phase III SAVOIR study, which compares sunitinib to savolitinib, is currently underway for treating *MET*-amplified PRCC (ClinicalTrials.gov identifier: [NCT03091192](https://clinicaltrials.gov/ct2/show/study/NCT03091192)).

As part of the gastric cancer–specific umbrella trial, the VIKTORY (Targeted Agent Evaluation in Gastric Cancer Basket Korea) trial, we enrolled *MET*-amplified patients into the following two separate trials: a savolitinib monotherapy trial (ClinicalTrials.gov identifier: [NCT02449551](https://clinicaltrials.gov/ct2/show/study/NCT02449551)) and a phase II docetaxel and savolitinib trial (ClinicalTrials.gov identifier: [NCT02447406](https://clinicaltrials.gov/ct2/show/study/NCT02447406)).¹¹ In the phase I trial of savolitinib plus docetaxel, we observed promising antitumor activity with a durable response that persisted for 297 days in patients with *MET*-amplified gastric cancer (unpublished data). Here, we report the first three consecutive patients with *MET* amplification who were administered savolitinib monotherapy as a second- or third-line treatment. We performed targeted sequencing of primary tumor and

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

We evaluated the mechanisms of acquired resistance to savolitinib in 3 patients with *MET*-amplified gastric cancer who showed a clinical response and then cancer progression using tumor and circulating tumor DNA (ctDNA) sequencing.

Knowledge Generated

ctDNA is a powerful tool for identifying potential genomic aberrations that emerge during therapy to confer resistance to targeted therapies. Using a next-generation sequencing 100-gene panel, we identified the target mechanisms of resistance *MET* D1228V/N/H and Y1230C mutations or high copy number *MET* gene amplifications that emerge when resistance to savolitinib develops in patients with *MET*-amplified gastric cancer.

Relevance

We demonstrated the utility of ctDNA in gastric cancer and confirmed this approach using baseline tumor tissue or rebiopsy. We identified various *MET* mutations previously unidentified in gastric cancer upon progression to savolitinib, as well as *MET* amplifications as drivers of savolitinib resistance during monotherapy treatment of patients with *MET*-amplified gastric cancer.

circulating tumor DNA (ctDNA) from baseline until progression every 2 cycles to evaluate the tumor biology during treatment with targeted agents. We identified newly emerged *MET* somatic mutations and increased copy numbers of the *MET* gene as potential resistance mechanisms in responders to the MET inhibitor in gastric cancer.

PATIENTS AND METHODS

Patients

Patients enrolled in this study had measurable, histologically confirmed, refractory, metastatic gastric cancer. The trial was conducted in accordance with the Declaration of Helsinki and Guidelines for Good Clinical Practice (ClinicalTrials.gov identifier: [NCT02447406](#)). The trial protocol was approved by the Institutional Review Board of Samsung Medical Center (Seoul, Korea), and all patients provided written informed consent before enrollment. The human investigations were performed after approval by a local human investigations committee (institutional review board) and in accordance with an assurance filed with and approved by the health authority in Korea. This trial was part of the VIKTORY trial (ClinicalTrials.gov identifier: [NCT02299648](#)). This prospective open-label trial was designed as a single-arm phase II study at an academic cancer center (ClinicalTrials.gov identifier: [NCT02449551](#)). Treatment was administered as follows: savolitinib 800 mg once a day until disease progression or unacceptable toxicity. The regimen is currently modified to 600 mg once a day, and 1 cycle of treatment is 3 weeks. Tumor tissue and plasma ctDNA samples from 3 patients enrolled in the VIKTORY trial were obtained before treatment, during therapy, and at the time of progression.

Next-Generation Sequencing

A targeted next-generation sequencing 100-gene panel was used to analyze matched tumor DNA at baseline and progression and longitudinal ctDNA to determine the

mechanisms of acquired resistance to savolitinib (AZ Translational Genomics Labs, Boston, MA). Genes included in this panel are provided in Appendix [Figure A1A](#). Sequencing libraries were prepared using the KAPA Biosystems HyperPrep kit (Wilmington, MA) and Roche NimbleGen Hybridization Capture reagents (Basel, Switzerland). Sequencing was performed at AstraZeneca (Gaithersburg, MD) on a HiSeq4000 instrument (2 × 150 base pairs). Raw sequencing data were processed using the bcbio framework.¹² The quality of sequencing data was assessed using the MultiQC report.¹³ FASTQ files were aligned to the reference genome hg38 using bwa mem aligner,¹⁴ yielding a median depth of coverage of 2,000× (range, 1,300-6,600×) in plasma samples and 1,200× (range, 1,100-1,400×) in tissue samples. Variants were called using VarDict¹⁵ and classified into the following 3 tiers from high to low based on the likelihood of the variant contributing to cancer-relevant processes: known, likely, and unknown.¹⁵ Copy number analysis of sequencing data were performed using the copy number caller Seq2C.¹⁶ All plasma samples were analyzed together as a plasma cohort, whereas tissue samples were processed together as a tissue cohort. Mapping and sequencing statistics for formalin-fixed paraffin-embedded tumor tissue ([Fig A1B](#)) and ctDNA from frozen plasma ([Fig A1C](#)) indicated that > 90% and 96% of the 100 genes were sequenced with 500× coverage in tumor and ctDNA, respectively, providing a quality data set for analysis.

ET Immunohistochemistry and *MET* Fluorescent In Situ Hybridization

MET immunohistochemistry (IHC) was performed using the rabbit monoclonal primary antibody, CONFIRM anti-total Met (SP44; Ventana Medical Systems, Tucson, AZ) according to the manufacturer's protocol. Overexpression was defined as 3+ according to previously published criteria.⁵ Fluorescence in situ hybridization (FISH) was

performed using dual-color DNA-specific *MET/CEP7* probes (Abnova, Walnut, CA), as described previously.^{5,8}

RESULTS

We analyzed the first 3 patients enrolled in the savolitinib trial, which enrolls patients with gastric cancer with *MET* amplification in the salvage setting. Patients were screened for the presence of *MET* amplification by tumor sequencing as part of the VIKTORY trial and are confirmed wild type for *MET*.¹¹ The VIKTORY trial screened 715 patients with genomic sequencing, and the incidence of *MET* amplification was 3.5% (25 of 715 patients). In this study, we tested the feasibility of using a 100-gene panel in patients with *MET*-amplified gastric cancer serially from baseline until disease progression using both matched biopsies and ctDNA. The first patient was a 35-year-old man diagnosed with metastatic gastric cancer at presentation. The patient had microsatellite-stable and HER2-negative gastric cancer (Fig 1A) and experienced rapid progression after 5 cycles of capecitabine plus oxaliplatin (initially stable disease) to extensive dissemination in the lymph nodes (Fig 1B). Pathologic findings were typical tubular adenocarcinoma, poorly differentiated, and a *MET/CEP7* ratio of 10 (Fig 1A). At baseline before savolitinib treatment, the patient's ctDNA showed *TP53* P190L (44%), *MET* 3.0 copy number, and *MYC* 5.6 copy number. The patient responded dramatically to 2 cycles of savolitinib (Fig 1B, middle panel), which was concordant with the decrease in the allele frequency of *TP53* P190L (7%), *MET* 1.4 copy number, and *MYC* 3.4 copy number in the ctDNA. The tumor size was reduced by 68.5% per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) compared with the size at baseline. Notably, although the patient maintained a radiologic and clinical partial response (PR) to savolitinib, low frequencies of *MET* D1228H (5%), *MET* D1228N (5%), *MET* D1228V (35%), and *MET* Y1230C (3%) were newly detected. After 3.5 months of savolitinib, the patient experienced rapid progression, and the patient's ctDNA showed persistently low copy numbers of *MET* and *MYC* compared with those at baseline; however, the allele frequencies of *MET* D1228H (31%) and *MET* D1228N (12%) significantly increased compared with those in ctDNA collected during the PR. The *MET* D1228V (1%) and *MET* Y1230C (1%) mutations were also observed at progression. *MET* D1228N is a resistant mutation to crizotinib in lung cancer.¹⁷ In addition, *MET* D1228V has been shown to induce resistance to type 1 *MET* tyrosine kinase inhibitors in lung cancer.¹⁸ Finally, *MET* Y1230C is associated with resistance to crizotinib in non-small-cell lung cancer.¹⁹ The high concordance for the copy number level between ctDNA 100-gene sequencing and tumor rebiopsy specimen sequencing supports the usefulness of copy number evaluation from longitudinal plasma ctDNA (Appendix Fig A2). This patient's samples showed decreases in *MET* and *MYC* levels compared with those at baseline, suggesting that the *MET* copy number did not drive

resistance in this patient. At disease progression, the patient had mostly experienced progression to bone and bone marrow metastases rather than previous lymph nodes, suggesting the emergence of an aggressive clone that spread to the bone and bone marrow at progression. Our data suggest that, rather than *MET* amplifications, *MET* mutations drove the rapid progression observed in this patient (Fig 1C).

The second patient was a 74-year-old woman who was diagnosed with gastric cancer with extensive liver metastases at presentation and enrolled in the VIKTORY trial at diagnosis. The patient's disease rapidly progressed to liver and peritoneal seeding after 1 cycle of first-line titanium silicate-1 (TS-1) chemotherapy, and she was enrolled in the VIKTORY trial in the savolitinib monotherapy arm as a second-line treatment because the cancer panel identified *MET* amplification (11.6 copies; log₂ ratio, 2.54). *MET* FISH data were not available because of the limited tumor specimen. The patient had tubular adenocarcinoma that was poorly differentiated, HER2 negative, and microsatellite stable. In accordance with the savolitinib response, ctDNA showed no *MET* amplification when the patient achieved a PR. After 4 cycles of savolitinib, with her PR confirmed radiologically, the *MET* copy number was 2.7 and showed a slight increase from diploid, and a *TP53* G245D (2%) variant began to emerge as detected in the ctDNA. The tumor size was reduced by 83.5% per RECIST 1.1 compared with baseline. After 6 months (7 cycles of savolitinib), the patient developed radiologic progression. Interestingly, in contrast to the newly emerging mutations conferring resistance to savolitinib in patient 1 (Fig 1), the second patient developed a high level of *MET* amplification of 13 copies and *CDK6* copy number of 3.9 at progression (Fig 2A). Although both genes are proximal to one another on chromosome 7, they are not part of the same amplicon because the *TRAP* and *PIK3CG* gene loci are between *MET* and *CDK6*, and they remained at a normal copy number in both the ctDNA and tumor samples at progression (Fig 2B, top panel). High concordance was observed between the ctDNA and tumor tissue DNA (liver metastasis) with a reincrease in *MET* amplification and *CDK6* amplification that was not detected at PR. In addition, the ctDNA sample at progression indicated that the *TP53* G245D substitution containing the clone had expanded, as suggested by an increase from a 2% allele fraction (at the third follow-up) to a 22% allele fraction (at progression). This was confirmed in the progression rebiopsy, where the same *TP53* G245D substitution that was not detected in normal DNA had subsequently increased to an 82% allele fraction (Fig 2B, bottom panel).

The third patient was a 31-year-old woman who underwent total gastrectomy for gastric cancer in 2013. The pathologic diagnosis of the surgical specimen was pT4N3M0. The patient had poorly differentiated tubular adenocarcinoma and was microsatellite stable and HER2 negative. The

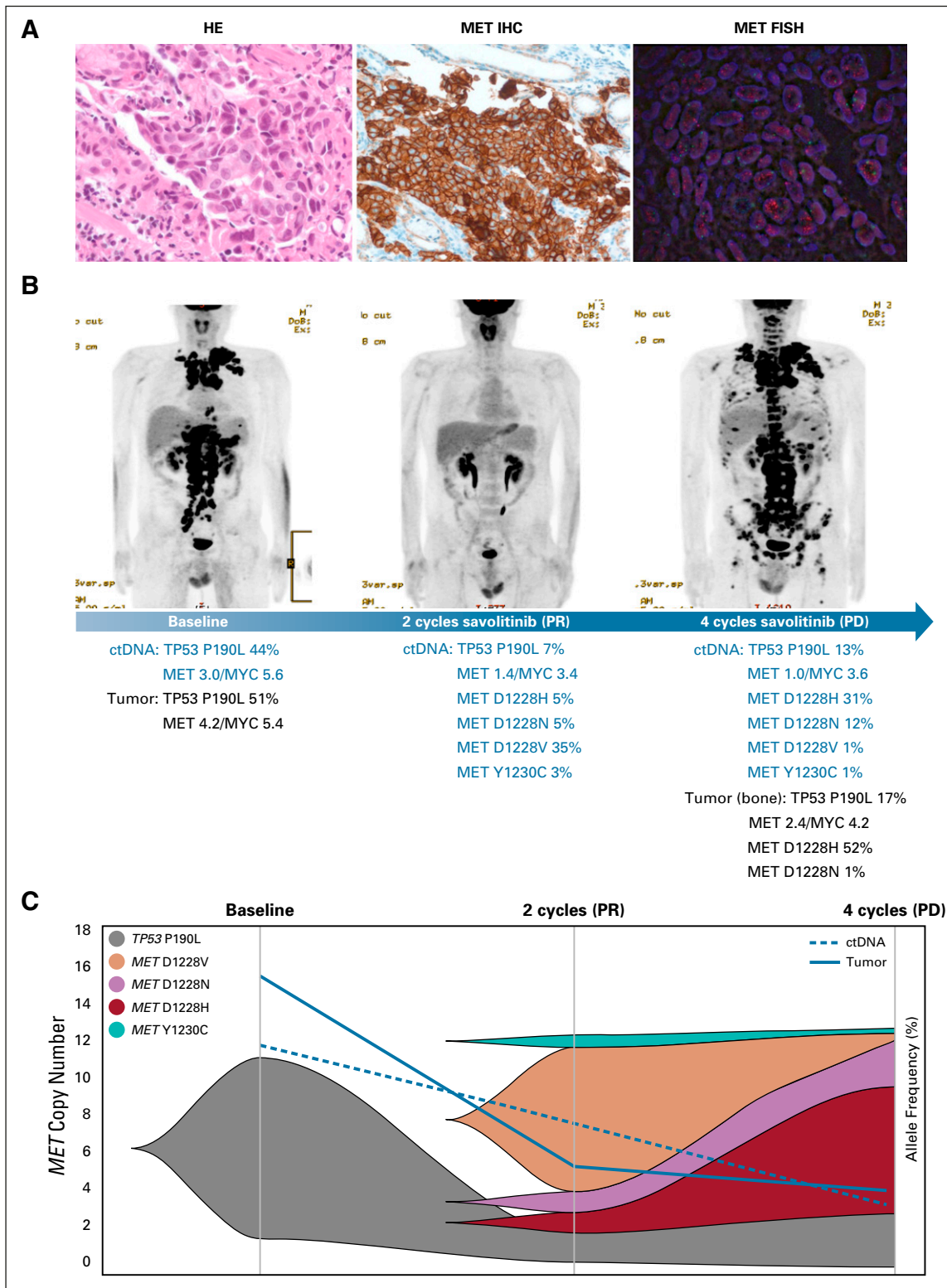


FIG 1. Pathology, clinical assessment, and circulating tumor DNA (ctDNA) monitoring for patient 1 (B5-002) identifies *MET* D1228 and Y1230 mutations as mechanism of resistance to savolitinib. (A) Hematoxylin and eosin (HE), MET immunohistochemistry (IHC), and *MET* fluorescence in situ hybridization (FISH; left to right) from baseline tumor tissue. (B) Tumor assessment by radiologic assessment at baseline before savolitinib treatment, after 2 cycles at time of partial response (PR), and after 4 cycles at time of progressive disease (PD). (C) Graphical representation of ctDNA mutation evolution in patient 1. Evolution of *TP53* and *MET* mutations by changes in allele frequency (%) from ctDNA in longitudinal samples using next-generation sequencing. Solid line represents tumor *MET* copy number, and dashed line represents ctDNA plasma *MET* copy number.

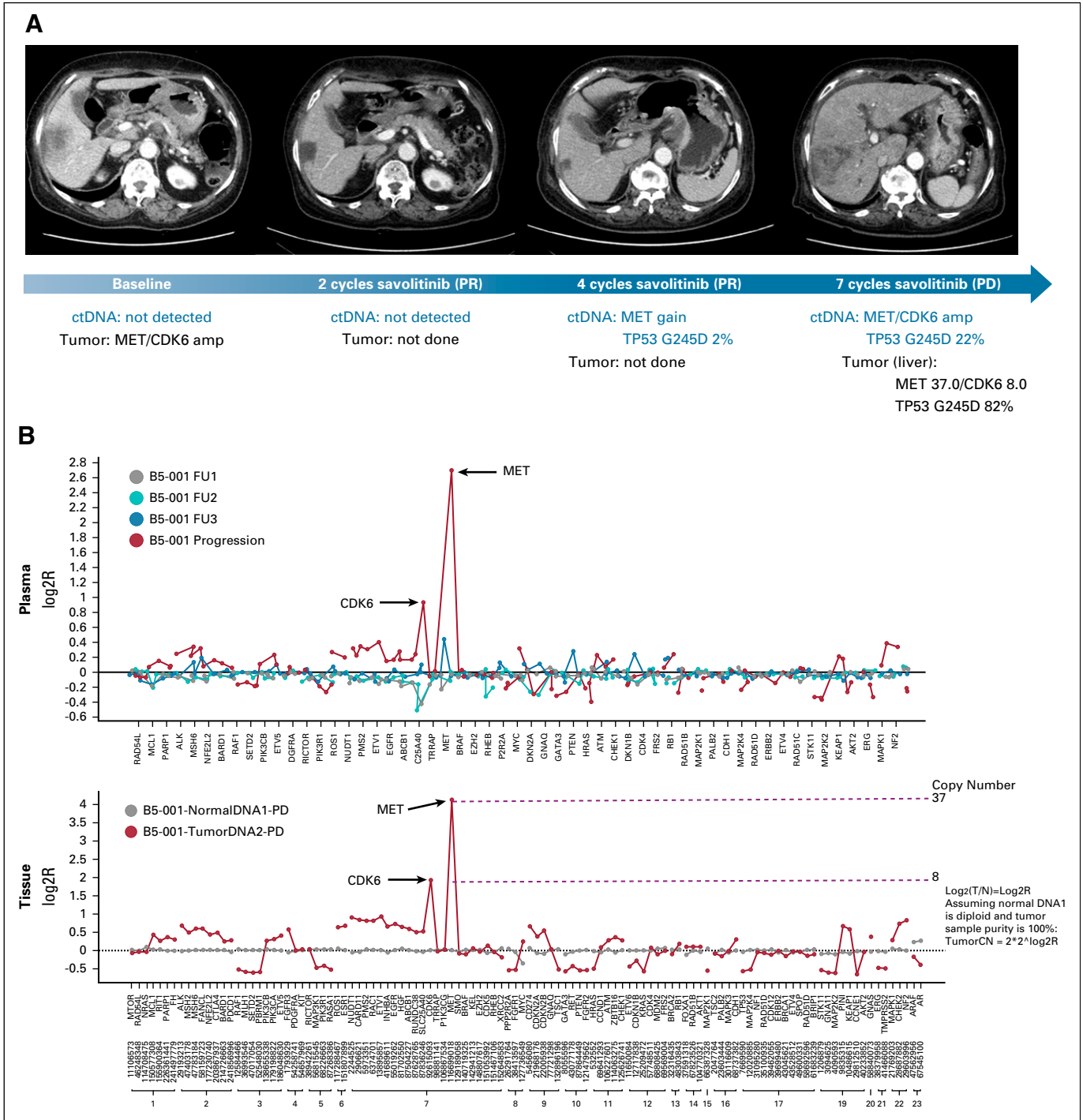


FIG 2. Clinical assessment and circulating tumor DNA (ctDNA) monitoring for patient 2 (B5-001) identify on-target high-level *MET* amplification (amp) as mechanism of resistance to savolitinib. (A) Radiologic assessment of tumor at baseline before savolitinib treatment (follow-up [FU] 1); after 2 cycles (FU2) and 4 cycles (FU3), both at time of partial response (PR); and after 7 cycles at time of progressive disease (PD). (B) Copy number profile of ctDNA plasma (top) and tumor tissue or normal DNA at progression (bottom), where each sample is represented by a different color at baseline (FU1), after 2 cycles (FU2) and 4 cycles (FU3), and after 7 cycles at time of progression.

patient was administered postoperative chemoradiation therapy with a TS-1 plus oxaliplatin regimen and developed recurrent metastases to both ovaries 18 months later. After undergoing bilateral oophorectomy, the patient was

administered 6 cycles of a postoperative capecitabine plus cisplatin regimen. Ten months later, the patient developed a peritoneal seeding mass (Fig 3B). Although this patient's tumor tissue sequencing failed, her tumor at the time of

oophorectomy showed MET IHC 3+ and *MET* amplification by FISH (MET/SEP7 ratio, 5.6; Fig 3A). Her tumor decreased in volume during savolitinib therapy, achieving a PR for 6 months with a maximal diameter decrease from baseline computed tomography of 47.7%. Genomic

sequencing of the ctDNA sample suggested that this patient had a nonshedding tumor; no sequencing variants were detected from across the 100-gene panel, including *MET* amplification, mutation, or other genomic aberrations (Fig 3C).

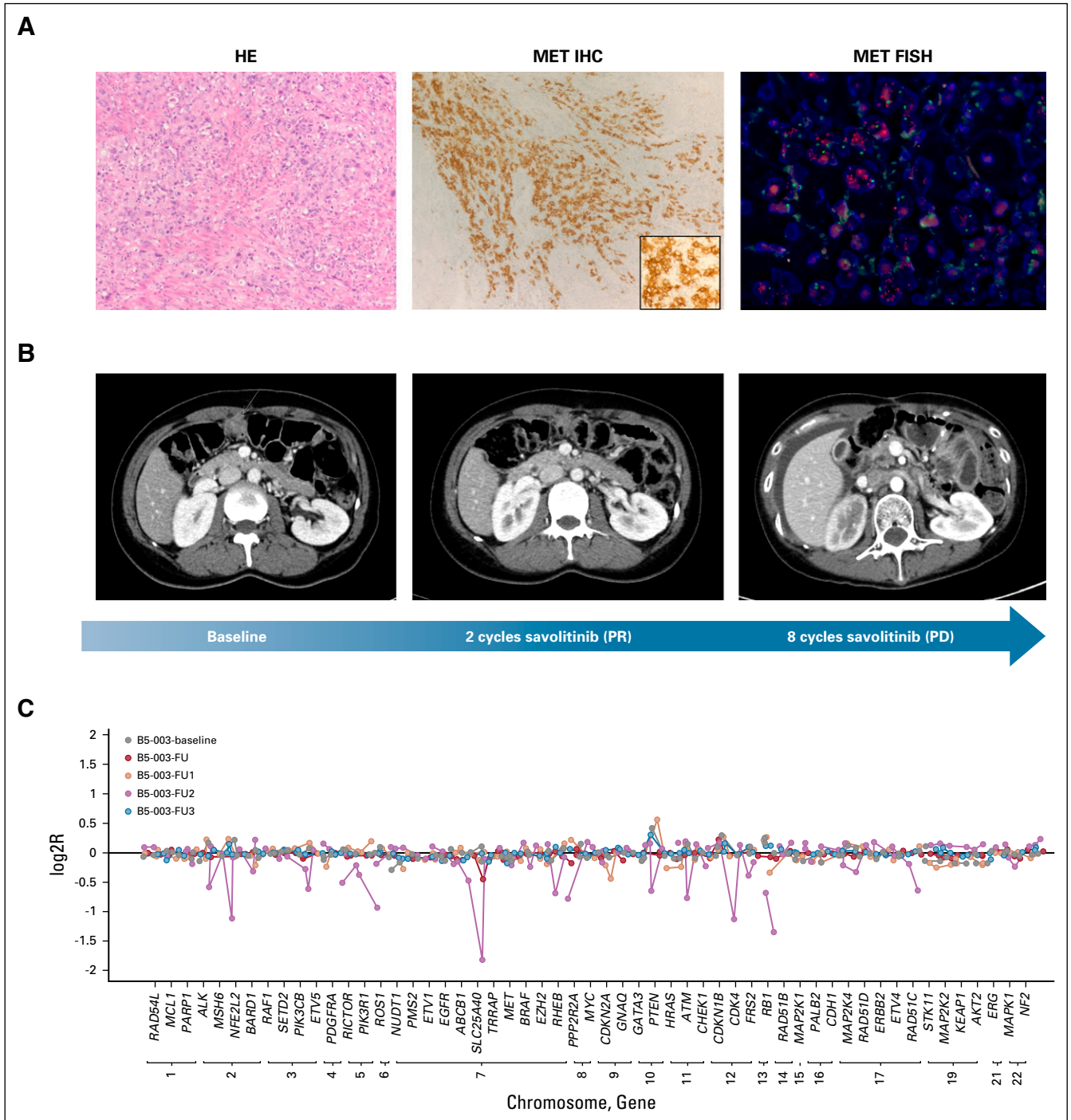


FIG 3. Pathology and clinical assessment of patient 3 (B5-003), who did not shed circulating tumor DNA (ctDNA). (A) Hematoxylin and eosin (HE), MET immunohistochemistry (IHC), and MET fluorescence in situ hybridization (FISH; left to right) from baseline tumor tissue. (B) Tumor assessment by radiologic assessment at baseline before savolitinib treatment, after 2 cycles at time of partial response (PR), and after 8 cycles at time of progressive disease (PD). (C) Copy number profile of ctDNA plasma where each time point is represented by a different color. FU, follow-up.

DISCUSSION

The aim of this exploratory study was to determine whether relapses on savolitinib in patients with gastric cancer after an initial PR resulted from the development of on-target or bypass mechanisms of resistance. Furthermore, we established the utility of ctDNA from plasma as a matrix for monitoring changes in DNA alterations during the course of treatment of gastric cancer and demonstrated the concordance of genomic alterations in ctDNA to tumor biopsy both before treatment with a targeted therapy and at the time of progression. This is a preliminary report of the first 3 patients with *MET*-amplified gastric cancer who were enrolled in a phase II savolitinib monotherapy study. The efficacy of the trial will be reported once the trial completes recruitment; here, we focused on the utility of ctDNA sequencing to identify potential resistance mechanisms in gastric cancer after MET inhibition. We identified the following two potential mechanisms of acquired resistance at progression following a dramatic response to savolitinib in *MET*-amplified gastric cancer: newly developed *MET* mutations previously only described in lung cancer and an increase in the *MET* copy number at resistance, which decreased during the clinical response but increased again at disease progression.

In the first patient, newly emerging *MET* D1228H (31%), *MET* D1228N (12%), *MET* D1228V (1%), and *MET* Y1230C (1%) mutations were detected with the sudden onset of disease progression to multiple bones and the bone marrow. At the time of progression, *MET* amplifications seemed to be persistently inhibited by savolitinib (Fig 1B). Thus, the 4 mutations, particularly *MET* D1228H and *MET* D1228N, were profoundly expanded compared with samples collected previously. These resistance mutations, located in the MET kinase domain, are likely responsible for genomic aberrations in newly developed disseminated bone and bone marrow metastases. This is an important finding because newly emerged mutations have not been described in patients with gastric cancer, particularly in association with inhibitors targeting amplifications such as *MET*. We previously reported several genomic aberrations that emerged with acquired resistance to lapatinib.²⁰ During lapatinib resistance, most patients showed concurrent amplifications beyond *HER2* amplification using ctDNA. This patient died of rapid progression immediately after developing resistance to savolitinib therapy.

Mutations in *MET*, including at positions D1228 and Y1230, are among the reported mechanisms of acquired resistance in preclinical models^{21,22} and have been reported to confer clinical resistance to MET inhibition in lung cancer.^{17,19} The cocrystal structure of the MET kinase domain and MET kinase inhibitor revealed an important binding interaction of the MET inhibitor and Y1230 and explains the abrogation of compound binding with the emergence of mutations at this residue. In addition, D1228

is engaged in interactions with other residues to maintain the activation loop in a conformation that enables the critical interaction between Y1230 and the MET inhibitor.²¹ These results demonstrate that the on-target resistance mutations emerge in *MET*-amplified gastric cancer treated with a selective MET kinase inhibitor. *MET* mutations at D1228 and Y1230 can impair the activity of class I selective MET inhibitors such as savolitinib, whereas class II MET inhibitors can inhibit mutants at these positions in the MET kinase domain but are not as selective as class I inhibitors. Thus, it is important to understand the underlying cause of resistance to optimize subsequent treatment options for each patient.²³

In the second patient, another on-target acquired mechanism of resistance was found in which there was further upregulation of the *MET* gene amplification, which was being controlled during the patient's clinical response. Mechanistic studies of gastric cancer cells demonstrated that a marked increase in Met protein expression and association of Met with E-cadherin in cell lines made them resistant to MET inhibitors, which is thought to drive cell migration and invasion signals.²⁴ Another Met pathway-specific mechanism of resistance for MET inhibitors has been described in which hepatocyte growth factor overexpression was reported to induce resistance to MET inhibitors in gastric cancer cell lines.²⁵ In the second patient's tissue and plasma analysis at progression, hepatocyte growth factor levels were increased to 3 copies; however, this gain was not as pronounced as *CDK6* and *MET* amplifications (Fig 2B). Therefore, upregulation of MET (in a savolitinib-responsive tumor) similar to HER2 (in a lapatinib-responsive tumor) may confer resistance via epithelial to mesenchymal transition and by promoting a metastatic phenotype.^{26,27} An on-target *MET* copy number gain as a mechanism of resistance has been reported in lung cancer, where one *MET* exon 14–skipping patient became a responder to a MET inhibitor in the clinic; however, at the time of progression, the exon 14 *MET* allele was amplified, but not the wild-type allele of *MET*,²⁸ suggesting that high-level amplification of the *MET* gene is a mechanism of resistance to MET inhibitors across indications.

In 2 of 3 patients studied, ctDNA was detectable in the circulation, harboring somatic alterations in the MET pathway that changed over time and were confirmed by rebiopsy at recurrence. In addition to *MET* mutations or amplifications, amino acid substitutions in *TP53* were also detected in longitudinal ctDNA plasma samples. A clinical report of patients with gastric cancer in which the ctDNA *TP53* fraction correlated with disease status²⁹ suggested that *TP53* is a surrogate for tumor size. Our data agree with this hypothesis; in the first patient, *TP53* P190L changed from a fraction of 44% at baseline to 7% during PR and then back to 13% at disease progression, which was further confirmed in tumor tissue with a 17% allele fraction harboring the same mutation in *TP53*. The second patient

contained no detectable ctDNA *TP53* until after 4 cycles, at which point a G245D amino acid substitution was detectable at a 2% allele fraction and increased to 22% at progression. The same alteration was identified by rebiopsy at an 82% allele fraction.

In 1 of 3 patients studied, no mutations in any of the 100 genes on the high-coverage deep-sequencing gene panel were detected, and therefore, this patient's tumors may not be shedding ctDNA into circulation. This patient demonstrates the limitations of the use of ctDNA to the 57%-87% of patients with gastric cancer who are shedding ctDNA from their tumor into circulation.^{30,31} This patient highlights that tumor collection at the time of progression will still

be important for identifying resistance mechanisms to targeted treatments in nonshedders and to understand tumor heterogeneity.

In summary, ctDNA is a powerful tool for identifying potential genomic aberrations that emerge during therapy to confer resistance to targeted therapies. In gastric cancer, ctDNA monitoring of shedding tumors may have clinical utility and provide dynamic assessments that are challenging to conduct by rebiopsy. In addition, we identified various *MET* mutations previously unidentified in gastric cancer, as well as *MET* amplifications as drivers of savitinib resistance during monotherapy treatment of patients with *MET*-amplified gastric cancer.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

A

<i>ABCB1</i>	<i>CDH1</i>	<i>ETV4</i>	<i>HRAS</i>	<i>MSH2</i>	<i>PIK3CG</i>	<i>RICTOR</i>
<i>AKT1</i>	<i>CDK12</i>	<i>ETV5</i>	<i>INHBA</i>	<i>MSH6</i>	<i>PIK3R1</i>	<i>RIT1</i>
<i>AKT2</i>	<i>CDK4</i>	<i>ETV6</i>	<i>KEAP1</i>	<i>MTOR</i>	<i>PIN1</i>	<i>ROS1</i>
<i>ALK</i>	<i>CDK5</i>	<i>EZH2</i>	<i>KEL</i>	<i>MYC</i>	<i>PMS2</i>	<i>RUNDC3B</i>
<i>AR</i>	<i>CDK6</i>	<i>FANCL</i>	<i>KIT</i>	<i>NF1</i>	<i>PPP2R2A</i>	<i>SETD2</i>
<i>ARAF</i>	<i>CDKN1B</i>	<i>FGFR1</i>	<i>KRAS</i>	<i>NF2</i>	<i>PTEN</i>	<i>SLC25A40</i>
<i>ATM</i>	<i>CDKN2A</i>	<i>FGFR2</i>	<i>MAP2K1</i>	<i>NFE2L2</i>	<i>RAC1</i>	<i>SMO</i>
<i>BARD1</i>	<i>CDKN2B</i>	<i>FGFR3</i>	<i>MAP2K2</i>	<i>NRAS</i>	<i>RAD51B</i>	<i>SPOP</i>
<i>BRAF</i>	<i>CHEK1</i>	<i>FH</i>	<i>MAP2K4</i>	<i>NUDT1</i>	<i>RAD51C</i>	<i>STK11</i>
<i>BRCA1</i>	<i>CHEK2</i>	<i>FOXA1</i>	<i>MAP3K1</i>	<i>PALB2</i>	<i>RAD51D</i>	<i>TMPRSS2</i>
<i>BRCA2</i>	<i>CTLA4</i>	<i>FRS2</i>	<i>MAPK1</i>	<i>PARP1</i>	<i>RAD54L</i>	<i>TP53</i>
<i>BRIP1</i>	<i>EGFR</i>	<i>GATA3</i>	<i>MAPK3</i>	<i>PBRM1</i>	<i>RAF1</i>	<i>TRRAP</i>
<i>CARD11</i>	<i>ERBB2</i>	<i>GNA11</i>	<i>MCL1</i>	<i>PDCD1</i>	<i>RASA1</i>	<i>TSC1</i>
<i>CCND1</i>	<i>ERG</i>	<i>GNAQ</i>	<i>MDM2</i>	<i>PDGFRA</i>	<i>RB1</i>	<i>TSC2</i>
<i>CCNE1</i>	<i>ESR1</i>	<i>GNAS</i>	<i>MET</i>	<i>PIK3CA</i>	<i>RET</i>	<i>XRCC2</i>
<i>CD274</i>	<i>ETV1</i>	<i>HGF</i>	<i>MLH1</i>	<i>PIK3CB</i>	<i>RHEB</i>	<i>ZBTB16</i>

B

Sample Name	Average Target Read Coverage	Mean GC Content (%)	≥ 200 (%)	≥ 500 (%)	≥ 1,000 (%)
B5-001-NormalDNA1	1,408.41	49	99.60	97.40	77.80
B5-001-TumorDNA2	1,480.14	48	99.40	92.90	57.60
B5-002-NormalDNA1	1,220.55	49	99.50	96.10	66.00
B5-002-TumorDNA1	1,225.51	53	97.70	68.60	30.70
B5-002-TumorDNA2	1,250.74	50	99.40	92.20	51.60
B5-003-NormalDNA1	1,141.28	50	99.60	94.90	58.80

C

Sample Name	Average Target Read Coverage	Mean GC Content (%)	≥ 200 (%)	≥ 500 (%)	≥ 1,000 (%)
B5-001-FU-PD	6,626.67	48	99.70	99.00	98.10
B5-001-FU1	3,245.16	49	98.90	96.80	93.50
B5-001-FU2	3,985.48	48	99.80	99.40	98.40
B5-001-baseline	1,535.74	49	98.00	94.40	83.40
B5-002-FU-PD	6,094.55	49	99.80	99.60	99.20
B5-002-FU1	2,400.78	50	98.40	95.20	89.00
B5-002-baseline	5,234.76	49	99.60	98.70	97.30
B5-003-FU	1,601.29	49	98.40	94.70	84.20
B5-003-FU1	1,340.89	48	98.90	96.20	80.90
B5-003-FU2	1,602.19	51	94.00	87.00	75.60
B5-003-FU3	1,582.06	49	99.10	96.80	86.90
B5-003-baseline	1,865.95	48	99.40	98.10	93.00

FIG A1. Next-generation sequencing 100-gene panel and with sequencing and mapping statistics. (A) List of genes with all coding regions captured in 100-gene panel. (B) Sequencing and mapping statistics of tissue samples and (C) plasma circulating tumor DNA (ctDNA) describing number of sequenced reads, percentage of aligned reads, percentage of duplicated reads, percentage of reads mapped on targets (+200 base pairs), percentage of usable reads, average depth of coverage, GC content (percentage of nitrogenous bases that are either guanine or cytosine), average insert size, and percentage of targeted bases with given coverage. Samples with elevated percent GC are more likely to show artificial copy number variant decreases (sample B5-003 follow-up [FU] 2). PD, progressive disease.

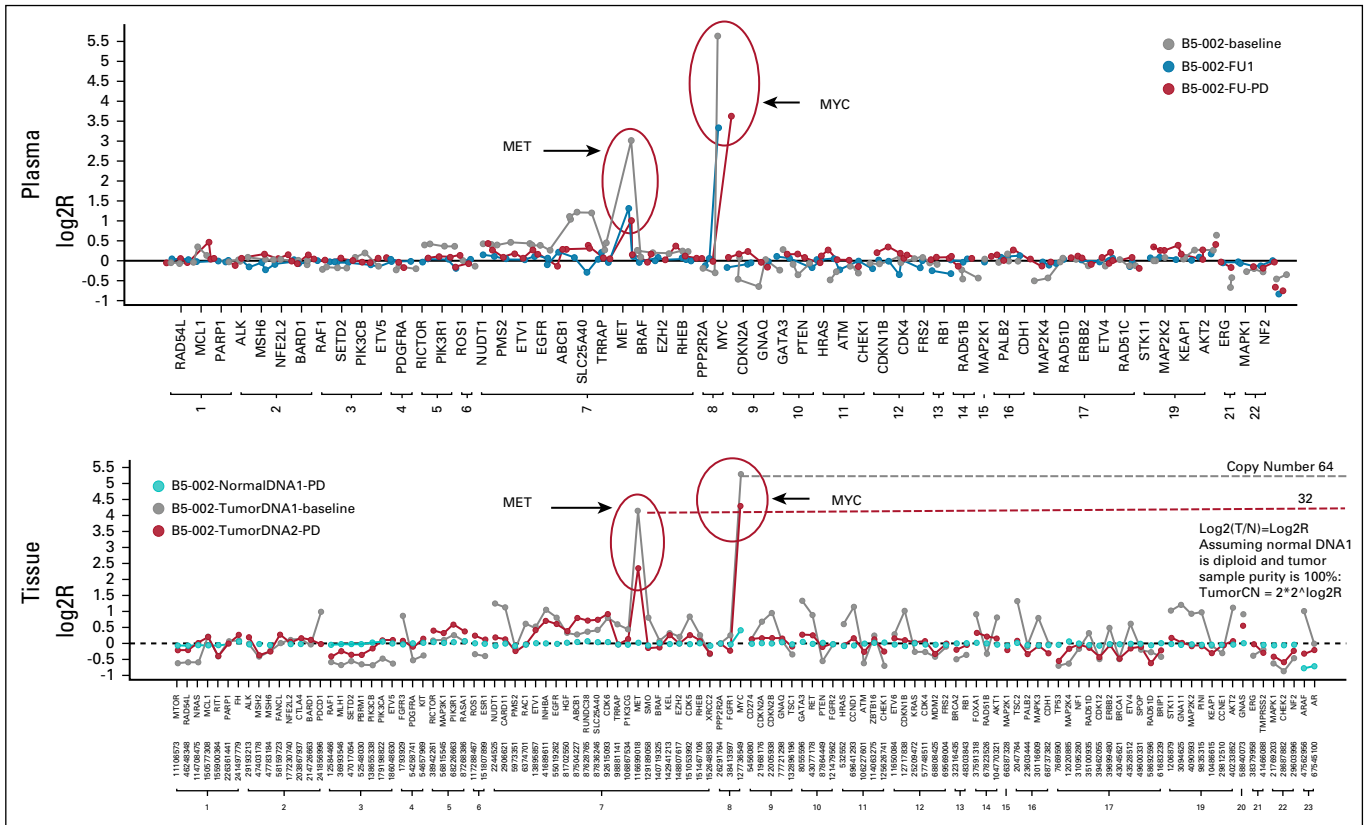


FIG A2. Next-generation sequencing 100-gene panel copy number profile for patient 1 (B5-002). Copy number profile of circulating tumor DNA (ctDNA) plasma (top) and tumor tissue at baseline and progression and normal DNA at progression (bottom), where each sample is represented by a different color. FU, follow-up; PD, progressive disease.