CLINICAL AND LABORATORY OBSERVATIONS

Use of cabozantinib to treat *MET*-amplified pediatric colorectal cancer

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Word count: Abstract, 96 words; main text, 1,277 words
Number of tables and figures: 3 figures, 1 table
Running title: Cabozantinib for *MET*-amplified pediatric CRC
Keywords: colorectal cancer, pediatric, cancer gene panel testing, *MET* inhibitor, cabozantinib

ABBREVIATIONS:

CA, cancer antigen; CEA, carcinoembryonic antigen; CRC, colorectal cancer; CT, computed tomography; EGFR, epidermal growth factor receptor; FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOX6, fluorouracil, leucovorin, and oxaliplatin; modified; TCGA, The Cancer Genome Atlas.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest associated with this manuscript.

ABSTRACT

Pediatric colorectal cancer (CRC) is extremely rare, with little information about genetic profiles compared with adult CRC. Here, a 13-year-old male with advanced CRC underwent cancer gene panel testing, which detected four genetic abnormalities (*MET* amplification in addition to *TP53*, *SMAD4*, and *CTNNA1* mutations) that might be associated with a poor prognosis. Based on high-level *MET* amplification, he received a multi-kinase inhibitor, cabozantinib, after failure of first- and second-line chemotherapy, resulting in transient disease stabilization. Tailored targeted therapy based on molecular profiling can be an effective treatment strategy for rare cancers such as pediatric CRC.

INTRODUCTION

Colorectal cancer (CRC) is very rare in the pediatric population, accounting for only 1% of all pediatric malignancies.[1] The predominance of high-grade tumors and advanced stage suggest that the biology of pediatric CRC is different from that of adult CRC.[2] In contrast to CRC associated with inherited cancer syndromes (e.g., familial adenomatous polyposis and Lynch syndrome) the genetics of pediatric non-inherited CRC remain to be elucidated. Whole-exome sequencing demonstrates that adolescent and young adult CRC patients have a higher frequency of damaging mutations than adult CRCs, while very few data are available for pediatric CRCs due to lack of patients.

In general, pediatric CRCs are treated in accordance with adult CRC treatment algorithms;[1] however, treatment of refractory cases, especially the efficacy of molecular target therapy, remains unknown.

Here, we report a pediatric case of advanced CRC who, based on the results of cancer gene panel testing, received the multi-kinase inhibitor cabozantinib after failure of first- and second-line chemotherapy.

CASE REPORT

A 13-year-old male presented with recurrent abdominal pain and bloody stool. He did not have an identifiable family history of inherited cancer syndromes. Abdominal computed

tomography (CT) revealed moderate ascites and global wall thickening in the splenic flexure (Fig. 1A), and colonoscopy revealed a circumferential mass in the descending colon (Fig. 1B). Serum cancer antigen (CA)19-9 and CA125 were elevated to 161.8 U/mL (normal upper limit, 37.0 U/mL) and 335.3 U/mL (normal upper limit, 35 U/mL), respectively, but carcinoembryonic antigen (CEA) was within the normal range (3.6 ng/ml; normal upper limit, 5.0 ng/mL). There were no physical findings suggestive of inherited cancer syndromes. He underwent left hemicolectomy, during which broad peritoneal dissemination was observed (Fig. 1C). He was diagnosed histogenetically as *RAS/BRAF* wild type, microsatellite stable signet ring cell carcinoma (Fig. 1D). First-line (fluorouracil, leucovorin, and irinotecan; FOLFIRI) and second-line (fluorouracil, leucovorin, and oxaliplatin; modified FOLFOX6) systemic chemotherapy in combination with the anti-epidermal growth factor receptor (EGFR) antibody panitumumab maintained stable disease for 10 months. However, the tumor became refractory to chemotherapy, resulting in progressive peritoneal dissemination and intestinal obstruction, with a gradual increase in CEA (10.8 ng/mL), CA19-9 (111.2 U/mL), and CA125 (194.1 U/mL) levels (Fig. 2).

Due to the advanced stage (pT4aN0M1c, stage IV), the patient underwent cancer gene panel testing (Foundation One®) during first-line chemotherapy, which revealed that the tumor harbored pathogenic mutations in *CTNNA1*, *SMAD4*, and *TP53*, as well as a *MET* amplification (106 copies/cell). No secondary germline findings linked to CRC were found

(Table). Due to the high-level *MET* amplification, the patient received cabozantinib (60 mg/day), after approval for the use of unapproved drugs was received from the Patient Safety Unit at Kyoto University Hospital. Three weeks after cabozantinib treatment, his symptoms (abdominal pain, nausea, and vomiting) improved transiently, which allowed him to spend time at home and maintain a certain quality of life with the aid of analgesics and sedatives. Cabozantinib treatment had no severe adverse effects. However, 1 month after initiation of cabozantinib, his disease exacerbated and he succumbed 13 months after the initial diagnosis.

DISCUSSION

Due to aggressive histology and tumor behavior, the present case underwent cancer gene panel testing, which detected pathogenic mutations in *TP53*, *SMAD4*, and *CTNNA1*, as well as *MET* amplification.

TP53 mutation is the most common genetic alteration found in human cancers. In CRC, *TP53* mutations are associated with advanced stage, and mutations with loss of transcriptional activity are associated with a poor prognosis.[3] The *TP53* R196P mutation was found recurrently in CRC patients from the COSMIC database (http://cancer.sanger.ac.uk/cosmic).[4] This mutation has 1% of the transcriptional activity of the wild type, and has a dominant-negative effect.[5] Therefore, the *TP53* R196P mutation is considered pathogenic and associated with a poor prognosis. *SMAD4* mutations are common

in a variety of cancers. Loss of SMAD4 expression and loss of heterozygosity at the *SMAD4* locus are associated with a poorer prognosis for CRC.[6,7] In particular, the proportion of *SMAD4* mutations is higher in signet ring cell carcinoma than in other histological types. *SMAD4* R361H, recurrently found in adult CRC patients,[4] drives tumor progression by down-regulating TGF-β signaling.[8] CTNNA1 is a protein related to cell adhesion, and epithelial mesenchymal transition caused by loss of CTNNA1 function is closely related to cancer invasion and metastasis. A report showing that downregulation of CTNNA1 expression is associated with a poor prognosis for CRC[9] suggests that *CTNNA1* S349fs, which is likely a loss-of-function mutation, may contribute to tumor aggressiveness. *MET* amplification, found in about 1.7% of all CRCs, is a well-known poor prognostic factor.[10] While less common in primary CRC, up to 22.6% of adult CRCs that are refractory to anti-EGFR drugs harbor *MET* amplification.[11] To explore the characteristics of the mutational profile of this case, we compared the mutation patterns of the four genes (*TP53*, *SMAD4*, *CTNNA1*, and *MET*) with mutation and copy number data from 212 patients/samples in The Cancer Genome Atlas (TCGA) cohort, which were obtained from cBioPortal (https://www.cbioportal.org/).[12–14] We found no adult CRC cases harboring all four mutations concurrently（Fig 3A）, which may reflect the aggressive clinical behavior of the case reported herein.

The present case, who experienced disease progression after multiple courses of panitumumab-containing chemotherapy, was thought to be resistant to other EGFR

inhibitors. Furthermore, the objective response rate of other salvage therapies, such as multi-kinase inhibitor regorafenib or trifluridine and tipiracil, is extremely low for such refractory cases.[15] Cabozantinib, an oral multi-kinase inhibitor with activity against MET, RET, AXL, VEGFR2, FLT3, and c-KIT, was approved recently for unresectable or metastatic renal cell carcinoma, and for refractory hepatocellular carcinoma.[16] To date, several MET inhibitors with different kinase selectivity have been developed and used as monotherapy, or in combination with other drugs, in a variety of clinical trials for advanced solid tumors. Regarding CRC, MET inhibitors have excellent anti-tumor effects in tissues refractory to EGFR inhibitors, and subgroup analysis revealed that they are effective in patients with a high *MET* copy number.[17,18] In a phase II study, approximately 10% of patients who were resistant to EGFR inhibitors and had a high *MET* copy number showed an objective response to combined treatment with the MET inhibitor tivantinib and the anti-human EGFR antibody cetuximab.[19] Taken together, we considered *MET* amplification as a possible actionable molecular target in this case, and the patient received cabozantinib as third-line therapy, which had a transient therapeutic effect. The *MET* copy number in the present case was very high when compared with that of 157 tumors (including five CRCs) with *MET* amplification (a genomic copy number of five or higher) in the International Cancer Genome Consortium cohort from the COSMIC database (Fig. 3B).[4] Therefore, the limited therapeutic effect of cabozantinib in the present case was probably due to *MET* hyper-amplification-associated

MET inhibitor resistance, as previously described.[20] Other possible explanations include involvement of other genetic mutations (*TP53*, *SMAD4*, and *CTNNA1*) in resistance to treatment, and poor absorption of cabozantinib due to decreased gastrointestinal function resulting from ileus or peritoneal dissemination. If cabozantinib had been started earlier, or used in combination with other chemotherapies and molecular targeting drugs, it may have had a greater effect in terms of inhibiting tumor growth.

In conclusion, we experienced a rare case of pediatric CRC that had four genetic abnormalities associated with a poor prognosis. To clarify the pathogenesis and to improve the prognosis of pediatric CRC, it will be necessary to perform comprehensive genomic analysis immediately after histological confirmation of the disease.

FUNDING

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ETHICS STATEMENT

The patient's legal guardians provided written informed consent to publish photographs under the guidelines of the Kyoto University Graduate School and Faculty of Medicine Ethics Committee.

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FIGURE LEGENDS

FIGURE 1. (A) Abdominal computed tomography (CT) at onset. The arrow indicates intestinal wall thickening at the splenic curvature. (B) Colonoscopy findings at onset. (C, D) Macroscopic appearance (C) and hematoxylin and eosin staining (D) of the resected specimen (magnification, ×400).

FIGURE 2. Clinical course of the patient. He received fluorouracil, leucovorin, and irinotecan; (FOLFIRI) (a), FOLFIRI plus panitumumab (b), and fluorouracil, leucovorin, and oxaliplatin (modified FOLFOX6) plus panitumumab (c) for 10 months.

FIGURE 3. (A) Oncoprint showing the frequency and co-occurrence of alterations in four genes (*TP53, SMAD4, CTNNA1,* and *MET*) in our case and in 212 colorectal cancer patients from The Cancer Genome Atlas (TCGA). TCGA data were downloaded from cBioPortal (https://www.cbioportal.org/). The variant types are indicated in the color legend at the bottom of the Oncoprint. (B) Genomic copy number of *MET* in this case compared with that in the COSMIC database (https://cancer.sanger.ac.uk/cosmic). The red dot represents this case relative to n=5 other colorectal cancers (green) and n=152 other cancers with *MET* amplification (five or more copies) in the International Cancer Genome Consortium cohort (black).

Figure 1

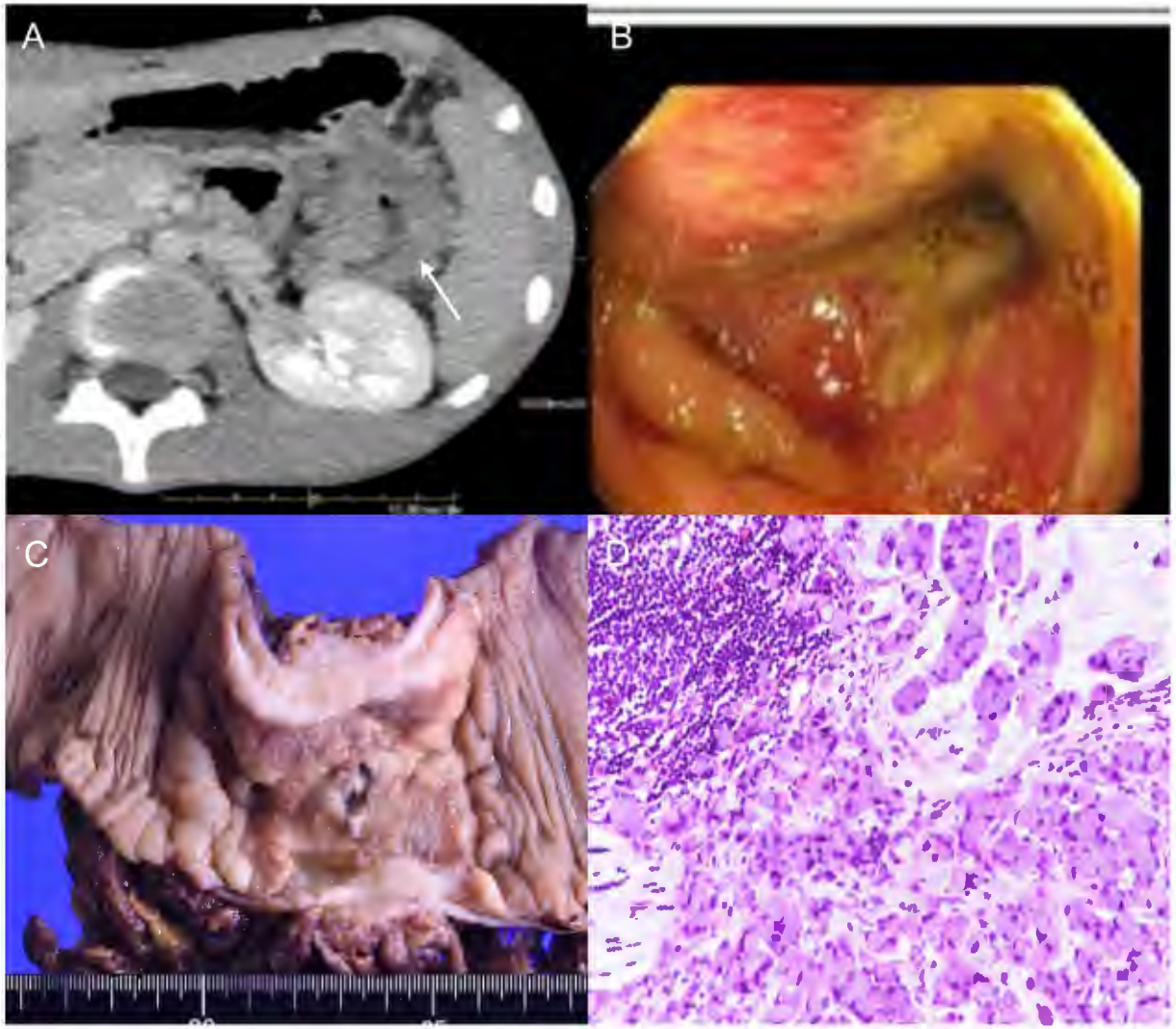


Figure 2

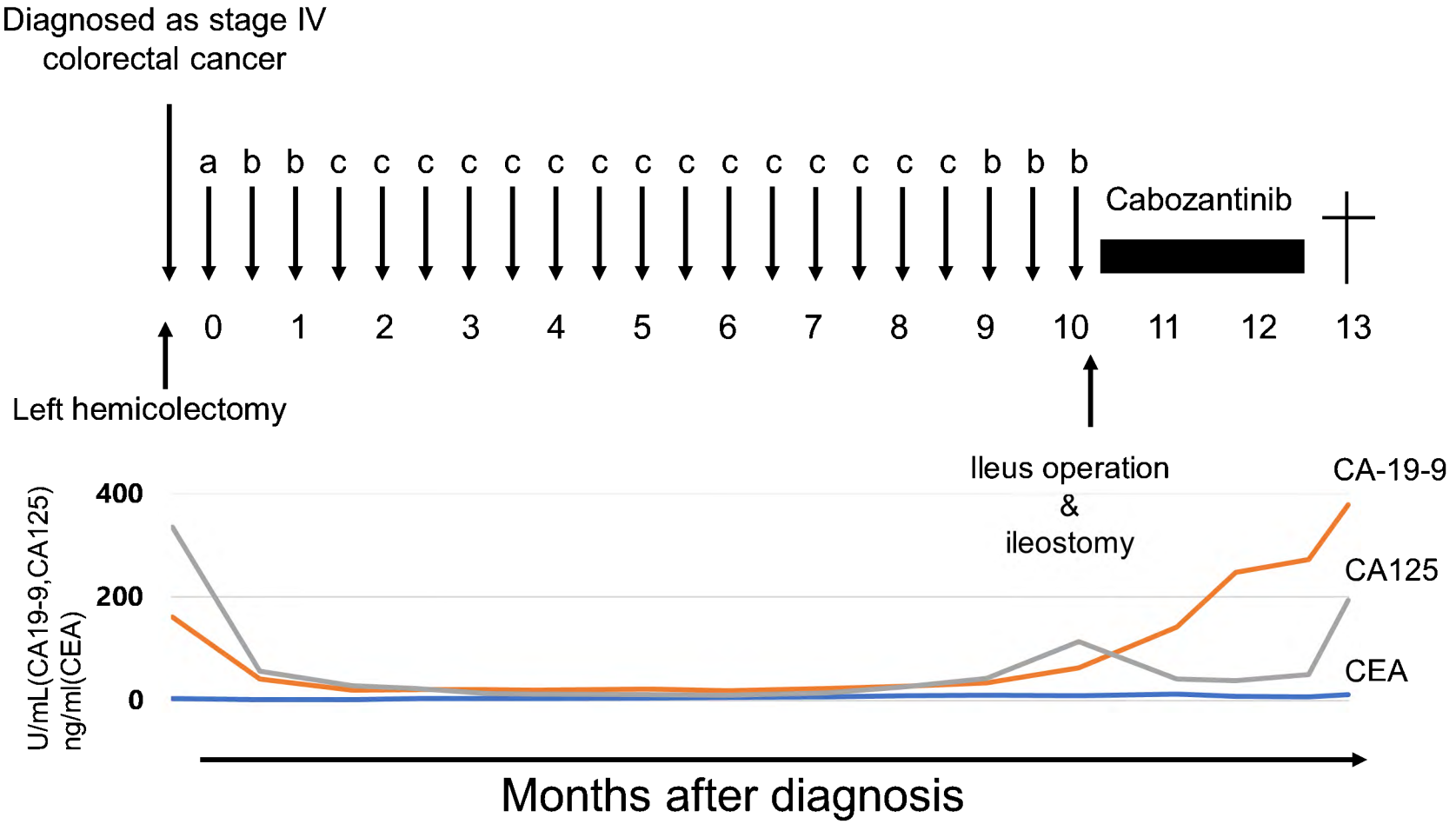
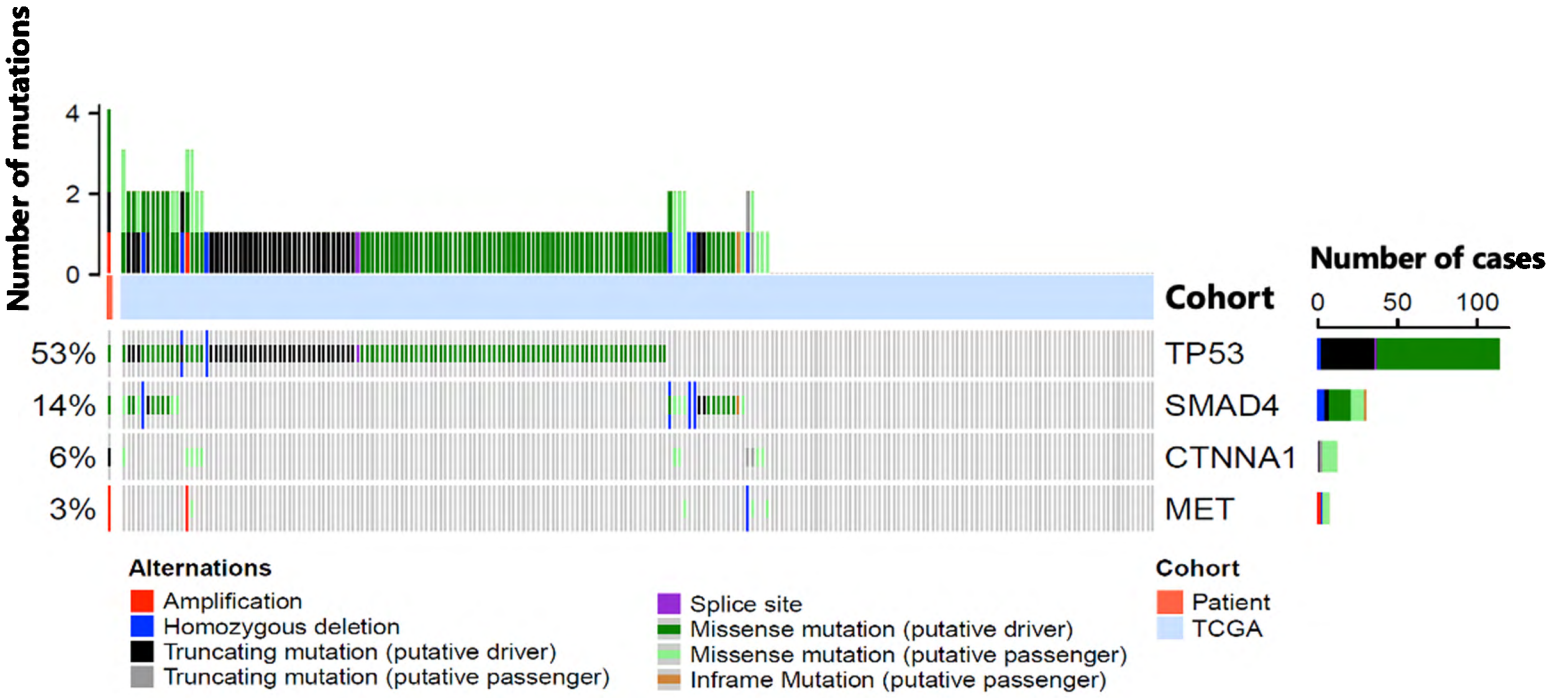

Diagnosed as stage IV colorectal cancer
a b b c c c c c c c c c c c c c c c b b b
Cabozantinib
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Left hemicolectomy
Ileus operation & ileostomy
CA-19-9
CA125
CEA
U/mL(CA19-9,CA125) ng/ml(CEA)
400
200
0
Months after diagnosis

Figure 3

A


Number of mutations
4
2
0
Cohort
Number of cases
0
50
100
53%
TP53
14%
SMAD4
6%
CTNNA1
3%
MET
Alternations
Amplification
Homozygous deletion
Truncating mutation (putative driver)
Truncating mutation (putative passenger)
Splice site
Missense mutation (putative driver)
Missense mutation (putative passenger)
Inframe Mutation (putative passenger)
Cohort
Patient
TCGA

B

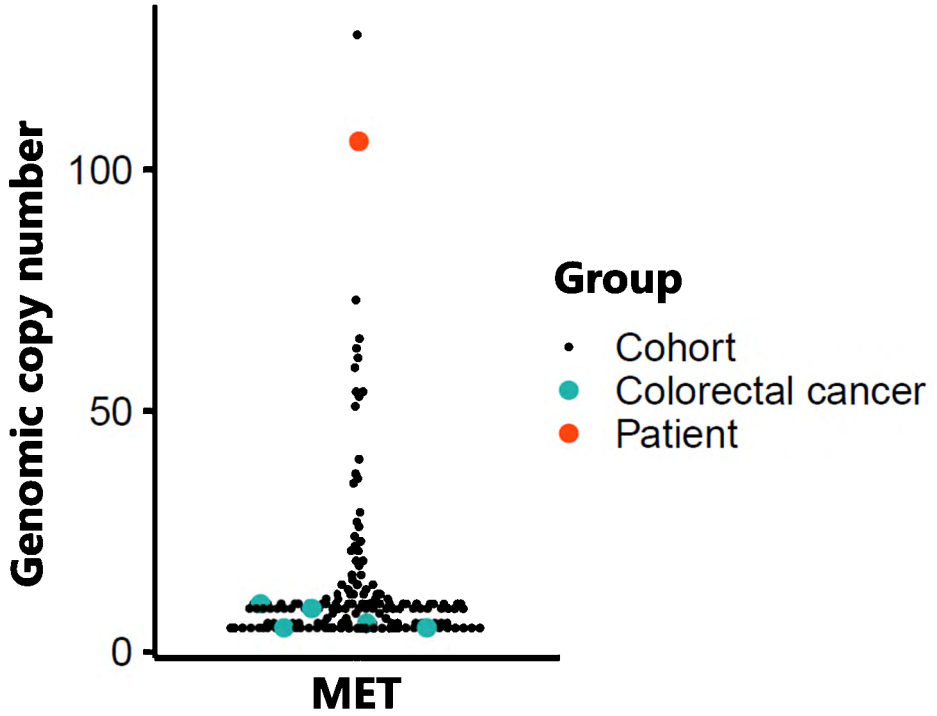

Genomic copy number
100
50
0
MET
Group
Cohort
Colorectal cancer
Patient

Table. Sequencing results from FoundationOne CDx

Biomarker findings			
Microsatellite status	stable		
Tumor mutational burden	1 Muts/Mb		
Genomic findings			
Short variants			
Gene	**Alteration**	**Variant allele frequency**	**COSMIC ID (Frequency of occurence in colorectal cancer)** [#]
SMAD4	R361H	0.3563	COSV61684056 (106)
TP53	R196P	0.3344	COSV52678297 (4)
CTNNA1	S349fs*10	0.318	None
KRAS	Wildtype	N.A	N.A
NRAS	Wildtype	N.A	N.A
Copy number variation			
Gene	**Alteration**	**Copy number**	
MET	Amplification	106	

Abbreviation: N.A, not applicable

[#] Based on COSMIC database (v94)