case reports

Targeting Secondary and Tertiary Resistance to BRAF Inhibition in BRAF V600E–Mutated Metastatic Colorectal Cancer

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CASE REPORT

A 30-year-old male presented with an obstructing left-sided colon tumor and extensive peritoneal carcinomatosis. Palliative resection revealed mucinous adenocarcinoma of the sigmoid colon, pT3 pN2a (5/6) pM1c (PER), according to the TNM Staging System 8th Edition (2017). Molecular tumor profiling (FoundationOne CDx) performed on primary tumor tissue identified microsatellite stability, a tumor mutational burden (TMB) of 8 mutations per megabase (muts/Mb), BRAF V600E (variant allele frequency [VAF]: 16%), TP53 R282W (VAF: 21%), and a low-level *GATA6* amplification (copy number variation (CNV 7); Fig 1, Appendix Fig A1).

The patient started first-line chemotherapy with FOLFOXIRI (fluorouracil, folinic acid, oxaliplatin, and irinotecan) in accordance with current guidelines for BRAF V600E–mutated metastatic colorectal cancer (mCRC).[1,2] No bevacizumab was given because of thrombosis of the superior mesenteric vein. Recurrent symptoms of subileus, enteritis, and systemic inflammation necessitated dose reductions. During first-line chemotherapy, carbohydrate antigen 19-9 (CA19-9) levels surged from initially 261 to 3,706 kU/L (Fig 2). Computed tomography (CT) imaging performed after four cycles of FOLFOXIRI confirmed disease progression with new-onset disseminated liver metastases and progressive peritoneal carcinomatosis (not shown).

For second-line treatment, therapy regimen was switched to the molecularly targeted drug combination of cetuximab, encorafenib, and binimetinib, analogous to the BEACON phase III trial.[3] Following treatment initiation, the patient experienced a transient relief of tumor-associated symptoms and improvement of his overall performance status (Eastern Cooperative Oncology Group [ECOG] 1-0). Treatment-related side effects included grade 2 skin toxicity, grade 2 diarrhea, grade 1 fever, and grade 2 anemia. Six weeks into second-line treatment, CA 19-9 levels dropped to a nadir of 411 kU/L. CT imaging performed after 8 weeks of second-line treatment showed an overall mixed response (not shown). Only 2 weeks later, CA 19-9 had risen again to 3,527 kU/L (Fig 2), and CT imaging now showed disseminated disease progression (not shown).

We collected a fresh tumor tissue biopsy from a liver lesion and switched treatment to third-line FOLFOX plus bevacizumab. Molecular tumor profiling (FoundationOne CDx) now showed a TMB of 8 muts/Mb, BRAF V600E (VAF: 42%) and TP53 R282W (VAF: 38%), and high amplification of *MET* (copy-number variation 31) (Fig 1, Appendix Fig A1). Immunohistochemical assessment confirmed c-MET positivity (score 3+).

After four cycles of FOLFOX plus bevacizumab, the patient experienced symptomatic disease progression requiring high doses of opioids for pain control. We therefore initiated personalized fourth-line treatment with the class I MET inhibitor capmatinib (400 mg twice a day) and encorafenib (incremental dosing from 75 to 225 mg qd). Thereunder, the patient's clinical condition rapidly improved, CA 19-9 levels dropped from 43,940 kU/L to a nadir of 1,835 kU/L (Fig 2), and fluorodeoxyglucose-positron emission tomography-CT imaging performed after 8 weeks of capmatinib plus encorafenib showed a partial metabolic and morphologic response (Fig 3). Capmatinib (400 mg twice a day) plus encorafenib was overall well tolerated with intermittent grade 2 diarrhea as the sole adverse event.

After 14 weeks on capmatinib plus encorafenib, FDG-PET-CT imaging again showed disease progression. We collected another tumor tissue biopsy from a liver lesion and, for the first time, a liquid biopsy. Key molecular findings in the tissue biopsy were TMB of 8 muts/Mb, BRAF V600E (VAF: 36%), TP53 R282W (VAF: 34%), absence of *MET* amplification, and emergence of MET D1228N (VAF: 21%), a well-characterized resistance mutation to class I MET inhibitors.[4] In the liquid biopsy, we found BRAF V600E (VAF: 5.6%), TP53 R282W (VAF: 6.7%), and KRAS G12D (VAF: 2.9%); however, no mutation or copy-number alteration in *MET* (Fig 1, Appendix Fig A1). Based on these findings, we undertook two further molecularly targeted treatment attempts. For fifth

ASSOCIATED CONTENT

Appendix

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

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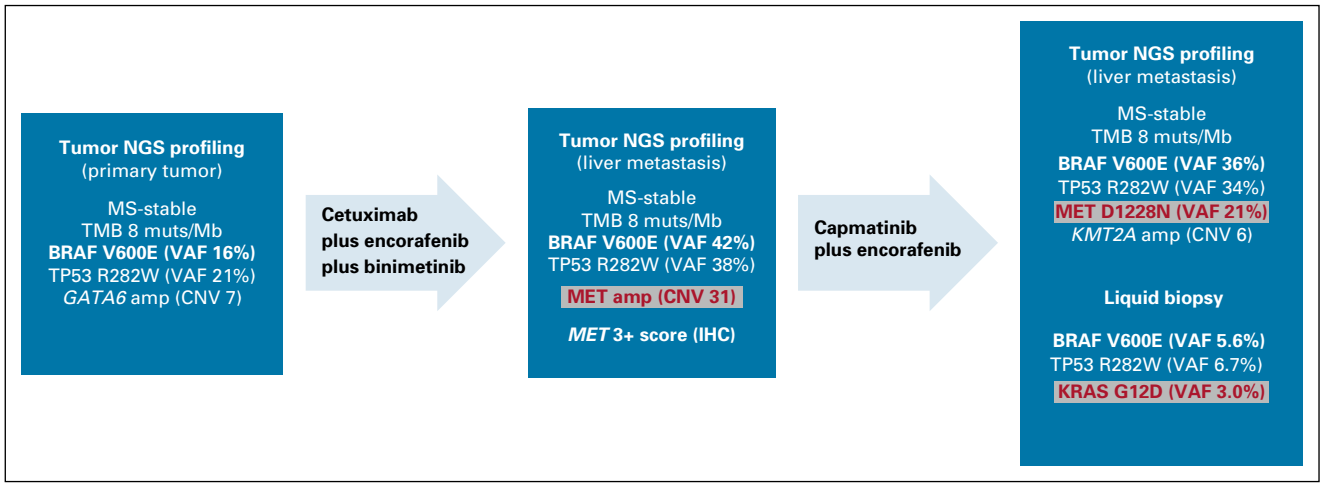

FIG 1. Serial comprehensive molecular profiling was performed in a 30-year-old male undergoing treatment for BRAF V600E–mutated mCRC. Three FoundationOne CDx assays were performed over disease course. Molecular testing results as well as molecularly targeted treatments are indicated. For liquid biopsy, cfDNA extracted from patient plasma was analyzed with Oncomine Pan-Cancer Cell-Free Assay (Life Technologies/Thermo Scientific [Waltham, MA]). amp, amplification; cfDNA, cell-free DNA; CNV, copy-number variation; IHC, immunohistochemistry; mCRC, metastatic colorectal cancer; MS-stable, microsatellite-stable; muts, mutations; NGS, next-generation sequencing, TMB, tumor mutational burden; VAF, variant allele frequency.

line, we switched treatment back to the BEACON triplet of cetuximab, encorafenib, and binimetinib, based on the absence of the *MET* amplification. For sixth-line treatment, we combined class II MET inhibitor cabozantinib with encorafenib in an attempt to target MET D1228N. However, there were no clinical or serologic responses to either of these combinations, and treatment had to be stopped early because of rapid clinical deterioration. No treatment-related adverse events higher than grade 2 occurred with the combination of cabozantinib and encorafenib. Treatment was continued with best supportive care and the patient ultimately succumbed to his disease.

The patient provided written consent for anonymized use of his clinical data. No identifiable images or data are included in this report.

DISCUSSION

This is, to our knowledge, the first report to combine the recently US Food and Drug Administration–approved MET inhibitor capmatinib with encorafenib to overcome MET-

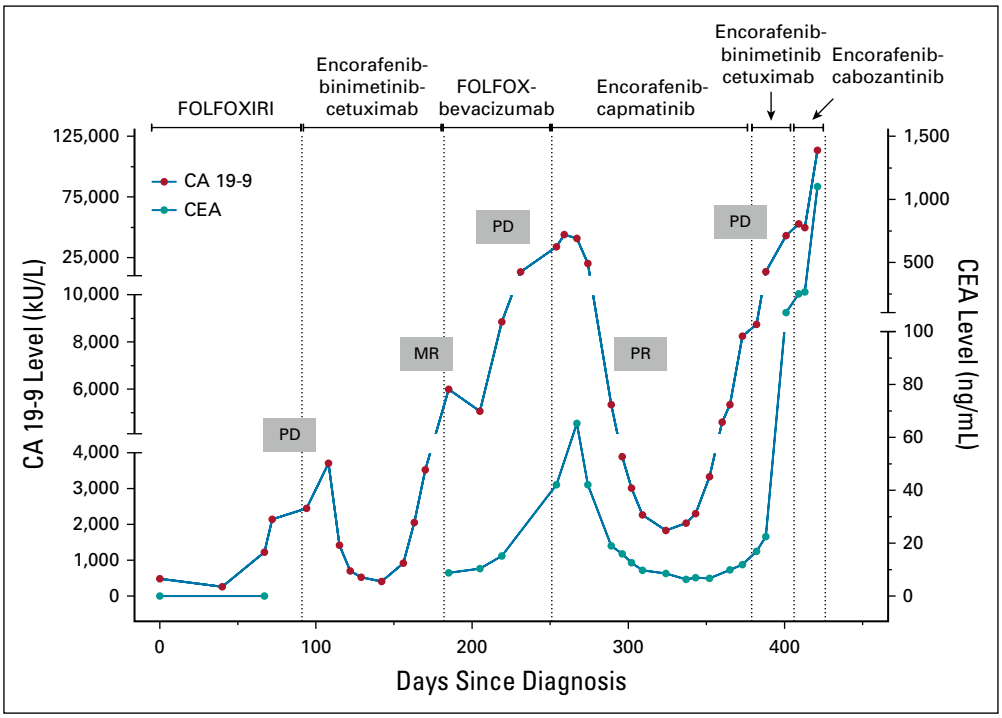

FIG 2. CA 19-9 and CEA monitoring during disease course. CA 19-9 and CEA levels over time as well as treatment lines are depicted. CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin; FOLFOXIRI, fluorouracil, folinic acid, oxaliplatin, and irinotecan; MR, mixed response; PD, progressive disease; PR, partial response.

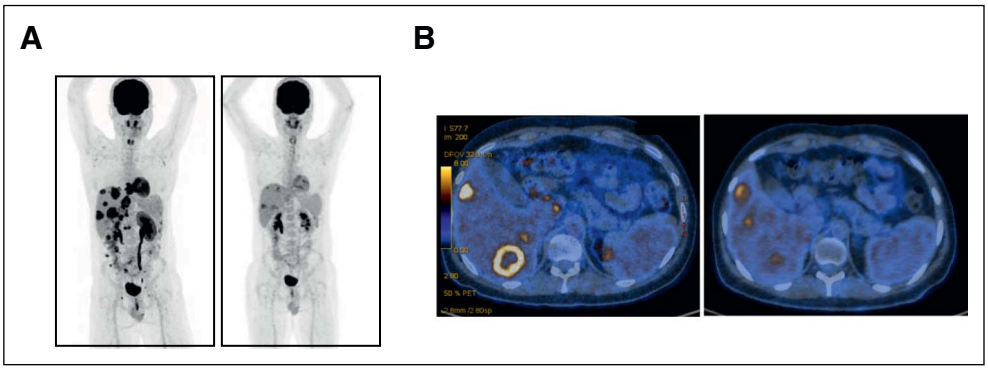

FIG 3. Targeting acquired *MET* amplification in BRAF V600E mCRC with capmatinib plus encorafenib. (A) FDG-PET scan, whole-body overview, before (left) and 8 weeks after (right) initiation of encorafenib and capmatinib. (B) Good partial metabolic and morphologic response of liver metastases in axial sections. FDG-PET, fluorodeoxyglucose-positron emission tomography; mCRC, metastatic colorectal cancer.

driven acquired resistance to molecularly targeted treatment for BRAF V600E–mutated mCRC. The case also illustrates how serial molecular monitoring can inform personalized later-line drug combinations for this difficult-to-treat molecular subgroup of mCRC.

BRAF V600E–mutated CRC accounts for 8%-12% of mCRCs and constitutes the clinically and biologically most aggressive subgroup of CRC with dismal prognosis.[5-9] In contrast to BRAF V600E–mutated malignant melanoma or lung cancer, however, BRAF V600E–mutated mCRCs are largely resistant to BRAF inhibitor monotherapy[10] and BRAF plus MEK inhibitor combination treatment.[11] Primary resistance is caused by an epidermal growth factor receptor (EGFR)-driven feedback loop leading to reactivation of the mitogen-activated protein kinase pathway and cross-activation of other receptor tyrosine kinase effector pathways including PI3K-mTOR.[12-14] Consequently, anti-EGFR and BRAF inhibitor treatment combinations have been developed for BRAF V600E–mutated mCRC.[15,16] The BEACON trial[3] was the first randomized phase III trial to show superior efficacy and improved overall survival for the molecularly targeted treatment combinations of anti-EGFR antibody cetuximab, in combination with BRAF inhibitor encorafenib (BEACON doublet), with or without MEK inhibitor binimetinib (BEACON triplet), over second-line chemotherapy regimens. Based on these data, the combination of cetuximab and encorafenib (BEACON doublet) gained US Food and Drug Administration approval for patients with pretreated BRAF V600E–mutated mCRC.[17]

Our patient experienced a very aggressive disease course with primary resistance to chemotherapy and only a mixed response to the BEACON triplet, followed by rapid disease progression under continued treatment. Notably, since no liver biopsy was taken before initiation of the BEACON triplet, we cannot rule out the presence of liver lesions with pre-existent *MET* amplification, accounting for the short-lived response.

There are no established treatment options following progression on combined anti-EGFR and BRAF blockade in BRAF V600E–mutated mCRC, which fuels a growing interest in developing a precision oncology approach to identify and—if possible—target acquired resistance to molecularly targeted treatment in BRAF V600E mCRC. Our current knowledge of the mechanisms driving primary and acquired resistance to combined EGFR and BRAF inhibition in BRAF V600E–mutated mCRC is still preliminary. Moreover, no clinical standards have been established of how to identify and target resistance in these patients. In a small series of patients treated with the triplet combination of panitumumab, dabrafenib, and trametinib, enrichment of mutated *KRAS* or *NRAS* in cell-free DNA was associated with resistance.[18,19] Other reports have identified targetable mechanisms of resistance, mainly amplification of the receptor tyrosine kinase *MET*.[20] In a series of patients, *MET* amplification was confirmed to drive resistance, and switching treatment from anti-EGFR and BRAF inhibition to an MET inhibitor plus BRAF inhibitor induced durable responses in patients.[21]

We followed the same approach for our patient and combined encorafenib with capmatinib for fourth-line treatment. The combination was well tolerated and achieved a partial response and the longest treatment duration (14 weeks) over disease course, highlighting the potential of this combination for targeting MET-driven resistance to the BEACON regimens and suggesting that, whenever clinically feasible, analysis for acquired *MET* alterations should be performed.

In our case, tertiary resistance to capmatinib and encorafenib proved more challenging than secondary resistance to cetuximab, encorafenib, and binimetinib. Loss of the *MET* amplification suggested that oncogenic signaling had rewired away from MET. However, the emergence of an established *MET* resistance mutation (MET D1228N) in the tissue biopsy suggested that MET was still a relevant driver and suggested a switch from capmatinib to a class II MET inhibitor (cabozantinib) to overcome resistance to capmatinib (Appendix Fig A2). In the liquid biopsy, however, MET D1228N was not detected and instead KRAS G12D was found. Our interpretation was that resistance had emerged in a polyclonal fashion, with MET D1228N not representing the predominant driver across a larger subset of lesions. Notably, BRAF V600E and TP53 R282W were detected in all tumor tissue samples analyzed and in the

liquid biopsy, confirming that those mutations remained the main drivers of the disease throughout disease course.

In this complex scenario, we first switched treatment back to cetuximab, encorafenib, and binimetinib. After a lack of response, we switched to cabozantinib plus encorafenib, aiming to target clones driven by MET D1228N, again without evidence of clinical or serologic response. In the future, the integration of an SHP2 inhibitor in combination with BRAF inhibition might be another promising therapeutic option to be considered for such patients.[22,23]

In summary, our case illustrates that acquired resistance to molecularly targeted agents in BRAF V600E mCRC is a highly dynamic, yet potentially targetable process, driven by clonal evolution under selective pressure.[24] Novel diagnostic tools of precision oncology including more comprehensive analyses of ctDNA to track clonal evolution as well as in vivo and ex vivo monitoring of oncogenic signaling and drug responses might provide novel insights into the mechanisms of resistance and help guide treatment choices for future patients.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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REFERENCES

1. Loupakis F, Cremolini C, Salvatore L, et al: FOLFOXIRI plus bevacizumab as first-line treatment in BRAF mutant metastatic colorectal cancer. Eur J Cancer 50:57-63, 2014
2. Van Cutsem E, Cervantes A, Adam R, et al: ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol 27:1386-1422, 2016
3. Kopetz S, Grothey A, Yaeger R, et al: Encorafenib, binimetinib, and cetuximab in BRAF V600E–mutated colorectal cancer. N Engl J Med 381:1632-1643, 2019
4. Baltschukat S, Engstler BS, Huang A, et al: Capmatinib (INC280) is active against models of non–small cell lung cancer and other cancer types with defined mechanisms of MET activation. Clin Cancer Res 25:3164-3175, 2019
5. Sanz-Garcia E, Argiles G, Elez E, et al: BRAF mutant colorectal cancer: Prognosis, treatment, and new perspectives. Ann Oncol 28:2648-2657, 2017
6. Souglakos J, Philips J, Wang R, et al: Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer 101:465-472, 2009
7. Yokota T, Ura T, Shibata N, et al: BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer. Br J Cancer 104:856-862, 2011
8. Tie J, Gibbs P, Lipton L, et al: Optimizing targeted therapeutic development: Analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. Int J Cancer 128:2075-2084, 2011
9. Petrowsky H, Fritsch R, Guckenberger M, et al: Modern therapeutic approaches for the treatment of malignant liver tumours. Nat Rev Gastroenterol Hepatol 17:755-772, 2020
10. Kopetz S, Desai J, Chan E, et al: Phase II pilot study of vemurafenib in patients with metastatic BRAF-mutated colorectal cancer. J Clin Oncol 33:4032-4038, 2015
11. Corcoran RB, Atreya CE, Falchook GS, et al: Combined BRAF and MEK inhibition with dabrafenib and trametinib in BRAF V600–mutant colorectal cancer. J Clin Oncol 33:4023-4031, 2015
12. Corcoran RB, Ebi H, Turke AB, et al: EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. Cancer Discov 2:227-235, 2012
13. Samuels ML, McMahon M: Inhibition of platelet-derived growth factor- and epidermal growth factor-mediated mitogenesis and signaling in 3T3 cells expressing delta Raf-1:ER, an estradiol-regulated form of Raf-1. Mol Cell Biol 14:7855-7866, 1994

14. Prahallad A, Sun C, Huang S, et al: Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 483:100-103, 2012
15. Corcoran RB, Andre T, Atreya CE, et al: Combined BRAF, EGFR, and MEK inhibition in patients with BRAF(V600E)-mutant colorectal cancer. Cancer Discov 8:428-443, 2018
16. Kopetz S, McDonough SL, Lenz H-J, et al: Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG S1406). J Clin Oncol 35, 2017 (suppl; abstr 3505)
17. Bettegowda C, Sausen M, Leary RJ, et al: Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 6:224ra24, 2014
18. Strickler JH, Loree JM, Ahronian LG, et al: Genomic landscape of cell-free DNA in patients with colorectal cancer. Cancer Discov 8:164-173, 2018
19. Corcoran RB, Chabner BA: Application of cell-free DNA analysis to cancer treatment. N Engl J Med 379:1754-1765, 2018
20. Oddo D, Siravegna G, Gloghini A, et al: Emergence of MET hyper-amplification at progression to MET and BRAF inhibition in colorectal cancer. Br J Cancer 117:347-352, 2017
21. Pietrantonio F, Oddo D, Gloghini A, et al: MET-driven resistance to dual EGFR and BRAF blockade may Be overcome by switching from EGFR to MET inhibition in BRAF-mutated colorectal cancer. Cancer Discov 6:963-971, 2016
22. Ahmed TA, Adamopoulos C, Karoulia Z, et al: SHP2 drives adaptive resistance to ERK signaling inhibition in molecularly defined subsets of ERK-dependent tumors. Cell Rep 26:65-78.e5, 2019
23. Hao HX, Wang H, Liu C, et al: Tumor intrinsic efficacy by SHP2 and RTK inhibitors in KRAS-mutant cancers. Mol Cancer Ther 18:2368-2380, 2019
24. Siravegna G, Mussolin B, Buscarino M, et al: Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med 21:795-801, 2015

APPENDIX

Cetuximab plus encorafenib plus binimetinib

Capmatinib plus encorafenib

Primary — FoundationOne CDx®

Gene	Nucleotide	Amino Acid	Coverage	Reads (%)	CNV
BRAF	c.1799T>A	p.Val600Glu	873	15.69	
TP53	c.844C>T	p.Arg248Trp	1,252	20.53	
GATA6					7

Liver metastasis — FoundationOne CDx®

Gene	Nucleotide	Amino Acid	Coverage	Reads (%)	CNV
BRAF	c.1799T>A	p.Val600Glu	506	42.29	
TP53	c.844C>T	p.Arg248Trp	1,211	38.07	
MET					31

Liver metastasis — FoundationOne CDx®

Gene	Nucleotide	Amino Acid	Coverage	Reads (%)	CNV
BRAF	c.1799T>A	p.Val600Glu	726	35.95	
TP53	c.844C>T	p.Arg248Trp	1,059	33.99	
MET	c.3682G>A	p.Asp1228Asn	689	21.19	
KMT2A					6

cfDNA — Oncomine™ Pan-Cancer Cell-Free

Gene	Nucleotide	Amino Acid	Coverage	Reads (%)	% LOD
BRAF	c.1799T>A	p.Val600Glu	3,774	5.59	0.11
TP53	c.844C>T	p.Arg248Trp	3,476	6.70	0.12
KRAS	c.35G>A	p.Gly12Asp	3,652	2.98	0.12

FIG A1. Raw sequencing data from all three tumor tissue FoundationOne CDx NGS analyses and the liquid biopsy assay (Oncomine Pan-Cancer Cell-Free Assay) as indicated. cfDNA, cell-free DNA; CNV, copy-number variation; LOD, limit of detection; NGS, next-generation sequencing.

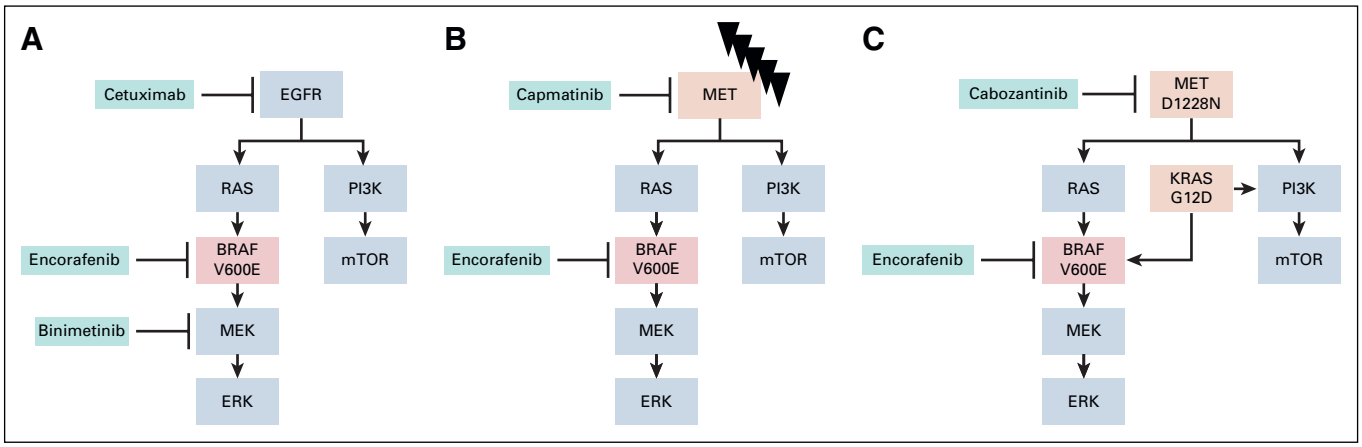

FIG A2. Schematic illustration of signaling pathways and molecularly targeted therapeutic approach in (A) second-line, (B) fourth-line, and (C) fifth-line treatments, together with the molecular alterations detected at the respective time points.