original reports

Randomized Trial of Irinotecan and Cetuximab With or Without Vemurafenib in BRAF-Mutant Metastatic Colorectal Cancer (SWOG S1406)

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abstract

PURPOSE *BRAF*^{V600E} mutations are rarely associated with objective responses to the BRAF inhibitor vemurafenib in patients with metastatic colorectal cancer (CRC). Blockade of *BRAF*^{V600E} by vemurafenib causes feedback upregulation of EGFR, whose signaling activities can be impeded by cetuximab.

METHODS One hundred six patients with *BRAF*^{V600E}-mutated metastatic CRC previously treated with one or two regimens were randomly assigned to irinotecan and cetuximab with or without vemurafenib (960 mg PO twice daily).

RESULTS Progression-free survival, the primary end point, was improved with the addition of vemurafenib (hazard ratio, 0.50, $P = .001$). The response rate was 17% versus 4% ($P = .05$), with a disease control rate of 65% versus 21% ($P < .001$). A decline in circulating tumor DNA *BRAF*^{V600E} variant allele frequency was seen in 87% versus 0% of patients ($P < .001$), with a low incidence of acquired RAS alterations at the time of progression. RNA profiling suggested that treatment benefit did not depend on previously established BRAF subgroups or the consensus molecular subtype.

CONCLUSION Simultaneous inhibition of EGFR and BRAF combined with irinotecan is effective in *BRAF*^{V600E}-mutated CRC.

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INTRODUCTION

Colorectal cancer (CRC), the second most common cancer in the United States, is associated with a high prevalence of mutations in the mitogen-activated protein kinase (MAPK) pathway, including KRAS and NRAS, which are mutated in approximately 50% of cancers, and BRAF in approximately 10% of cancers. The V600E mutation in BRAF is the most common of the BRAF mutations and is associated with a uniquely aggressive phenotype. Previous studies have demonstrated higher rates of peritoneal and metastatic lymph node involvement, less benefit from standard chemotherapy treatment, and shorter overall survival (OS) in patients with *BRAF*^{V600E}-mutated tumors.[1,2] Inhibitors of EGFR such as cetuximab and panitumumab do not routinely demonstrate response in patients with *BRAF*^{V600E} mutation as a single agent or in combination with cytotoxic chemotherapy.[3]

Responses to single-agent BRAF inhibitors or in combination with MEK inhibition are minimal, in contrast to the activity in other tumor types such as melanoma.[4,5] A key finding was the identification of adaptive feedback after BRAF inhibition, resulting in increased signaling through the EGFR pathway in colon cancer.[6,7] This led to studies combining BRAF and EGFR inhibitors, demonstrating improved activity.[8-10] Preclinical studies and coclinical trials with patient-derived xenografts demonstrated the ability of irinotecan to augment the activity of BRAF and EGFR inhibition, similar to the clinical benefits seen with EGFR inhibition + irinotecan demonstrated in *KRAS* wild-type tumors.[11] This led to a phase IB study of the combination of vemurafenib, a *BRAF*^{V600}-specific inhibitor, with cetuximab, and irinotecan, which demonstrated a 35% response rate and encouraging progression-free survival.[9] Based on these preliminary data, a randomized study of cetuximab and irinotecan with or without vemurafenib was initiated to evaluate the benefit of this combination with a primary end point of progression-free survival. The trial integrated a comprehensive translational research plan to investigate the relative benefit of the various components of the regimen to the clinical outcomes of the matched patients.

METHODS

Patients and Random Assignment

Patients enrolled in SWOG1406 were required to have histologically or cytologically documented adenocarcinoma

ASSOCIATED CONTENT

See accompanying editorial on page 259

Appendix

Protocol

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

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CONTEXT

Key Objective

BRAF^{V600E} colorectal cancer (CRC) has limited activity with single-agent BRAF inhibition, and combination with EGFR inhibitor cetuximab and the standard-of-care chemotherapy irinotecan has demonstrated benefit preclinically. This trial evaluates the impact of the addition of vemurafenib to cetuximab and irinotecan on outcomes for patients with BRAF^{V600E} CRC.

Knowledge Generated

The addition of vemurafenib improved progression-free survival and treatment response. Unlike other single-arm studies with targeted therapy alone, the mechanisms of resistance did not involve acquired RAS mutations, and benefit was independent of RNA-based subtypes.

Relevance

The combination of BRAF- and EGFR-targeted therapy with chemotherapy improves outcomes for patients with BRAF^{V600E} CRC.

of the colon or rectum that was either metastatic or locally advanced and unresectable (Protocol, online only). Patients also needed to have a *BRAF^{V600E}* mutation and the absence of NRAS and KRAS mutations. Brain metastases were allowed if they had been adequately treated with radiotherapy or surgery and stable for at least 90 days prior to step I initial registration. Patients were allowed to have one or two prior regimens of systemic chemotherapy for metastatic disease; patients who experienced disease recurrence during or within 6 months of completion of adjuvant chemotherapy were also eligible. Patients must not have been previously treated with EGFR, BRAF, or MEK inhibitors. Previous chemotherapy, immunotherapy, or radiation therapy must have been completed at least 14 days prior to eligibility screening. Patients were required to have adequate hepatic, renal, hematologic, and cardiac function; Zubrod performance status of 0-1; no known history of Gilbert's syndrome; or known homozygosity for the UGT1A*28 allele. In this open-label phase II study, patients were randomly assigned in a 1:1 manner into the two treatment groups by using a dynamic balancing algorithm with stratification by prior treatment with irinotecan (yes *v* no). This trial was approved by the institutional review board at each center. All patients provided informed consent.

Treatment

Treatment consisted of irinotecan at 180 mg/m^2 and cetuximab at 500 mg/m^2, both administered IV every 2 weeks. In the experimental arm, vemurafenib was administered orally at a daily dose of 960 mg. Upon documented disease progression, control arm patients were allowed to cross over onto the experimental regimen, assuming that appropriate eligibility criteria were still met.

Tumor and Plasma Studies

Local testing for BRAF using sequencing methodologies was allowed for eligibility determination. Where local testing was not available, central testing was offered using BRAF^{V600E}-specific immunohistochemistry (IHC), with confirmatory PCR testing.[12] Retrospectively, next-generation sequencing (NGS) was performed with a target panel including *BRAF* (Foundation Medicine, Cambridge, MA), as previously described.[13] Plasma was extracted from blood samples from patients while on study treatment at baseline, each restaging, and at the time of progression. Somatic mutations from circulating tumor DNA were identified from 2 mL of plasma by a 68-gene NGS panel using an Illumina Hi-Seq 2500 platform in a CLIA-certified setting (Guardant Health, Redwood City, CA).[14] Evaluable samples were defined as those with at least one gene alteration with a variant allele frequency $> 1\%$. MSI was determined by IHC with MLH1 in the tissue samples, as previously described.[1] Microsatellite status was also assessed as part of the ctDNA NGS assay.[15] RNA sequencing was performed on clinical paraffin samples (Illumina, Hayward, CA).[16] RNA signatures were constructed based on previously published gene sets.[17-19]

Outcomes

The primary outcome, PFS per local evaluation by the investigator, was defined by time from random assignment to disease progression, symptomatic deterioration, or death. Patients were censored at the time of last contact. Secondary outcomes included toxicity, OS, overall response rate (ORR; confirmed and unconfirmed, complete response, and partial response), and ORR and PFS in patients who crossed over to the experimental regimen after disease progression on the control arm. Patients were observed until death or 3 years after random assignment, whichever occurred first.

Statistical Considerations

Based on prior publications, median PFS for second- and third-line regimens was estimated at 2.4 months.[2]

Hypothesizing that the targeted agent would offer an increase of 2.4 months in median PFS (hazard ratio [HR], 0.5), the study required 94 eligible patients, based on 6 months of follow-up, a two-sided type 1 error of 5% and 90% power. Analyses of PFS and OS were conducted in all eligible patients according to a modified intent-to-treat principle. Probabilities of OS and PFS were estimated using the Kaplan-Meier method. Statistical differences in event rates between treatment arms were assessed via the stratified Cox regression model, with stratification by prior treatment with irinotecan (yes *v* no). Disease response was described by waterfall plots, separately by treatment arms, and ORR was compared across randomized treatment arms via the χ^2 test in patients with measurable disease. Statistical tests are reported as two-sided, with significance defined as $P < .05$. No adjustments were made for multiple comparisons. Eligible patients receiving at least one dose of a drug on any arm were included in the assessment of adverse events. OS, ORR, and PFS among patients who received the experimental treatment regimen after disease progression on the control arm were summarized using descriptive statistics as described previously.

The presence of a PIK3CA mutation was prespecified as a potential modifier of the PFS treatment effect; a test for interaction between PIK3CA status and treatment assignment was performed within a Cox regression model. The following factors were considered post hoc as potential PFS treatment effect modifiers: prior irinotecan treatment, tumor location, microsatellite status, consensus molecular subtype (CMS), high versus low MAPK signature, and previously described BM classes that represent two subtypes of $BRAF^{V600E}$ tumors based on transcriptomic analysis.[17-19] Tests for interaction were performed as described previously.

Sensitivity and specificity of BRAF detection methods (local mutation testing, IHC, ctDNA, and central NGS) were estimated relative to the tissue-based testing.

RESULTS

Clinical Efficacy

A total of 106 patients were randomly assigned and 100 were eligible (50/arm), with demographics as shown in Table 1 (Appendix Fig 1 [online only] for CONSORT diagram). Eligibility was determined for most patients by local testing of BRAF, with 4% of patients enrolling on the basis of central testing. Of the enrolled patients, 67 and 66 patients had tissue available for orthogonal IHC and NGS validation testing, with 97% and 98% concordance for $BRAF^{V600E}$, respectively (Appendix Table 1, online only).

PFS was significantly greater with the addition of vemurafenib, with an HR of 0.50 (95% CI, 0.32 to 0.76, $P = .001$). Median PFS was 4.2 and 2.0 months in the experimental and control arms, respectively, with 80% and 39% of patients free of progression and death at week 9 (Fig 1A). The response rate and disease control rate were 17% and 65% in the experimental arm, compared with 4% and 21% in the control arm (Figs 2A and 2B). Twenty-one patients on the control arm (42%) crossed over to the experimental regimen after disease progression. OS was not significantly different between the two arms (HR, 0.77, 95% CI, 0.50 to 1.18, $P = .23$; Fig 1B). Median PFS following crossover in this cohort was 5.4 months, with a response rate and disease control rate of 19% and 76%, respectively (Figs 1C and 2C).

Safety

Grade 3 and 4 adverse events higher in the experimental arm than those in the control arm included neutropenia (30% *v* 7%), anemia (13% *v* 0%), and nausea (19% *v* 2%). Eleven of 50 (22%) patients in the experimental arm discontinued treatment because of adverse events, compared with 4 of 50 (8%) in the control arm. Other adverse events of interest are shown in Table 2.

Predictors of Efficacy

PIK3CA mutations were present in 17% (11 of 66) and were associated with a numerically improved PFS HR 0.3 compared with HR 0.6 in patients with wild-type mutation, although sample size precludes definitive conclusions (Appendix Fig 2, online only). Similarly, the clinical benefit was seen without respect to the MSI-H status, which was present in 13 of 72 (18%) evaluable patients (six in the experimental arm and seven in the control arm); the PFS treatment effect was HR 0.5 in patients with MSI-H and MSS status. The treatment effect varied little according to prior irinotecan treatment or tumor location.

Circulating Tumor DNA

Circulating tumor DNA was evaluable in 69 patients, with $BRAF^{V600E}$ detected in 61 baseline samples at a median variant allele fraction (VAF) of 5.0% (range, 0.06%-49.6%) and a sensitivity of 88% (95% CI, 78% to 95%) among evaluable cases (Appendix Table 1). In one of the discordant cases, an $NRAS^{G13D}$ was instead seen at a moderate allele frequency, despite tissue testing demonstrating a $BRAF^{V600E}$ mutation in the baseline tissue. Thirteen cases were considered to be inevaluable because of the lack of any detectable mutations in the available plasma; when these cases are considered, the sensitivity of ctDNA is 74%. Although patients were enrolled based on tissue-detected $BRAF^{V600E}$, a strong treatment effect was also observed in the patient subset with baseline plasma detected $BRAF^{V600E}$ (HR, 0.2, 95% CI, 0.11 to 0.42, $P < .001$).

Serial ctDNA testing of $BRAF^{V600E}$ has been previously validated as a sensitive marker of treatment response.[9] In patients with at least two ctDNA time points, 87% demonstrated a reduction in VAF of $BRAF^{V600E}$ in the experimental arm, whereas no patients in the control arm demonstrated reduction in ctDNA levels ($P \leq .001$, Fig 3).

TABLE 1. Patient Characteristics

Patient Characteristics	Cetuximab + Irinotecan (n = 50)		Vemurafenib + Cetuximab + Irinotecan (n = 50)	
Age (y)				
Median (range)	61.9 (30-82)		59.7 (34-82)	
Sex				
Male	13	26%	29	58%
Female	37	74%	21	42%
Ethnicity				
Hispanic	2	4%	2	4%
Non-Hispanic	48	96%	47	94%
Unknown	0	0%	1	2%
Race				
White	49	98%	44	88%
Black	0	0%	1	2%
Asian	1	2%	4	8%
Unknown	0	0%	1	2%
Zubrod performance status				
0	23	46%	24	48%
1	27	54%	26	52%
Primary site				
Right colon	30	60%	36	72%
Left colon	17	34%	8	16%
Rectum	3	6%	6	12%
Sites of involvement				
Distant lymph nodes	14	28%	13	26%
Lung	21	42%	21	42%
Liver	28	56%	33	66%
Bone	2	4%	2	4%
Peritoneum	16	32%	16	32%
Prior treatment with irinotecan				
Yes	19	38%	21	42%
No	31	62%	29	58%
Prior adjuvant chemotherapy				
Yes	26	52%	25	50%
No	24	48%	25	50%
Number of prior chemotherapy regimens for advanced or metastatic disease				
Prior adjuvant therapy only	8	16%	3	6%
1	25	50%	27	54%
2	17	34%	20	40%

ctDNA also allows the identification of potential genomic mechanisms of resistance upon disease progression, within the limited context of the panel of evaluated genes. Acquired KRAS mutations (G12V and Q61L) were only identified in one patient on the experimental arm with progression samples available (Appendix Fig 3, online only).

RNA Profiling Subgroup Analyses

RNA sequencing was performed on the baseline specimens, and 36 of 71 evaluable patients (51%) had tumors with CMS1 (Appendix Fig 2). The treatment effect of vemurafenib on PFS varied little with CMS or a previously reported signature of MAPK pathway activation (Appendix

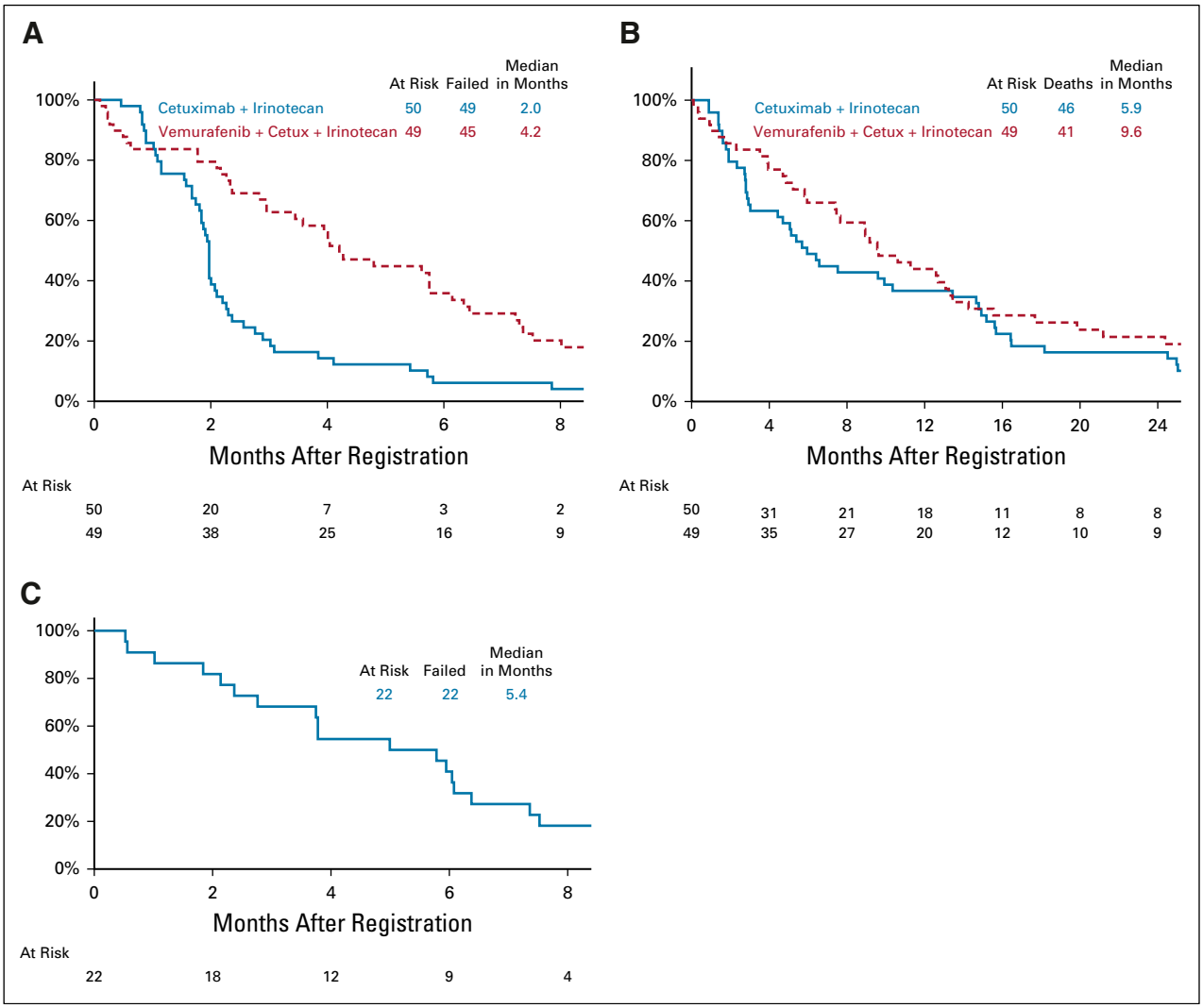

FIG 1. (A) Progression-free survival, (B) overall survival, and (C) progression-free survival in the crossover arm.

Fig 2).[19] While there was improved activity with the BM1 relative to BM2 subtype, this difference was not statistically significant.[18]

DISCUSSION

In this prospective randomized phase II study, the addition of vemurafenib to irinotecan and cetuximab (VIC regimen) improved PFS. This supports the finding that the combination of BRAF and EGFR inhibition can provide clinical benefit in a setting where the activity of either BRAF or EGFR inhibition alone is minimal. Indeed, in the control arm, the median PFS of 2 months corresponded with the first restaging and no patients demonstrated any regression in ctDNA with the control therapy, reiterating the need for novel approaches for this population. While the OS demonstrated improvement with the vemurafenib combination, this was not statistically significant and was impacted by crossover to the experimental regimen at the time of progression on the control arm. The activity of the crossover arm was consistent with the experimental arm, suggesting that prior irinotecan and cetuximab exposure did not meaningfully preclude benefit from the VIC regimen. Since the design and conduct of this study, guidelines have been updated to limit cetuximab treatment to patients with $BRAF^{wt}$ tumors, on the basis of meta-analyses failing to show benefit.[20,21] These findings as well as the performance of the S1406 control arm reiterate the limited standard-of-care options available in this setting.

The SWOG 1406 trial was the first cooperative group study in CRC to prospectively enrich for a molecular biomarker addressing a small subset. For sites where BRAF testing was not standard of care, screening was provided centrally with IHC testing. This was subsequently confirmed by

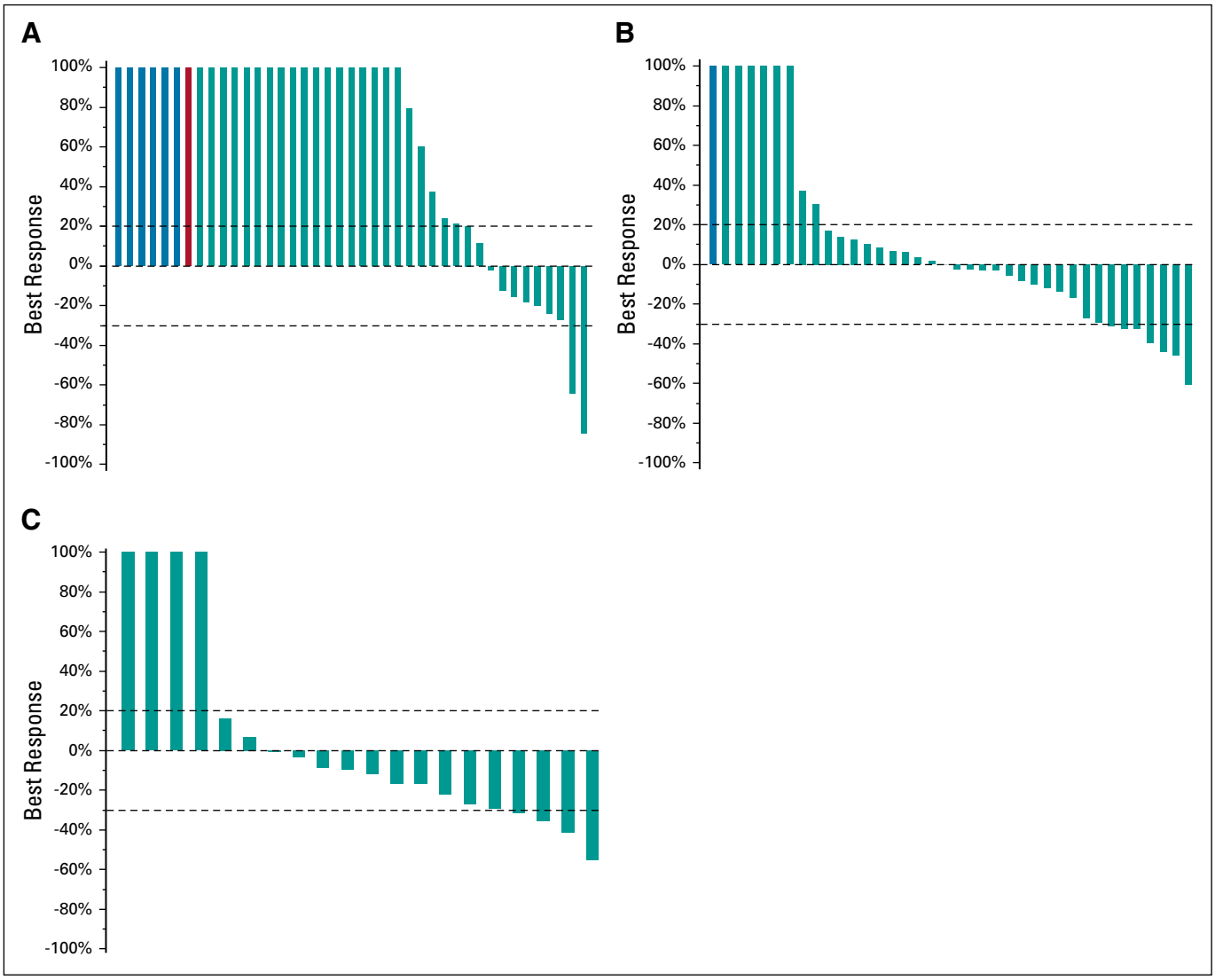

FIG 2. RECIST waterfall plots: (A) cetuximab + irinotecan arm, (B) vemurafenib + cetuximab + irinotecan arm, and (C) crossover arm. The bars on each plot represent the largest decrease under baseline of the sum of longest diameters of all target measurable lesions, or if no decrease was observed, smallest increase in the sum of longest diameters of target measurable lesions. The patient whose increase was greater than 100% over baseline has been truncated at 100% and is represented by a left-slanted bar pattern. Patients whose best response was progression due to new lesions, death (due to disease), or clear worsening of nonmeasurable disease are represented by a bar showing a 100% increase. Patients whose best response could not be determined because of symptomatic deterioration or early death (prior to any follow-up assessments and clearly not due to disease) are represented by a cross-hatched bar showing 100% increase. Patients whose best response could not be determined because of inadequate assessment are represented on the far-left side of the plot with a solid bar showing a 100% increase. Note: two cetuximab + irinotecan and four vemurafenib + cetuximab + irinotecan patients had nonmeasurable disease; six cetuximab + irinotecan arm and seven vemurafenib + cetuximab + irinotecan arm patients with measurable disease at baseline were not represented in these plots because of inadequate assessment during follow-up.

several alternate methodologies, including NGS testing and ctDNA. The concordance between these methods was high, with limitations of available tissue and plasma precluding additional tissue testing in 34% and 18% of patients, respectively, and lack of detectable ctDNA in approximately 13% of patients (Appendix Table 1). The rates of tissue collection for correlative efforts was lower than those in other studies, perhaps reflecting the higher rates of synchronous disease and limited clinical material available in this population. Future analyses will evaluate the correlation of ctDNA detection with tumor burden, location, and clinical characteristics. Recognizing the prognostic and predictive application, current biomarker guidelines for CRC recommend BRAF mutation testing on tumors of all metastatic patients.[22]

Alternative strategies for targeting *BRAF*^{V600E} have been reported, including a phase III study of encorafenib, cetuximab, with or without binimetinib, and a single-arm phase II study of dabrafenib, trametinib, and panitumumab.[8,23,24] Collectively, these approaches suggest several active

TABLE 2. Adverse Events

	Cetuximab + Irinotecan (n = 46) Grade				Vemurafenib + Cetuximab + Irinotecan (n = 47) Grade			
Adverse Events	**1 + 2**	**3**	**4**	**5**	**1 + 2**	**3**	**4**	**5**
Abdominal pain	5	1	0	0	8	2	0	0
Alkaline phosphatase increased	6	2	0	0	3	0	0	0
Anemia	9	0	0	0	15	6	0	0
Anorexia	7	1	1	0	10	4	0	0
Arthralgia	0	0	0	0	9	3	0	0
Dehydration	5	3	0	0	2	5	0	0
Diarrhea	20	5	1	0	16	11	0	0
Fatigue	21	7	0	0	22	8	0	0
Febrile neutropenia	0	1	1	0	0	4	1	0
Hypokalemia	8	1	0	0	9	5	0	0
Hypomagnesemia	17	2	0	0	13	0	0	0
Hyponatremia	0	1	0	0	6	3	0	0
Mucositis oral	7	0	0	0	16	2	0	0
Myalgia	0	0	0	0	4	2	0	0
Nausea	20	1	0	0	19	9	0	0
Neutrophil count decreased	8	2	1	0	5	7	7	0
Rash acneiform	23	3	0	0	21	0	0	0
Rash maculopapular	6	0	0	0	13	1	0	0
Sepsis	0	0	0	0	0	0	1	1
Vomiting	9	1	0	0	17	5	0	0
White blood cell decreased	7	0	0	0	8	3	4	0
Maximum grade any adverse event	17	23	3	0	5	31	7	1

strategies for these patients. While the ability to draw conclusions from cross-trial comparison is limited, the PFS estimates from these studies are similar to what is reported here and the observed OS is similar to the result of the randomized phase III study of binimetinib, encorafenib, and cetuximab. The VIC regimen is notable for the addition of irinotecan to the treatment, in contrast to other strategies only using targeted therapies. Preclinical data have demonstrated the ability of irinotecan to increase the depth of response and induce apoptosis in the models.[11] This is consistent with low response rates seen in prior studies of vemurafenib and cetuximab and suggests strategies to combine optimal BRAF and EGFR targeting with irinotecan.[25]

Our ctDNA results do suggest differences between this regimen and alternate *BRAF*^{V600E}-targeting strategies. In contrast to prior reports of acquired KRAS and NRAS mutations at the time of progression on regimens with targeted therapy alone, this study found only one acquired KRAS, no acquired NRAS mutations, and low rates of other identifiable genomic mechanisms of resistance.[26] Patterns of ctDNA on treatment were consistent with prior reports, with rapid declines in *BRAF*^{V600E} allele frequencies in the majority of patients with increasing allele frequency prior to and at the time of progression.[9] Our ability to detect these resistance mechanisms may have been hampered by the limited plasma volume available for analyses, although the variant allele frequencies seen suggest that this is not a major limitation. Conversely, it is possible that the mechanisms of resistance induced by a combination of cytotoxic chemotherapy with targeted therapy may differ from regimens consisting of targeted therapy alone. This is consistent with recent data suggesting that targeted therapies may be increased genomic instability.[27] Further evaluation of this is warranted, including the assessment of mechanisms of resistance from first-line study of BRAF, EGFR, and standard-of-care chemotherapy as it may have impacts on cross-resistance between these different treatment strategies.

The presence of a *BRAF*^{V600E} mutation is associated with poor outcomes and unique biology, as has been previously described.[28] Importantly, these findings do not apply to the non-V600 mutations in BRAF that have different signaling and clinical implications, as vemurafenib is specific to mutations in codon 600 of BRAF.[29,30] However, within *BRAF*^{V600E}-mutated tumors, it has been suggested

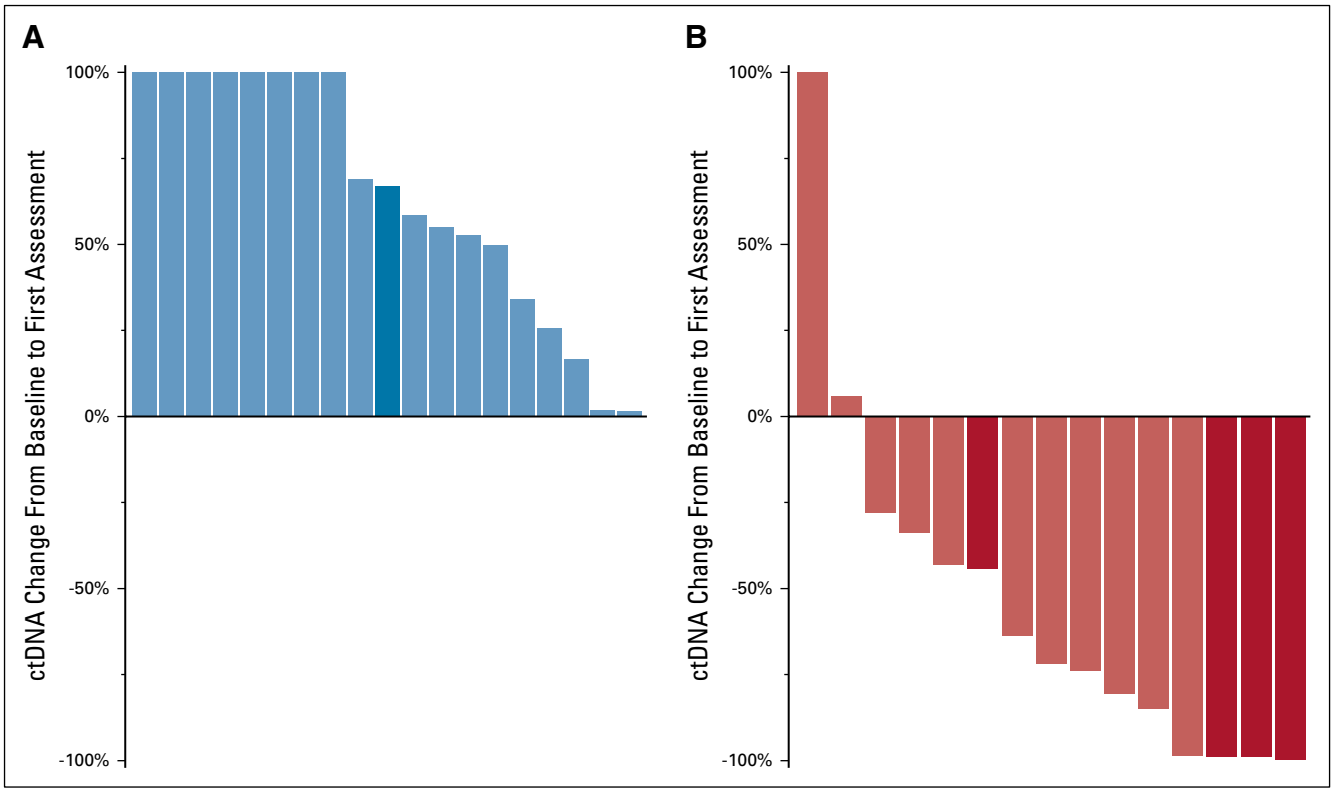

FIG 3. ctDNA waterfall plots: (A) control arm and (B) experimental arm. This plot only includes patients with baseline and follow-up data where ctDNA alterations were detected (N = 34). Patients with > 100% increase were truncated at 100% (one in vemurafenib + cetuximab + irinotecan and nine in cetuximab + irinotecan cohort). Dark shading reflects patients with confirmed partial responses.

that there is heterogeneity that can be evaluated by gene expression signatures. The CMS is one such framework that has been used to describe biological subgroups. Approximately half of the enrolled patients had a CMS1 subtype, consistent with the enrichment of *BRAF*^{V600E} in this subgroup. However, there was no discernable difference in the treatment effect on PFS between the CMS1 and other subtypes. A stronger treatment effect was observed in patients with BM1, defined by KRAS/AKT pathway activation and epithelial mesenchymal transition features relative to BM2, which displays deregulation of the cell cycle, but there was an insufficient sample size to confirm a predictive effect as has previously been suggested in nonrandomized cohorts.[18,31] Similarly, concurrent PIK3CA mutation was previously shown to be positively associated with treatment response to BRAF and MEK inhibition.[5] While we observed evidence of greater treatment effect in patients harboring PIK3CA mutation compared with wild-type mutation, the test for interaction was not statistically significant. The high rate of CMS1, even among the microsatellite stable patients, reflects an increased recognition of the immune-activated phenotype of these tumors. While not predictive of benefit to the therapies in this study, this finding supports current ongoing trials with inhibitors of BRAF, EGFR, and PD1 (ClinicalTrials.gov identifier: NCT04017650) or BRAF, MEK, and PD1 (ClinicalTrials.gov identifier: NCT03668431).

Vemurafenib treatment was associated with an increase in grade III/IV adverse events, notably anemia, neutropenia, and nausea or vomiting. Rash was not substantially increased with the combination, and the rate of secondary keratoacanthomas was lower than that reported with single-agent BRAF inhibition. Known vemurafenib-specific side effects of arthralgia and myalgia were observed but were typically able to be symptomatically managed with supportive medications and treatment interruptions.

In conclusion, the addition of vemurafenib to cetuximab and irinotecan represents an active combination that improves PFS. This represents a rationally designed study building on a foundation of understanding of the mechanisms of adaptive resistance in CRC and provides insights for further combination studies in the CRC field in the future.

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DISCLAIMER

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Randomized Trial of Irinotecan and Cetuximab With or Without Vemurafenib in BRAF-Mutant Metastatic Colorectal Cancer (SWOG S1406)

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APPENDIX

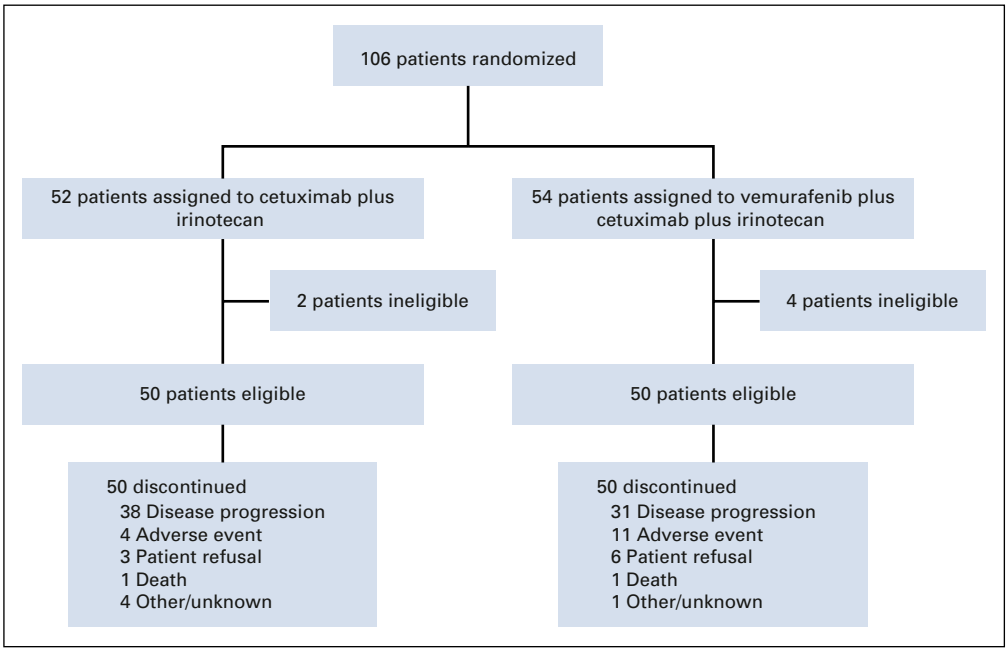

FIG A1. CONSORT flow diagram. Six patients were deemed ineligible due to: inadequate hematologic function (2), not having *BRAF*^{V600E} mutation (3), and receiving chemotherapy within 14 days prior to randomization (1).

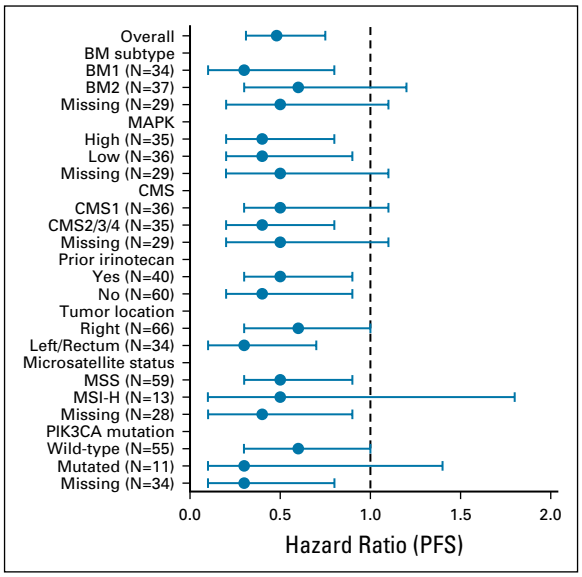

FIG A2. Treatment arm comparisons of progression-free survival in patient subgroups. *PIK3CA* mutations were limited to known somatic variants.

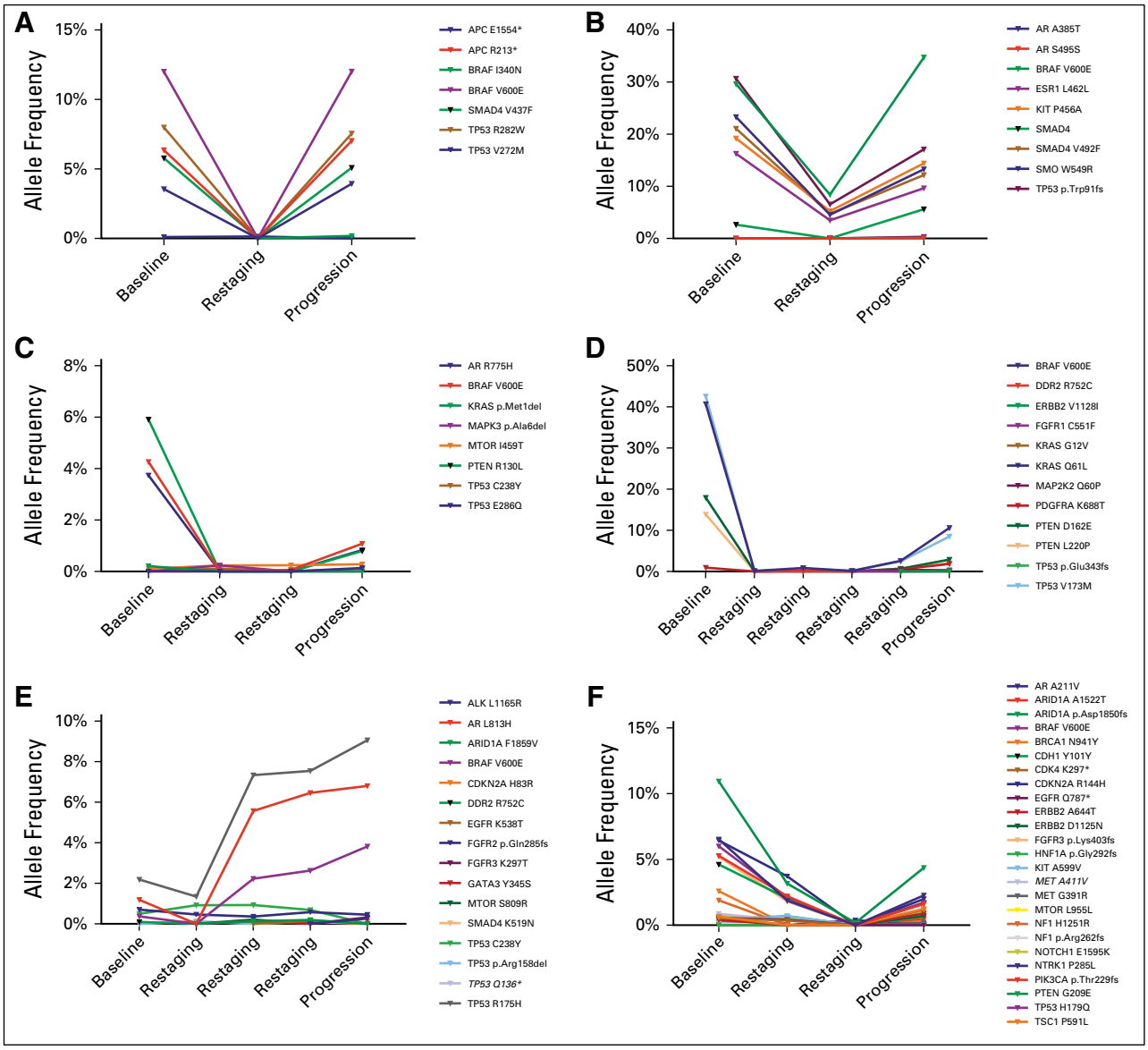

FIG A3. Patterns of ctDNA response and resistance in six patients who received vemurafenib + cetuximab + irinotecan. (A,B) Rapid restitution of existing clonal pattern with progression at 4 months. (C,D) Prolonged disease control, with rising *BRAF*^{V600E} allele frequency preceding radiologic progression by 2-4 months, (E) Rapid rebound in *BRAF*^{V600E} allele frequency followed by slow subsequent increases prior to radiographic progression, (F) Development of a large number of new variants at the time of progression in a patient with MSI-H. These patterns are reflective of the broader population.

TABLE A1. Mutation Testing Results for *BRAF*^{V600E} by Immunohistochemistry, Next-Generation Sequencing, and Circulating Tumor DNA in the Eligible Population (n = 100)

Testing Methodology	Mutated	Wild-Type	Inevaluable	No Sample
Central IHC	65	2		33
Central NGS	65	1		34
ctDNA	61	8	13	18

NOTE. No sample because of missing or insufficient quantity of tissue or plasma. Invaluable because of insufficient detectable ctDNA.
Abbreviations: IHC, immunohistochemistry; NGS, next-generation sequencing.