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Expanded low allele frequency *RAS* and *BRAF* V600E testing in metastatic colorectal cancer as predictive biomarkers for cetuximab in the randomized CO.17 trial

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Abstract

Background: Expanded *RAS/BRAF* mutations have not been assessed as predictive for single-agent cetuximab in metastatic colorectal cancer (mCRC) and low mutant allele frequency (MAF) mutations are of unclear significance. We aimed to establish cetuximab efficacy in optimally selected patients using highly sensitive BEAMing, capable of detecting alterations below standard clinical assays.

Methods: CO.17 compared cetuximab versus best supportive care (BSC) in *RAS/BRAF* unselected mCRC. We performed *RAS/BRAF* analysis on micro-dissected tissue of 242 patients in CO.17 using BEAMing for *KRAS/NRAS* (codons 12/13/59/61/117/146) and *BRAF* V600E.

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Clinical Trial Identifier: NCT00079066

Patients without BEAMing but with previous Sanger sequencing detected mutations were included.

Results: *KRAS*, *NRAS*, and *BRAF* mutations were present in 53%, 4%, and 3% of tumors, respectively. Cetuximab improved overall survival (OS) (HR 0.51, 95% CI 0.32–0.81, P=0.004) and progression free survival (PFS) (HR 0.25, 95% CI 0.15–0.41, P<0.0001) compared to BSC in *RAS/BRAF* wild type patients. Cetuximab did not improve OS/PFS for *KRAS*, *NRAS*, or *BRAF* mutated tumors and tests of interaction confirmed expanded *KRAS* (P=0.0002) and *NRAS* (P=0.006) as predictive, while *BRAF* mutations were not (P=0.089). BEAMing identified 14% more tumors as *RAS* mutant than Sanger sequencing and cetuximab lacked activity in these patients. Mutations at MAF<5% were noted in 6/242 patients (2%). One patient with a *KRAS* A59T mutation (MAF=2%) responded to cetuximab. More *NRAS* than *KRAS* mutations were low MAF (OR 20.50, 95% CI 3.88–96.85, P=0.0038).

Conclusions: We establish single-agent cetuximab efficacy in optimally selected patients and show that subclonal *RAS/BRAF* alterations are uncommon and remain of indeterminate significance.

Keywords

colon; rectal; metastatic; mutation; KRAS; NRAS; subclonal

Introduction:

The anti-epidermal growth factor receptor (anti-EGFR) antibodies cetuximab and panitumumab are important treatment options for patients with metastatic colorectal cancer (mCRC). *KRAS/NRAS* (*RAS*) mutation status and primary tumor location guide treatment selection, with left sided *RAS* wild type tumors showing greatest benefit from anti-EGFR antibodies.[1–7] Patients with *BRAF* V600E mutations may also have reduced benefit from anti-EGFR therapy.[8] However, it is unclear whether *BRAF* mutations obviate all benefit, and a test of interaction for the predictive utility of *BRAF* V600E mutations has not been established.

Though *RAS* and *BRAF* V600E sequencing helps identify the optimal population to treat with anti-EGFR antibodies, eventually patients develop resistance through acquired *RAS* mutations, which appear to be expanded from rare clones pre-existing in the tumor.[9] Longitudinal assessment of circulating tumor DNA (ctDNA) has provided evidence that resistant clones decay with time, creating an opportunity for anti-EGFR re-challenge.[10] In the CRICKET phase 2 trial, patients previously treated with anti-EGFR antibodies who subsequently progressed were re-challenged with cetuximab + irinotecan after intervening therapy. Among patients who were *RAS* wild type by ctDNA preceding re-challenge, progression free survival (PFS) was 4.0 months.[11] Numerous other re-challenge protocols are underway, many with drug combinations, and it is essential to understand the magnitude of benefit of single-agent anti-EGFR therapy to establish a bench mark for re-challenge.[9] Expanded *RAS* and *BRAF* V600E have not been previously assessed in a randomized single-agent cetuximab trial to establish predictive capacity and their utility are extrapolated from multi-agent or panitumumab trials.

It is also unclear whether mutations below the 5% mutant allele frequency (MAF) limit of detection of standard assays are of importance.[12] Although historic PCR and Sanger sequencing methods identified mutations occurring at MAFs above 10–20%, newer techniques have sensitivities down to 0.1% and may lead to improved outcomes.[13] In the CAPRI-GOIM trial evaluating FOLFIRI + cetuximab in mCRC, next generation sequencing revealed an additional 15.9% of patients with *KRAS* exon 2 mutations beyond Sanger sequencing. These patients had inferior outcomes compared to patients with *RAS* wild-type tumors and similar prognosis to high allele frequency *RAS* mutations.[14] These findings have been replicated by many retrospective studies, however it remains unclear whether low allele frequency mutations obviate all benefit.[15–17] In the CRYSTAL trial evaluating cetuximab and FOLFIRI in the first line, the use of high sensitivity BEAMing demonstrated a relationship between the *RAS* MAF and anti-EGFR efficacy and it was unclear whether low MAF mutations prevent all benefit.[18]

Given the current gaps in knowledge, we undertook a retrospective analysis of the CO.17 trial comparing cetuximab with best supportive care (BSC) to establish 1) the efficacy of single-agent cetuximab in optimally selected *RAS/BRAF* wild type patients relative to BSC, and 2) the frequency and clinical relevance of low allele frequency *RAS* mutations detected by an ultra-sensitive assay.

Methods:

Patient population:

CO.17 was a phase III clinical trial that randomized patients 1:1 to receive either cetuximab or BSC after institutional review board approval (NCT00079066).[1] The study was IRB approved, with written consent for all subjects, and conducted in accordance with the Declaration of Helsinki and International Ethical Guidelines for Biomedical Research Involving Human Subjects. Patients consented to enrollment and correlative studies and had either progressed on or were intolerant of a fluoropyrimidine, oxaliplatin, and irinotecan. No prior anti-EGFR therapy was allowed. Enrollment was unselected for *RAS/BRAF*.

This correlative analysis assessed all patients with remaining evaluable tissue (N=242). Median time from tissue collection to randomization was 2.2 years for patients who underwent analysis with BEAMing and did not differ between arms of the study (P=0.22). Of 211 samples with known site of origin, 207 arose from primary tumors (98.1%), while 4 were from metastases (1.9%). There were 84 patients without remaining tissue that were historically identified to have a *KRAS* exon 2 (N=76) or *BRAF* V600E (N=8) mutation using Sanger sequencing from previously published analyses that were included.[1,19] Two additional patients were not analyzable for *KRAS* in the BEAMing assay but historically had a *KRAS* exon 2 which was used to fill in the missing result. Patients with a prior mutation were included, however patients with no remaining tissue and a prior result that did not identify a mutation were excluded, as the previous assessments lacked coverage of all *KRAS/NRAS* codons.

Treatment:

Cetuximab treatment consisted of an intravenous loading dose of 400 mg/m2 followed by 250 mg/m2 given weekly until progression.

RAS and BRAF Testing:

Archival formalin fixed and paraffin embedded (FFPE) blocks were evaluated for sample quality prior to sectioning five slides for DNA extraction. Areas of highest tumor content were selected and micro-dissected. DNA was extracted using QIAMP DNA FFPE tissue kits with barcoding to maintain sample continuity.

Prior to sequencing, samples underwent a repair step using the New England Biolabs PreCR repair mix. DNA isolated from FFPE was subjected to LINE-1 qPCR for quantification and quality control.[20] Only PCR-accessible, inhibition-free and amplifiable target regions qualified for subsequent analysis. For any sample with amplicons exhibiting insufficient amplification, PCR products underwent additional analysis on an agarose gel to confirm successful and target-specific amplification before BEAMing analysis was performed. 2/242 samples had one or more amplicons in *RAS* that was not analyzable due to unamplifiable DNA. These samples were still analyzed for *BRAF* mutations.

A previously described highly sensitive beads, emulsion, amplification, and magnetics (BEAMing) analysis was utilized to detect mutations in *KRAS/NRAS* (codons 12, 13, 59, 61, 117, & 146) and *BRAF* V600E with coverage outlined in Supplemental table 1 and a 1% MAF limit of detection. Sequencing was carried out by Sysmex Inostics (Baltimore, MD).[15,18,21]

Statistical methods:

Survival was summarized with Kaplan-Meier curves and compared using stratified log-rank tests adjusted for performance status at randomization. Hazard ratios and 95% confidence intervals (95% CI) were calculated from stratified Cox-regression models with treatment group as the single factor. Overall survival (OS) was defined as the time from randomization until death from any cause. Progression free survival (PFS) was defined as the time from randomization until progression or death from any cause. To determine whether expanded *RAS* and *BRAF* V600E mutations were predictive, we used a Cox model with treatment, mutation status, and their interaction term as covariates. Objective response rate (ORR) was defined according to modified Response Evaluation Criteria in Solid Tumors.[22] Between group comparisons used Kruskal-Wallis tests for continuous variables or a $\chi 2$/Fisher's exact test as appropriate.

Results:

Patient Population:

Of 572 patients, 242 (42%) underwent analysis with BEAMing. BEAMing was successful in all samples for *BRAF,* but 3 had inconclusive *RAS* analysis. Baseline characteristics are summarized in Table 1. Prevalence in the BEAMing population was 97 (41%) *RAS/BRAF* V600E wild type, 126 (53%) *KRAS*, 9 (4%) *NRAS*, and 7 (3%) *BRAF* V600E mutated,

with specific mutations noted in Supplemental Table 2. There were 5 patients with 2 concurrent *KRAS* mutations, while 1 patient had 3 concurrent *KRAS* mutations. Patients with multiple alterations frequently had second mutations of low allele frequency. These cases were excluded from analysis of low allele frequency variants as they had both high and low allele frequency alterations (Supplemental Figure 1).

Overall Survival:

OS was significantly improved with cetuximab compared to BSC (median 10.1 vs 4.8 months, HR 0.51, 95% CI 0.32–0.81, P=0.004) in patients with *RAS/BRAF* V600E wild type tumors (Figure 1A). No improvement in OS was noted following cetuximab in patients with *KRAS* (HR 0.86, 95% CI 0.63–1.16, P=0.32), *NRAS* (HR 3.93, 95% CI 0.65–23.89, P=0.11), combined *RAS* (HR 0.91, 95% CI 0.68–1.23, P=0.55) or *BRAF* V600E mutated tumors (HR 0.71, 95% CI 0.22–2.27, P=0.56) compared to BSC (Figure 1B). A test of interaction was positive for combined *RAS* (P=0.037) and *NRAS* (P=0.026) but not *KRAS* alone (P=0.067) or *BRAF* V600E mutations (P=0.24) as predictive biomarkers for OS following cetuximab. Among *RAS/BRAF* V600E wild-type patients, left sided tumors (median 10.4 vs 4.8 months, HR 0.55, 95% CI 0.33–0.91, P=0.019) had improved OS with cetuximab relative to BSC but this was not significant for right sided tumors (median 5.7 vs 3.7 months, HR 0.35, 95% CI 0.11–1.07, P=0.055). We repeated our analysis but included patients with mutations <5% MAF as wild type (N=6) and noted no differences in results (Supplemental Table 3).

Progression Free Survival:

Among patients with *RAS/BRAF* V600E wild type tumors, PFS improved following cetuximab relative to BSC (median 5.4 vs 1.8 months, HR 0.25, 95% CI 0.15–0.41, P<0.0001) (Figure 2A). There did not appear to be any prolongation of PFS with the use of cetuximab for patients with *KRAS* (HR 1.03, 95% CI 0.78–1.35, P=0.86), *NRAS* (HR 1.26, 95% CI 0.28–5.74, P=0.76), combined *RAS* (HR 1.04, 95% CI 0.79–1.37, P=0.76) or *BRAF* V600E mutations (HR 0.75, 95% CI 0.26–2.19, P=0.60) (Figure 2B). *KRAS* (P=0.0002), *NRAS* (P=0.006), and combined *RAS* mutations (P=0.0001) were predictive of lack of benefit from cetuximab for PFS using a test of interaction, while *BRAF* V600E mutations neared significance for predictive utility (P=0.089). Left sided *RAS/BRAF* V600E wild type tumors had prolonged PFS following cetuximab (median 5.5 vs 2.0, HR 0.20, 95% CI 0.10–0.37, P<0.0001), while right sided tumors did not meet significance (median 3.6 vs 1.8 months, HR 0.48, 95% CI 0.17–1.36, P=0.16). Similar to OS, when we categorized the 6 patients with mutations occurring at MAF<5% as wild type and repeated the analysis, we noted no change in the PFS end point (Supplemental Table 3).

Response Rate:

ORR (19% vs 0%, P=0.002) was significantly improved with cetuximab compared to BSC in *RAS/BRAF* V600E wild type CRC (Figure 4). Among patients with *KRAS* (ORR 2%), *NRAS* (ORR 0%), combined *RAS* (ORR 2%), and *BRAF* V600 mutations (ORR 0%) there was no difference in ORR relative to BSC where ORR was 0% in all molecular groups. In left sided *RAS/BRAF* V600E wild type tumors ORR was higher than right sided tumors (23% vs 0%, P=0.18) but not significantly different. Categorizing mutations <5% MAF as

wild type did not change the ORR for patients with *RAS/BRAF* V600E wild type tumors but did decrease *KRAS* and combined *RAS* ORR to 1%.

Low Mutant Allele Frequency (MAF) Mutations:

Low allele frequency mutations (MAF<5%) occurred in 6/242 patients (2%). In these six tumors, 3 *KRAS* (G12V, A59T, A59T) and 3 *NRAS* mutations (G13R, A146T, A59T) were identified. Mutations in *NRAS* were more likely to occur at low allele frequency than *KRAS* (OR 20.5, 95% CI 3.9–96.9, P=0.0038). A59T *RAS* mutations were present in 3/6 patients (2 with *KRAS* and 1 *NRAS*) with low MAF alterations compared with 0/136 patients with mutations occurring at MAF>5% (OR ∞, 95% CI 26.28−∞, P<0.0001).

Most mutations occurred at high allele frequencies consistent with a clonal mutation (Figure 3). *KRAS* variants trended towards higher MAF than *NRAS* (P=0.058), but did not differ from *BRAF* V600E (P=0.69). *NRAS* and *BRAF* V600 allele frequencies did not differ (P=0.32). There were 34 (14%) patients who had results for *KRAS* exon 2 available from Sanger sequencing who were previously wild-type but now had a mutation in *KRAS* exon 2 detected with BEAMing. The median MAF for these 34 patients was 20% (range 2%−60%) and treatment with cetuximab did not improve OS (median 6.8 vs 5.4 months, HR 0.60, 95% CI 0.27–1.35, P=0.21), PFS (median 1.9 vs 1.8 months, HR 0.72, 95% CI 0.36–1.46, P=0.36) or response rate (7% vs 0% with BSC, P=0.41) among these patients, suggesting they were clinically relevant. Seven patients (2.9%) previously had Sanger detected *KRAS* mutations but were re-classified as wild type. All discordant cases had high quality assay results with BEAMing and were reviewed.

Of the 3 patients with *RAS* mutations at MAF <5% who received cetuximab, two progressed after 2.7 and 3.7 months with only one patient having a partial response that lasted 11.2 months, while those with low MAF *RAS* mutations in the BSC arm progressed after 1.9 and 3.6 months with one patient withdrawing and none having a response. The one response to cetuximab occurred in a male patient with a *KRAS* A59T mutation (MAF=2%) occurring in a left sided tumor. The patient had received prior fluoropyrimidine, oxaliplatin, and irinotecan and had liver limited metastatic disease. OS for patients with low MAF *RAS* mutations was 11.6, 18.2, and 12.4 months following cetuximab and 2.7, 10.7, and 12.0 months following BSC.

Discussion:

This updated analysis of CO.17 refines our understanding of the magnitude of benefit from single-agent cetuximab in optimally selected patients. Compared to the previous assessment of only *KRAS* exon 2 mutations (mPFS of 3.7 months with cetuximab), mPFS increased to 5.4 months with improved molecular profiling.[1] Using highly sensitive BEAMing we identified an additional 14% of patients who were wild type by Sanger sequencing and lacked benefit from cetuximab, highlighting the utility of more sensitive assays. This work also enhances our knowledge of predictive biomarkers in mCRC. Previously, a test of interaction for anti-EGFR interacting with expanded *RAS* mutations was only available for panitumumab, not cetuximab[3]. Additionally, despite the non-significant (P=0.089) test of interaction for *BRAF* V600E mutations being a predictive biomarker, this work highlights

reduced benefit from anti-EGFR therapy in this population. These findings support early incorporation of combination *BRAF* directed treatment rather than single agent anti-EGFR therapy.[23]

Our prevalence estimate of expanded *RAS* mutations (56%) agrees with other series, where pooled estimates suggest *RAS* mutations occur in 55.9% of mCRC.[24] By combining *RAS* and *BRAF* V600E alterations, the population expected to benefit from single-agent anti-EGFR therapy drops to only 41% in our study. Interestingly, we only detected an additional 6 (2%) patients with low allele frequency mutations. This is lower than others have reported, and may reflect the impact of tumor micro-dissection or utilization of a threshold for the assay associated with low rates of false-positive results (1% instead of 0.1%). Improved methodologies for high-depth sequencing have been developed, although the clinical relevance of such higher sensitivity approaches remain unclear given the low prevalence of this population and difficulty confirming lack of benefit.

In our study, 3 individuals had tumors harboring mutations at MAF<5% who received anti-EGFR therapy. One of these patients had a response to cetuximab, suggesting a potential gradient of efficacy based on the MAF of mutant *RAS* in a tumor. This is supported by the CRYSTAL trial, where a gradient of activity was noted among patients based on allele frequency of *RAS* mutations.[18] By using BEAMing technology, we were able to provide better stratification of patients. Not only were there 6 patients with mutations occurring between 1–5% MAF, but we also identified 34 patients that were *KRAS* wild type by Sanger sequencing. This suggests there were “intermediate” allele frequency mutations not detected with Sanger sequencing (threshold for detection between MAF 10–20%), however current next generation sequencing assays may have identified them.[1,13] Indeed, the median MAF of these 34 discordant cases was 20%. Although many of these samples should have had variants detected by Sanger’s threshold, an important distinction between the original assessment of *KRAS* for CO.17 and our current analysis is that microdissection was performed in our updated analysis. Therefore, the detected allele frequencies are likely higher than would have been noted in the original analysis that used whole slides. Taken together, our results lend further support to the need for high sensitivity assays in the clinic.

Current guidelines suggest assays need a 5% MAF limit of detection for *RAS* mutations and our work suggests the number of additional patients identified with more sensitive assays is relatively small.[12] While only 3 patients with low MAF *RAS* mutations were treated with cetuximab, 1 of these patients had a response and a PFS of 11.2 months, while 2 others with low MAF mutations had PFS of 2.7 and 3.7 months. In the CRYSTAL trial of FOLFIRI +/− cetuximab, high sensitivity BEAMing identified 23/430 (5.3%) patients with *RAS* mutations outside of codon 12/13 occurring at allele frequencies of 0.1–5%. In this group, the addition of anti-EGFR agents provided a signal towards benefit (HR 0.57, 95% CI 0.33–1.01).[25] While the low allele frequency of the responding patient’s mutation in CO.17 may explain the activity of cetuximab, the mutation was *KRAS* A59T which has previous case reports of response and is one of these least well studied *RAS* mutations, with only 7 patients harboring this alteration in the PRIME trial that defined expanded *RAS* as a biomarker.[3,26] Taken together, both low allele frequency mutations and certain expanded *RAS* mutations are sufficiently uncommon that it is unlikely we will ever conclusively establish their role as

predictive biomarkers. Hopefully increasing use of ctDNA will provide further insights into subclonal *RAS* dynamics.

ctDNA provides great promise for detecting acquired resistance to targeted therapies and evaluating evolutionary changes in cancers. Previous work has demonstrated that *RAS* mutant clones develop during anti-EGFR therapy[27,28] These variants tend to be lower allele frequency than mutations present at baseline, and in Morelli et al's report, 35% of them were found in primary tissue when assessed with high sensitivity BEAMing with sensitivity beyond standard clinical tests. It remains unclear whether the utility of ctDNA may better select baseline *RAS* status compared to tissue, however it does allow dynamic surveillance of resistance which is unique. In mCRC, many acquired resistance mechanisms have been shown to decay over time, allowing anti-EGFR re-challenge as a treatment consideration.[10,29] The improvement in median PFS from 1.8 to 5.4 months in *RAS/BRAF* V600 wild type patients in CO.17 sets a target for these re-challenge efforts. Given that many anti-EGFR re-challenge concepts include additional agents and in the context of the ever rising costs of oncology drugs, it is essential that combinatorial strategies demonstrate clear superiority to single agent re-challenge.[9]

This study also further supports the combinatorial treatment strategy for *BRAF* V600E mCRC as single agent anti-EGFR does not improve PFS in *BRAF* V600E mutated mCRC.[23] Unfortunately, only 15 patients with *BRAF* V600 mutations were evaluable in CO.17 for a test of interaction, which neared significance (P=0.089) despite small numbers. As CO.17 accrued in the treatment refractory population, it is not surprising that we saw a low prevalence of *BRAF* V600 mutations given their poor prognosis. Given the lack of benefit to date with anti-EGFR therapy, patients with *BRAF* V600E mutations should be prioritized for combinatorial strategies which have shown significant activity in this population.[23]

Despite the important findings of our study, it must be interpreted in the context of several limitations. As CO.17 completed enrollment over a decade ago, previous correlative analyses have exhausted much of the tissue and we could only analyze a subset of patients. Bias may be introduced into some analyses by the fact that certain patients had remaining tissue while others did not. However, we noted no differences in OS, PFS, or RR between the historic analysis and the updated analysis when assessing the best supportive care arm for prognosis in either the *RAS/BRAF* mutant group or the wild type group. The small number of patients who had *BRAF* or low allele frequency mutation means that findings among these groups must be interpreted in the context of the wide confidence interval surrounding treatment effect. When the trial was planned, the importance of *RAS* was not understood and as such our analyses are post-hoc and were not part of the original statistical plan. This is often the case with biomarker discovery, and all current evidence supporting *RAS* mutations as predictive are post-hoc.

In conclusion, we provide updated evidence that patients with mCRC harboring expanded *RAS* or *BRAF* V600E mutations lack benefit following single agent cetuximab. Our work demonstrates improved patient selection with the use of a high sensitivity assay that re-classified 14% of tumors as *RAS* mutated compared to Sanger sequencing. Subclonal mutations <5% MAF were uncommon, occurring in 2% of patients and remain of unclear

significance. We hope this updated work informs future anti-EGFR combinatorial strategies by setting a benchmark for the activity of single agent cetuximab using a modern high sensitivity assay.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance:

The predictive utility of expanded *RAS* and *BRAF* for anti-EGFR therapy in colorectal cancer arises from panitumumab trials and it is unclear whether low mutant allele frequency mutations in these genes impact efficacy. We evaluated tissue from the CO.17 trial that randomized patients to cetuximab or best supportive care with a high sensitivity assay (BEAMing) and micro-dissection for expanded *RAS/BRAF* mutations (MAF>1%). Cetuximab improved overall and progression free survival in patients with *RAS/BRAF* V600E wild type tumors relative to supportive care and a test of interaction confirmed *RAS* (P<0.01) but not *BRAF* (P=0.089) mutations as predictive for cetuximab benefit. BEAMing showed increased sensitivity and identified 14% more *KRAS* mutations than historic Sanger sequencing. Mutations in *RAS* with allele frequency <5% were noted in 2% of patients, one of whom responded to cetuximab.

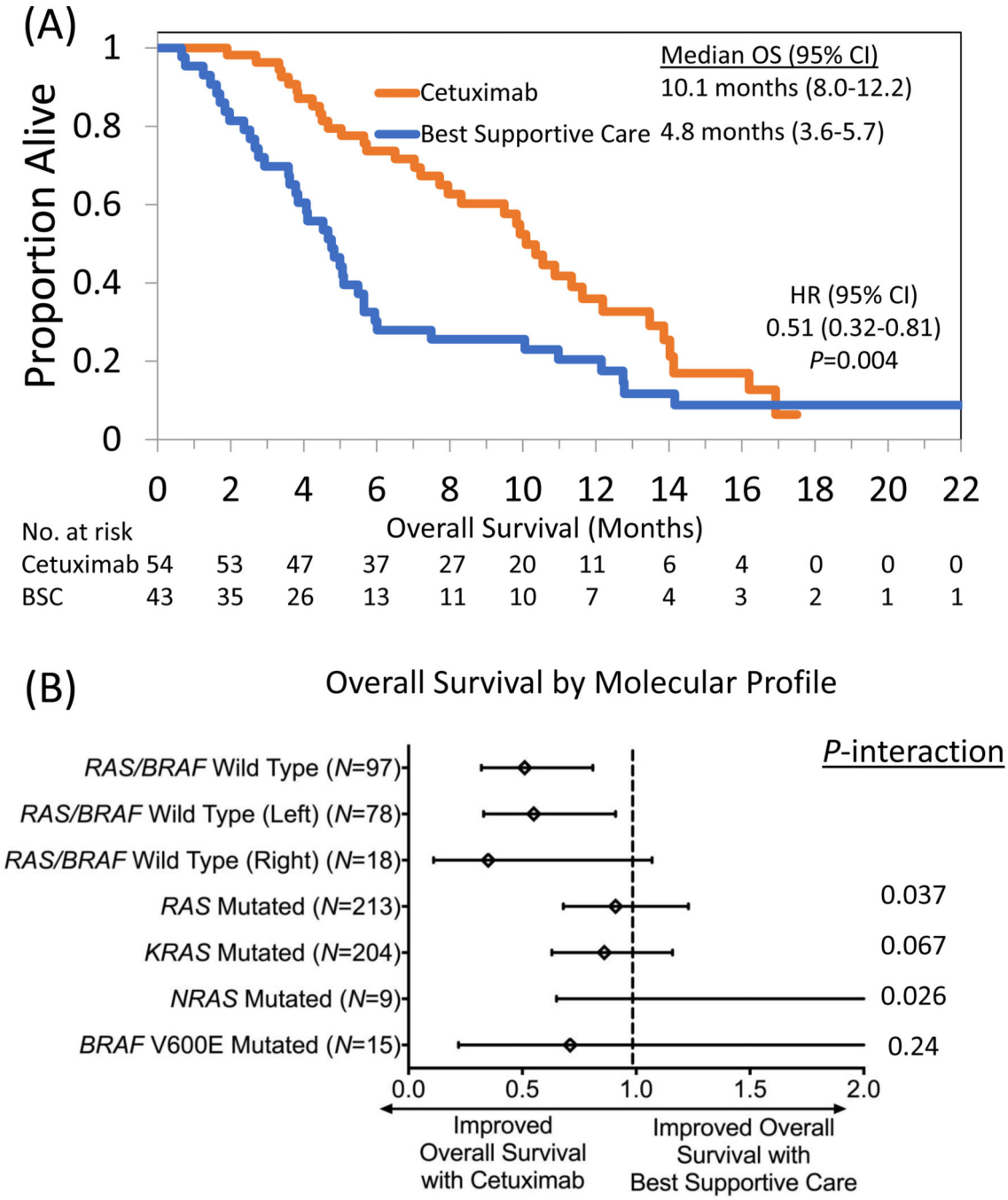

Figure 1.

Impact of cetuximab on overall survival in patients with (A) *RAS/BRAF* V600E wild type metastatic colorectal cancer compared to best supportive care in the CO.17 trial and stratified by (B) molecular subgroup.

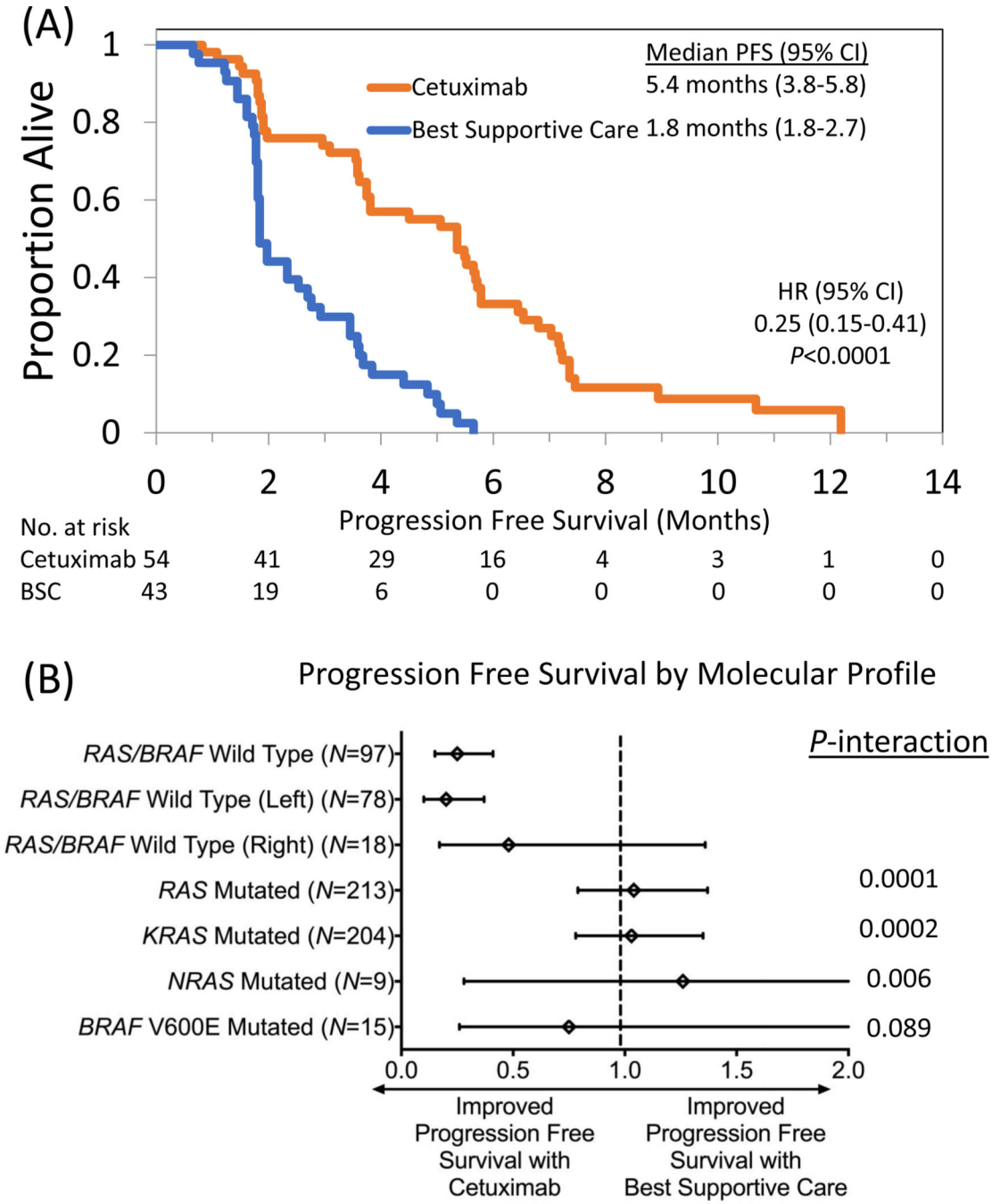

Figure 2.

Impact of cetuximab on progression free survival in patients with (A) *RAS/BRAF* V600E wild type metastatic colorectal cancer compared to best supportive care in the CO.17 trial and stratified by (B) molecular subgroup.

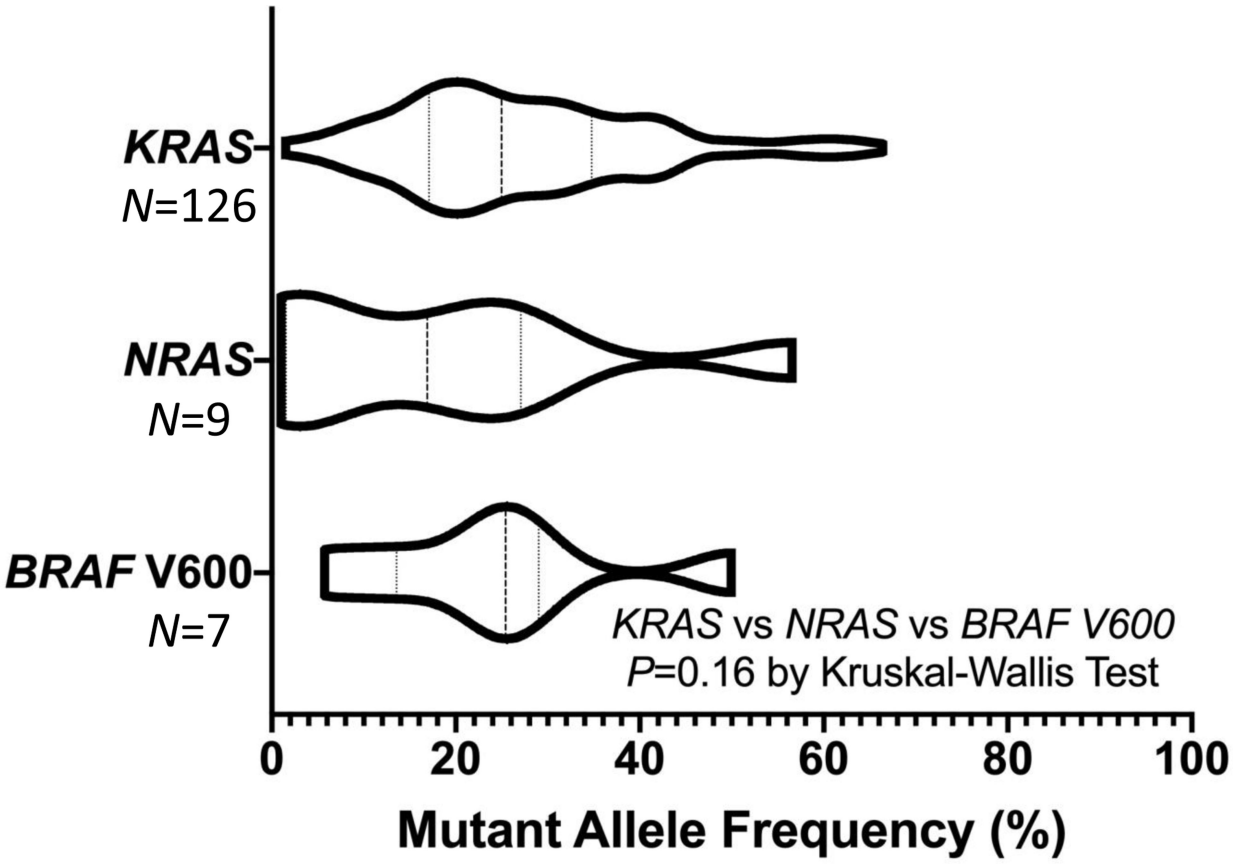

Figure 3.
Violin plot displaying the mutant allele frequency distribution density of detected mutations in *KRAS, NRAS* and *BRAF*.

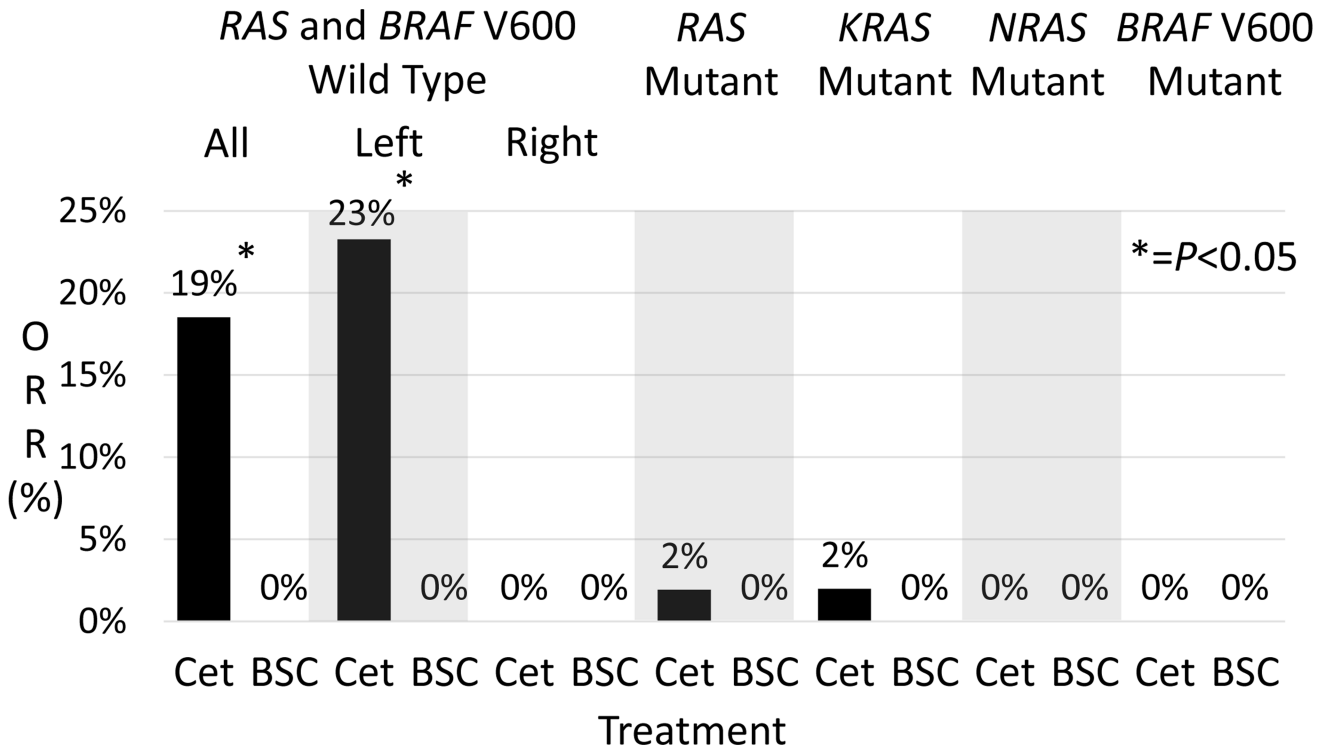

Figure 4.
Objective response rate of patients in CO.17 receiving cetuximab or best supportive care (BSC).

Table 1.

Baseline patient characteristics

Characteristic					
	***RAS* and *BRAF* V600E Wild Type (*N*=97)**	***KRAS* Mutated (*N*=204)**	***NRAS* Mutated (*N*=9)**	***BRAF* V600E Mutated (*N*=15)**	***P***
Median Age (range)	64 (29–88)	63 (37– 86)	69 (47–75)	64 (39– 77)	0.79
Gender					
Female	28 (29)	72 (35)	2 (22)	5 (33)	0.63
Male	69 (71)	132 (65)	7 (78)	10 (67)	
ECOG					
0	26 (27)	46 (23)	1 (11)	1 (7)	0.29
1	58 (60)	117 (57)	5 (56)	9 (60)	
2	13 (13)	41 (20)	3 (33)	5 (33)	
Side of tumor					
Right	18 (19)	71 (37)	3 (38)	10 (67)	0.0005
Left	78 (81)	123 (63)	5 (63)	5 (33)	
Prior Treatment					
5-FU	97 (100)	204 (100)	9 (100)	15 (100)	1
Irinotecan	92 (95)	199 (98)	8 (89)	14 (93)	0.36
Oxaliplatin	96 (99)	201 (99)	9 (100)	15 (100)	0.93
Site of Disease					
Liver	87 (90)	159 (78)	8 (89)	10 (67)	0.038
Lung	56 (58)	133 (65)	5 (56)	10 (67)	0.60
Nodes	51 (53)	80 (39)	3 (33)	7 (47)	0.16
Treatment					
Cetuximab	54 (56)	101 (50)	3 (33)	7 (47)	0.45
BSC	43 (44)	103 (51)	6 (67)	8 (53)	

*Percentages represent the % of known, *N*=number, BSC = best supportive care