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ORIGINAL REPORT

Mutant *KRAS* Codon 12 and 13 Alleles in Patients With Metastatic Colorectal Cancer: Assessment As Prognostic and Predictive Biomarkers of Response to Panitumumab

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ABSTRACT

Purpose
Panitumumab, a fully human monoclonal antibody targeting the epidermal growth factor receptor (EGFR), has demonstrated significant improvements in progression-free survival (PFS) in patients with wild-type *KRAS* metastatic colorectal cancer (mCRC) in studies 20050203 (first line), 20050181 (second line), and 20020408 (monotherapy). Mutations in *KRAS* codons 12 and 13 are recognized biomarkers that predict lack of response to anti-EGFR antibody therapies. This retrospective analysis of three randomized phase III studies assessed the prognostic and predictive impact of individual mutant *KRAS* codon 12 and 13 alleles.

Patients and Methods
Patients were randomly assigned 1:1 to FOLFOX4 (infusional fluorouracil, leucovorin, and oxaliplatin) in study 20050203, FOLFIRI (fluorouracil, leucovorin, and irinotecan) in study 20050181, or best supportive care in study 20020408 with or without panitumumab 6.0 mg/kg once every 2 weeks. In all, 441 (20050203), 486 (20050181), and 126 (20020408) patients with mutant *KRAS* codon 12 or 13 alleles were included in the analysis.

Results
No mutant *KRAS* allele in patients treated on the control arm emerged as a consistent prognostic factor for PFS or overall survival (OS). In addition, no mutant *KRAS* allele was consistently identified as a predictive factor for PFS or OS in patients receiving panitumumab treatment. Significant interactions for individual mutant *KRAS* alleles were observed only in study 20050203 with G13D negatively and G12V positively associated with OS in the panitumumab-containing arm. Pooled analysis indicated that only G12A was associated with a negative predictive effect on OS.

Conclusion
In this retrospective analysis, results across three treatment regimens suggest that patients with mutant *KRAS* codon 12 or 13 mCRC tumors are unlikely to benefit from panitumumab therapy. Currently, panitumumab therapy should be limited to patients with wild-type *KRAS* mCRC.

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INTRODUCTION

KRAS is a small G protein that acts as a transducer in the epidermal growth factor receptor (EGFR) pathway. Acquired *KRAS* codon 12 or 13 gain-of-function mutations lead to constitutive signaling through the EGFR pathway and to downstream activation of MAPK- and PI3K-dependent pathways.[1,2] In approximately 40% of all metastatic colorectal cancer (mCRC) tumors, one of several heterozygous *KRAS* codon 12 or 13 mutations is detected.[3] In individual patients, these point mutations are frequently detected in both primary and metastatic lesions,[4,5] consistent with the notion that *KRAS* mutations are acquired early during colorectal tumorigenesis.

Panitumumab is a fully human monoclonal antibody that targets the extracellular region of EGFR and effectively blocks ligand-dependent signaling downstream of the receptor. In the first-line 20050203 study,[6] the second-line 20050181 study,[7] and the monotherapy 20020408 study,[8,9] panitumumab significantly improved progression-free survival (PFS) and response rate in patients with wild-type (wt) *KRAS* mCRC but not in their mutant *KRAS* mCRC counterparts. Collectively, mutant

KRAS codon 12 and 13 alleles are established biomarkers for lack of response to anti-EGFR monoclonal antibodies in patients with mCRC.[6,7,9-11]

However, recent retrospective analyses[12,13] have suggested that patients whose tumors harbor a specific *KRAS* exon 2 mutation, a glycine (G; single-letter amino acid code) to aspartate (D) mutation at codon 13 (G13D), may derive clinical benefit from an anti-EGFR monoclonal antibody therapy in chemorefractory settings and in first-line combination therapy with irinotecan or oxaliplatin. In this analysis of 1,053 patients with mutant *KRAS* codon 12 or 13 alleles, we retrospectively examined the seven most common mutant *KRAS* codon 12 and 13 alleles for their prognostic and predictive impact on outcomes in patients with mCRC receiving panitumumab-containing therapy across three randomized phase III studies.

PATIENTS AND METHODS

Data Sets

Studies 20050203, 20050181, and 20020408 were open-label, multicenter, controlled phase III trials.[6-8] Patients in these trials were randomly assigned 1:1 to receive FOLFOX4 (infusional fluorouracil, leucovorin, and oxaliplatin) in study 20050203, FOLFIRI (fluorouracil, leucovorin, and irinotecan) in study 20050181, or best supportive care (BSC) in study 20020408 with or without panitumumab 6.0 mg/kg intravenously every 2 weeks. In studies 20020408 and 20050203, randomization was stratified by geographic region and Eastern Cooperative Oncology Group (ECOG) performance status. The primary end point for both studies was PFS. Key secondary end points included overall survival (OS), response rate, and safety. In study 20050181, randomization was stratified by prior oxaliplatin treatment, prior bevacizumab treatment, and ECOG performance status. The coprimary end points were PFS and OS. Key secondary end points included response rate and safety.

For all three phase III trials, key eligibility criteria included age $\geq$ 18 years, metastatic adenocarcinoma of the colon or rectum, measurable disease, ECOG performance status of 0 to 2, no prior anti-EGFR therapy, and paraffin-embedded tumor tissue available for central biomarker analyses. The *KRAS* status of patients' tumors was neither required nor assayed at study entry but was performed after all patients had been enrolled.

KRAS testing was conducted by a blinded central laboratory and determined by using the TheraScreen K-RAS Mutation Kit (Qiagen, Manchester, United Kingdom) that detects the seven most common mutations in *KRAS* codons 12 and 13 (*KRAS* G12A, G12C, G12D, G12R, G12S, G12V, and G13D). Individual *KRAS* allele testing was performed without knowledge of patient clinical outcomes. Descriptive statistics were provided for patient demographics and baseline characteristics in studies 20050203 and 20050181 but were not conducted in study 20020408 because of the relatively low number of patients with each mutant *KRAS* allele.

Statistical Analysis

The primary objective of this study was to examine the prognostic and predictive impact of the seven most common mutations in *KRAS* codons 12 and 13 on PFS, OS, and response rate in patients with mCRC who received panitumumab or control therapy. The analysis was conducted separately for each *KRAS* allele, for each study, and for all three studies combined. For prognostic analyses, comparisons were made between the outcomes of patients whose tumors harbored a specific *KRAS* mutation and the remaining patients whose tumors harbored any of the remaining six mutant *KRAS* alleles. Prognostic analyses were performed exclusively on patients who received control therapy (ie, non–panitumumab-containing). For analyses of the predictive impact of mutant *KRAS* alleles, relative treatment effects of panitumumab-containing and non–panitumumab-containing therapies were estimated among patients whose tumors harbored wt *KRAS*, any of the indicated mutant *KRAS* alleles (analyzed together as a group), or the specified individual mutant *KRAS* allele. Hazard ratios (HRs) and 95% CIs for PFS and OS were obtained by using the Cox proportional hazards model. A descriptive quantitative interaction test[14] was conducted to assess the relative treatment effect on PFS and OS between the specific mutant *KRAS* codon 12 or 13 allele and the other *KRAS* mutations. No adjustments were made for multiple testing. HRs were stratified by study for the pooled analysis. All statistical evaluations were performed with SAS software, version 11 (SAS Institute, Cary, NC).

RESULTS

Patients

KRAS status was ascertained in mCRC tumors from 1,096 (93%) of 1,183 patients in study 20050203 (panitumumab plus FOLFOX4 *v* FOLFOX4 alone), 1,083 (91%) of 1,186 patients in study 20050181 (panitumumab plus FOLFIRI *v* FOLFIRI alone), and 427 (92%) of 463 patients in study 20020408 (panitumumab plus BSC *v* BSC). This analysis of patients with mutant *KRAS* codon 12 or 13 mCRC included 441 (40%) of 1,096 patients in study 20050203, 486 (45%) of 1,083 patients in study 20050181, and 126 (30%) of 427 patients in study 20020408. The distribution of mutant *KRAS* codon 12 and 13 alleles was conserved across these three phase III studies and was equally balanced between the treatment and control arms (Table 1).

Table 1. Distribution of Patients With Mutant *KRAS* Codon 12 and 13 mCRC Included in the Current Analysis From Studies 20050203, 20050181, and 20020408, Segregated by Treatment Arm

	Study 20050203				Study 20050181				Study 20020408			
	Pmab + FOLFOX4 (n = 221)		FOLFOX4 (n = 220)		Pmab + FOLFIRI (n = 238)		FOLFIRI (n = 248)		Pmab + BSC (n = 56)		BSC (n = 70)	
KRAS Allele	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
G12D	68	31	57	26	77	32	70	28	21	38	22	31
G12V	57	26	64	29	62	26	79	32	10	18	18	26
G13D	46	21	52	24	39	16	45	18	9	16	11	16
G12C	16	7	19	9	26	11	19	8	6	11	6	9
G12A	21	10	13	6	17	7	17	7	6	11	5	7
G12S	13	6	14	6	12	5	13	5	4	7	6	9
G12R	0	0	1	< 1	5	2	4	2	0	0	2	3

Abbreviations: BSC, best supportive care; FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOX4, infusional fluorouracil, leucovorin, and oxaliplatin; mCRC, metastatic colorectal cancer; Pmab, panitumumab.

The results are consistent with published mCRC *KRAS* mutation analysis[15-18] and are comparable to the more than 9,000 primary colon and rectum adenocarcinoma cases in the public Catalogue of Somatic Mutations in Cancer (COSMIC) mutation database.[19] Together, *KRAS* G12D, G12V, and G13D comprised more than 70% of all mutant *KRAS* codon 12 and 13 alleles in each of the three studies. *KRAS* G12R was detected in less than 2% of mutant *KRAS* tumors and was not analyzed further.

Baseline demographic and clinical features were generally balanced in all mutant *KRAS* allele subgroups in studies 20050203 (Appendix Fig A1, online only) and 20050181 (Appendix Fig A2, online only) with the percentage of white patients, ECOG performance status, primary tumor site, incidence of liver plus other metastatic sites, prior surgery, and intensity of study therapy being similar across *KRAS* allelic subgroups and by treatment arm.

Prognostic Impact of KRAS *Alleles*

To evaluate the prognostic impact of *KRAS* codon 12 and 13 mutations, HRs with corresponding 95% CIs were plotted for the non–panitumumab-containing control arms of the first-line (20050203), second-line (20050181), and monotherapy (20020408) studies (Fig 1). HRs for patients whose tumors harbored each of the individual mutant alleles were ordered by allele frequency and were compared with the other mutant *KRAS* codon 12 and 13 alleles combined.

The 95% CIs for the calculated HRs did not cross unity for mutant *KRAS* allele G12C (HR, 2.06; 95% CI, 1.16 to 3.65), which appeared as a negative prognostic factor for PFS but not for OS in study 20050203. None of the mutant *KRAS* alleles in study 20050181 were associated with a prognostic impact. In study 20020408, the 95% CIs for the calculated HRs did not cross unity for alleles *KRAS* G12C (HR, 2.47; 95% CI, 1.04 to 5.90) and *KRAS* G12A (HR, 5.30; 95% CI, 1.96 to 14.34), which both appeared as negative prognostic factors for OS but not for PFS. Taken together, no single mutant *KRAS* allele was a consistent negative or positive prognostic factor for both PFS and OS or across lines of mCRC therapy.

Predictive Impact of KRAS *Alleles on Panitumumab Efficacy*

The predictive effect of mutant *KRAS* codon 12 and 13 alleles on PFS and OS was also evaluated in all three phase III panitumumab studies (Fig 2). HRs with 95% CIs were plotted for patients whose

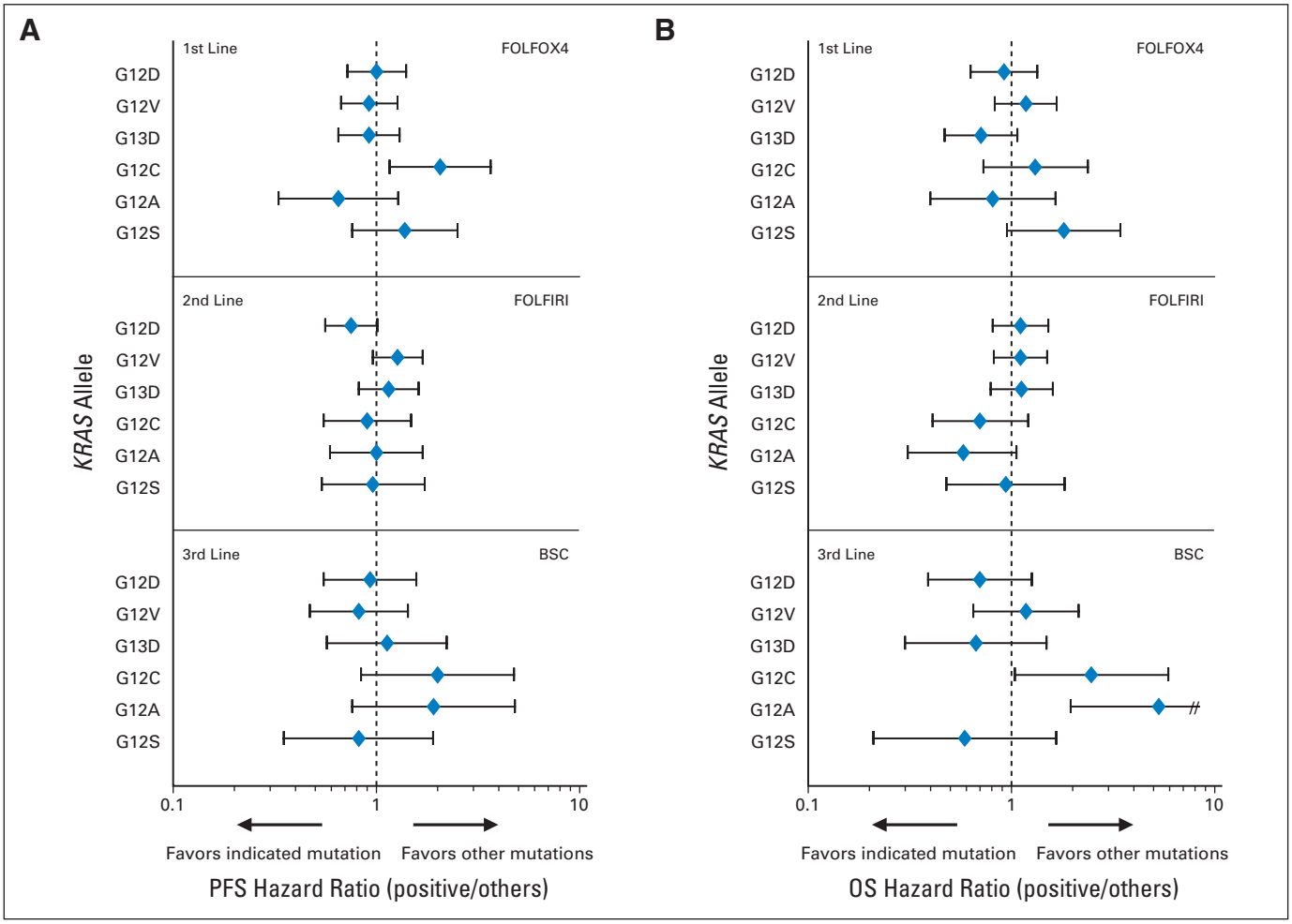

Fig 1. Prognostic impact of mutant *KRAS* codon 12 and 13 alleles (A) progression-free survival (PFS) and (B) overall survival (OS) in patients receiving control (non–panitumumab-containing) therapy. Point estimates for hazard ratios and their corresponding 95% CIs are plotted for the indicated mutant *KRAS* codon 12 and 13 alleles and are compared with the other mutant *KRAS* codon 12 and 13 alleles as a group. BSC, best supportive care; FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOX4, infusional fluorouracil, leucovorin, and oxaliplatin.

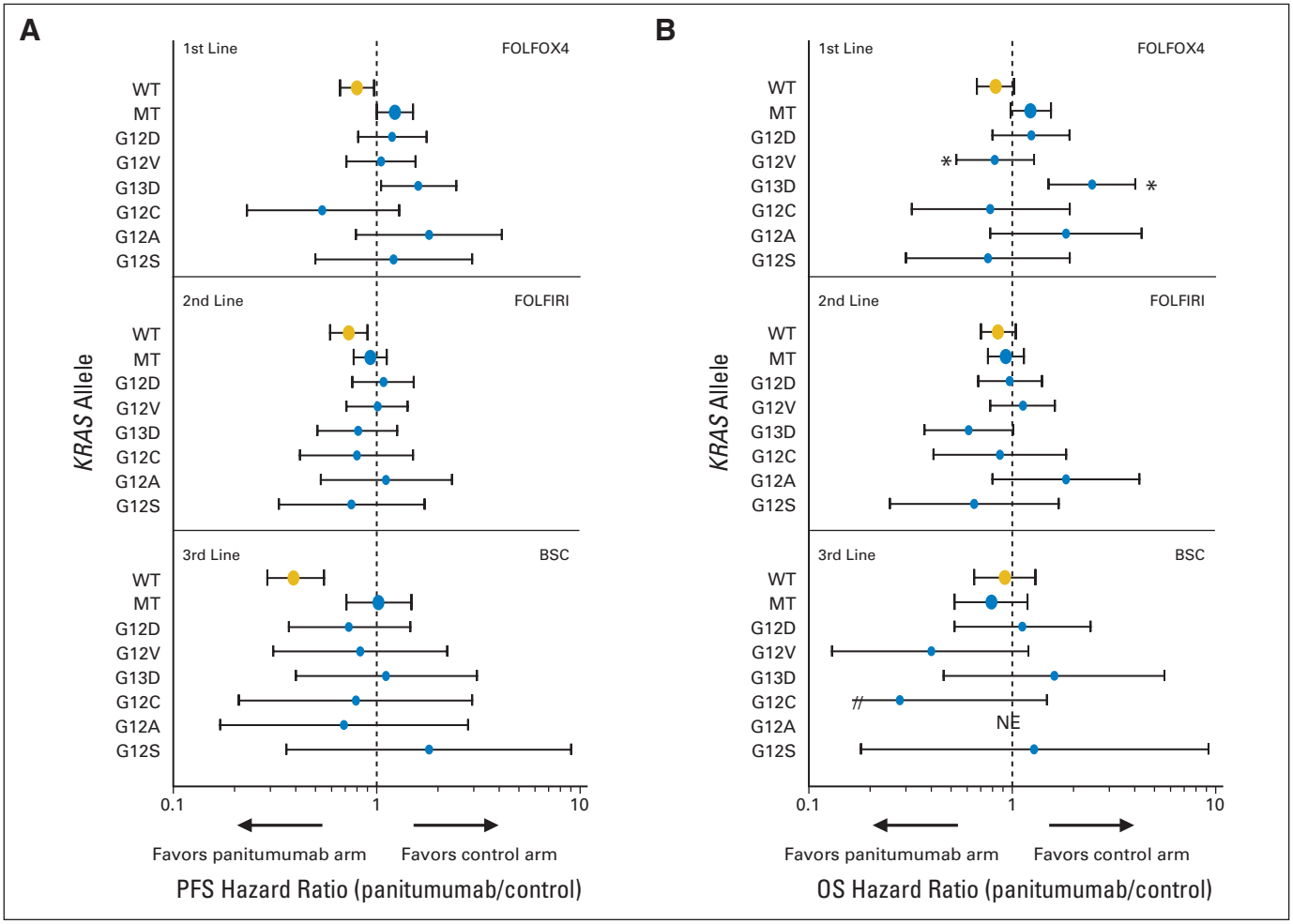

Fig 2. Predictive impact of mutant *KRAS* codon 12 and 13 alleles on (A) progression-free survival (PFS) and (B) overall survival (OS) in patients receiving either control (non–panitumumab-containing) or panitumumab-containing therapy. Point estimates for hazard ratios and their corresponding 95% CIs are plotted for wild-type (WT) *KRAS* and for the indicated mutant (MT) *KRAS* codon 12 and 13 alleles and are compared with the other mutant *KRAS* codon 12 and 13 alleles as a group. (*) Positive interaction test between indicated mutant *KRAS* allele and therapy. BSC, best supportive care; FOLFIRI, fluorouracil, leucovorin, and irinotecan; FOLFOX4, infusional fluorouracil, leucovorin, and oxaliplatin; NE, not estimable.

tumors harbored either wt *KRAS* or the indicated individual mutant *KRAS* alleles and were compared with the entire collection of mutant *KRAS* alleles. *KRAS* G13D was the only allele for which the 95% CIs for the calculated HRs did not cross unity, and it appeared as a negative predictive factor for both PFS (HR, 1.60; 95% CI, 1.05 to 2.46) and OS (HR, 2.47; 95% CI, 1.51 to 4.03) in the panitumumab-containing arm of the first-line 20050203 study. However, when a quantitative interaction test was conducted (Table 2), *KRAS* G13D was significantly associated only with a negative impact on OS ($P = .0018$) but not PFS ($P = .1609$). A borderline statistically significant positive impact on OS in study 20050203 was observed by interaction testing for *KRAS* G12V ($P = .0369$), but the 95% CIs for the calculated OS HR crossed unity (Fig 2). Taken together, across three studies, none of the individual mutant *KRAS* alleles were consistently associated with panitumumab treatment effects on PFS or OS outcomes. However, consistent with previous reports,[6,7,9] mutant *KRAS* alleles as a collective group were a negative predictive factor for both PFS and OS in panitumumab-containing therapies.

Response rates with 95% CIs were plotted for patients whose tumors harbored wt *KRAS*, any mutant *KRAS* codon 12 and 13 allele, or any indicated individual mutant *KRAS* allele (Appendix Fig A3, online only). In studies 20050203 and 20050181, objective responses for patients with mutant *KRAS* mCRC included only partial responses, and no complete responses were observed.[6,7] Response rates were similar across all mutant *KRAS* allele subgroups within each of the first- and second-line mCRC trials; 95% CIs for response rates of all individual mutant *KRAS* allele subgroups overlapped with each other, indicating no predictive effects of individual mutant *KRAS* codon 12 and 13 alleles on response rates. In study 20020408, no patient whose tumor harbored a mutant *KRAS* codon 12 or 13 allele responded to panitumumab therapy. As a collective group, mutant *KRAS* alleles were a negative predictive factor for response rate in the panitumumab-containing arms of all three trials.

Pooled Analysis of the Predictive Effect on PFS and OS by KRAS *Alleles Across Panitumumab Studies*

Pooled analysis of the three phase III trials was performed to increase sample size and to detect any significant trends that were not detectable in the individual studies. The analysis used individual patient-level data stratified by study. HRs and 95% CIs for PFS and OS

Table 2. *P* Values Determined From Quantitative Interaction Testing Exploring the Interaction Between the Specified Mutant *KRAS* Allele and Therapy on Either OS or PFS

KRAS Allele	Study 20050203		Study 20050181		Study 20020408	
	OS	PFS	OS	PFS	OS	PFS
G12D	.9870	.8692	.7351	.3658	.42	.41
G12V	.0369*	.4229	.2449	.7023	.48	.56
G13D	.0018*	.1609	.0665	.4736	.37	.90
G12C	.3005	.0590	.8457	.6291	N/D†	N/D†
G12A	.3362	.3279	.0974	.6547	N/D†	N/D†
G12S	.2866	.9641	.4437	.5878	N/D†	N/D†

Abbreviations: N/D, not determined; OS, overall survival; PFS, progression-free survival.
*Quantitative interaction tests with $P < .05$.
†Not performed because of limiting number of patients in these *KRAS* allele subgroups.

were plotted for 1,053 patients whose tumors harbored one of the *KRAS* codon 12 or 13 alleles pooled from studies 20050203, 20050181, and 20020408 (Fig 3). Only a single mutant *KRAS* allele, G12A, emerged as a predictive factor and was associated with a negative panitumumab treatment effect on OS but not on PFS. The earlier noted impacts of mutant *KRAS* G12V and *KRAS* G13D alleles on patient outcomes were no longer observed in the pooled analysis.

DISCUSSION

Preclinical studies have suggested that individual *KRAS* codon 12 or 13 alleles have displayed quantitative and/or qualitative differences in transforming capacity and other biologic phenotypes. Specifically, *KRAS* codon 12 mutations have displayed greater in vitro transforming ability when compared with *KRAS* codon 13 mutations,[20-22] and individual mutant *KRAS* codon 12 alleles have had a differential impact on cellular transformation.[23] Furthermore, the signaling networks activated downstream of individual mutant *KRAS* alleles have varied significantly.[22,23] Despite these intrinsic biologic differences observed in defined experimental systems, the differential prognostic or predictive impact of individual mutant *KRAS* codon 12 or 13 alleles in a genetically complex and heterogeneous disease such as mCRC have remained untested by a systematic approach.

This study is the largest retrospective analysis evaluating the seven most common mutations in *KRAS* codons 12 and 13 for prognostic and predictive impact in patients with mCRC receiving an anti-EGFR therapy. Enrollment was completed in trials 20050203, 20050181, and 20020408 before *KRAS* was established as a predictive marker for outcomes in patients with mCRC. *KRAS* allele status was ascertained in more than 90% of the patients in each of the three phase III trials. A total of 1,053 patients were included in this analysis from these three open-label, multicenter, randomized, controlled phase III trials. The frequency and distribution of mutant *KRAS* codon 12 and 13 alleles were conserved across the trials, equally balanced between the treatment and control arms, and consistent with public domain data and prior publications.[15-19] Baseline demographics and clinical features were also balanced by treatment arm and comparable between all mutant *KRAS* allelic subgroups in trials 20050203 and 20050181.

Analysis of mutant *KRAS* codon 12 and 13 alleles on prognosis in the control arms of the three phase III trials suggested a trend toward a negative prognostic factor for *KRAS* G12C on PFS for patients receiving FOLFOX4 and on OS for patients receiving BSC. A trend as a negative prognostic factor was also observed for *KRAS* G12A on OS for patients receiving BSC. However, no single mutant *KRAS* allele was a consistent prognostic factor on both PFS and OS or across lines of mCRC therapy.

The prognostic significance of *KRAS* mutations has been assessed in a multitude of studies, with conflicting results.[24] Several studies have suggested that *KRAS* mutations are a negative prognostic indicator in CRC.[25] When considering the five largest studies, a prognostic impact was reported by four of these studies.[26] The RASCAL II meta-analysis indicated that *KRAS* G12V in Duke's C CRC patients was associated with a significant reduction in disease-free survival and OS.[17,27] Samowitz et al[28] performed the first population-based study on *KRAS* mutations in CRC, and results suggested that mutations in *KRAS* codon 13 were associated with poor OS. In addition, De Roock et al[12] recently reported that *KRAS* G13D mCRC tumors had worse OS compared with wt *KRAS* tumors and compared with tumors

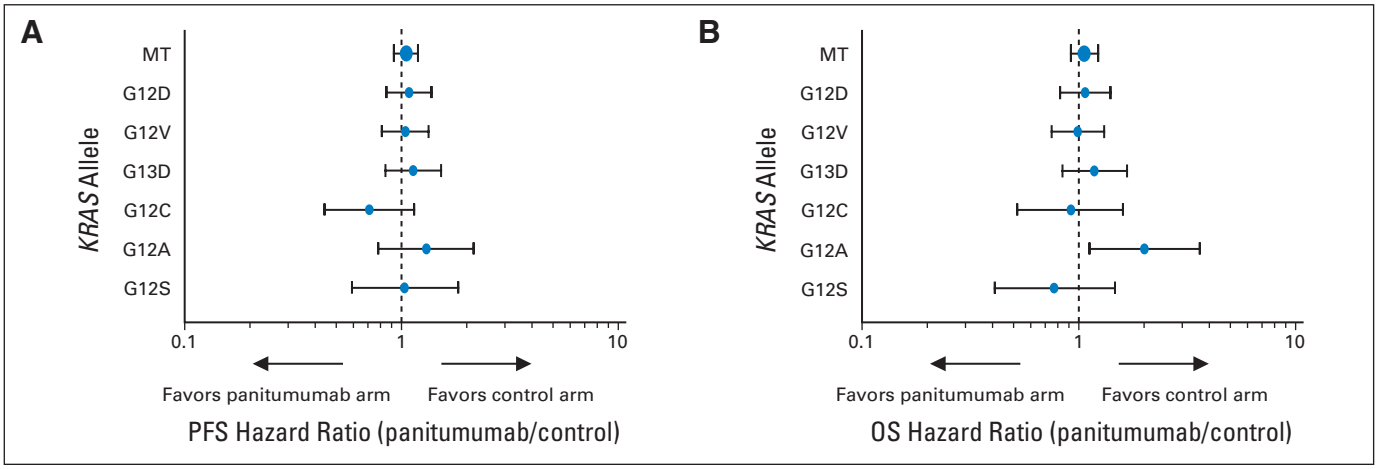

Fig 3. Pooled analysis of studies 20050203, 20050181, and 20020408: Predictive impact of mutant (MT) *KRAS* codon 12 and 13 alleles on (A) progression-free survival (PFS) and (B) overall survival (OS) in patients receiving either control (non–panitumumab-containing) or panitumumab-containing therapy. Point estimates for hazard ratios and their corresponding 95% CIs are plotted for the indicated mutant *KRAS* codon 12 and 13 alleles and are compared with the other mutant *KRAS* codon 12 and 13 alleles as a group.

bearing other *KRAS* mutations, although significance was lost in multivariate analysis.[12]

In contrast, a large number of reported studies (reviewed in Klump et al[26]) have found no association of *KRAS* gene mutations with survival, either in isolation or in combination with other tumor suppressor genes. Interpretation of the various studies published on the prognostic role of *KRAS* in mCRC may be challenging because of the different *KRAS* mutations investigated and variations in data collection, staging techniques, and mutant *KRAS* detection methodologies. In patients with mCRC treated with an anti-EGFR monoclonal therapy, it has been suggested that *KRAS* mutations are unlikely to be prognostic (independent of any specific treatment) and are likely predictive (attributable to treatment).[29]

In the analysis reported here, there was no consistent evidence that any individual mutant *KRAS* allele, compared with the remaining mutant *KRAS* alleles or the entire mutant *KRAS* group, had a differential impact on response rate, PFS, or OS. Only in the first-line FOLFOX4 treatment setting of study 20050203 were statistically significant differences observed for individual mutant *KRAS* alleles: *KRAS* G12V was favorably and *KRAS* G13D was unfavorably associated with panitumumab treatment effects on OS but not on PFS or response rate. Because associations with OS were observed only in the FOLFOX4 treatment setting and because other *KRAS* mutations have been associated with platinum sensitivity,[30] it is possible that selected mutant *KRAS* alleles may have a differential impact on patient outcomes in the specific context of coadministration with oxaliplatin-containing chemotherapy.

High *KRAS* ascertainment rates and consistent *KRAS* testing methodology permitted the pooling of data from all three phase III trials to potentially identify predictive trends across three lines of therapy that may not have been observed from analysis of any single trial. Pooled analyses indicated that no individual mutant *KRAS* codon 12 and 13 allele was associated with outcomes for both PFS and OS, relative to other *KRAS* mutations. A trend was observed for *KRAS* G12A, which was associated with a negative panitumumab predictive effect only on OS. *KRAS* alleles G12V and G13D were no longer associated with outcomes in the pooled analysis, suggesting there were no predictive trends across lines of therapy.

These results are in contrast with reported cetuximab data,[12,13] which have suggested patients with *KRAS* G13D responded to an anti-EGFR monoclonal antibody therapy. However, this improved survival in patients with *KRAS* G13D was not significant in the cetuximab monotherapy arm, and therefore the confounding effect of the chemotherapy backbone cannot be excluded.[12] In addition, a recent retrospective analysis of 110 patients treated with cetuximab[31] reported that patients whose tumor harbored a *KRAS* G13D allele did not benefit from cetuximab treatment (n = 12) and had a trend toward lower OS compared with patients whose tumors harbored either wt *KRAS* or one of the other *KRAS* mutations. Although panitumumab and cetuximab recognize similar epitopes,[32] they are of different antibody isotypes and may have different abilities to bind to EGFR mutations.[33] It is unclear whether these or other characteristics may have contributed to the conflicting results reported between these antibodies.

The cetuximab studies[12,13,31] and the analysis reported here were limited by their retrospective nature, they used subset analysis, and were subject to chance observations. None of the studies made adjustments for multiple testing. Other possible limitations were the low frequency and low number of patients in the specific mutant *KRAS* allelic subgroups, such as in the De Roock et al study[12] which had a total of 32 patients in the *KRAS* G13D subgroup in pooled analyses of patients in cetuximab monotherapy (n = 10) and cetuximab plus chemotherapy studies (n = 22).

On the basis of all of the available data and consistent with current clinical treatment guidelines, we suggest that patients with mCRC tumors that harbor any of the most common mutant *KRAS* codon 12 or 13 alleles are unlikely to benefit from panitumumab therapy. Therefore, only mCRC patients with wt *KRAS* tumors should be treated with panitumumab therapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Provision of study materials or patients: Eric Van Cutsem
Collection and assembly of data: Kathy Zhang, Richard Williams
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors

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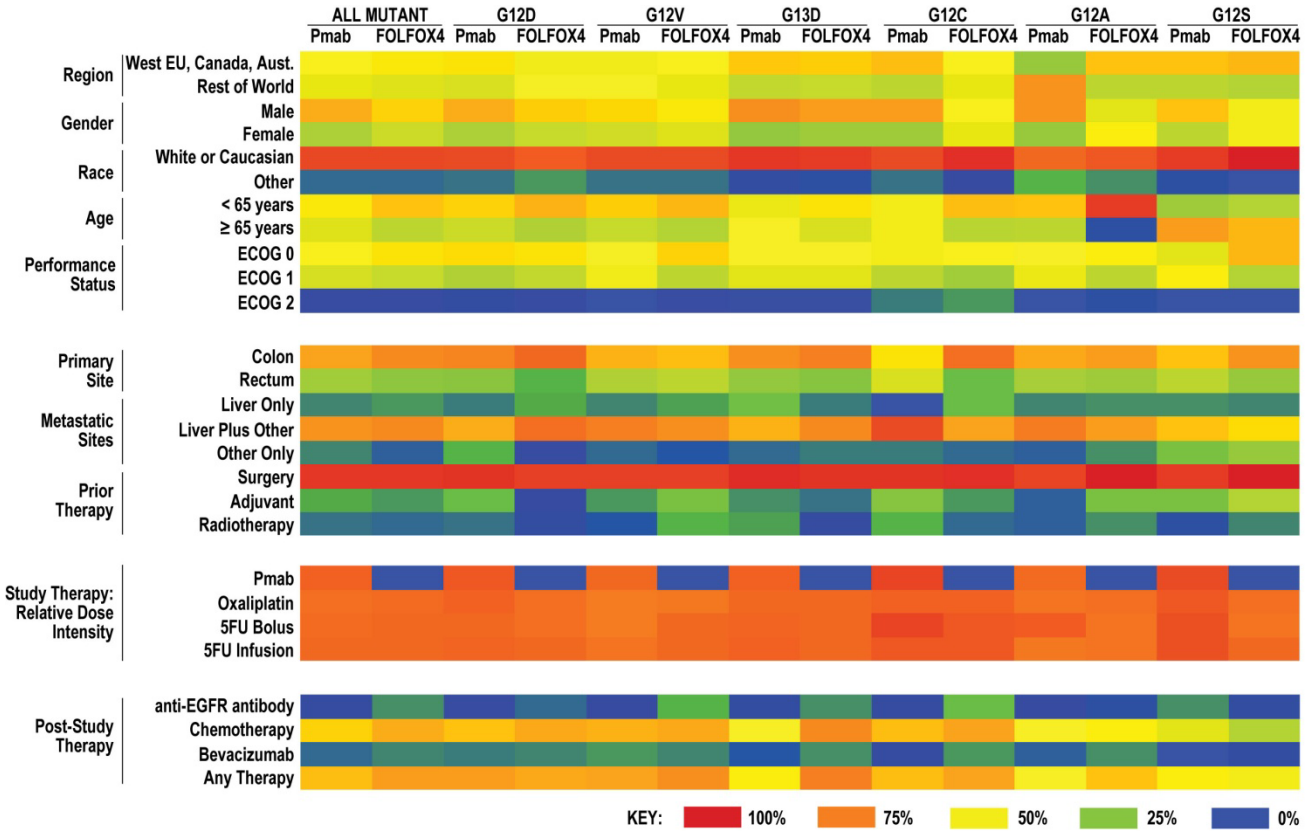
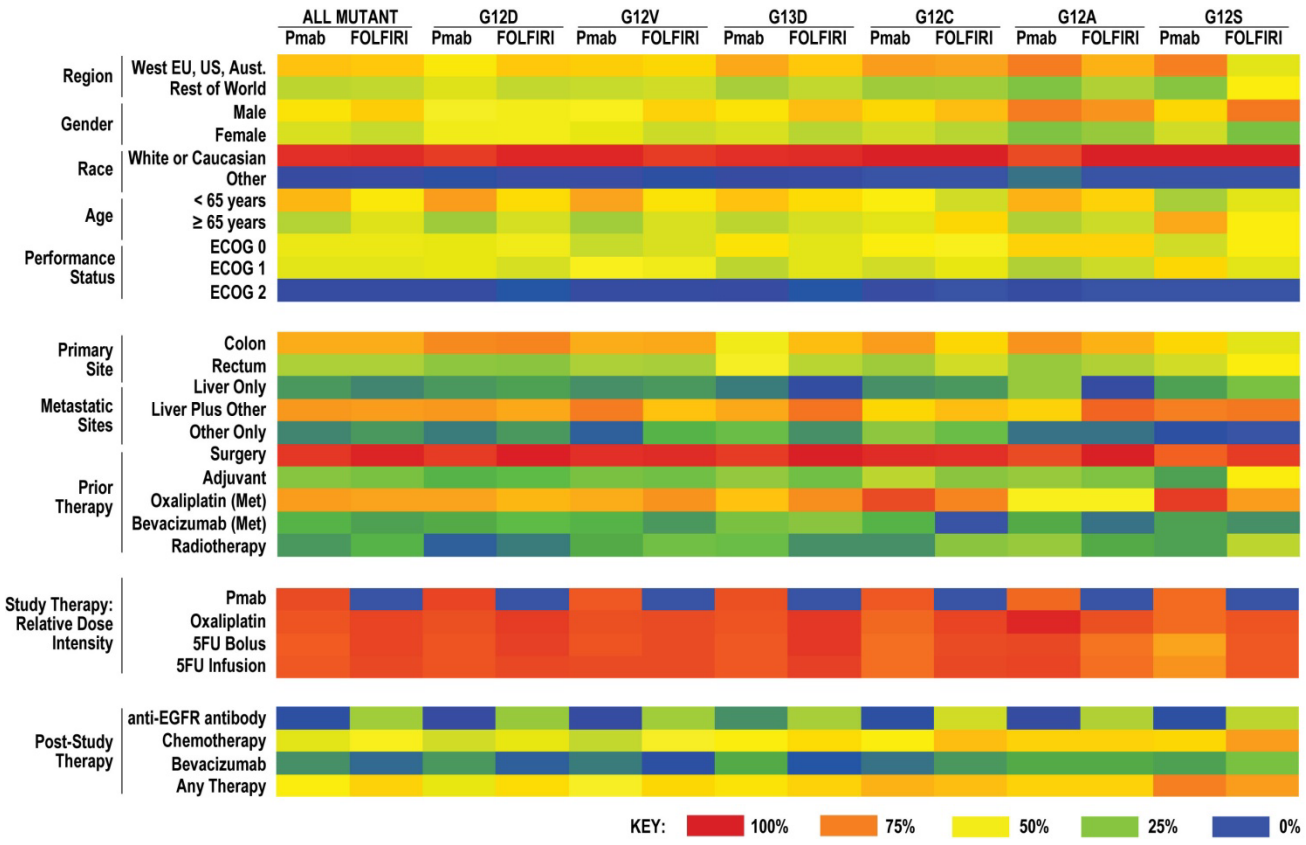
Supplementary Fig 1.



Supplementary Fig 2.



Supplementary Fig 3.