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KRAS-mutation status in relation to colorectal cancer survival: the joint impact of correlated tumour markers

A I Phipps*[,1], D D Buchanan[2], K W Makar[1], A K Win[3], J A Baron[4], N M Lindor[5], J D Potter[1,6] and P A Newcomb[1]

[1]*Public Health Sciences Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N., Seattle, WA 98109, USA;* [2]*Cancer and Population Studies Group, Queensland Institute for Medical Research, 300 Herston Road, Herston, Queensland 4006, Australia;* [3]*Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, The University of Melbourne, 207 Bouverie Street, Parkville, Victoria 3010, Australia;* [4]*Department of Medicine, University of North Carolina, 130 Mason Farm Road, Chapel Hill, NC 27599, USA;* [5]*Department of Laboratory Medicine and Pathology, Mayo Clinic, 13400 E. Shea Boulevard, Scottsdale, AZ 85259, USA and* [6]*Centre for Public Health Research, Massey University, Wellington, New Zealand*

Background: Mutations in the Kirsten Ras (*KRAS*) oncogene are common in colorectal cancer (CRC). The role of *KRAS*-mutation status as a prognostic factor, however, is unclear. We evaluated the relationship between *KRAS*-mutation status and CRC survival, considering heterogeneity in this association by tumour and patient characteristics.

Methods: The population-based study included individuals diagnosed with CRC between 1998–2007 in Western Washington State. Tumour specimens were tested for *KRAS* exon 2 mutations, the *BRAF* p.V600E mutation, and microsatellite instability (MSI). We used Cox regression to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association between *KRAS*-mutation status and disease-specific and overall survival. Stratified analyses were conducted by age, sex, tumour site, stage, and MSI. We conducted additional analyses combining *KRAS*-mutation, *BRAF*-mutation, and MSI status.

Results: Among 1989 cases, 31% had *KRAS*-mutated CRC. Kirsten Ras (*KRAS*)-mutated CRC was associated with poorer disease-specific survival (HR = 1.37, 95% CI: 1.13–1.66). This association was not evident in cases who presented with distant-stage CRC. Cases with *KRAS*-wild-type/*BRAF*-wild-type/MSI-high CRC had the most favourable prognosis; those with CRC exhibiting a *KRAS*- or *BRAF*-mutation and no MSI had the poorest prognosis. Patterns were similar for overall survival.

Conclusion: Kirsten Ras (*KRAS*)-mutated CRC was associated with statistically significantly poorer survival after diagnosis than *KRAS*-wild-type CRC.

The Kirsten Ras (*KRAS*) proto-oncogene encodes for a guanosine triphosphate (GTP)/guanosine diphosphate binding protein downstream of the epidermal growth factor receptor (EGFR) in the RAS/RAF/MAPK pathway. Mutations in *KRAS* are evident in 30–40% of colorectal tumours (Andreyev *et al*, 1998; Samowitz *et al*, 2000; Gnanasampanthan *et al*, 2001; Wang *et al*, 2003; Lee *et al*, 2008; De Roock *et al*, 2010a, 2010b; Nash *et al*, 2010; Roth *et al*, 2010; Hutchins *et al*, 2011; Imamura *et al*, 2012; Inoue *et al*, 2012). Based on evidence that the benefits of adjuvant treatment with anti-EGFR chemotherapy for distant-stage metastatic colorectal cancer (CRC) are limited to patients with *KRAS*-wild-type disease (Lin *et al*, 2011; Bokemeyer *et al*, 2012), testing for *KRAS* mutations is increasingly common in clinical practice in order to better direct treatment of CRC (Allegra *et al*, 2009). Although the role of *KRAS*-mutation status as a predictive biomarker for response to anti-EGFR-targeted therapy is well supported, the role of *KRAS* as a

*Correspondence: Dr AI Phipps; E-mail: aphipps@fhcrc.org

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prognostic biomarker for CRC survival, independent of anti-EGFR therapy, is less clear.

A number of previous studies have evaluated the relationship between *KRAS*-mutation status and survival after CRC diagnosis (Andreyev *et al*, 1998; Samowitz *et al*, 2000; Andreyev *et al*, 2001; Gnanasampanthan *et al*, 2001; Wang *et al*, 2003; Lee *et al*, 2008; Ogino *et al*, 2009a; Nash *et al*, 2010; Roth *et al*, 2010; De Roock *et al*, 2010a; Hutchins *et al*, 2011; Imamura *et al*, 2012). In the largest study to date, Andreyev *et al* (2001) reported that the presence of a somatic *KRAS* mutation was associated with statistically significantly poorer disease-free and overall survival after CRC diagnosis, but only among patients with Dukes' C CRC and only among those with the *KRAS* p.G12V mutation (Andreyev *et al*, 2001). This latter finding was supported by results from a recent study in which *KRAS* codon 12 mutations, particularly the p.G12V mutation, but not *KRAS* codon 13 mutations were associated with poorer survival (Imamura *et al*, 2012). Thus, observed inconsistencies in the literature regarding the association between *KRAS*-mutation status and CRC survival may be related to differences in the distribution of specific *KRAS* mutations, stage at diagnosis, or other characteristics.

Correlations between *KRAS*-mutation status and other tumour characteristics of prognostic relevance may further complicate the study of this marker in relation to prognosis. In particular, *KRAS*-mutated CRC is less likely to exhibit microsatellite instability (MSI) than *KRAS*-wild-type CRCs (Ogino *et al*, 2009a; Nash *et al*, 2010; Roth *et al*, 2010; Hutchins *et al*, 2011; Imamura *et al*, 2012) and is almost never *BRAF*-mutated (Lee *et al*, 2008; De Roock *et al*, 2010a; Hutchins *et al*, 2011; Imamura *et al*, 2012). The presence of high MSI (MSI-H) is associated with a more favourable prognosis (Guastadisegni *et al*, 2010), whereas *BRAF*-mutated CRC has a poorer prognosis than *BRAF*-wild-type disease (Ogino *et al*, 2009b; Roth *et al*, 2010; De Roock *et al*, 2010a). Failure to account for these attributes of *KRAS*-mutated CRC could thus obscure an association between *KRAS*-mutation status and CRC survival.

To better understand the relationship between *KRAS*-mutation status and survival after CRC diagnosis, we used data from two concurrent population-based studies of incident invasive CRC conducted in Western Washington State.

MATERIALS AND METHODS

Study population. Details of the population-based study samples have been published elsewhere (Newcomb *et al*, 2007a, b). Briefly, eligible participants included men and women diagnosed with invasive CRC between January 1998 and June 2002 who, at the time of diagnosis, were aged 20–74 years and resided in King, Pierce, or Snohomish counties in Western Washington State. Women who resided in 10 additional Washington counties and were diagnosed during the same time period at ages 50–74 years were also eligible. During a second phase of study recruitment, we identified eligible participants as men and women with invasive CRC in this 13-county ascertainment area who were diagnosed at ages 18–49 years between April 2002 and July 2007. All cases were identified through the Surveillance, Epidemiology, and End Results (SEER) cancer registry serving Western Washington State. Study eligibility was limited to English speakers with a publicly available telephone number. Of 3585 individuals contacted and identified as eligible, 463 (13%) were deceased, 351 (10%) refused participation, 128 (4%) could not be reached, and 24 (0.7%) completed only a partial interview. In total, 76% of eligible cases were enrolled in the study ($N=2708$).

At an average of 8.6 months after diagnosis, participants completed a structured telephone interview in which they were asked to provide detailed information on a number of potential risk factors, including smoking history, body mass index (BMI), family history of CRC, and use of selected medications. At the conclusion of the interview, participants were asked for consent to access diagnostic tumour specimens. Adequate tumour specimens were obtained for 78% of enrolled participants ($N=2120$).

This study was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center in accordance with assurances filed with and approved by the US Department of Health and Human Services.

***KRAS*-mutation testing and additional tumour characterisation.** DNA was extracted from paraffin-embedded formalin-fixed tumour tissue. In cases for whom tumour DNA was successfully extracted ($N=1989$), the coding sequence of *KRAS* exon 2 was amplified (Oliner *et al*, 2010). Mutations in exon 2 were identified via forward and reverse sequencing of amplified tumour DNA (Alsop *et al*, 2006). Cases for whom *KRAS* testing failed ($N=36$) or produced equivocal results ($N=30$) were classified as having unknown *KRAS*-mutation status. For quality control purposes, sequencing was also conducted on three cell-line controls (one containing the p.G12V mutation, one containing the p.G13D mutation, and one wild-type cell line).

Tumour specimens were also assayed for *BRAF*-mutation status and for the presence of MSI. Tumour DNA was tested for the c.1799 T>A (p.V600E) *BRAF* mutation using a fluorescent allele-specific PCR assay as described previously (Buchanan *et al*, 2010). With respect to MSI status, testing for cases enrolled in earlier years of recruitment ($N=1430$) was based on a 10-gene panel assayed in tumour DNA and in DNA extracted from normal surrounding tissue (BAT25, BAT26, BAT40, MYCL, D5S346, D17S250, ACTC, D18S55, D10S197, and BAT34C4) (Boland *et al*, 1998; Newcomb *et al*, 2007b); tumours were classified as MSI-H if instability was observed in $\geqslant$30% of markers, and as MSS if instability was observed in $<$30% of markers. For more recently enrolled cases ($N=470$), MSI status was based on immunohistochemistry (IHC) testing of four markers: MLH1, MSH2, MSH6, and PMS2 (Lindor *et al*, 2002; Shia, 2008); cases whose tumour tissue exhibited positive staining for all markers were considered MSS, whereas cases negative for at least one marker were considered MSI-H. High concordance between IHC and PCR-based MSI testing has been demonstrated elsewhere (Cicek *et al*, 2011). Cases for whom test results were equivocal or for whom testing was not completed ($N=80$) were classified as having unknown MSI status.

Information on tumour site and stage at diagnosis was available from SEER. Tumours located in the caecum through the splenic flexure were grouped together as proximal colon cancers (ICD-O-3 codes C180, C182, C183, C184, and C185) (World Health Organisation, 2000). Tumours located in the descending (C186) and sigmoid colon (C187) were classified as distal colon cancer, and tumours in the rectosigmoid junction (C199) and rectum (C209) were grouped together as rectal cancer. Stage at diagnosis was recorded according to SEER summary staging conventions (localised-, regional-, distant-stage).

Survival information. Vital status was determined via linkage to SEER and the National Death Index. For cases who died during study follow-up, information was obtained on the date and cause of death, classified according to ICD-10 conventions (World Health Organisation, 2007). Deaths with an underlying cause attributed to ICD-10 codes C18.0-C20.0 or C26.0 (that is, CRC) were classified as disease-specific mortality events. Vital-status linkage was performed periodically, with the most recent linkage capturing deaths occurring through September 2010.

Statistical analysis. We used Cox proportional hazards regression to evaluate the association between *KRAS*-mutation status and survival after CRC diagnosis. The time axis for analysis was defined

as days since diagnosis, with left censoring of participants until the date of study enrollment. We conducted separate survival analyses for disease-specific survival and overall survival. In all analyses, participants still alive at their last vital-status assessment were censored at that date. In analyses of disease-specific survival, we also censored persons who died due to causes other than CRC at the time of death. We evaluated associations between *KRAS*-mutation status and survival outcomes in the full cohort and within strata defined by patient characteristics (age at diagnosis, sex) and tumour characteristics (tumour site, stage, MSI status). In light of the fact that somatic mutations in *KRAS* and *BRAF* rarely co-occur (Davies *et al*, 2002), and given that *BRAF*-mutated CRC has been shown to have a poorer prognosis than *BRAF*-wild-type CRC (Ogino *et al*, 2009b; Roth *et al*, 2010; De Roock *et al*, 2010a), we conducted separate analyses: (1) in all cases irrespective of *BRAF*-mutation status; (2) restricted to *BRAF*-wild-type cases; and (3) combining information on *KRAS* and *BRAF* mutations to evaluate relative differences in survival for cases with a mutation in either *vs* neither gene. We also evaluated relative differences in survival between case groups defined by joint *KRAS*/MSI status, and by joint *KRAS*/*BRAF*/MSI status. Finally, we explored associations between different classes of *KRAS* mutations and survival outcomes, examining associations with specific mutations evident in $\geqslant 5\%$ of cases, and, more generally, with codon 12 mutations and codon 13 mutations separately; differences in codon-specific associations were evaluated via tests for heterogeneity. For all analyses, proportional hazards assumptions were assessed by testing for a non-zero slope of the scaled Schoenfeld residuals on ranked failure times (Therneau and Grambsch, 2000).

Regression models included adjustment terms for age (5-year categories), sex, and study phase. We also assessed potential confounding by several patient and tumour characteristics: cigarette smoking (never, former, current); BMI 2 years before diagnosis (<25.0, 25.0–29.9, $\geqslant 30.0\,kg\,m^{-2}$); race (white, non-white); regular use of non-steroidal anti-inflammatory drugs at baseline (no, yes); family history of CRC in first-degree relatives (no, yes); and tumour site (proximal colon, distal colon/rectum). Of these additional factors, only cigarette smoking and BMI were retained in our final analytic model as adjustment for other variables had minimal impact on effect estimates ($<5\%$ change).

We conducted sensitivity analyses using alternative approaches to assess the potential impact of excluding enrolled cases with unknown *KRAS*-mutation status. Specifically, we replicated analyses: (1) including all cases with missing *KRAS*-mutation status as *KRAS* wild-type; (2) including cases with missing *KRAS*-mutation status as *KRAS*-mutated; and (3) using multiple imputation for missing *KRAS* status. The multiple imputation model was based on all covariate variables from the multivariate model, as well as family history of CRC, tumour site, MSI status, *BRAF*-mutation status, race, survival time, and the survival outcome of interest (Moons *et al*, 2006; Sterne *et al*, 2009). All analyses were conducted in STATA SE version 12.0 (College Park, TX, USA).

RESULTS

Characteristics of the study population are presented in Table 1 by *KRAS*-mutation status. Approximately 31% of cases had *KRAS*-mutated CRC. Compared with cases with *KRAS*-wild-type CRC, cases with *KRAS*-mutated disease were statistically significantly less likely to have MSI-H or *BRAF*-mutated CRC (P-value <0.001). There was no statistically significant difference in the distribution of age at diagnosis, sex, tumour site, or stage according to *KRAS*-mutation status. Overall, 38% ($N=728$) of cases died during the study follow-up period (mean = 6.5 years, range = 5.3 months to 13.7 years). Of those cases who died, ~62% ($N=449$) died because of CRC.

Table 1. Study population characteristics by *KRAS*-mutation status

	***KRAS* wild-type (*N* = 1330)**	***KRAS*-mutated (*N* = 593)**	***P*-value***
Age at diagnosis			
<50	346 (26)	147 (25)	0.65
50–59	291 (22)	143 (24)	
60–69	415 (31)	188 (32)	
70–74	278 (21)	115 (19)	
Sex			
Male	609 (46)	264 (45)	0.61
Female	721 (54)	329 (55)	
Tumour site			
Proximal colon	505 (39)	255 (44)	0.10
Distal colon	364 (28)	147 (25)	
Rectal	424 (33)	183 (31)	
Unknown	37	89	
Stage at diagnosis[a]			
Localised	553 (42)	220 (37)	0.12
Regional	610 (46)	293 (50)	
Distant	144 (11)	75 (13)	
Unknown	23	5	
MSI status[a]			
MSS/MSI-L	1042 (82)	509 (90)	<0.001
MSI-H	236 (18)	56 (10)	
Unknown	52	28	
***BRAF* mutation status[a]**			
Wild-type	1083 (82)	580 (99)	<0.001
Mutated	232 (18)	6 (1)	
Unknown	15	7	
Vital status			
Alive	843 (63)	352 (59)	0.09
Deceased	487 (37)	241 (41)	
Mean years of follow-up (s.d.)	6.7 (3.9)	6.3 (4.1)	

Abbreviations: KRAS = Kirsten Ras; MSI = microsatellite instability; MSI-H = high microsatellite instability. *P-value for χ^2.
[a]% distribution excludes cases with unknown value of characteristic.

Multivariate-adjusted analyses of disease-specific survival yielded estimates nearly identical to those from unadjusted analyses, and provided evidence of statistically significantly poorer survival in cases with *KRAS*-mutated *vs* *KRAS*-wild-type CRC (Table 2) (hazard ratio (HR) = 1.37, 95% confidence interval (CI): 1.13–1.66). The magnitude of this association was similar when cases with *BRAF*-mutated disease were excluded or combined with the *KRAS*-mutated case group. Interaction terms by age at diagnosis, sex, tumour site, stage, and MSI status were not statistically significant ($P>0.05$); however, point estimates did vary slightly by stage and age at diagnosis. In particular, *KRAS*-mutation status was not associated with survival in cases who presented with distant-stage disease (P-interaction by stage = 0.07). Additionally, *KRAS*-mutated CRC was associated with statistically significantly poorer disease-specific survival in cases aged $\geqslant 50$ years at diagnosis but not in those aged <50 (P-interaction by age = 0.15).

Table 2. *KRAS*-mutation status and disease-specific survival after colorectal cancer diagnosis by patient and tumour characteristics, with and without consideration of *BRAF*-mutation status

	All cases			*BRAF*-wild-type CRC only			Joint *KRAS/BRAF* mutation status		
	***KRAS*-wild-type deaths/cases**	***KRAS*-mutated deaths/cases**	**HR (95% CI)[a]**	***KRAS*-wild-type deaths/cases**	***KRAS*-mutated deaths/cases**	**HR (95% CI)[a]**	***KRAS*- and *BRAF*-wt deaths/cases**	***KRAS*- or *BRAF*-mut deaths/cases**	**HR (95% CI)[a]**
Overall (unadjusted)	287/1330	162/593	1.36 (1.12–1.65)	238/1098	161/587	1.39 (1.14–1.70)	234/1083	214/834	1.31 (1.09–1.58)
Overall (adjusted)	287/1330	162/593	1.37 (1.13–1.66)	238/1098	161/587	1.40 (1.14–1.72)	234/1083	214/834	1.34 (1.11–1.63)
By age at diagnosis									
<50 years	79/346	34/147	1.03 (0.69–1.54)	67/320	34/147	1.14 (0.76–1.73)	65/317	48/176	1.40 (0.96–2.03)
⩾50 years	208/984	128/446	1.48 (1.18–1.85)	171/778	127/440	1.49 (1.18–1.88)	169/766	166/658	1.33 (1.07–1.66)
By sex									
Male	134/609	74/264	1.35 (1.02–1.79)	119/545	73/261	1.37 (1.03–1.85)	118/542	89/329	1.36 (1.03–1.79)
Female	153/721	88/329	1.38 (1.06–1.81)	119/553	88/326	1.43 (1.07–1.89)	116/541	125/505	1.35 (1.04–1.75)
By tumour site									
Proximal	104/505	72/255	1.44 (1.06–1.95)	68/322	71/252	1.45 (1.04–2.04)	67/315	109/444	1.25 (0.92–1.71)
Distal/rectal	178/788	87/330	1.29 (1.00–1.68)	166/745	87/327	1.33 (1.02–1.73)	163/737	101/376	1.35 (1.04–1.73)
By stage at diagnosis									
Localised	29/553	19/220	1.55 (0.85–2.82)	28/463	19/216	1.38 (0.75–2.54)	28/458	20/311	1.06 (0.58–1.93)
Regional	146/610	83/293	1.35 (1.03–1.78)	111/487	82/291	1.47 (1.10–1.96)	109/480	121/424	1.50 (1.15–1.96)
Distant	111/144	59/75	1.02 (0.73–1.41)	98/129	59/75	1.06 (0.76–1.49)	96/126	72/90	1.11 (0.80–1.53)
By MSI									
MSS	257/1042	143/509	1.24 (1.00–1.52)	221/943	142/504	1.33 (1.07–1.65)	217/933	180/613	1.40 (1.15–1.72)
MSI-H	22/236	11/56	2.06 (0.93–4.52)	10/115	11/55	2.17 (0.89–5.31)	10/112	25/181	1.67 (0.77–3.61)

Abbreviations: CI = confidence interval; CRC = colorectal cancer; HR = hazard ratio; KRAS = Kirsten Ras; MSI = microsatellite instability; MSI-H = high microsatellite instability. All associations are relative to the *KRAS* wild-type case group. All *P*-values for tests of interaction across strata indicate a lack of statistically significant interaction ($P > 0.05$).
[a]Adjusted for age at diagnosis, sex, study population, body mass index, and history of cigarette smoking.

These non-significant differences in the strength of association across stage and age strata were diminished in analyses combining cases with *BRAF*-mutated and *KRAS*-mutated CRC. Associations were similar but attenuated in analyses of overall survival (Table 3).

In analyses considering *KRAS* in combination with MSI status (Table 4), disease-specific and overall survival were statistically significantly more favourable in cases with *KRAS*-wild-type/MSI-H CRC (HR = 0.35, 95% CI: 0.23–0.55, and HR = 0.78, 95% CI: 0.60–1.00, respectively) and statistically significantly poorer in cases with *KRAS*-mutated/MSS CRC (HR = 1.24, 95% CI: 1.01–1.52, and HR = 1.21, 95% CI: 1.02–1.43, respectively) compared with cases with *KRAS*-wild-type/MSS disease. Results were similar after excluding cases with *BRAF*-mutated CRC. Patterns of association also changed very little when combining cases with *KRAS*- and/or *BRAF*-mutated disease: cases with *KRAS*- and *BRAF*-wild-type/MSI-H disease had the most favourable prognosis, and those with *KRAS*- or *BRAF*-mutated/MSS disease had the poorest survival.

Among cases with *KRAS*-mutated CRC, 75% ($N = 444$) had a mutation in codon 12 and 22% ($N = 132$) in codon 13 (Supplementary Table 1). Compared with cases with a codon 12 *KRAS* mutation, those with a codon 13 mutation were statistically significantly more likely to have CRC located in the proximal colon (54% *vs* 40%) and to have MSI-H disease (19% *vs* 7%). We found no statistically significant differences in the association between *KRAS*-mutation status and survival when we evaluated associations with mutated codon 12 *vs* mutated codon 13 (*P*-heterogeneity = 0.54 and *P*-heterogeneity = 0.30 for disease-specific and overall survival, respectively). The presence of a somatic p.G13D mutation was associated with statistically significantly poorer disease-specific (HR = 1.48, 95% CI: 1.04–2.04) and overall survival (HR = 1.38, 95% CI: 1.05–1.81) compared with *KRAS*-wild-type; neither p.G12D nor p.G12V mutations were significantly associated with survival outcomes when evaluated separately (Supplementary Table 2).

Compared with cases with known *KRAS*-mutation status, enrolled cases with unknown *KRAS* status were younger at diagnosis (median age = 52 years *vs* 60 years), more likely to have distant-stage disease (20% *vs* 12%), and had a lower 5-year overall survival (65% *vs* 74%) (not shown). In sensitivity analyses, we evaluated the effect of missing information on *KRAS* status ($N = 728$, 29%). In analyses based on our primary analytic model with no exclusion of *BRAF*-mutated cases, including all cases with unknown *KRAS*-mutation status as *KRAS*-mutated cases increased point estimates to HR = 1.53 (95% CI: 1.13–1.79) for disease-specific survival and HR = 1.39 (95% CI: 1.23–1.57) for overall survival. When we instead included these 728 cases as *KRAS*-wild-type, point estimates fell to HR = 1.12 (95% CI: 0.94–1.34) and HR = 1.06 (95% CI: 0.92–1.23) for disease-specific and overall survival, respectively. Thus, our point estimates comparing survival in *KRAS*-mutated *vs* *KRAS*-wild-type cases are subject to some uncertainty due to the exclusion of cases with missing *KRAS* data.

Table 3. *KRAS*-mutation status and overall survival after colorectal cancer diagnosis by patient and tumour characteristics, with and without consideration of *BRAF*-mutation status

	All cases			*BRAF*-wild-type CRC only			Joint *KRAS*/ *BRAF* mutation status		
	KRAS-wild-type deaths/ cases	*KRAS*-mutated deaths/ cases	HR (95% CI)[a]	*KRAS*-wild-type deaths/ cases	*KRAS*-mutated deaths/ cases	HR (95% CI)[a]	*KRAS*- and *BRAF*-wt deaths/ cases	*KRAS*-or *BRAF*-mut deaths/ cases	HR (95% CI)[a]
Overall (unadjusted)	487/1330	241/593	1.22 (1.05–1.43)	391/1098	239/587	1.28 (1.09–1.51)	386/1083	341/834	1.27 (1.10–1.47)
Overall (adjusted)	487/1330	241/593	1.24 (1.06–1.45)	391/1098	239/587	1.27 (1.08–1.50)	386/1083	341/834	1.24 (1.07–1.44)
By age at diagnosis									
<50 years	98/346	41/147	1.00 (0.70–1.45)	86/320	41/147	1.07 (0.74–1.55)	84/317	55/176	1.22 (0.87–1.72)
⩾50 years	389/984	200/446	1.31 (1.10–1.56)	305/778	198/440	1.33 (1.11–1.60)	293/766	286/658	1.24 (1.05–1.47)
By sex									
Male	238/609	115/264	1.20 (0.96–1.50)	207/545	114/261	1.24 (0.98–1.56)	205/542	146/329	1.25 (1.00–1.55)
Female	249/721	126/329	1.30 (1.04–1.61)	184/553	125/326	1.32 (1.05–1.67)	181/541	195/505	1.25 (1.01–1.53)
By tumour site									
Proximal	195/505	109/255	1.21 (0.95–1.54)	121/322	107/252	1.24 (0.95–1.62)	120/315	185/444	1.13 (0.90–1.43)
Distal/rectal	276/788	128/330	1.22 (0.99–1.51)	258/745	128/327	1.23 (0.99–1.53)	254/737	148/376	1.21 (0.99–1.49)
By stage at diagnosis									
Localised	130/553	60/220	1.19 (0.87–1.63)	107/463	59/216	1.15 (0.83–1.59)	106/458	83/311	1.05 (0.78–1.42)
Regional	227/610	117/293	1.24 (0.99–1.55)	171/487	116/291	1.33 (1.05–1.69)	169/480	177/424	1.36 (1.09–1.68)
Distant	118/144	62/75	1.01 (0.73–1.38)	104/129	62/75	1.05 (0.75–1.45)	102/126	76/90	1.09 (0.80–1.49)
By MSI									
MSS	391/1042	213/509	1.21 (1.02–1.43)	344/943	212/504	1.26 (1.06–1.51)	340/933	261/613	1.28 (1.09–1.51)
MSI-H	79/236	17/56	1.20 (0.68–2.12)	32/115	16/55	1.20 (0.63–2.30)	32/112	67/181	1.05 (0.67–1.64)

Abbreviations: CI = confidence interval; CRC = colorectal cancer; HR = hazard ratio; KRAS = Kirsten Ras; MSI = microsatellite instability; MSI-H = high microsatellite instability; mut = mutated; wt = wild-type. All associations are relative to the *KRAS* wild-type case group. All *P*-values for tests of interaction across strata indicate a lack of statistically significant interaction ($P > 0.05$).
[a]Adjusted for age at diagnosis, sex, study population, body mass index, and history of cigarette smoking.

However, when we implemented a multiple imputation model to account for missingness in *KRAS*, our results based on the analysis of known and imputed *KRAS* data indicated statistically significantly poorer disease-specific (HR = 1.35, 95% CI: 1.12–1.63) and overall survival (HR = 1.22, 95% CI: 1.05–1.42) associated with the presence of a *KRAS* mutation.

DISCUSSION

In this large population-based cohort of men and women with incident invasive CRC, the presence of a somatic *KRAS* mutation was associated with statistically significantly poorer survival, specifically in those without distant-stage disease. Patients with *KRAS*-mutated CRC, whose tumours were also MSS, had the poorest prognosis. These patterns of association were relatively unchanged when limited to *BRAF*-wild-type cases and when grouping *BRAF*-mutated and *KRAS*-mutated cases. Contrary to some previous reports, we did not find the association between *KRAS*-mutation status and survival to be limited to the p.G12V *KRAS*-mutation specific identified mutations.

Activating mutations in *KRAS* are among the most common mutations in human cancers (Ikediobi *et al*, 2006). Mutations in *KRAS* codons 12 and 13 have been shown to result in an altered RAS protein with greater resistance to GTPase activity (Bollag and McCormick, 1995; Al-Mulla *et al*, 1999). By remaining in an active GTP-bound state for longer, mutated RAS contributes to enhanced cellular growth and proliferation (Al-Mulla *et al*, 1999), activating the RAS/RAF/MAPK and the phosphoinositide 3-kinase-AKT pathways. The relationship between constitutive activation of the RAS/RAF/MAPK signalling pathway and CRC prognosis has previously also been supported by studies evaluating the association between the *BRAF* p.V600E activating mutation and CRC survival (Ogino *et al*, 2009b; Roth *et al*, 2010; De Roock *et al*, 2010a). Mutations in *BRAF* and *KRAS* are both thought to occur early in colorectal carcinogenesis, and are rarely observed together. Here, we found that only 1% ($N = 6$) of CRC cases with a somatic *KRAS* mutation harboured a *BRAF* mutation, compared with 18% of *KRAS*-wild-type CRC cases; this is consistent with data from The Cancer Genome Atlas (Cerami *et al*, 2012) and recent reports from other large studies (Hutchins *et al*, 2011; Imamura *et al*, 2012). When we combined information on *KRAS* and *BRAF* status to compare survival in CRC cases with a somatic mutation in at least one *vs* neither of these genes, we found only modest differences from our analyses where *BRAF*-mutation status was not considered.

The presence of a somatic *KRAS* mutation is also inversely associated with the presence of MSI (Ogino *et al*, 2009a; Nash *et al*, 2010; Imamura *et al*, 2012). MSI-H CRC is known to have a statistically significantly more favourable prognosis than MSS CRC (Guastadisegni *et al*, 2010), and to have a distinct

Table 4. *KRAS*-mutation status, in combination with MSI and *BRAF*-mutation status, in relation to disease-specific and overall survival after colorectal cancer diagnosis

	Disease-specific survival		Overall survival	
	Deaths/cases	HR (95% CI)[a]	Deaths/cases	HR (95% CI)[a]
Joint *KRAS* and MSI status				
KRAS wt/MSI-H	22/236	0.35 (0.23–0.55)	79/236	0.78 (0.60–1.00)
KRAS mut/MSI-H	11/56	0.77 (0.42–1.41)	17/56	0.87 (0.53–1.42)
KRAS wt/MSS	257/1042	1.00 (ref)	391/1042	1.00 (ref)
KRAS mut/MSS	143/509	1.24 (1.01–1.52)	213/509	1.21 (1.02–1.43)
Joint *KRAS* and MSI status (*BRAF* wild-type only)				
KRAS wt/MSI-H	10/112	0.34 (0.18–0.65)	32/112	0.74 (0.51–1.07)
KRAS mut/MSI-H	11/53	0.87 (0.47–1.60)	16/53	0.92 (0.55–1.52)
KRAS wt/MSS	217/933	1.00 (ref)	340/933	1.00 (ref)
KRAS mut/MSS	141/501	1.36 (1.09–1.68)	210/501	1.27 (1.07–1.52)
Joint *KRAS*, *BRAF*, and MSI status				
KRAS and BRAF wt/MSI-H	10/112	0.34 (0.18–0.65)	32/112	0.75 (0.52–1.08)
KRAS or BRAF mut/MSI-H	25/181	0.60 (0.39–0.91)	67/181	0.91 (0.69–1.20)
KRAS and BRAF wt/MSS	217/933	1.00 (ref)	340/933	1.00 (ref)
KRAS or BRAF mut/MSS	180/613	1.41 (1.15–1.73)	261/613	1.28 (1.08–1.51)

Abbreviations: CI = confidence interval; HR = hazard ratio; KRAS = Kirsten Ras; MSI = microsatellite instability; MSI-H = high microsatellite instability; mut = mutated; wt = wild-type.
[a]Adjusted for age at diagnosis, sex, study population, body mass index, and history of cigarette smoking.

clinicopathology: the distribution of MSI follows a clear gradient of decreasing prevalence from the ascending colon to the rectum (Yamauchi *et al*, 2012) and is less prevalent in cases diagnosed at later stages (Ogino *et al*, 2009b; Nash *et al*, 2010). Although we found that the prevalence of MSI was statistically significantly lower in *KRAS*-mutated *vs* *KRAS*-wild-type cases, we found no difference in the distribution of tumour site or stage at diagnosis according to *KRAS* status. We also found no statistically significant interaction in the association between *KRAS*-mutation status and survival according to MSI status, tumour site, or stage at diagnosis. However, our results did suggest that *KRAS*-mutation status was not associated with survival in cases who presented with distant-stage disease, as has been suggested by at least two previous studies (Nash *et al*, 2010; Inoue *et al*, 2012). Thus, although the prevalence of somatic *KRAS* mutations does not appear to differ by stage at diagnosis, the prognostic role of *KRAS* may differ by stage.

Several studies in the distant-stage, metastatic setting have demonstrated the utility of *KRAS*-mutation status as a predictive marker for response to anti-EGFR therapy (Lin *et al*, 2011; Bokemeyer *et al*, 2012). In a recent meta-analysis, Lin *et al* (2011) reported that the presence of a *KRAS* mutation had a positive likelihood ratio of 2.0 (95% CI: 1.45–2.76) for predicting non-response to anti-EGFR in distant-stage CRC . However, the role of *KRAS* as a predictive marker has not been demonstrated for less advanced disease: recently published findings from a phase III randomized trial of patients with stage III colon cancer indicated no benefit in 3-year disease-free survival with the addition of cetuximab to standard chemotherapy, regardless of *KRAS*-mutation status (HR = 1.21, 95% CI: 0.98–1.49 in *KRAS*-wild-type and HR = 1.12, 95% CI: 0.86–1.46 in *KRAS* mutated) (Alberts *et al*, 2012). Results from that trial did, however, provide support for the role of *KRAS*-mutation status as a prognostic factor, independent of anti-EGFR therapy: 3-year disease-free survival ranged from 72–75% across treatment arms in participants with *KRAS*-wild-type disease *vs* 65–67% in participants with *KRAS*-mutated disease (Alberts *et al*, 2012).

Previous studies focused on *KRAS*-mutation status as a potential prognostic factor has been mixed in their findings. In the largest study of *KRAS*-mutation status and survival to date, the Kirsten Ras Colorectal Cancer Collaborative Group study (RASCAL, $N = 2721$), Andreyev *et al* (1998) reported statistically significantly poorer overall survival for *KRAS*-mutated *vs* *KRAS*-wild-type disease at a magnitude similar to that observed here (HR = 1.22, 95% CI: 1.07–1.40). The majority of other, smaller studies have also indicated a poorer prognosis in patients with *KRAS*-mutated CRC (Nash *et al*, 2010; De Roock *et al*, 2010a; Hutchins *et al*, 2011; Imamura *et al*, 2012). Several studies, however, have failed to find an association between *KRAS* and patient outcomes (Samowitz *et al*, 2000; Gnanasampanthan *et al*, 2001; Wang *et al*, 2003; Lee *et al*, 2008; Ogino *et al*, 2009a,b; Roth *et al*, 2010). The basis for these inconsistencies is unclear, but may be related to limited sample size and differences in the distribution and consideration of other factors, such as age at diagnosis, stage, and MSI status.

Prior studies have also differed in their consideration of specific *KRAS* mutations in relation to CRC survival. In an update of the original RASCAL study (RASCAL II, $N = 4268$), Andreyev *et al* (2001) found the association between *KRAS*-mutation status and survival was largely confined to the p.G12V mutation. Imamura *et al* (2012) recently reported a similar finding, and found that mutations in *KRAS* codon 13 were not associated with CRC survival. Unlike these reports, we did not find a statistically significant association between the p.G12V *KRAS* mutation and prognosis. Although experimental evidence has suggested that mutations in *KRAS* codon 12, particularly p.G12V, confer lower GTPase activity (Bollag and McCormick, 1995; Al-Mulla *et al*, 1999), which may translate to greater transforming potential, our data are not consistent with a clear difference in the prognostic significance of somatic *KRAS* mutations by codon.

Results presented here should be interpreted in the context of study limitations. Only limited information on first course of treatment was available and it is possible that treatment could have differed according to *KRAS*-mutation status; however, 95% of cases

were diagnosed before 2006 at a time before *KRAS*-mutation status might have been used to decide on anti-EGFR therapy. *KRAS*-mutation status does not appear to be associated with differential response to other chemotherapies (Richman *et al*, 2009; Ogino *et al*, 2009a; Hutchins *et al*, 2011). In addition, *KRAS*-mutation status was not determined for 29% of enrolled cases. Although these cases differed from cases with known *KRAS*-mutation status on several factors that could be related to prognosis, we obtained point estimates similar to those in our primary analyses in sensitivity analyses using multiple imputation to account for these missing data. *KRAS*-mutation status also could not be determined in cases who were not enrolled in the present study because of refusal, death before enrollment, or loss to follow-up. If *KRAS*-mutated CRC is truly associated with poorer prognosis, the prevalence of *KRAS* mutations is likely to have been higher in those cases who died before they could be enrolled in the study: exclusion of deceased cases would thus have attenuated, rather than inflated our estimates of the strength of association.

Important strengths of the present study include the population-based design and large sample size. Our consideration of both MSI and *BRAF*-mutation status in evaluating the relationship between *KRAS*-mutation status and CRC survival also represents an important strength. Here, we confirm previous reports that *KRAS*-mutated CRC is less likely to be MSI-H and is very rarely *BRAF* mutated. When we evaluated these three markers in combination in relation to survival, we found a strong gradient in risk, particularly with respect to disease-specific survival. Those individuals with CRC that was *KRAS*-wild-type, *BRAF*-wild-type, and MSI-H had the most favourable disease-specific survival; individuals with CRC that was *KRAS*- or *BRAF*-mutated and MSS experienced a statistically significantly poorer prognosis than other case groups defined by combinations of these three markers. These results support the prognostic significance of *KRAS*-mutation status beyond its now established role as a predictive marker in distant-stage CRC.

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