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

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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...
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.1797T>G-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, DSS46, 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 $\geq 30\%$ 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 (Cieck 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 C180-C200 or C206 (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 by the date of CRC diagnosis.
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
coccur (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 ≥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 hetero-
genicity. 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, ≥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.

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 ≥50 years at
diagnosis but not in those aged <50 (P-interaction by age = 0.15).

| Table 1. Study population characteristics by KRAS-mutation status |
|---------------------------------------------------------------|
| **Age at diagnosis**                                          | KRAS wild-type (N = 1330) | KRAS-mutated (N = 593) | P-value* |
| <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**                                       |                            |                        |          |
| Localised                                                   | 553 (42)                  | 220 (37)              | 0.12     |
| Regional                                                    | 610 (46)                  | 293 (50)              |          |
| Distant                                                     | 144 (11)                  | 75 (13)               |          |
| Unknown                                                     | 23                        | 5                     |          |
| **MSI status**                                              |                            |                        | <0.001   |
| MSS/MSI-L                                                   | 1042 (82)                 | 509 (90)              |          |
| MSI-H                                                       | 236 (18)                  | 56 (10)               |          |
| Unknown                                                     | 52                        | 28                    |          |
| **BRAF mutation status**                                    |                            |                        | <0.001   |
| Wild-type                                                   | 1083 (82)                 | 580 (99)              |          |
| Mutated                                                     | 232 (18)                  | 6 (1)                 |          |
| Unknown                                                     | 15                        | 7                     |          |
| **Vital status**                                            |                            |                        | 0.09     |
| Alive                                                       | 843 (63)                  | 352 (59)              |          |
| 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 χ².
*% distribution excludes cases with unknown value of characteristic.
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, 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/MSI 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/MSI-H 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/MSI-H 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.
KRAS codons 12 and 13 have been shown to result in an altered 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)* | KRAS-wild-type deaths/cases | KRAS-mutated deaths/cases | HR (95% CI)* | KRAS-and BRAF-wt deaths/cases | KRAS-or BRAF-mut deaths/cases | HR (95% CI)* |
| 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 | ≥50 years |
|----------|-----------|-----------|
| Male     | 238/609   | 249/721   |
| Female   | 115/264   | 126/329   |

By sex

|                | Male | Female |
|----------------|------|--------|
| Proximal      | 195/505 | 115/264 |
| Distal/rectal | 276/788 | 126/329 |

By tumour site

|                | Localised | Regional | Distant |
|----------------|-----------|----------|---------|
|                | 130/553   | 227/610  | 118/144 |

By stage at diagnosis

|                | MSS        | MSI-H     |
|----------------|------------|-----------|
|                | 391/1042   | 79/236    |

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.

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)* | Deaths/cases | HR (95% CI)* |
| 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. *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; 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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