Specific mutations in KRAS codon 12 are associated with worse overall survival in patients with advanced and recurrent colorectal cancer

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Background: Activating mutations in KRAS have been suggested as potential predictive and prognostic biomarkers. However, the prognostic impact of specific point mutations remains less clear. This study assessed the prognostic impact of specific KRAS mutations on survival for patients with colorectal cancer.

Methods: Retrospective review of patients KRAS typed for advanced and recurrent colorectal cancer between 2010 and 2015 in a UK Cancer Network.

Results: We evaluated the impact of KRAS genotype in 392 patients. Mutated KRAS was detected in 42.9% of tumours. KRAS mutations were more common in moderate vs well-differentiated tumours. On multivariate analysis, primary tumour T stage (HR 2.77 (1.54–4.98), $P = 0.001$), N stage (HR 1.51 (1.01–2.26), $P = 0.04$), curative intent surgery (HR 0.51 (0.34–0.76), $P = 0.001$), tumour grade (HR 0.44 (0.30–0.65), $P = 0.001$) and KRAS mutation (1.54 (1.23–2.12), $P = 0.005$) were all predictive of overall survival. Patients with KRAS codon 12 mutations had worse overall survival (HR 1.76 (95% CI 1.27–2.43), $P = 0.001$). Among the five most common codon 12 mutations, only p.G12C (HR 2.21 (1.15–4.25), $P = 0.01$) and p.G12V (HR 1.69 (1.08–2.62), $P = 0.02$) were predictive of overall survival.

Conclusions: For patients with colorectal cancer, p.G12C and p.G12V mutations in codon 12 were independently associated with worse overall survival after diagnosis.

Colorectal cancer represents a heterogeneous group of diseases, and its molecular classification is increasingly important. A number of key genetic and epigenetic alterations have been identified (Colussi et al, 2013; Kudryavtseva et al, 2016), with early activating mutations in the KRAS gene reported in ~40% of tumours (Downward, 2003).

KRAS is a protein and downstream effector of epidermal growth factor receptor (EGFR), with binding of the EGF ligand to the receptor triggering downstream signalling via the PI3K/AKT/MTOR and RAF/MEK/ERK cellular proliferation pathways (Fearon, 2011). Approximately 90% of mutations occur within codon 12 and 13 (Janakiraman et al, 2010), with well-characterised

single-base substitution point mutations (Neumann *et al*, 2009). Patterns of *KRAS* mutation vary according to tumour location, with *KRAS* mutations twice as common in lesions proximal to the splenic flexure (Rosty *et al*, 2013). This concept of distinct genetic and epigenetic profiles of proximal and distal lesions was further evolved with the finding that the frequencies of CIMP-high, MSI-high and *BRAF* mutation gradually increased from rectum to ascending colon, suggesting the classic proximal *vs* distal classification may be oversimplistic (Yamauchi *et al*, 2012). By contrast, *KRAS* mutations did not follow this trend but were most common in caecal lesions.

The predictive role of *KRAS* mutation on efficacy of anti-EGFR therapy is well recognised. However, reports on prognostic value remain uncertain. It is now recognised that specific point mutations can have profoundly differing effects on *KRAS* function. It was long assumed that any *KRAS* mutation meant patients derived no advantage from treatment with anti-EGFR therapies, but there is now growing evidence that those with specific mutations in codon 13 (p.G13D) may derive a survival benefit in contrast to patients with codon 12 mutations (De Roock *et al*, 2010; Tejpar *et al*, 2012). These fundamental differences in tumour phenotype within the *KRAS*-mutant population have lead to a re-assessment of the prognostic value of *KRAS* mutations. Numerous studies have compared outcomes in patients with codon 12, 13 and 61 mutations, with mixed results (Samowitz *et al*, 2000; Andreyev *et al*, 2001; Bazan *et al*, 2002; Roth *et al*, 2010; Yokota *et al*, 2011; Imamura *et al*, 2012, 2014). However, these studies group mutations by codon and so the impact of specific amino acid changes remains unclear. Laboratory studies have suggested that specific *KRAS* point mutations in codon 12 may confer increased oncogenic potential through the inhibition of apoptosis, loss of contact inhibition and increased contact-independent growth when compared to codon 13 mutations (Al-Mulla *et al*, 1999; Guerrero *et al*, 2000; Smith *et al*, 2010), but the clinical relevance of these findings have yet to be clarified.

This study therefore aimed to assess the prognostic impact of specific point mutations in *KRAS* on overall survival in a mixed cohort of advanced and recurrent colorectal cancer patients.

MATERIALS AND METHODS

Study design. Patients who underwent *KRAS* typing on surgically resected or biopsied specimens between May 2010 and February 2015 in the Cheshire and Merseyside Cancer Network were identified from a prospectively collected database. Standard demographic and clinicopathological data were retrieved from paper and electronic case notes on each patient including age, sex, ASA grade, tumour location, stage and grade at presentation (date of curative intent surgery if appropriate, or date of diagnosis with irresectable disease), surgical management, date of last follow-up, date and pattern of recurrence, and date of death. Surgery was considered curative when all identifiable disease was resected with curative intent. Patients were treated with systemic chemotherapy according to contemporary NICE guidance (first-line treatment with systemic FOLFOX; Poston *et al*, 2011). Survival was calculated from date of curative intent surgery or presentation with irresectable metastatic disease to date of last follow-up. Where synchronous metastatic disease was resected with curative intent, survival time was calculated from date of final resection.

KRAS mutation analysis. *KRAS* testing was performed centrally in the Merseyside & Cheshire Regional Genetics Laboratories using resected or biopsied primary colorectal cancer. DNA was extracted using standard methodology from formalin-fixed paraffin-embedded tumour samples and analysed for mutations in codons 12, 13 and 61 of the *KRAS* gene using a Pyrosequencing-based assay (Qiagen,

Venlo, The Netherlands), capable of detecting all somatic mutations in codons 12, 13 and 61 of the *KRAS* gene. The assay will detect all possible base substitutions at the specified codons plus more complex insertion–deletion mutations, with a limit of detection of 5–10% mutant DNA (dependent on the base substitution identified).

Statistical analysis. Demographic, clinicopathological and perioperative details were stratified according to *KRAS* mutation. Quantitative and qualitative variables were expressed as medians (with range) and frequencies. Comparisons between the groups were analysed with the χ^2-test or Fisher exact test for proportions and the Mann–Whitney *U*-test for continuous variables. Overall- and disease-free survival were compared using the Kaplan–Meier method. Comparisons were made using log-rank test. To identify factors associated with survival in the entire cohort, variables were assessed using univariate analysis. All variables associated with $P < 0.05$ in the univariate proportional hazards model were entered into a Cox proportional hazards multi-variate model using a forward step wise procedure. $P < 0.05$ was considered significant. All statistical analyses were performed using IBM SPSS Statistics (v.22, Armonk, NY, USA).

RESULTS

Frequency of KRAS mutations and association with clinico-pathological factors. The total study population consisted of 495 patients in whom *KRAS* data were available. *KRAS* mutations were identified in 40% ($n = 198$) of samples, with the majority (31.5%, $n = 156$) in codon 12 and codon 13 (7.3%, $n = 36$). Only 1.2% ($n = 6$) had a mutation in codon 61.

About 392 (79.2%) patients in whom adequate retrospective data could be obtained were further assessed for clinicopathological and survival analysis. Table 1 summarises the baseline clinico-pathological characteristics of these patients stratified by *KRAS* mutation status. *KRAS* mutations were present in (42.9%, ($n = 168$), with the majority occurring in codon 12 (34.6%, $n = 136$) and 13 (7.1%, $n = 28$). Within codon 12, p.G12D was the most common point mutation (36.0%, $n = 49$ out of 136) followed by p.G12V (30.1%, $n = 41$ out of 136). Within codon 13, p.G13D was most common (92.9%, $n = 26$ out of 28). All other codon 12 and 13 mutations had a frequency <10% (Table 2).

Median patient age was 65.2 years (IQR 25–78) and most patients were female ($n = 242$, 61.7%). Most patients had a colonic primary tumour ($n = 243$, 61.9%), with the majority of lesions demonstrating moderately differentiated adenocarcinoma ($n = 282$, 71.9%). There was no difference in frequency of *KRAS* mutation and site of primary lesion. Of the 298 patients who had undergone curative intent surgery, 76% ($n = 226$) developed recurrence. Of those 226, 64 (21.5%) underwent resection of recurrent disease. Fifty-eight were treated with liver resection, five underwent lung resection and one underwent a further colorectal procedure for local recurrence. *KRAS* mutation was significantly correlated only with tumour grade ($P = 0.01$), and was not associated with stage at presentation, pattern of metastases or curative intent surgery (Table 2). The presence of a *KRAS* 12 mutation was not associated with any specific clinicopathological characteristics. When codon 61 mutations were excluded from analysis, no differences were observed between patients with wild-type *KRAS* and mutations in codon 12 or 13.

Overall survival. At a median follow-up of 22 months (IQR 3–100 months), 220 patients (56.1%) had died. Median overall survival for the entire patient cohort was 31.3 (IQR 28.6–33.9) months, with a nominal 1-, 3- and 5-year survival of 82%, 41% and 17%, respectively.

Univariate analysis identified stage and grade of tumour, curative intent surgery, pattern of metastasis and *KRAS* status as predictive of overall survival (Table 3). On multivariate analysis controlling for other factors, *KRAS* status remained statistically

Table 1. Association of clinicopathological features with KRAS mutational status

Clinicopathological feature	KRAS WT	KRAS mutant			P-value
	n = 224	Codon 12 n = 136	Codon G13 n = 28	Codon G61 n = 4	
Age (median, range)	64.2 (24–93)	65.6 (25–91)	64.6 (35–78)	72.4 (55–84)	
Gender					
Male	91 (40.6%)	47 (34.6%)	9 (32.1%	3 (75.0%)	0.26
Female	133 (59.4%)	89 (65.4%)	19 (67.9%)	1 (25.0%)	
ASA					
1	77 (34.4%)	47 (34.6%)	8 (28.6%)	2 (50.0%)	0.70
2	114 (50.9%)	73 (53.7%)	18 (64.3%)	1 (25.0%)	
3	33 (14.7%)	16 (11.8%)	2 (7.1%)	1 (25.0%)	
Curative intent surgery					
Yes	168 (75.0%)	106 (77.9%)	20 (71.4%)	4 (100.0%)	0.58
No	56 (25.0%)	30 (22.1%)	8 (28.6%)	0 (0%)	
T stage					
1	2 (0.9%)	5 (3.7%)	0 (0.0%)	0 (0.0%)	0.65
2	22 (9.8%)	11 (8.1%)	4 (14.3%)	0 (0.0%)	
3	116 (51.8%)	73 (53.7%)	12 (42.9%)	2 (50.0%)	
4	84 (37.5%)	47 (34.6%)	12 (42.9%)	2 (50.0%)	
N stage					
0	48 (21.4%)	44 (32.4%)	6 (21.4%)	2 (50.0%)	0.13
1	80 (35.7%)	50 (36.8%)	8 (28.6%)	1 (25.0%)	
2	96 (42.9%)	42 (30.9%)	14 (50.0%)	1 (25.0%)	
M					
0	121 (54.0%)	76 (55.9%)	13 (46.4%)	4 (100%)	0.24
1	103 (46.0%)	60 (44.1%)	15 (53.6%)	0 (0%)	
Grade					
NA	17 (7.6%)	12 (8.8%)	2 (7.1%)	0 (0.0%)	0.01
Poor	30 (13.4%)	10 (7.4%)	3 (10.7%)	1 (25.0%)	
Moderate	166 (74.1%)	91 (66.9%)	22 (78.6%)	3 (75.0%)	
Well	11 (4.9%)	23 (16.9%)	1 (3.6%)	0 (0.0%)	
Tumour location					
Caecum	37 (56.1%)	24 (36.4%)	4 (6.0%)	1 (0.2%)	0.28
Ascending	18 (38.3%)	22 (46.8%)	6 (12.8%)	1 (2.1%)	
Transverse	15 (68.2%)	2 (9.1%)	5 (22.7%)	0 (0%)	
Left	64 (28.6%)	39 (28.7%)	4 (14.3%)	1 (25.0%)	
Rectum	90 (40.2%)	49 (36.0%)	9 (32.1%)	1 (25.0%)	
Site of metastases					
No recurrence	52 (23.2%)	29 (21.3%)	7 (25.0%)	1 (25.0%)	0.80
Liver only	88 (39.3%)	45 (33.1%)	8 (28.6%)	1 (25.0%)	
Lung only	22 (9.8%)	11 (8.1%)	2 (7.1%)	1 (25.0%)	
Liver and lung only	23 (10.3%)	21 (15.4%)	4 (14.3%)	1 (25.0%)	
Widespread	39 (17.4%)	30 (22.1%)	7 (25.0%)	0 (0.0%)	

Abbreviations: ASA = American Society of Anaesthesiologists; NA = not applicable; Wt = wild type.

Table 2. Frequency of KRAS mutations

Somatic mutation	N (%)
c.35G > A p.G12D	49 (29.2%)
c.35G > C p.G12A	15 (8.9%)
c.34G > T p.G12C	15 (8.9%)
c.34_35delinsTT p.G12F	1 (0.6%)
c.34G > A p.G12S	16 (9.5%)
c.35G > T p.G12V	41 (24.4%)
c.38G > A p.G13D	26 (15.5%)
c.37G > T p.G13C	2 (1.2%)
c.183A > T p.Q61H	4 (2.4%)

and codon 13 was 24.8 and 22.4 months, respectively ($P = 0.002$ for codon 12, $P = 0.08$ for codon 13; Figure 1). Multivariate analysis confirmed patients with mutations in codon 12 had worse OS (HR 1.76 (95% CI 1.27–2.43, $P = 0.001$). In contrast, mutations in codon 13 did not appear to impact on survival (HR 1.7 (95% CI 0.93–3.46, $P = 0.06$).

The five most commonly identified codon 12 mutations were then further analysed, with worse overall survival associated with p.G12V (univariate HR 1.69 (95% CI 1.08–2.62, $P = 0.02$) and p.G12C (univariate HR 2.21 (95% CI 1.15–4.25, $P = 0.01$) point mutations (Table 4). Patients with p.G12V ($n = 41$) and p.G12C ($n = 15$) mutations both had a median survival of 24.9 months compared with 35.1 months for wild-type KRAS ($P < 0.02$; Figure 2).

DISCUSSION

This study assessed the impact of KRAS mutation on prognosis in advanced and recurrent colorectal cancer. Within our cohort,

significant (HR 1.54 (95% CI 1.23–2.12), $P = 0.005$). Median overall survival for patients with wild-type KRAS was 35.1 months compared with 25.8 for those with mutant KRAS ($P = 0.006$). Median overall survival for patients with mutations in codon 12

Table 3. Univariate and multivariate analysis of overall survival stratified by clinicopatholgical features

	Hazard ratio (95% CI)			
	Univariate	*P*-value	Multivariate	*P*-value
Age > 65	1.14 (0.87–1.34)	0.32		
Female	0.88 (0.77–1.03)	0.11		
ASA > 3	0.92 (0.75–1.13)	0.42		
AJCC stage at presentation				
T1/T2	1 (Reference)			
T2/T3	3.57 (2.06–6.2)	<0.001	2.77 (1.54–4.98)	0.001
N0	1 (Reference)			
N1/N2	2.20 (1.53–3.18)	<0.01	1.51 (1.01–2.26)	0.04
M0	1 (Reference)			
M1	2.78 (2.1–3.66)	<0.001	1.43 (0.96–2.13)	0.07
Location				
Caecum	1 (Reference)			
Ascending	0.89 (0.67–1.2)	0.23		
Transverse	0.94 (0.56–2.63)	0.12		
Descending	1.02 (0.87–1.54)	0.3		
Rectum	0.76 (0.54–1.76)	0.15		
Curative intent surgery	0.29 (0.22–0.39)	<0.001	0.51 (0.34–0.76)	0.001
Tumour grade				
Poor	1 (Reference)			
Moderate	0.49 (0.34–0.72)	<0.001	0.44 (0.30–0.65)	0.001
Well	0.57 (0.32–1.02)	0.59		
Metastatic site				
None	1 (Reference)			
Liver only	2.49 (1.59–3.90)	<0.001	1.33 (0.80–2.21)	0.27
Lung only	1.27 (0.69–2.34)	0.43		
Liver/lung only	2.88 (1.72–4.81)	<0.001	1.13 (0.62–2.05)	0.69
Widespread	2.71 (1.68–4.34)	<0.001	1.21 (0.71–2.07)	0.47
KRAS				
Wild type	1 (Reference)			
Mutant	1.48 (1.11–1.96)	0.007	1.54 (1.23–2.12)	0.005
All codon 12 mutants	1.55 (1.17–2.07)	0.002	1.76 (1.27–2.43)	0.001
All codon 13 mutants	1.65 (0.89–2.68)	0.06	1.7 (0.93–3.46)	0.06
All codon 61 mutants	0.85 (0.21–3.44)	0.82		

Abbreviations: AJCC = American Joint Committee on Cancer; ASA = American Society of Anaesthesiologists; CI = confidence interval.
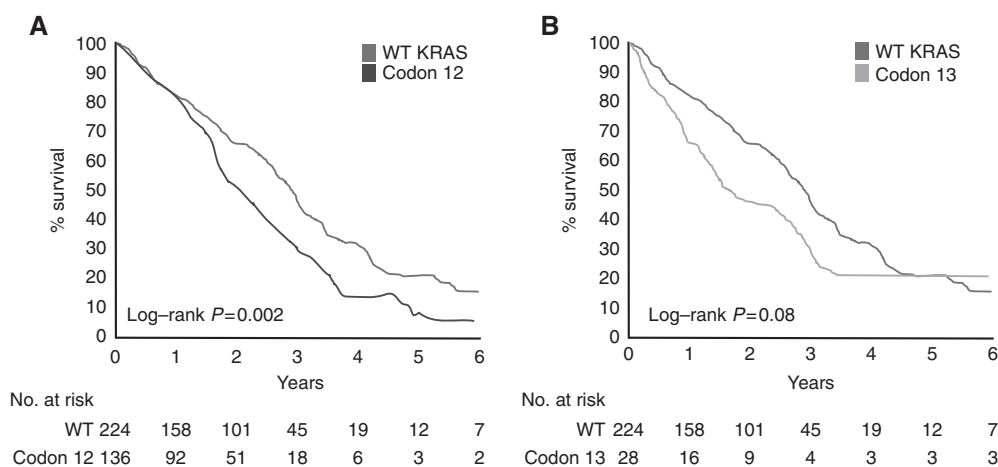
Figure 1. Overall survival for patients with advanced or recurrent colorectal cancer stratified by codon mutation (**A**) Wild type *vs* codon 12 (**B**) Wild type *vs* codon 13.

mutations in *KRAS* codon 12 were independently associated with a worse OS when compared with *KRAS* wild-type tumours. By contrast, mutations in codon 13 were not associated with worse OS. When outcome was further stratified by specific point mutations within codon 12, p.G12C and p.G12V mutations were both independently associated with worse OS compared with *KRAS* wild-type tumours.

KRAS mutations were identified in 42.9% of patients included for survival analysis, similar to other reports of both stage III and IV colorectal cancer (Yokota *et al*, 2011; Yoon *et al*, 2014), with similar rates of p.G12C (8.9 *vs* 10.0%) and p.G12V (24.4 *vs* 21.1%) mutation (Imamura *et al*, 2012). Rates of codon 61 mutation were low (1.5%), in keeping with other published series (Imamura *et al*, 2014). The advanced and recurrent nature of this patient cohort

implies more aggressive disease, with lower rates of *KRAS* mutation in general and p.G12V and p.G12C mutations in particular reported in groups of patients with earlier stage disease and long-term disease-free survival (Margonis *et al*, 2015).

Although the predictive role of *KRAS* is well recognised, its precise prognostic value remains controversial. Mutations in *KRAS* have been clearly demonstrated to confer resistance to systemic anti-EGFR therapies in large prospective studies (Van Cutsem *et al*, 2009, 2011; Bokemeyer *et al*, 2009). However, retrospective reports on the prognostic value of *KRAS* have failed to provide a clear answer (Samowitz *et al*, 2000; Castagnola and Giaretti, 2005). One potential source of error may be that most historical reports have compared *KRAS* wild type with any *KRAS* mutant, rather than mutations in specific codons. There is growing recognition that specific mutations in *KRAS* may alter tumour phenotype. For example, retrospective subgroup analysis of large randomised trials of anti-EGFR therapy have identified that in contrast with other *KRAS*-mutant patients, those with p.G13D mutations may actually derive benefit from anti-EGFR therapy (Tejpar *et al*, 2012). Somatic mutations in codon 12 and 13 have also been associated with more aggressive stage at presentation and worse DFS in resected stage III colon cancer and OS in stage IV colorectal cancer compared with wild-type disease (Andreyev *et al*, 2001; Yokota *et al*, 2011; Imamura *et al*, 2012; Yoon *et al*, 2014; Li *et al*, 2015). However, the prognostic value of specific point mutations has not yet been fully clarified.

This study clearly demonstrates that p.G12C (HR 2.21 (95% CI 1.15–4.25), $P = 0.01$) and p.G12V (HR 1.69 (95% CI 1.08–2.62), $P = 0.02$) were both strongly associated with worse overall survival. By contrast, other mutations in codon 12 and mutations in codon 13 and 61 did not impact on survival. These data are consistent with previous laboratory studies, which have suggested that

mutations in *KRAS* codon 12 confer a greater oncogenic capacity (Guerrero *et al*, 2000) and are in keeping with the concept that mutations in a single gene can lead to a specific tumour phenotype (Ogino *et al*, 2012). The negative impact of codon 12 mutation is also biologically plausible. Binding of GTP to KRAS results in protein activation, triggering downstream signalling and cellular proliferation. The enzyme GTPase regulates this process, causing KRAS-GTP deactivation and is regulated by Rho-GTPase-activating proteins and Rap guanine-nucleotide exchange factors (Karnoub and Weinberg, 2008). RAS mutants are resistant to this GTPase-controlled regulatory step, with mutations in codon 12 associated with higher thresholds for induction of apoptosis (Guerrero *et al*, 2000). Specifically, p.G12V mutations have been associated with more aggressive cellular transformation than other codon 12 mutations *in vitro*, in keeping with the findings of this study (Al-Mulla *et al*, 1999).

This study found no correlation between clinicopathological disease features, including tumour location and *KRAS* status, in contrast to other larger series, which identified higher rates of *KRAS* mutation in proximal disease (Cancer Genome Atlas Network, 2012; Yamauchi *et al*, 2012; Yoon *et al*, 2014). Proximal disease does appear to be more aggressive, with patients undergoing curative surgery for proximal tumours who develop recurrence less likely to be treatable with curative intent (Pugh *et al*, 2016). These apparently contradictory findings highlight the complex interplay between aberrant pathways in the pathogenesis of colorectal cancer.

Strengths of this study include a relatively large cohort of patients with advanced and recurrent colorectal cancer managed in contemporary Western practice. It also provides an accurate description of mutational frequency in metastatic colorectal cancer outside a selective clinical trial. Direct interrogation of patient notes, rather than reliance on clinical coding, also ensured a high degree of clinical accuracy.

Weaknesses of the study include the lack of testing for *BRAF* codon 600 mutations (a downstream molecule of KRAS), which is known to be a very poor prognostic indicator (Yokota *et al*, 2011). However, *KRAS* and *BRAF* mutations are recognised as being mutually exclusive, *BRAF* codon 600 mutations have a relatively low incidence (<10%) in Western populations (Rajagopalan *et al*, 2002) and it is well recognised that the respective malignancy of the codon 12 and 13 mutations is independent of BRAF (Colussi *et al*, 2013). Given the consistently demonstrated negative prognostic impact of *BRAF* codon 600 mutations on patient survival (Roth *et al*, 2010; Yokota *et al*, 2011) and their potential inclusion in the *KRAS* wild-type cohort, inclusion of *BRAF*-mutant

Table 4. Univariate analysis of overall survival according to codon 12 KRAS mutation

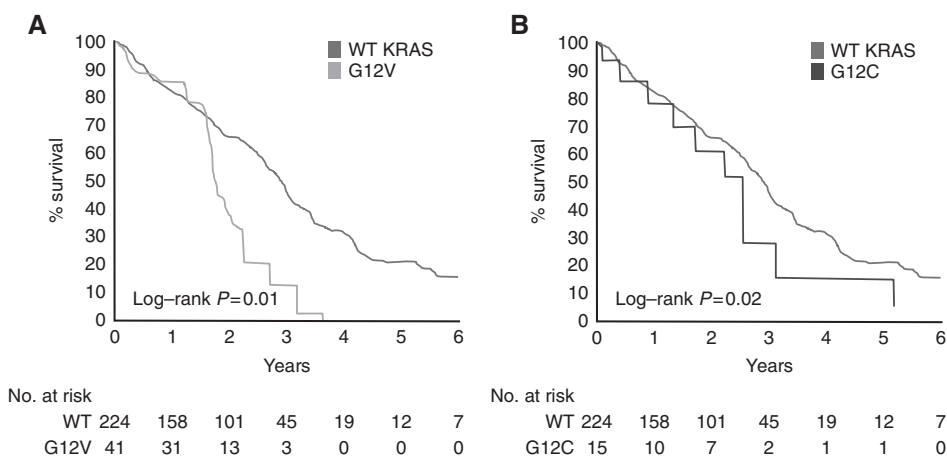| Somatic mutation	Univariate hazard ratio	P-value
WT	1 (Reference)	
c.35G>A p.G12D	1.28 (0.84–1.94	0.24
c.35G>C p.G12A	1.90 (0.99–3.68)	0.05
c.34G>T p.G12C	2.21 (1.15–4.25)	0.01
c.34G>A p.G12S	1.43 (0.77–2.67)	0.26
c.35G>T p.G12V	1.69 (1.08–2.62)	0.02
Abbreviation: Wt=wild type.		

Figure 2. Overall survival for patients with advanced or recurrent colorectal cancer stratified by codon 12 point mutation (**A**) Wild type *vs* p.G12V (**B**) Wild type *vs* p.G12C.

cancers would be unlikely to affect the key findings of this study although the possibility of an under estimation of the magnitude of effect of *KRAS* mutation on overall survival cannot be discounted. In addition, this study did not assess other less common mutations in *KRAS*, *NRAS* or *HRAS*. The importance of these mutations has only been identified in the last few years (Douillard *et al*, 2013; Colussi *et al*, 2013), and *KRAS*-only testing was contemporary clinical practice at the time of analysis. Subgroup analysis of biologically important but relatively low incidence mutations such as G12A and codon 61 mutations may also not have sufficient numbers to achieve statistical power. This phenomenon is not unique to this study, and likely explains in discrepancies in the reported importance of uncommon mutations between series (Margonis *et al*, 2015; Kim *et al*, 2016; Passot *et al*, 2016). Meta-analysis will be required to better define clinical importance.

This study included patients who presented with stage IV disease, as well as patients who had undergone curative intent surgery. The overwhelming majority of patients who had undergone surgical resection developed recurrence, reflecting the selection of this group for KRAS testing, and so the number of patients 'cured' by surgery was low. Patient characteristics were well matched between these groups, with concordance between primary and metastatic tumours in other key oncogenic mutations (such as NRAS, BRAF, PIK3CA and TP53) of over 90% (Vakiani *et al*, 2012), and so it seems the potential impact of this mixed cohort is likely limited. In addition, the key findings of this study are in line with the findings of the PETACC8 trial in stage III (non-metastatic) colorectal cancer that showed codon 12/13 mutations were associated with shorter time to recurrence after curative intent surgery (Blons *et al*, 2014).

The other major limitation of the current study surrounds the lack of data on subsequent cancer treatment. It is well recognised that treatment with systemic chemotherapy can have a significant impact on disease progression and overall survival in metastatic colorectal cancer, and it is impossible to exclude potential differences in treatments between subgroups, although all patients would have been treated according to contemporary UK NICE guidance (Poston *et al*, 2011). In addition, the proportion of patients treated with curative intent surgery were the same for each subgroup based on *KRAS* status. If patients are considered fit enough to tolerate curative intent surgery, it seems likely that they would be fit enough to receive systemic chemotherapy. The prognostic advantage enjoyed by *KRAS* wild-type tumours may also be partly explained by the use of anti-EGFR therapy. However, during the study period this was limited by UK NICE guidance to liver-limited irresectable metastatic disease (NICE (National Institute for Health and Care Excellence), 2009).

In conclusion, this study clearly demonstrates that mutations in *KRAS* codon 12 are independently associated with overall survival in recurrent and metastatic colorectal cancer, with specific somatic mutations within codon 12 (p.G12V and p.G12C) appearing to be prognostically deleterious. Analysis of *KRAS* mutation status may help guide clinical decision-making and prognostication in patients with advanced and recurrent colorectal cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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