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, MSL-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 contrast, KRAS classification may be oversimplistic (Yamauchi et al vs ascending colon, suggesting the classic proximal 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.

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significant (HR 1.54 (95% CI 1.23–2.12), \( P = 0.005 \)). Median overall survival for patients with wild-type \( \text{KRAS} \) was 35.1 months compared with 25.8 for those with mutant \( \text{KRAS} \) (\( P = 0.006 \)).

Median overall survival for patients with mutations in codon 12 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 1.70 (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 \( \text{KRAS} \) (\( P < 0.02 \); Figure 2).

## DISCUSSION

This study assessed the impact of \( \text{KRAS} \) mutation on prognosis in advanced and recurrent colorectal cancer. Within our cohort,
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).

### Table 3. Univariate and multivariate analysis of overall survival stratified by clinicopathological 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 (Reference)    |             |         |              |         |
| T2/T3                 | 3.57 (2.06–6.2)  | <0.001  | 2.77 (1.54–4.98) | 0.001  |
| N0 (Reference)        |             |         |              |         |
| N1/N2                 | 2.20 (1.53–3.18) | <0.01   | 1.51 (1.01–2.26) | 0.04   |
| M0 (Reference)        |             |         |              |         |
| M1                    | 2.78 (2.1–3.66)  | <0.001  | 1.43 (0.96–2.13) | 0.07   |
| Location              |             |         |              |         |
| Caecum (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 (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 (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 (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.
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, 2010; Yokota et al, 2011). Given the consistently demonstrated negative prognostic impact of KRAS 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
cancers would be unlikely to affect the key findings of this study although the possibility of an underestimation 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 surgery 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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