K-Ras mutation status as a predictive biomarker in metastatic colorectal cancer

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'In the last 5 years, cancer therapy has undergone a major revolution characterized by the introduction of targeted drugs that inhibit specific molecules.'

Colorectal cancer (CRC) is the third most common cancer worldwide, with approximately 1 million new cases diagnosed every year. It is estimated that approximately half of the patients with CRC will develop metastasis. Current use of irinotecan, oxaliplatin and bevacizumab, combined with the long-time standards 5-fluorouracil and leucovorin as first- or second-line treatment for metastatic CRC (mCRC), has resulted in median overall survival (mOS) figures of greater than 20 months. However, most patients develop resistance to these therapies and finally die owing to progression of their disease.

Additional improvements in treatment are likely to be facilitated by the use of rationally selected therapeutic agents that target functionally important proteins in tumor cells, such as the EGF receptor (EGFR), expressed in 75–90% of mCRC specimens [1]. The activation of EGFR leads to the activation of intracellular effectors involved in intracellular signaling pathways such as the G-protein K-Ras. K-Ras is a self-inactivating signal transducer, cycling from a GDP-bound 'off' state to GTP-bound 'on' state in response to receptor activation [2]. This response is transient owing to the intrinsic GTPase activity. Interestingly, *K-Ras* oncogene may harbor activating mutations yielding proteins with reduced GTPase activity. These activating *K-Ras* mutations are among the most common oncogenic alterations in cancer [3].

Monoclonal antibodies designed to bind to the ectodomain of the EGFR have shown activity in chemorefractory mCRC. Several Phase II studies have demonstrated the activity of cetuximab (a chimeric monoclonal IgG1 antibody) and panitumumab (a fully human monoclonal IgG2 antibody) in refractory mCRC. In a randomized Phase II study, increased activity with cetuximab plus irinotecan, compared with

cetuximab alone, was seen in patients with mCRC cancer who were refractory to irinotecan-based chemotherapy and had EGFR-expressing tumors [4].

Mutations in *K-Ras* oncogene should affect the clinical response to EGFR inhibition. Several studies have evaluated the potential predictive role of *K-Ras* mutational status in patients with mCRC treated with anti-EGFR monoclonal antibodies (Table 1). Lièvre *et al.* screened tumors for *K-Ras* mutations by direct sequencing from 30 patients with mCRC treated with cetuximab in order to define the usefulness of *K-Ras* mutations as a predictor of response to anti-EGFR therapy. *K-Ras* mutations were found in 13 tumors and the presence of these mutations was significantly associated with the absence of response to cetuximab. The mOS of patients without *K-Ras* mutations was significantly higher compared with that of those patients harboring a mutation (16.3 vs 6.9 months) [5]. Recently, Lièvre *et al.* presented the results of a larger series of 89 patients [6]. The aim of this study was to validate the predictive value of *K-Ras* mutations in the response to cetuximab treatment and survival. *K-Ras* mutations were present in 27% of the patients and they were associated with resistance to cetuximab-based treatment (0% of responders among the 24 patients with mutated tumors vs 40% of responders in 65 patients with nonmutated tumors), a poorer median progression-free survival (mPFS; 10.1 vs 31.4 weeks in patients with and without tumor mutations) and a worse mOS (10.1 vs 14.3 months in patients bearing tumors with and without mutations, respectively). When the authors pooled these 89 patients with patients from the previous study, the multivariate analysis showed that *K-Ras* mutation status was an independent prognostic factor associated with OS and PFS.

Several other retrospective analyses evaluating the role of *K-Ras* as a biomarker, predictive for activity with EGFR-directed monoclonal antibodies in patients with mCRC, have been published [7–10]. Benvenuti *et al.* analyzed specimens from 48 patients with mCRC bearing EGFR-expressing tumors enrolled into clinical trials

Table 1. Response rate for cetuximab or panitumumab ($\pm$ chemotherapy) according to the *K-Ras* mutation status in the different studies.

Treatment	n (WT:MT)	RR n (%)		Ref.
		MT	WT	
Cmab plus CT	89 (65:24)	0 (0)	26 (40)	[6]
Pmab or Cmab or Cmab plus CT	48 (32:16)	1 (6)	10 (31)	[7]
Cmab or Cmab plus irinotecan	113 (67:46)	0 (0)	27 (40)	[9]
Cmab $\pm$ CT	81 (49:32)	2 (6)	13 (26)	[11]
Cmab plus CT	59 (43:16)	0 (0)	12 (28)	[8]
Cmab	80 (50:30)	0 (0)	5 (10)	[10]
Pmab	427 (124:84)*	0 (0)	21 (17)	[12]
Cmab	48 (32:16)	0 (0)	9 (28)	[13]
Cmab plus FOLFIRI		5 (32)	18 (55)	[13]

*Only panitumumab arm.

Cmab: Cetuximab; CT: Chemotherapy; FOLFIRI: Folinic acid, fluorouracil and irinotecan; MT: Mutant; Pmab: Panitumumab; RR: Response rate; WT: Wild-type.

with panitumumab or cetuximab [7]. This retrospective analysis detected *K-Ras* mutations in 16 of the 48 (33.3%) tumors. The most frequent *K-Ras* alterations in the samples were single amino acid substitutions in codon 12. The presence of *K-Ras* mutations was not significantly linked to an objective response to therapy; however, a trend toward a negative association with response (one of 11 patients that achieved an objective response had tumors with *K-Ras* mutations compared with 15 of 37 nonresponding patients) was observed. Di Fiore *et al.* published the efficacy results of 59 patients with chemotherapy-refractory mCRC treated with cetuximab plus chemotherapy, according to *K-Ras* mutational status [8]; *K-Ras* mutations were detected in 28 of 59 tumors. Remarkably, no *K-Ras* mutation was found in the 12 patients with a clinical response. *K-Ras* mutations were associated with disease progression, and time to progression (TTP) was significantly decreased in patients bearing tumors with mutated *K-Ras* (3 vs 5.5 months). A similar experience was reported by De Roock *et al.* These authors studied the *K-Ras* mutation status of 113 patients with irinotecan-refractory mCRC treated with cetuximab with or without irinotecan [9]. An objective response was seen in 27 of 66 patients with wild-type (WT) *K-Ras* tumors versus 0 of 42 patients bearing tumors with mutant-type (MT) *K-Ras*. mOS was significantly improved in patients with WT *K-Ras* tumors versus patients with MT tumors (43.0 vs 27.3 weeks, respectively). Khambata-Ford *et al.* published the

results of a prospective trial conducted to identify biomarkers that are associated with disease control in patients treated with cetuximab as a single agent [10]. A total of 110 patients with mCRC were enrolled in this study. Transcriptional profiling was conducted on RNA from mandatory pretreatment metastatic biopsies to identify genes in which expression correlated with best clinical response. *K-Ras* mutation analysis was performed on DNA from pretreatment biopsies. The authors observed that patients whose tumors do not have *K-Ras* mutations had a significantly higher disease control rate than patients with *K-Ras* mutations (48 vs 10%, respectively). In addition, patients with tumors that had high gene expression levels of epiregulin and amphiregulin were more likely to have disease control with cetuximab treatment.

Finocchiaro *et al.* presented the results of a study aimed to identify biological predictors for sensitivity or resistance to cetuximab treatment in patients with mCRC at the 2007 American Society of Clinical Oncology (ASCO) meeting [11]. The authors analysed the *K-Ras* mutation status in paraffin-embedded tumor blocks from 85 patients with mCRC treated with cetuximab. Compared with patients with WT *K-ras* (n = 49), patients bearing tumors with *K-Ras* mutations (n = 32) had a significantly lower response rate (RR; 6.3 vs 26.5%), shorter TTP (3.7 vs 6.3 months) and shorter OS (8.3 vs 10.8 months).

Amado *et al.* presented the predictive effect of the *K-Ras* mutational status in the patients included in a Phase III randomized, controlled trial of panitumumab plus best supportive care (BSC) versus BSC alone in patients with chemotherapy-refractory mCRC at the 2008 Gastrointestinal Cancers Symposium [12]. *K-Ras* mutation status was assessed in tumor samples of 427 patients: 43% of patients had MT *K-Ras*. mPFS of patients treated with panitumumab was 12.3 weeks for those with WT *K-Ras* tumors and 7.4 weeks for those with MT *K-Ras*. mPFS for BSC patients was 7.3 weeks in both groups. In the experimental arm, 17% of patients treated with panitumumab and with WT *K-Ras* tumors had an objective response and 34% presented stable disease (SD) compared with 0% with an objective response and 12% with SD in those bearing MT *K-Ras* tumors. When the treatment arms were combined, OS was longer in patients with WT *K-Ras* tumors versus patients with MT *K-Ras* tumors; this difference demonstrated that *K-Ras* is also a prognostic factor of poorer survival.

Tabernero *et al.* presented the first series in which the *K-Ras* mutational status was correlated with efficacy in patients with mCRC treated in the first-line setting with cetuximab as a single agent and associated with the irinotecan-based chemotherapy schedule of folinic acid, fluorouracil and irinotecan (FOLFIRI). In this Phase I/II study, 48 patients were initially treated with cetuximab as single agent for 6 weeks, and thereafter with cetuximab combined with FOLFIRI [13]. *K-Ras* mutations were detected in 16 (41%) of the tumors. In the cetuximab monotherapy part of the study, patients with *K-Ras* WT tumors had an objective response rate (ORR) of 27.6% compared with 0% for those with *K-Ras* MT tumors ($p = 0.015$). In the combination part of the study (cetuximab plus FOLFIRI), patients with *K-Ras* WT tumors had an ORR of 55.2% compared with 31.6% for those with *K-Ras* MT tumors. Conversely, mPFS was 9.4 months in patients with WT *K-Ras* tumors versus 5.6 months in those with MT *K-Ras* tumors ($p = 0.0475$).

In the last 5 years, cancer therapy has undergone a major revolution characterized by the introduction of targeted drugs that inhibit specific molecules. Among these, monoclonal antibodies (cetuximab and panitumumab) targeting the EGFR have shown remarkable efficacy in the

treatment of mCRC. Similar to other targeted therapies, anti-EGFR drugs are active only in a fraction of patients and most of them subsequently become resistant to the treatment. These recent clinical data confirm that the efficacy of cetuximab and panitumumab is confined to patients bearing tumors with WT *K-Ras*. These results suggest that *K-Ras* tumor profiling should be considered in patients with mCRC who are candidates for treatment with anti-EGFR monoclonal antibodies. Ongoing studies in mCRC in the first- and second-line setting will prospectively examine the effect of cetuximab and panitumumab in combination with chemotherapy in patients whose tumors contain WT and MT *K-Ras*. Future studies should investigate the role of *K-Ras* mutational status in the adjuvant setting in CRC and in other indications.

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Bibliography

- Goldstein NS, Armin M: Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer Stage IV colon adenocarcinoma: implications for a standardized scoring system. *Cancer* 92, 1331–1346 (2001).
- Stites EC, Trampont PC, Ma Z *et al.*: Network analysis of oncogenic ras activation in cancer. *Science* 318, 463–467 (2007).
- Malumbres M, Barbacid M: *RAS* oncogenes: the first 30 years. *Nat. Rev. Cancer* 3, 7–13 (2003).
- Cunningham D, Humblet Y, Siena S *et al.*: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med.* 351, 337–345 (2004).
- Lièvre A, Bachet JB, Le Corre D *et al.*: KRAS mutations status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res.* 66, 3992–3995 (2006).
- Lièvre A, Bachet JB, Boige V *et al.*: KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J. Clin. Oncol.* 26, 374–379 (2008).
- Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F *et al.*: Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res.* 67, 2643–2648 (2007).
- Di Fiore F, Blanchard F, Charbonnier F *et al.*: Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. *Br. J. Cancer* 96, 1166–1169 (2007).
- De Roock W, Piessevaux H, De Schutter J *et al.*: KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann. Oncol.* 19(3), 508–515 (2007).
- Khambata-Ford S, Garrett CR, Meropol NJ *et al.*: Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *J. Clin. Oncol.* 25, 3230–3237 (2007).
- Finocchiaro G, Cappuzzo F, Jänne PA *et al.*: EGFR, HER2 and Kras as predictive factors for cetuximab sensitivity in colorectal cancer. *Proc. Am. Soc. Clin. Oncol.* 25, 18S (2007).
- Amado RG, Wolf M, Freeman D *et al.*: Panitumumab (pmab) efficacy and patient-reported outcomes (PRO) in metastatic colorectal cancer (mCRC) patients (pts) with wild-type (WT) *KRAS* tumor status. Presented at: *ASCO Gastrointestinal Cancer Symposium*. Orlando, FL, USA, 25–27 January 2008 (Abstract 278).
- Tabernero J, Cervantes A, Ciardiello F *et al.*: Correlation of efficacy to KRAS status (wt vs mut) in patients (pts) with metastatic colorectal cancer (mCRC), treated with weekly (q1w) and q2w schedules of cetuximab combined with FOLFIRI. Presented at: *ASCO Gastrointestinal Cancer Symposium*. Orlando, FL, USA, 25–27 January 2008 (Abstract 435).