World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v20.i29.9732

World J Gastroenterol 2014 August 7; 20(29): 9732-9743
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (5): Colorectal cancer

Beyond KRAS: Predictive factors of the efficacy of anti-EGFR monoclonal antibodies in the treatment of metastatic colorectal cancer

Alfonso De Stefano, Chiara Carlomagno

Alfonso De Stefano, Chiara Carlomagno, Department of Clinical Medicine and Surgery, University of Naples Federico II, 80131 Naples, Italy
Author contributions: Both authors contributed to this paper equally.
Correspondence to: Alfonso De Stefano, MD, PhD, Department of Clinical Medicine and Surgery, University of Naples Federico II, Via Sergio Pansini, 5, 80131 Naples, Italy. alfonso.destefano@unina.it
Telephone: +39-817-463660 Fax: +39-812-203147
Received: October 27, 2013 Revised: February 13, 2014
Accepted: March 12, 2014
Published online: August 7, 2014

Abstract

Systematic analysis of the epidermal growth factor receptor (EGFR) pathway revealed that biomarkers could be used to predict the response to and outcome of anti-EGFR therapies in patients affected by metastatic colorectal cancer. We have conducted a review on the most recent findings and advances on this topic. To this aim, we searched the PubMed database for articles devoted to predictive and prognostic biomarkers for patients administered cetuximab- and panitumumab-based therapies. Here we review the state of the art and the controversies about the molecular factors known to be predictors of the efficacy of anti-EGFR therapy, namely, KRAS, BRAF, NRAS, PI3KCA and PTEN, and we discuss their prognostic value in colorectal cancer patients.

Key words: KRAS; Anti-epidermal growth factor receptor; Metastatic colorectal cancer; Biomarkers

Core tip: This is a review underlining the importance of biomarkers in the treatment of metastatic colorectal cancer. All most recent findings are described to offer a contribution to define a correct approach to anti-epidermal growth factor receptor based treatment.

De Stefano A, Carlomagno C. Beyond KRAS: Predictive factors of the efficacy of anti-EGFR monoclonal antibodies in the treatment of metastatic colorectal cancer. *World J Gastroenterol* 2014; 20(29): 9732-9743 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i29/9732.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i29.9732

INTRODUCTION

Thanks to the advent of targeted therapies for colorectal cancer (CRC), it is now possible to assign, more accurately than hitherto, therapy according to specific molecular profiles within a distinct tumour and subsequently to personalize treatment. Although discoveries such as *KRAS* gene mutations were a breakthrough in targeted therapy for CRC patients, various factors must be clarified to maximize the efficacy of this strategy. In particular, studies are required to identify other intracellular anomalous pathways involved in CRC so that drugs targeting them may be developed in order to have the right drug for the right patient.

Colorectal cancer is the third most common neoplastic disease worldwide[1]. It is one of the leading causes of cancer mortality, accounting for about 10% of all cancer deaths, with approximately 40%-50% of all cases diagnosed as metastatic[2]. Advances in the treatment of metastatic CRC (mCRC) over the last 20 years have improved overall survival (OS) from a median of 10 mo to approximately 24 mo[1]. Although CRC is considered curable if diagnosed at an early stage, 5-year survival is less than 10% in patients with unresectable metastatic

disease[3,4]. About 40%-50% of CRC patients develop metastases during their clinical history, and 80%-90% of them have liver secondary lesions[5].

Approximately 50% of mCRC patients present with a synchronous primary tumour and metastatic lesions, whereas the others develop metachronous relapses. Surgery is the only potentially curative therapy for mCRC. Resection of hepatic metastases results in 5-year survival rates ranging from 35% to 55%, although the outcome depends on tumour- and treatment-related variables such as number of lesions, the maximum diameter of lesions and resection margins[6,7]. Similarly, 5-year survival rates after resection of lung metastases from CRC range from 20% to 60%[8]. Chemotherapy is palliative in metastatic patients; although used as pre-operative conversion treatment it can be part of "curative" treatment[9]. In fact, in patients with initially unresectable liver metastases, conversion chemotherapy enabled liver resection in up to one-third of cases, with survival rates similar to those of patients with initially resectable liver metastases[10-12]. Unfortunately, most mCRC patients are not eligible for surgical resection and the only treatment option to prolong survival times is palliative chemotherapy.

For several decades, the mainstay of mCRC treatment was 5-fluorouracil (5-FU)/leucovorin (LV) chemotherapy which resulted in a median survival of about 11 mo[13]. During the last 15 years, combination regimens with oxaliplatin or irinotecan added to 5-FU/LV have led to a considerable improvement of the outcome of patients with mCRC[14,15]. Irrespective of the first-line chemotherapy regimen, an OS exceeding 2 years is currently achieved when patients receive all available active anti-CRC cytotoxic drugs[16]. The introduction of the so-called targeted or biological drugs (namely, bevacizumab, cetuximab, panitumumab, aflibercept and regorafenib) has further improved the survival of mCRC patients. In contrast to standard drugs that target cell proliferation, targeted agents interfere with processes that control cell growth, survival, angiogenesis and spread. Because these compounds act on selective pathways, their efficacy is limited when treatment selection is driven by particular molecular profiles.

Most of the targeted inhibitors currently under development or in clinical use are molecules with high affinity for growth factor receptors, *i.e.*, the fibroblast growth factor receptor, vascular endothelial growth factor receptor, platelet-derived growth factor receptor, mast/stem cell growth factor receptor and epidermal growth factor receptor (EGFR). The addition of monoclonal antibodies (mAbs) that bind the vascular endothelial growth factor and the EGFR to chemotherapy regimens in mCRC has been shown to be effective, thereby increasing treatment options[17,18].

It is now generally recognized that the large individual differences in treatment response among mCRC patients are due to the fact that tumours differ at the molecular level because of the unique genetic and environmental context of each specific patient. Therefore, it is essential to understand these different molecular properties in order to optimize treatment. Personalized treatment is one of the most challenging aspects of medicine, particularly oncology. It is defined as treatment that is based on the patient's individual genetic features, and it improves cancer OS and it reduces detrimental side effects. Several prognostic and predictive biomarkers have been identified over the past decade and they can be used to personalize treatment for patients affected by mCRC. Prognostic biomarkers identify patients with different disease outcomes regardless of treatment, and may provide details about the disease prognosis/behaviour. Predictive biomarkers help to categorize patients who are most likely to benefit from a specific treatment and can guide or support therapeutic decisions[19]. Thus, efforts have been made to identify tumour-related predictive factors that can suggest treatment response[20]. Patient-related aspects, such as age, gender and presence of comorbidities, are candidate prognostic factors.

This review focuses on the advances made in the personalized treatment of mCRC and discusses the potential of new markers in selecting patients. The results obtained in clinical trials are analyzed, particularly in view of how these may influence routine clinical practice

FACTORS PREDICTIVE OF THE EFFICACY OF ANTI-EGFR TREATMENT IN MCRC PATIENTS

The Erb family of cell membrane receptors includes HER1/erbB1 (EGFR), HER2/c-neu (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4)[21]. Since the *EGFR* gene was identified as an oncogene, it has become one of the major targets of biologic therapeutics, and prompted the development of anti-EGFR mAbs and tyrosine kinase inhibitors (TKIs). The mAbs cetuximab (anti-IgG1) and panitumumab (anti-IgG2) act by binding to the extracellular ligand site of the receptor, whereas erlotinib and gefitinib, the two major EGFR TKIs, compete with the binding of ATP to the TK domain of the receptor, thereby resulting in inhibition of EGFR autophosphorylation. Both strategies (mAb and TKI) interrupt the intracellular downstream signalling cascade.

The first clinical trials with anti-EGFR mAbs enrolled patients whose tumours expressed high levels of EGFR; however overall response rates (ORRs) were low[22], which suggested that other unidentified factors could affect response to these agents[23]. Lièvre *et al*[24] were the first to identify a link between KRAS mutations and lack of response to EGFR-targeted therapy. They analysed 30 patients receiving cetuximab plus irinotecan as second- or third-line treatment. *KRAS* mutations were observed in 13 of the 30 (43%) patients. None of the responders (0/11) presented *KRAS* mutations, whereas 68.4% (13/19) of non-responders did (P = 0.0003). The OS was significantly higher in KRAS-WT patients than in patients carrying a *KRAS* mutation (median OS: 16.3 mo

vs 6.9 mo, respectively, $P = 0.016$).

The next challenges were to understand why *KRAS*-mutated tumours do not respond to anti-EGFR mAbs, and to investigate if other components of the EGFR pathway could help to predict the effect of anti-EGFR therapies. In this context, studies focused on key signalling molecules downstream of the EGFR, including mutations in the *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* genes, and PTEN protein expression. *KRAS* mutations result in constitutive activation of downstream EGFR signalling pathways thereby determining an unfavourable prognosis and a poor treatment response. Mutations in the *BRAF* gene, which encodes a serine/threonine kinase that activates the RAS-MAPK pathway, have been found in 4%-15% of CRCs[25-27]. Cell lines harbouring *RAS*/*BRAF* mutations are more resistant to cetuximab *in vitro* compared with wild-type cells[28]. The *PIK3CA* gene encodes the p110α catalytic subunit of the phosphoinositide 3-kinase (PI3K) protein, which is a component of the PI3K-Akt signalling pathway downstream of ligand-induced EGFR activation. This catalytic subunit is activated consequent to its interaction with RAS proteins. *PIK3CA* mutations occur in 10%-18% of CRCs[25,28,29]. Cell lines with activating *PIK3CA* mutations have been found to be resistant to cetuximab as compared with wild-type cell lines[28]. However, two studies found a lack of correlation between mutational status and cetuximab response in patients with colon cancer[24,30]. As *PIK3CA* mutations are rare, these data were based on only five patients, which could explain the lack of association between *PIK3CA* mutations and treatment response. The phosphatase and tensin homolog (PTEN) acts as a tumour suppressor protein by inhibiting the PI3K-Akt signalling pathway. Cell lines deficient in PTEN expression are more resistant to cetuximab *in vitro* than those with normal PTEN expression[28]. The loss of PTEN protein expression is a negative predictive factor of the efficacy of cetuximab therapy in patients with mCRC[31]. Subsequently, Sartore-Bianchi *et al*[32] devised the Quadruple Index, which is based on changes in the four above-mentioned factors. They found that 70% of tumours harboured at least one molecular alteration, and that the probability of treatment response was 51% among patients with no alterations, 4% among patients with 1 alteration, and 0% for patients with $\geqslant 2$ alterations ($P < 0.0001$). Accordingly, PFS and OS decreased in the following order: patients with tumors harboring none, 1 or $\geqslant 2$ molecular alterations ($P < 0.001$).

KRAS AS PREDICTIVE FACTOR

Within the EGFR/RAS/RAF/MEK/ERK kinase signalling pathway, the KRAS protein is a GTPase normally bound to the internal part of the cell wall. It serves as a molecular switch that transmits extracellular signals from the EGFR to the nucleus, thereby regulating cell growth, proliferation, and apoptosis. The *KRAS* gene is located on the short arm of chromosome 12 and belongs to the Erb family of oncogenes. Patients with point mutations in the *KRAS* gene generally exhibit mutations within codon 12 at exon 2 (82%-87%), codon 13 (13%-18%), codon 61 (exon 3) and 146 (exon 4)[33]. In patients carrying a wild-type *KRAS* gene copy, the binding of anti-EGFR antibodies to the external part of receptor induces conformational changes with its internalization and a sequential direct inhibition of TK activity, and blockage of downstream RAS/RAF/MEK/ERK pathway. *KRAS* mutations prevent the mAb-induced inhibition of EGFR activity because they induce constitutive activation of the intracellular domain of the KRAS protein. In CRC patients the incidence of *KRAS* mutations is about 30%-45%[34]. KRAS mutational status can be evaluated on samples of either the primary tumour or the metastases because *KRAS* mutations are highly concordant (approximately 95%) in the two tissue samples[35].

The proof of principle that patients carrying *KRAS* mutations do not benefit from treatment with either of the two anti-EGFR mAbs comes from two randomized clinical trials comparing panitumumab or cetuximab with best supportive care in heavily pretreated mCRC patients[33,36]. In the CO.17[36] and AMGEN[33] studies, a survival benefit over best supportive care was observed only in KRAS-WT patients treated with cetuximab (median OS: 9.5 mo *vs* 4.8 mo) or panitumumab (median PFS: 12.3 wk *vs* 7.3 wk), respectively. In patients with *KRAS* mutated tumours mAbs did not prolong PFS or OS as compared with best supportive care. Furthermore, the efficacy of first-[37-39] and second-line[40,41] chemotherapy improved when it was combined with anti-EGFR antibodies in the mCRC setting.

The cetuximab combined with irinotecan in first-line therapy for metastatic colorectal cancer phase Ⅲ study evaluated 599 patients receiving FOLFIRI with cetuximab and 599 patients receiving FOLFIRI alone[37]. Sixty-four per cent of the cases evaluated were exon 2-KRAS-WT, and both the risk of disease progression (HR of PFS: 0.68, 95%CI: 0.50-0.94) and death (HR of OS: 0.84, 95%CI: 0.64-1.11) were lower in cetuximab-treated patients. No difference in PFS or OS was noted when cetuximab was added to FOLFIRI in KRAS-MUT patients.

The fluorouracil, leucovorin, oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer study also evaluated the effect of *KRAS* mutational status on clinical outcome of patients[42]. This was a phase Ⅱ study in which 169 mCRC patients were randomized to receive FOLFOX-4 plus cetuximab, and 168 to receive FOLFOX-4 alone. Patients with *KRAS*-WT tumours treated with cetuximab had a significant increase in tumour response rate (61% *vs* 37%, OR = 2.54, $P = 0.011$) and a decrease in the risk of disease progression (HR = 0.57, $P = 0.016$) compared with patients receiving FOLFOX-4 alone. In the *KRAS* mutant population, patients treated with FOLFOX-4 plus cetuximab had a median PFS lower than patients administered FOLFOX-4 alone (8.6 mo *vs* 5.5 mo, respectively)[42].

Similarly, the addition of panitumumab to first-line chemotherapy with FOLFOX[39,43] and to second-line chemotherapy with FOLFIRI[41] significantly prolonged PFS and OS only in KRAS-WT mCRC patients.

KRAS AS PROGNOSTIC FACTOR

The role of KRAS as an independent prognostic marker in CRC is largely controversial. The CO.17 study[36] analysed the prognostic implications of KRAS status by assessing the interaction between KRAS status and survival in patients receiving best supportive care alone. There was no significant difference in median OS in patients with the KRAS-WT or KRAS-MUT (4.8 mo *vs* 4.6 mo, respectively, the HR for death in the KRAS mutant population was 1.01, $P = 0.97$).

Similarly, Kim *et al*[44] found that the response rate, PFS and OS did not differ between KRAS-WT and KRAS-MUT mCRC patients treated with chemotherapy alone. These findings suggested that KRAS is not a prognostic marker for CRC. In contrast, the results of a multivariate analysis of 89 mCRC patients treated with cetuximab after failure of irinotecan-based chemotherapy, suggested that *KRAS* mutational status is an independent prognostic factor in mCRC[45]. KRAS-MUT patients had a lower relative risk (RR) (0% *vs* 40%, $P < 0.001$), and a shorter PFS (median PFS: 10.1 wk *vs* 31.4 wk, $P = 0.0001$) and OS (median OS: 10.1 mo *vs* 14.3 mo, $P = 0.026$) than *KRAS*-WT patients. However, interpretation of these data should be tempered by the fact that it is difficult to differentiate the effect of *KRAS* mutational status on survival from the response to treatment in a patient population treated with cetuximab[46].

The RASCAL Collaborative Group evaluated thousands of patients with any-stage colorectal cancer to study the prognostic value of mutations in codons 12 and 13 of the *KRAS* gene[47,48]. They found that KRAS-MUT patients had a higher risk of disease progression and death compared with patients not carrying KRAS mutations, particularly when valine codon 12 was involved[47]. The RASCAL-2 study concluded that the G12V mutation in the *KRAS* gene at codon 12 increases the risks of recurrence or death only in Dukes' C colorectal tumours[48].

The retrospective analysis of mCRC patients enrolled in the MRC FOCUS trial[49] showed that KRAS mutations have a modest negative prognostic impact on OS (HR = 1.24, 95%CI: 1.06-1.46, $P = 0.008$) but not on PFS (HR, 1.14, 95%CI: 0.98-1.36, $P = 0.09$).

Contrasting results also emerged when the prognostic significance of *KRAS* mutations was evaluated in patients with non-metastatic colorectal cancer. In fact, in the retrospective analysis of more than 1050 stage Ⅱ-Ⅲ colon cancer patients enrolled in the PETACC-3 trial, *KRAS* mutations did not have any prognostic value regarding PFS or OS[50]. The QUASAR trial randomized stage Ⅱ-Ⅲ colon and rectal cancer patients to receive adjuvant 5FU-FA or nothing. Overall, *KRAS* mutant patients had a higher rate of recurrence (28% *vs* 21%, $P = 0.002$), but the difference was much more pronounced in a subgroup of rectal patients (43% *vs* 22%)[51].

TYPE OF *KRAS* MUTATION AND RESPONSE TO ANTI-EGFR MABS

Exon-2 *KRAS* mutations are highly specific negative biomarkers of the efficacy of anti-EGFR mAbs in mCRC. However, not all *KRAS* mutations are equal in terms of effect on cell proliferation and resistance to anti-EGFR inhibitors[52]. Preclinical data showed that cell lines with KRAS codon 13 glycine (G)-to-aspartate (D) mutations (p.G13D) respond to treatment with cetuximab similarly to WT clones[53]. Moreover, it has been reported that about 10% of patients carrying a *KRAS* mutation in tumour tissue respond to anti-EGFR mAbs[31,54,55] and a further 15% reach long-term disease stabilization[56]. In patients responding or showing long-term stabilization, codon 13 mutations are more frequent than in the overall KRAS-mutated tumour population.

A recent large retrospective analysis of 579 chemorefractory mCRC patients treated with cetuximab demonstrated that not all *KRAS* mutations are equally effective in predicting resistance to anti-EGFR mAbs[52]. Among patients receiving any cetuximab-based treatment, OS and PFS were significantly longer in patients bearing a p.G13D-mutation ($n = 32$) (median OS = 7.6 mo; median PFS = 4 mo) than in patients with other KRAS-mutated tumours (median OS = 5.7 mo; median PFS = 1.9 mo) in both univariate and multivariate analysis adjusted for potential prognostic factors and data set. Overall response rate did not differ significantly between patients with p.G13D mutations and other KRAS mutations (6.3% *vs* 1.6%, respectively, $P = 0.15$), There was a significant interaction between type of KRAS mutation (p.G13D *vs* other KRAS mutations) and OS benefit with cetuximab treatment (HR = 0.30, 95%CI: 0.14-0.67, $P = 0.003$).

The addition of cetuximab to first-line chemotherapy in patients with KRAS p.G13D mutations seemed to be beneficial in a pooled analysis of CRYSTAL and OPUS studies in which patients were randomized to receive FOLFIRI (CRYSTAL) or FOLFOX (OPUS) with or without cetuximab as first-line treatment for mCRC[57]. Among the 83 patients harbouring the *G13D* mutation, those receiving chemotherapy plus cetuximab had a better PFS and OS than did patients treated with chemotherapy alone, whereas patients with any other *KRAS* mutation did not benefit from combination therapy.

Peeters *et al*[58] examined the role of mutations in codons 12 or 13 of the *KRAS* gene in a pooled analysis of patients enrolled in three clinical trials in which panitumumab was added to FOLFOX4 in first-line treatment[59], to FOLFIRI in second-line treatment[41], or compared with best supportive care in heavily pre-treated mCRC patients[22]. They did not find that *G13D* mutation performs differently than codon 12 mutations, and thus concluded that codon 13 KRAS-MUT tumours are unlikely to benefit from panitumumab in the same way as

codon 12 KRAS-MUT tumours.

Two studies evaluated whether KRAS codon 61 and 146 mutations were associated with clinical outcome in mCRC patients treated with cetuximab[60,61]. Loupakis *et al*[60] found KRAS codon 61 and 146 mutations in 7 (8%) and 1 (1%) cases, respectively, among 87 patients with WT *KRAS* codons 12 and 13 treated with cetuximab plus irinotecan. None of the 8 patients with mutations in *KRAS* responded to the treatment, while 22 of the 68 WT cases (32.3%) did. *KRAS* 61 and 146 mutations caused a significantly shorter PFS (median PFS, 3.8 mo *vs* 5.1 mo, $P = 0.028$) as compared with *KRAS* 61/146 WT, whereas no significant differences were detected in OS (median OS, 9.7 mo *vs* 14.7 mo, respectively, $P = 0.39$). In the European Consortium study[61], 40% of patients harboured a *KRAS* mutation, 2.1% (16/747) in codon 61, and 2% (15/747) in codon 146. Patients with codon 61 mutations had a lower RR than WT patients.

In conclusion, assessment of KRAS codon 61/146 mutations might help to identify mCRC patients who may benefit from treatment with anti-EGFR mAbs. In fact, the recently updated guidelines on for cetuximab and panitumumab usage include the analysis of KRAS exon 3 codon 61 and exon 4 codon 146 mutations (www.emea.com).

BRAF

The BRAF protein is a cytoplasmic serine-threonine kinase that is mutated in approximately 7% of human cancers: specifically, in 8%-10% of sporadic CRCs[62]. The BRAF protein is one of the main effectors of KRAS, because it is immediately downstream of KRAS and it must be phosphorylated by KRAS to be activated. The point mutation V600E causes a classic CTG to CAG substitution at codon 600, which results in constitutive activation of the RAS/RAF/MEK/ERK pathway similar to *KRAS* mutations. *KRAS* and *BRAF* mutations are mutually exclusive in CRC[62].

Di Nicolantonio *et al*[62] performed a retrospective analysis of 113 tumour samples treated with cetuximab or panitumumab with or without chemotherapy. Seventy-nine patients with *KRAS*-WT were identified. In this cohort, 11 (13.9%) patients were *BRAF* mutants. None of them reported an objective tumour response.

In the CRYSTAL study[37], 9% (59 of 625) of patients had *BRAF* mutations and they had a shorter median OS in both the FOLFIRI (10.3 mo) and FOLFIRI/cetuximab (14.1 mo) arms compared with the *KRAS*-WT/*BRAF*-WT population in which survival was 21.6 and 25.1 mo, respectively. *BRAF*-MUT status was unrelated to cetuximab efficacy; thus the authors concluded that *BRAF* mutation is an indicator of poor prognosis, and that it did not predict the efficacy of cetuximab in their dataset. Also in the combined analysis of the CRYSTAL and OPUS results[63], *BRAF* mutation was found to be a negative prognostic marker. In fact, PFS and OS were lower in the *BRAF*-MUT population irrespective of treatment received.

Similarly, in a Dutch retrospective analysis of BRAF status conducted in patients treated with capecitabine/oxaliplatin/bevacizumab with or without cetuximab for mCRC, 8.7% of tested samples (45 of 519) had the BRAF V600E mutation[64,65]. Patients with BRAF mutations showed a shorter median PFS irrespective of treatment group. In a retrospective pooled study from the European Consortium that included 761 chemorefractory patients treated with cetuximab plus chemotherapy, De Roock *et al*[61] reported a 4.7% rate of BRAF mutation (35 patients with V600E mutations and 1 patient with mutation D548Gn). Compared with BRAF-WT subjects, *BRAF*-MUT patients had a significantly lower ORR (8.3% *vs* 38% for WT, OR = 0.15, $P = 0.0012$), and a shorter PFS (median, 8 wk *vs* 26 wk in WT, HR = 3.74, $P < 0.0001$) and OS (median, 26 wk *vs* 54 wk in WT, HR = 3.03, $P < 0.0001$).

BRAF mutation was a negative prognostic factor also in the PRIME trial[59]. In fact, patients with RAS-WT but *BRAF*-MUT tumours had a worse PFS and OS compared with subjects with both RAS and *BRAF* wild-type disease. In *RAS*-WT/*BRAF*-MUT subgroup, the addition of panitumumab to chemotherapy produced a small, statistically not significant benefit in term of DFS and OS ($P = 0.12$ and 0.76, respectively)[59].

The only trial to report that *BRAF* mutation plays both a prognostic and predictive role is the PICCOLO phase Ⅲ trial[66], which was designed to evaluate the addition of panitumumab to single-agent irinotecan as second- or subsequent-line therapy in 1198 prospectively tested *KRAS* WT mCRC patients. *BRAF*-MUT tumours (13.6%) showed a worse OS than *BRAF*-WT tumours, and the addition of panitumumab to irinotecan had a detrimental effect on survival (HR = 1.84, 95%CI: 1.10-3.08, $P = 0.029$).

In conclusion, *BRAF* mutation seems to be a negative prognostic factor rather than a negative predictive marker of the efficacy of anti-EGFR mAb. *BRAF*-MUT patients have a worse survival than *BRAF*-WT patients irrespective of treatment received. They may benefit from anti-EGFR mAB, but to a significantly lesser extent than B*RAF*-WT patients.

NEUROBLASTOMA-RAS

Neuroblastoma-ras (NRAS) is a member of the RAS oncogene family and is located on chromosome 1. The product of this gene is a GTPase enzyme membrane protein that shuttles between the Golgi apparatus and the cellular membrane. *KRAS*, *BRAF* and *NRAS* mutations are mutually exclusive[67]. In CRC, the *NRAS* mutation rate is 3%-5%[61]. *NRAS* mutations are associated with lack of response to treatment with cetuximab[61]. In the study by De Roock *et al*[61], *NRAS*-mutant patients treated with either cetuximab or panitumumab (2.6% of 644 *KRAS*-WT subjects) had a significantly lower ORR than *NRAS*-WT patients (7.7% *vs* 38.1%). Progression-free

survival and OS did not differ statistically between mutated and not mutated patients. In contrast, in the COIN trial[68], which failed to demonstrate a survival benefit with the addition of cetuximab to first-line oxaliplatin-based chemotherapy in exon 2 *KRAS*-WT mCRC patients, no benefit in OS was obtained with the combination treatment, even in the subgroup of KRAS/NRAS/BRAF-WT subjects. The presence of any mutation in the *KRAS, NRAS* or *BRAF* genes negatively affected OS, regardless of treatment received.

A retrospective evaluation of biomarkers in patients enrolled in the PRIME trial indicated that NRAS plays an important role in predicting the efficacy of panitumumab. Among the 656 patients with *KRAS*-WT exon 2, 108 (17%) had other mutations in *KRAS* exon 3 or 4, in *NRAS* exons 2, 3 or 4, or in *BRAF* exon 15[59]. Patients with *KRAS*-WT exon 2 tumours bearing any other *RAS* mutation did not benefit from the addition of panitumumab to FOLFOX (median OS 17.1 mo *vs* 17.1 mo, $P = 0.12$). In contrast, patients with "all RAS" wild-type tumours (namely, wild-type for *KRAS* exons 2/3/4 and for *NRAS* exons 2/3/4) significantly benefited from the combination treatment (median OS 25.8 mo *vs* 20.2 mo, HR = 0.77, 95%CI: 0.64-0.94, $P = 0.009$).

The FIRE-3 phase Ⅲ trial (AIO KRK-0306), conducted at 150 German and Austrian cancer centres, involved a head-to-head comparison of FOLFIRI plus either cetuximab or bevacizumab as first-line treatment in patients with mCRC. Of the 752 patients enrolled, *KRAS* wild-type tumours were identified in 592 patients who were then randomized 1:1 to receive first-line FOLFIRI every two weeks plus either cetuximab at 400 mg/m^2 on day 1 followed by 250 mg/m^2 weekly (arm A) or bevacizumab at 5 mg/kg every 2 wk (arm B). The primary analysis showed a median OS almost 4 mo longer in arm A *vs* arm B. The results of a pre-planned analysis of the effect of KRAS mutations in exon 3 (codon 59/61) and exon 4 (codon 117/146), of *NRAS* mutations in exon 2 (cordons 12/13), exon 3 (codons 59/61) and exon 4 (codons 117/146), and of the *BRAF* V600E mutation on treatment efficacy were presented at the 2013 ESMO Congress[69]. Mutational analyses were done by pyrosequencing in 396 of 592 randomised patients (66.9%). Among patients with "all RAS" wild-type tumours, OS was significantly longer with FOLFIRI/cetuximab than with FOLFIRI/bevacizumab (33.1 mo *vs* 25.6 mo, $P = 0.01$). The OS HR for adding cetuximab *vs* bevacizumab in patients with all RAS-WT tumours was 0.70 ($P = 0.01$). Overall survival was the same, 20.6 mo *vs* 20.3 mo, respectively, in patients with mutated RAS treated with either cetuximab or bevacizumab. A similar trend was observed in the association between PFS and mutational status; PFS was slightly longer in patients with RAS-WT treated with FOLFIRI/cetuximab than in those treated with FOLFIRI/bevacizumab: 10.4 mo *vs* 10.2 mo ($P = 0.54$) respectively. The HR for PFS in RAS-WT patients was 0.93 ($P = 0.63$). In the presence of any RAS mutation, PFS was significantly longer in patients administered bevacizumab therapy than in patients treated with cetuximab: 12.2 mo *vs* 6.1 mo ($P = 0.004$). These findings may impact on clinical decisions because RAS mutational analysis may identify the subgroup of patients who are more likely to benefit from first-line cetuximab with respect to bevacizumab.

PI3KCA

Activation of phosphatidylinositol 3-kinase (PI3K) caused by TK located at the cytoplasmic surface of the cell wall, results in the phosphorylation of PIP2 to phosphatidylinositol 3-kinase (PIP3). Phosphatidylinositol 3-kinase activates AKT, which in turn triggers downstream pathways thereby promoting cell survival and proliferation. The *PIK3CA* gene encodes the catalytic subunit for PI3K, and mutations within this gene result in aberrant AKT stimulation, which in turn promote the growth of various cancers[70,71]. Although *PIK3CA* mutations account for 10%-20% of CRC tumours, their effect on patient outcome is not yet clear[21,29,72].

Ogino *et al*[72] examined the tumour tissue of 450 patients with resected stage Ⅰ-Ⅲ colon cancer from two independent prospective cohort studies and found that 82 patients (18%) had *PIK3CA* mutations in exons 9 and 20[72]. They concluded that *PIK3CA* mutation increases cancer-specific mortality in patients with *KRAS*-WT tumours (HR = 3.80, 95%CI: 1.56-9.27), but seemed to have no significant effect on mortality in *KRAS*-MUT patients (HR = 1.25, 95%CI: 0.585-2.96). In contrast, in the metastatic setting, a *PIK3CA* mutation was identified in 17.7% (14/85) of cetuximab-treated mCRC patients, but ORR, time-to-progression and OS did not differ between mutated and non-mutated patients[73]. Moreover, the *PIK3CA* mutation was unrelated to outcome in *KRAS*-WT tumours treated in the cetuximab arm of the CAIRO 2 study[74]: 5-year survival was 90% in *PIK3CA*-WT and 82% in *PIK3CA* mutants (log-rank $P = 0.075$).

Two European groups published opposite results regarding the predictive role of *PIK3CA* mutations in anti-EGFR mAbs resistance. Sartore-Bianchi *et al*[70] analysed exons 9 and 20 *PIK3CA* mutational status in 110 mCRC patients treated with cetuximab or panitumumab. Fifteen patients (13.6%) had *PIK3CA* mutations, and none of them responded to treatment with anti-EGFR mAbs ($P = 0.038$). The correlation between *PIK3CA* mutations and lack of response was even stronger in the *KRAS*-WT subgroup of patients ($P = 0.016$). The authors concluded that *PIK3CA* mutations could help to distinguish potential non-responders to anti-EGFR mAbs within the KRAS-WT subpopulation. On the other hand, in a similar study, Prenen *et al*[71] analyzed *PIK3CA* mutations in 200 patients with chemorefractory mCRC, and did not find a link between PIK3CA and anti-EGFR mAb resistance. Thirteen percent (5/39) of their patients with *PIK3CA* mutations responded to cetuximab, and 11% (18/160) did not ($P = 0.78$). The data from the largest European analysis[61] conducted so far (773 DNA samples

from patients treated with cetuximab from 11 centres) explained the discrepancies between the Sartore-Bianchi *et al*[70] and Prenen *et al*[71] studies. In these studies, 14.5% of samples harboured *PIK3CA* mutations and a lower response rate to cetuximab was associated with *PIK3CA* exon 20 mutations but not to those with mutations in exon 9[61]. The Sartore-Bianchi *et al*[70] cohort had more exon 20 mutations (73%) than exon 9 mutations (27%), whereas the Prenen *et al*[71] cohort had the opposite - 13% and 78%, respectively[75].

Based on these results, *PIK3CA* mutations, even though promising, need further prospective studies before they can be used in clinical practice.

PTEN

PTEN functions as a key tumour suppressor gene that is involved in the homeostatic maintenance of PI3K/AKT signalling. In wild type cells, signal transduction events deriving from EGFR activation and directed *via* PI3K are balanced by the presence of this negative regulatory molecule. Loss of PTEN function causes increased levels of phosphatidylinositol-3,4,5-triphosphate (PIP-3), the major substrate of PTEN, thereby resulting in persistent activation of PI3K effectors[76,77]. PTEN activity can be altered by gene mutations (5%), allelic losses at chromosome 10q23 (23%), and epigenetic mechanisms such as hypermethylation of the PTEN promoter region (19.9% in CRC with MSI-H *vs* 2.2% in MSI-L)[78]. Loss of PTEN protein expression evaluated by immunohistochemistry is present in between 20% and 40% of unselected CRC cases[32]. Moreover, it is associated with *KRAS*, *BRAF* and *PIK3CA* mutations, and EGFR polysomy. Interestingly, while there is concordance of KRAS status in primary and distant tumours[79,80], PTEN expression is concordant in the primary tumour and metastases only in 60% of cases[35,79] because PTEN loss is more frequent in distant metastases than in the primary tumour. Preclinical analyses have also shown that PTEN loss provokes resistance to cetuximab-induced apoptosis in CRC cell lines[28].

Frattini *et al*[31] found that none of 11 patients expressing lower levels of PTEN responded to combination treatment with irinotecan and cetuximab, whereas 10 (63%) of 16 patients with intact PTEN protein expression had a partial response. Loupakis *et al*[35] recently reported that low expression of PTEN as measured by immunohistochemistry in the primary tumour did not affect response rate, PFS or OAS of mCRC patients treated with cetuximab plus irinotecan. By contrast, when PTEN was measured in the metastatic samples, ORR and PFS were significantly better in patients with high PTEN expression than in those with low PTEN expression: 26% *vs* 5% (P = 0.007) and 4.7 mo *vs* 3.3 mo (P = 0.005), respectively. Sartore-Bianchi *et al*[70] found that loss of PTEN was related to lack of response to cetuximab and panitumumab (P = 0.001), and to a shorter PFS and worse OS in 81 tumour specimens of mCRC. Differently, Laurent-Puig *et al*[81] showed PTEN protein expression did not affect ORR or PFS in 162 *KRAS*-WT mCRC patients treated with cetuximab, but PTEN loss was associated with a shorter OS (P = 0.013). The reasons for these discrepancies are not clear. It is difficult to interpret these data because of the small sample size, the presence of *KRAS* mutant tumours and the fact that PTEN expression was evaluated in primary tumours and/or in metastatic sites. Moreover, evaluation of PTEN protein expression by immunohistochemistryor other methods (*i.e.*, western blot) is influenced by the subjective parameter of the interpreter in the definition of cut-off levels. Therefore, these data should be considered exploratory and the value of PTEN as a predictive or prognostic marker in CRC cannot yet be established.

CHEMOTHERAPY BACKBONE FOR ANTI-EGFR MABS

The choice of chemotherapy backbone may influence the efficacy of EGFR-targeted mAbs. The COIN study[68] investigated the use of cetuximab in addition to oxaliplatin-based chemotherapy (66.1% with capecitabine and 33.9% with infusional 5FU) in the first-line setting for mCRC. Surprisingly, the addition of cetuximab to oxaliplatin-based chemotherapy did not increase OS or PFS compared with chemotherapy alone, even in the KRAS-WT subpopulation.

The CRYSTAL[37] and OPUS[42] studies, that demonstrated benefit from the addition of cetuximab to chemotherapy, both used infusional 5-FU regimens (FOLFIRI and FOLFOX, respectively). Similarly, in the COIN trial, the subgroup of patients treated with infusional 5FU benefited from cetuximab treatment (OS: HR = 0.72, 95%CI: 0.53-0.98), whereas those treated with capecitabine did not (OS: HR = 1.02, 95%CI: 0.82-1.26), although the formal test for interaction was not significant (P = 0.10)[68]. The NORDIC VII[82] trial also reported that no benefits came from adding cetuximab to oxaliplatin-based regimen (with bolus 5FU). The use of oral and bolus administration of fluoropyrimidines in COIN and NORDIC VII studies (instead of an infusional regimen) may explain the lack of benefit in survival. However, this hypothesis seems to be confuted by the FUTURE randomised phase Ⅱ study, which compared cetuximab plus oxaliplatin + UFT (oral fluoropyrimidine) with cetuximab plus FOLFOX4 (infusional fluoropyrimidine) as first-line treatment for mCRC patients. In fact, in the *KRAS*-WT population, median PFS and OS were very similar (PFS: 9.2 mo *vs* 6.8 mo, P = 0.18; OS: 20.8 mo *vs* 20.1 mo, P = 0.74) in the two arms using oral or infusional fluoropyrimidine[83].

Although the results of COIN and NORDIC VII studies seem to cast doubts on the efficacy of the oxaliplatin + cetuximab combination, significant benefits in survival from the addition of anti-EGFR mAbs to FOLFOX4 were reported in the PRIME[39] and OPUS[84] trials. Moreover, in the randomised phase Ⅱ AIO 0104[85] and CELIM[86] trials, which used oral and infusional fluoro-

Table 1 New approach integrates novel molecular biomarkers with the pathologic features

Biomarker	Incidence	Prognostic value	Predictive value
B-RAF mutations	4%-15%	Poor prognosis[38,49,62,64,66,68,72,74,83]	Controversial data
K-RAS mutations	40%	Controversial data[36,65,68,72,86]	Major predictor of resistance to anti-EGFR mAbs[24,37,39,56,61]
Mut G13D	15%-20%		Weaker resistance[52]
N-RAS mutations	3%-5%		Predictor of resistance[59,69]
PI3KCA mutations	10%-20%	Conflicting results[28,30,61,65,70,71,73]	Controversial data[61,70,71,73]
PTEN status	20%-40%	Conflicting results[31,32,35,81,87]	Controversial data[31,70]

pyrimidines, respectively, the activity of cetuximab was similar in oxaliplatin- and irinotecan-containing regimens.

Lastly, it is now clear that cetuximab or panitumumab should not be added to an oxaliplatin-based chemotherapy regimen in patients with any mutation in exon 2/3/4 *KRAS* or *NRAS* genes or in patients with unknown mutational status, because it exerts a detrimental effect on survival (EMA protocol number 000741-II/50, 25 July 2013; EMA protocol number 702774/2013, 21 November 2013).

CONCLUSION

Before the era of personalised medicine, cancer prognosis and treatment decisions were made mainly based on the histopathologic characteristics of the tumour. The new approach integrates novel molecular biomarkers with the pathologic features of a tumour to improve the prediction of prognosis and treatment efficacy (Table 1). During recent years, attempts have been made to understand the molecular mechanisms underlying resistance to EGFR inhibitors to refine the selection of candidates for these treatments, and improve the clinical outcome of patients treated with anti-EGFR mAbs. Encouraging progress has been made in our understanding the behaviour of mCRC at molecular level, which led to the discovery of other biomarkers of resistance to anti-EGFR mAbs besides KRAS. The development of new technologies, such as next-generation sequencing, will enable the simultaneous analysis of many genes, and lead to the identification and validation of predictive biomarkers for existing and new target therapies. Translating discoveries from bench to clinical practice is a costly and time-consuming process, but the benefits of personalised medicine justify this effort.

ACKNOWLEDGMENTS

We thank Jean Ann Gilder (Scientific Communication srl, Naples, Italy) for linguistic revision and editing.

REFERENCES

1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2013. *CA Cancer J Clin* 2013; **63**: 11-30 [PMID: 23335087 DOI: 10.3322/caac.21166]
2 **Koehler A**, Bataille F, Schmid C, Ruemmele P, Waldeck A, Blaszyk H, Hartmann A, Hofstaedter F, Dietmaier W. Gene expression profiling of colorectal cancer and metastases divides tumours according to their clinicopathological stage. *J Pathol* 2004; **204**: 65-74 [PMID: 15307139 DOI: 10.1002/path.1606]
3 **Ries L**, Melbert D, Krapcho M. SEER Cancer Statistics Review, 1975-2005. Bethesda, MD: National Cancer Institute, 2008: 1975-2005
4 **Greenwald P**. Colon cancer overview. *Cancer* 1992; **70**: 1206-1215 [PMID: 1511368]
5 **Field K**, Lipton L. Metastatic colorectal cancer-past, progress and future. *World J Gastroenterol* 2007; **13**: 3806-3815 [PMID: 17657834]
6 **Adam R**, Hoti E, Folprecht G, Benson AB. Accomplishments in 2008 in the management of curable metastatic colorectal cancer. *Gastrointest Cancer Res* 2009; **3**: S15-S22 [PMID: 20011559]
7 **Rees M**, Tekkis PP, Welsh FK, O'Rourke T, John TG. Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. *Ann Surg* 2008; **247**: 125-135 [PMID: 18156932 DOI: 10.1097/SLA.0b013e31815aa2c2]
8 **Fiorentino F**, Hunt I, Teoh K, Treasure T, Utley M. Pulmonary metastasectomy in colorectal cancer: a systematic review and quantitative synthesis. *J R Soc Med* 2010; **103**: 60-66 [PMID: 20118336 DOI: 10.1258/jrsm.2009.090299]
9 **Bilchik AJ**, Poston G, Adam R, Choti MA. Prognostic variables for resection of colorectal cancer hepatic metastases: an evolving paradigm. *J Clin Oncol* 2008; **26**: 5320-5321 [PMID: 18936470 DOI: 10.1200/JCO.2008.18.3152]
10 **Barone C**, Nuzzo G, Cassano A, Basso M, Schinzari G, Giuliante F, D'Argento E, Trigila N, Astone A, Pozzo C. Final analysis of colorectal cancer patients treated with irinotecan and 5-fluorouracil plus folinic acid neoadjuvant chemotherapy for unresectable liver metastases. *Br J Cancer* 2007; **97**: 1035-1039 [PMID: 17895897 DOI: 10.1038/sj.bjc.6603988]
11 **Pozzo C**, Basso M, Cassano A, Quirino M, Schinzari G, Trigila N, Vellone M, Giuliante F, Nuzzo G, Barone C. Neoadjuvant treatment of unresectable liver disease with irinotecan and 5-fluorouracil plus folinic acid in colorectal cancer patients. *Ann Oncol* 2004; **15**: 933-939 [PMID: 15151951]
12 **Wang CC**, Li J. An update on chemotherapy of colorectal liver metastases. *World J Gastroenterol* 2012; **18**: 25-33 [PMID: 22228967 DOI: 10.3748/wjg.v18.i1.25]
13 Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. Advanced Colorectal Cancer Meta-Analysis Project. *J Clin Oncol* 1992; **10**: 896-903 [PMID: 1534121]
14 **de Gramont A**, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F, Bonetti A. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000; **18**: 2938-2947 [PMID: 10944126]
15 **Douillard JY**, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alakl M, Gruia G, Awad L, Rougier P. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre ran-

domised trial. *Lancet* 2000; **355**: 1041-1047 [PMID: 10744089]
16 **Grothey A**, Sargent D. Overall survival of patients with advanced colorectal cancer correlates with availability of fluorouracil, irinotecan, and oxaliplatin regardless of whether doublet or single-agent therapy is used first line. *J Clin Oncol* 2005; **23**: 9441-9442 [PMID: 16361649 DOI: 10.1200/JCO.2005.04.4792]
17 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342 [PMID: 15175435 DOI: 10.1056/NEJMoa032691]
18 **Cunningham D**, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345 [PMID: 15269313 DOI: 10.1056/NEJMoa033025]
19 **Dienstmann R**, Vilar E, Tabernero J. Molecular predictors of response to chemotherapy in colorectal cancer. *Cancer J* 2011; **17**: 114-126 [PMID: 21427555 DOI: 10.1097/PPO.0b013e318212f844]
20 **Markman B**, Javier Ramos F, Capdevila J, Tabernero J. EGFR and KRAS in colorectal cancer. *Adv Clin Chem* 2010; **51**: 71-119 [PMID: 20857619]
21 **Heinemann V**, Stintzing S, Kirchner T, Boeck S, Jung A. Clinical relevance of EGFR- and KRAS-status in colorectal cancer patients treated with monoclonal antibodies directed against the EGFR. *Cancer Treat Rev* 2009; **35**: 262-271 [PMID: 19117687 DOI: 10.1016/j.ctrv.2008.11.005]
22 **Van Cutsem E**, Peeters M, Siena S, Humblet Y, Hendlisz A, Neyns B, Canon JL, Van Laethem JL, Maurel J, Richardson G, Wolf M, Amado RG. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol* 2007; **25**: 1658-1664 [PMID: 17470858 DOI: 10.1200/JCO.2006.08.1620]
23 **Siena S**, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 2009; **101**: 1308-1324 [PMID: 19738166 DOI: 10.1093/jnci/djp280]
24 **Lièvre A**, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, Côté JF, Tomasic G, Penna C, Ducreux M, Rougier P, Penault-Llorca F, Laurent-Puig P. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006; **66**: 3992-3995 [PMID: 16618717 DOI: 10.1158/0008-5472.CAN-06-0191]
25 **Barault L**, Veyrie N, Jooste V, Lecorre D, Chapusot C, Ferraz JM, Lièvre A, Cortet M, Bouvier AM, Rat P, Roignot P, Faivre J, Laurent-Puig P, Piard F. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. *Int J Cancer* 2008; **122**: 2255-2259 [PMID: 18224685 DOI: 10.1002/ijc.23388]
26 **Tan YH**, Liu Y, Eu KW, Ang PW, Li WQ, Salto-Tellez M, Iacopetta B, Soong R. Detection of BRAF V600E mutation by pyrosequencing. *Pathology* 2008; **40**: 295-298 [PMID: 18428050 DOI: 10.1080/00313020801911512]
27 **Calistri D**, Rengucci C, Seymour I, Lattuneddu A, Polifemo AM, Monti F, Saragoni L, Amadori D. Mutation analysis of p53, K-ras, and BRAF genes in colorectal cancer progression. *J Cell Physiol* 2005; **204**: 484-488 [PMID: 15702478 DOI: 10.1002/jcp.20310]
28 **Jhawer M**, Goel S, Wilson AJ, Montagna C, Ling YH, Byun DS, Nasser S, Arango D, Shin J, Klampfer L, Augenlicht LH, Perez-Soler R, Mariadason JM. PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal growth factor receptor inhibitor cetuximab. *Cancer Res* 2008; **68**: 1953-1961 [PMID: 18339877 DOI: 10.1158/0008-5472.CAN-07-5659]
29 **Velho S**, Oliveira C, Ferreira A, Ferreira AC, Suriano G, Schwartz S, Duval A, Carneiro F, Machado JC, Hamelin R, Seruca R. The prevalence of PIK3CA mutations in gastric and colon cancer. *Eur J Cancer* 2005; **41**: 1649-1654 [PMID: 15994075 DOI: 10.1016/j.ejca.2005.04.022]
30 **Moroni M**, Veronese S, Benvenuti S, Marrapese G, Sartore-Bianchi A, Di Nicolantonio F, Gambacorta M, Siena S, Bardelli A. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. *Lancet Oncol* 2005; **6**: 279-286 [PMID: 15863375 DOI: 10.1016/S1470-2045(05)70102-9]
31 **Frattini M**, Saletti P, Romagnani E, Martin V, Molinari F, Ghisletta M, Camponovo A, Etienne LL, Cavalli F, Mazzucchelli L. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. *Br J Cancer* 2007; **97**: 1139-1145 [PMID: 17940504 DOI: 10.1038/sj.bjc.6604009]
32 **Sartore-Bianchi A**, Di Nicolantonio F, Nichelatti M, Molinari F, De Dosso S, Saletti P, Martini M, Cipani T, Marrapese G, Mazzucchelli L, Lamba S, Veronese S, Frattini M, Bardelli A, Siena S. Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. *PLoS One* 2009; **4**: e7287 [PMID: 19806185 DOI: 10.1371/journal.pone.0007287]
33 **Amado RG**, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson SD, Chang DD. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 1626-1634 [PMID: 18316791 DOI: 10.1200/JCO.2007.14.7116]
34 **Wong R**, Cunningham D. Using predictive biomarkers to select patients with advanced colorectal cancer for treatment with epidermal growth factor receptor antibodies. *J Clin Oncol* 2008; **26**: 5668-5670 [PMID: 19001346 DOI: 10.1200/JCO.2008.19.5024]
35 **Loupakis F**, Pollina L, Stasi I, Ruzzo A, Scartozzi M, Santini D, Masi G, Graziano F, Cremolini C, Rulli E, Canestrari E, Funel N, Schiavon G, Petrini I, Magnani M, Tonini G, Campani D, Floriani I, Cascinu S, Falcone A. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from cetuximab plus irinotecan for patients with metastatic colorectal cancer. *J Clin Oncol* 2009; **27**: 2622-2629 [PMID: 19398573 DOI: 10.1200/JCO.2008.20.2796]
36 **Karapetis CS**, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008; **359**: 1757-1765 [PMID: 18946061 DOI: 10.1056/NEJMoa0804385]
37 **Van Cutsem E**, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 1408-1417 [PMID: 19339720 DOI: 10.1056/NEJMoa0805019]
38 **Van Cutsem E**, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P, Ciardiello F. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011; **29**: 2011-2019 [PMID: 21502544 DOI: 10.1200/JCO.2010.33.5091]
39 **Douillard JY**, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M, Gansert J. Ran-

domized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010; **28**: 4697-4705 [PMID: 20921465 DOI: 10.1200/JCO.2009.27.4860]

40 **Sobrero AF**, Maurel J, Fehrenbacher L, Scheithauer W, Abubakr YA, Lutz MP, Vega-Villegas ME, Eng C, Steinhauer EU, Prausova J, Lenz HJ, Borg C, Middleton G, Kröning H, Luppi G, Kisker O, Zubel A, Langer C, Kopit J, Burris HA. EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 2311-2319 [PMID: 18390971 DOI: 10.1200/JCO.2007.13.1193]

41 **Peeters M**, Price TJ, Cervantes A, Sobrero AF, Ducreux M, Hotko Y, André T, Chan E, Lordick F, Punt CJ, Strickland AH, Wilson G, Ciuleanu TE, Roman L, Van Cutsem E, Tzekova V, Collins S, Oliner KS, Rong A, Gansert J. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. *J Clin Oncol* 2010; **28**: 4706-4713 [PMID: 20921462 DOI: 10.1200/JCO.2009.27.6055]

42 **Bokemeyer C**, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, Donea S, Ludwig H, Schuch G, Stroh C, Loos AH, Zubel A, Koralewski P. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009; **27**: 663-671 [PMID: 19114683 DOI: 10.1200/JCO.2008.20.8397]

43 **Oliner K**, Douillard J, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocáková I, Ruff P, Blasinska-Morawiec M, Smakal M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson S. Analysis of KRAS/NRAS and BRAF mutations in the phase III PRIME study of panitumumab (pmab) plus FOLFOX versus FOLFOX as first-line treatment (tx) for metastatic colorectal cancer (mCRC). *J Clin Oncol* 2013; **31** (Suppl 15): Abstr3511

44 **Kim ST**, Park KH, Kim JS, Shin SW, Kim YH. Impact of KRAS Mutation Status on Outcomes in Metastatic Colon Cancer Patients without Anti-Epidermal Growth Factor Receptor Therapy. *Cancer Res Treat* 2013; **45**: 55-62 [PMID: 23613671 DOI: 10.4143/crt.2013.45.1.55]

45 **Lièvre A**, Bachet JB, Boige V, Cayre A, Le Corre D, Buc E, Ychou M, Bouché O, Landi B, Louvet C, André T, Bibeau F, Diebold MD, Rougier P, Ducreux M, Tomasic G, Emile JF, Penault-Llorca F, Laurent-Puig P. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 2008; **26**: 374-379 [PMID: 18202412 DOI: 10.1200/JCO.2007.12.5906]

46 **Garassino MC**, Farina G, Rossi A, Martelli O, Torri V. Should KRAS mutations be considered an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab? *J Clin Oncol* 2008; **26**: 2600; author reply 2601-2602 [PMID: 18487581 DOI: 10.1200/JCO.2008.16.8195]

47 **Andreyev HJ**, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J Natl Cancer Inst* 1998; **90**: 675-684 [PMID: 9586664]

48 **Andreyev HJ**, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, Young J, Walsh T, Ward R, Hawkins N, Beranek M, Jandik P, Benamouzig R, Jullian E, Laurent-Puig P, Olschwang S, Muller O, Hoffmann I, Rabes HM, Zietz C, Troungos C, Valavanis C, Yuen ST, Ho JW, Croke CT, O'Donoghue DP, Giaretti W, Rapallo A, Russo A, Bazan V, Tanaka M, Omura K, Azuma T, Ohkusa T, Fujimori T, Ono Y, Pauly M, Faber C, Glaesener R, de Goeij AF, Arends JW, Andersen SN, Lövig T, Breivik J, Gaudernack G, Clausen OP, De Angelis PD, Meling GI, Rognum TO, Smith R, Goh HS, Font A, Rosell R, Sun XF, Zhang H, Benhattar J, Losi L, Lee JQ, Wang ST, Clarke PA, Bell S, Quirke P, Bubb VJ, Piris J, Cruickshank NR, Morton D, Fox JC, Al-Mulla F, Lees N, Hall CN, Snary D, Wilkinson K, Dillon D, Costa J, Pricolo VE, Finkelstein SD, Thebo JS, Senagore AJ, Halter SA, Wadler S, Malik S, Krtolica K, Urosevic N. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer* 2001; **85**: 692-696 [PMID: 11531254 DOI: 10.1054/bjoc.2001.1964]

49 **Richman SD**, Seymour MT, Chambers P, Elliott F, Daly CL, Meade AM, Taylor G, Barrett JH, Quirke P. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol* 2009; **27**: 5931-5937 [PMID: 19884549 DOI: 10.1200/JCO.2009.22.4295]

50 **Roth AD**, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B, Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van Cutsem E, Bosman F. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol* 2010; **28**: 466-474 [PMID: 20008640 DOI: 10.1200/JCO.2009.23.3452]

51 **Hutchins G**, Southward K, Handley K, Magill L, Beaumont C, Stahlschmidt J, Richman S, Chambers P, Seymour M, Kerr D, Gray R, Quirke P. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol* 2011; **29**: 1261-1270 [PMID: 21383284 DOI: 10.1200/JCO.2010.30.1366]

52 **De Roock W**, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, Siena S, Lamba S, Arena S, Frattini M, Piessevaux H, Van Cutsem E, O'Callaghan CJ, Khambata-Ford S, Zalcberg JR, Simes J, Karapetis CS, Bardelli A, Tejpar S. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. *JAMA* 2010; **304**: 1812-1820 [PMID: 20978259 DOI: 10.1001/jama.2010.1535]

53 **Molinari F**, Felicioni L, Buscarino M, De Dosso S, Buttitta F, Malatesta S, Movilia A, Luoni M, Boldorini R, Alabiso O, Girlando S, Soini B, Spitale A, Di Nicolantonio F, Saletti P, Crippa S, Mazzucchelli L, Marchetti A, Bardelli A, Frattini M. Increased detection sensitivity for KRAS mutations enhances the prediction of anti-EGFR monoclonal antibody resistance in metastatic colorectal cancer. *Clin Cancer Res* 2011; **17**: 4901-4914 [PMID: 21632860 DOI: 10.1158/1078-0432.CCR-10-3137]

54 **Lee CN**, Chen HY, Liu HE. Favorable response to erlotinib in a lung adenocarcinoma with both epidermal growth factor receptor exon 19 deletion and K-ras G13D mutations. *J Clin Oncol* 2010; **28**: e111-e112 [PMID: 20008635 DOI: 10.1200/JCO.2009.24.0747]

55 **Benvenuti S**, Sartore-Bianchi A, Di Nicolantonio F, Zanon C, Moroni M, Veronese S, Siena S, Bardelli A. 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* 2007; **67**: 2643-2648 [PMID: 17363584 DOI: 10.1158/0008-5472.CAN-06-4158]

56 **De Roock W**, Piessevaux H, De Schutter J, Janssens M, De Hertogh G, Personeni N, Biesmans B, Van Laethem JL, Peeters M, Humblet Y, Van Cutsem E, Tejpar S. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 2008; **19**: 508-515 [PMID: 17998284 DOI: 10.1093/annonc/mdm496]

57 **Tejpar S**, Bokemeyer C, Celik I, Schlichting M, Sartorius U, Van Cutsem E. Influence of KRAS G13D mutations on outcome in patients with metastatic colorectal cancer (mCRC) treated with first-line chemotherapy with or without cetuximab. *J Clin Oncol* 2011; **29** (Suppl 15): Abstr3511

58 **Peeters M**, Douillard JY, Van Cutsem E, Siena S, Zhang K, Williams R, Wiezorek J. Mutant KRAS codon 12 and 13 alleles in patients with metastatic colorectal cancer: assessment as prognostic and predictive biomarkers of response to panitumumab. *J Clin Oncol* 2013; **31**: 759-765 [PMID: 23182985 DOI: 10.1200/JCO.2012.45.1492]

59 **Douillard JY**, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013; **369**: 1023-1034 [PMID: 24024839 DOI: 10.1056/NEJMoa1305275]

60 **Loupakis F**, Ruzzo A, Cremolini C, Vincenzi B, Salvatore L, Santini D, Masi G, Stasi I, Canestrari E, Rulli E, Floriani I, Bencardino K, Galluccio N, Catalano V, Tonini G, Magnani M, Fontanini G, Basolo F, Falcone A, Graziano F. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer* 2009; **101**: 715-721 [PMID: 19603018 DOI: 10.1038/sj.bjc.6605177]

61 **De Roock W**, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010; **11**: 753-762 [PMID: 20619739 DOI: 10.1016/S1470-2045(10)70130-3]

62 **Di Nicolantonio F**, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 5705-5712 [PMID: 19001320 DOI: 10.1200/JCO.2008.18.0786]

63 **Bokemeyer C**, Van Cutsem E, Rougier P, Ciardiello F, Heeger S, Schlichting M, Celik I, Köhne CH. Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. *Eur J Cancer* 2012; **48**: 1466-1475 [PMID: 22446022 DOI: 10.1016/j.ejca.2012.02.057]

64 **Tol J**, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. *N Engl J Med* 2009; **361**: 98-99 [PMID: 19571295 DOI: 10.1056/NEJMc0904160]

65 **Tol J**, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG, Erdkamp FL, Vos AH, van Groeningen CJ, Sinnige HA, Richel DJ, Voest EE, Dijkstra JR, Vink-Börger ME, Antonini NF, Mol L, van Krieken JH, Dalesio O, Punt CJ. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 563-572 [PMID: 19196673 DOI: 10.1056/NEJMoa0808268]

66 **Seymour MT**, Brown SR, Middleton G, Maughan T, Richman S, Gwyther S, Lowe C, Seligmann JF, Wadsley J, Maisey N, Chau I, Hill M, Dawson L, Falk S, O'Callaghan A, Benstead K, Chambers P, Oliver A, Marshall H, Napp V, Quirke P. Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. *Lancet Oncol* 2013; **14**: 749-759 [PMID: 23725851 DOI: 10.1016/S1470-2045(13)70163-3]

67 **Hawkes E**, Cunningham D. Relationship between colorectal cancer biomarkers and response to epidermal growth factor receptor monoclonal antibodies. *J Clin Oncol* 2010; **28**: e529-e531; author reply e532-e533 [PMID: 20679615 DOI: 10.1200/JCO.2010.29.5626]

68 **Maughan TS**, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, Idziaszczyk S, Harris R, Fisher D, Kenny SL, Kay E, Mitchell JK, Madi A, Jasani B, James MD, Bridgewater J, Kennedy MJ, Claes B, Lambrechts D, Kaplan R, Cheadle JP. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011; **377**: 2103-2114 [PMID: 21641636 DOI: 10.1016/S0140-6736(11)60613-2]

69 **Stintzing S**, Jung A, Rossius L, Modest DP, Fischer von Weikersthal L, Decker T, Möhler M, Scheithauer W, Kirchner T, Heinemann V. Analysis of KRAS/NRAS and BRAF mutations in FIRE-3: A randomized phase III study of FOLFIRI plus cetuximab or bevacizumab as first-line treatment for wild-type (WT) KRAS (exon 2) metastatic colorectal cancer (mCRC) patients. Europe: ECCO/ESMO, 2013

70 **Sartore-Bianchi A**, Martini M, Molinari F, Veronese S, Nichelatti M, Artale S, Di Nicolantonio F, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res* 2009; **69**: 1851-1857 [PMID: 19223544 DOI: 10.1158/0008-5472.CAN-08-2466]

71 **Prenen H**, De Schutter J, Jacobs B, De Roock W, Biesmans B, Claes B, Lambrechts D, Van Cutsem E, Tejpar S. PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. *Clin Cancer Res* 2009; **15**: 3184-3188 [PMID: 19366826 DOI: 10.1158/1078-0432.CCR-08-2961]

72 **Ogino S**, Nosho K, Kirkner GJ, Shima K, Irahara N, Kure S, Chan AT, Engelman JA, Kraft P, Cantley LC, Giovannucci EL, Fuchs CS. PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. *J Clin Oncol* 2009; **27**: 1477-1484 [PMID: 19237633 DOI: 10.1200/JCO.2008.18.6544]

73 **Cappuzzo F**, Varella-Garcia M, Finocchiaro G, Skokan M, Gajapathy S, Carnaghi C, Rimassa L, Rossi E, Ligorio C, Di Tommaso L, Holmes AJ, Toschi L, Tallini G, Destro A, Roncalli M, Santoro A, Jänne PA. Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. *Br J Cancer* 2008; **99**: 83-89 [PMID: 18577988 DOI: 10.1038/sj.bjc.6604439]

74 **Tol J**, Dijkstra JR, Klomp M, Teerenstra S, Dommerholt M, Vink-Börger ME, van Cleef PH, van Krieken JH, Punt CJ, Nagtegaal ID. Markers for EGFR pathway activation as predictor of outcome in metastatic colorectal cancer patients treated with or without cetuximab. *Eur J Cancer* 2010; **46**: 1997-2009 [PMID: 20413299 DOI: 10.1016/j.ejca.2010.03.036]

75 **Bardelli A**, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. *J Clin Oncol* 2010; **28**: 1254-1261 [PMID: 20100961 DOI: 10.1200/JCO.2009.24.6116]

76 **Cully M**, You H, Levine AJ, Mak TW. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat Rev Cancer* 2006; **6**: 184-192 [PMID: 16453012 DOI: 10.1038/nrc1819]

77 **Di Cristofano A**, Pandolfi PP. The multiple roles of PTEN in tumor suppression. *Cell* 2000; **100**: 387-390 [PMID: 10693755]

78 **Goel A**, Arnold CN, Niedzwiecki D, Carethers JM, Dowell JM, Wasserman L, Compton C, Mayer RJ, Bertagnolli MM, Boland CR. Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. *Cancer Res* 2004; **64**: 3014-3021 [PMID: 15126336]

79 **Molinari F**, Martin V, Saletti P, De Dosso S, Spitale A, Camponovo A, Bordoni A, Crippa S, Mazzucchelli L, Frattini M. Differing deregulation of EGFR and downstream proteins in primary colorectal cancer and related metastatic sites may

be clinically relevant. *Br J Cancer* 2009; **100**: 1087-1094 [PMID: 19293803 DOI: 10.1038/sj.bjc.6604848]

80 **Santini D**, Loupakis F, Vincenzi B, Floriani I, Stasi I, Canestrari E, Rulli E, Maltese PE, Andreoni F, Masi G, Graziano F, Baldi GG, Salvatore L, Russo A, Perrone G, Tommasino MR, Magnani M, Falcone A, Tonini G, Ruzzo A. High concordance of KRAS status between primary colorectal tumors and related metastatic sites: implications for clinical practice. *Oncologist* 2008; **13**: 1270-1275 [PMID: 19056857 DOI: 10.1634/theoncologist.2008-0181]

81 **Laurent-Puig P**, Cayre A, Manceau G, Buc E, Bachet JB, Lecomte T, Rougier P, Lievre A, Landi B, Boige V, Ducreux M, Ychou M, Bibeau F, Bouché O, Reid J, Stone S, Penault-Llorca F. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. *J Clin Oncol* 2009; **27**: 5924-5930 [PMID: 19884556 DOI: 10.1200/JCO.2008.21.6796]

82 **Tveit KM**, Guren T, Glimelius B, Pfeiffer P, Sorbye H, Pyrhonen S, Sigurdsson F, Kure E, Ikdahl T, Skovlund E, Fokstuen T, Hansen F, Hofsli E, Birkemeyer E, Johnsson A, Starkhammar H, Yilmaz MK, Keldsen N, Erdal AB, Dajani O, Dahl O, Christoffersen T. Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. *J Clin Oncol* 2012; **30**: 1755-1762 [PMID: 22473155 DOI: 10.1200/JCO.2011.38.0915]

83 **Douillard J**, Zemelka T, Fountzilas G. Randomized phase II study evaluating UFOX plus cetuximab vs FOLFOX4 plus cetuximab as first-line therapy in metastatic colorectal cancer. *Ann Oncol* 2012; **23** (Suppl 4): Abstr0-0017

84 **Bokemeyer C**, Bondarenko I, Hartmann JT, de Braud F, Schuch G, Zubel A, Celik I, Schlichting M, Koralewski P. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol* 2011; **22**: 1535-1546 [PMID: 21228335 DOI: 10.1093/annonc/mdq632]

85 **Moosmann N**, von Weikersthal LF, Vehling-Kaiser U, Stauch M, Hass HG, Dietzfelbinger H, Oruzio D, Klein S, Zellmann K, Decker T, Schulze M, Abenhardt W, Puchtler G, Kappauf H, Mittermüller J, Haberl C, Schalhorn A, Jung A, Stintzing S, Heinemann V. Cetuximab plus capecitabine and irinotecan compared with cetuximab plus capecitabine and oxaliplatin as first-line treatment for patients with metastatic colorectal cancer: AIO KRK-0104--a randomized trial of the German AIO CRC study group. *J Clin Oncol* 2011; **29**: 1050-1058 [PMID: 21300933 DOI: 10.1200/JCO.2010.31.1936]

86 **Folprecht G**, Gruenberger T, Bechstein WO, Raab HR, Lordick F, Hartmann JT, Lang H, Frilling A, Stoehlmacher J, Weitz J, Konopke R, Stroszczynski C, Liersch T, Ockert D, Herrmann T, Goekkurt E, Parisi F, Köhne CH. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. *Lancet Oncol* 2010; **11**: 38-47 [PMID: 19942479 DOI: 10.1016/S1470-2045(09)70330-4]

87 **Razis E**, Briasoulis E, Vrettou E, Skarlos DV, Papamichael D, Kostopoulos I, Samantas E, Xanthakis I, Bobos M, Galanidi E, Bai M, Gikonti I, Koukouma A, Kafiri G, Papakostas P, Kalogeras KT, Kosmidis P, Fountzilas G. Potential value of PTEN in predicting cetuximab response in colorectal cancer: an exploratory study. *BMC Cancer* 2008; **8**: 234 [PMID: 18700047 DOI: 10.1186/1471-2407-8-234]

P- Reviewer: Serafino A **S- Editor**: Zhai HH
L- Editor: O'Neill M **E- Editor**: Wang CH

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327